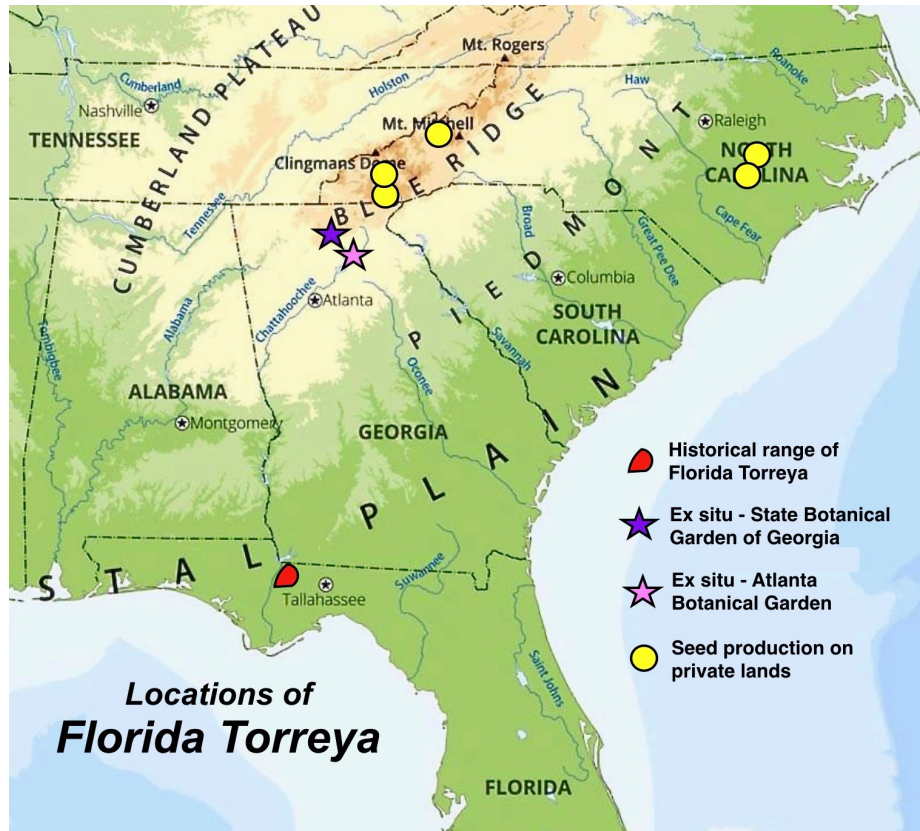


Applying the Plant and Seed Microbiome Paradigms to the endangered Florida *Torreya* tree

Compilation and recommendations by **CONNIE BARLOW**, June 2025

<http://www.torreyaguardians.org/plant-microbiome-torreya.html>



The **Florida Torreya tree, *Torreya taxifolia***, was categorized as a **glacial relict in 1905**.

It was officially listed as an **endangered species in 1984**.

In contrast to the dire situation in its historical range in Florida, ***Torreya taxifolia* is growing well more than two hundred miles northward.**

Two botanical gardens in Georgia established **ex situ "genetic safeguarding" plantings** in northern Georgia — where **seed production has been ongoing for nearly two decades**.

Additionally, private plantings of Florida torreya (which are independent of the official endangered species program) are **producing seeds in North Carolina at five sites**.

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ABSTRACT: Florida torreya (*Torreya taxifolia*), known as a glacial relict since 1905, is listed as an "endangered" tree under the U.S. Endangered Species Act of 1973. Recovery plans for listed species are to be formulated in accordance with "the best available science." The current recovery plan, adopted in 2020, is now under a 5-year review. Updating the science foundation to shift from a "latent pathogen" understanding of a fungal microbe (named *Fusarium torreyae* in 2013) to a plausible "mutualistic endophyte" interpretation would empower the U.S. Fish & Wildlife Service to disallow a decade-long policy of two botanical gardens in Georgia that has blocked distribution of tens of thousands of seeds produced annually in the ex situ plantings they control.

Instead, policy could be updated in accordance with the new "plant microbiome" and "seed microbiome" discoveries. These paradigm shifts expunge the fear of *Fusarium torreyae* spreading to other native tree species if "assisted migration" northward is utilized to remove the environmental stress of a warming climate that has destroyed torreya's viability in its historical range (a well-know glacial refugium) since the 1950s. Accordingly, this document excerpts a sampling of the new-paradigm papers and those that apply the new understanding to the genus *Fusarium*.

INTRODUCTION

THE PROBLEM: Credentialed and highly experienced staff in both of the botanical gardens **stopped distributing the ex situ seed production a decade ago — despite seeds numbering in the tens of thousands annually.** Because the seeds are "recalcitrant" and thus cannot be dried or frozen, they are impossible to store in bulk. The rationale for such wastage has been to **prevent the spread of a newly identified fungal pathogen** that causes lethal stem cankers in the historical range. **Two peer-reviewed papers (2011 and 2013) provided the scientific basis for that policy decision.** The microbial fungus was named in the 2013 paper: *Fusarium torreyae*. Note: The decision to end large-volume seed distribution was not mandated by the federal agency in charge of endangered species. It is not in the official recovery plan. Rather, the decision was made by the Atlanta Botanical Garden and the State Botanical Garden of Georgia.

THE OPPORTUNITY: Those two papers (2011 and 2013) are no longer "the best available science" (which is the legal grounding for endangered species policy at the federal level). An abundance of peer-reviewed papers have since firmly established the **PLANT MICROBIOME paradigm**, which includes an even more recent subset called the **SEED MICROBIOME**. Thus, instead of *Fusarium torreyae* being characterized as a "latent pathogen" (which expresses as disease only when its host is stressed), it must be reconsidered as a "mutualistic endophyte" — providing benefits to its host that would make no sense to re-engineer genetically or disable via fungicides. Indeed, a 2023 paper suggests:

"Given these growth-promoting roles of seed microbiome, **plant species conservation needs a paradigm shift** from the present approach of plant-specific protocols to envisaging a role for microbiome in the species conservation programmes. It implies that **a plant species cannot be conserved without conserving its integral seed microbiome** (Berg and Raaijmakers, 2018).... Laboratory experiments do not capture the complexity of seed-microbiome-plant interaction that exists in natural settings." — by Aadil Farooq War et al., 2023, "[Insights into the seed microbiome and its ecological significance in plant life](#)", in *Microbiological Research*.

THE EVIDENCE: Crucial support favoring the new plant paradigm for understanding the role of *Fusarium torreyae* is that **the genetic signature of this microbe has been detected in all tissues of Florida torreya that have been tested in sites more than 200 miles north of its Florida range. And yet the plants are healthy. *Fusarium torreyae* has also been detected in all tested seeds — and these, too, are healthy.**

THE NEED FOR A POLICY SHIFT: The old paradigm would indeed make restriction of seed distribution a wise baseline policy. However, **the new paradigm establishes that "vertical transmission" (via seeds) of the microbial endophyte is a fairly sure sign of mutualism.** The fungus engages in "horizontal transmission" of propagules via cankers only when its plant host is too stressed to produce seeds. And that is precisely what has been documented for the endangered Florida torreya tree: It produces cankers in Florida where it no longer produces seeds. Meanwhile, **Florida torreya produces seeds in canker-free northern Georgia and in long-established horticultural plantings in North Carolina.** Even more

astounding is the **annual seed production (sometimes tallying over 1,000 seeds) ongoing in Cleveland Ohio** since 2018.



ABOVE: Mature Florida torrey trees in the front yard of Fred Bess in Cleveland, OHIO, November 2024.

● **November 2023/ Fred Bess/ Parma, OHIO, photos of seed harvest from 3 female stems**



POLICY RECOMMENDATIONS: The "**Recommendations**" section in this document entails four items that urge **viewing the role of *Fusarium torreyae* through the lens of the plant and seed microbiome paradigm**. Central is the recommendation to then **apply the (new in 2023) ESA regulatory change that authorizes "experimental populations" outside of "historical range"**. Three existing plantings are suggested for official designation, each of which could be given seeds from the ex situ orchards to diversify their genetics: Shoal Sanctuary in the **Florida panhandle**, Caroline Dormon Nature Preserve in **Louisiana**, and a private forested site in southeastern Tennessee.

PART A

Birth of the Plant Microbiome Paradigm

- 2015 - **"Management of *Fusarium* Diseases Affecting Conifers"**, by Thomas R. Gordon, Cassandra L. Swett, and Michael J. Wingfield, *Crop Protection*.

EXCERPT 1: "Indications that **co-occurring microbes can have suppressive effects on pathogens affecting conifer seedlings** follow from the observation that although **decline and death of seedlings induced by *Fusarium oxysporum* is common in nurseries, disease caused by this fungus is not a problem in coniferous forest soils covered with needle litter**. Smith (1967) noted whereas *F. oxysporum* was recovered from the roots of 90% of sugar pines when trees were lifted from nursery soil, this fungus was not detectable on roots of sugar pines four years after outplanting in the forest.... *F. oxysporum* is rarely recovered from forest soils.... **Predictions of the model were consistent with the distribution of pitch canker in North America, where the disease has been established long enough to have approached ecological limits on its geographic range.**"

EXCERPT 2 (absent the internal citations): "**Pitch canker, caused by *Fusarium circinatum*, was first recognized as a disease in the state of North Carolina in the U.S. in 1945.** The pathogen was isolated from cankers that were characterized by extensive production of resin, which inspired the name 'pitch canker' for the disease. Pitch canker subsequently became more widespread in the southeastern U.S. (SE U.S.), where it continues to cause problems for production of pines in plantations, seed orchards, and seedling nurseries. **The disease was discovered in California in 1986, followed by confirmed reports from Japan, South Africa, Mexico, Spain, South Korea, Chile, Italy, Portugal, Colombia, and Brazil. The host range of *F. circinatum* extends to more than 60 species of *Pinus* and also includes Douglas-fir, the only conifer outside the pine genus known to be susceptible.** Under experimental conditions, *F. circinatum* is capable of colonizing maize (*Zea mays* L.) asymptotically, and **natural infections of grass hosts have been confirmed in the U.S. and in South Africa. No symptoms have been observed on infected grasses, suggesting the relationship is commensal.** The significance of an association with grasses has yet to be established but could influence disease development, as described below."

EXCERPT 3: *Fusarium circinatum* is not a common resident in soil and so is not likely to be a problem in seedling nurseries unless introduced with contaminated soil or seed. If the pathogen is not already established in soil nearby, **seed is the most likely vehicle for introduction. *F. circinatum* has been confirmed to occur both on and within seeds of several pine species.** Superficial contamination of seed is common in areas where pitch canker occurs, regardless of the disease status of the tree from which cones are obtained. Such infestations are presumably due to deposition of airborne microconidia and macroconidia, both of which *F. circinatum* can produce on infected host tissue. Spores might germinate on seed, but whatever subsequent growth occurs **appears not to extend beyond the seed coat because topical treatments with various anti-microbial materials will eliminate the pathogen.** For example, seeds collected from healthy *Pinus radiata* in stands affected by pitch canker commonly carry the pathogen, but the incidence of infestation can be reduced to zero by immersion of seeds in an aqueous solution of 1.0% sodium hypochlorite.... **Internally infested seeds** typically suffer high mortality rates from both pre- and post-emergence damping-off. However, some seed treatments will significantly reduce the rate of mortality, which indicates that **internally infested seed is capable of producing a healthy seedling if growth of the pathogen can be suppressed.** Both internal and external infestations of *P. radiata* seed were eliminated by treatment either with benomyl or a combination of benzimidazole, carboxin and thiram, which increased seedling emergence to 53 and 57%, respectively, compared to 43% for untreated seed. **No seedlings emerging from treated seed were infected with *F. circinatum*.**

EXCERPT 4: "The potential for seedlings to sustain **latent infections** poses a challenge for management, because **visual inspections are not sufficient to identify trees that may be carrying *F. circinatum*.** Furthermore, greenhouse studies have shown that extensive colonization of the root system by *F. circinatum* may not result in visible damage. **Shoot symptoms become apparent only after the fungus grows into the root collar and girdles the stem. Thereafter, *F. circinatum* grows more extensively in roots and can be isolated from necrotic tissue. Thus it appears that rotting of roots is a consequence of seedling death and not the cause** (Swett and Gordon, 2013). If this is typical of how disease

develops in seedling nurseries, inspection of roots may not be a reliable means of identifying cryptic infections."

• **Note by Torreya Guardians founder Connie Barlow:** The first sentence in Excerpt 1 above in red supports my own critique that **any lab experimentation in Florida** that injects *Fusarium torreyae* into potted plants or cuttings of conifer species native to the Appalachian region (especially the highest elevations, as in Red Spruce and Fraser Fir) are **ecologically negligent**, and thus likely would not pass peer review, and **certainly should not provoke botanical gardens in Georgia to refuse sending Florida torreya seeds northward from their own ex situ seed production in northern Georgia.**

Quite simply, *Fusarium torreyae*, like other species of the genus, **ramify into pathogenic agents only in conditions of stress.** As well, because techniques for discerning sophisticated morphological and molecular phylogenetic distinctions have only recently been developed, *Fusarium torreyae* is **not the only taxonomic splitting recently recognized.** (See papers excerpted on that topic in [Part C2: Recent papers on genus *Fusarium*](#), below.)

In 2003, *Fusarium commune* was distinguished from "its putative sister taxon, the *F. oxysporum* complex." Prior to publication of this discovery, the authors sampled not just their Denmark location but several other locales in the northern hemisphere — and thereby established its wide occurrence (Skovgaard et al., 2003, *Fusarium commune is a new species identified by morphological and molecular phylogenetic data*", *Mycologia*). In contrast, **the Aoki et al. 2013 paper announcing *F. torreyae* as a new species did not include any information on geographic range beyond its discovery locale.** Unfortunately, this seems to have ramped up opposition to assisted migration of Florida torreya northward beyond initial concerns that the tree itself might become "invasive" in northward recipient ecosystems. Now the new conjecture is that this newly identified *Fusarium* might not only invade new ecosystems but do so lethally. **This is a sophisticated conjecture that, when offered by respected professionals, is difficult for any non-professionals to speak against** — hence this lengthy list of linked papers and excerpts that I am posting on the Torreya Guardians website.

- 2016 - **"Editorial special issue: Soil, Plants and Endophytes"**, by Stephane Compant et al., *Plant and Soil*.

EXCERPTS: Despite the inception of the endophyte research dates back to the nineteenth century, **studies on this topic have only recently bloomed**, producing a fast-growing body of literature.... We currently describe **endophytes as any microbe that can be isolated from asymptomatic plant tissue.** **This definition includes neutral, commensal and/or beneficial microorganisms as well as dormant saprobes and pathogens during their latent phase of their life cycle.**

... Several studies related to endophytes are, moreover, mostly biased by experimental models evaluated under gnotobiotic conditions, that is, **far away from natural conditions.** We need to move beyond and to analyze how the whole plant and its associated microbiota are working together under multitrophic scenarios.... **SEED MICROBIOMES have not been studied until recently and many aspects in the ecology of seed endophytes remain elusive.** The sources of the seed microbiome, routes of transmission, possible heritability from one seed generation to the next and the role of the seed endophytes in the development of the microbiome in the emerging plant are still hardly understood.

Interestingly, the authors showed that despite differences found in soil bacterial assemblages, **seed and radicle endophytic communities were similar, and that the leaf endophytic assemblage was mainly derived from the environment and not from the seed.** Truysens et al. (2016) claimed that during plant growth, bacteria seem to be recruited from the environment to complement the endophytic population from which the seed endophytes of the next generation can again be selected, which is of special interest to better understand how plants select microbes eventually thriving as endophytes inside different tissues.... **The effect of harsh environmental conditions on the composition of microbial assemblages has not been studied well so far.**

- 2017 - **REVIEW: "Ecology and Genomic Insights into Plant-Pathogenic and**

Plant-Nonpathogenic Endophytes", by Gunter Brader et al., *Annual Review of Phytopathology*.

EXCERPTS: ... Pathogenicity is a complex phenomenon. The combination of many factors, such as pathogen and host genotypes and **abiotic and other environmental stresses**, as well as microbial interactions, determines the outcome of the reaction of a plant to a (pathogenic) microbe. Furthermore, **a single microbial species may comprise strains that are capable of exhibiting pathogenicity, mutualism, or no effect on their hosts**. For instance, *Fusarium oxysporum* is well known for its phytopathogenic properties. Collectively, strains within the *F. oxysporum* species complex can cause diseases on more than 100 plant species and exhibit strong host-specificity. However, **most isolates are nonpathogenic toward nonhosts, and for some isolates even biocontrol properties have been characterized**.

... **In this review, we address several aspects that determine the ecology and functioning of an endophyte that ultimately lead to pathogenicity or mutualism**. In particular, we elaborate on how the microbial environment may influence the function of an endophyte and elucidate **the differences between pathogenic and nonpathogenic endophytes, frequently mutualists**, in regard to plant response, pathogen colonization behavior, and genetic variation. Overall, we postulate that plant-colonizing microorganisms, irrespective of their function as a pathogen or nonpathogen, employ shared mechanisms to interact with their plant hosts, and that **pathogenicity is a consequence of fine-tuned interactions between host, environment, and other organisms**.

... "The *Fusarium oxysporum* Complex as an Interesting Model to Understand Pathogenic and Nonpathogenic Relationships" - ... Among many fungal pathosystems, the *F. oxysporum* species complex represents an interesting comparative model for **understanding pathogenic versus symbiotic relationships**. **Members within this species complex cause diseases in more than 100 plant species, but this species complex also comprises strains with biocontrol properties**. One particular nonpathogenic *F. oxysporum* strain, Fo47, has been studied as a **potential biocontrol agent of Fusarium wilt and other root diseases directly through several mechanisms, including nutrient competition, antibiosis, and mycoparasitism, or indirectly through inducing plant defense gene expression and alterations to root cell architecture**.... Even though the Fo47 genome lacks the pathogenicity related SIX genes, the genome encodes for many small secreted proteins that are actively induced during the course of interactions with the plant host *Arabidopsis thaliana* (L.-J. Ma, unpublished data). Effectors also play significant roles in mediating endophytic and symbiotic plant-fungal interactions; however, research in this field is scarce.

- 2019 - **"From pathogen to endophyte: an endophytic population of *Verticillium dahliae* evolved from a sympatric pathogenic population"**, by David Linnard Wheeler et al., *New Phytologist*

EXCERPTS from "Introduction": **Most plants are colonized by fungi** (Petrini, 1986; Wang & Qiu, 2006). These symbioses, defined by De Bary (1879) as 'the living together of dissimilar organisms', occur within the plant rhizosphere, endosphere, and phyllosphere. Regardless of the context, **the outcomes of symbioses vary across a continuum, from mutualism to parasitism** (Lewis, 1985). Likewise, the consequences of symbioses vary across a spectrum from terrestrialization to devastation of plants by mutualists and pathogens, respectively (Field et al., 2015; Strullu-Derrien et al., 2018). Although the consequences of symbioses are used to erect boundaries between categorical outcomes on the continuum, as well as the disciplines that study them, the fungi that interact with plants trespass these boundaries (Selosse et al., 2018).

... **Isolates of *Verticillium* and *Fusarium* spp., for example, can be pathogens or endophytes within** (Fordyce & Green, 1960; Schneider & Pendery, 1983) **or among hosts** (Wheeler & Johnson, 2016; Lofgren et al., 2018). For example, Fordyce & Green (1960) presented evidence that *V. albo-atrum* isolates were initially endophytic towards tomato and potato but, after several serial inoculations, incited symptoms on both hosts. Similarly, Wheeler & Johnson (2016) presented evidence that isolates of *V. dahliae* were both pathogens of potato and endophytes of several other species. Transitions between trophic states are temporally dependent (Schulz & Boyle, 2005) and governed by the physiological status of the host (Redman et al., 2001), fungus (Fordyce & Green, 1960), and/or environment (Alvarez-Loayza et al., 2011).

... At the evolutionary timescale, most endophytic representatives of the speciose fungal phylum Ascomycota arose from insect parasites (Rodriguez et al., 2008), plant pathogens

(Delaye et al., 2013), lichen-forming, endolichenic, and saprotrophic fungi (Arnold et al., 2009). **At the ecological timescale, some endophytes become mutualists under stressed conditions** (Redman et al., 2001), **while others become latent pathogens that cause disease over time** (Goodwin et al., 2011), or in other hosts (Ridout & Newcombe, 2018), or become saprotrophic (Promputtha et al., 2007). **The endophytic lifestyle therefore appears to be labile in most taxa studied.**

EXCERPT from "Discussion" section: Endophytic *Verticillium* spp. have been documented since the 1960s (Fordyce & Green, 1960; Woolliams, 1966). Despite this awareness, the prevalence of these fungi in nature, their evolutionary relationships with pathogenic isolates, their role in the maintenance of inoculum, disease expression, and their contribution to population structure are unknown. **This documentation is the first, to our knowledge, to describe emergence of an endophytic population of any *Verticillium* spp. from a pathogenic population.**

- 2020 - **"Bioprospecting endophytic fungi from *Fusarium* genus as sources of bioactive metabolites"**, by Rufin Marie Kouipou Toghueo, *Mycology*.

ABSTRACT: Endophytic fungi became an attractive source for the discovery of new leads, because of the complexity and the structural diversity of their **secondary metabolites**. **The genus *Fusarium* comprising about 70 species** is extremely variable in terms of genetics, biology, ecology, and consequently, secondary metabolism and have been isolated from countless plants genera from diverse habitats. These endophytic microbes may provide **protection and survival strategies in their host plants with production of a repertoire of chemically diverse and structurally unprecedented secondary metabolites reported to exhibit an incredible array of biological activities including antimicrobial, anticancer, antiviral, antioxidants, antiparasitics, immunosuppressants, immunomodulatory, antithrombotic, and biocontrol ability against plant pathogens and nematodes**. This review comprehensively highlights over the period 1981 to 2019, the bioactive potential of metabolites produced by endophytes from *Fusarium* genus.

EXCERPTS: ... Any species belonging to *Fusarium* genus, can be isolated as an endophyte of plants.... Undeniably, **endophytic *Fusarium* species living inside host plant tissues without causing any symptoms of disease** have proven over the years an outstanding potential by producing compounds actually approved for the treatment of several diseases including cancer, malaria, oxidative stress-related diseases, and inflammatory disorders.

- 2020 - **"REVIEW: "Biological control of *Fusarium* wilt in crop plants using non-pathogenic isolates of *Fusarium* species"**, by Suresh Patil & S. Sriram, *Indian Phytopathology*.

• **Note by Connie Barlow:** Below are lengthy excerpts to show the degree of technical understanding of differences between pathogenic and non-pathogenic strains of genus *Fusarium* that agricultural research has fostered. **Some of the later BOLD text reveals two things:** (1) It seems possible that a professional could use observational skills already developed to discern whether *Fusarium torreyae* is similar to the non-pathogenic strains and species. (2) It is clear than "non-pathogenic isolates differed in their performance at diverse temperatures" — hence **the reports that *Fusarium torreyae* is found at plantings in north Georgia and North Carolina but without pathogenic expression at those sites seem to confirm the prospect that this newly identified species is non-pathogenic in climates cooler than n. Florida.**

ABSTRACT: Many species of the genus *Fusarium* resemble each other morphologically. In many cases morphological differentiation is difficult, molecular tools are used. Pathogenic as well as non-pathogenic isolates have same habitat and colonize plant root system with equal measure. Since non-pathogenic isolates resemble pathogenic isolates in their nutritional and abiotic requirements, many attempts have been made to utilize them as biocontrol agents to manage *Fusarium* wilt diseases. Although these isolates colonize plants, they do not induce disease symptoms. They are primarily soil inhabiting in nature; once introduced in it remain in the soil for very long time providing significant and consistent disease control. Herewith we give **a review and summary of different reports of non-pathogenic *Fusarium* in different cropping systems**, mode of action (antibiosis, plant growth promotion, and induced systemic

resistance), molecular basis of bioassay and identification, environmental conditions, different formulations, cross protection as well as effects on non-target crops.

EXCERPTS: INTRODUCTION: ... **The taxon *Fusaria* is a complex one and more than one hundred formae speciales have been described.** It represents rhizosphere microflora that are plant pathogens or saprophytes. Even the pathogenic isolates survive as saprophytes and once they reach the corresponding host they infect and enter into parasitic life style. **Non-pathogenic isolates are capable of colonising root surfaces and protect even susceptible varieties from the highly virulent pathogenic isolates.** From asymptomatic roots, avirulent *Fusarium* isolates can be isolated.

INDUCED SYSTEMIC RESISTANCE (ISR): Induced systemic resistance has been identified as main mode of action in many of the non-pathogenic strains of *F. oxysporum*. First reported by Biles and Martyn (1989) ISR has been reported by many workers....

ANTIBIOSIS: ... Hua et al. (2015) observed the non-pathogenic *Fusarium* isolate CanR46 could produce anti-fungal compounds 5-hexenoic acid, limonene, octanoic acid and 3,4-2H-dihydropyran that inhibited mycelium growth, germination of conidia and germ-tube elongation in *Verticillium dahliae* and prevented wilt in cotton.... Rodriguez et al. (2006) reported another anti-fungal compound cyclosporine. It was produced by non-pathogenic *Fusarium* strain S6A inhibited the sclerotia formation in *Sclerotinia sclerotiorum* infecting soybean. Other compounds produced by non-infective isolate include saponins, phenol, flavonoid, tannins, alkaloids, anthroquinones and terpenoids in culture filtrates and some of them are toxic to fungi and bacteria (Nawar 2016).

ROOT COLONIZATION: ... Alterations in composition of root exudates due to non-pathogenic *Fusaria* was observed by Schouten et al. (2009) that reduced the nematode attraction and resulted in repellence to nematodes.

PLANT GROWTH PROMOTION (PGP): Besides inducing systemic resistance, these isolates also have ability to promote plant growth as observed by Pocasangre (2000) in banana. Treatment with non-pathogenic *Fusarium* isolates improved the tomato plant growth parameters viz., length and weight of root and shoot (Patil et al. 2011b). All these non-pathogenic *Fusarium* cultures were positive for phosphate solubilisation and production of IAA and GA....

