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FOREST PATHOLOGY (J WITZELL, SECTION EDITOR)

Complex Forest Tree Diseases – Diagnostics Beyond Koch's Postulates

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Abstract

Purpose of Review Forest tree diseases are a major contributor to forest degradation and loss of productivity. They are often quite complex in their causation (etiology), especially in the case of forest syndromes, i.e. diseases with multiple causes and concurrent symptoms. Traditionally, to prove pathogenicity of a microbial agent, and thus correctly diagnose the etiology of a disease, plant pathologists must satisfy all of the so-called Koch's postulates, as mandated by their deontological code. This review examines whether this approach is still current.

Recent Findings Koch's postulates state that a pathogen is a microorganism that, after being isolated in pure culture, can reproduce the disease when it is inoculated into a healthy plant. Over the decades, plant pathologists as well as medical scientists have discovered that these postulates are not always applicable in their entirety and that, furthermore, novel approaches based on molecular biology can be very helpful in uncovering relationships between microbes and diseases that are not easily proven using Koch's postulates.

Summary I conclude that Koch's postulates are not a viable approach for many forest tree diseases and propose a set of new guidelines, based on the *preponderance of the evidence* principle, to integrate this proven approach and bring it into the twenty-first century.

Keywords Causation · Fastidious · Syndrome · Molecular signature

Introduction

Forest pathogens, i.e. the biotic causal agents of forest tree diseases, can be among the most insidious and destructive sources of forest damage. Generally speaking, in the absence of a disturbance, endemic tree pathogens are just part of the ecological background; they contribute to generating much biodiversity, differentiating the genetic structure of host populations, and facilitate successional processes – for example, in old growth forests, they contribute to accelerating the death of older, senescing trees, in turn favoring regeneration by contributing to fostering gap phases in a dynamic equilibrium that defines the climax forest. Pathogens, therefore, also contribute to nutrient cycling through the dead biomass created when trees die and decompose. Decomposition of wood is also highly accelerated by the activity of decay fungi, which, under more disturbed conditions, e.g. active management for the purpose of resource extraction, can behave as pathogens, causing some of the most destructive forest diseases, such as annosus root rot (caused by *Heterobasidion* spp.) and Armillaria root rot (caused by *Armillaria* spp.).

Forest pathogens become an issue when human activities disrupt natural ecosystems. For example, silvicultural activities cause wounds that provide pathways of entry into the host; climate change alters the host–pathogen balance by both stressing the trees and triggering some otherwise innocuous endophytes into a pathogenic lifestyle; while the ever-increasing global trade and unfettered movement of people allow pathogens to break geographic barriers and be introduced into new environments, where trees are very often inherently susceptible due to a lack of co-evolutionary history with the novel, non-native invasive microorganism.

Therefore, the need for tree disease management and control is an issue that is completely dependent on human activities, but also, in turn, on the expectations we have for forest environments. In general, we notice when trees begin

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to suffer or die from diseases when this affects a product or ecosystem service humans anticipate from the forest (whether natural, managed, or urban), from timber production to soil retention, water purification to carbon sequestration, aesthetics to recreation, ecological integrity to biodiversity. At that point, we want the problem solved, and in order to even think of solutions aimed at disease management, we need to know what's going on, i.e. the causation of the problem (its etiology).

Koch's Postulates

Traditionally, and as prescribed by our own professional deontological code, to make an initial diagnosis of an unknown disease, a plant pathologist would apply the so-called Koch's postulates,[1] which are used to prove pathogenicity, i.e. the ability of a microorganism to cause disease. Koch's postulates state that an organism can be considered the causal agent of a disease, i.e. a pathogen, only if *all* of the following conditions apply ([1], as summarized by Fredricks and Relman [2]):

1. Postulate 1 (P1): "The parasite occurs in every case of the disease in question and under circumstances which can account for the pathological changes and clinical course of the disease."
2. Postulate 2 (P2): "The parasite occurs in no other disease as a fortuitous and nonpathogenic parasite;" and
3. Postulate 3 (P3): "After being fully isolated from the (plant) and repeatedly grown in pure culture, the parasite can induce the disease anew." [N.B.: Here I replaced the original reference to body (implicitly, human) with plant.]

