Fungal Endophytes in Mediterranean Oak Forests: A Lesson from *Discula quercina*

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ABSTRACT

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Fungal endophytes that colonize forest trees are widespread, but they are less well known than endophytes infecting grasses. The few studies on endophytes in trees mainly concern the tropical areas and the northernmost latitudes, while similar investigations in the Mediterranean region have so far been scarce and incidental. Endophytes are studied mostly in economically important forests suffering from diseases, such as oak forests. One common endophyte that has received some study on oak is the mitosporic *Discula quercina*. This paper, after first addressing some basic problems on tree endophytes, examines the ecology of *D. quercina* in Mediterranean oak stands. *D. quercina* is usually viewed as a symp-

tomless colonizer of healthy *Quercus cerris*, infecting new leaves early in the growing season, in an unstable equilibrium between transient mutualism/neutralism and latent pathogenesis. It is postulated here that climatic factors can change the endophytic nature of *D. quercina*, turning it into a weak pathogen or an opportunistic invader of senescing and indeed healthy trees. It is argued more generally that stochastic events can cause the lifestyle of an endophyte to switch from beneficial/neutral to pathogenic, transforming the tree-endophyte interaction, an interaction that depends in part on the matching genomes of the tree and endophyte, and on the environmental context.

Additional keywords: symbiosis, parasitism, fitness, drought, conflict of interest.

Fungal endophytes are mitosporic and meiosporic ascomycetes that reside asymptotically in the internal tissue of plants, beneath the epidermal cell layer, where they colonize healthy and living tissue by means of quiescent infections (123). The range of interactions between these microbes and living phototrophs extends from mutualistic to antagonistic depending on a variety of factors such as the mode of transmission, plant age, nutrition, the environmental conditions, allelochemistry, etc. (43,95). Asymptomatic, systemic, and seedborne fungal endophytes infecting grasses, for example, spend all, or nearly all, their lifetime in the host plant with only limited or no pathogenic effect (98), or they even benefit the host, protecting it from insect and microbial attacks and mammalian herbivores, and enhancing growth, mineral stress tolerance, etc. (29,67). Alternatively, in long-lived woody perennials the nonpathogenic occupation of the inner plant tissues may be ephemeral, and the fungus can switch to active growth, with saprobic exploitation of the substrate when the plant is subjected to physiological stress and/or senescence (14,23). In woody plants, an unpredictable period of fungal latency follows early, inconspicuous infections, but active invasion can resume if the tree is subjected to physiological stress (109). Tree or organ injury, stress or death may trigger endophytic fungi to grow actively into their hosts, producing symptoms and causing disease (56,77).

These hidden microorganisms occur in a range of natural ecosystems, and form associations with an array of members in the plant kingdom, from vascular plants (spermatophyta and pterophyta) to marine algae (thallophyta) and mosses (bryophyta) (5,88,107).

Fungal endophytism is not a new concept. As early as 1866 Anton de Bary (35), by joining two Greek words, coined the term endophyte to denote the mycoflora living in the inner tissues of plants. Despite their early discovery, these microbes were long considered a mere mycological curiosity, fulfilling no function. In the last decades, however, research has begun to shed a new and fascinating light on the origin of fungal endophytes and their biological significance. The new view considers endophytes important ecological agents, whose partnership with photosynthetic plants has been instrumental for the evolution of terrestrial flora (75). As a result, research on endophytes has burgeoned in the last few years.

The renewal of interest in fungal endophytes is in part also linked to the greatly improved investigative tools that now enable these fungi to be studied in much greater detail (60,74,100). Molecular markers have recently become a staple in laboratory work on endophytic fungi and have enabled this microbial community to be subjected to intense biological scrutiny. Conventional approaches, mainly based on the isolation of the fungi on culture media, followed by an evaluation of assayable morphological, physiological, or other phenotypic traits, are now being increasingly supplemented by highly discriminant DNA-based techniques. These methods are advancing our knowledge of taxonomic units, leading to a more accurate identification and phylogenetically congruent accommodation of sterile morphotypes and unculturable fungi, and expanding our appreciation of the breadth and depth of fungal endophytic diversity (2,3,54,55). Besides the fine-level partition of diversity provided by culture-independent DNA profiling techniques, other molecular surveys have examined host affinity, colonization patterns, the biochemical arsenal of endophytes, the genetic basis of adaptation to an endophytic lifestyle, and made possible functional genomic analyses of how endophytes interact with plants and plant enemies (33,38,61,99).