MOLECULAR CHARACTERIZATION OF NON-PATHOGENIC *FUSARIUM*: The sequence analysis with ITS region of the non-pathogenic *Fusarium* cultures along with ITS sequence of standard pathogenic isolates revealed that **the *Fusarium* isolates viz., *F. moniliforme*, *F. oxysporum*, *F. solani* and *F. merismoides* that did not infect tomato were distinct from not only the tomato pathogen, *F. oxysporum*, *f. sp. lycopersici*, but from all the other pathogenic *Fusarium* of banana, chilli, red gram, bengal gram, cucurbit, etc.** (Patil 2009).... Kurtz et al. (2009) used RFLP in the IGS region and found that **non-pathogenic isolates of *F. oxysporum f. sp. cubense* were similar irrespective of their diverse geographical location.**

EFFECT OF ENVIRONMENTAL CONDITIONS ON EFFICACY OF NON-PATHOGENIC *FUSARIUM*: Larkin and Fravel (2002) found that these isolates differed in their efficacy in different soil types. Isolate CS1 reduced wilt severity in tomato in sandy and loamy soil and not in clay soil. Isolate CS20 was effective at various environmental conditions. **Non-pathogenic isolates differed in their performance at diverse temperature regime....**

POSSIBILITY OF NON-PATHOGENIC ISOLATES TURNING INTO PATHOGENIC ISOLATES: Ma et al. (2010) experimentally proved that **a non-pathogenic *Fusarium* species could become pathogenic on tomato by horizontal chromosome transfer (HCT)** by transferring a pathogenicity chromosome from *Fusarium oxysporum f. sp. lycopersici* that causes tomato wilt. This transfer of lineage-specific chromosome between genetically distant strains indicated the polyphyletic origin of host specificity genes in *Fusarium* species. These lineage-specific chromosomes were rich in transposons and contained genes related to signal transduction and effector proteins related to pathogenicity and virulence. It was also hypothesized that **chromosome 14 from *F. oxysporum f. sp. lycopersici* was responsible for pathogenicity, and transfer of this chromosome can convert the non-pathogenic strains to pathogenic** to tomato. This chromosome 14 contained effector genes viz. SIX1, SIX2, SIX3, SIX5, SIX6 and SIX7, which are responsible for virulence of the pathogen.

- 2021 - **"An Ecological Insight into the Multifaceted World of Plant-Endophyte Association"**, by Sushma Mishra et al., *Critical Reviews in Plant Sciences*.

EXCERPTS: ... Based on the phylogenetic affiliation, metabolic potential, physiological features, and mode of transmission, endophytes can be classified into systemic and nonsystemic endophytes (Wani et al., 2015). **Systemic endophytes**, also known as true endophytes, represent the typical class of endophytes that exist in a symbiotic relationship with the host plant, and **do not cause disease at any stage of the life cycle**. The systemic endophytes are also co-cladogenetic, i.e. the plant may host the same set of endophytes irrespective of environmental conditions (Botella and Diez, 2011; Higgins et al., 2014). **Nonsystemic endophytes**, on the other hand, are the microorganisms that **live asymptotically within the plant tissues for at least a part of their life cycle**. Such associations are typically short-lived and seasonal; their diversity varies with changes in the host's physiological parameters and varying environmental conditions (Botella and Diez, 2011). **Another striking feature of nonsystemic endophytes is that they may turn pathogenic when the host plant is stressed or resource-limited** (Petrini, 1991).

A plant may attain its microbiota either through **horizontal transmission**, i.e. from the environment (mainly rhizosphere or phyllosphere), and/or **vertical transmission**, i.e. from one generation to the next, through seeds or vegetative propagules (Frank et al., 2017; Kumar et al., 2020).

In another study, the same microbe *Fusarium verticillioides*, demonstrated two modes of lifestyle in maize: pathogenic and endophytic, depending upon the environmental factors and the host genotype (Bacon et al., 2008). The authors reported that **the fungus switches to its pathogenic state when the plant is stressed**.... It needs to be emphasized that **the boundaries between the three types of symbiotic associations (mutualism, commensalism, and parasitism) are not well demarcated**. Depending on many factors including environmental conditions, biotic and abiotic stresses, and microbial interactions, the equation between the plant and microbe changes.

• 2021 - **"Endophytic fungi: a tool for plant growth promotion and sustainable agriculture"**, by Noemi Carla Baron & Everlon Cid Rigobelo, *Mycology*.

EXCERPTS: The association between plants and fungi is extremely common. Fossil records indicate that the existence of this union with endophytes and mycorrhizas have existed for more than 400 million years (Krings et al. 2007; Chadha et al. 2015) starting when plants colonised the soil, thus indicating the importance of this group in the evolution of this process (Rodriguez et al. 2009; Rai et al. 2014; Anjum et al. 2019). **The positive aspects of this interaction have always been noted and discussed, but in-depth studies evaluating the real benefit provided by these fungi have only been performed recently** (Busby et al. 2016; Card et al. 2016; Vega 2018; Quesada-Moraga 2020).

... The **endophytic interaction is defined as balanced antagonism** (Schulz et al. 2015) because the recognition of the plant as a host **requires the activation of virulence mechanisms for colonisation and the triggering of host defences** by these events. **While an equilibrium exists in this interaction, the fungus survives of nutrients from the host plant and, in exchange, provides benefits, including tolerance to biotic and abiotic stresses** (Bamisile et al. 2018).... Fungi are able to act as antagonists of plant pathogens through the use of a diverse range of mechanisms, such as the **production of metabolites (antibiotics, volatile compounds and enzymes), engagement in competition (for space, carbon sources, nitrogen and minerals) and parasitism, induction of systemic resistance by the plant and increases in plant growth, resulting in the reduction of the activity of the pathogens** (Vega et al. 2009; Vidal and Jaber 2015; Vega 2018; Lr 2018; Quesada- Moraga 2020).

... Some fungal endophytes are able to colonise a wide range of plant species, while others are more specific and occur only inside a restricted number of plants. Additionally, specificity can also be present in relation to the portion of the plant that is colonised (Aly et al. 2011; Bamisile et al. 2018). Apparently, **vertically transmitted fungi seem to present plant associations with a more mutualistic profile than horizontally transmitted fungi**, which are more likely antagonists (Aly et al. 2011).... Horizontal transmission occurs when vegetative propagules or spores are produced by the endophyte and spread to the plant population through the air or via some vector, while **vertical transmission consists of the transference of the fungi to the plant progeny via seeds**.

... **The genetic mechanisms involved in stress tolerance are poorly known, and an essential aspect is not considered in this process: the symbiotic association of plants and microorganisms** (Chadha et al. 2015). The fungal endophyte community that exists in

wild plants can be severely modified, and many representatives can be lost during domestication; therefore, fungi are harmed by losing their safe niche and plants are deprived of a partnership that could improve their ability to overcome environmental challenges (Lugtenberg et al. 2016). For a deeper discussion about the reasons why **endophytes can be lost during plant breeding**, see Lugtenberg et al. (2016). For endophytes, the inner part of the plant is a protected niche that contains the necessary nutrients for fungal survival and growth in addition to presenting low competition with other microorganisms. Therefore, **in exchange for this safe place, fungi improve plant fitness by several mechanisms** (Khan et al. 2015; Lugtenberg et al. 2016; Chitnis et al. 2020). The benefits of plant colonisation by endophytic fungi can occur directly and/or indirectly, and the differentiation among them is complex (Berg 2009). **Among the direct mechanisms of growth promotion, the most important are the acquisition of nutrients and the production of phytohormones, while tolerance to biotic and abiotic stresses, including combat against pathogens, is considered an indirect aspect in the promotion of growth** (Hardoim et al. 2015; Souza and dos Santos 2017).

Production of phytohormones: Endophytic fungi are able to produce auxins, gibberellins (GAs) and cytokinins. **The potential of phytohormone production by endophytic fungi is underexplored, especially for gibberellins**, even though these molecules are as important as chemical signalling and messengers for plant growth in different environmental conditions (Khan et al. 2015).... **Gibberellins are essential in several plant responses, including seed germination, stem elongation, sexual expression, flourishing, fruit formation and senescence** (Bomke and Tudzynski 2009). **The production of gibberellins by endophytic fungi** is described as occurring from acetyl-CoA by the mevalonic acid (MVA) pathway....

Activation of systemic resistance: Endophytic fungi can aid plants in improving their self-defence system, thus promoting the activation of induced systemic resistance (ISR) pathways, which may overlap with that of acquired systemic resistance (ASR) because both systems can improve plant growth (Berg 2009; Busby et al. 2016) and protect against pests and pathogens (Chadha et al. 2015).... The balanced interaction between fungal endophytes and their plant hosts occurs due to the lack of pathogenic properties. A good example is the comparison of Brassicaceae's endophytic strain *Colletotrichum tofieldiae* and the pathogenic *Colletotrichum incanum* in *Arabidopsis thaliana*. **Evolution has negatively selected genes of effector proteins in the endophytic strain, which are directly involved in the pathogenic action at the moment of plant colonisation, and the same did not occur with the pathogenic strain *C. incanum*.**

Production of antibiotics and secondary metabolites: In addition to stimulating the production of defence molecules by the plant itself, **endophytic fungi are a large reservoir of molecules that act in favour of their host**. They are excellent producers of compounds with activity against pathogens and herbivores, including **alkaloids, steroids, terpenoids, peptides, polyketones, flavonoids, quinols, phenols, chlorinated compounds and volatile organic compounds** (VOCs) (Card et al. 2016; Lugtenberg et al. 2016; Latz et al. 2018; Kaddes et al. 2019). Moreover, studies report the production of compounds with antiviral, antibacterial, antifungal and insect action (Card et al. 2016; Latz et al. 2018).... **An uncountable number of molecules are produced as secondary metabolites by endophytic fungi**; however, specific pathways and substances have not been well characterised thus far. The review of Lugtenberg et al. (2016) is recommended for deeper knowledge of the chemical structures of some secondary metabolites produced by endophytic fungi.... Among the wide range of secondary metabolites produced by endophytic fungi, **more than 300 of these molecules are VOCs** (Lugtenberg et al. 2016; Kaddes et al. 2019). They consist of small molecules, presenting high vapour pressure, and they are easily diffusible through the cell membrane, in the atmosphere and in the soil, which makes them **special agents of fungal communication with other organisms, including plants, in addition to presenting bioactivity against many pathogens** (Kaddes et al. 2019).

Protection against biotic and abiotic stresses: Environmental degradation by agricultural processes and **global climate change expose plants to increasingly challenging conditions** for their growth and maintenance.... **Endophytic fungi are able to combat abiotic stresses, including drought, high and low temperatures, salinity and toxic heavy metals** (Aly et al. 2011; Khan et al. 2015). For biotic stress protection, fungal endophytes are responsible for the activation of ISR and ASR, which produce metabolites against pathogens; moreover, parasitism or competition can occur to avoid disease and herbivory (Chadha et al. 2015; Chitnis et al. 2020).... **Endophytic fungi help host plants adapt to stress conditions through diverse mechanisms.** As reviewed by Khan et al.

(2015) and Yan et al. (2019), during oxidative stress, plants increase the activity of antioxidant enzymes, mainly catalases and peroxidases, which leads to the production of ROS, resulting in membrane attack causing the peroxidation of membrane lipids. By some not yet defined mechanisms, endophytic fungi confer tolerance to ROS, reducing lipid peroxidation. Another important problem caused by **abiotic stresses (drought, heat and salinity) in membranes is electrolyte leakage**, which is associated with the variation in the lipidic composition and the amount of these molecules of the cell membrane due to stress conditions. **Endophytic fungi are able to induce changes in the lipidic composition of the cell membrane**, preventing leakage (Khan et al. 2015; Yan et al. 2019).

... In relation to biotic stress, the main **defences against pathogens, herbivores and nematodes are the production of secondary metabolites and the activation of systemic resistance by endophytic fungi** (Latz et al. 2018; Yan et al. 2019; Poveda et al. 2020).

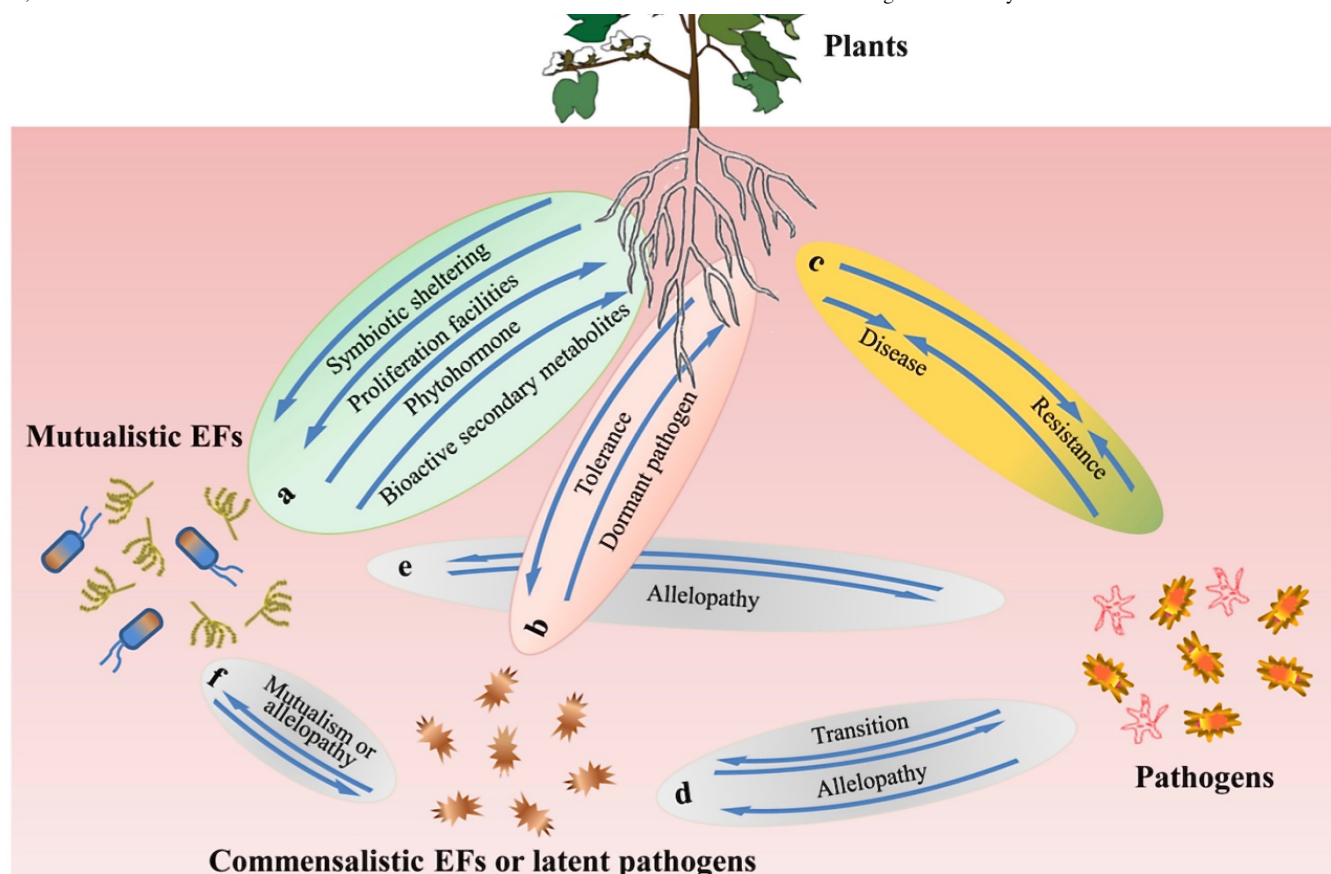
- 2021 - **"Protection to Tomato Wilt Disease Conferred by the Nonpathogen *Fusarium oxysporum* Fo47 Is More Effective Than that Conferred by Avirulent Strains"**, by Francisco J. de Lamo et al., *Phytopathology*.

ABSTRACT EXCERPT: **Although the vascular pathogen *Fusarium oxysporum* is notorious for being the causal agent of Fusarium wilt disease, the vast majority of *F. oxysporum* strains are harmless soil and root colonizers.** The latter *F. oxysporum*'s are often **endophytes** colonizing roots intracellularly without negatively affecting plant fitness. **Actually, most of them, like Fo47, are beneficial providing biological control to various root pathogens.**

EXCERPTS: *Fusarium oxysporum*, a filamentous ascomycete, is the causal agent of Fusarium wilt disease (Michielse and Rep 2009). This soilborne vascular fungus **ranks among the top 10 major fungal plant pathogens** (Dean et al. 2012). Each *F. oxysporum* pathogen harbors a specific set of effector genes required for pathogenicity on a specific host (van Dam et al. 2016). Nevertheless, **the ample majority of *F. oxysporum* strains are nonpathogenic saprotrophs able to endophytically colonize plant roots** (Bao et al. 2004). It has been well established that wilt disease-suppressive soils contain beneficial *F. oxysporum* endophytes that are responsible for protecting a susceptible host to pathogenic *F. oxysporum* strains (Alabouvette 1986; Tamietti et al. 1993). These *F. oxysporum* endophytes exert biological control by **directly affecting the invading pathogen and/or by inducing plant immune responses**.... In summary, compared with the endophyte Fo47, avirulent pathogens only weakly reduce susceptibility to *F. oxysporum* f. sp. *lycopersici*. A possible explanation for this observation is that the Fo47 endophyte is less, or even unable to suppress host immune responses. Presumably, **once immune responses are triggered by an endophyte they cannot be suppressed by the effectors present in a virulent strain**, or it is to a much lower extent not able to significantly contribute to resistance.

• **Note by Connie Barlow:** Thankfully, the genus *Fusarium* has pathogenic strains that so widely evoke disease symptoms in crop plants that funding has been available to **discern both the geographic span and the diversity of effects on host plants — ranging from beneficial to asymptomatic to pathogenic — that distinct strains of the same *Fusarium* species can evoke.** For those of us grounded in organismic or ecological aspects of the biological sciences, this ability of a known fungal pathogen to also express beneficially in a plant is surprising. Hence, the distinguishing and naming of a new *Fusarium* species (distinct from well-known *F. Lateritium*) and as later described as "the primary cause of Florida torreyia decline" (Dreaden et al. 2020) **evoked a great deal of fear of disease transmittal into other native trees, such that seed distributions from the ex situ official orchards in north Georgia were severely diminished.** It is therefore my hope that botanical garden and Native Plant Society staff (especially in Georgia and Florida) will familiarize themselves with the broader scientific literature on the *Fusarium* genus, such as evident in this paper.

- 2021 - **"REVIEW: "Endophytic Fungi: From Symbiosis to Secondary Metabolite Communications or Vice Versa?"**, by Beena Alam et al., *Frontiers in Plant Science*.



ABOVE: "EF" = Endophytic Fungi. "During infection, fungi form **three corresponding types of interactions with hosts: mutualistic (beneficial endophytes), commensalistic (non-beneficial/virulent endophytes), and pathogenic (virulent pathogens)**, depending on the physiological status or specific circumstances that host plants experience. According to these three modes of action, fungal strains can increase, have no palpable effects on, or decrease host fitness (Kogel et al., 2006).

EXCERPTS: ... According to their colonizing behaviors, endophytic microflora can be sorted into facultative and obligate categories. **Facultative endophytes** colonize plants at certain stages of their life cycles, but they may also reside outside the plant at other stages to form an association with the immediate rhizosphere soil of host plants (Abreu-Tarazi et al., 2010). In contrast, **obligate strains** live in plants throughout their entire life cycles. They usually proliferate across plant generations through **vertical transmission** and use or alter the metabolic machinery and products of plants for their own survival (Hardoim et al., 2008; Gouda et al., 2016).

... Among these endophytic microorganisms, endophytic fungi (EFs) have attracted much research interest because they have provided not only novel sources of cytotoxic compounds, such as anticarcinogenic molecules (Uzma et al., 2018) and antibacterial substances (Radic and Strukelj, 2012), but also biostimulants for essential oil biosynthesis (El Enshasy et al., 2019). **They may enhance nutrient solubilization in the plant rhizosphere (Mehta et al., 2019), promote plant growth (Poveda et al., 2021), act as biological control agents (Poveda and Baptista, 2021), or activate plant systemic resistances to biotic (Poveda et al., 2020a) or abiotic (Cui et al., 2021) stresses.**

... According to the reproductive pattern and host occurrence, EF communities can be sorted into **two categories**: the Clavicipitaceous/Balansiaceous group (C-group) and the non-Clavicipitaceous/non-Balansiaceous group (NC-group).... Some illustrative endophytic mycobiomes of the NC-group include **Fusarium spp.**, *Piriformospora indica*, and dark septate mycobiota (Varma et al., 2000; Schulz et al., 2002).

... Fungi that have been identified as endophytes that are also possible pathogens include *Cladosporium*, **Fusarium**, *Colletotrichum*, *Cordana*, *Deightonella*, *Periconiella*, *Verticillium*, *Curvularia*, *Nigrospora*, *Guignardia*, and *Phoma* (Photita et al., 2004; Cui et al., 2021). These EFs stay in latent or dormant state in the tissue of their host plants long before the outbreak of disease symptoms. In such cases, the dormancy phase is essential because it determines the

time when the fungus is harmless as an endophyte and when it is virulent as a pathogen. In the virulent phase, EFs cause obvious symptoms and change the morphology and physiology of host plants under adverse conditions (Figure 1d). It is precisely these hostile conditions, including malnutrition, disruption of ontogenetic state (Sieber, 2007; Rodriguez and Redman, 2008), biotic stresses, **drastic climate changes** (such as elevated temperature and excessive humidity), and senescence, that break the balance between EFs and their hosts and lead to the transition of EFs from latent mode to active virulent pathogens, although there are no obvious disease symptoms before transition (Romero et al., 2001; Photita et al., 2004; Poveda et al., 2020b). **There are also endemic fungal species, which typically include the majority of *F. oxysporum* strains, that live in host tissues without causing disease symptoms. Some strains even confer beneficial effects** (Imazaki and Kadota, 2015; Di et al., 2016), such as *C. tofieldiae*, which promotes plant growth and fertility as an endophyte under phosphorus-deficient conditions (Hiruma et al., 2016). It is assumed that a combination of effectors, enzymes, and secondary metabolites determines the outcome of an interaction; that is, whether it is endophytic or pathogenic (Di et al., 2016; Poveda et al., 2020b). Nutrient status may have facilitated the transition of *C. tofieldiae* from pathogenicity to symbiosis (Hiruma et al., 2016).

WHY DO ENDOPHYTIC FUNGI PRODUCE SECONDARY METABOLITES? When different microorganisms occupy the same habitat, they must compete for the resources of that habitat for nutrition, living space, reproduction, and other needs throughout their life cycles.... In order to survive, organisms have developed **two effective strategies to compete**. One is to **produce allelochemicals that inhibit the growth of their competitors** and eliminate toxic effects produced by their competitors in the vicinity (Macías- Rubalcava et al., 2008; Konarzewska et al., 2020; Poveda, 2021). The other is to **produce allelochemicals that help their producers form alliances through symbiotic relationships with symbionts or hosts**. These symbiotic relationships enable both parties to survive and reproduce safely, even in extremely adverse environments (Macías-Rubalcava et al., 2008). According to the hypothesis of long-term coevolution within biological communities (Ji et al., 2009), this mutual orientation of EFs and their hosts leads to each EF having developed a specific range of host species, enabling them to accumulate in a specific eukaryotic host group.

Most of these allelochemicals are SMs ["Secondary Metabolites"], a variety of usually low-molecular-weight and amazingly heterogeneous chemicals that were previously thought to have no direct functional effect on the growth, development, and reproduction of the organisms that produce them (Keller et al., 2005; Yu and Keller, 2005; Fox and Howlett, 2008; Shwab and Keller, 2008). Volatile organic compounds (VOCs) are a large group of such chemicals that allow their producers (including plants and microorganisms) to **defend themselves against attacks by pests or pathogens**, or to convey warnings intra- or inter-specifically during such attacks (for details of VOCs, please refer to Poveda, 2021). Historically, the term "secondary" used for such natural metabolites has been associated with their "inessentiality," but they have been demonstrated to play important roles in the growth and development of organisms in indirect ways (Pichersky and Gang, 2000; Deepika et al., 2016).

... The concept of coevolution has been applied to characterize the biochemical interactions between EFs and hosts. It is thought that the **coevolution of endophytes and their host plants shapes the production of SMs**, which play important roles in endophyte-host communication for mutual adaptation and their orientation to different environments (Debbab et al., 2011; Lind et al., 2017).... **Studies suggest that environmental stresses, sudden drastic climate changes, or senescence may facilitate the conversion of endophytes into pathogens in the host to adapt to these ecological changes** (Romero et al., 2001; Photita et al., 2004).

- 2019 - **REVIEW: "Bacterial and Fungal Endophytes: Tiny Giants with Immense Beneficial Potential for Plant Growth and Sustainable Agricultural Productivity"**, by Olawale Israel Omomowo and Olubukola Oluranti Babalola, *Microorganisms*.

• **Note by Connie Barlow:** Despite this being a 2019 paper, I placed it at the end of this section because it directly leads into the SEED MICROBIOME section that comes next. **MOST FUNGAL ENDOPHYTES ARE VERTICALLY TRANSMITTED THROUGH THE SEED.** That is a crucial statement in this 2019 paper. This understanding of **beneficial fungi in SEEDS** is such a recent scientific discovery that **even professional botanists in mid-career may be unaware of it.**

Access a later entry titled, [2019 - Audio podcast interview of a botanical garden staff person](#) to see an example. A staff person working for the State Botanical Garden in Georgia was so distraught by the recent discovery of *Fusarium torreyae* "within all tissues" and even "within seeds" that the policy she stated was that even the ex situ orchards "safeguarding" the wild genetics of Florida *Torreya* in n. Georgia might be removed and burned in order to ensure that the so-called pathogen would not spread northward.