Koch's Postulates are not Always Applicable

This procedure works in many cases, but not all, by far. Some organisms are simply not culturable and for this reason they are referred to as *fastidious*. These typically include pathogens such as viruses and phytoplasmas, but also some other bacteria. Some biotrophic fungi, like rust fungi or powdery mildews, are also typically fastidious. In these cases, specialized knowledge of all these systems allows pathologists to use indirect ways to get to a diagnosis. For example, with both viruses and phytoplasmas, consistent association with symptoms may be achieved using electron microscopy and nucleic acid-based tools for molecular identification, followed by inoculation of naïve plants with sap of virus- or phytoplasma-infected plants. On the other hand, inoculation of naïve plants with rust fungi can only be accomplished with spores collected from fruiting bodies on live plants, either in the field or in the greenhouse.

Thus, in one way or another, the technical constraint of obtaining a pure isolate of the organism can be obviated by specialized knowledge of the system. However, there is another case in which application of Koch's postulates is not straightforward, and that is the case of syndromes.

According to the Merriam-Webster dictionary, a syndrome is "a group of signs and symptoms that occur together and characterize a particular abnormality or condition." For those unfamiliar with plant pathology, a good example from human health would be acquired immune deficiency syndrome (AIDS). In this syndrome, common symptoms include headache, diarrhea, fever, or a sore throat. Clearly, these symptoms are common with many more familiar diseases, such as head colds and Strep throat. However, in more advanced stages, signs like persistent white spots or unusual lesions on the tongue or in the mouth, skin rashes or bumps, and cancers such as Kaposi's sarcoma, often become evident (Source: Mayo Clinic [3]). Because of all these complications in symptomatology (hence the use of *syndrome* to characterize the condition), it was initially extremely difficult for physicians to diagnose what would later be known as HIV (human immunodeficiency virus)/AIDS. Also note that if the Koch's postulates were to be applied to humans, scientists would have to intentionally inoculate people with HIV to demonstrate pathogenicity, which is obviously not ethical and therefore not allowed. In some cases, however, researchers can inoculate model systems, like mice and swine, to help them define human pathogenicity. However, since no such ethical constraints apply to plants, in plant pathology Koch's postulates must always be tested in their entirety to demonstrate pathogenicity.

The Challenge of Forest Syndromes

A classic example of forest syndrome is provided by the condition known as forest decline. In this condition, trees typically lose vigor over an extended period of time (a few to several years) in a way that is clearly different from normal senescence. Under decline conditions, trees display characteristic crown dieback symptoms, early leaf drop, wilting, and heart decay, leading to extensive tree mortality over large swaths of landscape. A currently accepted model for the causation and development of forest declines was first proposed by Wayne Sinclair [4] and later formalized by Paul Manion [5]. In this model, declines are the result of

[1] Robert Hermann Koch (11 December 1843 – 27 May 1910) was a German physician who developed Koch's postulates based on the insights of Jakob Henle. Koch's postulates were published by R.H. Koch in 1890.

a successional series of factors impacting trees in a given locale: (1) predisposing, i.e. long term, factors (e.g. climate change, poor soil quality, prolonged droughts), (2) inciting, i.e. short term, factors (e.g. summer defoliation by an insect, seasonal excessive precipitation), and (3) contributing factors, i.e. the *probable* (eventual) tree killers (e.g. root disease, attack by a wood boring insect). With such a combination of variable symptoms and probable (or just plausible) causes of death, diagnosing a forest decline has been the bane of forest pathologists for eons. In these cases, using Koch's postulates to demonstrate pathogenicity is basically non-sensical; at best, satisfaction of Koch's postulates may provide insights on the contributing factors (which could be multiple), but certainly not explain the entire process. Instead, in a classic whodunnit, forest pathologists use both evidence and their prior knowledge of systems and organisms to infer the most probable cause of ultimate death, be it e.g., a root or a wilt pathogen.

