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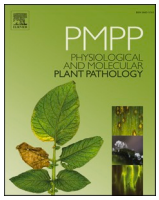

Unveiling the invisible threat: cutting-edge methods for detecting fungal and oomycete plant pathogens and mycotoxins

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ABSTRACT

Early detection of fungal and oomycete pathogens and accurate monitoring of mycotoxins are critical for ensuring food safety and sustaining agricultural productivity. This review provides a critical overview of current diagnostic tools, covering traditional morphological assessments, serological assays, and advanced molecular approaches such as PCR variants, isothermal amplification (LAMP, RPA), DNA hybridization, and next-generation sequencing. Analytical strategies for mycotoxin detection are also described, including solvent-based extraction, immunoassays, and liquid chromatography coupled with mass spectrometry, alongside emerging biosensor technologies. A distinctive contribution of this review is in the comparative assessment of these methodologies based on shared performance criteria sensitivity, specificity, turnaround time, field applicability, and cost, offering a transversal framework for method selection across phytosanitary contexts. Special attention is given to quarantine pathogens and mycotoxigenic fungi affecting the citrus supply chain, where early and accurate diagnosis is pivotal to prevent yield losses and trade restrictions. The review highlights that method selection should be guided by the specific diagnostic objective, available resources, and regulatory requirements, with integrated strategies often representing the most practical solution to ensure both food safety and phytosanitary security.

1. Introduction

Phytopathogenic fungi and oomycetes pose significant threats to global agriculture, causing severe losses in crop yield and quality [1–3]. Addressing these challenges requires precise and rapid detection methods for effective disease management. DNA-based methods have become increasingly popular for plant disease diagnosis due to their accuracy and specificity [4].

Fungi, in the broad sense of the term which includes both true fungi (fungi *sensu stricto*) and oomycetes, are the most numerous groups of plant pathogens [5]. Many of these microorganisms, which have been identified as causative agents of plant disease such as rusts, smuts, powdery mildews, downy mildews, fruit and vegetable rots, foot and root rots, wilts, anthracnoses and scabs, are responsible for severe pre- and post-harvest crop losses globally. Moreover, some fungi, particularly those belonging to the genera *Alternaria, Aspergillus, Fusarium* and *Penicillium*, produce noxious secondary metabolites collectively called mycotoxins, that may contaminate plant products and foods [6,7]. A few destructive fungal and oomycete plant pathogens are included in the lists of organisms of quarantine concern as they are considered a threat for agriculture and nature conservation. Restrictions are imposed to the trade of plants and plant products to prevent the spread of these noxious organisms. To this purpose, national phytosanitary services act to intercept them, primarily at territorial boundaries, to prevent their introduction and spread. Detection and identification of plant pathogenic fungi and mycotoxins have relevant implications for both food

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safety and human health [4,8]. In recent decades, the detection of plant pathogens has seen the development of increasingly sensitive and state-of-the-art methods [9–12]. Classical methods, based on observation of symptoms and isolation of the pathogen in the laboratory, are being replaced by new and rapid and cost-effective methods. Furthermore, due to the climate change in the last period, microorganisms considered until lately as secondary pathogens, are nowadays considered as emerging primary pathogens that may spread and threaten new areas [13–15]. Hence, a rapid detection is essential not only for identifying the cause of a disease, but also to facilitate the development and application of precise control strategies and reduce considerably intervention times [16]. Currently, conventional molecular methods are widely used for their accuracy and reliability [17,18]. However, these methods require unique, approved facilities, dedicated

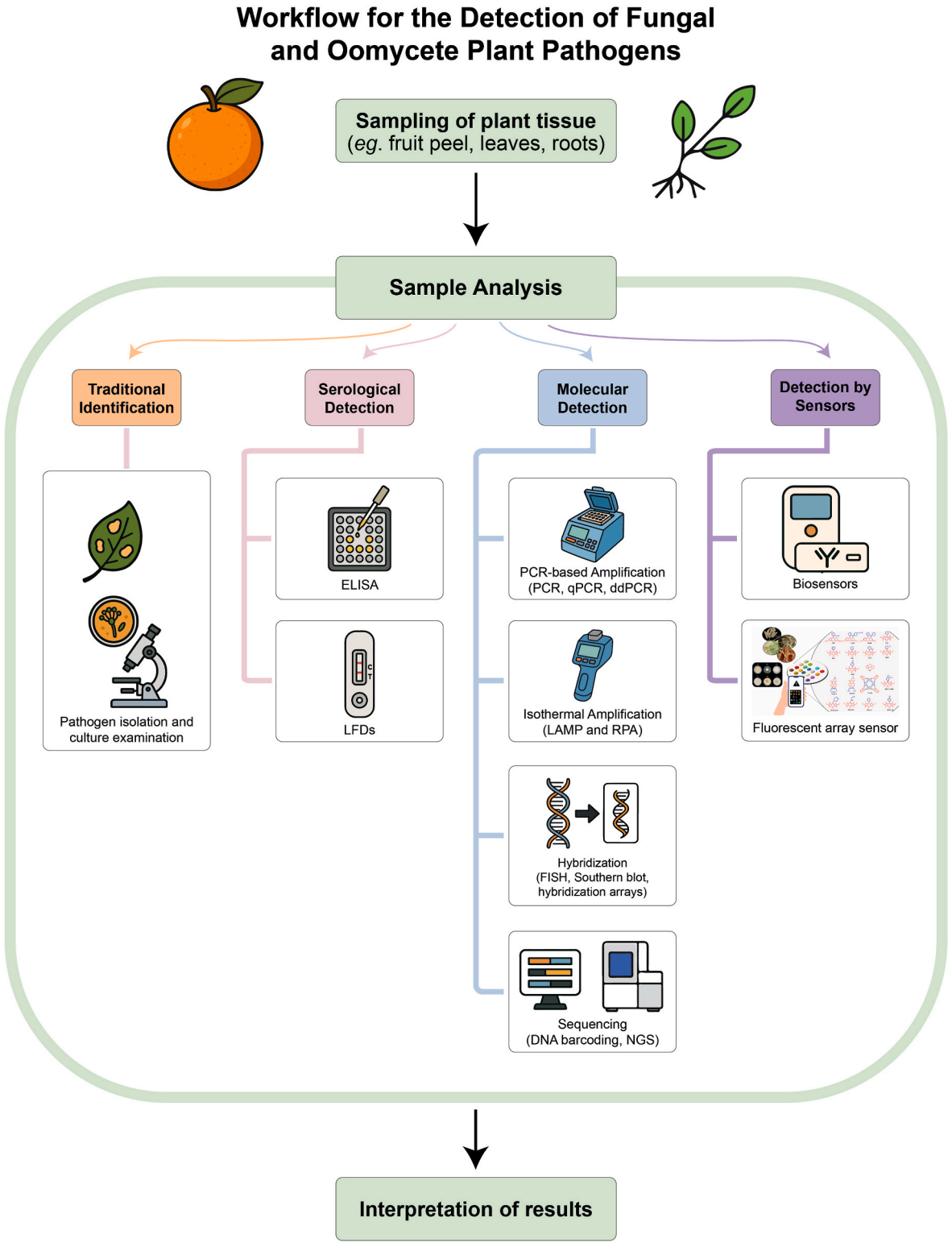

Fig. 1. Overview of diagnostic workflows for fungal and oomycete plant pathogens, from sampling to identification using traditional, serological, molecular, and biosensor-based methods.

equipment, and professional, skilled personnel to perform, while they are time-consuming and expensive.

This review aims to report the most effective methods currently available for detecting fungal and oomycete plant pathogens and their mycotoxins. It also includes a comparative assessment of these diagnostic approaches based on shared performance criteria, to support method selection in different practical contexts. Moreover, it provides a brief overview of phytopathological concerns of the citrus supply chain in the EPPO region, with a special focus on fungal and oomycete quarantine pathogens and mycotoxigenic fungi.

2. Detection of fungal and oomycete plant pathogens

Diagnostic approaches for fungal and oomycete pathogens have evolved from traditional morphology-based methods to advanced molecular and biosensor technologies. Traditional techniques, including symptom observation and pathogen isolation, remain foundational but are complemented by serological assays for rapid antigen detection and by molecular tools for highly specific and sensitive identification. More recently, biosensor platforms have emerged as portable solutions for field diagnostics. This section systematically explores these methods, outlining their principles, advantages, and limitations across different phytosanitary context (Fig. 1).

2.1. Traditional identification methods

Traditional methods for identifying disease-causing plant pathogens rely on visual examinations, culturing of the pathogen on artificial media and morphological (macro- and microscopic) identification [19]. Visual examination involves assessing symptoms and signs of the disease on the plant indicating the presence of a specific pathogen; for instance, the presence of leaf spots, blights, wilts, and mold growth are indicative of fungal/oomycete infections [16,20]. Culturing the pathogen involves isolating it from fresh plant tissue. For this purpose, in most cases, the infected plant material is surface-sterilized and placed on an artificial nutrient medium to stimulate fungal growth and sporulation [21,22]. Morphological identification involves the visual examination of the macro-morphological features and microscopic observations of micro-structures of the pathogen. For fungal and oomycete pathogens, this includes evaluating vegetative as well as sexual and asexual reproductive structures, such as gametangia, gametocysts, spore morphology, sporulation patterns, and the bi-dimensional arrangement of the mycelium [23,24]. Traditional method for pathogen identification has several limitations. One of the most relevant is the issue of variability and repeatability: laboratory identification of the pathogen is influenced by various factors, such as the type of substrate used, growth conditions, light exposure, and temperature; these variables can lead to significant variability and reduce the repeatability of results [23,25]. The ‘requirement of expertise’ includes accurate visual identification, time and microbiological skills, making the diagnosis laborious and time-consuming, and thus impractical for prompt disease management or interception of pathogens of quarantine concern [26]. A major limit of culture-based methods is the inapplicability to isolate obligate biotrophic plant pathogens onto artificial medium. Additionally, the morphological identification alone often fails to resolve taxa at the species level, as closely related species can share highly similar macro- and micro-morphological traits. Despite these challenges, culture methods remain widely used because they provide reliable and detailed descriptions of the physiological and morphological characteristics of fungal pathogens, encompassing both macroscopic colony features and microscopic structural traits.

2.2. Serological detection methods

Serological-based approaches rely on the specific recognition of pathogen antigens by antibodies [27,28]. Serological tests have been used to detect numerous plant pathogens [14,29,30]. ELISA [31] methods (Enzyme-Linked Immunosorbent Assay) are the simplest, fastest, and among the most sensitive immunological tests available. ELISA is widely used for viruses, less so for bacteria, and rarely for fungi [32].

Lateral Flow Devices (LFDs) were among the first widely used in-field test formats, offering simplicity and speed, taking less than 10 min, based on serological reagents [32]. LFDs use a porous nitrocellulose membrane with species-specific antibodies immobilized in a band and have been developed for viruses, bacteria, and some fungi like *Aspergillus* spp. [33]. Serological methods, such as ELISA and LFDs, have introduced speed and simplicity into pathogen detection; however, the low sensitivity of LFDs, which is lower than that of molecular methods, has limited their routine use and widespread adoption, particularly for fungal pathogens [34,35]. Despite these limitations, serological methods remain historically important and are still relevant in contexts where rapid and low-cost testing is required. For a much-detailed review of these methods see Cassedy et al. (2021) [34].

2.3. DNA-based detection methods

The advent of DNA-based methods, also known as molecular methods, has revolutionized the detection and identification of plant pathogens. Indeed, DNA sequences are excellent molecular markers for the detection and identification of pathogenic microorganism. The presence of a genetic sequence, which is unique to the target pathogen, can be detected either through its amplification by polymerase chain reaction (PCR)-based techniques, isothermal amplification techniques, or by hybridization-based techniques.

A prerequisite for DNA-based detection methods is the isolation, purification, and quantification of DNA [26,34,36]. Numerous protocols have been developed for fungal pathogens, typically involving freeze-drying of mycelium, disruption of the chitin cell wall (e.g., by milling), and DNA extraction in chemical buffers, often followed by protein removal with phenol–chloroform and DNA precipitation with propanol [37,38]. Although commercial extraction kits are increasingly popular due to convenience and reliability, many laboratories still employ these standard protocols, mostly because of their reduced cost. Purification methods, such as pellet collection, silica membrane columns, spin filters, or silica-coated magnetic particles, are then applied to remove contaminants and ensure DNA quality for downstream analyses [38–40]. The choice of extraction and purification method depends on the requirements of subsequent applications, available equipment, and operator expertise [40].

2.3.1. Polymerase chain reaction (PCR)-based techniques

Polymerase chain reaction (PCR) is a pivotal technique enabling the amplification of target nucleic acid fragments using oligonucleotide primers, a DNA polymerase enzyme, dNTPs, and a thermal cycler [41]. Detection of the PCR fragment (amplicons) of the expected size serves as confirmation of the target pathogen’s presence [42,43]. Conventional PCR typically employs endpoint detection methods such as agarose gel electrophoresis. Various variants of conventional PCR enhance specificity and sensitivity: Nested PCR utilizes two consecutive PCR reactions, ideal for detecting pathogens at low levels. Multiplex PCR enables simultaneous amplification of multiple target DNA sequences in a single reaction, facilitating the detection of several pathogens [42].

2.3.1.1. Quantitative real-time PCR (qPCR). The quantitative real-time PCR (qPCR) is an advanced technique that visualizes the accumulation of the amplified molecules during the amplification process [44]. Unlike conventional PCR, where products are detected during the plateau phase, qPCR provides real-time information on the initial concentration of the target molecules [42]. This is achieved through the use of fluorophores and quenchers in specific techniques such as TaqMan,

Molecular Beacon, and Scorpion, which emit fluorescent signals only when the molecular probe hybridizes with the target DNA, separating the fluorophore from the quencher [18]. The fluorescence intensity correlates with the number of amplicons generated, allowing for precise quantification of DNA. The SYBR Green I method is advantageous due to its simplicity and low cost but can generate false positives if non-specific sequences are amplified. The widespread adoption of qPCR as a diagnostic tool has been facilitated by the development of portable and rapid thermocyclers such as RAPID and Smart Cycler, initially designed for military applications to identify biological warfare agents [45]. qPCR operates on the same principle as conventional PCR, with the main difference being that the amplified DNA is measured in real-time during the reaction [42]. This allows for more sensitive and precise detection of the target DNA, eliminating the need for post-amplification steps and supporting high-throughput testing. Two main chemistries are used: non-specific (SYBR Green I) and specific (TaqMan, Molecular Beacons, Scorpion-PCR) [41].

One of the major limits of most molecular diagnostic approaches based on DNA sequencing and amplification is their inability to differentiate between dead and viable pathogens. To circumvent this limit, a strategy for the detection of plant pathogens, based on the use of mRNA as a viability marker was proposed. A real-time reverse-transcription PCR (RT-PCR) assay targeting the mRNA of the subunit I of the *cytochrome oxidase* gene was designed for *Phytophthora ramorum*, the causative agent of sudden oak death and ramorum blight [46,47].

2.3.1.2. Digital droplet PCR. Droplet digital PCR (ddPCR) is a next-generation quantitative PCR technique and a refinement of traditional polymerase chain reaction (PCR) methods [48]. It allows for the direct quantification and clonal amplification of nucleic acid strands, including DNA, cDNA, or RNA. RNA-based ddPCR offers high sensitivity, precision, and reproducibility [48–50]. Recently, the development of low-cost, portable, and user-friendly ddPCR devices has become a research hotspot due to the increasing demand for point-of-care detection and clinical diagnosis. A key feature of ddPCR is the use of microfluidic technology, which partitions the PCR mixture into thousands of uniform water-in-oil droplets that act as individual amplification chambers. This miniaturization and compartmentalization, achieved through microfluidic channels, ensures uniform droplet size, reduces reagent consumption, and enables precise downstream detection and quantification of amplified targets [51]. The ddPCR workflow consists of four main steps: droplet production, signal amplification, signal detection, and signal quantification. Digital droplet PCR (ddPCR) offers several advantages over real-time PCR (qPCR) [43,48]. ddPCR does not require a calibration curve for quantification, enabling direct measurement of DNA. This improves the reliability of quantification, as real samples can exhibit different amplification efficiencies compared to those used for calibration curves [50,52]. Additionally, ddPCR is more sensitive and more resistant to PCR inhibitors than qPCR [52]. And yet this method has not become widespread and widely used, mainly due to the high cost of the materials required for its implementation.

2.3.2. Isothermal amplification-based techniques

In contrast to PCR-based techniques, which require cycling between high and low temperatures, Isothermal amplification-based techniques make possible the DNA amplification at a single, constant (isothermal) temperature [53]. Within the options of isothermal technologies developed for the detection of pathogens, the loop-mediated isothermal amplification (LAMP) and the recombinase polymerase amplification (RPA) stand out for their simplicity, specificity, high sensitivity, and rapidity [54–56].

2.3.2.1. Loop-mediated isothermal amplification (LAMP). Loop-mediated isothermal amplification (LAMP) stands as a widely acknowledged method for DNA/RNA amplification, esteemed for its efficiency and robustness [56,57]. This technique relies on a combination of at least four primers (two inner and two outer) and a strand-displacing DNA polymerase to yield single-stranded DNA structures featuring dumbbell-like formations at both ends due to intramolecular complementarity [57,58]. The inner primers trigger self-priming, while the strand-displacing polymerase continuously generates fresh amplified DNA, yielding elongated concatemers of the target DNA segment [57,58]. Incorporating two loop primers can expedite the amplification process by targeting loop regions and providing additional polymerase initiation sites [57,59].

Operated at a consistent temperature range of 60–65 °C, LAMP typically provides results within 30 min and offers visualization through diverse methods, including gel electrophoresis, turbidity measurements, fluorescent DNA binding dyes, and lateral flow devices [56,58,60,61]. Employing a closed-tube strategy minimizes contamination risks by employing DNA-intercalating dyes or metal ion indicators such as SYBR Green, EvaGreen, calcein, and Hydroxynaphthol blue (HNB) [56,62,63]. While false positives may arise due to non-specific products, sequence-specific detection methods employing fluorescent probes can mitigate this issue, albeit at higher expenses [64].

LAMP's simplicity and resilience against PCR inhibitors makes it suitable for field applications with minimal sample preparation, often necessitating only crude extracts [57,65,66]. Though traditionally qualitative, recent advancements suggest quantification is achievable by correlating signal threshold times with initial DNA quantities, using tools like smartphone cameras or portable fluorescence devices. However, its quantification precision remains lower than qPCR, particularly at low concentration ranges [65,67]. LAMP assays have been tailored for various plant pathogens, showcasing notable sensitivity and swift detection capabilities [2,58,64,68–70]. A noteworthy instance includes a smartphone-based multiplex LAMP assay, effectively detecting *Phytophthora infestans* and tomato spotted wilt virus [71].

2.3.2.2. Recombinase polymerase amplification (RPA). RPA isothermal amplification technology is based on the recombinase-mediated hybridization of oligonucleotide primer pairs with double-stranded (ds) DNA target fragments [64]. At its core, RPA employs three key enzymes: i) a recombinase, ii) a single-stranded DNA-binding protein (SSB), and iii) a strand-displacing polymerase. The amplification process initiates as the recombinase binds to the primers, forming a recombinase/primers chimera that pairs with homologous sequences on the dsDNA target. The SSB then stabilizes this complex, while the strand-displacing polymerase catalyzes DNA synthesis from the primer binding sites [55,72]. This exponential amplification occurs through cyclic repetition of these steps at a constant, low temperature typically between 25 and 42 °C [64,72]. The simplicity of RPA's thermal requirements enables its utilization in portable thermal assay devices, rendering it both technically straightforward and cost-effective [2,55,73,74].

The specificity of RPA is ensured by employing primers of 28–35 nucleotides, with a GC content between 30 % and 70 %, and avoiding tandem repeats [73,75]. Furthermore, the chemistry involved in detecting the generated amplicons enhances specificity. Optimal RPA assays feature probes containing a tetrahydrofuran (THF) abasic site flanked by fluorophore-modified nucleotides (typically FAM) and a quencher, with a 3′-end block to prevent the probe from priming amplification. This probe chemistry adds another layer of specificity validation, as the probe's specific binding to complementary DNA activates an endonuclease, resulting in measurable fluorescence upon cleavage at the THF site [72].