The endophytes studied so far have mostly been those living in grasses, especially agricultural crop plants. Endophytes in the

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ascomycetous Clavicipitaceae (Hypocreales) living in symbiosis with grasses in the Poaceae have been studied more than any other (98).

Fungal endophytes in forest trees have been researched much less. The profits of forests are not as immediate or as substantial as those from agriculture and they are often indirect (providing ecological and social benefits). Some experiments have been conducted on endophytes in tropical forests (4,6,22), in coastal mangroves (63,72) and in evergreen forests at high latitudes (1,70,71,96), but endophytes of woody angiosperms and gymnosperms in the Mediterranean region have so far been relatively neglected (31,78,85).

This paper first addresses the main difficulties in attempting to determine the composition of the endophytic fungal assemblage in forest trees and examines why this microbial resource, while investigated in some native stands, has been overlooked in others. It then discusses on the biology and ecology of *D. quercina*, a frequent colonizer of healthy as well as senescent oaks in the Mediterranean Basin, with a preference for certain hosts and host organs. The role of *D. quercina* in particular, and of fungal endophytes in general, is discussed in the light of predictions that these specialized fungi may either act as mutualists, conferring benefits on their hosts, or may, under certain conditions, become pathogenic, causing damage to host structures and functions. An analysis of the oak-*D. quercina* interaction will provide a unifying paradigm of the forces driving the plant-endophyte relationship in natural forests generally.

PROBLEMS IN RESEARCH ON FUNGAL ENDOPHYTES OF FOREST TREES

Keeping track of the endophytes in trees is not an easy task, because it is beset by a number of technical difficulties and problems. The biology of the endophytes presents certain problems, for example the fact that they often cause no symptoms in the host, their sometimes uncertain mode of transmission, and variations in their infection rates. One important operational problem is that of the requisite sampling scale when working with woody hosts (48). The longevity of trees, the diversity of their organs and tissues (buds, flowers, leaves, twigs, branches, trunk, roots, bark), and the varied local environment of different tissues lead to many different niches which are colonized by more or less differentiated sets of endophytic fungi (12), in contrast to endophytes colonizing grasses which are generally systemic. This presents major challenges for measuring endophyte infection and for detailed studies of populations of endophytes in forest trees (106).

Several other drawbacks hamper the precise determination of endophytic diversity: the methods to isolate them and nutrient media may be selective (55); (i) some endophytes may be unculturable (28); (ii) slower-growing endophytes may be overgrown by those with faster growth (21); (iii) infrequent and rare fungi, which contribute largely to the diversity of the overall endophytic population, may not be picked up by the sampling grid, owing to their lower infectivity or a patchy occurrence (53); and (iv) the size of the sampling unit may exceed the size of individual thalli, with more than one taxon occurring within a single sample unit, so that some taxa remain unnoticed (32). As a result, a fair number of endophytes, especially those whose whole life-cycle is spent in apparently symptomless infections, may remain undetected and uncharacterized. When these endophytes are excluded from a survey, the fungal population size is necessarily underestimated (24,69). These problems pose a major challenge to a rigorous description of the distribution of fungal endophytes in forest trees. That conventional determination of endophyte levels is often inaccurate is confirmed by DNA-based detection: many endophytes baffle isolation/cultivation attempts and have only been identified by their molecular fingerprints (15,65).

MICROBIAL LIFE IN MEDITERRANEAN OAK TISSUES: PEEKING INTO A BLACK BOX

According to Shardl et al. (98), the most commonly investigated symbioses are those that cause disease to the plant, especially the aerial parts of vascular plants, which are exposed to a great variety of pathogenic microbes, in a constantly changing physical environment. However, much research has also been carried out on below-ground mutualistic symbioses, such as mycorrhizal fungi and nitrogen-fixing rhizobacteria (57,119).

