GENOME-WIDE ANALYSES OF THE DUTCH ELM DISEASE FUNGI

Louis Bernier[1]

Abstract.—The Ascomycete fungi *Ophiostoma ulmi* and *O. novo-ulmi* are the pathogens respectively responsible for the two successive pandemics of Dutch elm disease (DED) since the early 1900s. The advent of the highly fit and virulent *O. novo-ulmi* was a landmark event in the evolution of DED during the last 100 years. This, however, may not be the last major shift in the behavior of the pathogen and elm breeders must keep in mind this possibility as it bears consequences for ongoing elm improvement programs. This contribution reviews some of the current knowledge on the biology of the DED fungi, including findings from genome-wide analyses carried out during the last decade. Biological traits believed to be important for parasitic fitness of *O. novo-ulmi* are presented. Events that might allow the DED fungi to evolve further are proposed and discussed in the context of elm resistance breeding.

Introduction

Few diseases of trees have had as much impact in the last 100 years as Dutch elm disease (DED). Along with chestnut blight and white pine blister rust, DED is a textbook example of the destruction caused by fungal pathogens upon gaining access to new territories and host species. In the case of DED, two successive pandemics have killed an estimated 1 billion elms (Paoletti et al. 2006) native to Eurasia and North America, as well as elms introduced to New Zealand (Ganley and Bulman 2016). The sudden and spectacular development of the disease has led to prognostics of elm extinction or, at best, survival of elms as a scrub or understory population, with a few mature elms as escapees (Brasier 1983) in areas outside central and eastern Asia where native elm species are usually highly resistant to DED.

Breeding for disease resistance is one long-term proven approach for managing losses caused by pathogens and pests of trees. The first elm resistance breeding program was launched more than 80 years ago in the Netherlands by Christine Buisman and resulted in the selection and release of a few cultivars that were considered resistant to DED (Holmes and Heybroek 1990). Soon, however, this material had to be replaced with new cultivars as it became evident that the DED fungus had become more virulent. This situation is, of course, not unique to DED since resistance breeding faces a unique challenge as the pathogen, being a living organism with a short life cycle, has the potential to evolve and change within a few years. Nevertheless, there are many cases of successful resistance breeding programs in a wide variety of agricultural crops and forest tree species. In the case of DED, most published success stories concern European elm breeding programs, for reasons that are beyond the scope of this contribution. The 2016 American elm restoration workshop and proceedings, however, provides a unique opportunity to discuss the status of elms native to North America in the face of the ongoing pandemic of DED, revisit the genetics, biology, and physiology of elms, and showcase some of the most promising avenues for maintaining these taxa as part of the landscape. These topics will not be discussed here. Rather, the scope of this contribution is to provide an overview of the biology

[1] Professor of Forest Pathology and Fungal Genetics, Université Laval, Centre d'étude de la forêt (CEF) and Institut de biologie intégrative et des systèmes (IBIS), Pavillon Charles-Eugène-Marchand, 1030 Avenue de la Médecine, Québec (QC) Canada G1V 0A6. To contact, call 418-656-7655 or email at Louis.bernier@sbf.ulaval.ca.

of the DED pathogens and further discuss life traits that may contribute to parasitic fitness. Focus will be placed on the potential for the DED pathogens to adapt to changes occurring in their hosts following natural selection and resistance breeding. The discussion will thus include a retrospective look at changes that have occurred in the pathogen since the onset of DED, as well as a prospective assessment of what elm breeders may be faced with in the future. Some of the information included here was already presented in more detail in previous reviews (Bernier 2016, Bernier et al. 2015). On the other hand, several ideas are discussed for the first time in this contribution, ideas inspired by the latest findings in the molecular biology and genomics of the DED fungi and related species, as well as by unpublished results from ongoing investigations.