EXCERPTS: Endophytes are a group of ubiquitous and diverse microbes that are found in diverse ecological niches in the tissues of a plant. **They inhabit all plants asymptotically and serve as a treasure trove of biologically important metabolites that could be used for promoting plant growth, potent biocontrol against pathogens and pests, immune defense and fitness, and for conferring functional traits on the plant to allow it to withstand or tolerate external stresses.**

...All living plants are colonized by different endophytes, without any exceptions; indeed, plants are host to a diverse group of endophytic microbes in a mutualistic, beneficial way, which is vital to plant growth and health. Endophytic microbes can enter and colonize plants through the vertical seeding method or through horizontal transmission from the soil to the plants. Either way, mutualistic benefits are still conferred through the plant-microbe interactions. This highly beneficial mutualistic interaction makes it possible for endophytic microbes to confer the following benefits on their plant host: **they increase the nutrients made available to the plant host, act as protective defenders against pathogens and destructive pests, improve the capability of the plant to withstand or tolerate environmental stresses, help in modulating development of their plant host,** and are also helpful in tackling issues related to the growth of unwanted weeds.

... Fungal endophytes exist in close, mutually beneficial association with their plant host, in that they **provide ecological support to their host plants by allowing them to survive adverse biotic and abiotic stresses.** In their turn, the endophytes derive nutrients and protection from the plant. **Fungal endophytes colonize plant host tissues such as stems, fruits, flowers, roots, leaves and branches; this is done asymptotically, without any adverse effects.** These include the containment or mitigation of damage caused by pests or destructive insects. Moreover, reports indicate that plants colonized by these fungal endophytes are less susceptible to the destructive effects of pests. The endophytes confer these benefits on their host plant by interrupting the growth and development phases of the pest; they also affect the feeding pattern and reproductive stages of the pest, thereby affecting its overall survival.... The lessening of damage through the containment of pests by the fungal endophytes can be attributed to their **production of secondary mycotoxigenic metabolites in their host plants which are toxic to the pests.**

Another important mutualistic benefit of the endophytic fungal interrelationship with the plant host is **the ability of the endophyte to confer both abiotic- and biotic-stress-tolerant traits on its plant host, thereby helping the host to improve its growth and to be less susceptible to disease.** Fungal endophytes have also proven to be important sources of metabolically active compounds. They have the ability to **produce vital plant hormonal compounds such as piperine, gibberellic acid, and indole-3-acetic acid, which are required for the promotion of plant growth.** They are capable of inhibiting plant disease causing pathogens, and enable plants to tolerate salinity stress, among other stresses.

It can be asserted based on studies done in the last few years that **endophytic fungi play key functional roles in the ecosystem.** Their main effects in terms of beneficial interactions with plants include their ability to make vital growth-promoting nutrients available to the plant; they help suppress noxious plant pests, pathogens, nematodes, and other destructive insects; **they help in mitigating environmental stress; and they are also useful in the bioremediation of environmental contaminants.** They are able to achieve these effects by using different mechanisms.

... Endophytic fungi positively influence exudations in the roots of plants, thereby attracting beneficial rhizosphere microbiota that facilitate the transportation of minerals from the soil that are required by plants. They are also beneficial partners in promoting plant immunity and in ecological and physiological adaptations in their plant hosts, thus enabling them to adapt to environmental stresses and to fight against pathogens.

... Endophytic bacteria and fungi that are associated with the colonization of plant tissues are **transmitted horizontally (plant or soil to plant), vertically (parent plant to seed),**

or in a mixed way. **MOST FUNGAL ENDOPHYTES ARE VERTICALLY TRANSMITTED THROUGH THE SEED.** Endophytic bacteria, on the other hand, prefer horizontal transmission. Bacterial endophytes are optimally adapted to the horizontal transmission route, although there have been reports of vertically transmitted seed-borne bacterial endophytes that are of biotechnological importance.

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PART B

Birth of the Seed Microbiome Paradigm

- 2017 - **"Ecological patterns of seed microbiome diversity, transmission, and assembly"**, by Ashley Shade et al., *Current Opinion in Microbiology*.

Seeds are involved in the transmission of microorganisms from one plant generation to another and consequently act as the initial inoculum for the plant microbiota. The purpose of **this mini-review is to provide an overview of current knowledge on the diversity, structure and role of the seed microbiota.**

- 2018 - **"What Is There in Seeds? Vertically Transmitted Endophytic Resources for Sustainable Improvement in Plant Growth"**, by Raheem Shahzad et al., *Frontiers in Plant Science*.

EXCERPTS: [This paper is a "Mini-Review"; *Fusarium* genus is mentioned several times, both as pathogenic and as mutualistic.]

Seeds are not merely the carriers of a plant's hereditary information, but also both reservoirs for plant microbiota and vehicles for their vertical transmission. **The role of seed-associated microbes is of significance to plant growth and development because these microbial communities may secrete important phytohormones, such as cytokinins, that break seed dormancy and inhibit microbial invasions.**

Recent evaluations suggest that over 300,000 plant species are found worldwide, and that **every plant carries at least one endophyte.** Indeed, endophytic microbes have been found in every plant species examined to date.... It can be assumed that plants deprived of endophytes would be more vulnerable to environmental stress and pathogenic attacks. **Endophytic microorganisms (bacteria or fungi) are a key class of plant symbionts that live inside plant tissues without inducing any disease symptoms,** and which are associated with the plant throughout its life history, from seed germination to fruit development. **Endophytes are found in the roots (rhizosphere), leaves (phylloplane), stems (laimosphere and caulosphere), fruits (carposphere), seeds (spermosphere), and flowers (anthosphere),** as described by many scientists.

... The relationship between endophytes and plants is unique in the ability of the former to provide alternative sources of biologically active metabolites, such as enzymes, biofunctional chemicals, phytohormones, nutrients, and minerals, and to facilitate the distribution or production of these resources which contributed in the elimination of various stresses. In return, the host plant provides a protective sanctuary for the microbes within the plant tissues, in which they can grow and reproduce, but without compromising the plant's own growth resources.

... Seed-borne endophytes are of particular importance because they are passed between successive plant generations via vertical transmission, thus ensuring their presence in the next generation of seedlings.... The internal environment of a seed changes during maturation, which consequently affects the seed endophytic community. **The ability to reside in a seed and adapt to severe environmental conditions are special characteristics of seed endophytes that are rarely found in endophytes isolated from roots, shoots, or other plant tissues.** Seed endophytes have the ability to form endospores, thus providing protection from changing conditions inside the seed. They also maintain other features, such as cell motility and phytase activity, in order to be able to migrate

freely inside the plant and enter the seeds before they harden. There have however been relatively few studies examining biodiversity in seed-borne endophytes.... Conservation of vertically transmitted endophytes indicates an evolved form of mutualism.... Some studies indeed report that the rate of vertical transmission for many fungal endophytes is greater than 90%.

- 2019 - **REVIEW: "The seed microbiome: Origins, interactions, and impacts"**, by Eric B. Nelson, *Plant and Soil*

BACKGROUND: The development and dispersal of seeds as well as their transition to seedlings represent perhaps the most critical stages of a plant's life cycle. **The endophytic and epiphytic microbial interactions that take place in, on, and around seeds during these stages of the plant's life cycle may have profound impacts on plant ecology, health, and productivity.** While our understanding of the seed microbiota has lagged far behind that of the rhizosphere and phyllosphere, many advances are now being made.

EXCERPTS: In discussing seed microbiomes, it is important to distinguish between the endophytic microbiota (i.e., those microbial species that reside in internal seed tissues and **vertically-transmitted to progeny seedlings**) and epiphytic microbiota (i.e., those microbial species that colonize seed surfaces and may or may not become internalized within seed tissues and transmitted either vertically or horizontally). Although this is a rather artificial division, in part because endophytes can become epiphytes and vice versa, the reasoning for distinguishing them is that the endophytic microbiota may often originate from different seed tissues or environmental sources than those of the epiphytic microbiota. For example, **microbes associated with the embryo and endosperm are more likely to be transmitted vertically than those associated with the seed coat**, which are likely to be much more diverse and transmitted horizontally (Barret et al. 2016).

... For many years, the endophytic seed microbiota has been viewed as being composed of taxa that are strict commensals or mutualists (Hume et al. 2016; Malfanova et al. 2013; Muller et al. 2016; Porras-Alfaro and Bayman 2011; Santoyo et al. 2016; Truyens et al. 2015), despite our recognition of endophytic viral, bacterial, and fungal seed-borne pathogens as early as the 1940s (Munkvold 2009). The endophytic lifestyle does not necessarily imply ecology or a functional characterization of plant responses to the presence of the microbe (Schulz and Boyle 2005) and **we now clearly recognize that mutualism and pathogenicity are not inherent microbial properties and are only expressed within certain contexts** (Alvarez-Loayza et al. 2011; Eaton et al. 2011; Fesel and Zuccaro 2016; Malcolm et al. 2013). I believe this contemporary view better advances our understanding of the ecology of plant-microbe interactions in general but of seed-microbe interactions specifically. Therefore, for the purposes of this review, I will adopt this latter view.

... The other ascomycete classes contain the commonly described endophytic genera *Chaetomium*, *Fusarium* (and associated teliomorphs), *Microdochium*, *Stemphylium*, and *Xylaria*. In addition to being present in seeds, many of these fungal genera are commonly associated with soils where they are frequently transmitted horizontally to plants. In fact, recent studies have demonstrated that local site conditions and not host genotype may have a strong influence on the assembly of fungal seed microbiomes (Klaedtke et al. 2016). Although in many cases the physical location of fungi in the seed is not clear, they may largely reside on and in the seed coat (Rodriguez et al. 2009) where they may be both vertically and horizontally transmitted to subsequent generations.

... Seed germination and seedling growth is the stage of the life cycle where all the previous interactions with microbes potentially have their ultimate impact. **While microbial interactions during flowering, seed dispersal, and dormancy in the seed bank may all impact the microbial legacy of a seed, the interactions of seed-associated microbes with the soil microbiota during seed germination may have the strongest impacts on overall plant fitness.** Both the endophytic and epiphytic seed microbiota as well as the soil microbiota are activated during seed imbibition and germination to create a spermosphere environment either beneficial or detrimental to the critical seed-to-seedling phase of the life cycle. Whether the origin of the spermosphere and seedling microbiota arises from the endophytic seed microbiome, the soil microbiome, or from a combination of both remains unresolved. However, recent studies are revealing new insights into this question and improving our understanding of how these interactions may ultimately influence plant health management in agriculture and ecological dynamics in natural ecosystems.

... While it should be clear that **seeds associate with a large diversity of both endophytic and epiphytic microbes, the connections between the two and their interconnectedness with the soil are just beginning to be realized.** Research exploring the movement of seed endophytic microbes into the spermosphere and rhizosphere as well as recruitment from the spermosphere and rhizosphere and into the endophytic seed microbiome offer exciting possibilities for better understanding the dynamics of plant microbiomes.

- 2019 - **"Pine Seeds Carry Symbionts: Endophyte Transmission Re-examined"**, by R.J. Deckert et al., pp 335-361, Chapter 16 in *Seed Endophytes*, S. Verma and J. White, eds.

EXCERPTS: ABSTRACT: Observations from an increased scrutiny of **seed-borne microbes** are challenging our traditional concepts of endophyte transmission, but **few of these studies have focused on trees, particularly the conifers** that dominate many forested ecosystems.

16.9 VERTICAL TRANSMISSION: ... We think an important criterion to add, but one which is often missing when discussing vertical transmission, is that a genetically individual endophyte (i.e., a clone) **should persist in the host and offspring for more than one generational cycle**, that is, from a particular host down to its grandchildren.

16.12 RETICULATE TRANSMISSION: ... We propose a new category of symbiont transmission, *reticulate transmission*, where **symbionts are acquired horizontally from the environment and filtered by the host parent(s) for incorporation in the the seed microbiome** where it is passed on vertically to the following generation. The microbe may persist as a symbiont of the young plant or be discarded after use (e.g., as a germination promoter).

- 2020 - **"Extending Plant Defense Theory to Seeds"**, by James W. Dalling et al., *Annual Review of Ecology, Evolution, and Systematics*.

EXCERPTS: ... We also highlight recent insights into the mutualistic and antagonistic interactions between seeds and microbial communities, including fungi and endohyphal bacteria, that can influence seed survival in the soil and subsequent seedling vigor.

Seed bank persistence. Seeds that persist for prolonged periods before germination, and consequently become incorporated in the soil seed bank, may have a reduced risk of predation (Hulme 1998). However, once in soil they are increasingly susceptible to microbial infection from pathogens and decay of seed-protecting structures by saprophytes (Wagner & Mitschunas 2008). **Seed bank-forming species might therefore be expected to undergo selection for particularly effective antimicrobial defenses** relative to species with a more transient seed stage. **Effective defenses for persistent seeds may include indirect defenses via microbial mutualistic partnerships that exclude potential pathogens or promote germination** (Sarmiento et al. 2017) or the induction of biochemical defenses consisting of latent enzymes that are activated in the presence of pathogens (Fuerst et al. 2017).

... Within this framework, species with physical dormancy use physical defenses to exclude predators and germinate rapidly to evade pathogens. Species with physiological dormancy are de-fended with varying investments in chemical and physical defenses. **Quiescent seeds, which have comparatively limited chemical and physical defenses, may instead depend on beneficial seed-infecting microbes for protection.**

- 2021 - **"Seed-Transmitted Bacteria and Fungi Dominate Juvenile Plant Microbiomes"**, by David Johnston-Monje et al., *Frontiers in Microbiology*.

EXCERPTS: Over hundreds of millions of years, angiosperms have **coevolved with microbes that helped them acquire nutrients, resist stress, and combat pathogens. Today, plants are considered to be HOLOBIONTS, a community of microbes cooperating and coevolving with their host to stimulate its anatomy, physiology, development, immunity, behavior, and genetic variation....** It was not until the advent of high-throughput sequencing technologies at the beginning of this new millennium, however, that the immense diversity of plant-associated microbes began to be understood by the broader scientific community, highlighting the potential to discover many new beneficial plant-associated bacteria and fungi.... If plants are truly holobionts that have survived and coevolved with microbes for hundreds of millions of years (Vandenkoornhuyse et al., 2015), **it makes sense that their most important symbionts would be vertically transmitted through seed rather than gambling that all of the correct soil-dwelling microbes might be available at the**

germination site (Nelson, 2018).... Although much work needs to be done to better understand the importance of seed endophytes, it has been shown that they can aid in germination, provide protection from pathogens, and improve mineral nutrition and vigor of the seedling.... **Seeds and the area around the germinating seed (spermospheres) are increasingly being appreciated as microbial habitats contributing microbiota that can protect seeds against rotting in the soil, aid in germination, and increase seedling vigor** (Nelson, 2018).

... It is troubling to think that because of the use of vegetative propagation in plants like cassava, potatoes, and strawberries, in addition to the phytosanitary standards requiring the physical and chemical disinfestation of botanical or vegetative seeds in order to have pathogen-free crops, **the normal transmission of microbes from seeds to seedlings may have been interrupted by modern agriculture**. Regardless of provenance, with thousands of different species of microbe in the plant biome, how does one determine which are the most important to the plant's well-being and productivity?

... When trying to identify a **core microbiome**, occupancy (how often a microbe is observed in a sample) is most often considered the defining characteristic. For example, **one definition of a core microbiome are those bacteria and fungi that are closely associated with a particular species or genotype of plant (i.e., high occupancy), independent of environmental conditions. Core microbiomes are thought to contain key microbial taxa that have been important for plant survival and reproduction over evolutionary time**. Such microbes, must have over millions of years, developed a robust and efficient transmission strategy and retained the ability to colonize the plants and also to provide beneficial functions that contribute to plant growth, survival, and/or reproduction; traits which could be under positive selection in the holobiont.

... **The primary purpose of this study was to demonstrate the importance of seed transmission to the establishment of plant microbiomes, which have traditionally been assumed to acquire all their microbes from soil. Showing that seeds are dominant players in establishing plant microbiomes could lead to a paradigm shift in our understanding** of (and ability to manipulate) plant microbiome assembly, which has been assumed to depend largely on soil.

A secondary purpose of this study was to establish whether core seed-transmitted microbiomes might exist across these economically important plant species.... Our experiment attempts to document the common (appearing in over 60% of samples) and core (appearing in 100% of samples) microbes inhabiting seed interiors and seed surfaces (spermospheres) of a panel of 17 academically and economically important plant species, many of which have had their genomes sequenced and serve as model organisms.... **In seeds or spermospheres, only *Pantoea* (BactOTU1), *Enterobacter* (BactOTU2), *Pseudomonas* (BactOTU3), *Bacillus* (BactOTU8), and *Fusarium* (FungOTU1) appeared to be part of a core microbiome across the plant species.... Seed-borne *Fusarium* has been identified as one of the dominant members of the stem endosphere mycobiome of maize**.... In our experiment, **the dominant genera of seed-transmitted fungi in roots grown on sterile sand were *Fusarium*, *Alternaria*, *Pseudozyma*, *Sarocladium*, *Penicillium*, and *Phoma*, which also dominated soil-grown roots, although only *Fusarium* and *Pseudozyma* occurred in all samples. Both *Fusarium* and *Alternaria* have been identified as core root fungi in comparisons of poplar, oak, and pine.**

• **Note by Connie Barlow:** Note especially the text in purple. "**Holobiont, core microbiome, and paradigm shift**" are three terms of great importance here. As well, that a type of *Fusarium* has been found in seeds of Florida *torreyia* is strong evidence that the fungal type is not only a beneficial endophyte — but is part of the core microbiome. Hence, **if professionals in charge of the recovery plan for *Torreyia taxifolia* would take the time to read this and other recent papers about seed microbiomes, they too might experience the kind of "paradigm shift" that the authors of this paper set forth.**

- 2021 - **"Experimental Evidence of Microbial Inheritance in Plants and Transmission Routes from Seed to Phyllosphere and Root"**, by Ahmed Abdelfattah et al., *Applied Microbiology International*.

SUMMARY EXCERPTS: While the environment is considered the primary origin of the plant microbiome, the potential role of seeds as a source of transmitting microorganisms has not received much attention. Here **we tested the hypothesis that the plant microbiome is partially inherited through vertical transmission**. An experimental culturing device was constructed to grow **oak seedlings** in a microbe-free environment while keeping belowground and aboveground tissues separated. The microbial communities associated with the acorn's embryo and pericarp and the developing seedling's phyllosphere and root systems were analysed using amplicon sequencing of fungal ITS and bacterial 16S rDNA. Results showed that **the seed microbiome is diverse and nonrandomly distributed within an acorn**. The microbial composition of the phyllosphere was diverse and strongly resembled the composition found in the embryo, whereas the roots and pericarp each had a less diverse and distinct microbial community. **Our findings demonstrate a high level of microbial diversity and spatial partitioning of the fungal and bacterial community within both seed and seedling, indicating inheritance, niche differentiation and divergent transmission routes for the establishment of root and phyllosphere communities.**

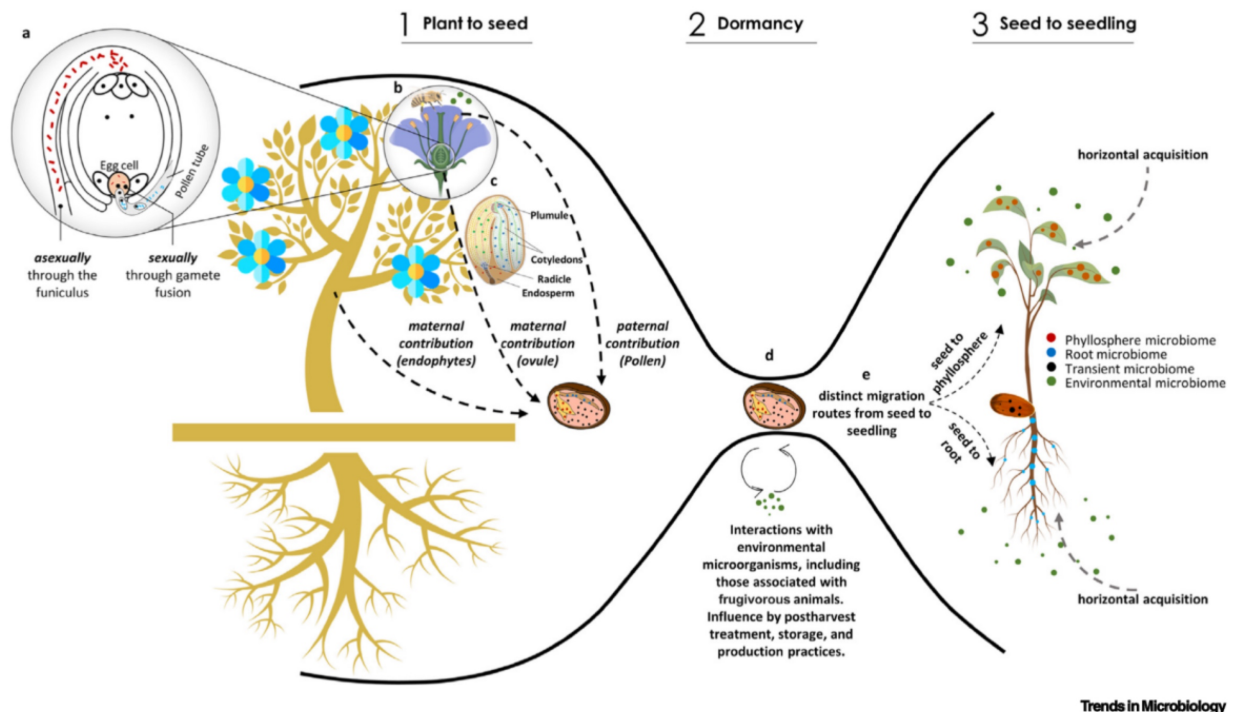
INTRODUCTION EXCERPTS: **All plant species, without exception, have been found to be associated with a diverse community of microbes.** The first known association between two microorganisms occurred around **1.45 billion years ago, between a prokaryote now known as a mitochondrion and an archaeon**, which led to the creation of eukaryotes (Roberts, 2017). A billion years later, **the merging of a eukaryote with a photosynthetic bacterium (now known as a plastid) represented the first step of plant evolution** (Sagan, 1967; Gray, 2017). In current ecological systems, plants have been shown to recruit, actively or passively, microbes that form a dynamic assemblage of species referred to as the **plant microbiome**. The fossil record indicates that **plants have been associated with endophytic fungi and bacteria for more than 400 million years** (Krings et al., 2007). Plants pass vital endosymbionts such as mitochondria and chloroplasts to their offspring through cytoplasmic inheritance; however, data on the inheritance and continuity of a distinct plant microbiome, and how the transfer occurs, are lacking. This raises a fundamental question, where do plant-associated microbes come from?

DISCUSSION EXCERPTS: ... **The fact that the transmitted microbiome represented a very large fraction of the seed microbiome further emphasizes the ecological role of seeds as a reservoir and source for community assembly in new seedlings** (Shade et al., 2017).... Although the fungal and bacterial community in the phyllosphere reflected the community present in the embryo, only part of the microbes present in the embryo and pericarp were identified in the roots of the developing seedling. As a result, **the microbial community of the phyllosphere differed significantly from the root community, despite originating from the same source**. These differences could be due to a combination of microbial life-history traits and plant regulatory factors that limit the migration of some members of the seed microbiome into the roots. **Whether direct or indirect, plant-mediated or microbe-mediated, results of the present study indicate that the distinct microbial communities commonly reported between above- and below-ground plant parts in natural and agricultural studies may originate during the transmission of microbes from seed to seedling, and not, as commonly assumed, due to inherent differences in the microbial communities in the soil and air**. Such initial seed-borne differences among the below- and above- ground plant tissues may cause strong priority effects during later development. Our demonstration of partial transmission of the seed microbiome to the roots confirms previous work suggesting that plant seeds are a repository for rhizosphere microbial communities (Kong et al., 2019), and **the high resemblance of the embryonic and phyllosphere microbiome indicates that seeds may play an even larger role as a repository for the phyllosphere microbial community.**

... We chose to focus on the **core microbiome** in an attempt to identify those taxa that have a consistent presence in the acorn, and that were consistently transmitted to the developing seedling. Such core taxa are expected to play a significant functional role in the respective compartments of the **holobiont** (Vandenkoornhuyse et al., 2015). In this regard, several taxa were found to be **vertically transmitted** include taxa with **various functions such as the production of antimicrobial compounds, detoxification, nutrient uptake and growth-promoting activities**.... The collective data provide **clear evidence of vertical transmission from seed to seedling and highlight the important role of vertical transmission during the assembly of the plant microbiome.**

- 2022 - **OPINION: "From seed to seed: the role of microbial inheritance in the assembly of the plant microbiome"**, by Ahmed Abdelfattah et al., *Trends in Microbiology*.

Microbial inheritance in plants



EXCERPTS: Despite evidence that the microbiome extends host genetic and phenotypic traits, information on how the microbiome is transmitted and maintained across generations remains fragmented. For seed-bearing plants, **seeds harbor a distinct microbiome and play a unique role by linking one generation to the next**. Studies on microbial inheritance, a process we suggest including both vertical transmission and the subsequent migration of seed microorganisms to the new plant, thus become essential for our understanding of host evolutionary potential and **host-microbiome coevolution**. We propose dividing the inheritance process into **three stages: (i) plant to seed, (ii) seed dormancy, and (iii) seed to seedling**. We discuss the factors affecting the assembly of the microbiome during the three stages, highlight future research directions, and emphasize the implications of microbial inheritance for fundamental science and society.