Much of the work on above-ground plant-microbe interactions has focused on those interactions that decrease host fitness (parasitism), because they were harmful and conspicuous in their effects. By contrast, nonpathogenic associations increasing host fitness (mutualism), have tended to be overlooked because they are internal and hidden. This has occurred in both agricultural systems and natural forests. The most intensively studied forests were those that suffered from dieback or decline. Decline symptoms, classified as manifestations of "complex diseases," often baffled investigators because it proved impossible to identify discrete etiological agents. Over the past few decades there has been widespread dieback and mortality of oak trees with no easily identifiable cause in many European forests (103). The symptoms and signs of the decline noted often did not conform to any known syndrome, as different biotic entities interacted with adverse abiotic factors such as water deficit, excess moisture, temperature extremes, large-scale flooding, etc., in a complex sequence of events whose final outcome also depended in part on the local environment. This mix of causes produced a variety of disorders in oaks, with symptoms that varied from site to site, but that were collectively recognized as defining an oak decline syndrome (102,114). Oak stands were thoroughly investigated to determine any changes that could have caused this decline. As part of these investigations, detailed surveys were carried out that included the fungi associated with declining trees.

These surveys revealed that oak forests harbored a vast array of fungi, many of which had not been known on oaks before, and whose identification is still far from being completed. In a literature survey of the microorganisms associated with oak decline in Europe, Ragazzi et al. (87) counted 210 fungi, 2 chromista, 14 bacteria, 1 phytoplasma, and 1 virus. The fungi included *Amphicytostroma quercinum* (Sacc.) Butin, *Apiognomonina quercina* (Kleb.) Höhn, *Armillaria* spp., *Biscogniauxia mediterranea* (De Not.) Ces. et De Not., *Colpoma quercinum* (Pers.) Wallr., *Diplodia mutila* Fr. apud Mont., *Phomopsis quercina* (Sacc.) Höhn., and several oomycetes, such as some *Phytophthora* spp. A multitude of other fungi, playing more or less important roles in the decline, was added to the list in later surveys (9,46,47,62). All these surveys distinctly show that the mycoflora associated with oaks, whether healthy or declining, was superabundant and extremely diverse. Recent studies have gradually made it clear that many of the fungi that were initially thought to be contributing factors in oak decline were in reality tree endophytes that caused symptomless infections in healthy oaks or at most maintained low levels of symptomatic infections on debilitated trees (49,82).

DISCULA QUERCINA: A CASE STUDY FOR OAK-ENDOPHYTE RELATIONSHIPS

Life history. The anamorphic coelomycete *D. quercina* (West.) Arx. has long been known as a common endophyte on oak (92). It belongs to a genus whose members exhibit considerable variation in morphology, physiology, and host specificity. Some *Discula* species have teleomorphs in the genera *Apiognomonina* or *Gnomoniella*, but most do not exhibit a known sexual stage and are only identified as asexually reproducing ascomycetes. *D. quercina* is recognized as the asexual state of *A. quercina* (Kleb.) Höhn (Valsaceae, Diaporthales).

Together with its teleomorph, *D. quercina* is responsible for a disease known as oak anthracnose. Anthracnose is the epithet commonly given to a group of diseases, caused by several related fungi that attack many woody hosts. Variations in morphology, behavior, and ecology exist among these anthracnose-causing fungi, and that is why they cannot be amalgamated into a single taxon. The anamorphic *D. quercina*, for example, is morphologically distinct from the fungi that cause anthracnose in other hardwoods, such as ash and sycamore. A fair degree of polymorphism has also recently been revealed between *D. quercina*, *D. umbrinella* (Berk. et Br.) Morelet, *D. destructiva* Redlin, and other *Discula* spp. by means of DNA fingerprinting (20).

D. quercina frequently occurs on the leaves and twigs of declining oaks. At its symptomatic stage, it causes irregular brown patches and spots on the leaves, particularly along the midrib. On the twigs it induces small, elongated cankers that develop in sequence, and that are characterized by some of the bark splitting along the border because of callus formation by the host. On both leaves and twigs *D. quercina* differentiates its conidial structures in minute black pycnidia. The teleomorph, like most Gnomoniaceae, remains inconspicuous in the leaf litter, producing rather larger black perithecia in the following spring. Some of the biological and epidemiological characteristics of these metagenetically related morphs have been determined: their growth rate on various substrates; (i) heterokaryon formation and occurrence of vegetative compatibility groups (VCGs); (ii) the infection ability of their ascospores and conidia; (iii) optimum conditions for host infection (temperature, light, relative humidity, and photoperiod); (iv) their specificity to various hosts and host organs; (v) their colonization pattern on different hosts and host tissues (in relation also to the host's physiological state and phenological phase); (vi) and their disease incidence and severity (78-81,83,84,86).

