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*Review*

# Rethinking tree disease aetiology: from classical pathogens to complex pathological systems and emerging diagnostic approaches

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**Abstract**

The aetiology of tree diseases developed from a classical single-pathogen-based model to one of greater complexity and integration, which acknowledges interaction between microbial communities, host physiology, and environmental stress. Conventional models based on Koch's postulates are often inadequate for forest pathologies due to multifactor interactions, e.g., non-cultivable organisms and synergistic ecological processes. More specific and detailed analysis of disease trends is now being possible because of new diagnostics like metagenomics, metabarcoding, and artificial intelligence-assisted remote sensing. This paper describes a systems approach of forest tree disease aetiology and discusses further the limitations of reductionist paradigms. For effective management and comprehension of emerging tree diseases, we recommend the unification of molecular technology, ecological habitats, and forecasting equipment with a focus on the necessity of holistic diagnostic methods.

**Key words:** artificial intelligence, environmental stress, Koch's postulates, metabarcoding, tree pathogens.
**Abbreviations:** BLD, beech leaf disease.

## Introduction

Knowledge of the aetiology of tree disease is central to management of forest health, conservation of biodiversity, and ecosystem stability. Diagnosis of tree disease had historically been based on a proven formula involving the use of Koch's postulates and the need to isolate, culture, inoculate, and re-isolate a putative pathogen to prove causality (Rivers 1937). This approach has led to landmark discoveries in plant pathology and assistance in the mitigation of major outbreaks in agricultural and forestry systems. However, the complexity of forest ecosystems along with diverse biotic communities, extended host life cycles, and exposure to fluctuating environmental conditions often renders Koch's criteria insufficient for modern tree disease diagnostics (Gillies 2016). Traditional research, e.g., Sinclair's (1965) on decline of white ash, oak, and sugar maple, and Manion's (1991), "decline disease" concept, stressed both biotic and abiotic predisposing elements. These concepts brought in the idea that trees tend to become susceptible as a result of climatic stresses such as drought, soil adversities, and pollution, which pave the entry for opportunistic pathogens. This multi-step development of disease challenges the notion of a singular causal agent and instead suggests that tree pathology is more accurately characterised by pathological systems that involve interacting networks of pathogens, hosts, and environmental conditions.

Recent outbreaks of forest diseases further illuminate the need for a revised etiological model. For example, beech leaf disease has been linked to a nematode, *Litylenchus crenatae mccannii*; but newer work incriminates shifts in the bacterial and fungal microbiomes as causatives also (Carta et al. 2020; Ewing et al. 2021). Likewise, the linkage of *Phytophthora cinnamomi* and white oak decline demonstrates how root pathogens can synergize with drought stress and with extreme temperatures, high or low (Nagle et al. 2010; Balci et al. 2007). These illustrations highlight increasing agreement that several tree diseases are the result of multifactorial and complex interactions. In this review, we evaluate the development of tree disease aetiology from traditional notions to recent knowledge of pathology in complex systems. We assess here the limitations of Koch's postulates using case studies of new tree diseases, and exploring the potential of emerging diagnostic methods like microbiome analysis, metabarcoding, and machine learning. The aim of this research is to promote an integrated, systems-based approach that captures forest disease dynamics' real-world complexity.

## The classical view on tree disease

The history of tree disease aetiology has its roots firmly planted in the basic principles of plant pathology. Formulation of Koch's postulates towards the end of the 19th century gave us a scientific benchmark for detecting causative organisms (pathogens) of infectious diseases. These postulates of pathogen isolation, culture, vaccination, and re-isolation were a gold standard for proof of microbial causation (Rivers 1937). In forest science, this technique allowed early investigations into disease epidemics, tree fall syndromes and multiple pathogenic fungi, bacteria and nematodes were discovered. Sinclair's (1965) ground-breaking work compares losses in white ash (*Fraxinus americana*) and oaks (*Quercus* spp.). Chinese maple (*Acer saccharum*) is one illustration of the classical method in north-eastern U.S. forests. Although his research recognized environmental stressors, it basically looked for a single pathogen as the responsible agent, mirroring the scientific consensus of the time. However, in many cases the inability to consistently isolate a certain pathogen undid the emerging complexity of disease causation in natural ecosystems. To overcome this complexity, Manian (1991) introduced the concept of "degenerative diseases," which he defined as those diseases that develop due to interactions between predisposing factors, provoking stress, and contributing pathogens. This model broadened the aetiological model to address additional stressors such as drought, nutrient stress, and pollution as factors diminishing tree resistance. It also emphasized the contributions of secondary organisms such as fungi and bacteria, which are not necessarily the primary pathogens but play a major role in advancing the disease.