... Spermatophytes (seed plants) have evolved as an adaptation to the terrestrial lifestyle during the late **Devonian period**. Seeds served as the plant's means of **survival during harsh periods, facilitated dispersal across the landscape, and provided plant embryos with the protection and nourishment necessary to withstand external disturbances, especially desiccation**. During the evolution of seed plants, plants formed symbiotic relationships with microorganisms, which facilitated their establishment on land and subsequent evolutionary divergence. Seeds themselves contain diverse microbial communities, which are present **within their tissues (seed endophytes) and on their surface (seed epiphytes)**. The existence and function of the seed microbiome, however, has remained unnoticed for decades. **The first suggestions regarding the presence of microorganisms in seeds came from studies that focused on pathogenic fungi, giving the impression that seeds free of microorganisms reflected higher seed quality. This gave rise to the widespread use of physical and chemical treatments of crop seeds.**

... Early evidence that seeds harbor beneficial microorganisms was described in a study on the symbiosis between endophytic fungi of the family Clavicipitaceae and tall fescue *Festuca arundinacea* [1994 and 2004]. These findings were pivotal in changing our view on seed-borne microorganisms and **altered the notion that seed sterilization processes would necessarily result in a healthier crop**. Since then, seed microbial communities — including fungi, bacteria, and archaea — have been repeatedly shown to be a reservoir of specialized microorganisms. **Currently it is evident that seeds contain mainly plant-beneficial microorganisms — many of which are involved, directly or indirectly, in fundamental physiological processes, such as seed dormancy and germination, environmental**

adaptation, resistance and tolerance against diseases, and growth promotion. Yet, compared to other plant compartments, such as the phyllosphere and rhizosphere, studies on the seed microbiome are lagging.

... Despite the variation in species richness among seeds belonging to different species, some microbial groups are commonly found in seeds, and are collectively referred to as **the seed core microbiome**. These include members of the orders Capnodiales and Pleosporales for fungi, Pseudomonadales and Enterobacteriales for bacteria, as well as very low abundances of the phyla Thaumarchaeota and Euryarchaeida for archaea. In a meta-study on 50 plant species, *Cladosporium perangustum*, *Alternaria* sp., *Pantoea agglomerans*, *Pseudomonas viridiflava*, and *Pseudomonas fluorescens* were found to be the dominant seed-associated taxa. When assessing microbial function through the perspective of the plant's fitness and survival, several functional characteristics were found to be congruent among seed endophytes. Those include **phosphorus solubilization, nitrogen fixation, antibiosis, acetoin secretion, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, the production of growth-promoting hormones such as indole acetic acid (IAA), siderophore production to transport iron inside the plant, as well as the ability to suppress growth of plant-pathogenic microorganisms**. Interestingly, both members and functions of the seed microbiota are typical for plant-beneficial microbes.

The transmission route of plant endophytes to seeds has been extensively reviewed and can be divided into two groups: sexual or asexual routes. The asexual route includes the vascular system and intracellular cavities connecting the maternal plant to the developing seed through the funiculus, chalaza, and micropyle. The sexual route exclusively includes microorganisms transmitted via male and female gametophytes, which subsequently colonize the embryo and endosperm. The transmission through gametophytes, however, is only hypothesized based on studies showing that flowers, pollen, and ovary contain microbial species similar to those found in seeds. Neither the actual transmission during sexual fertilization nor the subsequent fate and distribution of these microorganisms has been documented.... In the case of oak and melon, the authors showed that **embryonic tissues, including the cotyledons, harbored more diverse microbial communities compared to the seed coat.**

... A seed comprises the plant embryo, and, depending on the plant species, other compartments such as seed coat, endosperm, and perisperm. These compartments were previously shown to contain distinct communities of microorganisms, some of which can be transmitted to the developing seedling. A recent study on oak showed that seeds transmit a distinct community of microorganisms to the emerging seedling's phyllosphere and roots. This suggests that **the seed microbiome contains niche-specialized microbes that then migrate to their respective tissues.**

... From a natural perspective, the ecological consequences of inheritance of microorganisms are unexplored, and might turn out to be of major importance for our understanding of spatial and temporal variation in microbiome community structure, plant-microbe interactions, and plant fitness, with consequences for community dynamics and ecosystem functioning. From an evolutionary perspective, microbial inheritance is expected to play a role in evolutionary changes and speciation in both plants and microbes and affect host-symbiont coevolutionary dynamics. **From an applied conservation perspective, this offers new ways for the actual topic of ecosystem restoration and microbiome stewardship in preventing biodiversity loss.**

- 2023 - **Insights into the seed microbiome and its ecological significance in plant life**, by Aadil Farooq War et al., *Microbiological Research*.

EXCERPTS from "Introduction": ... To perpetuate their generation, seeds need to survive unfavourable conditions, sense their environment, and synchronize germination with favourable environmental conditions. **While seed coat, seed appendages, and endosperm play critical roles in seed biology and ecology, the hitherto often neglected seed microbiome is also an important determinant and driver of seed health and seed germination....** Seeds are not only carriers of the DNA for future generations but also act as a vector to transmit additional functionality in the form of microbes to the next plant generation. The **seed microbiome consists of a narrow range of microbial species**, which seems to have co-evolved with the host plant species and contributed to plant survival.... Although some information exists about the diversity of the seed microbiome, **its functional significance is yet to be fully understood.**

OTHER EXCERPTS: ... Most of the information about seed-borne microbes and their functional roles has come from culture-based studies on crop plants. **Since most of the seed-borne microbes are unculturable, these culture-based methods only document a fraction of the total microbial diversity** and hence the seed microbial catalogue remained underexplored and incomplete. **This limitation has been overcome due to advancements in molecular techniques** and metagenomic Next Generation Sequencing (NGS) which have helped in cataloguing the entire seed microbiome. It has also expanded our knowledge about the diversity of microbes living within seeds and on the seed surface, as well as their putative functional role in the host plants.

... Based on the review of relevant studies undertaken during the present study, **the fungal seed diversity is represented by 170 genera belonging to six phyla with the dominance of Ascomycota**. The fungi belonged mostly to the classes Dothideomycetes, Eurotiomycetes, Leotiomycetes, Pezizomycetes, and Sordariomycetes.... Fungi of the class Dothideomycetes predominate the fungal seed microbiome and **the most abundant genera** are *Alternaria*, *Aureobasidium*, *Cladosporium*, *Chaetomium*, *Epicoccum*, *Fusarium*, *Microdochium*, *Phaeosphaeria*, and *Xylaria*. It is noteworthy that arbuscular mycorrhizal fungi do not colonize seeds very often, except for a few *Glymeromycota* species which also have low prevalence. **Compared to the microbiome of other plant parts, only a small fraction of microbial taxa colonizes the seeds.**

... Fungal endophytes were traditionally believed to be only horizontally transmitted, but recent studies have shown **vertical transmission rates of over 90%**, for some fungal endophytes (Hodgson et al., 2014). Moreover, **vertical transmission is more effective in asexual fungi**. [Editor's note: My understanding is that *Fusarium torreyae* is asexual when sampled and studied in cankers.] ... Plants have the genetic tendency to accommodate those microbes in their microbiome that promote plant growth and are antagonistic towards soil-borne pathogens.... **The microbes present in pollen can be passed on directly through the gametes that finally end up in the embryo and endosperm.**

... Seed-associated microbes have been shown to promote the seedling establishment, growth and development of host plants in nutrient-poor soils and under stressful conditions through (1) optimization of nutrient uptake, assimilation through phosphate solubilization, siderophore production, ACC deaminase production and nitrogen fixation; (2) antagonism (e.g., biocontrol of phyto-pathogens) and competition (competitive exclusion of other plant species); (3) improved plant-water-soil relationship; (4) stimulation of plant metabolism (modulation of reactive oxygen species (ROS), auxin, cytokinin and gibberellin levels); and (5) tolerance to abiotic stresses, such as drought, salinity, and stress due to heavy metals. **Seed microbiome, therefore, improves the overall fitness of the host and provides it a competitive advantage over other plants, thereby influencing the outcome of competitive interactions** in a community and resultantly shaping the community assembly patterns.... In addition, beneficial seed-inhabiting microbes have **a profound role in mediating seed dormancy and preparing a suitable environment for germination by managing the hormone levels in the soil**.... The underlying mechanisms of germination enhancement are related to the alleviation of stressful conditions by **modulating seed hormone levels of gibberellic acid and cytokinins**. In addition to seed germination, root growth and drought tolerance under severe drought conditions are attributed to the seed microbiome.... Many plants experience high mortality at the seed stage because they are susceptible to predation by pathogens. The **seed microbiome can prevent seed pathogenic microbes** from degrading the seeds.... Further, seed-associated microbes may directly **produce secondary metabolites** or stimulate the production of secondary metabolites by producing elicitors. These secondary metabolites (such as ethylene, jasmonic acid, and salicylic acid) in turn **induce host defence against insects or pathogens** using induced systemic resistance (ISR) and systemic acquired resistance (SAR) pathways.

Given these growth-promoting roles of seed microbiome, plant species conservation needs a PARADIGM SHIFT from the present approach of plant-specific protocols to envisaging a role for microbiome in the species conservation programmes. It implies that a plant species cannot be conserved without conserving its integral seed microbiome (Berg and Raaijmakers, 2018).... **Laboratory experiments do not capture the complexity of seed-microbiome-plant interaction that exists in natural settings.**

• **2023 - REVIEW: "Uniting the Role of Endophytic Fungi Against Plant Pathogens and Their Interaction",** by Shazia Akram et al., *Journal of Fungi*.

EXCERPTS: **Endophytic fungi are used as the most common microbial biological control agents (MBCAs) against phytopathogens and are ubiquitous in all plant parts.** Most of the fungal species have roles against a variety of plant pathogens. Fungal endophytes provide different services to be used as pathogen control agents, using an important aspect in the form of enhanced plant growth and induced systemic resistance, produce a variety of antifungal secondary metabolites (lipopeptides, antibiotics and enzymes) through colonization, and compete with other pathogenic microorganisms for growth factors (space and nutrients).

... The plant can also induce the different genome expression in various endophytic microbes during their colonization. The progress of **symbiosis research** shows that through nutritional monitoring, plants can identify whether invading microorganisms are beneficial or pathogenic. The genotype of the host plant also significantly affects the endosphere microbiome community in host plants. In some endophytes, in addition to local abiotic stress factors, it has also been found that the change of their lifestyle to a pathogenic state also depends on the genotype of the host (i.e., *Ramularia colloocygni* can survive as an asymptomatic endophyte during the initial growth period, but in the later growth period, it switches to necrotic pathogen). At the ecological time scale, *Fusarium verticillioides* in maize plant can survive as endophytes or later become a **latent pathogen** that causes disease over a period of time. However, **external and endogenous factors that cause fungi to transform from endophytes to pathogens are not yet fully understood.** In order to better understand the dynamics of endophytes, comparative studies are needed to find the gene expression and conditions (in plants and endophytes) in which the same microorganisms behave as symbionts or pathogens. However, for endogenous lifestyles, a single mechanism or factor has not yet been determined, so further discoveries are needed.

• 2024 - **"Vertical transfer and functional characterization of cotton seed core microbiome"**, by Chongdie Wu et al., *Frontiers in Microbiology*.

CONCLUSION: This study provides an extensive perspective on **microbiome dynamics within cotton seeds throughout their growth and developmental cycles** by employing amplicon-based microbiome analysis. This revealed the **efficient migration of seed-derived bacteria into both aboveground (e.g., stems and leaves) and belowground (e.g., roots) plant structures**. The identification of core microorganisms intimately associated with the interior of cotton seeds, pivotal network nodes, and **core microbiome that underwent vertical transmission** provides a theoretical foundation for microbiome regulation aimed at enhancing plant adaptation....

OTHER EXCERPTS: This study aimed to comprehensively analyze the diversity, structure, and functional properties of microbiomes inhabiting different microhabitats within cotton (*Gossypium* spp.). **These microorganisms engage in diverse beneficial interactions with host plants, including mutualistic symbiosis, co-evolution, and benign parasitism** (Hardoim et al., 2008; Kaga et al., 2009). In addition, host plants can transfer beneficial endophytic microorganisms vertically to their progenies.... Our findings revealed that **microhabitats exerted a more substantial influence on microbiome diversity than genotypes**. This is consistent with prior research, such as the study of rice microbiome, which identified microhabitats as the primary determinant rather than environmental factors or host genotypes.... Analysis of microbiome diversity and network interactions revealed a **significant decrease in both microbiome diversity and network complexity from roots, stems, and leaves to seeds**. This trend may suggest a selective effect by the plant host, preferring to vertically transmit beneficial microorganisms to its progeny.... By comparing the microbiome composition of seed-P [parent] and seeds, we revealed the dynamics of **vertical transmission** of microbiomes within the cotton lifecycle. This finding suggests that **the adaptability and evolution of microbiomes are not only related to the genetic traits of the plant itself but may also be significantly influenced by external environmental factors**.... The results of this study offer a comprehensive and validated body of knowledge, enhancing our understanding of the vertical transfer of microbiomes within seeds and shedding light on the co-evolutionary dynamics between hosts and microorganisms.... **The core endophytic microbiome of seeds can engage in co-evolutionary processes with their host plants over the course of the plant life cycle and develop efficient dispersal mechanisms across multiple generations.**

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PART C

Applying the New Paradigms to the Florida *Torreya* Tree

Note: This section entails 5 titled subsections, each chronological:

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C2: Background: *Fusarium torreyae* as focal pathogen

C3: Background: Recent papers on genus *Fusarium*

C4: New paradigm papers applied to genus *Fusarium*

C5: New paradigm implications for managing Florida *Torreya*

C6: RECOMMENDATIONS for the 2025 RECOVERY PLAN UPDATE

PART C1

Background: Early analyses of Florida *torreya*'s rapid decline

- 2010 - **Florida *Torreya* Recovery Plan (2010 update)**, by U.S. Fish & Wildlife Service.

EXCERPTS (pp. 5-6): **Identify pathogen(s) responsible for the decline.** This is an ongoing action that goes back to 1967 (Alfieri et al. 1967). The authors observed that the **stem and needle blight disease** of *T. taxifolia* appears to be incited by a **fungus causal agent** implicating *Physalospora* and *Macrophoma*. Alfieri et al. (1987) isolated **six other fungi** from leaves and stems of *T. taxifolia*. Lee et al. (1995) **isolated more than 30 different endophytic fungi**, but consistently, *Pestalotiopsis microspora* (a fungus that **resides in the inner bark of symptomless *T. taxifolia* trees**) was found on diseased trees. They concluded that **the pathological activity of this fungus could be triggered by physiological and/or environmental stress**.

Herman and Schwartz (1997) studied the pathogenicity of *Scytalidium* sp. They inoculated both needles and stems causing needle spots and necrosis, but **couldn't provide evidence that it was the cause of the original decline of *T. taxifolia***. Soil-borne pathogens, such as *Phytophthora* sp., *Pythium* sp., *Rhizoctonia solani* and *Sclerotium rolfsii* have been reported on *T. taxifolia*. At present, Dr. Lydia Rivera (Univ. of Puerto Rico, 2009) is conducting a soil-borne pathogen survey, emphasizing the detection of *Phytophthora* spp. She isolated 102 fungi from TSP, FL, and Corps property, GA. Of the trees surveyed, 48% had root necrosis and stem cankers. She is **designing a pathogenicity test associated with potential disease outbreaks**.

Dr. Jason Smith (Univ. of Florida) is conducting an above-ground plant pathogen study. **He isolated numerous fungi from cankers and consistently found an undescribed *Fusarium* sp.**; he is working with a specialist in Japan to describe the new *Fusarium* species. According to Smith (2010, pers. comm.), **"inoculation experiments with seedlings and larger potted torreya's have provided ample evidence that *Fusarium* is the causal agent" for the current population decline**; the cause of the initial decline remains unknown. **When plants are inoculated with *Fusarium*, it leads to canker development, lesions, and mortality** (Smith 2010, pers. comm.). He proposes to elucidate the disease biology, as well as conductive epidemiological factors and treatment. Aaron Trulock is a graduate student under Smith and will be doing his research on the biology of canker disease of the *T. taxifolia*.

• **Note by Connie Barlow:** This 2010 official document was the first update since the original recovery plan of 1986. It provides a **useful summary of the search for ultimate and proximate causes of the severe decline of this species**. Close reading of the third paragraph excerpted above can be interpreted in a variety of ways. From my 2023 standpoint of how subsequent naming of the then-undescribed *Fusarium* sp. has affected recovery actions, several aspects are important to reflect on before delving into the published *Fusarium* papers.

The staff writer of this 2010 federal document followed standard scientific guidelines in presenting Smith's pre-publication statement as a direct quotation, and as a personal communication. Prior to peer-review and publication in a journal, it would be imprudent to present as factual the statement that there is "ample evidence" that a *Fusarium* species distinct from those already known and described is actually "the causal agent" for the current population decline. This would be a powerful finding — and **it would immediately require a rethinking of recovery actions from the standpoint of risk**, such as:

- (1) Is this *Fusarium* an EXOTIC that has arrived from another continent? If so, **could it spread to other native plant species? And how far has it spread already?**
- (2) If this *Fusarium* is NATIVE, **why did its disease potency suddenly ramp up** and on just one species occupying a tiny range?
- (3) **Whether exotic of native**, just HOW LARGE is its current RANGE?
- (4) Could it be PATHOGENIC TO OTHER NATIVE PLANTS within the **ecological conditions of their own native ranges and habitat profiles?**
- (5) Is it already ENDOPHYTIC or EPIPHYTIC in/on other plant species, but expressing pathogenically (as a "**latent pathogen**") only where the plants are **climatically stressed?**
- (6) If it is **already living in northward plantings of Florida torreya**, but not expressing pathogenically in those, should the RECOVERY PLAN be modified such that "experimental populations" in such cooler climates can be initiated, in accordance with the **NEW 2023 REGULATION** that eliminated "historical range" as a constraint for planting sites?
- (7) Overall, how should the RISK of **botanical garden ex situ plantings** be regarded in northern Georgia, and what about the **assisted migration projects ongoing by citizens** to locations even farther north?

The above are the practical **PRE-PARADIGM SHIFT QUESTIONS** that can be set aside as overly risk-averse once one evaluates management of this endangered species from the stance of the **PLANT AND SEED MICROBIOME discoveries**.

- 2010 - "**The Decline of Florida Torreya: An Endemic Conifer on the Edge of Extinction**", by Jason A. Smith and Aaron Trulock, University of Florida School of Forest Resources and Conservation, research paper.

EXCERPTS: Considered a common tree in its restricted habitat until just before WWII, by 1962 Florida torreya had declined so severely that the species was considered to be destined for extinction (Godfrey and Kurz, 1962). **The decline of Florida torreya was first observed around 1938 (Alfieri et al., 1967). By the 1960s, no adult individuals could be found and the outlook for the species seemed very bleak (Alfieri et al., 1967). The rapid decline of the species was then attributed to an unknown fungal disease** because of the abundance of leaf spots and stem cankers and the rapid nature of the decline (Godfrey and Kurz, 1962). Since then, Florida torreya has continued to decline and in addition to disease, has been subjected to changes in hydrology, forest structure, heavy browsing by deer and a loss of reproductive capability (Schwartz and Hermann, 1995). Despite these challenges, **Florida torreya stems which have been killed by disease often re-sprout from the stump in a manner reminiscent of American chestnut following chestnut blight, although seed reproduction has been non-existent for decades in the wild** (Schwartz and Hermann, 1999). Estimates show Florida torreya has declined 99% since pre-settlement population levels, from an estimated population of 357,500 individuals in 1914 to approximately 1,350 in the 1990s (Schwartz et al., 2000). It is believed that the population has declined further since 2000 to current estimates of 400-600 individuals (T. Spector, unpublished data, 2010).

Despite several attempts to conclusively determine the causal agent responsible,

disease etiology has not been previously elucidated (Alfieri et al., 1967; Alfieri et al., 1987; El-Gholl, 1985; Lee et al., 1995; and Schwartz et al., 1996). In the first pathology studies conducted on *T. taxifolia* (Alfieri et al., 1967), it was noted that disease symptoms of leaf spots, needle necrosis, defoliation and stem lesions were common on native and cultivated *T. taxifolia*. Several pathogens were isolated commonly from symptomatic needles (*Macrophoma* sp., *Rhizoctonia solani*, *Sphaeropsis* sp. and *Sclerotium rolfsii*), however, no pathogens were isolated from cankered stems and Koch's postulates (proof of pathogenicity) were not demonstrated.

About 20 years later, El-Gholl (1985) implicated *Fusarium lateritium* as a causal agent by demonstrating this species' capacity to cause leaf spots, however, the causal agent of the canker disease remained unknown. Alfieri et al. (1987) completed more pathogenicity studies with a *Phyllosticta* sp., *Xylocoremium flabelliforme* and *F. lateritium*. They also completed Koch's postulates with *F. lateritium* as a leaf spot pathogen, but the canker-causing organism remained elusive. **In 1991, Schwartz et al. implicated *Pestalotiopsis microspora* as the causal agent of the canker disease**, having isolated the pathogen from 56 symptomatic plants and completed Koch's postulates on 10 stems. However, no information was given on the canker development, morphology or ability to cause mortality. Typically ***Pestalotiopsis* spp. are considered opportunistic pathogens** (Sinclair, 2005). Lee et al. (1995) investigated the endophytic and pathogenic chemical attributes of *P. microspora* infection and artificial inoculations resulted in stem canker development, however, again no stem mortality was observed.

Subsequent studies by Hermann and Schwartz (1997) implicated a *Scytalidium* sp. due to frequent isolation from cultivated and naturally occurring Florida torreya. Inoculation attempts led to small lesions on needles, but cankers were not observed.

In addition to biotic causes of decline, researchers have looked into changes in soils, drought, **global warming**, sunlight exposure and fire regime as possible causes of decline (Schwartz et al., 1995). Some of these environmental changes are thought to have occurred because of the building of the **Woodruff Dam along the Apalachicola River in 1957** (Schwartz et al., 1995), and changing land uses in the surrounding areas. However, **none of these environmental hypotheses have been demonstrated as a cause of the decline. The rapid nature of the decline during the period of 1938 to 1945 and numerous observations of disease symptoms provides ample evidence that a pathogen, possibly non-native, was involved** (Schwartz et al., 1995).

• **Note by Connie Barlow:** This document is a helpful historical summary. It was not, however, published in a journal and it therefore may not have been peer-reviewed in the customary manner.

PART C2

Background: *Fusarium torreyae* as focal pathogen

- 2010 - **REVIEW: "Biogeography and Phylogeography of *Fusarium*"**, by Brett A. Summerell et al., *Fungal Diversity*.

• **Note by Connie Barlow:** This document was published one year before the 2011 first paper on a "novel fusarium" being identified as a canker cause on Florida Torreya. **As a review paper, it is helpful for putting in context (a) the recent upsurge in designating and naming more species within the large and global *Fusarium* genus complex, (b) demonstrating how much more difficult it is for definitive standards to be developed and agreed upon for this fungal genus, especially compared to taxonomic standards familiar to professional plant or animal biologists, and (c) revealing that the *Fusarium torreyae* discovery (2011) and naming (2013) papers, which are excerpted below, both entail a coauthor, Kerry O'Donnell, who had already published a number of papers leading the movement to split the large *Fusarium* genus into more species.** In contrast, the authors of this 2010 review paper urge caution against the surge in designating new species within the *Fusarium* genus, especially if (1) isolates are few and (2) the isolates used for discerning taxonomic relationships derive from samples taken from agricultural rather than

natural settings. As you will see, the 2013 *Fusarium torreyae* paper adheres to neither of these two precautions.

EXCERPTS: As genetic and molecular techniques have become more sophisticated, more widely available and more commonly applied, **formerly functional species concepts, definitions and relationships are being stretched, with numerous new species being described. The species concept for *Fusarium* depends to at least some degree on those describing the species, and there is no universally accepted rule or guideline currently followed across the entire *Fusarium* research community.** The *Dictionary of Fungi* (Ainsworth et al. 2001) notes that **500 species of *Fusarium*** have been reported, although the number of common and well-described species is considerably smaller. The number of new species and their documentation should continue to increase as new ecosystems are explored and as the broad species concepts for some species are redefined more precisely. Thus, a continuation of the ongoing reassessment of *Fusarium* species definitions and their boundaries is needed to accurately define existing species, and to ensure that all fungi with *Fusarium* names warrant inclusion within the genus.

Morphological species concepts: Defining a species depends on differences in morphology between species, i.e., the species look different (Mayr 1963). This approach has been used by fungal taxonomists for over 200 years, is well known and understood, and is supported by extensive technical literature....

Biological species concepts: The biological species concept as put forth by Mayr (1940; 1963) considers "... species as groups of populations that actually or potentially interbreed with each other." **There are practical problems in applying a biological species concept to many *Fusarium* species, as many strains of *Fusarium* are asexual and only rarely, if ever, produce a sexual stage, even under laboratory conditions.** Nonetheless, the biological species concept has been used quite profitably for some groups, most notably the *Gibberella fujikuroi* complex (Leslie 1995).

Phylogenetic species concepts: The phylogenetic species concept (Taylor et al. 2000) is an even more recent development in mycology that can clarify many taxonomic difficulties, but if inappropriately applied or misinterpreted can lead to confusion. This species concept is especially useful for species that do not regularly produce a sexual stage. Phylogenetic species concepts utilize multiple markers, usually differences in DNA sequences of selected genes, and can yield quantitative measures of genetic relatedness. When working with phylogenetic species concepts alone, a common problem is where to draw the line between "species", i.e. "How different must two strains be to belong to different taxa?" In practice, many fungal phylogenetic studies rely on DNA sequences of one or two loci, from one or a few representative or well-characterized isolates. **This process can result in problems that are best avoided by ensuring that enough loci and enough individuals are studied to obtain an accurate picture of the variation within the species while remaining true to the original definition of phylogenetic species, which relies on populations rather than individuals as the analytical unit....** We believe that before such groups are raised to species level solely on the basis of sequence differences that additional techniques that scan the genome for differences, or even better, that use a distinct species concept, should be employed to verify the existence and the significance of the observed sequence differences.

Biogeographic concepts in the origin of *Fusarium*: With the available evidence, it is difficult to identify a centre of origin for *Fusarium*. Members of the genus have been **found in virtually all environments and are dispersed globally.** The genus is thus likely to be ancient and could have appeared relatively early in the evolution of the ascomycetes. The diversity of the morphological and phylogenetic traits across the genus is consistent with this hypothesis. Taylor and Berbee (2006) calibrated a molecular clock for key groups of fungi, including *Fusarium*. Depending on the taxonomic group chosen as the calibration point **the minimum age for *Fusarium* could be 110, 250 or 420 million years ago. All of these dates precede the major splitting and creation of the current land masses and, as such, provide support for the diversity and early spread of *Fusarium* species to all types of ecosystems on a global basis.** Thus, climate and host(s) are likely to be informative about the origin of various *Fusarium* species as the geographic location from which they can be recovered.