Host and organ specificity. Mediterranean oak species vary in their response to *D. quercina* infection, with *Q. cerris* L. being the most susceptible (78,84). *D. quercina* isolates collected at distant geographic areas from *Q. cerris*, *Q. frainetto* Ten., *Q. pubescens* Willd. and *Q. robur* L. individuals, and from related hosts in the family Fagaceae (*Castanea sativa* Mill. and *Fagus sylvatica* L.) on which the fungus occurs in the same habitats (mixed stands), revealed a certain degree of host specialization when they were artificially inoculated back to seedlings of the same species. With the exception of isolates from beech and chestnut, neither of which infected the other, isolates from all the hosts infected all the other target hosts on which they were sprayed. Each species and provenance however was more prone to infection by its own source isolate, as indicated by the number of cankers produced and the mortality rate recorded at fixed time intervals. Measures of disease severity again proved *Q. cerris* to be the most susceptible to *D. quercina* (84). The predominant occurrence of a strain on a given host or range of hosts was already reported for *D. umbrinella* on hosts in the genera *Castanea*, *Fagus*, and *Quercus* (116,117), for the teleomorph of *D. umbrinella*, *Apiognomonia errabunda* (Rob.) Höhn on *Fagus sylvatica* L. (106), as well as for a number of other pathogenic, saprobic, and mutualistic fungi (18,26,77,105).

That some *D. quercina* strains were host-specific was further suggested by in vitro experiments (80). A cross-section of *D. quercina* isolates from several Italian locations was tested for growth rate, acervuli formation on various nutrient media, heterokaryon formation capacity, and vegetative compatibility group (VCG) occurrence. Isolates from *Q. cerris* always had the highest growth rate and always produced acervuli. Isolates from *Q. pubescens*, on the other hand, produced acervuli less often, and *Q. frainetto* and *Q. robur* never did so. Three phenotypic classes of nitrate nonutilizing (nit) mutants, nit₁, nit₃, and nit_M, were recognized on the basis of their ability to exploit different nitrogen sources, and five VCGs were identified which correlated with an oak host (80).

Additional findings demonstrated that *D. quercina* had a specific preference for *Q. cerris*. Investigations at several hierarchical levels (tissue, host, site, and stand) indicated that this fungus was a dominant component of the species-rich fungal assemblage colonizing this tree. It was isolated from both healthy and declining *Q. cerris*, from current-year twigs and buds, and from leaves at various phenological phases (budbreak, full vegetation, or abscised), irrespective of the soil, the altitude, or the floristic composition of the stand (81,83). A similar colonization pattern independent of site characteristics and stand composition was also reported for *D. umbrinella* (118), but with other fungi these factors did appear to have an influence on isolation frequency (IF) (105). The IF of *D. quercina* peaked in June, then declined gradually, with the lowest values being reached in October. Variations in IF during the growing season were also reported for a *Discula* sp. on *Fagus crenata* Blume (94), and for *A. errabunda* on *F. sylvatica* (106). The spatiotemporal colonization pattern of *D. quercina* on *Q. cerris* was mostly similar to that on *Quercus garryana* Dougl. (124).

The sexual and asexual forms of oak anthracnose differ by reproductive and ecological traits. The mitosporic *D. quercina* more frequently colonizes the twigs of (declining or healthy) oak trees, whereas the meiosporic *A. quercina* is mostly found on the leaves (78). Allotopic tissue colonization between forms (organ specificity) is not a novelty with this group of fungi: von Arx (121) differentiated several *Discula* spp. into leaf and twig forms and also into summer and winter forms.

The two forms also exhibited a different host preference: *D. quercina* was mainly restricted to *Q. cerris*, and occurred less frequently on *Q. pubescens*; whereas *A. quercina* infected a wider range of oak species: *Q. cerris*, *Q. frainetto*, *Q. pubescens*, *Q. robur*, and *Q. suber* L. (78). Host selectivity appears to be a common feature of members in the genus *Discula*, having also been reported for *D. umbrinella* on *Q. alba* and *Q. rubra* on the basis of estimates of endophytic infection, pathogenic response, and mycelial compatibility (30).