Taxonomical Status of the DED Fungi

For many years, DED was considered to be caused by one fungal pathogen referred to as *Ceratocystis ulmi*. In the late 1980s, the fungus was reassigned to the genus *Ophiostoma* and hence became known as *O. ulmi*. In 1991, however, researchers formally recognized the occurrence of two distinct DED pathogens, *O. ulmi* and *O. novo-ulmi*. *Ophiostoma ulmi* caused the first pandemic which started in the early 1900s and lasted until the end of the 1960s in most areas impacted by DED, whereas *O. novo-ulmi* is responsible for the second, ongoing pandemic of DED which is believed to have started in the late 1940s (Brasier 1991). Two geographic subspecies, designated *novo-ulmi* and *americana,* are recognized in *O. novo-ulmi* (Brasier and Kirk 2001). As indicated above, *O. ulmi* and *O. novo-ulmi* are responsible for the drastic decline in elm populations in Eurasia, North America, and other parts of the world where elms have been introduced. A third DED fungus, *O. himal-ulmi*, has also been reported in northeast India but is not associated with a disease epidemic (Brasier and Mehrotra 1995). The DED fungi are phylogenetically related to several species of saprophytic *Ophiostoma* species, which cause sapstain (or blue stain) in various deciduous and coniferous tree species (Fig. 1).

Because of changes in the taxonomy of the pathogens, discovery of cryptic taxa, as well as use of outdated nomenclature by some authors, the DED literature can be confusing for nonspecialists. For example, North American isolates of the highly aggressive strain of *Ceratocytis ulmi* mentioned in earlier reports likely represent isolates of *O. novo-ulmi* subsp. *americana*. The latter seems to be the only taxon found in North America in the last decades (e.g., Houston 1991), whereas both subspecies co-occur in several areas in Europe (e.g., Brasier and Kirk 2010, Tziros et al. 2017).

Salient Biological Traits of the DED Fungi and Other Ophiostomatales

All DED fungi are pathogens but exhibit varying degrees of virulence (used here in a quantitative sense). *O. ulmi* is less virulent and was previously referred to as the "nonaggressive strain." Nevertheless, *O. ulmi* can kill more susceptible elm species such as *U. americana*. *Ophiostoma novo-ulmi* is highly virulent and can kill elm species that were tolerant to *O. ulmi*. The third DED fungus, *O. himal-ulmi*, was recovered from symptomless *Ulmus wallichiana*. Technically, it could be considered as an endophyte on this host but has been shown to induce typical DED symptoms when inoculated to European elms (Brasier and Mehrotra 1995).

The DED fungi are vascular pathogens that have evolved several traits (discussed below) that allow them to colonize the water-conducting vessels in the xylem. There is evidence that these pathogens also have an effect (direct and/or indirect) on the parenchyma cells surrounding the vessels (Rioux and Ouellette 1991, Tippett and Shigo 1981). The DED fungi gain access to