Dispersal of *Fusarium*: There are almost as many dispersal processes in *Fusarium* as there are species, including human transport, which makes generalizations regarding dispersal difficult. Certainly there are long distance dispersal processes known today (Schmale et al.

2006) that could have contributed to the widespread dispersal of various members of the genus across millennia. When combined with anthropogenic dispersal of host plants and their associated products, e.g., soil, there is more than sufficient long-range movement to effectively mask much of the underlying biogeography of the genus. Long-range movement of *Fusarium* falls into three broad categories: air dispersal, spread in soil, and distribution with infected host plants.... Backhouse et al. (2001) argued that there are **four different types of distribution patterns found in species of *Fusarium*: cosmopolitan, host-associated, climate-mediated, and anthropogenic.**

Phylogeography of plant pathogenic species of *Fusarium*: Tracking form species often has been done to search for geographic origins of various pathogenic strains, e.g. *F. oxysporum* f. sp. *radicis-lycopersici*, which may have been imported to the United States from Israel (Rosewich et al. 1999) or *F. oxysporum* f. sp. *cubense* race 1 which appears to have been circulated worldwide with banana cuttings (Ploetz 1990). Without supporting evidence of the genetic identity or similarity of the strains involved, however, many of these reports are questionable. **Instead, the first query should be whether the form species pathotype in question might have arisen locally.**

Other exceptions to **the O'Donnell et al. biogeographic hypothesis** include *F. werrikimbe*, *F. succisae*, *F. bulbicola* and *F. sterilihyphosum*, all of which are associated with hosts that have proposed geographic origins that are outside the area predicted by the clade they are associated with (Adams 1955; Britz et al. 2002; Kvas et al. 2009; Nirenberg and O'Donnell 1998; Walsh et al. 2010). These exceptions have been **attributed to anthropological dispersal of economically important plants and host jumps by the fungi (O'Donnell et al. 1998a), but also may be an artefact of the small number of available isolates of these species, which may not accurately reflect the primary geographic range or the most important host substrate.** The source and manner in which the fungal isolates were recovered has a significant impact on the hypotheses that are developed based on them. If the isolates evaluated are primarily collected from agricultural ecosystems, then any biogeographic information may be masked by the anthropogenic distribution patterns caused by the distribution of agricultural germplasm. Consequently, artefacts will arise in and confuse the distribution patterns. **We have long argued that it would be more appropriate to define biogeographical clades that attempt to explain the evolution of species within the genus based on isolates derived from natural ecosystems.**

CONCLUDING REMARKS: Biogeographic surveys of non agro-ecosystems continue to prove their importance in understanding the taxonomy and evolutionary history of *Fusarium*. The application of robust phylogenetic analysis to *Fusarium* populations from non agro-ecosystems will continue to improve our understanding of the evolution of this remarkable genus.

- 2011 - **"A Novel *Fusarium* Species Causes a Canker Disease of the Critically Endangered Conifer, *Torreya taxifolia*"**, by Jason A. Smith, **Kerry O'Donnell**, Lacey L. Mount, Keumchui Shin, Kelly Peacock, Aaron Trulock, Tova Spector, Jenny Cruse-Sanders, and Ron Determann, *Plant Disease*, 7 pp. with photos.

ABSTRACT EXCERPT: **A canker disease of Florida torreyia (*Torreya taxifolia*)** has been implicated in the decline of this critically endangered species in its native range of northern Florida and southeastern Georgia. In surveys of eight Florida torreyia sites, **cankers were present on all dead trees and 71 to 100% of living trees**, suggesting that a fungal pathogen might be the causal agent. To identify the causal agent, nuclear ribosomal internal transcribed spacer region (ITS rDNA) sequences were determined for 115 fungi isolated from cankers on 46 symptomatic trees sampled at three sites in northern Florida. BLASTn searches of the GenBank nucleotide database, using the ITS rDNA sequences as the query, indicated that **a novel *Fusarium* species might be the etiological agent...**

[Final paragraph]: Florida torreyia faces numerous challenges to its future survival in its natural habitat. In addition to the canker disease, deer routinely cause damage to stems from antler rubbing. It is unclear whether they are attracted by the tree's aroma or seek out Florida torreyia for some other unknown reason. Whether the wounds caused by deer serve as infection courts for pathogens, including the new *Fusarium* sp. (Fsp-1), is unclear and warrants further study. Additionally, since lesions on the larger plants in IE3 resulted in less stem girdling and no mortality, **the host response to infection, particularly under different stress conditions, needs to be investigated.** In addition to more research on the biology and management of

CDFT, **more work is needed to assess the various factors involved in decline of Florida torreya and how the species can be protected from extinction.**

- 2012 - **"Host Range and Biology of *Fusarium torreyae*, Causal Agent of Canker Disease of Florida Torreya**, by Aaron J. Trulock, 55 pp., (master of science thesis, University of Florida).

ABSTRACT: Florida torreya is an endangered, endemic conifer with a limited range near the Apalachicola River. The species began to decline in the 1950's and the population has plummeted from an estimated 375,000 to approximately 1,000. Since 1967 many investigations have tried to determine the cause, including pathological and environmental factors. The **Torreya Guardians propose recovering the species through assisted migration, where the species would be "re-introduced" into the southern Appalachian Mountains, the potential "historic" range of Florida torreya.** In 2010 a previously unknown pathogen was discovered on Florida torreya, which causes stem cankers and stem girdling. **The potential host range was investigated via artificial inoculations. Species tested included conifers whose range overlaps with Florida torreya, other species from the Torreya genus, and conifers from the southern Appalachian Mountains.** One species with an overlapping range, Florida yew (*Taxus floridana*); two other *Torreya* species, California torreya (*Torreya californica*) and Chinese nutmeg yew (*Torreya grandis*), and five species from the Appalachian Mountains, Fraser fir (*Abies fraseri*), Red spruce (*Picea rubens*), White pine (*Pinus strobus*), Table mountain pine (*Pinus pungens*), and Eastern hemlock (*Tsuga canadensis*) were found to be potentially susceptible. The effect of temperature on the growth, sporulation, and spore dissemination was tested, also. Growth was maximized at 25 C, sporulation was maximized at 20 C, however, there was no significant difference in spore dissemination among the temperatures tested. The necessity of wounds for infection was investigated; it was found that ***F. torreyae* cannot infect leaf or stem tissue without the presence of wounds** FINAL PARAGRAPH: *Fusarium torreyae* appears to have the ability to complete its lifecycle if moved to a new, cooler location and would not be limited by the new environment. **The movement of infected *T. taxifolia* plants into the southern Appalachian Mountains may spread this pathogen to a new area, to stressed, injured susceptible hosts and create a new epidemic. What is the value in moving one species to potentially imperil others?**

• **Note by Connie Barlow** - Although this paper is not peer-reviewed, p. 18 and the final two paragraphs (pp. 46-47) have been used as a reputable source for **implicating northward plantings of Florida torreya by Torreya Guardians as dangerous for possibly spreading *Fusarium torreyae* to several Appalachian native trees, including Fraser Fir and Eastern Hemlock.** Because this warning is severe, it would be useful for the experiments leading to this conclusion to be published in a peer-reviewed journal, and thus with their methodologies made clear and deemed acceptable.

This reviewer judges **the laboratory parameters as ecologically unsound:** Trulock apparently discovered that the *Fusarium* "grows well and sporulates in the average summer and fall temperatures" of the southern Appalachians, but there is no mention as to whether fungal population dieback would occur in the kinds of severe winter conditions recurrent in mountain locations. Thus **there is no mention of testing the *Fusarium* under ecologically natural conditions of the two high-altitude tree species of the southern Appalachians, rather than short-term laboratory conditions in Gainesville, Florida.**

Importantly, **this paper was the first published statement that a significant pathogenic risk was associated with translocating Torreya plant specimens or tissues geographically.** A "novel *Fusarium*" (later named *Fusarium torreyae*) was first published as the definitive lethal disease the prior year (see the above, 2011, publication). **Prior to Torreya Guardians' first reception of seeds from the Biltmore Gardens ca 2005, movement had been routinely conducted by agents associated with the official USF&WS recovery plan, beginning in 1989.** No volunteer planter associated with Torreya Guardians has ever taken any material from the native site (Torreya State Park and surrounds) where canker expression is rampant on torreya stems. In contrast, **Atlanta Botanical Garden agents for many years have shuttled plant materials, tools, and boots**

directly between the diseased range and facilities in central Georgia. **It is unfair to isolate *Torreya* Guardians as having potentially moved the then-unknown *Fusarium*, without acknowledging the many years in which researchers, experimenters, and others might have unknowingly served as more direct vectors for northward movement of a variety of pathogens afflicting *Torreya taxifolia* in its native habitat.**

- 2012 - **"Somatic embryogenesis, plant regeneration, and cryopreservation for *Torreya taxifolia*, a highly endangered coniferous species"**, by X. Ma, K. Bucalo, R.O. Determann, J.M. Cruse-Sanders, and G.S. Pullman, *In Vitro Cellular & Developmental Biology - Plant*.

ABSTRACT EXCERPT: *Torreya taxifolia* Arn., an ancient evergreen tree, is on the brink of extinction from attack by a fungal disease, recently reported to be caused by a novel isolate of *Fusarium*. We report the development of a somatic embryogenesis tissue culture system that can be used for **cryogenic storage of *T. taxifolia* cultures and subsequent plant regeneration**.... Germination of somatic embryos ranged from 64 to 82 %. Embryogenic tissue cultures from 30 genotypes representing seed from six mother trees were cryopreserved, and culture recovery was demonstrated after freezing. In contrast to many other coniferous tree seeds, the measured water potential (-MPa) of *T. taxifolia* megagametophyte tissue rose greatly during seed after-ripening. Duplication of this rise in vitro allowed development of somatic embryos to the cotyledonary stage.

• **Note by Connie Barlow** - Because the large seed of *Torreya taxifolia* is "recalcitrant", it cannot be dried or stored for any length of time. Nor can it be freeze-dried. **The only proven method for long-term storage is by deep-freeze cryogenesis** — and not as entire seeds. Instead, the embryo must be removed and cryogenically preserved in pieces, which in turn **requires high-tech methods for restoring life back to the embryo pieces** and continuing their growth into seedlings. **This costly, professional storage method was necessary to develop when the two botanical gardens in charge of ex-situ groves in north Georgia continued to resist "assisted migration" poleward. Meanwhile, the groves had become so productive that TENS OF THOUSANDS OF SEEDS were annually produced (yet left on the ground and not officially counted) for lack of approved destinations, combined with inability to store seeds in the usual dry-storage manner.**

- 2013 - **"Root and Soil-borne Oomycetes (*Heterokontophyta*) and Fungi Associated with the Endangered Conifer, *Torreya taxifolia* in Georgia and Florida"**, by Lydia I. Rivera Vargas and Vivian Negron-Ortiz, article in Vol 1 of a new journal titled *Life: The Excitement of Biology* (25 pp; Dr. Vivian Negron-Ortiz was USF&WS staff person and primary writer/signer of the 2010 and 2020 recovery plan updates.).

EXCERPTS and TABLES: ... **Several *Fusarium* species have been shown pathogenic to *T. taxifolia*, by causing needle spots, i.e. *F. lateritium*** Nees (El-Gholl 1985, Alfieri et al. 1987), and has been associated with root rot (Alfieri et al. 1984). **More recently, a novel described species *F. torreyae*** Aoki, Smith, Mount, Geiser, and O'Donnell, was demonstrated to be **the causal agent of stem cankers** (Smith et al. 2011). Until now, no *Fusarium* spp. has been demonstrated to cause cankers comparable to those observed in the field. In other conifer species, ***F. oxysporum*** Schltdl. Emend. Snyder and Hansen has been associated with seedling death and root rot (Viljoen et al. 1992).

... In this study, various *Fusarium* species (anamorph of *Gibberella* spp.) were isolated in both locations from roots and associated to bark, plant litter and dying trees. From Georgia, *Fusarium oxysporum* was isolated from roots and plant litter. This species has been associated with seedling death and with root rot in *Pinus* and *Eucalyptus* seedling in South Africa (Viljoen et al. 1992). ***Fusarium solani*** was also isolated from roots of dying trees at this site (Table 3). Based on macroconidia and chlamydospores morphology, none *F. torreyae* was isolated during this study (Aoki et al. 2013). **Recently this species have been shown to cause *Torreya* canker disease in Florida (Smith et al. 2011).** DNA analysis of the ITS rDNA region showed that *Fusarium* spp. isolated during this study were closed to *F. subglutinans*, *F. oxysporum* and *Gibberella* spp. clades (Figure 5). However, amplification of other more informative genetic

regions such as RNA polymerase largest subunit (RPB1 and RPB2) was not employed during this study....

Table 1. Fungi previously reported associated with *Torreya* spp.

Species of Fungi	Symptoms	References
<i>Alternaria</i> sp.	needle spot	Alfieri et al. 1984
<i>Botryosphaeria</i> sp.	needle spot	Alfieri et al. 1984, Mount and Smith 2009
<i>Caeoma torreyae</i> Bonar, 1951	rust	Farr et al. 1995
<i>Diaporthe</i> sp.	associated to cankers	Mount and Smith 2010
<i>Diplodia natalensis</i> Pole-Evans, 1911	twig dieback	Alfieri et al. 1984
<i>Fusarium</i> sp.	root rot associated to cankers	Alfieri et al. 1984, Mount and Smith 2010
<i>Fusarium torreyae</i> Aoki, Smith, Mount, Geiser, and O'Donnell, 2013	canker	Smith et al. 2011, Aoki et al. 2013
<i>Fusarium lateritium</i> Nees, 1817	needle spot	El-Gholl 1985, Alfieri et al. 1987
<i>Hypoxylon</i> sp.	associated to cankers	Mount and Smith 2010
<i>Janetia bonarii</i> (M. B. Ellis) S. Hughes, 1983	associated to needles	Farr et al. 1995
<i>Macrophoma</i> sp.	needle and stem blight	Alfieri et al. 1984
<i>Lasiodiplodia theobromae</i> (Pat., 1892) Griffon and Maulb., 1909	associated to cankers	Mount and Smith 2010
<i>Pestalotiopsis microspora</i> (Speg., 1880) G. C. Zhao and N. Li, 1995	needle spots and stem cankers	Schwartz et al. 1996
<i>Phomopsis</i> sp.	associated to cankers	Mount and Smith 2010
<i>Phyllosticta</i> sp. ²	needle spot	Alfieri et al. 1987

Table 1. Fungi previously reported associated with *Torreya* spp.

Species of Fungi	Symptoms	References
<i>Phylospora</i> sp.	needle stem and twig blight	Alfieri et al. 1987
<i>Phytophthora cinnamomi</i> Rands, 1922	root rot	Alfieri et al. 1984
<i>Pythium</i> sp.	root rot	Alfieri et al. 1984
<i>Rhizoctonia solani</i> Kühn, 1858	root rot	Alfieri et al. 1984
<i>Sclerotium rolfsii</i> Sacc., 1911	southern blight	Alfieri et al. 1984
<i>Scytalidium</i> sp.	needle spot and necrosis	Hermann and Schwartz 1997
<i>Sphaeropsis</i> sp.	needle blight	Alfieri et al. 1984
<i>Sporidesmium fragillissimum</i> (Berk and M.A. Curtis, 1875) M. B. Ellis, 1958	associated needles	Farr et al. 1995
<i>Xylcoremium flabelliforme</i> ³ (Schwein., 1797) J. D. Rogers, 1984	associated to needles and stems	Alfieri et al. 1987

¹ Current name *Botryosphaeria rhodina* (Berk. and M. A. Curtis) Arx., 1970

² *Phyllosticta* sp., imperfect stage of *Guignardia* sp.

³ *Xylcoremium flabelliforme* is the imperfect stage of *Xylaria cubensis* (Mont.) Fr.

RESULTS AND DISCUSSION

Torreya taxifolia trees assessment

All *T. taxifolia* trees sampled showed moderate to severe degrees of decline (100% decline incidence) based on criteria such as poor development of trees, stunting and fragility (Figure 1, Table 2). In addition, feeder root necrosis and stem cankers were observed in 45.8 % of trees examined (Table 2). Disease severity was higher and trees were smaller with poor development at Florida sites, showing an average height of 89 cm, and an DBH of 5 cm compared to trees in Georgia's site (174 cm h and 10.6 cm DBH in average) (Figure 1, Table 2). In Florida sampling sites, soil samples were damped with higher humidity, compared to the soil at the Georgia site. Overall, four trees showed mycelial growth on cortex or on tree base (Figure 1). In Florida, the main trunk of two severe affected trees had cankers at the base of the tree.

- 2013 - "***Fusarium torreyae* sp. nov., a pathogen causing canker disease of Florida torreya (*Torreya taxifolia*), a critically endangered conifer restricted to northern Florida and southwestern Georgia**", by Takayuki Aoki, Jason A. Smith, Lacey L. Mount, David M Geiser, **Kerry O'Donnell**, *Mycologia*.

EXCERPTS (in which the *Fusarium* is named and distinguished from closest *Fusarium* relatives): **During a survey for pathogens of Florida torreya (*Torreya taxifolia*) in 2009, a novel *Fusarium* species was isolated from cankers** affecting this critically endangered conifer whose current range is restricted to northern Florida and southwestern Georgia. Published multilocus molecular phylogenetic analyses indicated that **this pathogen represented a genealogically exclusive, phylogenetically distinct species representing one of the earliest divergences within the *Gibberella* clade of *Fusarium*.** Furthermore, completion of Koch's postulates established that this novel species was **the causal agent of Florida torreya canker disease**. Here we formally describe this pathogen as a new species, ***Fusarium torreyae*.**

... The available data suggests these three fusaria can be distinguished by host range in that *F. torreyae* is known only from **Florida torreya** (Smith et al. 2011), *F. lunulosporum* has been isolated only from **grapefruit** (Gerlach and Nirenberg 1982) and **wheat** (Gert van Coller, A-L Boutigny, A Viljoen pers comm) in South Africa, and *F. tucumaniae* has been recovered only from **soybean** in Argentina and Brazil (Aoki et al. 2003, O'Donnell et al. 2010). **The identification of *F. torreyae* as *F. lateritium*** by El-Gholl (El-Gholl 1985) and subsequent confirmation by Paul E. Nelson (D. Geiser pers comm) illustrate **the daunting challenge presented by overly broad morphological concepts of fusaria**, especially when applied to species that produce only sporodochial conidia. **We speculate that the reported needle blight of Florida torreya induced by *F. lateritium* in a pathogenicity experiment (Alfieri et al. 1987) also can be attributed to *F. torreyae*;** however, no isolate from this study was accessioned so the identity of this pathogen cannot be verified. Other examples of misplaced *Fusarium* spp. pathogenic to trees are provided by the etiological agent of **coffee wilt disease** in Africa, *F. xylarioides* Steyaert, and **vascular wilt of pigeon pea** (*Cajanus cajan* (L.) Millsp.) in Africa and Asia caused by *F. udum* Butler. The latter two species were classified in section *Lateritium* by Booth (1971), but **molecular phylogenetic analyses** have clearly established that they are nested within the African clade of the *Gibberella fujikuroi* species complex (O'Donnell et al. 1998, Geiser et al. 2005, Lepoint et al. 2005)

... Most fusariologists included only fusaria that produce conidia that are mostly straight in ***F. lateritium*** (Wollenweber and Reinking 1935, Booth 1971, Gerlach and Nirenberg 1982, Leslie and Summerell 2006). However, Nelson et al. (1983) also included isolates from woody plants that produced macroconidia more curved in the midsection, which is consistent with his phenotypic identification of FRC L-212 (5 NRRL 54626 *F. torreyae*) as *F. lateritium*. **We speculate that El-Gholl's (1985) and Nelson's identification of FRC L212 *F. torreyae* as *F. lateritium* also was influenced by the fact that the latter species is broadly attributed to canker diseases on trees.** Our discovery that FRC L-212 is *F. torreyae*, and not *F. lateritium*, was made during a multilocus phylogenetic assessment of *F. lateritium* clade species accessioned in the FRC and the CBSKNAW Biodiversity Centre. As posited by Leslie and Summerell (2006), our preliminary assessment suggests this clade comprises at least 15 phylogenetically distinct species, not including *F. torreyae*, which was resolved as a distinct monotypic lineage. **Because the limited morphological characters produced by *F. torreyae* may pose difficulties in identifying it phenotypically**, we recommend conducting nucleotide BLAST queries of GenBank, Fusarium-ID (Geiser et al. 2004) or Fusarium MLST (O'Donnell et al. 2010), using portions of EF-1a, RPB1 or RPB2 gene sequences to obtain a definitive identification.

• **Notes by Connie Barlow:**

(1) This publication mentions a half-dozen *Fusarium* or other canker-causing pathogens that are all, with the exception of the Florida *Torreya* tree, **hosted on plants that are of food value**: a spice in China, cultivated grapefruit, wheat, a soybean cultivar in Argentina and Brazil, coffee bushes in Africa, and the pigeon pea that is cultivated in Africa and Asia. This indicates that **the majority of documented pathogenic species of genus *Fusarium* attack plants of commercial value**. Might one wonder: **How many *Fusarium* species exist in southeastern North America and sometimes cause cankers on trees and other native plants, but are undiscovered (or mislabelled as the early known *F. lateritium*) because no researcher has been funded to isolate them?** As well, **might *Fusarium torreyae* be found elsewhere in the southeastern USA, but has not been documented because of low virulence in regions where native plants are less stressed by a deteriorating climate?**

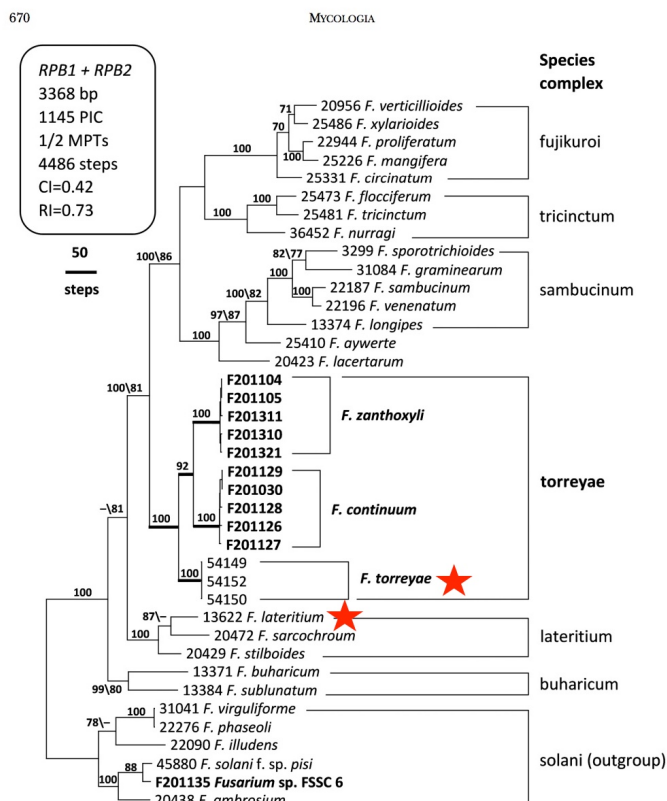
(2) In the "Comments" column of "Action Number 34" of the USF&WS matrix of **Record of Actions - Florida *Torreya***, a statement reports that a *Fusarium* canker has been found in the California *Torreya*. **Given that the *Fusarium torreyae* discovery and risk speculations have led to a shutting down of distribution of tens of thousands of seeds produced in the official ex situ orchards in n. Georgia, IT SHOULD BE A PRIORITY TO IMMEDIATELY IDENTIFY THE FUSARIUM ATTACKING CALIFORNIA TORREYA and then ecologically assessing whether upslope or northern populations show less or no damage from that pathogen.** The official USF&WS "Record of Actions" online document states:

"The recovery plan suggests grafting [asexual propagation where the tissues (vascular cambium) of one plant are fused with those of another] with *T. californica*. However, ***T. californica* is exhibiting some issues with cankers caused by pathogens with a different *Fusarium* species which is killing the cambium.**"

- 2016 - **"Two novel *Fusarium* species that cause canker disease of prickly ash (*Zanthoxylum bungeanum*) in northern China form a novel clade with *Fusarium torreyae*"**, by Zhou Xue, Kerry O'Donnell, Akayuki Aoki, Jason A. Smith, Matthe T. Kasson, Zhi-Min Cao, *Mycologia*.

EXCERPTS: **Canker disease** of prickly ash (*Zanthoxylum bungeanum*) has caused a decline in the production of this **economically important spice in northern China** in the past 25 y. To identify the etiological agent, 38 fungal isolates were recovered from symptomatic tissues from trees in five provinces in China. These isolates were identified by conducting BLASTN queries of NCBI GenBank and phylogenetic analyses of DNA sequence data ... Results of these analyses suggested that 30/38 isolates belonged to **two novel fusaria most closely related to the Florida torreya (*Torreya taxifolia* Arn.) pathogen, *Fusarium torreyae* in Florida and Georgia**. These three canker-inducing tree pathogens form a novel clade within *Fusarium* here designated the ***F. torreyae* species complex (FTOSC)**.

...Molecular clock estimates place the divergence of the FTOSC in the mid-Eocene, 40 Mya (O'Donnell et al. 2013), but **it remains an open question whether this clade first evolved in the Old or New World. Furthermore, it remains to be determined whether *F. torreyae* is native to North America and restricted to *T. taxifolia*.** Surveys for *F. torreyae* on *Torreya* endemic to China are warranted because it is the modern area of diversity of this genus (Li et al. 2001) and because the putative Asian origin of the CDZB pathogens could indicate that the most recent common ancestor of the FTOSC evolved in Asia.