A case I experienced first-hand involved a white oak (*Quercus alba* L.) decline along the Ohio river that had been documented for a few years, approximately between 2005–2010. Large numbers of mature overstory trees had been dying while regeneration was very poor; however, such stand decline was not explicitly associated with any specific, known pathogenic or pest agents. Instead, the affected stands had experienced more or less pronounced droughts in 1999–2002 (predisposing factors), followed by a few years of moderate to severe defoliation by the half-wing geometer (*Phigalia titea* Cramer), the forest tent caterpillar (*Malacosoma disstria* Hubner), and the common oak moth (*Phoberia atomaris* Hubner), which were considered inciting factors. Note that the identification of predisposing and inciting factors was an educated guess, i.e. based on prior experiences and knowledge, with no direct evidence of a causal role in the syndrome. Concurrently, work had detected the presence of various species of *Phytophthora* in eastern forest soils, comprising southern Ohio [6]. The list of *Phytophthoras* included *P. cinnamomi*, a well-known serious pathogen and killer of many woody plant species, including trees (e.g. jarrah decline in Australia, [7]). The presence of these organisms, in conjunction with a patchy distribution and main occurrence of the white oak decline in bottomland areas (i.e. tendentially more humid), suggested that *Phytophthoras* may be the contributing factors in the syndrome, i.e. the actual killers. So we tried to uncover a possible pathogenic role for *P. cinnamomi*. What we obtained was circumstantial evidence that *P. cinnamomi* could indeed be the killer: higher densities of propagules in declining stands, a positive, exponential relationship between propagule density and soil moisture on a seasonal scale (but only in one of two years of observations), and a positive association with sites with greater soil clay content [8]. When we tested the pathogenicity of *P. cinnamomi* on 1-year-old potted white oak seedlings grown in native soil mixes in the greenhouse, we found that root systems were significantly damaged by *P. cinnamomi*, especially under flooding conditions. Clearly, these were two very artificial conditions that are very different from the field situation (seedlings vs. mature trees; uniform, short-term greenhouse conditions vs. variable, long-term field conditions, etc.), but our results suggested that *P. cinnamomi* was indeed a contributing agent to the decline.

In this case we did not satisfy Koch's postulates: *P. cinnamomi* was not consistently associated only with symptomatic plants – in fact, it was isolated from the soil, not host tissue and moreover, it was found in soils of both declining and healthy stands, basically negating the first postulate. And while we demonstrated that we could reproduce *some symptoms* by inoculating naïve tissues, we did not even attempt to reisolate the pathogen, given the difficulty of doing so directly from plant tissues with many species of *Phytophthora*. At the end of day, by no means did we consider *P. cinnamomi* the smoking gun, but we had good reason to come to the conclusion that it was an important contributing factor, based on prior experiences and knowledge. In other words, we came to a reasonable, acceptable conclusion, based on the preponderance of the evidence, without satisfying *any* of the three Koch postulates.

Further above I have described how HIV can be considered a good example of the difficulty inherent in using Koch's postulates to demonstrate pathogenicity in medicine. Indeed, some of the more incisive re-considerations of Koch's postulates come from the medical field. For example, Fredricks and Relman [2] note how oftentimes there is no unique association between suspected pathogen and symptoms (P1), but rather a quantitative difference in pathogen "content" (AKA inoculum "load" or "titre"). This is particularly true for latent pathogens or in healthy carrier situation, which is also common in plant pathology. I can bring another personal example to this aspect. Beech leaf disease (BLD) [9] is an emerging disease of American beech (*Fagus grandifolia*) in the Northeastern United States. BLD has been attributed to a novel foliar nematode, *Litylenchus crenatae mccannii* (LCM) [10•] because (1) it was initially found *only* in symptomatic leaves (P1); (2) inoculation of beech leaves, and especially buds, with wild nematodes extracted from symptomatic leaves reproduced the symptoms (P2); and (3) LCM could be re-extracted from inoculated tissues (P3) [10•]. However, later phytobiome analysis showed that LCM was not uniquely associated with symptomatic leaves; rather, other microorganisms (mainly bacteria) were uniquely associated with the symptoms [11•]. To date, Koch's postulates have not been clearly demonstrated, either through unique association of microorganisms or pure cultures of LCM, because

no in vitro culturing system exists at present for this microscopic worm. Nevertheless, at this point, and for all intents and purposes, no one doubts that LCM is a least necessary, if not sufficient, to cause BLD, based on preponderance of the evidence.