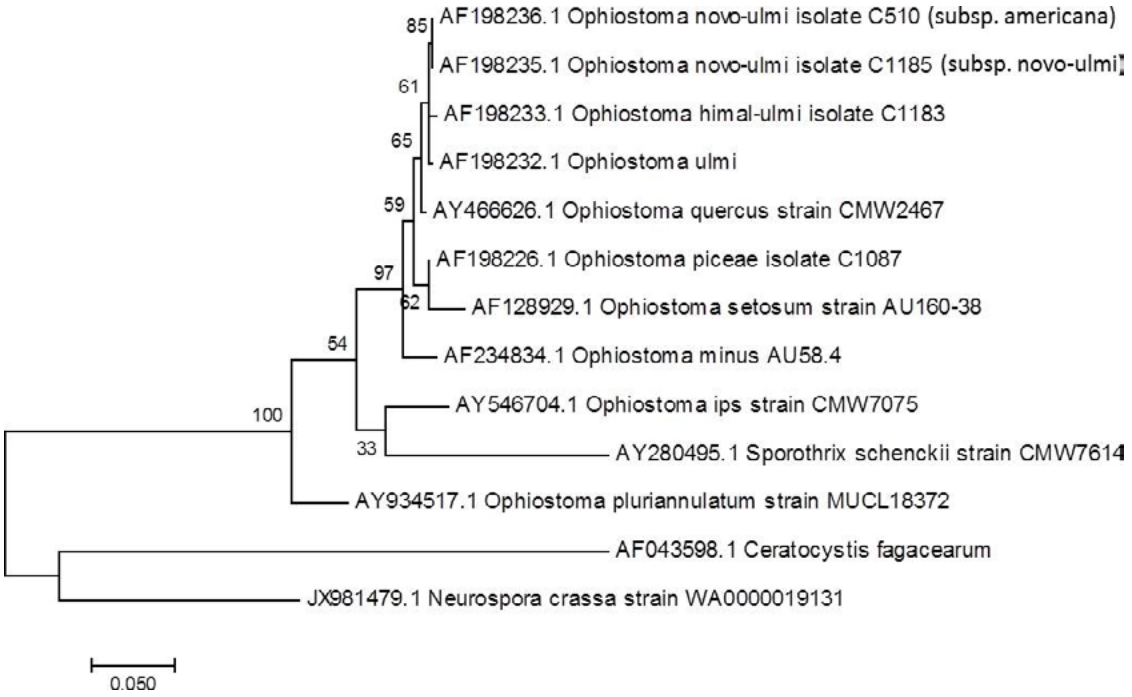

Figure 1.—Phylogenetic relationships between the Dutch elm disease (DED) fungi *Ophiostoma ulmi*, *O. novo-ulmi,* and *O. himal-ulmi*, and other Ascomycete species.

Internal Transcribed Spacer 1 (ITS1) sequences were downloaded from the National Center for Biotechnology Information (NCBI) database. Sequences were aligned and concatenated with BioEdit 7.2.5 software (Hall 1999). A maximum-likelihood tree was constructed with Mega6 with 1000 bootstraps (Tamura et al. 2013). Bootstrap support is indicated on the nodes. The DED pathogens are closely related to sapstaining *Ophiostoma* species, including *O. quercus*, and to the human pathogen *Sporothrix schenckii*. *Ceratocystis fagacearum*, the oak wilt pathogen, is a distant relative. Figure created by Martha Nigg. More exhaustive phylogenetic trees including the DED fungi and other Ophiostomatales can be found in De Beer et al. (2016) and Ploetz et al. (2013).

the xylem of healthy elms through their association with elm bark beetles in the genera *Scolytus* and *Hylurgopinus* (Fig. 2). In the field, the DED pathogens appear to be specific to species in the genus *Ulmus*. Results from controlled inoculations of *Prunus pensylvanica* with *O. novo-ulmi*, however, have shown that the potential host range of the DED fungi extends beyond the range of its vectors (Rioux and Ouellette 1989).

Based on the extensive mortality incurred by elms worldwide in the last 100 years, it is obvious that the DED fungi are very efficient pathogens. Not surprisingly, there have been several studies devoted to the identification of biological traits that contribute to parasitic fitness (*sensu* Andrivon 1993). Most studies have focused on pathogenicity and several mechanisms have been proposed over the years to explain how the DED fungi kill elms. These include the release of toxins, cell-wall degrading enzymes, inhibitors of elm resistance mechanisms, or a combination of these. None of these mechanisms, however, has been demonstrated conclusively to be a main contributor to pathogenesis. For example, a small secreted hydrophobin, designated cerato-ulmin (CU), was once described as a wilt-inducing toxin responsible for the high virulence of *O. novo-ulmi* (Stevenson et al. 1979, Takai et al. 1983). It is not considered a pathogenicity factor anymore based on the recovery of virulent *O. novo-ulmi* mutants lacking the ability to produce CU (Bowden et al. 1996, Brasier et al. 1995).

The DED fungi exhibit yeast-mycelium dimorphism, a feature found in several fungal pathogens of plants (e.g., *Ustilago maydis*, *Verticillium albo-atrum*) and animals (e.g., *Candida albicans*, *Histoplasma capsulatum,* and the Ophiostomatale species *Sporothrix schenckii*). Several environmental factors will prompt the DED fungi to switch from one growth form to the other