RPA has been successfully used for the development of early detection assays across a spectrum of plant pathogens, including oomycetes [76,77], and fungi [49,56,67,78–80].

2.3.3. DNA hybridization

DNA hybridization is a detection technology based on the ability of

DNA strands to hybridize with their complementary sequences. This technique utilizes labeled DNA probes for hybridization [81]. Probes are nucleic acids tagged with either radioactive or nonradioactive markers that complement the nucleic acid sequence under investigation. The marker within the probe allows the double-stranded molecule formed during hybridization to become visible. Consequently, the labeled probe enables the identification of the target DNA or RNA in an unlabeled mixture containing the DNA or RNA of interest [82]. Detection of hybridization products can be either direct or indirect. For direct and *in situ* detection, radioactive and fluorescent probes are used, a process known as Fluorescence *In Situ* Hybridization (FISH). Indirect detection employs enzyme reporters on solid media membranes such as nitrocellulose or nylon, as well as polymer particles. DNA blotting techniques, specifically Southern and Northern blotting, are the most common methods for indirect detection [83].

2.3.3.1. Fluorescence in situ hybridization (FISH). FISH is an advanced method for the *in situ* detection of actively growing organisms in environmental samples [84]. This technique can visualize the exact location of specific DNA or RNA sequences within the cytoplasm, organelles, or nuclei of biological materials. As a result, FISH can detect metabolically active fungi directly in the environment without the need for cultivation when RNA is present. The primary steps of FISH include preparing biological materials or environmental samples, labeling a nucleic acid sequence with a fluorescent marker to form a probe, and then hybridizing the probe to the DNA or RNA in biological materials under controlled experimental conditions to form a double-stranded molecule. Finally, the sites of hybridization are detected and visualized [85]. The first FISH probe targeting a living fungus was designed to detect *Aureobasidium pullulans* on the phylloplane of apple seedlings [86].

2.3.3.2. Nucleic acid blotting. The DNA blot, developed by Southern in 1975 [67], has long been considered the method of choice for reliable detection of specific DNA sequences among researchers [87]. In this technique, negatively charged, purified nucleic acid from prokaryotic or eukaryotic cells is separated by size using electrophoresis through an agarose gel matrix. The denatured nucleic acid is then transferred and immobilized onto a membrane made of nitrocellulose or nylon. The nucleic acids on the membrane are hybridized with a specific labeled probe, consisting of homologous single-stranded nucleic acids that carry molecules for detection and visualization. Hybridization between the immobilized nucleic acids and the labeled probe allows for the detection of specific DNA (Southern blot technique) or RNA (Northern blot technique) sequences within a complex mixture. The detection and visualization method depends on the nature of the labeled probe: radioactive probes enable autoradiographic detection, while enzyme-labeled probes facilitate chemiluminescent or colorimetric detection [87–89].

2.3.3.3. Hybridization arrays. The hybridization arrays allow the simultaneous detection of numerous (virtually unlimited) pathogens [90]. Array-based methods, including microarrays, microarrays, and the Luminex system, involve immobilizing many sequence-specific capture probes on a solid support [26]. These assays use a reverse hybridization approach, labeling target DNA instead of immobilized detector probes with fluorescence, radioisotopes, or enzyme tags. DNA is extracted from the sample, amplified, labeled, denatured and hybridized with the detector oligonucleotides on the solid support. The bound labeled amplicons reveal which DNA sequences and target pathogens are present in the sample. Microarrays and macroarrays are well-known hybridization arrays. The main differences between them lie in the density and number of immobilized probes and detection methods [91,92]. Microarrays have thousands of densely packed probe spots less than 200 μm in diameter on a glass slide. Detection typically involves fluorescent probes and laser-induced fluorescence with a scanning confocal microscope [93]. Macroarrays have larger spot sizes, greater than 300 μm in diameter, on a nylon membrane, with detection usually performed using chemiluminescent labels [45,94]. The Luminex xMAP system uses detector oligonucleotides bonded to microspheres, distinguishable by unique spectral properties [95]. Unlike flat support arrays with localized spots, the Luminex system is suspension-based, simplifying use, reducing costs and improving hybridization kinetics. You can use up to 100 different microbeads at the same time. After hybridization of the labeled target DNA with the respective microbead, the individual beads are analyzed as they pass through a series of lasers. The spectral properties identify the specific detector oligonucleotide, while the fluorescent signal indicates the presence of a complementary sequence and the target pathogen. Although hybridization arrays have the advantage of targeting multiple pathogens simultaneously, they require prior knowledge of the genetic sequences of the target pathogens [93]. The accuracy of the results depends on the severity of the hybridization conditions, which require careful optimization [96]. Furthermore, although microarrays are generally automated, macroarrays require a lot of labor and time, increasing costs. Several arrays have been developed to detect bacterial, fungal, and viral plant pathogens [45,92,94,97–100]. Commercial kits are available, such as a macroarray capable of simultaneously detecting eight potato viruses (BIOREBA) their use has decreased in recent years due to decreasing sequencing costs [93].

2.3.4. Barcode sequencing and next-generation sequencing (NGS)

Unlike purely amplification-based diagnostics, sequencing-based approaches such as DNA barcoding enable species-level identification by amplifying and sequencing short marker regions and comparing them with curated databases [101]. The core concept of DNA barcoding is to utilize a small DNA sequence from a sample for identification purposes. To serve as an effective barcode, a gene or genomic region must meet certain criteria: it should be universal and carry sufficient phylogenetic information, exhibit significant sequence variability, maintain sequence conservation in flanking regions, and be of manageable length for amplification, sequencing, and analysis [102]. The Internal Transcribed Spacer (ITS) region of ribosomal DNA stands out as the primary barcode for fungi and oomycetes identification, comprising ITS1 and ITS2 flanking the conserved 5.8S region. ITS possesses key characteristics, including a conserved region with a hypervariable segment, small size (ranging from 500 to 800 bp), and universality. ITS1 generally offers better resolution for Basidiomycetes, while ITS2 excels for Ascomycete [103,104]. Additional effective barcodes for fungi and oomycetes include cytochrome *c* oxidase subunit I (COI), nuclear ribosomal subunits, RNA polymerase II subunits, elongation factor 1-α, mitochondrial ribosomal operon subunits, actin, chitin synthase, calmodulin, and heat shock protein 90 [51,53,80,105–110]. Studies have shown that these genetic markers often provide superior species resolution compared to ITS [101]. To facilitate the accurate identification of some important and large groups of plant pathogens, like *Fusarium* and *Phytophthora*, web-accessible databases of phylogenetically informative gene sequences are available [111–113].

Next-generation sequencing (NGS), also known as high throughput sequencing (HTS), is a cutting-edge approach for diagnostics, enabling innovative detection and identification of phytopathogens [114]. The key steps in DNA-based NGS include DNA isolation and fragmentation, library preparation, massive parallel sequencing, bioinformatics analysis, and variant/mutation annotation and interpretation [115]. Advanced HTS methods include massively parallel signature sequencing, pyrosequencing, polony sequencing, and sequencing by oligonucleotide ligation detection (SOLID) [94]. RNA-Sequencing (RNA-Seq) provides advanced coverage and greater resolution of the transcriptome's dynamic nature. The Illumina HiSeq platform is the most widely used NGS platform for RNA-Seq, setting the standard for NGS, with its more recent release being the MiSeq desktop sequencer [116]. More recently, Oxford Nanopore Technologies (ONT) has emerged as a complementary platform offering long-read, real-time sequencing with portable devices suitable for field-based pathogen

diagnostics [117].

2.3.5. General considerations on DNA-based methods

DNA-based methods have transformed plant pathogen diagnostics by providing highly specific and sensitive detection capabilities. However, while their advantages are considerable, each methodological group also presents intrinsic limitations that influence their applicability in routine and large-scale contexts.

PCR-based methods, including conventional PCR, qPCR, and ddPCR, remain the benchmark for diagnostic reliability and quantitative accuracy. Their main strengths lie in the ability to detect low levels of target DNA and provide species-level identification. Yet, these methods require sophisticated thermal cyclers, controlled laboratory environments, and trained personnel, which can limit their use outside centralized facilities. Moreover, DNA-based assays do not discriminate between viable and non-viable cells, potentially leading to false-positive results in certain applications.

Isothermal amplification techniques such as LAMP and RPA represent a significant advance toward field-applicable diagnostics. Their ability to operate at constant temperatures eliminates the need for complex equipment and allows rapid detection directly from crude extracts. Nevertheless, despite their robustness to inhibitors, these techniques can suffer from non-specific amplification and may require meticulous primer design to ensure reliability. Quantitative assessment with LAMP and RPA also remains less precise compared to qPCR.

DNA hybridization methods, including FISH, Southern/Northern blotting, and hybridization arrays, provide high specificity and the capacity to screen multiple targets simultaneously. Hybridization arrays, in particular, enable parallel detection of a broad spectrum of pathogens. However, these approaches are labor-intensive, require prior knowledge of target sequences, and can be cost-prohibitive, especially when high-density arrays are employed.

Next-generation sequencing (NGS) offers unprecedented insights into pathogen populations, diversity, and evolutionary dynamics, enabling comprehensive diagnostic surveys. It allows the detection of both known and novel pathogens without prior target-specific probes. The availability of both short-read platforms (e.g., Illumina) and long-read platforms (e.g., Oxford Nanopore Technologies) broadens the range of possible applications but also introduces variability in accuracy, costs, and data analysis requirements. Despite these advances, NGS overall entails high costs, complex bioinformatics, and significant computational resources, which currently limit its routine use in standard diagnostic laboratories. Furthermore, interpreting metagenomic data can be challenging, particularly in distinguishing pathogenic from non-pathogenic taxa in environmental samples.

Overall, the choice of a DNA-based method should be guided by the diagnostic objective, required resolution, available infrastructure, and context of application (e.g., laboratory-based screening vs. on-site field testing). In many scenarios, an integrated approach that combines rapid field assays with confirmatory laboratory-based techniques provides the most robust and practical solution.

2.4. Biosensors and fluorescent array sensors

Biosensors have emerged as promising tools for point-of-care applications due to their low cost, ease of use, and ability to provide rapid results [118,119]. Biosensors typically consist of a biorecognition element linked to a physicochemical transducer, which generates a measurable signal when the target analyte binds to the biorecognition element [117,118,120]. Based on these fundamental components, three types of biosensors have been developed: i. electrochemical transducers detect binding events through changes in voltage, impedance, or conductance; ii. mass-based transducers detect changes in resonant frequency caused by the mass change when the target analyte binds to the biorecognition element; iii. optical transducers detect variations in the reflectance of incoming light following the binding of a target analyte to the biorecognition element [117,118]. The peculiarities of biosensors are determined by the simplicity of the elements that constitute them and their easy use. The support, generally inert, serves to contain the receptors that will subsequently react with the analyte sample; these receptors have a role in selecting the most suitable biorecognition element. The immobilization of biorecognition elements on the sensitive surface plays an important role in the effectiveness of biosensors [121]. There are several immobilization methods such as adsorption-based techniques, covalent attachment, avidin and biotin systems as well as self-assembled monolayers. The variety of different transducers and biorecognition elements allows the development of a wide range of different types of biosensors that can be used for the detection of plant pathogens [122]. The world of sensors is diverse and is advancing at a rapid pace due to the fact of its high demand and constant technological improvements [123]. The electrochemical sensors provide a low-cost and convenient solution for the detection of variable analytes and are widely utilized in agriculture, food, and oil industries as well as in environmental and biomedical applications. The electrochemical transducer reports changes in the shape of the electrical signal which is directly proportional to the concentration of the analyte. The binding of the analyte causes an ion discharge, which can then be measured as current or voltage, using appropriate transducers [123]. The optical biosensors detect changes in the phase, polarization, or frequency of the optical field within a biorecognition element when it interacts with an analyte [124]. These biosensors are categorized based on their transduction mechanisms into absorption, fluorescence, and luminescence-based types. Adsorption-based biosensors measure changes in light amplitude as it interacts with the sensing surface, comparing the bare surface to one with analytes present. This amplitude change can be correlated to determine the analyte concentration in a solution. When using visible light, these are known as colorimetric biosensors. Colorimetric biosensors that can be read by the naked eye, typically labeled, are ideal for low-resource settings as they do not require instrumentation [44,116].

Also, the fluorescent array sensor is designed to contain molecular probes with different excitation/emission ranges and the ability to establish various non-covalent interactions with analytes, which should be specific to each target pathogen. Specifically, four different classes of chromophores will be selected and analyzed. These probes possess multiple recognition elements capable of interacting with analytes through non-covalent interactions, including hydrogen bonds, ion-dipole interactions, dipole-dipole interactions, and the hydrophobic effect. The synthesis of these probes is performed using straightforward synthetic protocols to detect the selected pathogens [125–131].

3. Detection of mycotoxins

Among the secondary metabolites produced by phytopathogenic fungi, mycotoxins represent a major concern for food safety due to their toxicity to humans and animals and their frequent occurrence in both cereals and fruit-derived commodities [132]. Their detection is therefore a critical step in assessing contamination levels and preventing exposure along the food chain. Reliable analytical workflows must combine efficient extraction of target compounds from complex matrices with accurate quantification, while meeting the regulatory limits established for food and feed. The following sections summarize and critically discuss the main approaches for mycotoxin analysis, including extraction procedures, immunological assays, chromatographic techniques coupled with mass spectrometry, and emerging technologies (Fig. 2).

3.1. Extraction methods

Efficient extraction of mycotoxins from food and feed matrices is essential for achieving accurate quantification and minimizing matrix effects during subsequent analytical procedures [133]. The most

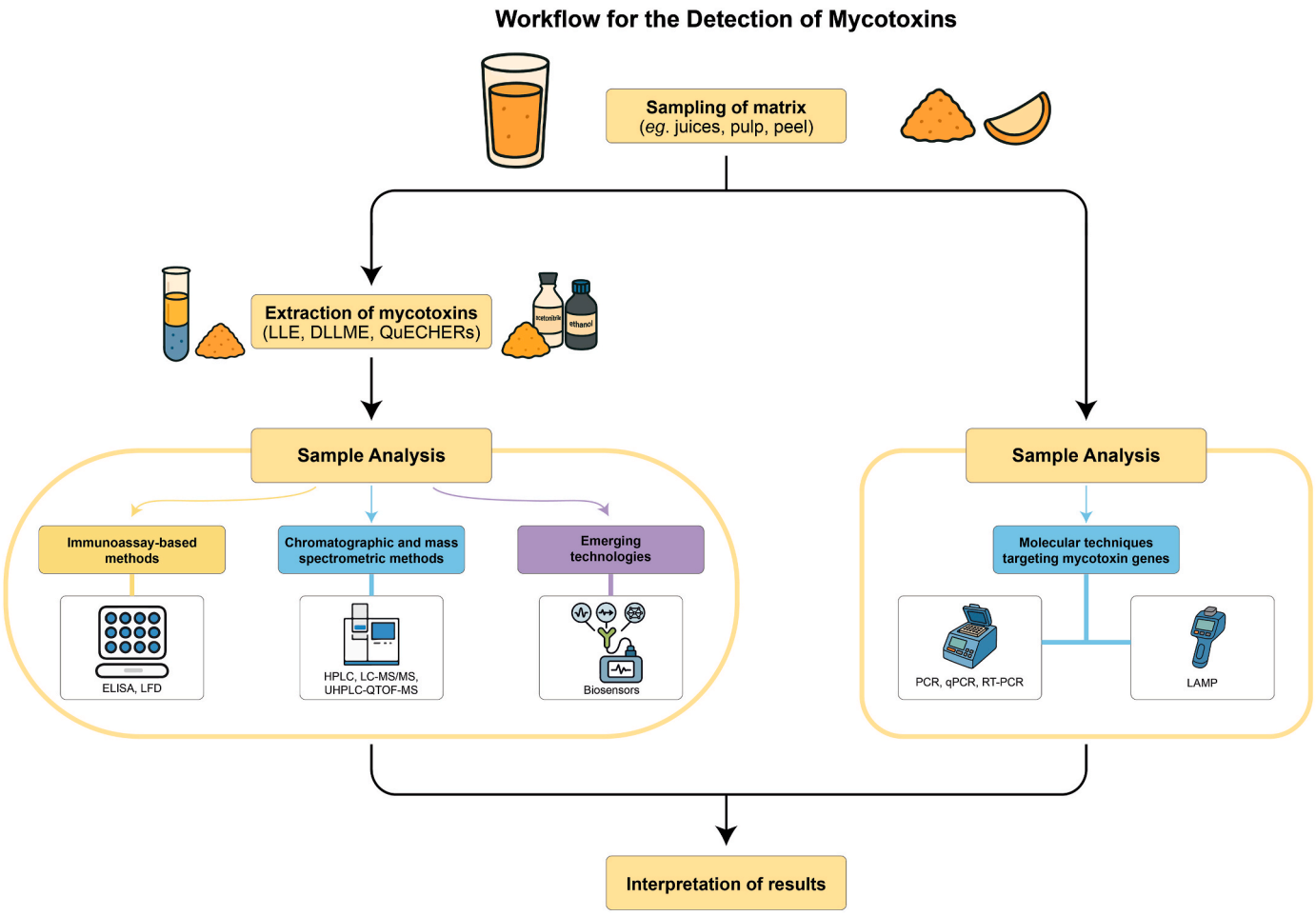

Fig. 2. Workflow for mycotoxin detection: from sampling and extraction to analysis using immunoassays, chromatographic–mass spectrometric techniques, biosensor platforms, and molecular assays targeting mycotoxin biosynthetic genes.

common techniques employed in the food sector are solvent-based extractions, which utilize organic solvents such as ethanol, methanol, acetonitrile, or chloroform to isolate and concentrate these compounds. Widely adopted protocols for solvent-based extraction include QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), conventional liquid–liquid extraction (LLE), and dispersive liquid–liquid microextraction (DLLME) [134,135]. DLLME, in particular, has become increasingly popular due to its operational simplicity, high recovery rates, low solvent consumption, and potential for automation [17,136]. This technique has been successfully adapted for a range of commodities, enabling detection of ochratoxin A in wines, zearalenone in beer, aflatoxins in edible oils, patulin in fruit juices, and combined aflatoxins/ochratoxin A in rice. Studies such as those by Tolosa et al. [137] and Rodríguez-Carasco et al. [138] demonstrated its applicability for multi-mycotoxin determination in complex matrices like fish plasma or tomato-based products, while Serrano et al. [139] explored its use in monitoring enniatin migration during pasta cooking. Although originally optimized for cereals and processed foods, these extraction approaches have been successfully adapted to fruit matrices, including citrus juices and pulps, where high acidity and sugar content can affect analyte recovery. For instance, DLLME has been extensively used to isolate mycotoxins from various fruit juices, including apple and orange blends, particularly in acidic and sugary environments [140,141]. In addition, solvent-based protocols have been employed for citrus products, such as methanol extraction for orange peel and ethyl acetate extraction for orange juice, demonstrating their suitability for subsequent mycotoxin analysis in these matrices [142]. These findings emphasize that extraction strategies must be tailored to the chemical properties of the target mycotoxin, matrix composition, and compatibility with downstream immunoassay or chromatographic methods.

3.2. *Immunoassay-based methods*

Immunological assays, particularly enzyme-linked immunosorbent assays (ELISA), are among the most widely employed screening methods for detecting mycotoxins in food and feed matrices. These assays rely on the high specificity of antigen–antibody interactions to identify target toxins, offering rapid analysis and the possibility to process numerous samples simultaneously. In the context of food and feed, ELISA has been extensively applied to detect patulin, aflatoxins, and ochratoxin A, which are among the most frequently occurring mycotoxins in these commodities [143–146]. Lateral flow devices (LFDs) represent a portable immunoassay format that enables on-site screening, typically providing results within minutes and requiring minimal operator training [147]. Their ease of use and low cost make them suitable for preliminary testing in production facilities or border inspections. Despite their advantages, immunoassay-based methods face limitations when applied to different food matrices, as different compounds can interfere with antibody binding or generate false positives [148]. Additionally, cross-reactivity among structurally related mycotoxins can compromise assay specificity [148]. For this reason, immunoassays are primarily used as first-line screening tools, while confirmatory quantification is routinely performed using liquid chromatography coupled with mass spectrometry (LC-MS/MS), as recommended by regulatory authorities such as FAO and WHO [149,150].