Towards niche restriction? Differences in host and organ preference suggest that subspecific taxa may occur within the oak anthracnose holomorph, though isolates from different sets of hosts are undistinguishable by their morphological attributes alone.

A recent investigation of the population structure of *D. quercina*, based on sequence analysis of traits of interest from the mitochondrial small subunit ribosomal DNA (mtSrDNA), from the nuclear rDNA region containing the internal transcribed spacers and the 5.8S rRNA gene (ITS1-5.8S-ITS2), and from genes coding for actin, β -tubulin, the elongation factor, and glyceraldehyde phosphate dehydrogenase (GPD), suggested that this could well be so: complete homology (100%) between isolates was found in the mtSrDNA and elongation factor sequences, but some nucleotide variation was detected in the rDNA internal transcribed spacer (ITS) region, and in the actin and β -tubulin genes, which supported the hypothesis that there was a selective strain occurrence in *Q. cerris* (68).

The factors governing this difference in host preference, with the anamorph colonizing *Q. cerris* and *Q. pubescens*, and the teleomorph occurring on a wider range of hosts, are still unclear and require further study. So far, only a fraction of the Mediterranean oak flora has been examined for fungal endophytes. A mere lack of records must not be taken to mean that these agents do not occur on any oak species that have not yet been examined for them. *Colletotrichum* spp. for example were found on only one host, *Q. ilex*, by Fisher et al. (44), but in that study these authors already speculated that this was probably due only to the paucity of other potential host species examined for this fungus. On the other hand, several pathogenic species in this genus are known to colonize nonpathogenically a number of nonhost plants, a behavior that is common to many plant pathogenic fungi (101).

In both antagonistic and mutualistic symbioses, host preference is a diffuse phenomenon in nature, residing at the interface between ecology and evolution, and still not fully understood. Classic evolutionary theory predicts that the ecological range and niche breadth of organisms living in symbiosis will be greater than those of free-living organisms (11). This is surely true for symbiotic metaorganisms (e.g., lichens), in which species co-operation guarantees greater range and breadth, with enhanced metabolic benefit to both partners. In the symbiosis of fungi and vascular plants, on the contrary, the adaptive process resulting in specificity leads to a restriction in the ecological range of symbionts. The microbial partner is constrained by its lifestyle to limit the niches it exploits, and ends up depending only on a few or a single resource (host) for its survival. In the fungal kingdom, a lack of specificity is considered a characteristic more typical of saprobic fungi (125), whereas the host selectivity displayed by *D. quercina* rather suggests a more specialized relationship.

Coevolution of matching traits. Host specificity, or the occurrence of a strain only on one specific host, or range of hosts (125), requires a close adaptation between the plant and its fungal partner. This suggests a mutual influence deriving from a long-standing cohabitation and coevolution, as the microbial partner depends in its own evolution largely on the evolutionary processes of its host. This protracted association in the long run becomes permanently imprinted in the genetic constitution of both partners that end up developing complementary genetic systems. Host specificity thus has a genetic basis, the plant-microbe relationship being dependent on a tight and specific interaction between the microbe genotype and the host genotype. The fungus-plant relation must therefore be viewed as a flexible interaction, whose directionality is determined by slight differences in fungal gene expression in response to the host, or conversely by the host recognizing and responding to the fungus. Subtle genetic differences in the genomes of both partners control the outcome (positive, negative, or neutral) of the symbiosis.

This type of interaction, which is a driving force in evolution, is well-documented in antagonistic symbioses. In many pathosystems, a co-evolutionary interplay has been demonstrated between disease resistance genes in the host and avirulence genes in the pathogen (45,59,115). That there is gene recognition and adaptation in such interactions is demonstrated also by diseases involving the production of host-specific toxins by a pathogen (16).

On the other hand, in mutualistic, nonpathogenic symbioses, the partners have a long-standing, coordinated association, as is witnessed by the widespread phenomenon of co-cladogenesis of host and symbiont (29,58). This allows the partners to evolve strategies and molecular machines for combined adaptation and defense. A clear sign of reciprocal recognition and adaptation is the lack of elicitation of plant-defense reactions following microbial infection (27). Evidence of coevolution is also provided by the metabolic affinity of the symbionts, as is shown by the fact that they commonly produce the same antimicrobials active against both phytopathogens and herbivores (108,111).