The decline disease model took tree pathology beyond the rigid application of Koch's postulates. This laid the groundwork for understanding tree diseases as processes rather than discrete events. Yet, even with this expanded model, focus has often remained on identifying a major provoking pathogen. This focus can make the aetiology of many tree diseases more simple, especially in cases with chronic or progressive decline over many years or decades. In addition, the reliance on pure enrichment techniques and trait-based identification limited the ability of researchers to uncover the entire group of organisms involved in tree health. Most microbial pathogens, such as viruses and phytoplasmas, are impossible or hard to grow in culture and thus incompatible with Koch's paradigm (Gillies 2016). This failing has led to a revaluation of causation in intricate systems such as forests, where several organisms can interact synergistically, competitively, and opportunistically inside the same ecosystem or host. As the subsequent sections will outline, new disease emergence has increasingly circumvented the traditional paradigm. The use of high-throughput technologies, environmental monitoring, and systems thinking is uncovering a new level of complexity in tree disease that necessitates redefining the causality itself. The mainstream trend in tree pathology science no doubt signifies an underlying shift away from traditional, single-pathogen aetiologies to systemic and multi-factorial disease causation paradigms. While Koch's postulates laid down the foundation for plant pathology and remain a helpful heuristic method in the controlled setting, their shortcomings in representing forest disease dynamics are increasingly evident (Rivers 1937; Gillies 2016).

## *Phytophthora* and decline syndrome

The *Phytophthora* genus contains some of the world's most notorious plant pathogens to have a significant impact on forest ecosystems. Of these, *Phytophthora cinnamomi* has emerged as a major cause in reducing the syndrome in oak, chestnut, and other hardwoods. Unlike acute diseases caused by invasive pathogens, phytophthora-related declines often result from long and complex interactions between the pathogen, host physiology, and environmental conditions (Weste, Marks 1987). Nagle et al. (2010) reported a high correlation between the existence of *P. cinnamomi* and the decline of white oak (*Quercus alba*) populations in southern Ohio (United States). Nevertheless, the research also recognized that the occurrence of the pathogen per se cannot account for the complete extent of the decline. Environmental stresses, particularly drought and poor site quality, played a key role in predisposing trees to infection and advancing disease severity. These findings are aligned with Manion's (1991) decline disease model. These early models, while still based on pathogen identification, brought with them precious conceptual tools, like predisposing, inciting, and contributing factors.

A similarly multifactorial pathology is seen in beech leaf disease (BLD), a rapidly emerging condition affecting *Fagus grandifolia* in eastern North America. Initially linked to the foliar nematode i.e. *Litylenchus crenatae mccannii,* subsequent research has shown that the affected trees also exhibit deep changes in their associated leaf microbial communities (Carta et al. 2020). Such changes suggest action by an extended microbial consortium or pathobiome rather than a single causative agent. In such cases, the nematode serves as a precipitating factor causing disruption of leaf tissue, preparing the site for colonization by secondary fungal and bacterial species. Decline induced by *Phytophtora* illustrates an overarching theme in contemporary tree pathology i.e. development of disease relies frequently on interactions among stress-susceptible hosts, microbial networks, and environmental factors. Pathogens do not act alone, but instead within ecological contexts that modulate their virulence, transmission, and overall impact. Recognition of this interdependence is needed to move beyond simplistic disease models and approach more accurate and relevant understanding of forest health.