3.3. Chromatographic and mass spectrometric methods

Chromatographic techniques, particularly those combined with spectrometric detection, share the common characteristic of providing highly accurate and quantitative data on mycotoxin levels, in contrast to immunological methods that are primarily suited for rapid screening. These approaches are based on two complementary principles: chromatographic separation of analytes according to their physicochemical properties (e.g., polarity, molecular weight, volatility) and subsequent spectrometric identification and quantification through mass-to-charge (m/z) ratio measurements. This combination ensures both specificity and sensitivity, enabling the detection of multiple mycotoxins even in complex fruit-derived matrices rich in sugars, acids, and pigments [151].

High-performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC-MS/MS), gas chromatography (GC), thin-layer chromatography (TLC) and ultra-performance liquid chromatography (UPLC) are widely used chromatographic techniques for the detection of mycotoxins in food and feed matrices [152–154]. Among them, liquid chromatography (LC) combined with mass spectrometry (MS) offers rapid, cost-effective, and precise detection, enabling simultaneous analysis of multiple mycotoxins with high sensitivity and specificity [152–154].

HPLC has been extensively applied for the analysis of mycotoxins in feeds, cheese, milk, bee pollen, cereal products, and wine [155–160]. In these contexts, HPLC coupled with fluorescence or UV detection was initially the method of choice [161–163]. However, the integration of HPLC with tandem mass spectrometry (LC-MS/MS) has dramatically enhanced selectivity and detection limits, particularly in complex matrices such as fruit juices and purees, which are rich in sugars and organic acids [164,165]. Multi-mycotoxin methods based on LC-MS/MS have been developed to simultaneously quantify regulated and emerging toxins, facilitating comprehensive risk assessments in citrus and apple-derived products [166,167].

Gas chromatography (GC) has also been employed, albeit less frequently, for the analysis of volatile or derivatizable mycotoxins such as trichothecenes [168]. Although GC offers high separation efficiency, its application to fruit-derived matrices is limited due to the need for derivatization and potential thermal degradation of certain toxins [169, 170]. Thin-layer chromatography (TLC), one of the earliest analytical approaches for mycotoxins, remains in use for preliminary screening in laboratories with limited resources [171].

In recent years, high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) has emerged as an advanced analytical platform offering high sensitivity, resolution, and fast data acquisition [137]. This approach has been applied to study changes in fungal metabolomic profiles induced by bio-preservative microorganisms in food-contaminating fungi [138], to determine pesticide residues on tomato peels [139], to identify secondary metabolites of *Alternaria alternata* in pomegranate fruit affected by heart rot [172], and to analyze polymethoxylated flavonoids (PMFs) in citrus peels [141] and other plant matrices [137]. Although not exclusively dedicated to mycotoxin analysis, these applications highlight the versatility of UHPLC-QTOF-MS for profiling both toxic and bioactive metabolites in complex food matrices, including citrus-derived products. Furthermore, this high-resolution technique enables the identification of masked and emerging mycotoxins that may escape detection by conventional LC-MS/MS workflows.

3.4. Emerging technologies

Several emerging technologies are being developed to complement conventional chromatographic and immunological methods for mycotoxin detection. Among them, biosensor-based platforms (electrochemical, optical, and nanomaterial-enhanced) offer rapid, portable, and highly sensitive detection, with potential for on-site screening of fruit-derived products [133,173–176]. Surface-Enhanced Raman Spectroscopy (SERS) and hyperspectral imaging are also gaining interest for their ability to provide non-destructive, high-throughput analyses directly on whole fruits or juices, minimizing sample preparation [177, 178].

In addition to direct detection, molecular approaches targeting mycotoxin biosynthetic genes have been explored as indirect indicators of contamination risk. For example, PCR-qPCR-, RT-PCR- and LAMP-based assays focusing on regulatory and structural genes within the aflatoxin biosynthesis cluster (aflR, aflD) in *Aspergillus* spp. can identify potentially toxigenic strains before toxin accumulation occurs [179–184]. Similar strategies have been proposed for patulin-producing *Penicillium expansum* and ochratoxin A-producing *Aspergillus* and *Penicillium* species [185–188]. While these methods cannot replace quantitative toxin analysis, they provide valuable early-warning tools for hazard assessment and are particularly useful when integrated with classical detection workflows.

4. Comparative assessment of diagnostic methods for fungal and oomycete pathogens and mycotoxins

The increasing complexity of plant health diagnostics requires not only a knowledge of available techniques but also an understanding of their operational performance under real-world constraints. Although methods for detecting fungal and oomycete pathogens and those for identifying mycotoxins rely on distinct biological targets, they are often evaluated and selected using a common set of performance criteria. These include analytical sensitivity and specificity, turnaround time, field applicability, instrumentation needs, regulatory validation, and overall cost-effectiveness. In this section, the diagnostic methods reviewed in the previous chapters are comparatively assessed based on these shared criteria. This integrative perspective is particularly useful for professionals operating at the interface between plant pathology and food safety, where both pathogen detection and mycotoxin monitoring are critical for timely decision-making. Table 1 summarizes the key diagnostic features of the two categories, providing a comparative framework that may support the design of fit-for-purpose detection strategies across different phytosanitary scenarios.

The features summarized above reveal important strengths and limitations associated with each methodological category. The comparative analysis presented in Table 1 highlights how diagnostic strategies for both pathogen and mycotoxin detection can be evaluated using shared operational dimensions. This enables a transversal approach to method selection, regardless of the biological nature of the target. Speed is often a primary constraint: for example, field-deployable

Table 1
Summary of key comparative operational and analytical requirements of tools for detection of plant pathogens (fungi/oomycetes) and mycotoxins.

Decision Criterion	Pathogen Detection (Fungi/Oomycetes)	Mycotoxin Detection
Time to result	Variable (from <1h with RPA to >12h with conventional PCR)	Variable (minutes with LFD to hours with LC-MS/MS)
Analytical sensitivity	High (especially with qPCR, ddPCR, NGS)	Very high (especially with LC-MS/MS)
Diagnostic specificity	High (especially with nucleic acid-based methods)	High (with LC-MS/MS, ELISA competitive formats)
Field applicability	High with isothermal methods (LAMP, RPA) and LFDs	Moderate with ELISA/LFDs, low with instrumental methods
Instrumental requirements	From minimal (LAMP and RPA) to high (NGS platforms)	High (HPLC, LC-MS/MS, some advanced biosensors)
Per-sample cost	Medium to low (depending on technology)	Medium to high
Availability of official standards	Broad (EPPO, ISO guidelines)	Extensive (EFSA, Codex Alimentarius)

isothermal amplification (LAMP, RPA) or lateral flow immunoassays allow for results within minutes to an hour [113]. whereas confirmatory diagnostics using qPCR or LC-MS/MS require several hours and laboratory conditions [189,190]. Analytical sensitivity and diagnostic specificity are both critical parameters for reliable detection. In plant pathogen diagnostics, nucleic acid-based assays such as qPCR and ddPCR offer high sensitivity and specificity due to the use of highly selective primers and probes targeting unique genomic regions of the pathogen [191]. Similarly, in mycotoxin detection, methods such as LC-MS/MS achieve exceptional specificity by separating structurally similar compounds based on retention time and mass-to-charge ratio, making them the gold standard for confirmatory analysis [192]. Competitive ELISA formats also offer high specificity when monoclonal antibodies are employed [193].

From a practical standpoint, field applicability is currently better developed for pathogen diagnostics, especially with portable nucleic acid tests and rapid antigen-based methods. Mycotoxin detection in field conditions is generally limited to semi-quantitative tests such as ELISA or LFDs, with quantitative confirmation requiring sample transport to analytical laboratories. Instrumental requirements vary widely across platforms. Pathogen diagnostics can be implemented with minimal equipment, such as a heating block for LAMP and RPA or a basic lateral flow reader, but may also involve highly complex systems like those employed in NGS platforms [80,194,195]. Mycotoxin analysis, in contrast, typically requires high-end laboratory instrumentation such as HPLC or LC-MS/MS, demanding advanced infrastructure, regular calibration, and trained personnel [196,197]. Per-sample cost is likewise dependent on the method used. LAMP, RPA and LFDs, whether for pathogen or toxin detection, offer low-cost solutions suitable for large-scale screening [198–200]. However, techniques such as qPCR, NGS, HPLC, and LC-MS/MS entail significantly higher costs due to reagents, instrument wear, and operator expertise [201–204]. ELISA and LFDs, whether for pathogen or toxin detection, provide low-cost, user-friendly tools for large-scale screening, although at the expense of accuracy [200,205].

Availability of official standards is generally broader for both domains, but with different focuses. For fungal and oomycete pathogen detection, validated protocols are widely available through EPPO and ISO, covering molecular (PCR, qPCR, LAMP) and serological methods for quarantine and regulated species [206,207]. In contrast, mycotoxin detection is mainly regulated through Codex Alimentarius standards, which define maximum residue levels in food and feed commodities [149]. In the European context, EFSA scientific opinions provide toxicological assessments and risk evaluations that inform these limits [208,209], while harmonized analytical methods for confirmation, particularly HPLC and LC-MS/MS, are standardized through ISO guidelines, such as ISO 16050:2003 for aflatoxin determination [210].

5. Quarantine and emerging pathogens in the citrus supply chain: fungal, oomycete, and mycotoxigenic concerns

The detection and identification of phytopathogenic fungi as well as dangerous biological substances has significant implications for both food safety and human health [4]. On a global scale, citrus fruits are grown in more than 140 countries [141,146]. Citrus fruits are primarily intended for human consumption, either as fresh fruit or derived beverages [144]. International citrus production mainly includes oranges (65 %), mandarins (19 %), lemons and limes (11 %), and grapefruits (5 %) [142]. The Mediterranean area is a major producer of fresh fruit for the international market worldwide [145]. One of the main obstacles to the success of the citrus trade, both nationally and internationally, is the occurrence of serious diseases affecting plants and fruits throughout the entire supply chain [143,147,148,211]. In this regard, citrus fruits are susceptible to infection by numerous fungal and oomycete pathogens, from the nursery stage to fruiting, resulting in yield losses varying on average between 30 and 50 % [114,149].

During recent years, due to the increased trade of plants, the introduction and spread of new parasites into new geographical areas has increased significantly. In this respect, international agencies for plant security are committed to preventing the introduction of parasites (including invasive and/or exotic alien plants) from other countries, in order to safeguard local agriculture and ensure the conservation of natural ecosystems and national biodiversity. National and international control authorities monitor the import and export trade of plants and produce reliable protocols to promptly detect pests before their introduction and spread [142]. The policies of the European Community define quarantine organisms as pests considered harmful to plant health, which are either not present in member countries or present but not widely distributed, with the aim to prevent the introduction and further spread of these organisms [142].

In order to better define the priority of actions and measures to be adopted and to allocate resources effectively, the EU, by the Implementing Regulation 2019/2072, divide plant parasites into four main categories: 1. Quarantine Pests: Not present or not widely distributed, under official control; 2. Regulated Non-Quarantine Pests: Present and economically impactful, requiring regulation; 3. Emerging Pests: Newly identified, with potential for significant damage if not controlled; 4. Established Pests: Widespread, requiring ongoing management. These regulated harmful organisms are listed at the Annex IV of EU Implementing Regulation 2019/2072 [212].

Among quarantine microorganism, fungi and oomycetes from the citrus supply chain, as *Plenodomus tracheiphilus*, *Phyllosticta citricarpa* and some *Phytophthora* spp. stand out as major concerns for citrus cultivation.

5.1. *Plenodomus tracheiphilus*

The pathogenic fungus *Plenodomus tracheiphilus* (Petri) de Gruyter, Aveskamp & Verkley [140,157,169,213] is the causative agent of the disease known as ‘Mal secco of citrus’ [156–160]. This pathogen is listed in Annex IIAII of Directive 2000/29/EC. *P. tracheiphilus* is a vascular pathogen of lemons (*Citrus limon* L.) and other species of the genera *Citrus, including C. medica*, *C. bergamia, C. aurantifolia*, *C. aurantium, C. jambhiri.* Other Rutaceae species susceptible to *P. tracheiphilus* are *Fortunella margarita*, *Poncirus trifoliata,* and their hybrids such as *C. x aurantium* and *C. x sinensis x P. trifoliata* [155,164–167]. The term Mal Secco refers to nonspecific symptoms and was originally used in a broad sense to indicate citrus diseases of various origins [168,214]. Later, Petri [215] used the term Mal Secco of citrus in a stricter sense to indicate a tracheomycosis widespread in lemon orchards in Sicily [214]. Early diagnosis of Mal secco can be done by identification of visual symptoms of the disease and signs of the pathogen. The first symptoms on lemon trees usually appear in spring as shoot and leaf vein chlorosis followed by a premature leaf shedding and a dieback of twigs and branches [171,214,216]. Although initially the disease affects individual twigs and branches, gradually it affects the whole tree, which eventually dies. On the symptomatic twigs, immersed, flask-shaped or globose pycnidia of *P. tracheiphilus* appear as scattered black points within lead-grey or ash-grey areas. On cutting into the twigs or after peeling off the bark of the branches, characteristic salmon-pink or orange-reddish discoloration of the wood can be observed. This internal symptom is associated with gum production within the xylem vessels [217]. The diagnosis of infection by *P. tracheiphilus* is not based only on the symptoms on plant, as they can be caused by other pathogenic agents or abiotic factors; furthermore, there may be cases in which the affected plant is asymptomatic or that the disease is in latent form. Therefore, the reliable detection of *P. tracheiphilus* is currently carried out by laboratory tests on plant tissues. In this respect, *P. tracheiphilus* can be isolated on artificial culture media (potato dextrose agar, carrot agar, malt extract + 1 μg/mL streptomycin) and can be identified on the basis of its cultural and morphological characters [156,157]. Molecular protocols for the detection of *P. tracheiphilus* include conventional PCR-based protocols

developed for both the identification of pure cultures of the pathogen and its detection in plant tissues (leaves, twigs, fruits) [156,216,218–220]. Rapid and sensitive molecular tests currently employed for the detection of *P. tracheiphilus* in the host and for its detection in latently infected (asymptomatic) plant tissues include qPCR and recombinase polymerase amplification (RPA) protocols [156,173–175]. The validation and large scale-application of the RPA detection method, recently developed by Rovetto et al. (2023) [71], for routine tests of the phytosanitary status of nursery plants might be a breakthrough in the prevention of Mal secco. In fact, there is mounting evidence that initial foci of this vascular disease in lemon orchards originate from infected propagation material.

5.2. *Phyllosticta citricarpa*

Phyllosticta citricarpa (McAlpine) is a fungal plant pathogen subject to quarantine regulations, as listed in Annex II Part A of the Commission Implementing Regulation (EU) 2019/2072. It is the causative agent of citrus black spot (CBS), a significant disease that affects citrus crops. The Commission Delegated Regulation (EU) 2019/17025 also listed *P. citricarpa* as a priority pest and it is included in the EPPO A1 List [221–223]. *P. citricarpa* is also a quarantine organism in the USA [224]. CBS is prevalent in several major citrus-producing countries, including Australia, Argentina, Brazil, India, and both North and South Africa, and recently has been reported from Tunisia [225,226]. Strict EPPO regulations impose market access restrictions on countries where CBS is present, affecting trade with regions free from the disease. Consequently, the development of sensitive and accurate detection methods for *P. citricarpa* is critical for managing CBS and ensuring market access [223,227–229]. The disease is of particular concern for regions heavily dependent on citrus exports, such as South Africa and Argentina, where interceptions at European Union (EU) borders have led to temporary suspensions of imports. Multiple interceptions of *P. citricarpa* in citrus shipments from South Africa and Argentina have increased EU vigilance against this pathogen. These interceptions have led to strict phytosanitary measures and temporary bans on citrus imports from these countries, aimed at preventing the establishment and spread of CBS within the EU. *P. citricarpa* has been also detected in sweet orange fruits exported from Egypt, a country where the disease is known to occur [225,226]. All these aspects highlight the global nature of the threat posed by CBS and the importance of robust inspection and quarantine protocols at international borders to safeguard agricultural production. Symptoms of CBS lack specificity, with the most common being 'hard spots' on fruit peels, characterized by sunken, pale brown necrotic lesions with dark reddish-brown borders often containing pycnidia. Additional fruit symptoms may manifest as virulent spots, false melanose, and freckle. On lemons, CBS also induces round, small, sunken necrotic lesions with yellow halos on leaves and twigs [223,230,231]. This non-specific symptomatology, which is also caused by other various *Phyllosticta* species [223,230] and by other ascomycetes, makes impossible the precise diagnosis solely based on the recognition of signs of the pathogen and symptoms of CBS. Additionally, the genetic traits of *P. citricarpa* make this pathogen particularly challenging to identify using molecular methods. This difficulty stems from its high genetic similarity to closely related taxa, such as *Phyllosticta paracapitalensis* and *Phyllosticta paracitricarpa,* which has historically led to misidentification or detection failures with commonly used rapid molecular assays [223]. Although numerous *Phyllosticta* species have been reported on citrus hosts, only *P. citricarpa* is classified as a quarantine pathogen in the European Union. Therefore, achieving accurate species-level identification is essential, particularly to avoid false positives involving morphologically and genetically similar species like *P. paracitricarpa*. Recent research has significantly clarified this taxonomic ambiguity. Van Ingen-Buijs et al. [232] performed a comprehensive phylogenomic analysis using 3000 single-copy orthologous genes and whole-genome comparisons. Their results showed that the genomic variation between *P. citricarpa* and *P. paracitricarpa* falls within the range of intraspecific diversity, concluding that these taxa are conspecific and should be considered synonyms [232].

In parallel, advances in molecular diagnostics have led to the development of more robust and specific tools. For instance, highly specific qPCR protocols have recently been validated for the reliable detection of *P. citricarpa,* overcoming the cross-reactivity limitations of earlier methods [233,234]. Nonetheless, the PCR amplification and sequencing of the *tef1* gene remains the gold standard for accurate identification, as its sequence offers species-level resolution [231].

Taken together, these findings highlight the critical need for continuous refinement of molecular diagnostics to support effective surveillance and management of Citrus Black Spot (CBS), particularly in the context of international phytosanitary regulations.

5.3. *Phytophthora hibernalis and Phytophthtora syringae*

Extra EU international agencies assign quarantine risk status to microorganisms not included in Annex IV of the EU Implementing Regulation 2019/2072. This is the case of *Phytophthora hibernalis* Carne and *Phytophthora syringae* (Kleb.) Kleb., two oomycetes which received attention as quarantine concerns in China as causative agents of the Phytophthora brown rot of citrus fruits [199]. Phytophthora brown rot is an important disease of citrus fruit worldwide, especially in areas and seasons with high rainfall [235–237]. Symptoms include brown discoloration of the rind, a leathery decay texture, and typically a distinctive pungent, rancid odor [238]. Worldwide, citrus brown rot is caused by several species of *Phytophthora*, including *Phytophthora citrophthora* (R. E. Sm. & E. H. Sm.) Leonian, *P. syringae* (Kleb.) Kleb., *P. nicotianae* Breda de Haan, *P. hibernalis* Carne, *P. palmivora* (E. J. Butler) E. J. Butler, *P. boehmeriae* Sawada, *P. cactorum* (Lebert & Cohn) J. Schröt., *P. cinnamomi* Rands, *P. citricola* Sawada, *P. prodigiosa* Cacciola & M.V. Tri and *P. mekongesis* Cacciola & Hoa [13,239–243].