[It is important to note here that some pathogens can be hard to find even by using molecular tools. This is especially true with phytoplasmas, whose populations can fluctuate during the season and in different tree organs, to the point of becoming undetectable at times even with the most sensitive molecular methods [12–15]. Assay timing can therefore also be crucial with phytoplasmas.]

The Experience with Koch's Postulates in Philosophy and the Medical Field

Probably due to the general ease and lack of ethical considerations characteristic of plant inoculations, I could not find much relevant conversation on the status of Koch's postulates in the primary phytopathological literature. In contrast, the debate in the medical and philosophical domains has been fairly active and quite interesting.

For example, Donald Gillies [16] brings us a very stimulating treatment of Koch's postulates in relation to the concept of causation in the philosophy of science, specifically with reference to what are called Action, Intervention, and Manipulation (AIM) theories of causality. Fundamentally, these theories predicate a necessary connection between causality and AIM. For example, we may have the basic statement "A causes B." Gillies discusses *deterministic* and *indeterministic* causes. Deterministic is when such cause is necessary and sufficient to determine an action: e.g. my turning of the spigot causes the sprinkler to come on and my grass to become wet [16]. On the other hand, the statement that smoke causes lung cancer is indeterministic, because smoking alone is not always sufficient to cause lung cancer; as we know, many heavy smokers never develop it. But if smoking were sufficient to cause lung cancer (a deterministic cause), then smoking could be considered a "productive" cause, as it alone is sufficient to produce disease. Koch's postulates really apply only to deterministic causes, as in microbe A causes disease B. If microbe A is a necessary condition to cause disease B, then removing A from the picture prevents B from occurring. The latter could be considered an "avoidance" proof of causality. However, another way to prove causality may be in "blocking" the effect. One way to think of this in plants is acquired resistance (analogous to vaccination in animals), where disease B is blocked from occurring in the first place, demonstrating causality for microbe A. As should be evident by now, this is where the name AIM for these theories of causality comes from. So, how does all this apply to Koch's postulates? Using our microbe A/disease B example, P3 states that disease B ought to be reproducible by inoculation with microbe A, a clear case of "productive" action. But P3 should also be extended to avoidance, as Gillies argues avoidance is more important than productive actions. Gillies suggests that P3 may be amended into a P3a clause (which is the same as the original P3), and a P3b clause, as follows: "It must be shown that if the microorganisms are prevented from multiplying in the (plant), then the (plant) will not have the disease." [16] (N.B.: Here I replaced the original reference to "patient" with "plant.").

Philosophical arguments with respect to Koch's postulates are quite interesting to study their embedded logic, but there are also very practical reasons for reevaluating the Koch's postulates in the modern day and age. Fredricks and Relman [2] provide another interesting perspective based on a historical analysis of the formulation of the postulates as they apply to something Koch himself could have never imagined: modern molecular diagnostics. For many of the reasons discussed further above, these authors find the postulates quite limited and limiting. Indeed, Rivers [17] reports that Koch himself had at some point decided that adherence to all three postulates would not be necessary to establish causality, the first two postulates being sufficient. But even this interpretation can be too limiting. For example, in many cases, disease is not the result of the mere presence of a microbe, i.e. its presence is not a sufficient condition to cause disease: inoculum load (as mentioned above) is also critical, meaning it may be present in asymptomatic tissues but at significantly lower titers than in symptomatic material. In other words, presence of a microbe may be necessary, but in itself insufficient to cause disease. This is particularly true with plants, where we are well trained in the concept of the disease triangle, which states that to have disease, the right combination of a virulent pathogen in sufficient load, a susceptible host, AND a conducive environment must all co-occur. The take home message from all this, according to [2], is that "A microbe that fulfills Koch's postulates is most likely the cause of the disease in question. A microbe that fails to fulfill Koch's postulates may still represent the etiologic agent of disease or may be a simple commensal. The power of Koch's postulates comes not from their rigid application but from the spirit of scientific rigor that they foster. The proof of disease causation rests on the concordance of scientific evidence, and Koch's postulates serve as guidelines for collecting this evidence [2]."