## Beech leaf disease and nematode pathology

Beech leaf disease (BLD) is an emerging forest epidemic in eastern North America that challenges conventional models of tree disease causality. First reported in Ohio in 2012, BLD affects American beech (*Fagus grandifolia*) and is characterised by dark green interveinal banding, leaf curling, bud failure, and progressive canopy thinning. In contrast to traditional disease models with established fungal or bacterial pathogens, the major agent responsible for BLD is a recently described foliar nematode i.e. *Litylenchus crenatae mccannii* (Carta et al. 2020). The nematode's presence in beech leaves (symptomatic), and its consistent coexistence with disease expression, has suggested a causal link. But difficulties in satisfying Koch's postulates, most notably the failure to regularly isolate and reproduce disease symptoms by inoculation create uncertainty (Carta et al. 2020). Recent surveys across Ohio have confirmed the widespread distribution of foliar nematode *L. crenatae mccannii* in forest stands exhibiting BLD symptoms, reinforcing its strong association with the disease (Burke et al. 2025). This uncertainty created the incentive for additional research into the BLD pathological system by means of high-throughput sequencing and microbiome profiling.

Ewing et al. (2021) also carried out a comprehensive analysis of the foliar microbiome on BLD-affected trees and found that disease expression coincided with critical changes in microbial community structure. Fungal and bacterial taxa were enriched or depleted in symptomatic tissues, indicating that BLD is not causally motivated by a single nematode but by an interconnected network of microbes potentially creating a "pathobiome." A pathobiome is a microbial consortium which together is responsible for disease establishment and progression (Ewing et al. 2021). Earlier studies by Ewing et al. (2019) had already characterised the rapid spread and ecological severity of BLD, noting its ability to cause decline and mortality in both mature trees and saplings within just a few years. This swift progression sets it apart from traditional decline syndromes such as those induced by Phytophthora, which highlights its epidemic potential.

BLD emphasizes the difficulties of using old-fashioned causal paradigms to explain new and intricate forest diseases. Inability to meet Koch's postulates does not deny biological reality in disease causation, especially in systems where synergistic relationships between more than one agent exist (Gillies 2016). The BLD case also illustrates the importance of molecular and ecological methods like metagenomics, microscopy, and foliar community profiling in diagnoses of diseases that elude classical identification methods. As BLD continues to spread across eastern North America, understanding its pathogenesis remains a high priority. Continued research into nematode behaviour, host responses, and microbial interactions will be essential to developing effective management strategies and rethinking disease diagnostics in complex pathological systems.

## Phytoplasmas and the complexity of causality

Phytoplasmas are phloem-limited, cell wall-lacking bacteria that are not cultivable *in vitro* and are thus recalcitrant to conventional disease diagnosis. Phytoplasmas live inside the sieve elements of the host plant and are usually transmitted via insect vectors, i.e., leafhoppers and psyllids (Bertaccini, Duduk 2010). Due to their association with most of the tree decline syndromes, attributing direct causality to them by conventional means is challenging. Several studies have emphasized the multifaceted expression of phytoplasma diseases in forest tree species. Sahashi et al. (1995) illustrated the seasonality of phytoplasma detection in *Paulownia* witches' broom trees, which showed the temporal fluctuation of the infections. Similarly, Errea et al. (2002) described pear tree phytoplasma transmission as strongly dependent on the season, making the identification of pathogens a matter of uncertainty and reducing accuracy of symptomatic indicators as a diagnostic tool.

In European hackberry (*Celtis australis*), Mittempergher et al. (1999) described decline as not being solely the result of phytoplasma infection. The results showed that *Aceria* mites and environmental stress, specifically drought, were synergistic with the pathogen. This synergy model is analogous to Manion's (1991) "decline disease" model, wherein phytoplasmas are not the causative agents but are one of the interacting factors compromising tree vigour. Even in phytoplasma detection, direct pathogenicity or opportunistic colonization of stressed hosts remain unexplored. Inability to meet Koch's postulates with phytoplasmas has been a cause of controversy over what is sufficient evidence for causality. Gillies (2016) had contended that the classical model, as helpful as it is, falls short in cases of non-culturable pathogens. Rather, a combination of molecular detection, vector association, symptomology, and ecological context should be employed to discern aetiological significance.