Traditionally, the diagnosis of *Phytophthora* spp. includes pathogen isolation and identification based on the morphological and molecular features. This process could be time-consuming. With the advent of molecular technology, other faster and more cost-effective techniques, including isothermal diagnostic assays, have become the mainstream in rapid detection of many *Phytophthora* species [62], such as *P. infestans* [243–245], *P. sojae* [65,246], *P. nicotianae* [57,224] *P. cinnamomi*. For detecting *P. hibernalis,* a LAMP and RPA protocols were developed by Li et al. (2019) [2] and Dai et al. (2019) [246] respectively.

5.4. *Mycotoxigenic fungi of the citrus supply chain*

Some pathogenic fungi of citrus crops, in addition to causing yield losses through direct damage to the fruit, can produce substances hazardous to human and animal health. Indeed, several citrus fungal pathogens have been reported to produce mycotoxins [169,247,248]. Numerous fungal species responsible for plant diseases such as rusts, smuts, powdery and downy mildews, fruit and vegetable rots, anthracnose, and scab-contribute to substantial pre- and post-harvest crop losses globally, representing a major economic burden for the agricultural sector. Mycotoxins are secondary metabolites produced by filamentous fungi that colonize crops either as pathogens or saprophytes and are toxic to humans and animals [213,248,249]. Food contamination with mycotoxins remains one of the primary causes of toxicosis in humans, posing a serious concern not only for public health but also for the agronomic sector and the entire vegetable production chain.

Phytopathogenic fungi are a significant threat to global food security, as they can severely reduce both the quantity and quality of crops [250,251]. Among the fungal genera known to produce mycotoxins are *Alternaria*, *Aspergillus*, *Fusarium*, *Claviceps*, and *Penicillium* [1,248,251,252]. These toxic compounds are commonly found in a wide range of food and feed commodities, including cereals, fruits, and vegetables [253,254], and their widespread occurrence poses considerable health

risks for both humans and animals. To date, hundreds of different mycotoxins have been identified in raw and processed materials intended for human and animal consumption [250,255,256]. A particularly relevant issue concerns fungal pathogens that are absent in certain geographical areas. These are classified as quarantine organisms, i.e., plant pathogens not yet present in the European Union, or present but not widely distributed, and officially recognized as harmful under EU legislation. The objective of quarantine policy is to prevent their introduction and limit their spread in order to protect plant health and agricultural productivity [257].

These organisms can cause, both post-harvest and during the various processing phases, alterations in the aesthetic and organoleptic characteristics of crops, but they can also compromise the healthiness of the product itself, leading to externally asymptomatic rotting. These rots are generally caused by the presence of toxigenic fungi that develop by producing mycotoxins on the organic matrix. Numerous contaminating fungal species synthesize, in environmental situations, some secondary metabolites with low molecular weight and high chemical stability, mycotoxins, which can cause acute to chronic toxicity depending on their toxic activity. For this reason, these substances are defined as toxic because they present a marked toxic activity towards humans and animals when accidently introduced through food [213,252,258]. However, the presence of mycotoxigenic fungi in the citrus production chain can result in the loss of up to 70 % of gross annual production, constituting an economically harmful, but severely reduced supply of seasonal produce to markets around the world. Although national and international control bodies monitor the storage, transport, and preservation chain, the shelf life of agri-food products is subject to many moments of contamination from the production field to consumption on the table. Therefore, to keep the properties of the product unaltered at every stage, but above all to reduce the economic loss linked to the deterioration of fresh products, rapid and non-invasive detection methods are necessary.

A wide range of mycotoxins are relevant to food safety due to their toxicity and frequent occurrence in fruit and cereal based products. Among the best characterized are aflatoxins (AFs), typically associated with dried fruits and nuts; ochratoxin A (OTA), prevalent in grapes and wine derivatives; and citrinin (CIT), mainly contaminating stored cereals but also occasionally detected in fruits [7,248,249,259]. Additional important toxins include zearalenone (ZEA), fumonisins (FBs), and deoxynivalenol (DON), the latter belonging to the trichothecenes (TCTs) group, produced by *Fusarium* spp. and widespread in cereal crops. Patulin (PAT), a polyketide metabolite primarily synthesized by *Penicillium expansum*, is commonly found in decaying apples and apple juice. Moreover, ergot alkaloids, belonging to the indole group, are produced by *Claviceps* spp. infecting various monocotyledonous hosts in the Poaceae, Juncaceae, and Cyperaceae families, including widely cultivated grasses such as corn, wheat, barley, oats, millet, sorghum, rice, and rye. Within the genus *Alternaria*, the dibenzopyrone derivative alternariol (AOH) is one of the most frequent contaminants of fruit and vegetables, and other *Alternaria* toxins (ATs) include alternariol monomethyl ether (AME), altenuene (ALT), perylene derivatives such as altertoxins (ATX-I, ATX-II, ATX-III), and the tetramic acid compound tenuazonic acid (TeA) [140,172,260]. The degree of contamination by these metabolites is highly variable and influenced by factors such as cultivar susceptibility, climatic conditions, storage environments, pest injuries, pesticide applications, geographic origin, and mechanical damage during handling or weather events [140,172,260]. Due to their high thermal stability, mycotoxins frequently survive industrial processing and are thus detected in both fresh and processed products, including juices. Several have been identified in apple, apricot, banana, berry, orange, peach, and pear juices [261–263]. To mitigate their impact on public health, regulatory limits have been established for major mycotoxins in foods and feeds. In the European Union, specific limits apply only to PAT and OTA in fruits and juices [140,264]. For PAT, Regulation (EU) No. 165/2010, which amends Regulation (EC) No. 1881/2006, sets a maximum level of 50 μg/kg in fruit juices, nectars, cider, and other fermented apple-based drinks; 25 μg/kg in processed solid apple products intended for direct consumption; and 10 μg/kg in apple-derived products intended for infants and young children [265]. For OTA, the maximum permissible level is 2 μg/kg in reconstituted concentrated grape juice, grape nectar, grape must, and similar products intended for direct human consumption [266] [EUR-Lex Regulation (EC) No. 1881/2006]. The EU also sets limits for aflatoxin B1 (AFB1) ranging from 2 to 12 μg/kg, depending on the commodity, and Recommendation 2006/576/EC5 defines thresholds for DON, ZEA, OTA, FBs, T-2, and HT-2 toxins in animal feeds [250][EFSA CONTAM Panel, 2020]. Despite EFSA's assessment of the risks associated with Alternaria toxins, no EU limits currently exist for this group [260,265,267,268]. Growing awareness of these health risks has stimulated the development of robust analytical methods aimed at efficiently extracting and quantifying these metabolites from complex plant matrices and processed foods, ensuring compliance with food safety standards [261–263]. The analytical workflows detailed in Section 3, including extraction, immunoassay-based screening, chromatographic–mass spectrometric techniques, and emerging technologies are equally applicable to citrus-derived matrices for the detection and quantification of these toxins.

6. Conclusions

Climate change is exacerbating the challenges faced in plant pathogen management. As global temperatures rise and weather patterns shift, pathogens that were once secondary concerns are emerging as primary threats, spreading to new regions. Moreover, globalization has intensified trade between distant countries and technological progress has reduced transport and delivery times, thus increasing the risk of introduction and spread of alien pathogens. This underscores the need for rapid and accurate detection methods, to face the risks posed by climate change and meet the needs of international trading.

Therefore, the early diagnosis of fungal and oomycete plant pathogens of quarantine concern, along with mycotoxins, is a cornerstone of modern agriculture. Indeed, in a broader perspective, the continuous refinement of diagnostic tools not only safeguards plant trade and food safety but also provides a fundamental knowledge base for developing and optimizing Integrated Pest Management (IPM) strategies. In recent years, molecular diagnostics and analytical chemistry have made rapid progress and a number of new effective and robust methods are available to intercept undesired organisms and detect the presence of mycotoxins even in traces. However, a constant effort has to be made to validate and homologate internationally recognized official diagnostic methods. An aspect that should not be overlooked by both mycologists and phytopathologists is that an efficient phytosanitary organization needs to be supported by robust, universally recognized taxonomic systems of fungi and oomycetes. Recent academic debates surrounding the genera *Fusarium* and *Phytophthora* underscore the critical importance of accurate pathogen identification and classification [94,236,269]. Similarly, a more in-depth knowledge of mycotoxins, their toxicological effects and mycotoxigenic potential of pathogenic or contaminant fungi is crucial to regulate the acceptable levels of these fungal secondary metabolites in agricultural products and their derivatives.

In addition to reviewing individual diagnostic approaches, this article has introduced a comparative assessment of methodologies for detecting both pathogens and mycotoxins, based on shared performance criteria such as sensitivity, specificity, turnaround time, field applicability, and cost-effectiveness. This transversal evaluation offers a practical framework for selecting fit-for-purpose techniques across diverse phytosanitary scenarios, thereby addressing one of the major gaps identified in previous reviews.

Comparative analysis highlights clear strengths and limitations among existing technologies. PCR-based methods (notably qPCR and ddPCR) remain the benchmark for laboratory diagnostics, providing high sensitivity and specificity, while isothermal amplification

techniques (LAMP and RPA) emerge as portable solutions for rapid field applications. Hybridization-based approaches retain value in multiplex detection, whereas next-generation sequencing, including long-read platforms such as Oxford Nanopore, enables unprecedented insight into pathogen diversity and emergence, though still limited by cost and analytical complexity. For mycotoxins, LC-MS/MS stands as the gold standard for quantitative analysis, complemented by immunoassays for rapid screening and biosensor technologies that are increasingly suitable for on-site applications.

Altogether, an integrated strategy that combines rapid field assays with confirmatory laboratory analyses emerges as the most effective approach to safeguard fruit quality, regulatory compliance, and consumer safety. This holistic framework is particularly relevant to the citrus supply chain, where pathogenic fungi and their secondary metabolites jointly threaten both yield and marketability, demanding timely interventions to ensure agricultural resilience in an era of global trade and accelerating climatic change.

CRediT authorship contribution statement

Rossana Parlascino: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Sebastiano Conti Taguali:** Visualization, Validation, Methodology. **Mario Riolo:** Visualization, Validation, Methodology. **Moricca Salvatore:** Visualization, Supervision. **József Bakonyi:** Visualization, Supervision. **David Ezra:** Visualization, Supervision. **Alessandra Benigno:** Visualization, Validation. **Federico La Spada:** Writing – review & editing, Writing – original draft, Validation, Methodology, Data curation, Conceptualization. **Antonella Pane:** Visualization, Supervision. **Santa Olga Cacciola:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

References

[1] S. Chatterjee, Y. Kuang, R. Splivallo, P. Chatterjee, P. Karlovsky, Interactions among filamentous fungi *Aspergillus niger, Fusarium verticillioides* and *Clonostachys rosea*: fungal biomass, diversity of secreted metabolites and fumonisin production, BMC Microbiol. 16 (2016) 83, https://doi.org/10.1186/s12866-016-0698-3.

[2] J. Li, J. Macdonald, F. von Stetten, Review: a comprehensive summary of a decade development of the recombinase polymerase amplification, Analyst 144 (2019) 31–67, https://doi.org/10.1039/C8AN01621F.

[3] Y. Peng, S.J. Li, J. Yan, Y. Tang, J.P. Cheng, A.J. Gao, X. Yao, J.J. Ruan, B.L. Xu, Research progress on phytopathogenic fungi and their role as biocontrol agents, Front. Microbiol. 12 (2021), https://doi.org/10.3389/fmicb.2021.670135.

[4] H. Shi, Y. Wang, Z. Zhang, S. Yu, X. Huang, D. Pan, Z. Wang, Q. Huang, Z. Zhu, Recent advances of integrated microfluidic systems for fungal and bacterial analysis, TrAC, Trends Anal. Chem. 158 (2023) 116850, https://doi.org/10.1016/j.trac.2022.116850.

[5] G.N. Agrios, Plant Pathology, fifth ed., Elsevier Academic Press, Amsterdam, 2008 [3rd print].

[6] F. Aloi, M. Riolo, F. La Spada, G. Bentivenga, S. Moricca, E. Santilli, A. Pane, R. Faedda, S.O. Cacciola, *Phytophthora* root and collar rot of *Paulownia*, a new disease for Europe, Forests 12 (2021) 1664, https://doi.org/10.3390/f12121664.

[7] C. Stracquadanio, C. Luz, F. La Spada, G. Meca, S.O. Cacciola, Inhibition of mycotoxigenic fungi in different vegetable matrices by extracts of *Trichoderma* species, J. Fungi 7 (2021) 445, https://doi.org/10.3390/jof7060445.

[8] P. Gladieux, E.J. Byrnes III, G. Aguileta, M.C. Fisher, J. Heitman, T. Giraud, Epidemiology and Evolution of Fungal Pathogens in Plants and Animals, first ed., Elsevier, 2017.

[9] A.K. Gautam, S. Kumar, Chapter 12 - techniques for the detection, identification, and diagnosis of agricultural pathogens and diseases, in: C. Egbuna, B. Sawicka (Eds.), Nat. Remedies Pest Dis. Weed Control, Academic Press, 2020, pp. 135–142, https://doi.org/10.1016/B978-0-12-819304-4.00012-9.

[10] W. Li, J.S. Hartung, L. Levy, Quantitative real-time PCR for detection and identification of *Candidatus liberibacter*species associated with Citrus Huanglongbing, J. Microbiol. Methods 66 (2006) 104–115, https://doi.org/10.1016/j.mimet.2005.10.018.

[11] W.J. Moss, Genetics and evolution of infectious diseases, Clin. Infect. Dis. 66 (2018) 643, https://doi.org/10.1093/cid/cix874.

[12] R. Patel, B. Mitra, M. Vinchurkar, A. Adami, R. Patkar, F. Giacomozzi, L. Lorenzelli, M.S. Baghini, A review of recent advances in plant-pathogen detection systems, Heliyon 8 (2022) e11855, https://doi.org/10.1016/j.heliyon.2022.e11855.

[13] S.O. Cacciola, M.L. Gullino, Emerging and re-emerging fungus and oomycete soil-borne plant diseases in Italy, Phytopathol. Mediterr. 58 (2019) 451–472, https://doi.org/10.14601/Phyto-10756.

[14] E. Piombo, A. Abdelfattah, S. Droby, M. Wisniewski, D. Spadaro, L. Schena, Metagenomics approaches for the detection and surveillance of emerging and recurrent plant pathogens, Microorganisms 9 (2021) 188, https://doi.org/10.3390/microorganisms9010188.

[15] S. Sankaran, A. Mishra, R. Ehsani, C. Davis, A review of advanced techniques for detecting plant diseases, Comput. Electron. Agric. 72 (2010) 1–13, https://doi.org/10.1016/j.compag.2010.02.007.

[16] S. Prencipe, F. Savian, L. Nari, P. Ermacora, D. Spadaro, M. Martini, First report of *Phytopythium vexans* causing decline syndrome of *Actinidia deliciosa* ‘hayward’ in Italy, Plant Dis. 104 (2020) 2032, https://doi.org/10.1094/PDIS-10-19-2101-PDN.

[17] X.L. Fang, D. Phillips, H. Li, K. Sivasithamparam, M.J. Barbetti, Severity of crown and root diseases of strawberry and associated fungal and oomycete pathogens in Western Australia, australas, Plant Pathol. 40 (2011) 109–119, https://doi.org/10.1007/s13313-010-0019-5.

[18] S.F. Ortega, S. Matic, D. Spadaro, Use of new molecular techniques for plant disease diagnostics, in: Prot. Delle Colt., 2018, pp. 9–14. https://www.cabidigitallibrary.org/doi/full/10.5555/20193169211. (Accessed 9 April 2025).

[19] L. Schena, F. Nigro, A. Ippolito, D. Gallitelli, Real-time quantitative PCR: a new technology to detect and study phytopathogenic and antagonistic fungi, Eur. J. Plant Pathol. 110 (2004) 893–908, https://doi.org/10.1007/s10658-004-4842-9.

[20] I.N. Gagarina, D.B. Borodin, I.A. Gneusheva, I.V. Gorkova, I.Y. Solokhina, E. V. Kostromicheva, A.V. Lushnikov, I.V. Yakovleva, N.Y. Ageeva, Agrobiological Substantiation of the Technology of Growing Vegetable Products Using Biological Means of Protection (Oryol: Orlov, State Agrarian University, 2018.

[21] A. Jain, S. Sarsaiya, Q. Wu, Y. Lu, J. Shi, A review of plant leaf fungal diseases and its environment speciation, Bioengineered 10 (2019) 409–424, https://doi.org/10.1080/21655979.2019.1649520.

[22] J.B. Sinclair, O.D. Dhingra, Basic Plant Pathology Methods, second ed., CRC Press, Boca Raton, 2019 https://doi.org/10.1201/9781315138138.

[23] S.D. Atkins, I.M. Clark, Fungal molecular diagnostics: a mini review, J. Appl. Genet. 45 (2004) 3–15.

[24] Y.S. Ooi, N.M.I. Mohamed Nor, G. Furusawa, M. Tharek, A.H. Ghazali, Application of bacterial endophytes to control bacterial leaf blight disease and promote rice growth, Plant Pathol. J. 38 (2022) 490–502, https://doi.org/10.5423/PPJ.OA.01.2022.0014.

[25] J. Guan, F.W. Nutter, Factors that affect the quality and quantity of sunlight reflected from alfalfa canopies, Plant Dis. 85 (2001) 865–874, https://doi.org/10.1094/PDIS.2001.85.8.865.

[26] F.W. Nutter, P.D. Esker, R.A.C. Netto, Disease assessment concepts and the advancements made in improving the accuracy and precision of plant disease data, Eur. J. Plant Pathol. 115 (2006) 95–103, https://doi.org/10.1007/s10658-005-1230-z.

[27] F.M. Dewey, C.R. Thornton, C.A. Gilligan, Use of monoclonal antibodies to detect, quantify and visualize fungi in soils, in: J.H. Andrews, I.C. Tommerup, J. A. Callow (Eds.), Adv. Bot. Res., Academic Press, 1997, pp. 275–308, https://doi.org/10.1016/S0065-2296(08)60076-2.

[28] P. Narayanasamy, Microbial Plant Pathogens-Detection and Disease Diagnosis, Springer Netherlands, Dordrecht, 2011, https://doi.org/10.1007/978-90-481-9735-4.

[29] G.E. Trujillo, A.W. Saettler, A combined semi-selective medium and serology test for the detection of Xanthomonas blight bacteria in bean seed, J. Seed Technol. 4 (1979) 35–41. https://www.jstor.org/stable/23432732. (Accessed 28 February 2025).

[30] S. Werres, C. Steffens, Immunological techniques used with fungal plant pathogens: aspects of antigens, antibodies and assays for diagnosis, Ann. Appl. Biol. 125 (1994) 615–643, https://doi.org/10.1111/j.1744-7348.1994.tb04998.x.

[31] J.W.L. Van Vuurde, G.W. Van den Bovenkamp, Routine methods for the detection of halo-blight (*Pseudomonas phaseolicola*) in beans, in: Rep. 17th Int. Workshop Seed Pathol, ISTA Zurich, 1981.

[32] N. Rajeshwari, M.D. Shylaja, M. Krishnappa, H.S. Shetty, C.N. Mortensen, S. B. Mathur, Development of ELISA for the detection of *Ralstonia solanacearum* in tomato: its application in seed health testing, World J. Microbiol. Biotechnol. 14 (1998) 697–704, https://doi.org/10.1023/A:1008892400077.

[33] G.A. Posthuma-Trumpie, J. Korf, A. van Amerongen, Lateral flow (immuno) assay: its strengths, weaknesses, opportunities and threats. A literature survey, Anal. Bioanal. Chem. 393 (2009) 569–582, https://doi.org/10.1007/s00216-008-2287-2.

[34] A. Cassedy, A. Parle-McDermott, R. O'Kennedy, Virus detection: a review of the current and emerging molecular and immunological methods, Front. Mol. Biosci. 8 (2021), https://doi.org/10.3389/fmolb.2021.637559.