Then there is the whole issue of fastidious microorganisms that can only be identified based on molecular sequences. Fredricks and Relman [2] propose the following

set of guidelines, which in my view offer a very contemporary and valid perspective on this issue:

(1) "A nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease. Microbial nucleic acids should be found preferentially in those organs or gross anatomic sites known to be diseased (i.e., with anatomic, histologic, chemical, or clinical evidence of pathology) and not in those organs that lack pathology.
(2) Fewer, or no, copy numbers of pathogen-associated nucleic acid sequences should occur in hosts or tissues without disease.
(3) With resolution of disease (for example, with clinically effective treatment), the copy number of pathogen-associated nucleic acid sequences should decrease or become undetectable. With clinical relapse, the opposite should occur.
(4) When sequence detection predates disease, or sequence copy number correlates with severity of disease or pathology, the sequence-disease association is more likely to be a causal relationship.
(5) The nature of the microorganism inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms. When phenotypes (e.g., pathology, microbial morphology, and clinical features) are predicted by sequence-based phylogenetic relationships, the meaningfulness of the sequence is enhanced.
(6) Tissue-sequence correlates should be sought at the cellular level: efforts should be made to demonstrate specific in situ hybridization of microbial sequence to areas of tissue pathology and to visible microorganisms or to areas where microorganisms are presumed to be located.
(7) These sequence-based forms of evidence for microbial causation should be reproducible."

Clearly, this is a much more nuanced outlook on proving causation of a disease than originally proposed by Koch. And with it, it also brings new questions. For example, what is the threshold of sequence copy numbers sufficient to determine disease? In some cases this can be determined empirically, but in the BLD example discussed above, LCM was found in both asymptomatic and symptomatic leaves. However, Koch's postulates led us astray, as we focused on P1, in a way that initially made us doubt that LCM was really the culprit. In reality, the threshold is probably unknowable, because disease, especially with plants, is predicated on the "Goldilocks conditions" determined by the disease triangle, i.e. it depends on the right environmental conditions and on the level of genetic host susceptibility to that particular pathogen.

Conclusion: Where does this Leave us with Tree Diseases?

In conversations with several forest and plant pathology colleagues on this subject, it is quite obvious that most of them still prefer some evidence of pathogenicity from artificial inoculations, at least under controlled conditions, such as on seedlings in a greenhouse. But as the discussion above demonstrates, this is clearly not always possible. Is it time for us plant (forest) pathologists to adopt something similar to the guidelines proposed by Fredricks and Relman [2], integrated with concepts of causality such as those expressed by Gillies [16]? I believe such refinement would be useful and here I will venture into proposing what I would consider more contemporary plant disease diagnostic guidelines (schematized in Fig. 1), based on [2] and [16]:

1. The gold standard: If isolation in pure culture and reinoculation with a putative causal agent is possible, then conduct artificial inoculations in the field, if feasible, otherwise under controlled environments and complete Koch's postulates.
2. If isolation in pure culture and reinoculation of a putative causal agent is not possible, or if multiple agents are suspected (e.g. in decline syndromes), then consider diagnosing a disease using the *preponderance of the evidence* principle (i.e. the microorganism is more likely than not the causal agent) with molecular/microbiome data (e.g. [18]):
    a. Molecular signatures of a putative pathogen should be the norm (=consistent) and not the exception, i.e. molecular signatures should always be present only in symptomatic trees or, at the very least, in statistically higher copy numbers in diseased than in asymptomatic tissues. This association should be reproducible.
    b. The molecular signatures should be consistent with expert knowledge with respect to similar, phylogenetically-related groups of organisms, i.e. they must be plausibly associated with pathogenic traits.
    c. If the diseases can be cured somehow (this is quite rare in plant pathology and basically unheard of with tree diseases) the frequency of pathogen molecular signatures should significantly decrease. With relapse, the frequency of pathogen molecular signatures should significantly increase.
    d. By adopting the avoidance/blocking principles, if the plant/tree is somehow protected, e.g. with prophylactic application of antimicrobials, or via pre-pathogen induction of defense or acquired resistance, then exposure to the putative pathogen will not result in disease, whereas lack of protection will result in disease.