Phytoplasmas make their contribution to the disease complexes by their interaction with other organisms, thus affecting the severity of diseases. For instance, it is very common to have mixed infections among viruses, fungi, and secondary bacterial pathogens, thus making causality demarcation difficult (Bertaccini, Duduk 2010). This requires the use of diagnostic systems that acknowledge the contribution of the community dynamics to the disease development. The growing body of literature on phytoplasma-associated disease highlights the need to move beyond the single-pathogen model. By incorporating variables like vector biology, seasonality, host physiology, and the environment, a more integrative model of disease causation can be developed. This is needed to account for the complexity and sometimes cryptic nature of

symptoms induced by phytoplasmas in forest ecosystems. Phytoplasmas stress the questions of disease causation attribution for unculturable pathogens and vector-borne symptom expression and season and environmental site dependence. Their presence in multifactorial infections makes diagnosis more difficult and demonstrates the limitation of reductionism models. Molecular detection, ecological information, and symptomology need to be integrated to determine causality in such systems.

## Emerging diagnostic technologies and integrated approaches

### *General aspects*

One of the most important contributory elements to the complexity of tree disease syndromes is the multi-dimensionality of host-pathogen-environment interactions. These interactions often include a multifaceted suite of microbial agents, e.g., bacteria, fungi, nematodes, and phytoplasmas, acting in complex ecological webs. Therefore, diagnosis of disease needs to move beyond general models and should prioritize single causal agents to include all the complexity of these systems (Gillies 2016; Ewing et al. 2021). Traditional culture-based methods, which have for centuries underpinned plant pathology, are now increasingly regarded as inadequate for the identification of fastidious, unculturable, or slowly growing organisms such as phytoplasmas and certain foliar nematodes (Bertaccini, Duduk, 2010; Carta et al. 2020). In addition, these methods ignore functional or ecological interactions in host microbiomes or rhizosphere microbiomes. Facing these challenges, a diversity of new tools has emerged to revolutionize the diagnosis, surveillance, and control of tree disease syndromes.

### *Metabarcoding and metagenomics*

High-throughput sequencing technologies have transformed microbial diagnostics through the ability to detect microbial communities in environmental samples without cultivation. Metagenomics and metabarcoding are two methods that generate different but complimentary insights. Metabarcoding has the ability to attain high taxonomic resolution at low financial and computational expense on the basis of amplification and sequencing of conserved genetic markers, for instance, 16S rRNA in bacteria (Beckers et al. 2016; Abdelfattah et al. 2017). When identifying endophyte-pathogen complexes in symptomatic and asymptomatic tree tissue, the process is of particular usefulness (Fearer et al. 2022). Metagenomics, however, is composed of untargeted sequencing of all genomic DNA present within an environmental sample with an even wider perspective that includes taxonomic identity along with gene content of processes. The general strategy allows identification of virulence factors, resistance genes, and disease manifestation-associated metabolic pathways but at the cost of higher financial expense and analytical complexity (Deiner et al. 2017; Nilsson et al. 2018). While metabarcoding is more popular for large-scale ecological surveys and diagnostic testing, metagenomics is considered more suitable for the functional characterization of intricate pathosystems involving, for instance, pathobiomes – microbial consortia causing disease and no single pathogens (Ewing et al. 2021; Román-Reyna et al. 2021). The objectives of research or management programs then dictate which of these approaches is optimal. For example, for beech leaf disease, metabarcoding has made it easy to easily detect trends in the structure of the microbial community, and metagenomic analysis is starting to discern the metabolic interactions between bacteria, fungi, and *L. crenatae mccannii* in infected tissues (Ewing et al. 2021; Burke et al. 2025).

### *Machine learning and artificial intelligence*

Rapid and scalable identification of plant diseases may be possible with the integration of artificial intelligence and machine learning. The two technologies possess high pattern recognition capability and the ability to process high, multi-dimensional data sets, e.g., hyperspectral images, to determine the health state of a plant. By detecting minute physiological alterations that are invisible to the naked sight, machine learning algorithms in forestry can differentiate between healthy and diseased trees (Wei et al. 2023). An interesting application is early detection of the Dutch elm disease using spectral and spatial information of leaf images. Wei et al. (2023) illustrated that the machine learning models of hyperspectral and multispectral imaging data can identify resistance characteristics in elm populations and predict disease prevalence. Non-destructive monitoring of this nature is particularly beneficial for preventive control and monitoring in natural and managed ecosystems. As such technologies evolve, they can also be used in real-time disease prediction systems that integrate multiple data streams – genomic, phenotypic, and environmental drivers – to forecast outbreaks before they occur.