[35] C.R. Thornton, Development of an immunochromatographic lateral-flow device for rapid serodiagnosis of invasive aspergillosis, Clin. Vaccine Immunol. CVI 15 (2008) 1095–1105, https://doi.org/10.1128/CVI.00068-08.

[36] D. Kethiravan, P. Mazumdar, B.C. Tan, J.A. Harikrishna, Phenotypic, molecular and serological detection of viral diseases in melon: a comprehensive review on progress and challenges, J. Plant Dis. Prot. 132 (2025) 55, https://doi.org/10.1007/s41348-024-01044-3.

[37] G. Doehlemann, B. Ökmen, W. Zhu, A. Sharon, Plant pathogenic fungi, Microbiol. Spectr. 5 (2017), https://doi.org/10.1128/microbiolspec.funk-0023-2016.

[38] M.C. Fisher, S.J. Gurr, C.A. Cuomo, D.S. Blehert, H. Jin, E.H. Stukenbrock, J. E. Stajich, R. Kahmann, C. Boone, D.W. Denning, N.A.R. Gow, B.S. Klein, J. W. Kronstad, D.C. Sheppard, J.W. Taylor, G.D. Wright, J. Heitman, A. Casadevall, L.E. Cowen, Threats posed by the fungal kingdom to humans, wildlife, and agriculture, mBio 11 (2020), https://doi.org/10.1128/mbio.00449-20.

[39] M. Kumar, M. Mugunthan, Evaluation of three DNA extraction methods from fungal cultures, Med. J. Armed Forces India 74 (2018) 333–336, https://doi.org/10.1016/j.mjafi.2017.07.009.

[40] S.K. Tripathy, M. Maharana, D.M. Ithape, D. Lenka, D. Mishra, A. Prusti, D. S. Ranjan Mohanty, K.R.R. Raj, Exploring rapid and efficient protocol for isolation of fungal DNA, Int. J. Curr. Microbiol. Appl. Sci. 6 (2017) 951–960, https://doi.org/10.20546/ijcmas.2017.603.113.

[41] J.H. Shin, Nucleic acid extraction techniques, in: Y.-W. Tang, C.W. Stratton (Eds.), Adv. Tech. Diagn. Microbiol., Springer US, Boston, MA, 2013, pp. 209–225, https://doi.org/10.1007/978-1-4614-3970-7_11.

[42] M. Husakova, P. Kralik, V. Babak, I. Slana, Efficiency of DNA isolation methods based on silica columns and magnetic separation tested for the detection of *Mycobacterium avium* subsp. *paratuberculosis* in milk and faeces, Materials 13 (2020) 5112, https://doi.org/10.3390/ma13225112.

[43] M. Venbrux, Current and emerging trends in techniques for plant pathogen detection, Front. Plant Sci. 14 (2023), https://doi.org/10.3389/fpls.2023.1120968.

[44] K. Nagamine, T. Hase, T. Notomi, Accelerated reaction by loop-mediated isothermal amplification using loop primers, Mol. Cell. Probes 16 (2002) 223–229, https://doi.org/10.1006/mcpr.2002.0415.

[45] Y. Mori, K. Nagamine, N. Tomita, T. Notomi, Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation, Biochem. Biophys. Res. Commun. 289 (2001) 150–154, https://doi.org/10.1006/bbrc.2001.5921.

[46] N. Naidoo, M. Ghai, K. Moodley, L. Mkize, L. Martin, S. McFarlane, S. Rutherford, Modified RS-LAMP assay and use of lateral flow devices for rapid detection of *Leifsonia xyli* subsp. *xyli*, Lett. Appl. Microbiol. 65 (2017) 496–503, https://doi.org/10.1111/lam.12799.

[47] N. Tomita, Y. Mori, H. Kanda, T. Notomi, Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products, Nat. Protoc. 3 (2008) 877–882, https://doi.org/10.1038/nprot.2008.57.

[48] X. Zhang, H. Zhang, J. Pu, Y. Qi, Q. Yu, Y. Xie, J. Peng, Development of a real-time fluorescence loop-mediated isothermal amplification assay for rapid and quantitative detection of *Fusarium oxysporum* F. sp. *cubense* tropical race 4 in soil, PLoS One 8 (2013) e82841, https://doi.org/10.1371/journal.pone.0082841.

[49] A.V. Ivanov, I.V. Safenkova, A.V. Zherdev, B.B. Dzantiev, The potential use of isothermal amplification assays for in-field diagnostics of plant pathogens, Plants 10 (2021) 2424, https://doi.org/10.3390/plants10112424.

[50] T.J. Moehling, G. Choi, L.C. Dugan, M. Salit, R.J. Meagher, LAMP diagnostics at the Point-of-Care: emerging trends and perspectives for the developer community, Expert Rev. Mol. Diagn. 21 (2021) 43–61, https://doi.org/10.1080/14737159.2021.1873769.

[51] B.J. Hindson, K.D. Ness, D.A. Masquelier, P. Belgrader, N.J. Heredia, A. J. Makarewicz, I.J. Bright, M.Y. Lucero, A.L. Hiddessen, T.C. Legler, T.K. Kitano, M.R. Hodel, J.F. Petersen, P.W. Wyatt, E.R. Steenblock, P.H. Shah, L.J. Bousse, C. B. Troup, J.C. Mellen, D.K. Wittmann, N.G. Erndt, T.H. Cauley, R.T. Koehler, A. P. So, S. Dube, K.A. Rose, L. Montesclaros, S. Wang, D.P. Stumbo, S.P. Hodges, S. Romine, F.P. Milanovich, H.E. White, J.F. Regan, G.A. Karlin-Neumann, C. M. Hindson, S. Saxonov, B.W. Colston, High-throughput droplet digital PCR system for absolute quantitation of DNA copy number, Anal. Chem. 83 (2011) 8604–8610, https://doi.org/10.1021/ac202028g.

[52] H.Q. Nguyen, V.D. Nguyen, H. Van Nguyen, T.S. Seo, Quantification of colorimetric isothermal amplification on the smartphone and its open-source app for point-of-care pathogen detection, Sci. Rep. 10 (2020) 15123, https://doi.org/10.1038/s41598-020-72095-3.

[53] S.V. Gomez-Gutierrez, S.B. Goodwin, Loop-mediated isothermal amplification for detection of plant pathogens in wheat (*Triticum aestivum*), Front. Plant Sci. 13 (2022), https://doi.org/10.3389/fpls.2022.857673.

[54] R. Ghosh, A. Nagavardhini, A. Sengupta, M. Sharma, Development of loop-mediated isothermal amplification (LAMP) assay for rapid detection of *Fusarium oxysporum* F. sp. *ciceris* - wilt pathogen of chickpea, BMC Res. Notes 8 (2015) 40, https://doi.org/10.1186/s13104-015-0997-z.

[55] V. Mancini, S. Murolo, G. Romanazzi, Diagnostic methods for detecting fungal pathogens on vegetable seeds, Plant Pathol. 65 (2016), https://doi.org/10.1111/ppa.12515.

[56] E. Paul, Soil Microbiology, Ecology and Biochemistry, Academic Press, 2014.

[57] L. Lillis, D. Lehman, M.C. Singhal, J. Cantera, J. Singleton, P. Labarre, A. Toyama, O. Piepenburg, M. Parker, R. Wood, J. Overbaugh, D.S. Boyle, Non-instrumented incubation of a recombinase polymerase amplification assay for the rapid and sensitive detection of proviral HIV-1 DNA, PLoS One 9 (2014) e108189, https://doi.org/10.1371/journal.pone.0108189.

[58] S. Cesbron, E. Dupas, M.-A. Jacques, Evaluation of the AmplifyRP® XRT+ kit for the detection of *Xylella fastidiosa* by recombinase polymerase amplification (RPA), PhytoFront. 3 (2022), https://doi.org/10.1094/PHYTOFR-03-22-0025-FI.

[59] M. Fernández-Fernández, P. Naves, J. Witzell, D.L. Musolin, A.V. Selikhovkin, M. Paraschiv, D. Chira, P. Martínez-Álvarez, J. Martín-García, E.J. Muñoz-Adalia, A. Altunisik, G.E.M. Cocuzza, S.D. Silvestro, C. Zamora, J.J. Diez, Pine pitch canker and insects: relationships and implications for disease spread in Europe, Forests 10 (2019) 627, https://doi.org/10.3390/f10080627.

[60] Y. Guo, H. Xia, T. Dai, T. Liu, RPA-CRISPR/Cas12a mediated isothermal amplification for visual detection of *Phytophthora sojae*, Front. Cell. Infect. Microbiol. 13 (2023), https://doi.org/10.3389/fcimb.2023.1208837.

[61] X. Lu, H. Xu, W. Song, Z. Yang, J. Yu, Y. Tian, M. Jiang, D. Shen, D. Dou, Rapid and simple detection of *Phytophthora cactorum* in strawberry using a coupled recombinase polymerase amplification-lateral flow strip assay, Phytopathol. Res. 3 (2021) 12, https://doi.org/10.1186/s42483-021-00089-8.

[62] T.D. Miles, F.N. Martin, M.D. Coffey, Development of rapid isothermal amplification assays for detection of *Phytophthora* spp. in plant tissue, Phytopathology 105 (2015) 265–278, https://doi.org/10.1094/PHYTO-05-14-0134-R.

[63] M. Munawar, A. Toljamo, F. Martin, H. Kokko, Recombinase polymerase amplification assay for fast, sensitive and on-site detection of *Phytophthora cactorum* without DNA extraction, Eur. J. Hortic. Sci. 84 (2019) 14–19, https://doi.org/10.17660/eJHS.2019/84.1.2.

[64] M.A. Munawar, A. Toljamo, F. Martin, E. Oksanen, H. Kokko, Development and evaluation of a recombinase polymerase amplification assay for rapid detection of strawberry red stele pathogen, Phytopathol. Res. 2 (2020) 26, https://doi.org/10.1186/s42483-020-00069-4.

[65] J.A. Rojas, T.D. Miles, M.D. Coffey, F.N. Martin, M.I. Chilvers, Development and application of qPCR and RPA Genus- and species-specific detection of *Phytophthora sojae* and *P. sansomeana* root rot pathogens of soybean, Plant Dis. 101 (2017) 1171–1181, https://doi.org/10.1094/PDIS-09-16-1225-RE.

[66] J. Yu, D. Shen, T. Dai, X. Lu, H. Xu, D. Dou, Rapid and equipment-free detection of *Phytophthora capsici* using lateral flow strip-based recombinase polymerase amplification assay, Lett. Appl. Microbiol. 69 (2019) 64–70, https://doi.org/10.1111/lam.13166.

[67] E.M. Southern, Detection of specific sequences among DNA fragments separated by gel electrophoresis, J. Mol. Biol. 98 (1975) 503–517, https://doi.org/10.1016/s0022-2836(75)80083-0.

[68] P. Changtor, W. Jaroenpol, K. Buddhachat, W. Wattanachaiyingcharoen, N. Yimtragool, Rapid detection of *Sclerotium rolfsii* causing dry stem and root rot disease in cassava by recombinase polymerase amplification technique (RPA) combined with CRISPR/Cas12a, Crop Prot. 172 (2023) 106340, https://doi.org/10.1016/j.cropro.2023.106340.

[69] Y. Ju, C. Li, P. Shen, N. Wan, W. Han, Y. Pan, Rapid and visual detection of *Verticillium dahliae* using recombinase polymerase amplification combined with lateral flow dipstick, Crop Prot. 136 (2020) 105226, https://doi.org/10.1016/j.cropro.2020.105226.

[70] R. Paul, E. Ostermann, Y. Chen, A.C. Saville, Y. Yang, Z. Gu, A.E. Whitfield, J. B. Ristaino, Q. Wei, Integrated microneedle-smartphone nucleic acid amplification platform for in-field diagnosis of plant diseases, Biosens. Bioelectron. 187 (2021) 113312, https://doi.org/10.1016/j.bios.2021.113312.

[71] E.I. Rovetto, M. Garbelotto, S. Moricca, M. Amato, F. La Spada, S.O. Cacciola, A portable fluorescence-based recombinase polymerase amplification assay for the detection of mal secco disease by *Plenodomus tracheiphilus*, Crop Prot. 184 (2024) 106825, https://doi.org/10.1016/j.cropro.2024.106825.

[72] R.K. Daher, G. Stewart, M. Boissinot, M.G. Bergeron, Recombinase polymerase amplification for diagnostic applications, Clin. Chem. 62 (2016) 947–958, https://doi.org/10.1373/clinchem.2015.245829.

[73] J. Xu, X. Yang, C. Wu, Z. Chen, T. Dai, Recombinase polymerase amplification-lateral flow dipstick assay for rapid detection of *Fusarium circinatum* based on a newly identified unique target gene, Plant Dis. 107 (2023) 1067–1074, https://doi.org/10.1094/PDIS-04-22-0864-RE.

[74] P. Zhao, J. Luo, W.-Y. Zhuang, Practice towards DNA barcoding of the nectriaceous fungi, Fungal Divers. 46 (2011) 183–191, https://doi.org/10.1007/s13225-010-0064-y.

[75] H. Aslan, A. Ekinci, İ. Aslan, Chapter 13 - nucleic acid–based methods in the detection of foodborne pathogens, in: C. Egbuna, B. Sawicka (Eds.), Nat. Remedies Pest Dis. Weed Control, Academic Press, 2020, pp. 143–161, https://doi.org/10.1016/B978-0-12-819304-4.00013-0.

[76] J.C. Alwine, D.J. Kemp, G.R. Stark, Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl-paper and hybridization with DNA probes, Proc. Natl. Acad. Sci. U. S. A. 74 (1977) 5350–5354, https://doi.org/10.1073/pnas.74.12.5350.

[77] S.L. Foley, K. Grant, Molecular techniques of detection and discrimination of foodborne pathogens and their toxins, in: S. Simjee (Ed.), Foodborne Dis., Humana Press, Totowa, NJ, 2007, pp. 485–510, https://doi.org/10.1007/978-1-59745-501-5_20.

[78] H.M.U. Aslam, H. Riaz, N. Killiny, X.-G. Zhou, L. Thomashow, N. Peters, A. Chanda, Microarray Technology for Detection of Plant Diseases (2022) 203–223, https://doi.org/10.1007/978-981-19-5896-0_11.

[79] R. Bumgarner, Overview of DNA microarrays: types, applications, and their future, Curr. Protoc. Mol. Biol. Chapter 22 (2013), https://doi.org/10.1002/0471142727.mb2201s101. Unit 22.1.

[80] I.M. Lobato, C.K. O'Sullivan, Recombinase polymerase amplification: basics, applications and recent advances, TrAC, Trends Anal. Chem. 98 (2018) 19–35, https://doi.org/10.1016/j.trac.2017.10.015.

[81] J.R. Úrbez-Torres, P. Haag, P. Bowen, T. Lowery, D.T. O'Gorman, Development of a DNA macroarray for the detection and identification of fungal pathogens causing decline of young grapevines, Phytopathology 105 (2015) 1373–1388, https://doi.org/10.1094/PHYTO-03-15-0069-R.

[82] S.A. Dunbar, Applications of luminex xMAP technology for rapid, high-throughput multiplexed nucleic acid detection, Clin. Chim. Acta Int. J. Clin. Chem. 363 (2006) 71–82, https://doi.org/10.1016/j.cccn.2005.06.023.

[83] B. Sassolas, C. Haddad, M. Mockenhaupt, A. Dunant, Y. Liss, K. Bork, U. F. Haustein, D. Vieluf, J.C. Roujeau, H. Le Louet, ALDEN, an algorithm for assessment of drug causality in Stevens-Johnson syndrome and toxic epidermal necrolysis: comparison with case-control analysis, Clin. Pharmacol. Ther. 88 (2010) 60–68, https://doi.org/10.1038/clpt.2009.252.

[84] R. Charlermroj, O. Himananto, C. Seepiban, M. Kumpoosiri, N. Warin, M. Oplatowska, O. Gajanandana, I.R. Grant, N. Karoonuthaisiri, C.T. Elliott, Multiplex detection of plant pathogens using a microsphere immunoassay technology, PLoS One 8 (2013) e62344, https://doi.org/10.1371/journal.pone.0062344.

[85] B. Krawczyk, L.L. Minku, J. Gama, J. Stefanowski, M. Woźniak, Ensemble learning for data stream analysis: a survey, Inf. Fusion 37 (2017) 132–156, https://doi.org/10.1016/j.inffus.2017.02.004.

[86] A.S.R.S. Rao, S.G. Krantz, T. Kurien, R. Bhat, S. Kurapati, Model-based retrospective estimates for COVID-19 or coronavirus in India: continued efforts required to contain the virus spread, Curr. Sci. 118 (2020) 1023–1025. https://www.jstor.org/stable/27226407. (Accessed 10 April 2025).

[87] Z.G. Zhang, Y.Q. Li, H. Fan, Y.C. Wang, X.B. Zheng, Molecular detection of *Phytophthora capsici* in infected plant tissues, soil and water, Plant Pathol. 55 (2006) 770–775, https://doi.org/10.1111/j.1365-3059.2006.01442.x.

[88] P. Choudhary, B.N. Singh, H. Chakdar, A.K. Saxena, DNA barcoding of phytopathogens for disease diagnostics and bio-surveillance, World J. Microbiol. Biotechnol. 37 (2021) 54, https://doi.org/10.1007/s11274-021-03019-0.

[89] W.J. Kress, D.L. Erickson, DNA barcodes: genes, genomics, and bioinformatics, Proc. Natl. Acad. Sci. 105 (2008) 2761–2762, https://doi.org/10.1073/pnas.0800476105.

[90] E. Banchi, C.G. Ametrano, D. Stanković, P. Verardo, O. Moretti, F. Gabrielli, S. Lazzarin, M.F. Borney, F. Tassan, M. Tretiach, A. Pallavicini, L. Muggia, DNA metabarcoding uncovers fungal diversity of mixed airborne samples in Italy, PLoS One 13 (2018) e0194489, https://doi.org/10.1371/journal.pone.0194489.

[91] B. Buyck, S. Zoller, V. Hofstetter, Walking the thin line… ten years later: the dilemma of above- versus below-ground features to support phylogenies in the *Russulaceae* (Basidiomycota), Fungal Divers. 89 (2018) 267–292, https://doi.org/10.1007/s13225-018-0397-5.

[92] F.W. Nutter, P.D. Esker, R.A.C. Netto, Disease assessment concepts and the advancements made in improving the accuracy and precision of plant disease data, Eur. J. Plant Pathol. 115 (2006) 95–103, https://doi.org/10.1007/s10658-005-1230-z.

[93] S.A. Balajee, A.M. Borman, M.E. Brandt, J. Cano, M. Cuenca-Estrella, E. Dannaoui, J. Guarro, G. Haase, C.C. Kibbler, W. Meyer, K. O'Donnell, C. A. Petti, J.L. Rodriguez-Tudela, D. Sutton, A. Velegraki, B.L. Wickes, Sequence-based identification of *Aspergillus, Fusarium*, and *Mucorales* species in the clinical mycology laboratory: where are we and where should we Go from here? J. Clin. Microbiol. 47 (2009) 877–884, https://doi.org/10.1128/jcm.01685-08.

[94] D.M. Geiser, M. del Mar Jiménez-Gasco, S. Kang, I. Makalowska, N. Veeraraghavan, T.J. Ward, N. Zhang, G.A. Kuldau, K. O'donnell, FUSARIUM-ID V. 1.0: a DNA sequence database for identifying *Fusarium*, Eur. J. Plant Pathol. 110 (2004) 473–479, https://doi.org/10.1023/B:EJPP.0000032386.75915.a0.

[95] S.-B. Hong, H.-D. Shin, J. Hong, J.C. Frisvad, P.V. Nielsen, J. Varga, R.A. Samson, New taxa of *Neosartorya* and *Aspergillus* in *Aspergillus* section *fumigati*, Antonie Leeuwenhoek 93 (2008) 87–98, https://doi.org/10.1007/s10482-007-9183-1.