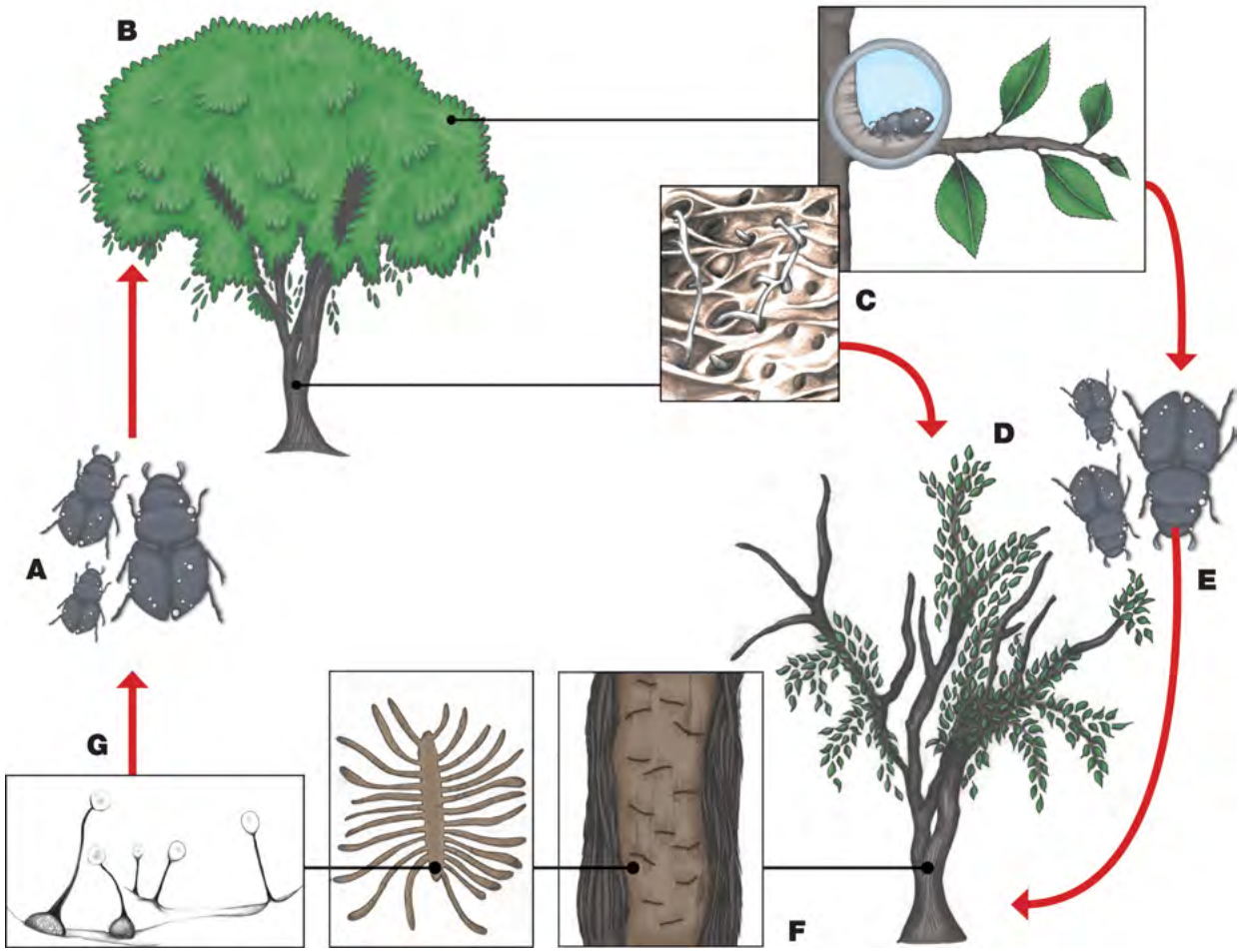

Figure 2.—Dutch elm disease (DED) cycle.

Young elm bark beetles carrying spores of DED fungi (A) and feeding on healthy trees (B) provide the pathogens with access to water-conducting vessels within the xylem. The DED fungi produce both yeast-like spores and mycelium when invading the vascular system (C). Infection of susceptible elms results in wilting and eventually death (D). Virgin female bark beetles looking for suitable breeding sites are attracted by trees that have been killed or weakened by DED. Many of these beetles carry spores of DED fungi (E), which will readily colonize galleries in which females have oviposited after mating (F). Within the galleries, the DED fungi produce reproductive structures (G) including asexual synnemata and sexual perithecia. Spores produced by both structures are embedded in a drop of sticky mucilage and will easily attach to the exoskeleton of young elm bark beetle adults (A) emerging from the galleries. While candidate genes associated with pathogenicity, yeast-mycelium dimorphism, and formation of fruiting bodies have been identified, the molecular bases of the host-pathogen interaction leading to DED remain unknown. Figure created by Marilou Desharnais and reprinted from Comeau et al. 2015, with permission.