### *Internet of things and remote sensing*

Remote sensing technologies like satellite images, aerial monitoring using drones, and ground based LiDAR technology enable us in holistic and continuous monitoring of forest health metrics like canopy reflectance, chlorophyll fluorescence, and leaf water content (Bassuk et al. 2009). When combined with internet of things-based sensor networks – consisting of temperature, humidity, and soil moisture probes – the technologies give rise to real-time diagnostic platforms that are adept at identifying both abiotic and biotic stressors in large geographic areas. The integration of internet of things and remote sensing allows for data-informed forestry management through the early identification of disease incidence, monitoring the efficacy

of treatment, and informing species choice for restoration (Nagle et al. 2010; Feacher et al. 2022). In addition, the technologies can be used to improve metabarcoding and metagenomic diagnostics through targeted sampling in areas with suspected disease hotspots.

*Diagnostic limitations and challenges*

Although cutting-edge diagnostic tools promise great potential, they are confronted with significant technical and logistical challenges. High-throughput screening and artificial intelligence platforms generate high-throughput data sets which need to be expertly curated and subject to challenging statistical analysis. Inefficient processing can lead to spurious conclusions (Pollock et al. 2018; Zinger et al. 2019). Sensitive methods will detect non-pathogenic or transient organisms that are not considered to be pathogens. The incorporation of contextual ecological information is still relevant for correct interpretation of results (Janda, Abbott 2007). Sequencing, imaging technologies, and computing hardware costs may preclude the use of these technologies in economically disadvantaged regions (Sapkota, Nicolaisen 2015). To maximize reproducibility and reliability of diagnostic outputs, it is necessary to embrace standardized sampling protocols, prime set validation (e.g., Beckers et al. 2016; Usyk et al. 2017), and rigorous experimental protocols.

*Proposed integration framework*

To counter urgent limitations and realize the full potential of novel technologies, we suggest an integrated diagnostic platform for forest tree diseases. The platform consists of classical observation techniques (symptomatology, culturing of pathogens, and microscopy), molecular detection methods (polymerase chain reaction, metabarcoding, metagenomics, and transcriptomics), microbial ecology techniques (pathobiome and microbial network analysis), and predictive analytics tools (artificial intelligence or machine learning models, remote sensing, and environmental metadata). The multi-disciplinary platform enables timely, precise, and environmentally friendly diagnosis of complex forest diseases. In addition, it conforms to the new paradigm towards evidence-based predictive plant health management, enabling stakeholders to transition from reactive interventions to proactive, evidence-based interventions (Manion 1991; Burke et al. 2025). With tree disease syndromes growing in number due to climate change, globalization, and loss of biodiversity, the demand for integrated diagnostic tools will grow exponentially. Fig. 1 shows graphical representation of the interplay of emerging diagnostic techniques in detection of tree diseases.

## Limitations of study

This review integrates recent advancements in tree disease aetiology, diagnostics, and microbiome-based methods; however, it has some major drawbacks. The literature reviewed is dominated by temperate climates that compromises broader ecological applicability. Many of the new diagnostic methods, including metabarcoding and machine learning techniques, are at early experimental phases with poor field validation. The complex host-microbe-environment interaction complicates causative interaction definitions, especially in diverse forest ecosystems. In addition, a lack of long-term monitoring data limits us from assessing development of diseases