[96] G.P. Robideau, A.W.a.M. DeCock, M.D. Coffey, H. Voglmayr, H. Brouwer, K. Bala, D.W. Chitty, N. Désaulniers, Q.A. Eggertson, C.M.M. Gachon, C.-H. Hu, F. C. Küpper, T.L. Rintoul, E. Sarhan, E.C.P. Verstappen, Y. Zhang, P.J.M. Bonants, J. B. Ristaino, C. André Lévesque, DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer, Mol. Ecol. Resour. 11 (2011) 1002–1011, https://doi.org/10.1111/j.1755-0998.2011.03041.x.

[97] A.D. Roe, A.V. Rice, S.E. Bromilow, J.E.K. Cooke, F.a.H. Sperling, Multilocus species identification and fungal DNA barcoding: insights from blue stain fungal symbionts of the Mountain pine beetle, Mol. Ecol. Resour 10 (2010) 946–959, https://doi.org/10.1111/j.1755-0998.2010.02844.x.

[98] R. Samson, K. Seifert, A. Kuijpers, J. Houbraken, J. Frisvad, Phylogenetic analysis of *Penicillium* subgenus *Penicillium* using partial P-tubulin sequences, Stud. Mycol. 2004 (2004) 175–200.

[99] K.A. Seifert, Progress towards DNA barcoding of fungi, Mol. Ecol. Resour. 9 (2009) 83–89, https://doi.org/10.1111/j.1755-0998.2009.02635.x.

[100] Z. Zeng, P. Zhao, J. Luo, W. Zhuang, Z. Yu, Selection of a DNA barcode for *Nectriaceae* from fungal whole-genomes, Sci. China Life Sci. 55 (2012) 80–88, https://doi.org/10.1007/s11427-012-4266-2.

[101] C.-H. Shen, Amplification of Nucleic Acids (2019) 215–247, https://doi.org/10.1016/B978-0-12-802823-0.00009-2.

[102] X. Zhao, C.-W. Lin, J. Wang, D.H. Oh, Advances in rapid detection methods for foodborne pathogens, J. Microbiol. Biotechnol. 24 (2014) 297–312, https://doi.org/10.4014/jmb.1310.10013.

[103] B. Lievens, M. Brouwer, A.C.R.C. Vanachter, C.A. Lévesque, B.P.A. Cammue, B.P. H.J. Thomma, Design and development of a DNA array for rapid detection and identification of multiple tomato vascular wilt pathogens, FEMS Microbiol. Lett. 223 (2003) 113–122, https://doi.org/10.1016/S0378-1097(03)00352-5.

[104] X. Wang, F. Li, Z. Cai, K. Liu, J. Li, B. Zhang, J. He, Sensitive colorimetric assay for uric acid and glucose detection based on multilayer-modified paper with smartphone as signal readout, Anal. Bioanal. Chem. 410 (2018) 2647–2655, https://doi.org/10.1007/s00216-018-0939-4.

[105] J. Chen, A.M. Montanari, Isolation and identification of new polymethoxyflavonoids from dancy tangerine leaves, J. Agric. Food Chem. 46 (1998) 1235.

[106] A. Chimento, S.O. Cacciola, M. Garbelotto, Detection of mRNA by reverse-transcription PCR as an indicator of viability in *Phytophthora ramorum*, For. Pathol. 42 (2012) 14–21, https://doi.org/10.1111/j.1439-0329.2011.00717.x.

[107] R.T. Hayden, Z. Gu, J. Ingersoll, D. Abdul-Ali, L. Shi, S. Pounds, A.M. Caliendo, Comparison of droplet digital PCR to real-time PCR for quantitative detection of *Cytomegalovirus*, J. Clin. Microbiol. 51 (2020) 540–546, https://doi.org/10.1128/jcm.02620-12.

[108] L. Schena, K.J. Hughes, D.E. Cooke, Detection and quantification of *Phytophthora ramorum*, *P. kernoviae*, *P. citricola* and *P. quercina* in symptomatic leaves by multiplex real-time PCR, Mol. plant path. 7 (5) (2006) 365–379. https://doi.org/10.1111/j.1364-3703.2006.00345.x.

[109] S. Panno, S. Matić, A. Tiberini, A.G. Caruso, P. Bella, L. Torta, R. Stassi, A. S. Davino, Loop mediated isothermal amplification: principles and applications in plant virology, Plants Basel Switz 9 (2020) 461, https://doi.org/10.3390/plants9040461.

[110] S.C. Taylor, G. Laperriere, H. Germain, Droplet digital PCR versus qPCR for gene expression analysis with low abundant targets: from variable nonsense to publication quality data, Sci. Rep. 7 (2017) 2409, https://doi.org/10.1038/s41598-017-02217-x.

[111] L. Becherer, N. Borst, M. Bakheit, S. Frischmann, R. Zengerle, F. von Stetten, Loop-mediated isothermal amplification (LAMP) – review and classification of methods for sequence-specific detection, Anal. Methods 12 (2020) 717–746, https://doi.org/10.1039/C9AY02246E.

[112] P. Craw, W. Balachandran, Isothermal nucleic acid amplification technologies for point-of-care diagnostics: a critical review, Lab Chip 12 (2012) 2469–2486, https://doi.org/10.1039/C2LC40100B.

[113] O. Piepenburg, C.H. Williams, D.L. Stemple, N.A. Armes, DNA detection using recombination proteins, PLoS Biol. 4 (2006) e204, https://doi.org/10.1371/journal.pbio.0040204.

[114] Z.G. Abad, T.I. Burgess, A.J. Redford, J.C. Bienapfl, S. Srivastava, R. Mathew, K. Jennings, IDphy: an international online resource for molecular and

morphological identification of *Phytophthora*, Plant Dis. 107 (2023) 987–998, https://doi.org/10.1094/PDIS-02-22-0448-FE.
[115] K. O'Donnell, B.K. Whitaker, I. Laraba, R.H. Proctor, D.W. Brown, K. Broders, H.-S. Kim, S.P. McCormick, M. Busman, T. Aoki, T.J. Torres-Cruz, D.M. Geiser, DNA sequence-based identification of *Fusarium*: a work in progress, Plant Dis. 106 (2022) 1597–1609, https://doi.org/10.1094/PDIS-09-21-2035-SR.
[116] D. Chalupowicz, B. Veltman, S. Droby, E. Eltzov, Evaluating the use of biosensors for monitoring of *Penicillium digitatum* infection in citrus fruit, Sensor. Actuator. B Chem. 311 (2020) 127896, https://doi.org/10.1016/j.snb.2020.127896.
[117] E.N. Kudjordjie, A.S. Schmidt-Høier, M.-B. Brøndum, M.G. Johnsen, M. Nicolaisen, M. Vestergård, Early assessment of fungal and oomycete pathogens in greenhouse irrigation water using Oxford nanopore amplicon sequencing, PLoS One 19 (2024) e0300381, https://doi.org/10.1371/journal.pone.0300381.
[118] Q. Dahui, Next-generation sequencing and its clinical application, Cancer Biol. Med. 16 (2019) 4–10, https://doi.org/10.20892/j.issn.2095-3941.2018.0055.
[119] T. Rajesh, M. Jaya, 7 - next-generation sequencing methods, in: P. Gunasekaran, S. Noronha, A. Pandey (Eds.), Curr. Dev. Biotechnol. Bioeng., Elsevier, 2017, pp. 143–158, https://doi.org/10.1016/B978-0-444-63667-6.00007-9.
[120] K.R. Kukurba, S.B. Montgomery, RNA sequencing and analysis, Cold Spring Harb. Protoc. 2015 (2015), https://doi.org/10.1101/pdb.top084970 pdb.top.084970.
[121] H. Bridle, M. Desmulliez, Chapter 7 - biosensors for the detection of waterborne pathogens, in: H. Bridle (Ed.), Waterborne Pathog, second ed., Academic Press, 2021, pp. 189–235, https://doi.org/10.1016/B978-0-444-64319-3.00007-1.
[122] A. Yadav, K. Yadav, Portable solutions for plant pathogen diagnostics: development, usage, and future potential, Front. Microbiol. 16 (2025) 1516723, https://doi.org/10.3389/fmicb.2025.1516723.
[123] S. Hameed, L. Xie, Y. Ying, Conventional and emerging detection techniques for pathogenic bacteria in food science: a review, Trends Food Sci. Technol. 81 (2018) 61–73, https://doi.org/10.1016/j.tifs.2018.05.020.
[124] Y. Fang, R.P. Ramasamy, Current and prospective methods for plant disease detection, Biosensors 5 (2015) 537–561, https://doi.org/10.3390/bios5030537.
[125] J. Baranwal, B. Barse, G. Gatto, G. Broncova, A. Kumar, Electrochemical sensors and their applications: a review, Chemosensors 10 (2022) 363, https://doi.org/10.3390/chemosensors10090363.
[126] S.M. Borisov, O.S. Wolfbeis, Optical biosensors, Chem. Rev. 108 (2008) 423–461, https://doi.org/10.1021/cr068105t.
[127] R.M. Cardoso, T.S. Pereira, M.H.M. Facure, D.M. dos Santos, L.A. Mercante, L.H. C. Mattoso, D.S. Correa, Current progress in plant pathogen detection enabled by nanomaterials-based (bio)sensors, Sens. Acutators Rep. 4 (2022) 100068, https://doi.org/10.1016/j.snr.2021.100068.
[128] M.L. Giuffrida, E. Rizzarelli, G.A. Tomaselli, C. Satriano, G.T. Sfrazzetto, A novel fully water-soluble Cu(I) probe for fluorescence live cell imaging, Chem. Commun. 50 (2014) 9835–9838, https://doi.org/10.1039/C4CC02147A.
[129] R. Santonocito, N. Tuccitto, V. Cantaro, A.B. Carbonaro, A. Pappalardo, V. Greco, V. Buccilli, P. Maida, D. Zavattaro, G. Sfuncia, G. Nicotra, G. Maccarrone, A. Gulino, A. Giuffrida, G. Trusso Sfrazzetto, Smartphone-assisted sensing of trinitrotoluene by optical array, ACS Omega 7 (2022) 37122–37132, https://doi.org/10.1021/acsomega.2c02958.
[130] R. Santonocito, N. Tuccitto, A. Pappalardo, G. Trusso Sfrazzetto, Smartphone-based dopamine detection by fluorescent supramolecular sensor, Mol. Basel Switz. 27 (2022) 7503, https://doi.org/10.3390/molecules27217503.
[131] S. Yüksel, L. Schwenkbier, S. Pollok, K. Weber, D. Cialla-May, J. Popp, Label-free detection of *Phytophthora ramorum* using surface-enhanced raman spectroscopy, Analyst 140 (2015) 7254–7262, https://doi.org/10.1039/C5AN01156F.
[132] C.G. Awuchi, E.N. Ondari, C.U. Ogbonna, A.K. Upadhyay, K. Baran, C.O. R. Okpala, M. Korzeniowska, R.P.F. Guiné, Mycotoxins affecting animals, foods, humans, and plants: types, occurrence, toxicities, action mechanisms, prevention, and detoxification strategies—A revisit, Foods 10 (2021) 1279, https://doi.org/10.3390/foods10061279.
[133] R. Khan, F. Anwar, F.M. Ghazali, A comprehensive review of mycotoxins: toxicology, detection, and effective mitigation approaches, Heliyon 10 (2024) e28361, https://doi.org/10.1016/j.heliyon.2024.e28361.
[134] R. Santonocito, I.M. Caruso, G. Trusso Sfrazzetto, N-isobutyl-1,8-bis (isobutylamino)-naphthalimide, molbank 2023. https://doi.org/10.3390/M1568, 2023.
[135] N. Tuccitto, G. Catania, A. Pappalardo, G. Trusso Sfrazzetto, Agile detection of chemical warfare agents by machine vision: a supramolecular approach, Chem. Weinh. Bergstr. Ger. 27 (2021) 13715–13718, https://doi.org/10.1002/chem.202102094.
[136] L. Zeng, E.W. Miller, A. Pralle, E.Y. Isacoff, C.J. Chang, A selective turn-on fluorescent sensor for imaging copper in living cells, J. Am. Chem. Soc. 128 (2006) 10–11, https://doi.org/10.1021/ja055064u.
[137] J. Tolosa, F.J. Barba, N. Pallarés, E. Ferrer, Mycotoxin identification and in silico toxicity assessment prediction in Atlantic salmon, Mar. Drugs 18 (2020) 629, https://doi.org/10.3390/md18120629.
[138] Y. Rodríguez-Carrasco, D. Heilos, L. Richter, R.D. Süssmuth, P. Heffeter, M. Sulyok, L. Kenner, W. Berger, R. Dornetshuber-Fleiss, Mouse tissue distribution and persistence of the food-born fusariotoxins enniatin B and beauvericin, Toxicol. Lett. 247 (2016) 35–44, https://doi.org/10.1016/j.toxlet.2016.02.008.
[139] A.B. Serrano, G. Font, J. Mañes, E. Ferrer, Dispersive liquid-liquid microextraction for the determination of emerging fusarium mycotoxins in water, Food Anal. Methods 9 (2016) 856–862, https://doi.org/10.1007/s12161-015-0257-9.
[140] D. Carballo, G. Font, E. Ferrer, H. Berrada, Evaluation of mycotoxin residues on ready-to-eat food by chromatographic methods coupled to mass spectrometry in tandem, Toxins 10 (2018) 243, https://doi.org/10.3390/toxins10060243.
[141] I. Sadok, A. Stachniuk, M. Staniszewska, Developments in the monitoring of patulin in fruits using liquid chromatography: an overview, Food Anal. Methods 12 (2019) 76–93, https://doi.org/10.1007/s12161-018-1340-9.
[142] E.I. Rovetto, C. Luz, F. La Spada, G. Meca, M. Riolo, S.O. Cacciola, Diversity of mycotoxins and other secondary metabolites recovered from blood oranges infected by *Colletotrichum, Alternaria*, and *Penicillium* species, Toxins 15 (2023) 407, https://doi.org/10.3390/toxins15070407.
[143] M.H. Boshra, G.S. El-Housseiny, M.M.S. Farag, K.M. Aboshanab, Evaluation of ELISA and immunoaffinity fluorometric analytical tools of four mycotoxins in various food categories, AMB Express 13 (2023) 123, https://doi.org/10.1186/s13568-023-01629-5.
[144] A. Nazir, M. Asif, I. Kalim, M. Iqbal, N. Ahmad, Quantification of aflatoxins in dry fruits using enzyme linked immunosorbent assay with reference to standards for human consumption. https://doi.org/10.5281/ZENODO.6801794, 2022.
[145] M.A. Pavon, I. Gonzalez Alonso, R. Martín, T. Garcia, Competitive direct ELISA based on a monoclonal antibody for detection of ochratoxin A in dried fig samples, Food Agric. Immunol. - FOOD AGRIC IMMUNOL 23 (2012) 83–91, https://doi.org/10.1080/09540105.2011.604769.
[146] A. Przybylska, A. Chrustek, D. Olszewska-Słonina, M. Koba, S. Kruszewski, Determination of patulin in products containing dried fruits by enzyme-linked immunosorbent assay technique patulin in dried fruits, Food Sci. Nutr. 9 (2021) 4211–4220, https://doi.org/10.1002/fsn3.2386.
[147] L. Anfossi, C. Baggiani, C. Giovannoli, G. D'Arco, G. Giraudi, Lateral-flow immunoassays for mycotoxins and phycotoxins: a review, Anal. Bioanal. Chem. 405 (2013) 467–480, https://doi.org/10.1007/s00216-012-6033-4.
[148] B. Cvak, B. Warth, J. Atehnkeng, A. Parich, A. Moritz, M. Sulyok, R. Krska, Evaluating the performance of lateral flow devices for total aflatoxins with special emphasis on their robustness under sub-saharan conditions, Toxins 13 (2021) 742, https://doi.org/10.3390/toxins13110742.
[149] Food and Agriculture Organization of the United Nations and World Health Organization, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2021, 2022 anno: 1995. Codex Alimentarius: General Standard for Contaminants and Toxins in Food and Feed CXS 193-1995 Amended in 2010, 1995.
[150] J.Y. Zhang, N. Li, Y. Zhou, Y. Jiang, P.F. Tu, Simultaneous qualitative and quantitative determination of major polymethoxylated flavonoids in the leaves of *Murraya paniculata* by RRLC-DAD-ESI-MSn, Anal. Methods 4 (2012) 3399.
[151] H.U. Melchert, E. Pabel, Reliable identification and quantification of trichothecenes and other mycotoxins by electron impact and chemical ionization-gas chromatography-mass spectrometry, using an ion-trap system in the multiple mass spectrometry mode. Candidate reference method for complex matrices, J. Chromatogr. A 1056 (2004) 195–199.
[152] C. Ruan, X. Diao, N. Li, H. Zhang, Y. Pang, C. Liu, Determination of ochratoxin A and citrinin in fruits using ultrasound-assisted solvent extraction followed by dispersive liquid–liquid microextraction with HPLC with fluorescence detection, Anal. Methods 8 (2016) 1586–1594, https://doi.org/10.1039/C5AY03219A.
[153] M. Vaclavikova, Z. Dzuman, O. Lacina, M. Fenclova, Z. Veprikova, M. Zachariasova, J. Hajslova, Monitoring survey of patulin in a variety of fruit-based products using a sensitive UHPLC–MS/MS analytical procedure, Food Control 47 (2015) 577–584, https://doi.org/10.1016/j.foodcont.2014.07.064.
[154] A. Zgoła-Grześkowiak, T. Grześkowiak, Dispersive liquid–liquid microextraction, TrAC, Trends Anal. Chem. 30 (2011) 1382–1399, https://doi.org/10.1016/j.trac.2011.04.014.
[155] C. Brera, S. Grossi, B. De Santis, M. Miraglia, Automated HPLC method for the determination of ochratoxin A in wine samples, J. Liq. Chromatogr. Relat. Technol. 26 (2003) 119–133, https://doi.org/10.1081/JLC-120017157.
[156] R.J. Garcia-Villanova, C. Cordón, A.M. González Paramás, P. Aparicio, M. E. Garcia Rosales, Simultaneous immunoaffinity column cleanup and HPLC analysis of aflatoxins and ochratoxin A in Spanish bee pollen, J. Agric. Food Chem. 52 (2004) 7235–7239, https://doi.org/10.1021/jf048882z.
[157] S. Guan, M. Gong, Y. Yin, R. Huang, Z. Ruan, T. Zhou, M. Xie, Occurrence of mycotoxins in feeds and feed ingredients in China, J. Food Agric. Environ. 9 (2011).
[158] A.Ž. Kostić, D.D. Milinčić, T.S. Petrović, V.S. Krnjaja, S.P. Stanojević, M.B. Barać, Ž.L. Tešić, M.B. Pešić, Mycotoxins and mycotoxin producing fungi in pollen: review, Toxins 11 (2019) 64, https://doi.org/10.3390/toxins11020064.
[159] D. Morais, K. Massarolo, E. Grinfelder Ardohain, J. Lima, F. Ferreira, D. Drunkler, Method for determination of multi-mycotoxins in milk: QuEChERS extraction modified followed by HPLC-FL analysis, Food Anal. Methods 17 (2024) 47–60, https://doi.org/10.1007/s12161-023-02550-0.
[160] M.N. Pascale, Detection methods for mycotoxins in cereal grains and cereal products, Zb. Matice Srp. Za Prir. Nauke (2009) 15–25. https://doiserbia.nb.rs/Article.aspx?ID=0352-49060917015P.
[161] S.M. Herzallah, Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors, Food Chem. 114 (2009) 1141–1146, https://doi.org/10.1016/j.foodchem.2008.10.077.
[162] H.J. Kim, M.J. Lee, H.J. Kim, S.K. Cho, H.J. Park, M.H. Jeong, Analytical method development and monitoring of aflatoxin B1, B2, G1, G2 and ochratoxin A in animal feed using HPLC with fluorescence detector and photochemical reaction device, Cogent Food Agric. 3 (2017) 1419788, https://doi.org/10.1080/23311932.2017.1419788.
[163] H.E. Ok, S.Y. Lee, H.S. Chun, Occurrence and simultaneous determination of nivalenol and deoxynivalenol in rice and bran by HPLC-UV detection and immunoaffinity cleanup, Food Control 87 (2018) 53–59, https://doi.org/10.1016/j.foodcont.2017.12.005.
[164] S.Y. Woo, S.Y. Lee, T.K. Jeong, S.M. Park, J.H. Auh, H.-S. Shin, H.S. Chun, Natural occurrence of *Alternaria* toxins in agricultural products and processed foods