A striking proof of such a coevolutionary path is also provided by the coordination of the fungal and of the host reproductive systems, a strategy that again reduces the potential for conflict among the partners (17,42,97). This is especially evident in vertical transmission, where both partners benefit from successful reproduction by the host. In the long run however, this trend leads to a reduction of the genetic diversity of the microbial symbiont, by excluding inputs of external genetic material, and by creating a bottleneck at each generation. The genetic homogeneity of the symbiotum thus increases, and selection for traits such as to increase competitive ability, that may be detrimental to the partner, is reduced (58).

An unstable relationship. Mutualism is ubiquitous and dominant in nature, and it is profoundly influential at all levels of biological organization, from ecosystems to molecules. Even at

the most minute scale of life, at the subcellular level, from the mitochondria providing energy for eukaryotic cells, to the single gene, the smallest "symbiotic unit," working within the gene pool to perform critical cellular functions, parallel and cooperative stories abound (34).

Mutualistic fungal symbiosis is widespread both geographically (occurring in a wide range of ecosystems and at different latitudes and altitudes), and taxonomically (forming partnerships with representatives from different kingdoms). Mutualistic fungi form relations with: plant roots (mycorrhizae) (50), the rumen of mammalian herbivores (51), algae to form lichens (93), ants in ant gardens (25), insect pollinators (e.g., some rust fungi, in order to reproduce sexually) (91), as well as grasses and woody plants (endophytes). The varied benefits provided by this last group of fungi to their hosts (growth promotion, insect feeding suppression, metal tolerance, etc.) have been pointed out by several researchers (19,73,76,90,110) and have been amply reviewed by Saikkonen et al. (95).

Mutualistic interactions are generally conceived of as a sort of exchange of goods and services (e.g., food in exchange for protection). This view is attractive but misleading. Mutualism does not involve any cleverness or foresight on the part of the interacting partners—microbes, cell organelles, and genes obviously do not have a conscious intention to create a reciprocally beneficial relationship. Most mutualistic associations originate in a one-sided exploitation that, as a matter of pure chance, ends up benefiting both partners (37). It is the overall balance between the costs and benefits to each partner that determines the outcome of the interaction, i.e., whether the association is mutualism or a more competitive form of interaction.

It has become increasingly evident of late that the symbiosis fungi establish with some eukaryotes is subject to variations over time in response to spatial and/or temporal changes in the physical or biotic environment, in a continuum of oscillations between mutualism and antagonism (95).

The plant-endophyte association is intriguing in that its members spend most or all of their lives in close physical contact. By its very nature, such partnerships will be intimate and complex, with mutualism being just one of a number of possible outcomes (93). Conflicts of interest in a plant-endophyte association arise when the survival and reproduction of one member of an interaction do not conform with those of the partner. This is what seems to have happened in the oak-*D. quercina* relationship in the Mediterranean area in recent times. The Mediterranean climate has an irregular annual rainfall pattern, peaking in autumn and winter and with a long dry period in late spring and summer. Drought stress in the hotter months may have a major impact on the growth and health of Mediterranean oaks, causing dieback and mortality to the younger individuals and the less tolerant genotypes. Various studies have found that in grasses endophytes have a beneficial effect enhancing drought tolerance (in terms of hormonal control of stomata, negative stomatal conductance, osmotic adjustment, etc.) (7,41,67,98). Such endophyte-mediated recovery or repair has been largely unexplored in other plants and has not been studied in trees. However, the seasonal pattern of tree tissue colonization indirectly suggests that endophytes might alleviate drought stress. In some oak stands, the infection load of *D. quercina* generally peaks in spring, when rainfall is not a limiting factor, and then becomes lower in the hotter and drier summer months, when rainfall is much less and trees tend to suffer from a water deficit (81,124). The same seasonal infection pattern has also been reported for the related *Apiognomonium errabunda* on beech (8). Such a colonization pattern, however, does not mean that the endophyte reduces its aggressiveness because the host is water stressed, but rather that drought has a direct negative effect on the endophyte itself (36). Endophytic fungi thrive in spring and early summer, when rainfall and relative humidity are high and the host is not physiologically stressed by water shortage. At