EXCERPTS CONTINUE: ... **Several species of *Fusarium* are capable of causing cankers on woody plants, and mixed infections frequently occur.** For example, a particular FSSC species occasionally was found **co-occurring with *F. torreyae* in cankers on Florida torrey (Torreya taxifolia) (Smith et al. 2011).** Although both species could induce cankers, ***F. torreyae* is considered to be the primary pathogen due to increased virulence and consistent isolation from a large number of cankers.** By contrast the available data indicates the FSSC taxon should be regarded as an opportunistic (J. Smith pers comm).

• Notes by Connie Barlow:

(1) The figure above is published in the paper as "one of two most-parsimonious phylograms inferred from a combined RPB1-RPB2 dataset that strongly supported the monophyly of seven species complexes within *Fusarium*, including a novel clade of canker pathogens here designated the *F. torreyae* species complex...." **I added the two RED STARS to highlight how the "torreyae" complex is newly split off from the long-established "lateritium" complex (which was the species identification of the only canker-causing *Fusarium* listed in the initial, 1986, recovery plan and 2010 update prior to designation of *F. torreyae* as a separate species).** As well, the 2013 *Fusarium* paper, as excerpted above, concludes that the early *F. lateritium* complex was overly broad — presumably because "multilocus molecular phylogenetic analyses" had not been available in earlier years.

(2) A survey to determine whether a *Fusarium* is present and causes lethal cankers on the only other *Torreya* species in North America, **California Torreya (*Torreya californica*), is surely "warranted",** as well as the authors' suggestion that such an endeavor is warranted in China.

(3) The first sentence in this paper reports that "Canker disease of prickly ash ... has caused a decline in the production of this economically important spice in northern China." **Yet no suggestion is made that the decline in China owes to the canker being exotic; rather, the fusarium is presumed to be native. In contrast, instead of pondering that CLIMATE CHANGE IMPACTS ON A GLACIAL RELICT might be the most likely cause of a canker's rise to lethality ON JUST ONE PLANT SPECIES in Florida,** the naming of the canker as a new species, *Fusarium torreyae*, prompted speculation of an "exotic" origin as the most-mentioned ultimate cause of the stem

dieback. This, in turn, fomented **speculative fears of its spreading into northward tree species**. Even in 2023, when "climate change" is finally acknowledged in Endangered Species regulation, the exotic-origin presumption continues to impair access to ex situ seed sources for recovering Florida torreyia.

- 2017 - "Wilt, Crown, and Root Rot of Common Rose Mallow (*Hibiscus moscheutos*) Caused by a Novel *Fusarium* species", by S. L. Lupien, F. M. Dugan, K. M. Ward, and K. O'Donnell, *PLant Disease*.

EXCERPTS: ... the *Hibiscus* pathogen was nested within the *Fusarium buharicum* species complex (FBSC).... The FBSC was strongly supported as **sister to a clade comprising members of the *F. lateritium* and *F. torreyae* species complexes** (Fig. 3A to D), which are primarily canker-inducing tree pathogens (Smith et al. 2011; Zhou et al. 2016).

• **Note by Connie Barlow:** This paper is excerpted here because (a) the *Fusarium torreyae* species complex is mentioned in it and (b) by putting *F. torreyae* into a "species complex" that neighbors the *F. lateritium* species complex, this is yet another indication that **the *F. torreyae* isolates associated with Florida torreyia are a new name for what had previously been listed as the globally distributed *F. lateritium* pathogen affecting *Torreya taxifolia*.**

- 2018 - "Symptomatic Citrus trees reveal a new pathogenic lineage in *Fusarium* and two new *Neocosmospora* species", by M. Sandoval-Denis et al., *Persoonia*

ABSTRACT: The diversity of fusaria in symptomatic Citrus trees in Greece, Italy and Spain was evaluated using morphological and molecular multi-locus analyses ... **Three new *Fusarium* species are described** ... belonging to the newly described *F. citricola* species complex and the *F. fujikuroi* species complex...."

EXCERPTS: ... Currently, **more than 1,400 *Fusarium* names** are listed in the Index Fungorum and MycoBank databases.... **Positive ecological interactions between fusaria and *Citrus* spp. have been recorded for species formerly included in *Fusarium***, i.e., *Microcera coccophila* (Syn *Fusarium coccophilum*) and *Microcera larvarum* (Syn *Fusarium larvarum*), successfully employed as biocontrol agents against citrus fruit attacking armoured scales. While *Fusarium* taxonomy is actively changing, with numerous species being described each year mostly based in molecular phylogenetic approaches, **just a handful of studies deal with the distribution of *Fusarium* spp. in *Citrus*, and there is scant data for the Mediterranean basin**.... More studies are therefore needed on these new taxa in order to elucidate their host range, specificity, and global distribution, as well as their potential impact on the Citrus industry.

• **Note by Connie Barlow:** This is a short, technical paper in which *Fusarium torreyae* is one of the internationally typed strains that appears on a relational phylogram, but not among the isolates sampled from citrus trees along the Mediterranean. Importantly, the paper mentions that **some cohabiting species of genus *Fusarium* confer "positive ecological interactions" to their plant hosts — thus recognizing the PLANT MICROBIOME paradigm, rather than the earlier "latent pathogen" paradigm.**

- 2018 - "Heterothallic sexual reproduction in three canker-inducing tree pathogens within the *Fusarium torreyae* species complex", by Xue Zhou, Kerry O'Donnell, Hye-Seon Kim, Robert H. Proctor, Gail Oehring, Zhi-Min Cao, *Mycologia*.

EXCERPT of ABSTRACT: *Fusarium zanthoxyli* and *F. continuum* are sister taxa that are the etiological agents of canker disease of prickly ash (*Zanthoxylum bungeanum*) in northern China. These two pathogens, together with *F. torreyae*, the causal agent of canker disease of the critically endangered conifer **Florida torreyia (*Torreya taxifolia*)** from northern Florida and southwestern Georgia, constitute a novel clade, the *F. torreyae* species complex.... Genotyping

of 34 - 40 progeny from the *F. zanthoxyl* and *F. continuum* crosses confirmed that they were the products of sexual reproduction. However, only 36% of the progeny in the *F. torreyae* cross were recombinant, which was roughly half of the nonparental progeny expected with three markers segregating.

• **Note by Connie Barlow:** Notice that in 2018, *Fusarium torreyae* is still regarded as the lead species in a "complex" of closely related sister species of the genus in northern China that was delineated in the 2016 paper immediately above.

- 2019 - **"Antiplasmodial and Cytotoxic Cytochalasins from an Endophytic Fungus, *Nemania* sp. UM10M, Isolated from a Diseased *Torreya taxifolia* Leaf"**, by Mallika Kumarihamy et al., *Molecules*.

EXCERPTS: Plant-like metabolic pathways in apicoplasts serve as promising targets for antimalarial drug discovery. We have screened a number of plant pathogenic fungal extracts with phytotoxic activity for their antiplasmodial activity. Some of these extracts yielded compounds with good antiplasmodial activity. As part of this program, **we investigated endophytic fungi isolated from a diseased leaf of cultivated *Torreya taxifolia***. One of the fungal extracts showed potent phytotoxic activity and selective antiplasmodial activity. This fungus (UM10M) was identified as **a species of the genus *Nemania*** (Xylariaceae). Some members of this family have been identified as **endophytes and plant pathogens....** It showed weak suppressive activity but its high toxicity to animals would preclude it as a potential malaria drug lead.

• **Note by Connie Barlow:** This is yet another example of how *Torreya taxifolia* carries **non-pathogenic "endophytic" fungi as beneficial mutualisms** — in this case, coevolved in a way that suppresses a single-celled protozoan.

- 2019 - **Audio podcast interview of a botanical garden staff person.**

• **Note by Connie Barlow:** Although not a published document, this podcast is important for revealing the degree to which the *Fusarium torreyae* concern had constrained recovery actions: In November 2019 an audio interview with a primary institutional implementer of the Florida *Torreya* official recovery plan, **JENNIFER CESKA (State Botanical Garden of Georgia)**, indicated that **the fear of native conifer contamination in northward plantings** (including official ex situ plantings in north Georgia) had ramped up to a level that "if we do see a problem, we would remove and burn that material." Yet, century-old groves of Florida *Torreya* in the vicinity of **Asheville NC** and **Highlands NC** confirm that the original trees and their nearby offspring show no disease expression, nor do other conifers in their surrounds. (*Torreya* Guardians is unique in **documenting the existence and health of Florida *Torreya* planted outside of native historical range.**)

It is therefore clear that even credentialed and highly experienced staff people may not be familiar with recent papers on plant pathology that confirm **the ubiquity of benign and mutualistic expressions of known fungal pathogens when host plants are not suffering from environmental or other stresses.**

Links to the **"In Defense of Plants" audio podcast and a transcript of key excerpts** can be found in a November 2019 entry on the *Torreya* Guardians Reports webpage. For a lengthy argument on the importance of historic groves, see page 5 of the 26-page **"Petition to Downlist Florida *Torreya* to Threatend"** by Connie Barlow, September 2019.

EXCERPTS (JC = Jennifer Ceska):

28:31 JC: [Recent debates about whether "commercialization of endangered plants" was helpful for the plants] ... *Torreya* was the godchild; you could grow an

endangered tree in your garden and it's a beautiful horticulture tree ... that document never saw the light because **we learned from Jason Smith at the University of Florida [what the pathogen was]**.

30:03 JC: ... We thought we were taking the torreyia away from the disease; we thought this was in the soil. We thought it could be introduced or always been there. Well, no. **It's in the torreyia; it is part of torreyia. He named it; it's a fusarium named for torreyia. It's in all of its tissues; it's passed from mother to child. And if the tree is healthy, everything's fine; they live together. But if the tree is weak in some way horticulturally, then this fusarium can express and it can spread. And in the lab Jason Smith has learned, he's done inoculations in the lab, that it can jump in the lab to different plant families.**

30:48 JC: So we had these conversations in '95, these triple promises that we would track and monitor and check for disease and plant in cultivated areas. But we brought the disease up here. We brought the disease to our botanic gardens. **Now, our trees are healthy; that is something we are very careful to track. But that means we brought the disease up here. So, no: The Georgia Plant Conservation Alliance is not going to produce plant material or share plant material for production because we don't know.** There's so much we don't know about critically imperilled plants, and torreyia is the example that we refer to. So we have been on hold to that for a good fifteen years....

- 2020 - **"Detection method for *Fusarium torreyae* the canker pathogen of the critically endangered Florida torreyia, *Torreya taxifolia*"**, by Tyler J. Dreaden, Tania Quesada, Jason A. Smith, *Forest Pathology*.

EXCERPT OF ABSTRACT: ... Recently, **a canker disease caused by *Fusarium torreyae* was identified as the primary cause of Florida torreyia decline**. Efforts to restore and preserve the species in situ and ex situ are hampered by lack of pathogen-free planting stock, and there exists an interest in **methods to verify pathogen presence in seeds and seedlings prior to collection and transport for planting**. This paper presents a new species-specific diagnostic method that enables detection of *F. torreyae* and may allow for conservation programmes to ensure germplasm is free of the pathogen prior to planting.

EXCERPTS: **The pathogen that might have caused the rapid decline in the 1950s is not known, but the Florida torreyia is currently being affected by a canker disease caused by *F. torreyae*** and it is hypothesized that this disease might be responsible for the species' rapid decline (Aoki et al., 2013; Smith et al., 2011). **An ex situ conservation strategy was initiated with one cutting from each of 150 trees sampled and placed in four botanical gardens** (Schwartz, 1993). Ex situ collections are ongoing (Smith et al., 2011), with the Atlanta Botanical Garden (Atlanta, GA) continuing to acquire new accessions and maintaining a large collection. **Many of the botanical gardens were unable to maintain the collections, and most of the samples are currently found only at the Atlanta Botanical Garden.** There is therefore **a need to have ramets of these accessions at other locations to ensure their preservation. This is hampered by the necessity to ensure that the canker pathogen, *F. torreyae*, is not found in seed or seedlings that will be sent to the new locations where that pathogen is currently not found.** This study was undertaken to develop a rapid and inexpensive PCR-based method to identify *F. torreyae* from culture to help screen samples for the pathogen before propagules of *T. taxifolia* are moved to new locations.

... Current *T. taxifolia* conservation/restoration efforts include ex situ collections. While this will help maintain the genotypes, **the movement of the plants is a pathway for also spreading the canker pathogen to new locations**. The assay developed here can be used to screen *T. taxifolia* plants or seed before they are moved to new locations and thus **limit the spread of the damaging canker pathogen that could affect other hosts in new environments** (Trulock, 2013).

- **Notes by Connie Barlow:** Despite its publication in 2020, this paper appears vacant of any notion of the PLANT & SEED MICROBIOME PARADIGM SHIFTS. Because this paper is peer-

reviewed, **the prospect that the official recovery program for Florida *torreya* will ever entail establishment of an "experimental population" northward of Georgia is therefore remote.** Peer-review is the stamp of credibility — unless another peer-reviewed paper is published that refutes any of the statements implied as factual and/or the conclusions directly stated. Below I first point to the **two phrases that can supply reasons for arguing against any officially sanctioned translocation of seeds or seedlings northward of Georgia.** Second, I point to the use of a master's thesis (itself not peer-reviewed in a professional sense) that is the sole reference supporting the paper's final and most fear-laden sentence.

(1) In the paper's abstract, *Fusarium torreyae* is said to have been "identified as the **PRIMARY CAUSE of Florida *torreya* decline.**" Yet, in my own close reading of the series of *Fusarium* papers, there is no peer-reviewed paper that undergirds that statement. It is true that the Aoki et al. 2013 paper does use the phrase "primary pathogen" in referring to *F. torreyae*, but here is the full sentence: "Although both species could induce cankers, *F. torreyae* is considered to be the primary pathogen due to increased virulence and consistent isolation from a large number of cankers." Thus the context for the statement was which of two canker types is the primary pathogen — and that is a far more constrained quest than the search for **ULTIMATE CAUSE.** Thus, because Dreaden et al. use "primary" to modify the word "cause" rather than to modify "pathogen", **the long-standing quest to distinguish effective pathogens from a possibly different ultimate, or root, cause becomes unavailable for reader consideration.** Hence the prospect that **environmental change (notably, locally induced or regional CLIMATE CHANGE) could be the root cause** of a variety of previously identified sources of pathogenicity, including *Fusarium torreyae*, attacking a known "glacial relict" is not acknowledged.

(2) This is the only peer-reviewed *Fusarium torreyae* paper that cites a master's thesis (Trulock 2013) in its references. Notably, that is the only reference given to support the paper's final sentence, which reads: "The assay developed here can be used to screen *T. taxifolia* plants or seed before they are moved to new locations and thus limit the spread of the damaging canker pathogen that could affect other hosts in new environments (Trulock, 2013)." Aaron Trulock's short-term and lab-based research and speculations as presented in his thesis on the possibility of *F. torreyae* being able to injure or kill other native trees entails the only record of an empirical study on this topic to date. Normally, such risks for terrestrial plant hosts on the same continent are seriously considered only if the pathogen of concern has crossed an ocean or some intracontinental dispersal barrier. **A geographic threshold determination of nativity has not, however, been established for this newly distinguished species of the vast and global *Fusarium* genus.** At minimum, Trulock's speculations should have been balanced in this paper by drawing upon the ecologically based conclusions in the **2015 Gordon et al. paper** (excerpted in Part A above) as to the prevalence of *Fusarium* sp. that are both endophytic and non-symptomatic, unless the host is stressed.

- 2020 - **Florida Torreya Recovery Plan (2020 update)**, by U.S. Fish & Wildlife Service.

FULL TEXT of **"Ongoing Action: Identify pathogen(s) responsible for the decline"**, p.5.

2020: An ongoing project at ABG [Atlanta Botanical Garden] is focused on determining **whether the fungal pathogen** (see 2011, 2013 Smith & collaborators findings) **has infected outplanted material at Vogel State Park and Smithgall Woods State Park, GA and if it has moved into surrounding trees.** A preliminary inspection of **trees at Vogel State Park revealed fungal cankers on outplanted *T. taxifolia* material, and fungal infections on surrounding *Tsuga caroliniana* and *Carya* sp. Fungal material from these trees has yet to be sequenced to determine if the species infecting these trees is *Fusarium torreyae*.**

2019: **Kumarihamy et al. (2019)** investigated **endophytic fungi** isolated from a diseased leaf of cultivated *T. taxifolia*; compounds isolated showed potent in vitro activity against *P. falciparum* D6 and W2 strains (protozoan parasite that causes malaria).

2013: A **systematic survey** (soil-borne pathogen survey of roots, soil and plant litter

associated with *T. taxifolia*) was conducted from three sites at TSP, Florida, and one site in Decatur, Georgia. About 102 fungi were isolated: 27 isolates (26%) were from TSP and 75 (74%) from Georgia. All *T. taxifolia* trees sampled showed moderate to severe levels of decline; 48% had root necrosis and stem cankers. Composition of fungal community included plant pathogens, lignin and cellulose decomposers, endophytes and saprophytes.

2011, 2013: Smith et al. (2011) conducted an above-ground plant pathogen study. They **isolated numerous fungi from cankers and consistently found *Fusarium torreyae*** (Aoki et al. 2013), as possible etiological agent. According to the studies, when *Torreya* plants were inoculated with *F. torreyae*, it leads to canker development, lesions, and mortality. Dr. Smith's lab identified ***F. circinatum* in a canker on one of the lower branches of a permanently planted individual at the Bok Tower Garden** (Bok Garden; P. Lynch, Bok Garden, 6/03/2020, pers. comm.).

1997: Herman and Schwartz (1997) studied the pathogenicity of *Scytalidium sp.* They inoculated both needles and stems causing needle spots and necrosis, but **couldn't provide evidence that it was the cause of the original decline of *T. taxifolia*.**

1967: This ongoing action goes back to 1967 (Alfieri et al. 1967). The authors observed that **the stem and needle blight disease of *T. taxifolia* appears to be incited by a fungus causal agent implicating *Physalospora* and *Macrophoma*.** Alfieri et al. (1987) isolated **six other fungi from leaves and stems of *T. taxifolia*.** Lee et al. (1995) isolated **more than 30 different endophytic fungi**, but consistently, ***Pestalotiopsis microspora* (a fungus that resides in the inner bark of symptomless *T. taxifolia* trees) was found on diseased trees. They concluded that the pathological activity of this fungus could be triggered by physiological and/or environmental stress.**

• **Note by Connie Barlow:** The final two sentences in the above chronological list by USF&WS hark back to the paradigm that preceded the 2013 naming of *Fusarium torreyae*. The prevailing PARADIGM prior to 2013 was this: **While one or more fungal species is clearly the PROXIMATE cause of torreya stem dieback, the ULTIMATE cause is environmental stress of some sort that provokes benign endophytes to shift into a pathogenic phase.** Sadly, a lack of scientific interest (perhaps owing to a lack of funding?) to provide answers to **two crucial questions** will continue to support **RISK ANALYSES that privilege the fear of pathogen spread over any translocation form of climate adaptation** that could prevent extinction of this endangered tree. The two crucial questions that need to be explored and results then published in peer-reviewed papers are:

- (1) What is the full geographic extent of *Fusarium torreyae* range today?
- (2) At what geographic locales (northward and/or at higher altitudes) is *Fusarium torreyae* present but manifests as a benign and symptomless endophyte of Florida *Torreya* specimens and perhaps other native conifers?

- 2021 - **"Antimalarials and Phytotoxins from *Botryosphaeria dothidea* Identified from a Seed of Diseased *Torreya taxifolia*"**, by Mallika Kumarihamy and 8 coauthors (including **Edward M. Croom, Jr**, descendant of the EuroAmerican who first reported the existence of this tree, Hardy Bryan Croom), *Molecules*.

EXCERPTS: ... **As part of our program to search for new antimalarials from plant pathogenic fungi, we investigated FUNGI FROM SEEDS OF A DISEASED TORREYA TAXIFOLIA** Arnott. (Taxaceae).... **The decline of the native population during the recent past has been attributed to both abiotic and biotic causes, including fungal diseases.** Several fungi have been isolated from diseased *T. taxifolia* and some of them have been shown to cause **leaf spots and canker disease** in healthy plants. For this study, **SEEDS of *T. taxifolia* were collected from a tree with disease symptoms cultivated on the Biltmore Estate in Asheville, North Carolina.**

From fragments of a surface-sterilized SEED, several ENDOPHYTIC FUNGI were isolated. An EtOAc extract of the broth of one of these fungi grown in potato-dextrose liquid medium showed phytotoxic and antiplasmodial activities. This fungus (UM124) was identified as

Botryosphaeria dothidea (Botryosphaeriaceae) by DNA analysis. Members of the family Botryosphaeriaceae (Botryosphaerales, Ascomycota) **cause leaf spots, fruit and root rots, and cankers in a variety of hosts**, and *B. dothidea* has specifically been isolated from a large number of diseased and healthy woody plants, including many economically important crops. **A *Botryosphaeria* sp. strain has previously been isolated from *T. taxifolia* LEAVES infected with needle-spot disease.**

... This is the first report of the isolation of a fungus producing phytotoxins from the SEEDS of diseased *T. taxifolia*. *B. dothidea* might play a significant role in decreasing the population of the endangered *T. taxifolia*.... Dothilactaene B was isolated from the active fraction, which showed moderate in vitro antiplasmodial activity with high selectivity index. In spite of this activity, its instability and various other biological activities shown by related compounds would **preclude it from being a viable antimalarial lead.**

• **Note by Connie Barlow:** This is a highly technical paper, written by researchers intent on analyzing natural compounds as potential "natural products" to fight disease and instill health in humans. A Google Scholar search for technical papers related to the naming of a distinct *Fusarium* linked with canker disease in Florida *torreya* has taught me that **the evolutionary advantages for the plant itself that underly herbal remedies** is that plants will naturally form symbiotic relationships with coevolved species of fungi and bacteria in their above-ground tissues — just as they do with mycorrhizal fungi and bacterial shields in the soil (rhizosphere). This paper also helps me understand why **the processed seeds of cultivated *Torreya* species in Asia are used for medicinal purposes as well as food.**

Most important is that **THIS PAPER SHOULD REDUCE THE NOW-WIDESPREAD FEARS that distributing sanitized SEEDS from the ex situ "safeguarding" orchards in northern Georgia could "spread the disease" (specifically, *Fusarium torreyae*).** Rather, in accordance with many papers drawing upon the **SEED MICROBIOME paradigm shift**, we now know that (1) **endophytes coevolved beneficial mutual relationships with plant hosts** and (2) **endophytes found in healthy seeds almost certainly provide benefits to the host.** In this paper we have learned that the fungus *Botryosphaeria dothidea* produces metabolites that deter other fungi or bacteria from entering a seed. A crucial question then arises: **Might *Fusarium torreyae*, which is known to be benignly present in seeds as well as harmful when producing stem cankers, also produce a helpful metabolite at some stage and location when the plant is still healthy enough to produce seeds?**

THE NEED FOR CROSS-DISCIPLINARY EDUCATION OF CONSERVATION PROFESSIONALS: Botanists, ecologists, and conservation biologists who have great influence over the public perception of a disease-riddled endangered plant, and who determine the outcome of official recovery plan updates, will likely **continue to resist "assisted migration" northward of Florida *torreya* SEEDS** until a thorough review of (a) the pathology papers is undertaken, alongside (b) reading papers outside of plant pathology that review the new understandings of **PLANT & SEED MICROBIOMES**, and (c) followed by discussion amongst that range of professionals. I hope this compilation and excerpting of papers from both realms will encourage professionals to undertake such effort in behalf of this endangered tree.

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PART C3

Background: Recent papers on genus *Fusarium*

• 2011 - **REVIEW: "Fifty years of *Fusarium*: how could nine species have ever been enough?"**, by Brett Anthony Summerell & John F. Leslie, *Fungal Diversity*.

• **Note by Connie Barlow:** As with the **2010 review paper above** (by the same lead author, Summerell), excerpts here are primarily to inform readers that **the naming of new species within**

genus *Fusarium* entailed a controversial surge around the time that *Fusarium torreyae* was distinguished from *Fusarium lateritium* as a fungal type associated with stem cankers among the native population of Florida *Torreya*.

EXCERPTS:... The genus is well known for taxonomic difficulties and has been composed of anywhere between 9 and >1,000 species depending on the authority queried and the time when the question was asked. **Definition and identification of species remains problematic, with problems at both ends of the scale** — when are too many things grouped into a single species, e.g. *F. oxysporum* (e.g. Laurence et al. 2011) and *F. solani*, and when have the separations gone too far, e.g. the 13 phylogenetic species within *F. graminearum* (O'Donnell et al. 2000) or the separation of *F. brevicatenulatum* from *F. pseudoanthophilum* (Amata et al. 2010). Morphological characters often are indicative, but may not be definitive, while molecular markers need to be checked against other characters since where to draw the line between species when only molecular markers are used is not always clear (Summerell et al. 2003).

... *Fusarium* is a genus of ascomycete fungi first described by Link (1809) as *Fusisporium*. Members of the genus are numerous and can be recovered from plants and soil worldwide as pathogens, endophytes and saprobes (Summerell et al. 2010). **Members of the genus are notorious for their capabilities as plant pathogens, although work with native plants and soil in undisturbed areas suggests that the number of species not associated with known diseases may far outnumber those that cause disease.** Most members of the genus produce an array of secondary metabolites, which vary widely in chemical form (Desjardins 2006).

... the recognition of biological species and the development of DNA technology radically changed the way species concepts were viewed in *Fusarium*, and indeed in all fungi. Species concepts in *Fusarium* have been discussed in some depth (Leslie et al. 2001), and most current workers now use a combination of morphological, biological and phylogenetic markers in a polyphasic approach based on a relative weighting of the available data and markers.... **From 1998 onwards there was a proliferation of new species described;** this was a consequence of the recognition that a number of the traditional morpho-species are species complexes composed of many species combined with the exploration of both crops and **natural vegetation in poorly understood ecosystems.**

... *Fusarium* can cause various types of diseases, including vascular wilts, head and seed blights, stem rots, root and crown rots and **canker diseases**, with some species capable of simultaneously causing multiple or overlapping disease syndromes depending on the host and the environment.... Root and crown rots are probably the most widespread type of disease caused by *Fusarium* species. **In many cases the causal agents of these diseases may be difficult to determine, as they may occur in plants affected by other factors** that are simultaneously infected by weak pathogens or saprophytes.