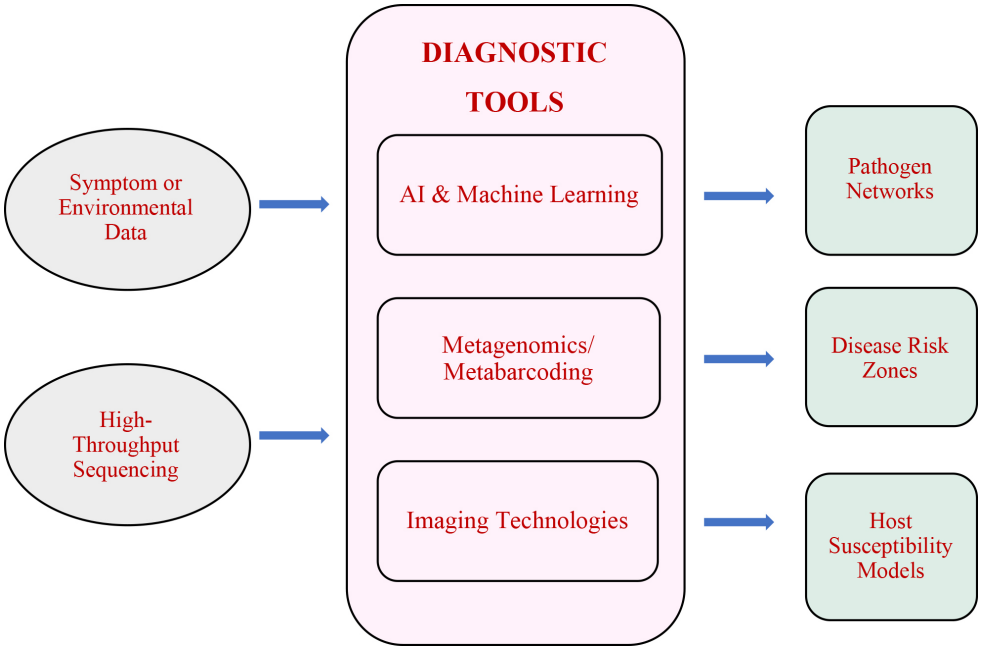


**Fig. 1.** Interplay of emerging diagnostic techniques in detection of tree diseases.

and pathobiome dynamics over longer timescales. More standardized protocols and greater geographical representation are now required in follow-up studies to enhance relevance and efficiency.

## Conclusions

Tree disease pathobiological origin has shifted from pathogen-focused models (as defined by Koch's postulates) to more complex paradigms that take into consideration multiple causes and ecological niches. Plant pathogens like *Phytophthora*, nematodes, and phytoplasmas usually interact with abiotic stress factors and microbial communities, thus making conventional diagnostic methods cumbersome. Enhanced molecular tools, microbiome studies, and artificial intelligence-based remote sensing provide greater accuracy and predictive power for the monitoring, understanding, assessment and management of forest diseases. The conceptualization of disease as a result of ecological dissonance rather than discrete infection events facilitates the formulation of holistic, adaptive, and sustainable forest management strategies. Adopting the pathobiome perspective is not only scientifically imperative but is also ecologically critical with the rapid pace of global environmental change development.

### References


Abdelfattah A., Malacrinò A., Wisniewski M., Cacciola, S.O., Schena L. 2017. Metabarcoding: A powerful tool to investigate microbial communities and shape future plant protection strategies. *Biol. Contr.* 120: 1–10.

Aoun M., Jacobi V., Boyle B., Bernier L. 2010. Identification and monitoring of *Ulmus americana* transcripts during *in vitro* interactions with the Dutch elm disease pathogen *Ophiostoma novo-ulmi*. *Physiol. Molec. Plant Pathol.* 74: 254–266.

Aoun M., Rioux D., Simard M., Bernier L. 2009. Fungal colonization and host defense reactions in *Ulmus americana* callus cultures inoculated with *Ophiostoma novo-ulmi*. *Phytopathology* 99: 64:2–650.

Balci Y., Balci S., Eggers J., MacDonald W.L., Juzwik J., Long R.P., Gottschalk K. W. 2007. *Phytophthora* spp. associated with forest soils in eastern and north-central U.S. oak ecosystems. *Plant Dis.* 91: 705–710.

Bassuk N. 2009. *Recommended Urban Trees: Site Assessment And Tree Selection for Stress Tolerance.* Cornell University, Urban Horticulture Institute.

Bates D., Mächler M., Bolker B., Walker S. 2015. Fitting linear mixed-effects models using lme4. *J. Statist. Softw.* 67: i01.

Beckers B., De Beeck M.O., Thijs S., Truyens S., Weyens N., Boerjan W., Vangronsveld J. 2016. Performance of 16s RDNA primer pairs in the study of rhizosphere and endosphere bacterial microbiomes in metabarcoding studies. *Front. Microbiol.* 7: 650.

Beier G.L., Blanchette R.A. 2018. Defence responses in the xylem of *Ulmus americana* cultivars after inoculation with *Ophiostoma novo-ulmi*. *Forest Pathol.* 48: e12453.