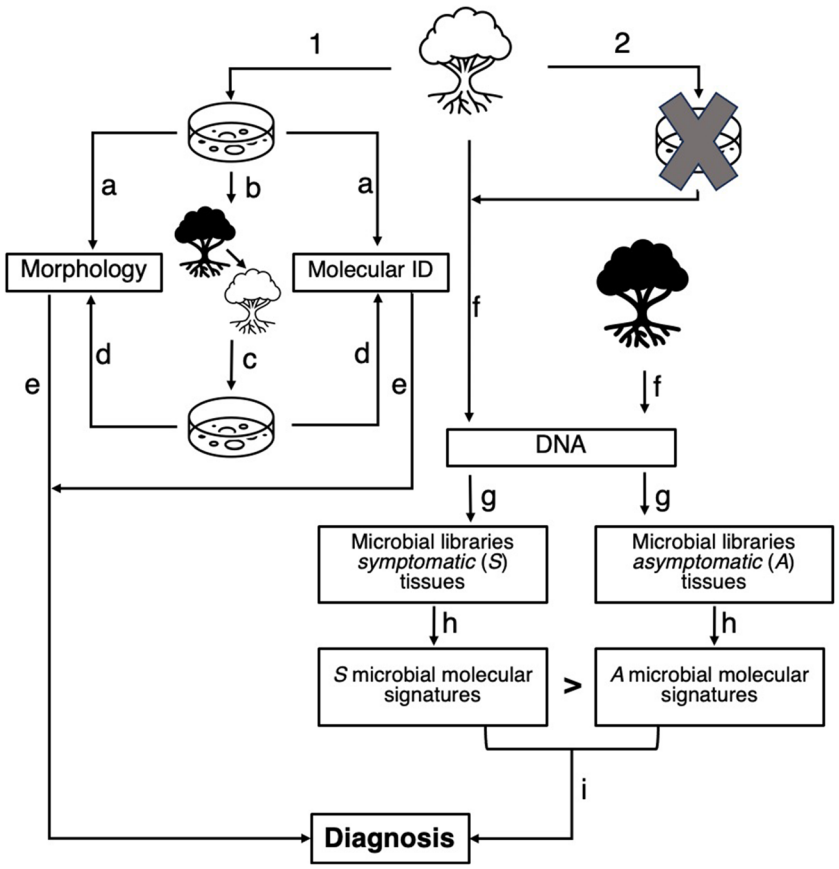

Fig. 1 Schematic representation of proposed guidelines for diagnosis of complex tree diseases. In the presence of a diseased tree (empty tree icon) there are two potential routes: (1) a putative pathogen can be isolated in pure culture, or (2) the putative pathogen is fastidious. In the first case, a diagnostician would follow Koch's postulates as closely as possible: the pathogen can be characterized morphologically and/or molecularly (**a**) and inoculated (**b**) into a healthy tree (filled tree icon). If the tree becomes symptomatic (empty tree icon), then the putative pathogen is reisolated (**c**) and confirmed to be the same as the original isolate by morphology and/or molecular data (**d**). Such confirmation would be sufficient to come to a diagnosis (**e**). If the putative pathogen is fastidious (2), then the diagnostician proceeds to extract total DNA for microbial profiling from both symptomatic and asymptomatic trees (**f**), continuing to library construction (**g**), for example by Kingdom group: fungi, bacteria/phytoplasmas, viruses, nematodes, etc. Following high throughput sequencing (**h**), molecular signals present in significantly higher levels in symptomatic material would point to the likely causal agent(s) (**i**). This may or may not warrant more in-detail investigations to firm up the diagnosis