... **Canker diseases involve the formation of lesions on stems and branches and most prominent on woody perennial species in horticulture and forestry.** Pathogens such as ***F. circinatum*, the cause of pitch canker in pine**, and *F. decemcellulare*, which causes cankers on tropical fruit trees, are good examples of the pathogens in this category.... **Chemical control measures have been spectacularly unsuccessful for *Fusarium* diseases** and there are very few fungicides currently available that could be described as economically effective control strategies for these diseases.

- 2012 - "**34. *Fusarium* Root and Stem Diseases**", by Robert L. James, pp. 117-120 in **USDA Forest Nursery Pests.**

- **Note by Connie Barlow** - Written from a fungal pathology perspective, this chapter is helpful for reporting the large number of *Fusarium* species listed as infesting tree seedling nurseries with various diseases, including root rot manifesting as foliage, needle tip, and twig dieback, along with stem necrosis.

EXCERPTS: *Fusarium* diseases are caused by several *Fusarium* species, especially *F. oxysporum*, *F. proliferatum*, *F. solani*, and several species within the 'roseum' complex, including *F. acuminatum*, *F. avenaceum*, *F. sambucinum*, *F. sporotrichioides*, and *F. equiseti*. Recent

evidence indicates that some highly virulent strains previously identified as *F. oxysporum*, should be classified as *F. commune*. **Although all conifer and many hardwood species may be affected, Douglas-fir, pines, larch, and true firs are most susceptible to *Fusarium* diseases.** Spruce is often damaged less and species of cedar and cypress are relatively resistant to *Fusarium* diseases.

... *Fusarium* species cause **seedling root decay, stem cankers (including hypocotyl rot), seed decay, and wilt** of some plant species within nurseries. Infected seedlings are often killed or have reduced growth and vigor. This pattern often results in increased numbers of culls and reduced survival after outplanting. Mortality is more common during the first growing season on bareroot stock and toward the end of the growth cycle on container seedlings. Losses can vary greatly depending on nursery management practices, seedling species, seedlot, geographic area, soil type, and environmental factors.

.... Many different plant seeds may be contaminated with *Fusarium* and may provide an important pathogen introduction source for nurseries.

- 2014 REVIEW PAPER, **"Systematics of key phytopathogenic *Fusarium* species: Current status and future challenges"**, by Takayuki Aoki, Kerry O'Donnell, and David M. Geiser, in *Journal of General Plant Pathology*

• **Note by Connie Barlow:** This is a crucial paper for learning about the **the exponential increase in taxonomic splitting of existing *Fusarium* species into many more species**. It is an important historical understanding, as it **can affect the degree to which one regards a newly described *Fusarium* as a possible non-native — and thus capable of spreading a possibly exotic plant disease** to other regions and other species if the Florida *Torreya* tree is experimentally planted in outlying regions where climate may be less stressful to its relictual character. Also, recognize that **this review paper is by three of the coauthors of the 2013 *F. torreyae* naming paper**.

ABSTRACT: This review is intended to provide plant pathologists and other scientists with a current overview of the most important *Fusarium* phytopathogens and mycotoxin producers. Knowledge of *Fusarium* species diversity and their evolutionary relationships has increased dramatically due to the application of multilocus molecular phylogenetics and genealogical concordance phylogenetic species recognition over the past 15 years. **Currently *Fusarium* is estimated to comprise at least 300 genealogically exclusive phylogenetic species; however, fewer than half have been formally described.** The most important plant pathogens reside in the following four groups: the *F. fujikuroi* species complex noted for **Bakanae of rice, ear rot of maize, pitch canker of pine and several species that contaminate corn and other cereals** with fumonisin mycotoxins; the *F. graminearum* species complex including the primary agents causing ***Fusarium* head blight of wheat and barley** that contaminate grain with trichothecene mycotoxins; the *F. oxysporum* species complex including vascular **wilt agents of over 100 agronomically important crops**; and the *F. solani* species complex, which includes many economically destructive foot and **root rot** pathogens of diverse hosts. Several other *Fusarium* phytopathogens reported from Japan and nested within other species complexes are reviewed briefly....

- 2016 - **"First report of *Fusarium* crown and root rot on *Torreya grandis* caused by *Fusarium oxysporum* species complex in China"**, by Zhang Chuangqing, Shuya Zhang, X.L. Chen, Q.Q. Qi, H.Z. Lou, *Plant Disease*.

ABSTRACT EXCERPT: ***Torreya grandis* cv. Merrillii** is an important economic forest crop with a price as high as \$75/kg in nut markets throughout humid regions of South China. **In 2009, sporadic occurrence of crown rot disease was recorded** in Shaoxing City, which produces 80% yields of *T. grandis* in China. In 2014, nearly 37% of orchards and 4.5% of trees were affected in Shaoxing. Symptoms were crown rot characterized by light-brown discoloration of the cambium, with brownish black necrotic areas which appeared on the roots and often advanced to the collar on most plants. Diseased trees showed reduced vigor and chlorosis on the foliage and eventually died.... **To our knowledge, this is the first report of *T. grandis* crown and root rot caused by fungus belonging to the *F. oxysporum* species complex**

worldwide. Further work is needed to determine the clade of *F. oxysporum* to which the isolates pathogenic to *T. grandis* belong.

• **Note by Connie Barlow:** The sudden occurrence and rapid rise of pathogenicity by a **NATIVE FUSARIUM SPECIES** injuring a **NATIVE TORREYA SPECIES IN CHINA** should provoke the scientifically minded in Florida and Georgia to reconsider their presumption that *Fusarium torreyae* is possibly (or even likely) an exotic, and hence inherently risky to other native tree species.

A 2023 paper is titled, "First report of *Fusarium commune* causing *Torreya grandis* crown and root rot in China", and another 2023 paper is titled, "Root Rot Disease of *Torreya grandis* Caused by *Fusarium fujikuroi* in China".

- 2018 - "First report of canker disease in *Dalbergia tonkinensis* caused by *Fusarium lateritium* and *Fusarium decemcellulare*", by N.P. Nhung, *Australasian Plant Pathology*.

EXCERPT: This is the first report of ***Fusarium lateritium*** and ***Fusarium decemcellulare*** causing canker disease on *D. tonkinensis* [a precious tree for furniture] in Vietnam. The symptoms of *D. tonkinensis* canker disease caused by *F. lateritium* and *F. decemcellulare* were **canker lesions**, cracks, and dents in the bark with associated darkening of the wood and bark. Severe infestation resulted in leaf fall and shoot and tree death.

- 2018 - "New *Fusarium* species from the Kruger National Park, South Africa", by Marcelo Sandoval-Denis, Wijnard J Swart, and Pedro W. Crous, *MycoKeys*.

EXCERPTS: The genus ***Fusarium*** includes a vast number of species, commonly recovered from a variety of substrates including soil, air, water and decaying plant materials; being also able to colonise living tissues of plants and animals, including humans; **acting as endophytes, secondary invaders or becoming devastating plant pathogens** (Nelson et al. 1994). In addition to their ability to colonise a multiplicity of habitats, *Fusarium* is a cosmopolitan genus, present in almost any ecosystem in the world, including human-made settings such as air and dust in the indoor environment or even in hospitals (Perlroth et al. 2007; Aydogdu and Asan 2008; Pinheiro et al. 2011).... **Little attention has however been given to the occurrence of non-pathogenic fungal species, including *Fusarium* spp. in root microbial communities** (Zakaria and Ning 2013; Jumpponen et al. 2017; LeBlanc et al. 2017), while comprehensive DNA sequence-based surveys have been directed mostly to the study of highly relevant and abundant rhizosphere fungal genera such as *Trichoderma* Pers., *Verticillium* Nees or mycorrhizal fungi (Zachow et al. 2009; Bent et al. 2011; Ruano-Rosa et al. 2016; Saravanakumar et al. 2016).

... The analysis of the FBSC included sequences of EF-1a, RPB1 and RPB2 loci from **18 isolates representing 10 taxa**, including members of the ***Fusarium torreyae*** T. Aoki, J.A. Sm., L.L. Mount, Geiser & O'Donnell species complex (FTYSC) and ***Fusarium lateritium*** Nees species complex (FLSC) as outgroup.

... **The three *Fusarium* species, described here, were not associated with any visible symptomatology on their hosts.** However, they cannot be ruled out as pathogens since they were not assessed for pathogenicity against the sampled plants nor any other putative host species at the same locations. **Likewise, it is unknown if these fungi exert any beneficial or deleterious effect on their ecosystems.** These are important unsolved questions that need further evaluation. However, as shown by phylogenetic analyses, each of the three new species was in close genetic proximity with well-known plant pathogenic *Fusarium* spp. on their respective species complexes, which could suggest a potential pathogenic role.

LAST PARA: **This study is a new example of how easily new *Fusarium* spp. can be found when mycological studies are directed to neglected natural ecosystems of minimal anthropogenic disturbance** (Phan et al. 2004; Leslie and Summerell 2011; Summerell et al. 2011; Burgess 2014; Laurence et al. 2015). Although irrelevant for some researchers, finding and properly describing new species, regardless of whether they have little or no pathogenic or mycotoxigenic potential, is of utmost importance to improve our understanding on the diversity, biogeographic and phylogeographic patterns of such a complex and heterogeneous genus as

Fusarium. In addition, this study remarks on the significance and need to further stimulate the exploration of conserved, non-manipulated natural environments (supersites) and their potential impact on biodiversity research on the fungal kingdom.

• **Note by Connie Barlow:** I found this study via an advanced search in Google Scholar for the term *Fusarium torreyae* appearing anywhere in a published paper. Thanks to this paper, I was able to put the controversy regarding *Fusarium torreyae* into perspective. Key learnings for me are: (1) Research at the level of discerning new species of this global and species-rich genus generally owes to funding for understanding instances where *Fusarium* is injuring a commercially important plant species. (2) A pathogenic *Fusarium* species is generally also found in symptomless endophytic associations outside of commercial monocultural settings. (3) Funding is rare for *Fusarium* research in nonpathogenic settings. (4) Even when discovered in plant tissues as a nonpathogenic endophyte, this genus is generally not explored for possible benefits to its host plant, nor to find and catalogue other host species beyond the single host for which funding is obtained. (5) Research funding tends to not include an exploration of the geographic range of a newly identified fungal species, except when it is also recognized as pathogenic on a crop plant in a distant region or continent.

I thus sadly conclude that, it is very unlikely that any research funding will become available to ramify the scientific understanding of the newly identified *Fusarium torreyae*. Yet, without additional studies — or at least attempts to educate agency and botanical garden staff whose voices have the highest credibility in endangered species management — there will be no reduction in the fear that assisted migration northward of Florida torreyae might spread a purportedly new, possibly exotic, and possibly geographically isolated fungal pathogen to other tree species (as hypothesized in the Trulock 2012 masters thesis).

Specifically, there will be no search for the wider geographic range of this species. There will be no testing for whether other native trees in eastern North America for which other *Fusarium* species have already been catalogued might instead (or also) be nonsymptomatic hosts to *F. torreyae* when tested in their native ecosystems. There will be no search for nonsymptomatic endophytic (possibly even beneficially symbiotic) instances of *F. torreyae* associated with any of the tissues and seeds of the tree *Torreya taxifolia*, as quality research would require studies in ecological settings over its entire range — not in a Florida lab setting.

BOTTOM LINE: While it made sense to fund high-level research that eventuated in naming a new species of *Fusarium* as distinct from the well-known and globally ranging pathogen affecting Florida torreyae (*Fusarium lateritium*), the default decision will likely be that the risks of spreading a possibly pathogenic, possibly exotic disease to other native trees in eastern North America will outweigh the potential benefits of authorizing assisted migration northward of seeds for establishing "experimental populations" of the Florida torreyae tree. Accordingly, as climate continues to warm, it is likely that currently asymptomatic associations of the tree with its namesake *Fusarium* species (such as those in the ex situ orchards of north Georgia, and also at the Biltmore Gardens in NC) would eventually shift to pathogenic expression, as had already occurred in its glacial refugial native range in n. Florida by mid-20th century. Ideally, the sudden deaths of whole mountain slopes of climate-stressed native conifers in the western USA, effected by native bark beetles and their fungal symbionts, will eventually serve as important evidence to managers in charge of Florida torreyae. Such managers would then have evidential reason to consider that sudden death of mature stems of this species in Torreya State Park from the 1930s to 1960s should not automatically be attributed to introduction of an exotic disease. Sudden death can happen via native agents when a climate shift reaches a physiological tipping point of stress for a plant.

• 2025 - **"WEBPAGE: A Novel *Fusarium* Database for Fusarioid Fungi"**.

EXCERPT: In the past two decades, the application of phylogenetic species recognition based on genealogical concordance and non-discordance (GCPSR) has resulted in the

identification of approximately 400 phylogenetic species in *Fusarium*. However, **the majority of these phylogenetic species remain unnamed due to their cryptic morphology**.

Historically, *Fusarium* taxonomy has focused on **the asexual morph** (anamorph), the more frequently encountered morph in nature by biologists, as **the sexual morph (teleomorph) is mostly unknown for most species**. The implementation of the International Code of Nomenclature for algae, fungi and plants (ICN; Melbourne) in 2011, in which the asexual and sexual morphs are granted equal status with names assigned based on priority, has revolutionised the taxonomic treatment of fungi, including *Fusarium*. This has resulted in the recent segregation of *Fusarium*, with the resurrection and introduction of sexual morph generic names, which have been met with mixed emotions by the international *Fusarium* working community.

Fusarium taxonomic studies in the past two decades have seen the use of numerous variant characterisation protocols (morphology and DNA-based), sometimes making comparative studies difficult or even impossible, with several key *Fusarium* strains lodged in collections not accessible to the international *Fusarium* working community. Thus, it has become clear that a standardized research protocol for the treatment of fusarioid fungi in the fields of molecular taxonomy, pathology and morphology is urgently needed.

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PART C4

New paradigm papers applied to genus *Fusarium*

- 2019 - **"Three novel Ambrosia *Fusarium* Clade species producing clavate macroconidia known (*F. floridanum* and *F. obliquiseptatum*) or predicted (*F. tuaranense*) to be farmed by *Euwallacea* spp. (Coleoptera: Scolytinae) on woody hosts"**, by Takayuki Aoki, Jason A. Smith, and 5 coauthors, *Mycologia*

EXCERPTS: **Ambrosia *Fusarium* Clade (AFC) mutualists are carried in specialized cavities called mycangia by female *Euwallacea* beetles, which farm them in galleries they construct in the xylem of their woody hosts** and use them as a source of nutrition for themselves and their larvae (Hulcr and Stelinski 2017). This mutualism has attracted considerable attention by agricultural scientists because several xyleborine *Fusarium*-farming *Euwallacea* beetles cause dieback and death of economically important hosts, including **Chinese tea (*Camellia sinensis*)**, **cacao (*Theobroma cacao*)**, **rubber tree (*Hevea brasiliensis*)**, **citrus (*Citrus* spp.)** (Brayford 1987), and **avocado (Mendel et al. 2012)**. **The discovery of invasive Asian *Euwallacea* farming *Fusarium* associated with wilt and dieback of avocado (*Persea americana*)**, as well as diverse woody hosts in managed urban landscapes and native forests in the United States, Israel, Australia, Mexico, and South Africa, **during the past decade** has spurred intensive research efforts focused on understanding the genetic diversity of these exotic **mutualists**...

The present study reports on multilocus molecular phylogenetic analyses and detailed phenotypic data that were used to **distinguish three unnamed AFC species** ... Herein, these species are formally described as ***Fusarium floridanum* (AF-3) farmed by *E. interjectus* on boxelder (*Acer negundo*) in Gainesville, Florida** ... Of these, *Fusarium floridanum* and *F. obliquiseptatum* are known to cause limited **cankers associated with beetle attacks** on boxelder and avocado, respectively.

Due to the significant threat the invasive *Euwallacea*-*Fusarium* mutualists pose to urban landscapes, native forests, and the avocado industry worldwide, extensive pathogen surveys and multilocus molecular phylogenetic analyses conducted over the past 6 years led to **the surprising discovery of 15 additional phylospecies within the AFC....** Although **the majority of the fungus-farming *Euwallacea* are not known to cause widespread damage**, the likelihood that they might switch symbionts offers the real **potential of creating more aggressive and economically destructive *Euwallacea*-*Fusarium* pest-pathogen associations**.

• **Note by Connie Barlow:** Here is yet another paper centered on the recent discovery and naming of new species of genus *Fusarium* sampled from diseased plant tissue. I highlighted in red the newly named *Fusarium floridanum* because it was found in Gainesville FL (likely by second author Jason A. Smith) on box elder trees, *Acer negundo*. Samples were acquired at Loblolly Woods Nature Park, which is at the southern border of the large northward native range of box elder. This is an important detail, as there is no argument that native trees in eastern North America are already the most stressed at their southern borders (except when attacked by invasive exotic insects and pathogens). And it is also a given that benign endophytic fungi can become pathogenic when their host tree is stressed.

This point certainly applies to *Torreya taxifolia* as a glacial relict, already stressed by Holocene warming decades before the exponential rise in anthropogenic carbon-dioxide. I wonder: Would anyone have seriously considered a non-native origin for a newly distinguished (and named) pathogen (*Fusarium torreyae*) attacking a critically endangered relictual tree, if its discovery in 2010 had been delayed a decade? After all, by the 2020s, even the public was aware that NATIVE BARK BEETLES were carrying NATIVE FUNGI into climate-stressed native conifers in the Rocky Mountains and Pacific Coast Ranges — and whole slopes were suddenly turning brown as their spruces, pines, and firs succumbed to the beetle/fungi attacks.

• 2020 - **"Fighting *Fusarium* Pathogens in the Era of Climate Change: A Conceptual Approach"**, by Salme Timmusk et al., *Pathogens*.

EXCERPTS: ***Fusarium* head blight (FHB) caused by *Fusarium* pathogens is one of the most devastating fungal diseases of small grain cereals worldwide, substantially reducing yield quality and food safety. Its severity is increasing due to the climate change caused by weather fluctuations....** *Fusarium* is one of the most renowned genera in the Fungi kingdom. It includes, in a broad sense, a large number of morphologically and phylogenetically diverse fungi, commonly found as air-, soil-, or water-borne saprobic organisms, but also found in dead or living plant material as endophytes or epiphytes. Many *Fusarium* spp. are effective plant pathogens or secondary invaders of worldwide concern.... Based on the perceived scientific and economic importance, *Fusarium* spp. were recently included in the top 10 globally most important genera of plant pathogenic fungi.

Plants harbor different species- and cultivar-specific microbial communities that grow as epiphytes and endophytes in the rhizosphere and phyllosphere. The phyllosphere microbiome is composed of various microorganisms but bacteria are the most abundant cellular organisms in the phyllosphere community, and the most efficient colonizers are present in numbers between 10⁶ and 10⁷ cells cm² of leaf tissue. The bacteria in phyllosphere are often vertically transmitted as endophytes and they form patchy assemblages on plant surface called biofilms or microbial consortia. **While the rhizosphere and endorhizosphere have been thoroughly studied for centuries, less is known about the drivers that influence the phyllosphere, i.e., any aerial part of the plant, such as stems, leaves, leaf ears, or flowers.** This is a significant limitation as phyllosphere microbiome is of critical importance for dispersal of *Fusarium* pathogens. Although *Fusarium* spp. can survive several years on plant debris, even if located deep in the soil, **the pathogens themselves, however, develop only on the upper parts of living plants.** Hence, the review focuses on the phyllosphere, which has a large potential for the design of beneficial microbial biofilm layers.... Competitive exclusion of pathogens by the broader phyllosphere community may play an important role in plant pathogen resistance.

Biofilm formation is an environmental microbiology concept, denoting microbial cell adaptation to multicellular lifestyle. The temporal sequence of events in biofilm formation starts with planktonic cell attachment, followed by microcolony formation and cell detachment. Biofilm formation maximizes nutrient delivery to all viable cells and increases resistance to environmental stress factors. The attachment is initiated by exuding extracellular polysaccharide material that entraps cells and debris within a glue-like matrix.

Despite importance of plant health for forestry and agriculture, its meaning is not fully conceptually developed. The reductionist approach is rooted in investigating small parts of pathosystems. Interactions with pathogens as well as beneficial microbes have been studied for years in different academic fields. **The reductionist approach is rooted in investigating**

small parts of pathosystem, and the broad scientific field is currently highly fragmented. In particular, interactions of plants with pathogens and with beneficial microbes have been studied for years in different academic fields. Microbial community interaction networks have been studied by microbial ecologists, volatiles by chemical ecologists, environmental signals by climate and soil scientists, and interactions with harmful microorganisms are in the domain of plant pathologists. As a result, over the years, each of the fields has developed its own approach and language. It is clear that **our current challenge is to strengthen the connections between ecological and agricultural studies.... As a general practice, most of the plant-pathogen interaction research has been performed under a few static conditions that do not allow capturing the pathosystem dynamics as it occurs in the nature.**

• **Note by Connie Barlow:** This is a helpful background paper for appreciating two things: (1) It is not surprising that a technologically sophisticated study of the *Fusarium* association with cankers on Florida *torreya* stems would lead to the naming of a new species distinct from *Fusarium lateritium*, which had long been recognized as a disease agent on this endangered tree. (2) It is not surprising that the forest pathology research team limited their research to morphological uniqueness coupled with plausible taxonomic relations within the genus *Fusarium*. **Ecological studies might have demonstrated that pathogenicity derived from a warming climate rather than putative translocation of a possibly non-native fungus, but such studies have not been accomplished.**

- 2020 - **"Biological control of Fusarium wilt in crop plants using non-pathogenic isolates of *Fusarium* species"**, by Suresh Patil and s. Sriram, *Indian Phytopathology*.

ABSTRACT: Many species of the genus *Fusarium* resemble each other morphologically. In many cases morphological differentiation is difficult, molecular tools are used. Pathogenic as well as non-pathogenic isolates have same habitat and colonize plant root system with equal measure. Since non-pathogenic isolates resemble pathogenic isolates in their nutritional and abiotic requirements, many attempts have been made to utilize them as biocontrol agents to manage *Fusarium* wilt diseases. Although these isolates colonize plants, they do not induce disease symptoms. They are primarily soil inhabiting in nature; once introduced they remain in the soil for very long time providing significant and consistent disease control. Herewith we give **a review and summary of different reports of non-pathogenic *Fusarium* in different cropping systems**, mode of action (antibiosis, plant growth promotion and induced systemic resistance), molecular basis of bioassay and identification, environmental conditions, different formulations, cross protection as well as effects on non-target crops.

EXCERPTS: The taxon *Fusaria* is a complex one and more than one hundred *formae speciales* have been described. It represents rhizosphere microflora that are plant pathogens or saprophytes. **Even the pathogenic isolates survive as saprophytes, and once they reach the corresponding host they infect and enter into parasitic life style. Non-pathogenic isolates are capable of colonising root surfaces and protect even susceptible varieties from the highly virulent pathogenic isolates.** From asymptomatic roots, avirulent *Fusarium* isolates can be isolated.

• **Note by Connie Barlow:** This is yet another paper that suggests **the importance of conducting an ECOLOGICAL study of the newly named *Fusarium torreyae***. There may well be ecological situations and other plant hosts in which *Fusarium torreyae* is benign or even beneficial — as seems to be the case for species of the *Fusarium* genus at large. Notably, **is this particular *Fusarium* non-pathogenic on Florida *Torreya* already living in northward realms, such as the ex situ orchards of north Georgia and at the Biltmore Gardens near Asheville? As well, is this *Fusarium* benign on or within trees native to the Appalachian Mountains in their natural ecological settings** — despite having expressed disease symptoms when potted versions were inoculated with the *Fusarium* in a Florida lab setting (Trulock 2012)?

- 2021 - **"Phylogenomic Analysis of a 55.1-kb 19-Gene Dataset Resolves a Monophyletic *Fusarium* that Includes the *Fusarium solani* Species Complex"**, by David M. Geiser et al, *Phytopathology*.

EXCERPT: Three additional species complexes recognized within genus *Fusarium* since the publication of the rpb1 1 rpb2 phylogeny (O'Donnell et al. 2013) are represented in the dataset: the **F. torreyae species complex** (Zhou et al. 2018), the *F. newnesense* species complex (Laurence et al. 2016), and the *F. burgessii* species complex (here represented by *F. beomiforme*; Laraba et al. 2018; Laurence et al. 2011; Nelson et al. 1987), bringing the total to 23 species complexes recognized within the genus.

- 2022 - **"Response of the plant core microbiome to *Fusarium oxysporum* infection and identification of the pathobiome"**, by Zhiguang Qiu et al, *Environmental Microbiology*.

EXCERPT: **Plant core microbiomes consist of persistent key members that provide critical host functions, but their assemblages can be interrupted by biotic and abiotic stresses.** The pathobiome is comprised of dynamic microbial interactions in response to disease status of the host. Hence, identifying variation in the core microbiome and pathobiome can significantly advance our understanding of microbial interactions and consequences for disease progression and host functions.... This study advances key understanding of core microbiome responses and existence of plant pathobiomes, which provides a novel framework to better manage plant diseases in agriculture and natural settings.... The core microbiome is comprised of members of microbial assemblages commonly present in hosts or within particular niches of a broad host community (Turnbaugh et al., 2007).... It has been reported that **beneficial members of the core microbiome are critically involved in plant performance**.... The pathobiome, which is an emerging concept, considers disease as the manifestation of multiple interactions among several microbial species, including pathogens, which affect the health and disease status of the host.... These findings potentially support the recent evidence for **the 'cry for help' strategy of plants under pathogen attacks (Liu et al., 2020)** and may suggest that such a strategy of accumulating beneficial microbes in plant microbiomes could be common in many plant species.... The pathobiome concept has expanded our view from a single microorganism as a disease agent to a broader perspective of communities that co-affect a particular disease.