marketed in South Korea by LC–MS/MS, Toxins 14 (2022) 824, https://doi.org/10.3390/toxins14120824.
[165] Y. Zha, C. Yang, J. Ye, X. Wang, L. Lin, S. Zeng, Y. Huang, Y. Su, Determination of mycotoxin contaminants in juice by using Lc-Ms/Ms, in: Mach. Mater. Sci. Eng. Appl., CRC Press, 2017.
[166] Éder de Vilhena Araújo, F.F. Souza Jesus, B. Lederman Valente, M. Cristianini, C. C. Montagner, T.P. Fill, Analytical determination of tryptoquialanines A and B: ensuring the quality and safety of Orange juices, Food Res. Int. 174 (2023) 113607, https://doi.org/10.1016/j.foodres.2023.113607.
[167] N.P. Kalogiouri, E.-N. Papadakis, M.G. Maggalou, G.S. Karaoglanidis, V. F. Samanidou, U. Menkissoglu-Spiroudi, Development of a microwave-assisted extraction protocol for the simultaneous determination of mycotoxins and pesticide residues in apples by LC-MS/MS, Appl. Sci. 11 (2021) 10931, https://doi.org/10.3390/app112210931.
[168] T. Tanaka, A. Yoneda, S. Inoue, Y. Sugiura, Y. Ueno, Simultaneous determination of trichothecene mycotoxins and zearalenone in cereals by gas chromatography-mass spectrometry, J. Chromatogr. A 882 (2000) 23–28, https://doi.org/10.1016/s0021-9673(00)00063-7.
[169] A. Alshannaq, J.-H. Yu, Occurrence, toxicity, and analysis of major mycotoxins in food, Int. J. Environ. Res. Publ. Health 14 (2017) 632, https://doi.org/10.3390/ijerph14060632.
[170] F. Orata, Derivatization reactions and reagents for gas chromatography analysis, in: Adv. Gas Chromatogr. - Prog. Agric. Biomed. Ind., Appl., IntechOpen, 2012, https://doi.org/10.5772/33098.
[171] P.M. Scott, J.W. Lawrence, W. van Walbeek, Detection of mycotoxins by thin-layer chromatography: application to screening of fungal extracts, Appl. Microbiol. 20 (1970) 839–842, https://doi.org/10.1128/am.20.5.839-842.1970.
[172] N. Pallarés, D. Carballo, E. Ferrer, M. Fernández-Franzón, H. Berrada, Mycotoxin dietary exposure assessment through fruit juices consumption in children and adult population, Toxins 11 (2019) 684, https://doi.org/10.3390/toxins11120684.
[173] G. Evtugyn, A. Porfireva, T. Kulikova, T. Hianik, Recent achievements in electrochemical and surface plasmon resonance aptasensors for mycotoxins detection, Chemosensors 9 (2021) 180, https://doi.org/10.3390/chemosensors9070180.
[174] A. Rico-Yuste, R. Abouhany, J.L. Urraca, A.B. Descalzo, G. Orellana, M.C. Moreno-Bondi, Eu(III)-Templated molecularly imprinted polymer used as a luminescent sensor for the determination of tenuazonic acid mycotoxin in food samples, Sensor. Actuator. B Chem. 329 (2021) 129256, https://doi.org/10.1016/j.snb.2020.129256.
[175] D.R. Thévenot, K. Toth, R.A. Durst, G.S. Wilson, Electrochemical biosensors: recommended definitions and classification, Biosens. Bioelectron. 16 (2001) 121–131, https://doi.org/10.1016/s0956-5663(01)00115-4.
[176] W.-W. Zhao, J.-J. Xu, H.-Y. Chen, Photoelectrochemical immunoassays, Anal. Chem. 90 (2018) 615–627, https://doi.org/10.1021/acs.analchem.7b04672.
[177] M.M. Hassan, M. Zareef, Y. Xu, H. Li, Q. Chen, SERS based sensor for mycotoxins detection: challenges and improvements, Food Chem. 344 (2021) 128652, https://doi.org/10.1016/j.foodchem.2020.128652.
[178] F. Xing, H. Yao, Y. Liu, X. Dai, R.L. Brown, D. Bhatnagar, Recent developments and applications of hyperspectral imaging for rapid detection of mycotoxins and mycotoxigenic fungi in food products, Crit. Rev. Food Sci. Nutr. 59 (2019) 173–180, https://doi.org/10.1080/10408398.2017.1363709.
[179] F. Degola, E. Berni, C. Dall'Asta, E. Spotti, R. Marchelli, I. Ferrero, F.M. Restivo, A multiplex RT-PCR approach to detect aflatoxigenic strains of *Aspergillus flavus*, J. Appl. Microbiol. 103 (2007) 409–417, https://doi.org/10.1111/j.1365-2672.2006.03256.x.
[180] M. Ferrara, A.F. Logrieco, A. Moretti, A. Susca, A loop-mediated isothermal amplification (LAMP) assay for rapid detection of fumonisin producing *Aspergillus* species, Food Microbiol. 90 (2020) 103469, https://doi.org/10.1016/j.fm.2020.103469.
[181] W. Mellikeche, A. Ricelli, G. Casini, M. Gallo, N. Baser, G. Colelli, A.M. D'Onghia, Development of loop-mediated isothermal amplification (LAMP) assays for the rapid detection of toxigenic *Aspergillus flavus* and *A. carbonarius* in nuts, Int. J. Mol. Sci. 25 (2024) 3809, https://doi.org/10.3390/ijms25073809.
[182] L. Niessen, J. Bechtner, S. Fodil, M.H. Taniwaki, R.F. Vogel, LAMP-Based group specific detection of aflatoxin producers within *Aspergillus* section *Favi* in food raw materials, spices, and dried fruit using neutral red for visible-light signal detection, Int. J. Food Microbiol. 266 (2018) 241–250, https://doi.org/10.1016/j.ijfoodmicro.2017.12.013.
[183] S.F. Ortega, I. Siciliano, S. Prencipe, M.L. Gullino, D. Spadaro, Development of PCR, LAMP and qPCR assays for the detection of aflatoxigenic strains of *Aspergillus flavus* and *A. parasiticus* in hazelnut, Toxins 12 (2020) 757, https://doi.org/10.3390/toxins12120757.
[184] M.J. Sweeney, P. Pàmies, A.D. Dobson, The use of reverse transcription-polymerase chain reaction (RT-PCR) for monitoring aflatoxin production in *Aspergillus parasiticus* 439, Int. J. Food Microbiol. 56 (2000) 97–103, https://doi.org/10.1016/s0168-1605(00)00277-4.
[185] L.M. Frisch, L. Niessen, Development and optimization of a group-specific loop-mediated isothermal amplification (LAMP) assay for the detection of patulin-producing *penicillium* species, Int. J. Food Microbiol. 298 (2019) 20–30, https://doi.org/10.1016/j.ijfoodmicro.2019.03.010.
[186] B. Patiño, A. González-Salgado, M.T. González-Jaén, C. Vázquez, PCR detection assays for the ochratoxin-producing *Aspergillus carbonarius* and *Aspergillus ochraceus* species, Int. J. Food Microbiol. 104 (2005) 207–214, https://doi.org/10.1016/j.ijfoodmicro.2005.02.011.
[187] M. Storari, R. von Rohr, I. Pertot, C. Gessler, G.a.L. Broggini, Identification of ochratoxin A producing *Aspergillus carbonarius* and *A. niger* clade isolated from grapes using the loop-mediated isothermal amplification (LAMP) reaction, J. Appl. Microbiol. 114 (2013) 1193–1200, https://doi.org/10.1111/jam.12139.
[188] J. Tannous, A. Atoui, A. El Khoury, S. Kantar, N. Chdid, I.P. Oswald, O. Puel, R. Lteif, Development of a real-time PCR assay for *Penicillium expansum* quantification and patulin estimation in apples, Food Microbiol. 50 (2015) 28–37, https://doi.org/10.1016/j.fm.2015.03.001.
[189] G. Maussion, R.A. Thomas, I. Demirova, G. Gu, E. Cai, C.X.-Q. Chen, N. Abdian, T. J.P. Strauss, S. Kelaï, A. Nauleau-Javaudin, L.K. Beitel, N. Ramoz, P. Gorwood, T. M. Durcan, Auto-qPCR; a python-based web app for automated and reproducible analysis of qPCR data, Sci. Rep. 11 (2021) 21293, https://doi.org/10.1038/s41598-021-99727-6.
[190] F. Rupprecht, S. Enge, K. Schmidt, W. Gao, R. Miller, Automating LC–MS/MS mass chromatogram quantification: wavelet transform based peak detection and automated estimation of peak boundaries and signal-to-noise ratio using signal processing methods, Biomed. Signal Process Control 71 (2022) 103211, https://doi.org/10.1016/j.bspc.2021.103211.
[191] Y. Liu, J. Li, Z. Guo, C. Feng, Y. Gao, D. Liu, D. Wang, Development and validation of a droplet digital PCR assay for sensitive detection and quantification of *Phytophthora nicotianae*, Front. Plant Sci. 16 (2025), https://doi.org/10.3389/fpls.2025.1573949.
[192] J. Masquelier, E.K. Tangni, P. Becker, J. Sanders, J. Laporte, B. Mertens, Validation of an LC-MS method for quantification of mycotoxins and characterization of fungal strains occurring in food and feed, Chemosensors 13 (2025) 106, https://doi.org/10.3390/chemosensors13030106.
[193] K. Garg, F. Villavicencio-Aguilar, F. Solano-Rivera, L. Gilbert, Analytical validation of a direct competitive ELISA for multiple mycotoxin detection in human serum, Toxins 14 (2022) 727, https://doi.org/10.3390/toxins14110727.
[194] J.S. Mandlik, A.S. Patil, S. Singh, Next-generation sequencing (NGS): platforms and applications, J. Pharm. BioAllied Sci. 16 (2024) S41, https://doi.org/10.4103/jpbs.jpbs_838_23.
[195] T. Notomi, Y. Mori, N. Tomita, H. Kanda, Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects, J. Microbiol. 53 (2015) 1–5, https://doi.org/10.1007/s12275-015-4656-9.
[196] M. Sulyok, R. Krska, R. Schuhmacher, A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples, Anal. Bioanal. Chem. 389 (2007) 1505–1523, https://doi.org/10.1007/s00216-007-1542-2.
[197] N.W. Turner, S. Subrahmanyam, S.A. Piletsky, Analytical methods for determination of mycotoxins: a review, Anal. Chim. Acta 632 (2009) 168–180, https://doi.org/10.1016/j.aca.2008.11.010.
[198] B. Babu, F.M. Ochoa-Corona, M.L. Paret, Recombinase polymerase amplification applied to plant virus detection and potential implications, Anal. Biochem. 546 (2018) 72–77, https://doi.org/10.1016/j.ab.2018.01.021.
[199] Y. Mori, T. Notomi, Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases, J. Infect. Chemother. 15 (2009) 62–69, https://doi.org/10.1007/s10156-009-0669-9.
[200] J. Pedreira-Rincón, L. Rivas, J. Comenge, V. Skouridou, D. Camprubí-Ferrer, J. Muñoz, C.K. O'Sullivan, A. Chamorro-Garcia, C. Parolo, A comprehensive review of competitive lateral flow assays over the past decade, Lab Chip 25 (2025) 2578–2608, https://doi.org/10.1039/D4LC01075B.
[201] A. Ali, High-performance liquid chromatography (HPLC): a review, Ann. Adv. Chem. 6 (2022) 10–20, https://doi.org/10.29328/journal.aac.1001026.
[202] K.S.-Y. Leung, B.M.-W. Fong, LC–MS/MS in the routine clinical laboratory: has its time come? Anal. Bioanal. Chem. 406 (2014) 2289–2301, https://doi.org/10.1007/s00216-013-7542-5.
[203] H. Ma, K.N. Bell, R.N. Loker, qPCR and qRT-PCR analysis: regulatory points to consider when conducting biodistribution and vector shedding studies, Mol. Ther. - Methods Clin. Dev. 20 (2021) 152–168, https://doi.org/10.1016/j.omtm.2020.11.007.
[204] M. Mirza, L. Goerke, A. Anderson, T. Wilsdon, Assessing the cost-effectiveness of next-generation sequencing as a biomarker testing approach in oncology and policy implications: a literature review, Value Health 27 (2024) 1300–1309, https://doi.org/10.1016/j.jval.2024.04.023.
[205] M.E.E. Alahi, S.C. Mukhopadhyay, Detection methodologies for pathogen and toxins: a review, Sensors 17 (2017) 1885, https://doi.org/10.3390/s17081885.
[206] Introduction to PM 7 Standards on Diagnostics, EPPO Bull. 54 (2024) 310–311, https://doi.org/10.1111/epp.13065.
[207] ISO 22174:2024, ISO 22174:2024 microbiology of the food chain — polymerase chain reaction (PCR) for the detection and quantification of microorganisms — general requirements and definitions. https://www.iso.org/obp/ui/#iso:std:iso:22174:ed-2:v1:en, 2024.
[208] E.P. on C, In the F. Chain (CONTAM), scientific opinion on risks for animal and public health related to the presence of nivalenol in food and feed, EFSA J. 11 (2013) 3262, https://doi.org/10.2903/j.efsa.2013.3262.
[209] E.P. on C, In the F. Chain (CONTAM), scientific opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed, EFSA J. 9 (2011) 2481, https://doi.org/10.2903/j.efsa.2011.2481.
[210] ISO 16050:2003, ISO 16050:2003 foodstuffs — determination of aflatoxin B1, and the total content of aflatoxins B1, B2, G1 and G2 in cereals, nuts and derived products — high-Performance liquid chromatographic method, ISO - Int. Organ. Stand. (2003) 1–12. https://www.iso.org/standard/29628.html.
[211] D. Zhang, B. Sun, Y. Luan, X. Zhou, J. Huang, Y. Sun, S. Feng, H. Yang, Simultaneous broad-spectrum detection of over 50 mycotoxins in maize silage via