Bertaccini A., Duduk B. 2010. *Phytoplasma* and phytoplasma diseases: a review of recent research. *Phytopathol. Medditer.* 48: 355–378.

Brasier C.M., Webber J.F. 2019. Is there evidence for post-epidemic attenuation in the Dutch elm disease pathogen *Ophiostoma novo-ulmi*? *Plant Pathol.* 68: 921–929.

Büchel,K., Fenning T., Gershenzon J., Hilker M., Meiners T. 2015. Elm defence against herbivores and pathogens: morphological, chemical and molecular regulation aspects. *Phytochem. Rev.* 15: 961–983.

Burke D.J., Colbert-Pitts M., Macy T., Carrino-Kyker S.R., Martin D. 2025. The presence and distribution of nematode *Litylenchus crenatae* ssp. *mccannii*, the causative agent of beech leaf disease, in forest stands across Ohio. *Environ. Monit. Assessm.* 197: 478.

Campanella T.J. 2003. *Republic of Shade: New England and the American Elm*. Yale University Press.

Carta L.K., Handoo Z.A., Li S., Kantor M., Bauchan G., McCann D., Gabriel C.K., Yu Q., Reed, S., Koch J., Martin D., Burke D. J. 2020. Beech leaf disease symptoms caused by newly recognized nematode subspecies *Litylenchus crenatae mccannii* (Anguinata) described from *Fagus grandifolia* in North America. *Forest Pathol.* 50: e12580.

Creer S., Deiner K., Frey S., Porazinska D., Taberlet P., Thomas W.K., Potter C., Bik H.M. 2016. The ecologist's field guide to sequence-based identification of biodiversity. *Methods Ecol. Evol.* 7: 1008–1018.

Deiner K., Bik H.M., Mächler E., Seymour M., Lacoursière-Roussel A., Altermatt F., Creer S., Bista I., Lodge D.M., De Vere N., Pfrender M.E., Bernatchez L. 2017. Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Mol. Ecol.* 26: 5872–5895.

Errea P., Aguelo V., Hormaza J.I. 2002. Seasonal variations in detection and transmission of pear decline phytoplasma. *J. Phytopathol.* 150: 439–443.

Ewing C.J., Hausman C.E., Pogacnik J., Slot J., Bonello P. 2018. Beech leaf disease: An emerging forest epidemic. *Forest Pathol.* 49: e12488.

Ewing C.J., Slot J., Benítez M., Rosa C., Malacrinò A., Bennett A., Bonello E. 2021. The foliar microbiome suggests that fungal and bacterial agents may be involved in the beech leaf disease pathosystem. *Phytobiomes J.* 5: 335–349.

Fearer C.J., Malacrinò, A., Rosa C., Bonello P. 2022. Phytobiome Metabarcoding: a tool to help identify prokaryotic and eukaryotic causal agents of undiagnosed tree diseases. In: Luchi N. (Ed.) *Plant Pathology: Method and Protocols.* Springer Science+Business Media, New York, pp. 347–366.

Foster Z.S.L., Sharpton T.J., Grünwald N.J. 2017. Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. *PLoS Comput. Biol.* 13: e1005404.

Gillies D.A. 2016. Establishing causality in medicine and Koch's postulates. *Int. J. Hist. Philos. Medic.* 6: 10603.

Hadziavdic K., Lekang K., Lanzen A., Jonassen I., Thompson E.M., Troedsson C. 2014. Characterization of the 18S RRNA gene for designing universal eukaryote specific primers. *PLoS One* 9: e87624.

Horton M.W., Bodenhausen N., Beilsmith K., Meng D., Muegge B.D., Subramanian S., Vetter M.M., Vilhjálmsson B.J., Nordborg M., Gordon J.I., Bergelson J. 2014. Genome-wide association study of *Arabidopsis thaliana* leaf microbial community. *Nature Commun.* 5: 5320.

Janda J.M., Abbott S.L. 2007. 16S RRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *J. Clin. Microbiol.* 45: 2761–2764.

Kembel S.W., Cowan P.D., Helmus M.R., Cornwell W.K., Morlon H., Ackerly D.D., Blomberg S.P., Webb C.O. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26: 1463–1464.

Kim M.-J., Oh J.J., Kim G.-H. 2022. Comparison of fungal diversity on woods with different fungal susceptibility during above-ground exposure. *BioResources* 17: 6740–6760.