(Berrocal et al. 2012, Kulkarni and Nickerson 1981, Naruzawa and Bernier 2014, Naruzawa et al. 2016, Wedge et al. 2016). Results by Richards (1994) suggest that the ability to transition between the mycelium and yeast phases is important for pathogenicity of *O. novo-ulmi*. Ongoing transcriptomic analyses of yeast-mycelium dimorphism in *O. novo-ulmi* have shown that at least 10 percent of the nuclear genes undergo a significant shift in transcription during the transition *in vitro* (Nigg et al. 2015) and that the remodeling of the transcriptome occurs early in the transition (Nigg and Bernier 2016). Furthermore, several genes that may be key regulators of dimorphism, and possibly pathogenicity, have been identified as good candidates for further studies.

The interaction between the DED fungi and elm bark beetles is likely another key component of overall fitness. In contrast to other vector-fungus associations in which the two organisms live in symbiosis, elm bark beetles and DED fungi seem to enjoy a more unidirectional interaction

from which the fungus is the main benefactor. It has been estimated that an individual elm bark beetle may carry up to 350,000 spores on its exoskeleton (Webber 1990). Cerato-ulmin appears to be a key molecule in the interaction between elm bark beetles and *Ophiostoma* spp., as mutants of *O. novo-ulmi* impaired in CU production were shown to be less efficient than wild-type isolates in attaching to insects (Temple et al. 1997). McLeod et al. (2005) suggested that the DED fungi manipulated elms to make them more attractive to elm bark beetles. This hypothesis, if verified, would put the elm bark beetle-*Ophiostoma* interaction in a context very different from the unidirectional view mentioned above.

Dutch elm disease fungi survive as saprophytes in dead or dying elms. This part of the fungal life cycle has received limited attention compared to the pathogenic phase, even though it plays an important role in both the short- and long-term evolution of the disease. Breeding galleries that are dug by beetles under the bark are colonized by mycelium of DED fungi. As reviewed by Santini and Faccoli (2015), fungal isolates found in these galleries may originate either from the pathogenic phase (through movement of the pathogen from the xylem towards the phloem) or from new inoculations by female beetles when they colonize the bark. Not surprisingly, mosaics of genotypes have been reported in populations of DED isolates recovered from inner elm bark (Webber et al. 1986). When they colonize elm beetle galleries, the DED fungi undergo another important developmental change as they produce synnemata, asexual structures bearing synnematal spores. Sexually compatible individuals within the same gallery may mate and produce perithecia containing recombinant ascospores. Both synnemata and perithecia exhibit features that favor acquisition of the pathogen by elm bark beetles, as masses of synnematal spores and ascospores are embedded in droplets of sticky mucilage. Furthermore, the synnema head fluid and the ostiolar hairs on perithecia of *O. novo-ulmi* have been shown to contain high amounts of CU (Svircev et al. 1988, Takai et al. 1980). Fungal genotypes able to dominate the populations occurring within breeding galleries are likely to be preferentially inoculated to healthy elms by young beetles emerging from the galleries during maturation feeding time (Fig. 2).

Origin of the DED Fungi

Based on pollen counts, the first documented case of massive decline of elm populations occurred in western Europe during the early Neolithic, around 5,500 calibrated years before present (Batchelor et al. 2014). This decline has been attributed by some authors to interactions among causal factors including paludification, deforestation for agriculture, and disease. Although macrofossils of two known vectors of DED fungi, *S. scolytus* and *S. multistriatus*, have been found at some sites, there is no evidence that these insects had already acquired the DED pathogens at the time. Therefore, the Dutch elm disease pandemics that have been developing since the early part of the 20th century in Eurasia and North America are, unless new evidence arises to the contrary, a "modern" disease that resulted from the introduction of exotic pathogens. It was widely believed that *O. ulmi*, responsible for the first pandemic, originated from China (Horsfall and Cowling 1978) but the fungus was never found in that country (e.g., Brasier 1990). A more recent hypothesis is that *O. ulmi* originated from Japan (Masuya et al. 2010). Likewise, the geographic origin of *O. novo-ulmi,* responsible for the current pandemic, remains the object of much speculation. Subspecies *novo-ulmi* and *americana* are thought to have originated in central Europe and around the North American Great Lakes, respectively (Brasier 1996). The identity and geographic origin of their common ancestor, however, is unknown. Based on available genetic and genomic data (Comeau et al. 2015, Khoshraftar et al. 2013), it is nevertheless clear that *O. novo-ulmi* is not a mutant that arose recently from *O. ulmi* and that the two species in fact diverged some time ago (Fig. 1). The third DED pathogen, *O. himal-ulmi*, is, so far, reported only on the Indian side of the Himalayas (Brasier and Mehrotra 1995). The full extent of its geographic range in Asia is unknown.