liquid chromatography-tandem mass spectrometry: method development and application, Food Control 172 (2025) 111143, https://doi.org/10.1016/j.foodcont.2025.111143.
[212] Commission Implementing Regulation (EU) 2019/2072, Commission implementing regulation (EU) 2019/2072 establishing uniform conditions for the implementation of regulation (EU) 2016/2031 of the european parliament and the council, as regards protective measures against pests of plants, and repealing commission regulation (EC) no. 690/2008 and amending commission implementing regulation (EU) 2018/2019. | FAOLEX. https://www.fao.org/faolex/results/details/en/c/LEX-FAOC192043/, 2019. (Accessed 4 September 2024).
[213] H. Shi, S. Li, Y. Bai, L.L. Prates, Y. Lei, P. Yu, Mycotoxin contamination of food and feed in China: occurrence, detection techniques, toxicological effects and advances in mitigation technologies, Food Control 91 (2018) 202–215, https://doi.org/10.1016/j.foodcont.2018.03.036.
[214] Y. Zha, C. Yang, J. Ye, X. Wang, L. Lin, S. Zeng, Y. Huang, Y. Su, Determination of mycotoxin contaminants in juice by using LC-MS/MS,, in: Mach. Mater. Sci. Eng. Appl., CRC Press, 2017.
[215] L. Petri, Sulla posizione sistematica del fungo parassita delle piante di limone affette da "malsecco", Bolletino Della Stazione Patol. Veg. 9 (1929) 393–396 (in Italian).
[216] T. He, J. Zhu, Y. Nie, R. Hu, T. Wang, P. Li, Q. Zhang, Y. Yang, Nanobody technology for mycotoxin detection: current status and prospects, Toxins 10 (2018) 180, https://doi.org/10.3390/toxins10050180.
[217] V. Dopazo, C. Luz, J. Mañes, J.M. Quiles, R. Carbonell, J. Calpe, G. Meca, Bio-preservative potential of microorganisms isolated from red grape against food contaminant fungi, Toxins 13 (2021) 412, https://doi.org/10.3390/toxins13060412.
[218] M. Riolo, C. Luz, C. Bua, S. Barreca, M.C. Tambè, J. Calpe, M. Masi, A. Cimmino, G. Tirrò, G. Meca, D. Ezra, S.O. Cacciola, Qualitative and relative abundance analysis of *Alternaria alternata* metabolites in pomegranate (*Punica granatum* L.) cultivars affected by heart rot using UHPLC-Q-TOF-MS, Food Control 172 (2025) 111204, https://doi.org/10.1016/j.foodcont.2025.111204.
[219] E.M. Thurman, I. Ferrer, A.R. Fernández-Alba, Matching unknown empirical formulas to chemical structure using LC/MS TOF accurate mass and database searching: example of unknown pesticides on tomato skins, J. Chromatogr. A 1067 (2005) 127–134, https://doi.org/10.1016/j.chroma.2004.11.007.
[220] T.T. Xing, X.J. Zhao, Y.D. Zhang, Y.F. Li, Fast separation and sensitive quantitation of polymethoxylated flavonoids in the peels of citrus using UPLC-Q-TOF-MS, J. Agric. Food Chem. 65 (2017) 2615–2627, https://doi.org/10.1021/acs.jafc.6b05821.
[221] R.B. Baldassari, E. Wickert, A. de Goes, Pathogenicity, colony morphology and diversity of isolates of *Guignardia citricarpa* and *G. mangiferae* isolated from *citrus* spp, Eur. J. Plant Pathol. 120 (2008) 103–110, https://doi.org/10.1007/s10658-007-9182-0.
[222] EFSA, Evaluation of a paper by Guarnaccia, on the first report of *Phyllosticta citricarpa* in Europe (2017). https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2018.5114. (Accessed 25 February 2025).
[223] EPPO, PM 7/017 (3, *Phyllosticta citricarpa* (Formerly *Guignardia citricarpa*) - EPPO Bulletin, Wiley Online Libr, 2020. https://onlinelibrary.wiley.com/doi/10.1111/epp.12700. (Accessed 25 February 2025).
[224] A. Schirmacher, J. Tomlinson, A. Barnes, V. Barton, Species-specific real-time PCR for diagnosis of Phyllosticta citricarpa on citrus species, EPPO Bull. 49 (2019), https://doi.org/10.1111/epp.12555.
[225] N. Boughalleb-M'Hamdi, A. Fathallah, N. Benfradj, S.B. Mahmoud, A.B.H. Ali, L. Medhioub, I. Jaouadi, J. Huber, C. Jeandel, R. Ioos, First report of citrus black spot disease caused by *Phyllosticta citricarpa* on *citrus Limon* and *C. sinensis* in Tunisia, new dis, Rep 41 (2020), https://doi.org/10.5197/j.2044-0588.2020.041.008.
[226] M. Spósito, L. Amorin, R. Bassanezi, P.T. Yamamoto, M.R. Felippe, A.B. C. Czermainski, Relative importance of inoculum sources of *Guignardia citricarpa* on the citrus black spot epidemic in Brazil, Crop Prot. 30 (12) (2011) 1546–1552.
[227] A. Garibaldi, A. Minuto, V. Grasso, M.L. Gullino, Application of selected antagonistic strains against *Phytophthora cryptogea* on gerbera in closed soilless systems with disinfection by slow sand filtration, Crop Prot. 22 (2003) 1053–1061, https://doi.org/10.1016/S0261-2194(03)00139-X.
[228] J. Kotze, Epidemiology and control of Citrus Black Spot in South Africa Plant dis. 65 (12) (1981).
[229] I. Paul, A.S. van Jaarsveld, L. Korsten, V. Hattingh, The potential global geographical distribution of citrus black spot caused by *Guignardia citricarpa* (kiely): likelihood of disease establishment in the european union, Crop Prot. 24 (2005) 297–308, https://doi.org/10.1016/j.cropro.2004.08.003.
[230] V. Guarnaccia, T. Gehrmann, G.J. Silva-Junior, P.H. Fourie, S. Haridas, D. Vu, J. Spatafora, F.M. Martin, V. Robert, I.V. Grigoriev, J.Z. Groenewald, P.W. Crous, *Phyllosticta citricarpa* and sister species of global importance to citrus, Mol. Plant Pathol. 20 (2019) 1619–1635, https://doi.org/10.1111/mpp.12861.
[231] V. Guarnaccia, J.Z. Groenewald, H. Li, C. Glienke, E. Carstens, V. Hattingh, P. H. Fourie, P.W. Crous, First report of *Phyllosticta citricarpa* and description of two new species, *P. paracapitalensis* and *P. paracitricarpa*, from citrus in Europe, Stud. Mycol. 87 (2017) 161–185, https://doi.org/10.1016/j.simyco.2017.05.003.
[232] V.A. van Ingen-Buijs, A.C. van Westerhoven, P. Skiadas, X.C.L. Zuijdgeest, S. Haridas, C. Daum, K. Duffy, J. Guo, H. Hundley, K. LaButti, A. Lipzen, J. Pangilinan, R. Riley, J. Wang, M. Yan, F. Martin, K. Barry, I.V. Grigoriev, J. Z. Groenewald, P.W. Crous, M.F. Seidl, *Phyllosticta paracitricarpa* is synonymous with the EU quarantine fungus *P. citricarpa* based on phylogenomic analyses, Fungal Genet. Biol. 175 (2024) 103925, https://doi.org/10.1016/j.fgb.2024.103925.
[233] R. Ioos, A. Puertolas, C. Renault, A. Ndiaye, I. Cerf-Wendling, J. Hubert, W. Wang, C. Jiao, H. Li, J. Armengol, J. Aguayo, Harnessing the power of comparative genomics to support the distinction of sister species within *Phyllosticta* and development of highly specific detection of *Phyllosticta citricarpa* causing citrus Black spot by real-time PCR, PeerJ 11 (2023) e16354, https://doi.org/10.7717/peerj.16354.
[234] J. Zajc, Z. Kogej Zwitter, S. Fišer, C. Gostinčar, A. Vicent, A.G. Domenech, L. Riccioni, N. Boonham, M. Ravnikar, P. Kogovšek, Highly specific qPCR and amplicon sequencing method for detection of quarantine citrus pathogen *Phyllosticta citricarpa* applicable for air samples, Plant Pathol. 72 (2023) 548–563, https://doi.org/10.1111/ppa.13679.
[235] C. Brasier, B. Scanu, D. Cooke, T. Jung, *Phytophthora*: an ancient, historic, biologically and structurally cohesive and evolutionarily successful generic concept in need of preservation, IMA Fungus 13 (2022) 12, https://doi.org/10.1186/s43008-022-00097-z.
[236] C.M. Brasier, N.J. Grünwald, T.B. Bourret, F. Govers, B. Scanu, D.E.L. Cooke, T. Bose, D.L. Hawksworth, Z.G. Abad, M.V. Albarracin, W. Alsultan, A. E. Altamirano-Junqueira, A.R. Arifin, M.J. Arnet, H.D.R. Aumentado, J. Bakonyi, W.H. Belisle, A. Benigno, J.C. Bienapfl, G.J. Bilodeau, J.E. Blair, L. Botella, A. Brandano, S.O. Cacciola, I. Carbone, V.L. Castroagudin, N. Chaendaekattu, J. D. Consford, T. Corcobado, P.A. Covey, H.A. Daniels, A. Deidda, A.E. Dorrance, E. N. Dort, A. Drenth, F. Drizou, E. Evangelisti, S.N. Fajardo, Y. Fang, C.M. Ference, S.J. Frankel, E.M. Goss, D.I. Guest, G.E.S.J. Hardy, A.R.H. Harris, M.D. Hawku, K. Heungens, C. Hong, I.J. Horner, M.H. Jung, O.J. Iyanda, B.-A. Jamieson, S. N. Jeffers, H.S. Judelson, M. Junaid, E. Kalogeropoulou, S. Kamoun, S. Kang, T. Kasuga, T. Kudláček, J. LeBoldus, C.A. Lee, D. Li, A.K. Llanos, H.D. Lopez-Nicora, H. Machado, G.M. di San Lio, C. Maia, K. Mandal, P. Manosalva, F. N. Martin, M.E.H. Matson, R.L. McDougal, J.M. McDowell, R.W. Michelmore, I. Milenković, S. Moricca, R. Mostowfizadeh-Ghalamfarsa, Z.Á. Nagy, E. V. Nikolaeva, P. Ortega-López, T. Paap, C.H. Parada-Rojas, F.P. Hand, A. Pérez-Sierra, M. Pettersson, P. Prasad, A.S. Puig, M. Raco, N.A. Rajput, J.B. Ristaino, S. Rooney-Latham, M.F. Seidl, S.F. Shamoun, A. Solla, C.F.J. Spies, M. A. Sudermann, T.J. Swiecki, M. Tian, S. Tripathy, S. Uematsu, K. Van Poucke, A. E. Vichou, M. Walter, J.F. Webber, N.M. Williams, M.J. Wingfield, D. Yadav, X. Yang, T. Jung, Preserving the biologically coherent generic concept of *Phytophthora*, "Plant Destroyer," Phytopathology® 115 (2025) 573–586, https://doi.org/10.1094/PHYTO-11-24-0372-LE.
[237] W. Hao, T.D. Miles, F.N. Martin, G.T. Browne, H. Förster, J.E. Adaskaveg, Temporal occurrence and niche preferences of *Phytophthora* spp. causing brown rot of citrus in the central valley of California, Phytopathology 108 (2018) 384–391, https://doi.org/10.1094/PHYTO-09-17-0315-R.
[238] E.I. Rovetto, F. La Spada, F. Aloi, M. Riolo, A. Pane, M. Garbelotto, S.O. Cacciola, Green solutions and new technologies for sustainable management of fungus and oomycete diseases in the citrus fruit supply chain, J. Plant Pathol. 106 (2024) 411–437, https://doi.org/10.1007/s42161-023-01543-6.
[239] P.W. Crous, M.J. Wingfield, T.I. Burgess, G.E.S.T.J. Hardy, P.A. Barber, P. Alvarado, C.W. Barnes, P.K. Buchanan, M. Heykoop, G. Moreno, R. Thangavel, S. Van der spuy, A. Barili, S. Barrett, S.O. Cacciola, J.F. Cano-Lira, C. Crane, C. Decock, T.B. Gibertoni, J. Guarro, M. Guevara-Suarez, V. Hubka, M. Kolařík, C. R.S. Lira, M.E. Ordoñez, M. Padamsee, L. Ryvarden, A.M. Soares, A.M. Stchigel, D. A. Sutton, A. Vizzini, B.S. Weir, K. Acharya, F. Aloi, I.G. Baseia, R.A. Blanchette, J. J. Bordallo, Z. Bratek, T. Butler, J. Cano-Canals, J.R. Carlavilla, J. Chander, R. Cheewangkoon, R.H.S.F. Cruz, M. Da silva, A.K. Dutta, E. Ercole, V. Escobio, F. Esteve-Raventós, J.A. Flores, J. Gené, J.S. Góis, L. Haines, B.W. Held, M. Horta jung, K. Hosaka, T. Jung, Ž. Jurjević, V. Kautman, I. Kautmanova, A.A. Kiyashko, M. Kozanek, A. Kubátová, M. Lafourcade, F. La spada, K.P.D. Latha, H. Madrid, E. F. Malysheva, P. Manimohan, J.L. Manjón, M.P. Martín, M. Mata, Z. Merényi, A. Morte, I. Nagy, A.-C. Normand, S. Paloi, N. Pattison, J. Pawłowska, O. L. Pereira, M.E. Petterson, B. Picillo, K.N.A. Raj, A. Roberts, A. Rodríguez, F. J. Rodríguez-Campo, M. Romański, M. Ruszkiewicz-Michalska, B. Scanu, L. Schena, M. Semelbauer, R. Sharma, Y.S. Shouche, V. Silva, M. Staniaszek-Kik, J.B. Stielow, C. Tapia, P.W.J. Taylor, M. Toome-Heller, J.M.C. Vabeikhokhei, A. D. van Diepeningen, N. Van Hoa, M. Van Tri, N.P. Wiederhold, M. Wrzosek, J. Zothanzama, J.Z. Groenewald, Fungal planet description sheets: 558–624, Persoonia - Mol. Phylogeny Evol. Fungi 38 (2017) 240–384, https://doi.org/10.3767/003158517X698941.
[240] D.C. Erwin, O.K. Ribeiro. *Phytophthora* Diseases Worldwide, American Phytopathological Society (APS Press, St. Paul, MN, USA, 1996.
[241] T.E. Graham, E. Hibbert, P. Sathasivam, Metabolic and exercise endurance effects of coffee and caffeine ingestion, J. Appl. Physiol. Bethesda Md 1985 85 (1998) 883–889, https://doi.org/10.1152/jappl.1998.85.3.883.
[242] L.P.N.M. Kroon, H. Brouwer, A.W.A.M. deCock, F. Govers, The genus *Phytophthora*, Phytopathology 102 (2012) 348–364, https://doi.org/10.1094/PHYTO-01-11-0025.
[243] Z.R. Hansen, B.J. Knaus, J.F. Tabima, C.M. Press, H.S. Judelson, N.J. Grünwald, C. D. Smart, Loop-mediated isothermal amplification for detection of the tomato and potato late blight pathogen, *Phytophthora infestans*, J. Appl. Microbiol. 120 (2016) 1010–1020, https://doi.org/10.1111/jam.13079.
[244] M. Khan, B. Li, Y. Jiang, Q. Weng, Q. Chen, Evaluation of different PCR-based assays and LAMP method for rapid detection of *Phytophthora infestans* by targeting the Ypt1 gene, Front. Microbiol. 8 (2017), https://doi.org/10.3389/fmicb.2017.01920.
[245] M. Si Ammour, G.J. Bilodeau, D.M. Tremblay, H. Van der Heyden, T. Yaseen, L. Varvaro, O. Carisse, Development of real-time isothermal amplification assays for On-Site detection of *Phytophthora infestans* in potato leaves, Plant Dis. 101 (2017) 1269–1277, https://doi.org/10.1094/PDIS-12-16-1780-RE.

[246] T. Dai, T. Hu, X. Yang, D. Shen, B. Jiao, W. Tian, Y. Xu, A recombinase polymerase amplification-lateral flow dipstick assay for rapid detection of the quarantine citrus pathogen in China, *Phytophthora hibernalis*, PeerJ 7 (2019) e8083, https://doi.org/10.7717/peerj.8083.

[247] K. Aslam, S.Z. Iqbal, A.F.A. Razis, S. Usman, N.B. Ali, Patulin contamination of citrus fruits from Punjab and northern Pakistan and estimation of associated dietary intake, Int. J. Environ. Res. Publ. Health 18 (2021) 2270, https://doi.org/10.3390/ijerph18052270.

[248] H.P. Bartholomew, M. Bradshaw, W.M. Jurick, J.M. Fonseca, The good, the bad, and the ugly: mycotoxin production during postharvest decay and their influence on tritrophic host–pathogen–microbe interactions, Front. Microbiol. 12 (2021), https://doi.org/10.3389/fmicb.2021.611881.

[249] D. Tsitsigiannis, M. Dimakopoulou, P. Antoniou, E. Tjamos, Biological control strategies of mycotoxigenic fungi and associated mycotoxins in Mediterranean Basin crops, Phytopathol. Mediterr. 51 (2012) 158–174, https://doi.org/10.14601/Phytopathol_Mediterr-9497.

[250] P. Battilani, R. Palumbo, P. Giorni, C. Dall'Asta, L. Dellafiora, A. Gkrillas, P. Toscano, A. Crisci, C. Brera, B. De Santis, R. Rosanna Cammarano, M. Della Seta, K. Campbell, C. Elliot, A. Venancio, N. Lima, A. Gonçalves, C. Terciolo, I. P. Oswald, Mycotoxin mixtures in food and feed: holistic, innovative, flexible risk assessment modelling approach, EFSA Support. Publ. 17 (2020) 1757E, https://doi.org/10.2903/sp.efsa.2020.EN-1757.

[251] M. Nan, H. Xue, Y. Bi, Contamination, detection and control of mycotoxins in fruits and vegetables, Toxins 14 (2022) 309, https://doi.org/10.3390/toxins14050309.

[252] S. Stankovic, J. Levic, T. Petrovic, A. Logrieco, A. Moretti, Pathogenicity and mycotoxin production by *Fusarium proliferatum* isolated from onion and garlic in Serbia, Eur. J. Plant Pathol. 118 (2007) 165–172, https://doi.org/10.1007/s10658-007-9126-8.

[253] A. Amiri, R. Dugas, A.L. Pichot, G. Bompeix, *In vitro* and *in vitro* activity of eugenol oil (*Eugenia caryophylata*) against four important postharvest Apple pathogens, Int. J. Food Microbiol. 126 (2008) 13–19, https://doi.org/10.1016/j.ijfoodmicro.2008.04.022.

[254] B. Prakash, R. Shukla, P. Singh, A. Kumar, P.K. Mishra, N.K. Dubey, Efficacy of chemically characterized *Piper betle* L. essential oil against fungal and aflatoxin contamination of some edible commodities and its antioxidant activity, Int. J. Food Microbiol. 142 (2010) 114–119, https://doi.org/10.1016/j.ijfoodmicro.2010.06.011.

[255] J.O. Beer, C. Van Poucke, Ensuring the quality of results from food control laboratories: laboratory accreditation, method validation and measurement uncertainty. Determining Mycotoxins Mycotoxigenic Fungi Food Feed, 2011, pp. 194–222, https://doi.org/10.1533/9780857090973.2.194.

[256] M. Masi, J.G. Zorrilla, S. Meyer, Bioactive metabolite production in the genus *Pyrenophora* (Pleosporaceae, Pleosporales), Toxins 14 (2022) 588, https://doi.org/10.3390/toxins14090588.

[257] FAO, The future of food and agriculture: trends and challenges, Food and Agriculture Organization of the United Nations (2017).

[258] L.B. Bullerman, A. Bianchini, Stability of mycotoxins during food processing, Int. J. Food Microbiol. 119 (2007) 140–146, https://doi.org/10.1016/j.ijfoodmicro.2007.07.035.

[259] G. Bentivenga, A. Spina, K. Ammar, M. Allegra, S.O. Cacciola, Screening of durum wheat (*Triticum turgidum* L. subsp. *durum* (desf.) husn.) Italian cultivars for susceptibility to Fusarium head blight incited by *Fusarium graminearum*, Plants 10 (2021) 68, https://doi.org/10.3390/plants10010068.

[260] A. Laganà, Introduction to the toxins special issue on LC-MS/MS methods for mycotoxin analysis, Toxins 9 (2017) 325, https://doi.org/10.3390/toxins9100325.

[261] A. Del Fiore, M. Reverberi, A. Ricelli, F. Pinzari, S. Serranti, A.A. Fabbri, G. Bonifazi, C. Fanelli, Early detection of toxigenic fungi on maize by hyperspectral imaging analysis, Int. J. Food Microbiol. 144 (2010) 64–71, https://doi.org/10.1016/j.ijfoodmicro.2010.08.001.

[262] J.F. Leslie, A. Moretti, Á. Mesterházy, M. Ameye, K. Audenaert, P.K. Singh, F. Richard-Forget, S.N. Chulze, E.M.D. Ponte, A. Chala, P. Battilani, A.F. Logrieco, Key global actions for mycotoxin management in wheat and other small grains, Toxins 13 (2021) 725, https://doi.org/10.3390/toxins13100725.

[263] C. Nobili, A. Del Fiore, P. De Rossi, V. Tolaini, Development of methods for the "early detection" toxigenic fungi in food matrices 61 (2015) 158–159. https://inis.iaea.org/records/4vnv7-33q30. (Accessed 7 May 2025).

[264] F. Malir, V. Ostry, A. Pfohl-Leszkowicz, J. Toman, I. Bazin, T. Roubal, Transfer of ochratoxin A into tea and coffee beverages, Toxins 6 (2014) 3438–3453, https://doi.org/10.3390/toxins6123438.

[265] L. Escrivá, G. Font, L. Manyes, H.L. Berrada, Studies on the presence of mycotoxins in biological samples: an overview, Toxins 9 (2017) 251, doi: 10.3390/toxins9080251.

[266] Commission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxinsText with EEA relevance, (2010).

[267] S. Drusch, W. Ragab, Mycotoxins in fruits, fruit juices, and dried fruits, J. Food Protect. 66 (2003) 1514–1527, https://doi.org/10.4315/0362-028X-66.8.1514.

[268] L Escrivá, S Oueslati, G Font, L Manye, *Alternaria* mycotoxins in food and feed: an overview, J. Food Qual. 1569748 (2017 (2017), https://doi.org/10.1155/2017/1569748.

[269] P.W. Crous, L. Lombard, M. Sandoval-Denis, K.A. Seifert, H.-J. Schroers, P. Chaverri, J. Gené, J. Guarro, Y. Hirooka, K. Bensch, G.H.J. Kema, S. C. Lamprecht, L. Cai, A.Y. Rossman, M. Stadler, R.C. Summerbell, J.W. Taylor, S. Ploch, C.M. Visagie, N. Yilmaz, J.C. Frisvad, A.M. Abdel-Azeem, J. Abdollahzadeh, A. Abdolrasouli, A. Akulov, J.F. Alberts, J.P.M. Araújo, H. A. Ariyawansa, M. Bakhshi, M. Bendiksby, A. Ben Hadj Amor, J.D.P. Bezerra, T. Boekhout, M.P.S. Câmara, M. Carbia, G. Cardinali, R.F. Castañeda-Ruiz, A. Celis, V. Chaturvedi, J. Collemare, D. Croll, U. Damm, C.A. Decock, R.P. de Vries, C.N. Ezekiel, X.L. Fan, N.B. Fernández, E. Gaya, C.D. González, D. Gramaje, J.Z. Groenewald, M. Grube, M. Guevara-Suarez, V.K. Gupta, V. Guarnaccia, A. Haddadji, F. Hagen, D. Haelewaters, K. Hansen, A. Hashimoto, M. Hernández-Restrepo, J. Houbraken, V. Hubka, K.D. Hyde, T. Iturriaga, R. Jeewon, P. R. Johnston, Ž. Jurjević, I. Karalti, L. Korsten, E.E. Kuramae, I. Kušan, R. Labuda, D.P. Lawrence, H.B. Lee, C. Lechat, H.Y. Li, Y.A. Litovka, S.S. N. Maharachchikumbura, Y. Marin-Felix, B. Matio Kemkuignou, N. Matočec, A. R. McTaggart, P. Mlčoch, L. Mugnai, C. Nakashima, R.H. Nilsson, S.R. Noumeur, I. N. Pavlov, M.P. Peralta, A.J.L. Phillips, J.I. Pitt, G. Polizzi, W. Quaedvlieg, K. C. Rajeshkumar, S. Restrepo, A. Rhaiem, J. Robert, V. Robert, A.M. Rodrigues, C. Salgado-Salazar, R.A. Samson, A.C.S. Santos, R.G. Shivas, C.M. Souza-Motta, G. Y. Sun, W.J. Swart, S. Szoke, Y.P. Tan, J.E. Taylor, P.W.J. Taylor, P.V. Tiago, K. Z. Váczy, N. van de Wiele, N.A. van der Merwe, G.J.M. Verkley, W.a.S. Vieira, A. Vizzini, B.S. Weir, N.N. Wijayawardene, J.W. Xia, M.J. Yáñez-Morales, A. Yurkov, J.C. Zamora, R. Zare, C.L. Zhang, M. Thines, *Fusarium*: more than a node or a foot-shaped basal cell, Stud. Mycol. 98 (2021) 100116, https://doi.org/10.1016/j.simyco.2021.100116.