- 2023 - **"Root Rot Disease of *Torreya grandis* Caused by *Fusarium fujikuroi* in China"**, by L. Zheng et al., *Plant Disease*

EXCERPT: ***Torreya grandis* is an evergreen plant endemic to China** and widely grown in Southern China.... **From 2018 to 2020, typical root rot symptoms of *T. grandis* were found in plantations** in Huangshan and surrounding areas of Huangshan, Anhui province, China. About 15 to 32% of root rot disease incidence was recorded. Diseased plants were observed with symptoms such as yellow to brownish leaves without lesions and later drying, and rotten roots that looked dark brown and **eventually led to the death of the diseased plant**. The root rot symptomatic plants were collected in June of 2020.... Eight isolates with similar morphology were isolated from single spores.... According to the culture and conidial characteristics, the isolates were tentatively **identified as *Fusarium* species**.... From symptomatic roots, the pathogen was reisolated and RPB1 and RPB2 were amplified and

sequenced to confirm the molecular identity of the reisolated pathogen as *F. fujikuroi*, satisfying Koch's postulates. **To the best of our knowledge, this is the first report of *Fusarium fujikuroi* causing root rot of *T. grandis* in China.**

• **Note by Connie Barlow:** In this paper, disease expression was not attributed to any named cause. The publication was, rather, a very short "Disease Note." Nonetheless, if **climate change** is a possible cause of the sudden occurrence of disease symptoms by the *fujikuroi* species of genus *Fusarium* expressed on an Asian species of genus *Torreya*, then it is **parsimonious to entertain the notion that Holocene and subsequent anthropogenic warming in North America is also a causal factor — perhaps the ultimate cause — that provoked a native *Fusarium* species into pathogenic consequences on a climate-weakened glacial relict plant that had been unable to disperse northward from Florida as the glacial age came to a close.**

• 2023 - **"First Report of Stem Rot in *Cymbidium sinense* Caused by *Fusarium oxysporum* in China"**, by Peng-yu Liange et al., *Plant Disease*.

EXCERPTS: *Cymbidium sinense* (Jackson ex Andr.) Willd. is a **perennial terrestrial plant in the orchid family** mainly in China, Japan, India, and Southeast Asia that occupies a strong position in the flower market (Zhang et al. 2013), and its roots have anti-asthmatic properties (Ke et al. 2004). **In August 2020, approx. 15% stem rot on 2-year-old *C. sinense* with varying severity was observed in five nursery gardens** in Enshi city (30–∞169N, 109–∞299E), Hubei province, China. Typical symptoms included roots and the inner part of the pseudobulbs changing from white to brown and rotting. Leaves became brown and withered from bottom to top, and there was an obvious bright yellow halo at the junction of diseased and healthy tissue, which eventually caused the whole plant to wilt and die.... *Fusarium oxysporum* was reisolated from the infected plants to fulfill Koch's postulates.... **Members of the *Fusarium oxysporum* species complex are notorious for causing many diseases, including stem rot of *Sulcorebutia heliosa* and root rot of *Torreya grandis* (Garibaldi et al. 2020; Zhang et al. 2016). This is the first report of stem rot caused by *F. oxysporum* on *C. sinense* in China.** The finding of this pathogen provides a clear target for stem rot control.

• **Note by Connie Barlow:** This Asian-based study of a *Fusarium* species suddenly bringing disease symptoms to a commercially important plant is published in the same year and same journal as the Asian study excerpted immediately above. My same conclusions apply to this paper, and are amplified.

• 2023 - **"Genome Sequence and Assembly of 18 *Fusarium* Isolates from Florida Citrus under High Huanglongbing Disease Pressure and California Citrus under Low Huanglongbing Disease Pressure"**, by Tania Kurbessoian et al., *Microbiology Resource Announcements*.

EXCERPT: Fungal isolates were cultured from leaf, stem, and root tissue of California (Riverside County) and Florida (Marion, Lake, and Martintown Counties) citrus trees.

• **Note by Connie Barlow:** This is a very short paper. It is included in this list on the Torreya Guardians website because it appears to be **the first published documentation of *Fusarium torreyae* (along with other *Fusarium* species) being present within a host plant other than Florida Torreya — and outside of Florida Torreya's native range.** As presented in Table 1, *Fusarium torreyae* is the identity of one of the fungal species or strains sampled from a CITRUS grove located in "Martintown County". I could not find that name on google maps, but the town of Martin is a bit south of Gainesville, which puts it at about 180 miles SE of Torreya's native range. The other species of *Fusarium* collected from various citrus groves in Florida were *equiseti*, *irregulare*, and *oxysporum*. *F. falciforme* is the only species found in both Florida and California citrus groves. Crucially, the researchers made no distinction of which of the "18 *Fusarium* isolates"

were pathogenic v. those that were benign or even mutualistic. So, while there is evidence that *F. torreyae* has a geographic range larger than previously documented, there is no conclusion as to whether and how it affects any agricultural species of citrus tree.

QUESTIONS TO CONSIDER: If there is genuine fear that transport of Florida *Torreya* seeds into northward states might spread the disease to other native trees, what about THE SHIPMENT OF EXOTIC CITRUS FRUITS NORTHWARD? And might *Fusarium torreyae* therefore already occupy a very large range as a result, with as-yet no evident disease symptoms in northern plant species significant enough to have been recognized?

It is thus plausible that, whatever the geographic range of *Fusarium torreyae*, it is a benign epiphyte and/or endophyte on stems of one or more native tree species where climate does not (yet) induce pathogenicity. As *Fusarium torreyae* is closely related to (even formerly identified as) the *Fusarium lateritium* global complex, another 2023 paper (below) is important to consider:

"Acuminatums E and F, two new cyclic lipopeptides from *Fusarium lateritium* HU0053 and their antifungal activity", by Zhi-Juan Zhong et al., *Journal of Asian Natural Products Research*. Their analysis found one particular strain/sample of *Fusarium lateritium* to "exhibit antifungal activities against *Penicillium digitatum*."

- 2024 - "New insights into decoding the lifestyle of endophytic *Fusarium lateritium* Fl617 via comparing genomes", by Yan Zhao et al., *Genomics*.

EXCERPTS: The surface, interior, rhizosphere and phyllosphere of plants are occupied by complex microbial communities that include beneficial endophytes, commensal bacteria as well as harmful pathogens [1,2]. However, the type of binding (endophytic or pathogenic) that microbial-plant interactions will establish is governed by complex mechanisms that depend greatly on factors such as the genetic structure, compatibility, and environment of both parties [3]. In particular, there is a continuum between endophytes and pathogens: some organisms can infest or cause disease in some hosts, while being endogenous and perhaps even mutually beneficial in others, and vice versa [3,4]. However, compared to the investigation of the molecular mechanisms of pathogen-plant interactions, there is still room for further elucidation regarding the endophytic lifestyle of endophytes.

These include *Serendipita* spp., *Colletotrichum* spp., and *Fusarium* spp. with biphasic colonization strategies. *Fusarium* species are commonly known as pathogens and are also widely present as plant endophytic fungi. *F. oxysporum* exhibit biphasic colonization strategies and establish root compatibility on different hosts, which makes them important tools for studying the molecular basis and evolution of fungal endogeny [2]. However, the events involved in their endophytic processes remain largely unknown. *F. lateritium* adopts different colonization strategies in different hosts and thus behaves either pathogenic or endophytic. Fungi produce a series of **secondary metabolites (SMs)** that act as virulence factors to help pathogens infect host plants. In addition, these SMs also play a role in the establishment of beneficial endophytic relationships between endophytic fungi and plant hosts and are potential determinants of endophytic lifestyle.... It is hypothesized that endophytic fungi may have fewer secondary metabolite gene clusters than pathogenic fungi, which may be one of the reasons why **fungi ultimately maintain an endophytic lifestyle**.

Endophytic *Fusarium lateritium* lacks pathogenic factor SIX... *Fusarium lateritium* has been widely reported for its pathogenicity in a wide range of plants [29-31]. And **endophytic *F. lateritium* has also been found in different plant species with good antagonistic activity and biocontrol potential against pathogens**.

- 2024 - "Known from trees and the tropics: new insights into the *Fusarium lateritium* species complex", by M.M. Costa et al., *Studies in Mycology*.

EXCERPTS: The ***Fusarium lateritium* species complex (FLSC)** currently comprises **11 phylogenetic species** ... In the present study, we examined 179 strains related to *F. lateritium* from different countries and substrates.... Phylogenetically, **the FLSC resolves as a sister clade to the *F. buharicum* and *F. torreyae* species complexes** (Crous et al. 2021, Geiser et al. 2021). The FLSC comprises fungi commonly found associated with arboreal plants

such as *Araucaria cunninghamii*, *Cassia fistula*, *Citrus* spp., and *Coffea* spp., but also on *Camellia* leaves (Geiser et al. 2005, Cavalcanti et al. 2020, Perera et al. 2020, Crous et al. 2022, Hyde et al. 2023).

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PART C5

New paradigm implications for managing Florida *Torreya* (*Fusarium torreyae* as an endophytic MUTUALIST in northward habitats)

• **Note by Connie Barlow:** Papers excerpted in the previous sections repeatedly stated or implied that **"environmental stress" can transform asymptomatic endophytes within the tissues of plants into hostile agents**. Prior to the new paradigms, these were labelled rather simply as **"latent pathogens"** — thereby sidestepping any need to distinguish between asymptomatic commensals and mutualistic members of the microbiome. (See, for example, the 2017 paper **"*Botryosphaeria dothidea*: a latent pathogen of global importance to woody plant health"**.) Even today, few publications articulate the exact nature of positive roles played by recognized fungal endophytes.

Fortunately, **there are several papers that document instances in which endophytic *Fusarium lateritium* offered benefits to its plant host**. This long-recognized species (now a "species complex") is closely related to *Fusarium torreyae*.

Indeed, **"*Lateritium*" was the species identification given to the documented *Fusarium* expressing pathogenically on Florida *torreya* decades before *Fusarium torreyae* became the newly branded name for this canker-causing fungus**.

BELOW ARE 5 PAPERS documenting flexibility in pathogen expression and outright beneficial aspects of ***Fusarium lateritium***, culminating in a sixth paper published in 2025 that attributes this same kind of flexibility to the ***Fusarium torreyae* species complex**.

- 2018 - **"Diversity and Characterization of Endophytic Fungi Isolated from the Tropical Mangrove Species, *Rhizophora mucronata*, and Identification of Potential Antagonists Against the Soil-Borne Fungus, *Fusarium solani*"**, by Tuan Noraida Tuan Hamzah et al., *Frontiers in Microbiology*.

EXCERPTS: The current study was designed to determine species diversity of the **endophytic fungal community in the leaf tissue of *R. mucronata*, a tropical mangrove species** commonly found at the Matang Mangrove Forest Reserve (MMFR), Perak, Malaysia, and to characterize biological activities of several selected species. These fungal endophytes might offer novel species or strains that possess valuable bioactive compounds, which demonstrates the significance of their study.... **Endophytic fungi live internally within various tissues of a host plant, asymptotically, without causing any negative effect to the host plant** (Aly et al., 2011). **When a host plant harbors endophytes, their concurrence may help the host to adapt to biotic and abiotic stress factors**.

... Different parts of *R. mucronata* such as the bark and leaves have been used traditionally as astringent and antiseptic, and have been shown to possess activities against bacteria, ulcers, and inflammation (Kokpol et al., 1990; Suganthi and Pandima Devi, 2016).... Apart from focusing on the plant itself, several studies have been conducted on microorganisms associated with *R. mucronata*, including fungal endophytes. Endophytic fungi from the genera *Penicillium*, *Ampelomyces*, and ***Fusarium***, **isolated from *R. mucronata*, were found to be active against *E. coli*** (Prihanto et al., 2011)....

... From the preliminary qualitative antibacterial screening of the six fungal isolates, ***Fusarium lateritium* showed inhibition to all four bacterial pathogens tested**, while *Xylaria* sp. to three.... *Fusarium solani* is a common tree pathogen and has been isolated from diseased mangrove plants (Umechuruba, 2005). We showed that endophytes ***Fusarium lateritium***, *N. oryzae*, *Phoma* sp., and *Xylaria* sp., **successfully inhibited the pathogenic**

Fusarium solani* in the dual culture assay....** In contrast to *Xylaria* sp., ***F. lateritium did not display distinct scavenging activity. This result however is not as observed by previous studies, which regarded ***Fusarium*** sp. as a potent source of antioxidant based on their significant antioxidant activity recorded (Vasundhara et al., 2016). Such example include *Fusarium oxysporum*, an endophytic fungus from the rhizomes of *Dioscorea zingiberensis* (Li et al., 2012) and *Fusarium proliferatum*, from pigeon pea (Zhao et al., 2012b).

• **Note by Connie Barlow:** If *Fusarium torreyae*, newly named from isolates within diseased tissue of *Torreya taxifolia*, is (as evident in a later entry) a new name for what had been identified during past decades as the well-known and global *Fusarium lateritium*, then **this 2018 paper offers strong evidence that *Fusarium torreyae* could have been endophytically present (either benign or mutualistic) in the host plant Florida torreya before pathogenicity developed** — which, in turn, was likely triggered by environmental stress on the host plant. Alternatively, a distinct strain of *Fusarium lateritium* could have arrived during the 20th Century on this continent from an exotic source, but was only recognized as a distinct strain, divergent enough to gain a new species name, in 2012.

Either way, the key insight in this paper is this: *Fusarium lateritium* clearly has evolved the capacity to be a beneficial endophyte in at least the mangrove studied, and so it is possible that the form of *Fusarium lateritium* now called *Fusarium torreyae* would likely also have the capacity to exist as a benign or beneficial endophyte in one or more of its host plants, so long as the host remains healthy enough to maintain the mutualism.

• 2022 - **"Apple Endophytic fungi and their antagonism against apple scab disease"**, by Leila Ebrahimi et al., *Frontiers in Microbiology*.

EXCERPT: Endophytic fungi are microorganisms with the ability to colonize plants for the entire or at least a significant part of their life cycle asymptotically, establishing a plant-fungus association. They play an important role in balancing ecosystems, as well as **benefiting host through increasing plant growth, and protecting the host plants from abiotic and biotic stresses** using various strategies. In the present study, endophytic fungi were isolated from wild and endemic apple cultivars, followed by characterizing their antifungal effect against *Venturia inaequalis*.

• **Note by Connie Barlow:** Here the authors conclude that the 61S2 isolate of *Fusarium lateritium* benefitted the host (apple) plant in two ways: (1) reducing the severity of the apple scab pathogenic fungus and (2) solubilizing inorganic phosphate such that the plant could make use of this essential mineral.

• 2023 - **"Acuminatums E and F, two new cyclic lipopeptides from *Fusarium lateritium* HU0053 and their antifungal activity"**, by Zhi-Juan Zhong et al., *Journal of Asian Natural Products Research*.

Editor's note: I could not access the full article where *F. lateritium* was presented at depth.

ABSTRACT: Two new cyclic lipopeptides, acuminatums E (1) and F (2), together with four known cyclic lipopeptides, acuminatums A - D (3 - 6) were **isolated from the corn culture of endophytic *Fusarium lateritium* HU0053**. Their structures were elucidated by spectroscopic and advanced Marfey's amino acid analysis. All compounds were found to exhibit **antifungal activities against *Penicillium digitatum***. Acuminatum F (2), a new cyclic lipopeptide containing an unusual 3, 4-dihydroxy-phenylalanine unit exhibited the strongest antifungal activities with inhibition zone of 6.5 mm at the dose of 6.25 ug. Therefore, acuminatum F might be a potential **environmental-friendly preservative for citrus fruits**.

• 2023 - **REVIEW: "The endophytic *Fusarium* strains: A treasure trove of**

natural products", by Arwa Mortada Ahmed et al., *Royal Society of Chemistry*.

EXCERPTS from "Introduction": ... *Fusarium* is considered as a member of the most dominant endophytic fungal genera in the world, characterized genetically with extraordinary discrepancy, together with its ability to grow on a wide range of substrates and their efficient mechanisms for dispersal, which affect their biology and interaction with their surrounding organisms, together with secondary metabolism that makes *Fusarium* an important group of fungi....

***Fusarium* is considered to be a rich source of bioactive compounds, including more than one hundred compounds with unique chemical structures**, among more than three hundred compounds of various classes such as butenolides, alkaloids, terpenoids, cytochalasins, phenalenones, xanthenes, sterols, and diphenyl ether and anthraquinone derivatives, with multidimensional bioactivities such as antimicrobial, antiviral, anticancer, antioxidant, antiparasitic and immunomodulating activity.... **This review covers the literature on the isolated natural products from the fungal endophyte *Fusarium* species and their various bioactivities** from April 1999 to April 2022.

EXCERPTS from "Conclusion and future perspectives": Fungal endophytes are the fungal population of the internal tissues of plants causing no apparent symptoms of disease. **These endophytes are able to produce certain phytoconstituents originally attributed to their host plant**, which is thought to be due to genetic recombination between the endophyte and the host plant that occurred in the evolutionary period. In addition, fungal endophytes are reported to produce new secondary metabolites, which may be totally different from those of the host plant, so they are a precious source of novel bioactive natural products and an alternative source for phytochemicals initially produced by higher plants. The *Fusarium* genus is among the well-known genera of fungal endophytes with many different species. *Fusarium* endophytes are considered to be a rich source of new secondary metabolites with valuable biological activities and a promising basis for drug discovery.... *Fusarium* species have the ability to produce a varied array of secondary metabolites such as sterols, polyketides, alkaloids, terpenes, peptides and other compounds.

• **Note by Connie Barlow:** This is a lengthy paper containing very technical short subsections on a number of species of the *Fusarium* genus, including **beneficial characteristics of *Fusarium lateritium***.

- 2024 - **"New insights into decoding the lifestyle of endophytic *Fusarium lateritium* FI617 via comparing genomes"**, by Yan Zhao et al., *Genomics*.

Editor's note: This paper was excerpted at greater lengths above, [here](#).

EXCERPTS: ***F. lateritium* adopts different colonization strategies in different hosts and thus behaves either pathogenic or endophytic.... Endophytic *Fusarium lateritium* lacks pathogenic factor SIX... *Fusarium lateritium* has been widely reported for its pathogenicity in a wide range of plants [29-31]. And **endophytic *F. lateritium* has also been found in different plant species with good antagonistic activity and biocontrol potential against pathogens.****

- 2025 - **"Genomic features and evolution of lifestyles support the recognition of distinct genera among fusarioid fungi"**, by Bartosz Ulaszewski et al., *Mycological Progress*

Editor's note: This is the paper that confirms the **flexibility in expression of *Fusarium torreyae* and used the word "climate" as one of the factors in its manifestation as an "asymptomatic endophyte" or a "virulent pathogen"**.

EXCERPT: **The common ancestor of *Fusarium* (node F3) was most likely a plant pathogen. However, except for the most early-diverging clades of *Fusarium* (*F. buharicum*, *F. lateritium* and *F. torreyae* species complexes) all the other major clades are inferred to have likely evolved from an endophytic or plant pathogenic lifestyle, with plant pathogenic species present throughout the genus. The reason for this might**

be the ambiguity between the two lifestyles. **Many studies have shown that fungi can switch between their growth as an asymptomatic endophyte and a virulent pathogen depending on environmental conditions** (Kogel et al. 2006; Fesel and Zuccaro 2016; Rai and Agarkar 2016). Based on these data, it is apparent that in *Fusarium* s. str., **most plant pathogens will also be isolated as endophytes**, and that its species have adaptable lifestyles, underlining their potential to act as generalists and opportunists in various niches. Furthermore, definitions of lifestyles are known to be too rigid, and frequently cannot incorporate the flexibility as observed in nature (Buijs et al. 2022). For fusarioid fungi specifically, in line with our conclusions, Hill et al. (2022) therefore suggested that **multiple lifestyle hypotheses need to be considered to incorporate their various interactions, which depend on host, climate, and environment**. Thus, especially **the classification into endophytes vs pathogens should be considered ambiguous**.

Endophytism appears to be a lifestyle derived from plant parasitism, and vice versa. Both lifestyles have evolved in many separate occasions in most genera included here. Endophytism is however markedly more common in *Fusarium*, but it also appears as a more recent evolutionary event, or as secondary lifestyle in *Calonectria*, *Corinectria*, *Cylindrodendrum*, *Dactylonectria*, *Ilyonectria*, *Neocosmospora*, *Neonectria*, *Scolecofusarium* and *Thelonectria*.

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PART C6

RECOMMENDATIONS for the 2025 RECOVERY PLAN UPDATE

by Connie Barlow

THE PROBLEM: It is clear that **even credentialed and highly experienced staff in the botanical gardens that have blocked access to ex situ seed production (numbering in the tens of thousands annually) may not be familiar with recent papers on the PLANT and SEED MICROBIOME PARADIGM SHIFTS**. If they were aware of the new paradigm, then the **2019 statement by Jennifer Ceska** that ***Fusarium torreyae* has been found in all of the tissues of Florida torreyae, yet is not expressing pathogenically in northern Georgia**, would have indicated its **status as a MUTUALISTIC ENDOPHYTE rather than a dangerous pathogen**. This, in turn, would have enabled a return to authorizing distribution of seeds from the two ex situ plantings in northern Georgia.

* * *

• **RECOMMENDATIONS:** Therefore, during the 2025 preparation of the update to the FLORIDA TORREYA RECOVERY PLAN, I recommend that U.S. Fish & Wildlife Service staff:

(1) **Read and then reference the NEW PARADIGM papers** excerpted here as **"the best available science"** for discerning **whether *Fusarium torreyae* is more likely to be a "mutualistic endophyte"** rather than a "latent pathogen" as long presumed.

If there is pushback from qualified agency and botanical garden scientists against that new characterization, then (a) ensure that they are familiar with the new paradigm papers and (b) **clarify whether the claimed documentation of *Fusarium torreyae* at the Biltmore Gardens in North Carolina and in the ex situ groves and/or seeds in northern Georgia is based on genetic findings on tissues sampled, rather than isolated from externally apparent stem cankers**. If the latter, what is the extent of disease expression? Does the context indicate recent environmental stress? Have cankers been found on nearby trees of other species, and are they keyed to *Fusarium torreyae*? (Note: My own last visit to the torreyas at Biltmore Gardens was in 2018 — and I saw no evidence of cankers.)

(2) **Restore a paragraph pertaining to the breadth of disease expressions in the historical range** that appeared in the **2010 plan update** (pp. 5-6):

Identify pathogen(s) responsible for the decline. This is an ongoing action that goes back to 1967 (Alfieri et al. 1967). The authors observed that the **stem and needle blight disease**

of *T. taxifolia* appears to be incited by a **fungus causal agent** implicating *Physalospora* and *Macrophoma*. Alfieri et al. (1987) isolated **six other fungi** from leaves and stems of *T. taxifolia*. Lee et al. (1995) **isolated more than 30 different endophytic fungi**, but consistently, *Pestalotiopsis microspora* (a fungus that **resides in the inner bark of symptomless *T. taxifolia* trees**) was found on diseased trees. They concluded that **the pathological activity of this fungus could be triggered by physiological and/or environmental stress**.

(3) **Apply the (new in 2023) ESA regulatory change that authorizes "experimental populations" outside of "historical range."** Consider that the tens of thousands of seeds produced annually in the ex situ groves would make possible a great number of experiments, without risking the fundamental importance of ongoing "genetic safeguarding." **I recommend 3 existing plantings documented on the Torreya Guardians website:**

Begin by visiting the March 2025 entry on the **"Learnings"** webpage of the Torreya Guardians website. Summarized there are proposals for two ongoing experiments in southern states that could greatly benefit from the planting of genetically wild seed stock: **Shoal Sanctuary** in the **Florida panhandle** and **Caroline Dornon Nature Preserve** in **Louisiana** — both of which are under protective conservation easements.

A third highly recommended site is in **southeastern Tennessee**. Crucially, it is barely northward of the latitude of the ex situ orchard managed by the State Botanical Garden of Georgia (at Smithgall Woods). This site is not yet under conservation easement protection, however, but its forested setting, absence of deer herbivory, large acreage, and diverse genetic stocking of Florida torreyia (and of Florida yew!) make it ideal for official recognition and therefore access to wild genetic seed stock. East of Chattanooga, it is in the **Ocoee watershed, along Greasy Creek**. Clint Bancroft is the private landowner. For many years he has contributed photos and text observations that have already contributed a great deal to our documentation of best planting practices.

(4) Consult the **22-page PDF I submitted to FWS 25 July 2024 titled "Utilizing Torreya Guardians Experience for SSA and Recovery Planning"**. Subtitle: "Comment for Five-Year Revision re Florida Torreya." It is also **available on Researchgate**. Although I wrote it expressly to assist FWS staff preparation of the "Species Status Assessment (SSA)", which is the first stage of the updating process, it also contains **technical recommendations and support for policy consideration** during the writing of the Recovery Plan Update itself. Below are two of the most relevant for policy-level decisionmaking.

- ACTION RECOMMENDATION 1E: When developing the Recovery Plan itself for posting in the *Federal Register*, **consider reaching back to the 2010 plan update to restore the "experimental populations" language used on p. 19:**

Translocation (introduction of a species to a site outside the known historical range), could offer a best management option if the site provides the only place safe from the threats that brought the species to endangerment, and should only be considered if it can be shown that there is a net gain for the species conservation, i.e., recovery unit. This management option should be carefully evaluated, and planning should be done with the very best biological science. **If a population has been already translocated, it could potentially be evaluated as an experimental population.**

- ACTION RECOMMENDATION 4A: **Insist that the two botanical gardens in charge of the ex situ groves in northern Georgia submit annual seed production reports to the agency.** Annual estimates of numbers of seeds produced is a crucial factor for (a) monitoring the health of the groves and (b) deciding whether existing and potential experimental plantings can be awarded seeds numbering in the thousands, such that direct seed planting is a practical and low-cost option. Note: Substantial support for this mandate exists in the set of communications that ensued in 2018 when I launched a **FOIA request for numbers of annual seed production** from both ex situ plantings in northern Georgia (from 2007 through 2017).

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