Love M.I., Huber W., Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15: 550.

Manion P.D. 1991. *Tree Disease Concepts.* 2nd Ed. Prentice-Hall, Englewood Cliffs, New Jersey. 402 p.

McMurdie P.J., Holmes S. 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8: e61217.

Mittempergher L., Sfalanga A., Vibio M., Bertaccini A. 1999. Phytoplasmas, *Aceria bezzii* and drought in declining european hackberry *(Celtis australis* l.). *Acta Horticult.* 496: 87–92.

Nagle A.M., Long R.P., Madden L.V., Bonello P. 2010. Association of *Phytophthora cinnamomi* with white oak decline in Southern Ohio. *Plant Dis.* 94: 1026–1034.

Nilsson R.H., Anslan S., Bahram M., Wurzbacher C., Baldrian P., Tedersoo L. 2018. Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nature Rev. Microbiol.* 17: 95–109.

O'Brien H.E., Parrent J.L., Jackson J.A., Moncalvo J., Vilgalys R. 2005. Fungal community analysis by large-scale sequencing of environmental samples. *Appl. Environ. Microbiol.* 71: 5544–5550.

Paradis E., Schliep K. 2018. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35: 526–528.

Pollock J., Glendinning L., Wisedchanwet T., Watson M. 2018. The madness of microbiome: attempting to find consensus "best practice" for 16S microbiome studies. *Appl. Environ. Microbiol.* 84: e02627-17.

Ramakodi M.P. 2021. Effect of amplicon sequencing depth in environmental microbiome research. *Curr. Microbiol.* 78: 1026–1033.

Rivers T.M. 1937. Viruses and Koch's postulates. *J. Bacteriol.* 33: 1–12.

Román-Reyna V., Dupas E., Cesbron S., Marchi G., Campigli S., Hansen M.A., Bush E., Prarat M., Shiplett K., Ivey, M. L.L., Pierzynski J., Miller S.A., Hand F.P., Jacques M., Jacobs J.M. 2021. Metagenomic sequencing for identification of *Xylella fastidiosa* from leaf samples. *mSystems* 6: e00591-21.

Sahashi N., Nakamura H., Yoshikawa N., Kubono T., Shoji T., Takahashi T. 1995. Distribution and seasonal variation in detection of *Phytoplasma* in bark phloem tissues of single *Paulownia* trees infected with witches' broom. *Jap. J. Phytopathol.* 61: 481–484.

Sapkota R., Nicolaisen M. 2015. High-throughput sequencing of nematode communities from total soil DNA extractions. *BMC Ecol.* 15: 3.

Sinclair W.A., Ross E., Hibben C., Staley J. 1965. Comparisons of recent declines of white ash, oaks and sugar maple in northeastern woodlands. *Cornell Plantations* 20: 62–64.

Usyk M., Zolnik C.P., Patel H., Levi M.H., Burk R.D. 2017. Novel ITS1 fungal primers for characterization of the mycobiome. *mSphere* 2: e00488-17.

Wei X., Zhang J., Conrad A.O., Flower C.E., Pinchot C.C., Hayes-Plazolles N., Chen Z., Song, Z., Fei, S., Jin J. 2023. Machine learning-based spectral and spatial analysis of hyper- and multi-spectral leaf images for Dutch elm disease detection and resistance screening. *Artific. Intellig. Agricult.* 10: 26–34.

Weste G., Marks G.C. 1987. The biology of *Phytophthora cinnamomi* in Australasian forests. *Annu. Rev. Phytopathol.* 25: 207–229.

Zinger L., Bonin A., Alsos I.G., Bálint M., Bik H., Boyer F., Chariton A.A., Creer S., Coissac E., Deagle B.E., De Barba M., Dickie I.A., Dumbrell A.J., Ficetola G.F., Fierer N., Fumagalli L., Gilbert M.T.P., Jarman S., Jumpponen A., Kauserud H., Orlando L., Pansu J., Pawlowski J., Tedersoo L., Thomsen P.F., Willerslev E., Taberlet P. 2019. DNA metabarcoding—Need for robust experimental designs to draw sound ecological conclusions. *Mol. Ecol.* 28: 1857–1862.

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