It is important to remark that, even with these extended guidelines, uncertainty remains an inescapable feature of the process. For example, the poor quality of some reference databases can lead the molecular diagnostician astray. Thus, it is essential that the most curated databases available be used in this process. Even so, in most cases we will never be 100% certain of the true causal agent of a disease, especially with syndromes. Furthermore, the pathogen may not be present in symptomatic tissues when a disease is expressed as secondary symptoms, e.g. foliar symptoms associated with root diseases or caused by some wilts; or other non-pathogenic agents may also have higher biomass, and therefore more reads, in symptomatic tissues. Finally, some disorders have nothing to do with biotic agents and instead are caused by adverse environmental factors (e.g. nutrient deficiency, soil pH, soil salt contamination, extreme temperatures, water logging or, conversely, drought, etc.), i.e. are of an abiotic nature. In all these instances, and others that can certainly arise, expert knowledge, as described further above, is critical to discriminate possibilities and plausibility.

As a final note, in addition to molecular signatures, we may not be far from being able to rely on spectroscopy and artificial intelligence to use chemical fingerprints as very rapid diagnostics in place of molecular signatures (e.g. [19••21, 22, 20••,]). Thus, the proposed procedures should be considered living guidelines, rather than some fixed standards – after all, diagnostics is more of an art than an exact science. It is my hope that these recommendations will stimulate further conversations and perhaps formalization by a professional society after the necessary refinements. Ultimately, a more realistic approach to disease diagnosis will better serve our profession as well as society at large as we adapt to rapid developments in science and technology.