this time trees are actively vegetating, with shoot sprouting and leaf expansion, and the xylem vessels are large enough to ensure an optimal sap flow. Free water and high moisture in the oaks obviously favor fungal activity and host colonization (64,112, 120). But this favorable situation changes as the onset of drought induces water scarcity. A first effect of water shortage is to reduce water availability from the apoplast. A low water potential reduces overall fungal viability, as shown by lower mycelium development and infection levels (39,40,52,113); this explains the reduced endophyte load at the end of the growing season. In the Mediterranean area, where drought is a cyclical summer phenomenon, such seasonal infection patterns are the norm.

A different scenario emerges however when drought extends beyond summer to parts of spring and autumn and persists for a number of years in succession. Persistent droughts have become more and more common in the Mediterranean area over the last three or four decades as a consequence of global warming (104). Many fungal endophytes have a much greater plasticity than the plants harboring them, and remain viable and active at water potentials well below those critical to their hosts (13,39,66). This means that a seasonally prolonged and yearly repeated drought cause a disruption of the plant water regime and, soon after, of the very delicate tree–endophyte relationship. The endophyte then ceases to be neutral or mutualistic and becomes pathogenic. In the case of *D. quercina*, the endophyte switches from intercellular growth to epiphytic growth, colonizes drying tree tissues (weakened and senescing from water stress) more aggressively, spreads out over the tree surface, and sporulates. These drought-related changes in the nature of fungal infection have already been observed in oaks (122) and also in other forest tree species (36). Other possible consequences of exceptional drought episodes that merit investigation are the favored survival of the more thermophilic fungal genotypes, and of hypervirulent strains from low-virulence populations (10).

All these aspects, though recognized as being crucial, have been poorly studied in forest trees, especially in the Mediterranean Basin. This represents an evident gap in our knowledge, as the effects of drought in the long term (spanning several years) on the plant–endophyte relationship can be properly investigated only on perennial plants, preferably on trees growing in unmanaged forests. On annual crops, and on all cultivated plants in general, such investigations can provide only limited insights because of the short lifespan of plants and of the human manipulation of agricultural systems.

CONCLUSIONS

The behavior of *D. quercina* in native oak forests support the hypothesis that factors external to the oak–endophyte association can alter the very delicate relationship between oak and endophyte. Depending on the tree physiology and local environmental conditions, innocuous endophytes can turn into phytopathogens inducing infection symptoms in their hosts when these become senescent or water stressed. The damage ranges from a reduction in the growth rate and reproductive ability to mortality (89). Stochastic events, such as abiotic or biotic constraints, thus can have a strong effect on the nature of the interaction between these two partners, causing it to shift in the direction of antagonism. The oak anthracnose agents usually induce quiescent and asymptomatic infections in healthy trees. Fungus fructifications and disease symptoms (leaf necroses and twig cankers) develop only after the host organs undergo a certain degree of natural senescence (78). The transition phase between quiescent infection and active host colonization is temporally indefinite, suggesting that for oak anthracnose, the distinction between pathogenic and nonpathogenic colonization is a mere academic exercise. But this delicate balance is not fixed forever across all populations and years. Spatial or temporal variation in water availability may

cause physiological stress in oaks and this in turn may induce agents of anthracnose to colonize their hosts and incite disease.

Ultimately, the lesson that is being learned by the study of the epidemiology of *D. quercina* in Mediterranean oak forests is that the elements shaping the plant–endophyte interactions in nature are complex. The genetic systems of the partners clearly play a key role in becoming adapted to each other, but the partners are also acted upon by a multiplicity of extraneous factors, such as environmental or ecological variables, other microbes, etc. Their life history traits are thus shaped by external forces that act multidirectionally, involving multi-species interactions and a great many levels of causation (95). The capacity of Mediterranean oak forest ecosystems to withstand natural disturbances is limited, since their ecological integrity has become much impaired by millennial anthropic pressure. A disturbance, like an extended drought period, can have profound immediate effects on these fragile ecosystems, greatly altering the communities of organisms living inside. A consequence of the stress caused by drought is to channel the evolutionary trajectory of plant–endophyte relationships in the direction of detriment.

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