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Author Contributions P.B. conceived and wrote the manuscript and prepared Fig. 1.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The author declares no competing interests.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Koch R. Uber bakteriologische Forschung, in Xth International Congress of Medicine, 1890. Berlin: Hirschwald, Berlin; 1891. p. 35.
2. Fredricks DN, Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. Clin Microbiol Rev. 1996;9:18–33. https://doi.org/10.1128/cmr.9.1.18.
3. Anonymous. HIV/AIDS. 2024 [cited 2024 Jan. 3]; Available from: https://www.mayoclinic.org/diseases-conditions/hiv-aids/symptoms-causes/syc-20373524.
4. Sinclair WA. Comparisons of recent declines of white ash, oaks and sugar maple in Northeastern woodlands. The Cornell Plantations. 1965;XX:62–7.
5. Manion PD. Tree Disease Concepts. 2nd ed. Englewood Cliffs, New Jersey: Prentice-Hall; 1991. p. 402.
6. Balci Y, Balci S, Eggers J, MacDonald WL, Juzwik J, Long RP, Gottschalk KW. Phytophthora spp. associated with forest soils in eastern and north-central US oak ecosystems. Plant Dis. 2007;91:705–10. https://doi.org/10.1094/pdis-91-6-0705.
7. Weste GA, Marks GC. The biology of *Phytophthora cinnamomi* in Australasian forests. Ann Rev Phytopathol. 1987;25:207–29. https://doi.org/10.1146/annurev.py.25.090187.001231.
8. Nagle AM, Long RP, Madden LV, Bonello P. Association of *Phytophthora cinnamomi* with white oak decline in Southern Ohio. Plant Dis. 2010;94:1026–34. https://doi.org/10.1094/PDIS-94-8-1026.
9. Ewing CJ, Hausman CE, Pogacnik J, Slot J, Bonello P. Beech leaf disease: An emerging forest epidemic. For Pathol. 2019;49:e12488. https://doi.org/10.1111/efp.12488
10.• Carta LK, Handoo ZA, Li S, Kantor M, Bauchan G, McCann D, Gabriel CK, Yu Q, Reed S, Koch J, Martin D, Burke DJ. Beech leaf disease symptoms caused by newly recognized nematode subspecies *Litylenchus crenatae mccannii* (Anguinata) described from *Fagus grandifolia* in North America. For Pathol. 2020;50:e12580. https://doi.org/10.1111/efp.12580. **This paper shows how a convincing case can be made for the etiological agent of a forest disease even without completely satisfying Koch's postulates.**
11.• Ewing CJ, Slot J, Benítez M-S, Rosa C, Malacrinò A, Bennett A, Bonello E. The foliar microbiome suggests that fungal and bacterial agents may be involved in the beech leaf disease pathosystem. Phytobiomes J. 2021;5:335-49. https://doi.org/10.1094/pbiomes-12-20-0088-r. **This paper shows how focusing on strictly applying Koch's postulates in complex tree diseases can lead scientists astray for the true causal agent.**
12. Sahashi N, Nakamura H, Yoshikawa N, Kubono T, Shoji T, Takahashi T. Distribution and seasonal variation in detection of phytoplasma in bark phloem tissues of single Paulownia trees infected with witches' broom. Jpn J Phytopathol. 1995;61:481–4. https://doi.org/10.3186/jjphytopath.61.481.
13. Mittempergher L, Sfalanga A, Vibio M, Bertaccini A. Phytoplasmas, *Aceria bezzii* and drought in declining European hackberry (*Celtis australis* L.). Acta Horticult. 1999;496:87–92. https://doi.org/10.17660/ActaHortic.1999.496.9.
14 Errea P, Aguelo V, Hormaza JI. Seasonal variations in detection and transmission of pear decline phytoplasma. J Phytopathol-Phytopathol Z. 2002;150:439–43. https://doi.org/10.1046/j.1439-0434.2002.00767.x.
15. Bertaccini A, Duduk B. Phytoplasma and phytoplasma diseases: a review of recent research. Phytopathol Mediterr. 2010;48:355–78. https://doi.org/10.14601/Phytopathol_Mediterr-3300.
16. Gillies DA. Establishing causality in medicine and Koch's postulates. Int J Hist Philos Med. 2016;6:10603. https://doi.org/10.18550/ijhpm.0603.
17. Rivers TM. Viruses and Koch's postulates. J Bacteriol. 1937;33:1–12. https://doi.org/10.1128/jb.33.1.1-12.1937.
18. Fearer CJ, Malacrinò A, Rosa C, Bonello P, Phytobiome metabarcoding: a tool to help identify prokaryotic and eukaryotic causal agents of undiagnosed tree diseases. In: N. Luchi, Ed. Plant Pathology: Method and Protocols. New York, NY: Springer US, 2022. pp 347–66. https://doi.org/10.1007/978-1-0716-2517-0_19
19.• Wei X, Zhang J, Conrad AO, Flower CE, Pinchot CC, Hayes-Plazolles N, Chen Z, Song Z, Fei S, Jin J. Machine learning-based spectral and spatial analysis of hyper- and multi-spectral leaf images for Dutch elm disease detection and resistance screening. Artif Intell Agricult. 2023;10:26–34. https://doi.org/10.1016/j.aiia.2023.09.003. **This paper shows how modern, machine learning-based, culture and nucleic acid-independent approaches could be the future of not only detection but also diagnostics.**
20.• Fearer CJ, Conrad AO, Marra RE, Georskey C, Villari C, Slot J, Bonello P. A combined approach for early in-field detection of beech leaf disease using near-infrared spectroscopy and machine learning. Front For Global Change. 2022;5. https://doi.org/10.3389/ffgc.2022.934545. **This paper shows how modern, machine learning-based, culture and nucleic acid-independent approaches could be the future of not only detection but also diagnostics.**
21. Conrad AO, Li W, Lee D-Y, Wang G-L, Rodriguez-Saona L, Bonello P. Machine learning-based presymptomatic detection of rice sheath blight using spectral profiles. Plant Phenomics. 2020;2020:8954085. https://doi.org/10.34133/2020/8954085
22. Mukrimin M, Conrad AO, Kovalchuk A, Julkunen-Tiitto R, Bonello P, Asiegbu FO. Fourier-transform infrared (FT-IR)

spectroscopy analysis discriminates asymptomatic and symptomatic Norway spruce trees. Plant Sci. 2019;289:110247. https://doi.org/10.1016/j.plantsci.2019.110247.