Shifts That Have Happened in the DED Fungi and Their Consequences

The 100 years of DED pandemics have been marked by biological events with dire consequences for elms and elm resistance breeding programs. One such event was the advent of *O. novo-ulmi*, a highly fit and aggressive pathogen able to attack and kill native European elm species, as well as early releases of elm cultivars that were tolerant to *O. ulmi*. Thus, years of breeding efforts were lost within a short timespan because of an event that nobody had foreseen. The high fitness of *O. novo-ulmi* has allowed it to rapidly displace the less competitive *O. ulmi* from most areas impacted by the disease. Another significant event, however, took place while both species co-occurred in the same geographic areas: *O. novo-ulmi* acquired "useful" alleles from *O. ulmi* through interspecific hybridization. One such acquisition is the *MAT1-1* mating-type allele, which was not detected in initial surveys of *O. novo-ulmi* (Brasier 1988, Paoletti et al. 2006). Therefore, reproduction in earlier-day populations of *O. novo-ulmi* was essentially clonal. This is no longer the case, since the *MAT1-1* and *MAT1-2* alleles both occur in current populations of the fungus and allow sexual recombination to take place. A third event with biological importance was the rise of two genetically distinct subspecies within *O. novo-ulmi*, followed by the occurrence of sexual recombination between the subspecies in areas of Europe where they are both present. As documented by Brasier and Kirk (2010), many of the resulting hybrids exhibit high pathogenic fitness.

Will There Be Other Important Shifts in the DED Fungus Species and Populations?

The evolution of fungal populations is dependent on their size, occurrence of mutations that affect the phenotype, and frequency of asexual and sexual reproduction (Zeyl 2009). Mutations typically result from errors in DNA repair mechanisms during the normal life cycle or following external stresses (Rattray and Strathern 2003). Mutations can also result from the insertion and movement of foreign DNA such as transposons (Daboussi and Capy 2003). Many biological features of the DED fungi should favor the accumulation and combination of mutations that may contribute to new genotypes, including some with improved parasitic fitness. Large populations of synnematal spores produced in bark beetle galleries are expected to contain a proportion of spontaneous mutants with altered phenotypes which can be recombined through sexual crosses to yield recombinants with higher fitness. Some novel phenotypes may have been caused by insertion or excision of mobile elements such as the OPHIO DNA transposons (Bouvet et al. 2007, 2008) and SWING retrotransposons (Comeau et al. 2015). With the rapid demise of *O. ulmi*, interspecific hybridization events are less likely to occur. However, introgression of hybrids to *O. novo-ulmi* could yield highly fit individuals. For example, the progeny from a laboratory cross between highly virulent *O. novo-ulmi* strain H327 and less virulent introgressant strain AST 27 (Et-Touil et al. 1999) included a few highly virulent individuals with an extended growth temperature range (Et-Touil 2000).

In nature, *O. ulmi* and *O. novo-ulmi* are geographically isolated from *O. himal-ulmi*. Results from laboratory crosses led Brasier and Mehrotra (1995) to conclude that there was also strong (but incomplete) reproductive isolation between *O. himal-ulmi* and the other two DED fungi. When crosses occurred, F_1 progeny were reported to display strong negative interactions for mycelial growth rate compared to parental growth rate means (Brasier and Mehrotra 1995). To our knowledge, however, other components of parasitic fitness such as pathogenicity and virulence, were not examined by these authors. Furthermore, all *O. himal-ulmi* strains tested originated from the same location in northern Himachal Pradesh. Therefore, one cannot

rule out the possibility that highly virulent and reasonably fit hybrids might arise from some encounters between *O. himal-ulmi* and the other DED fungi.

Although other known members of the '*Ophiostoma piceae-Ophiostoma ulmi*' complex are considered to be saprophytes, the possibility that one of them might hybridize with any of the DED fungi is a relevant issue. According to Brasier (1993), there is strong reproductive isolation between the DED pathogens (*O. ulmi* and *O. novo-ulmi*) and the saprobes *O. piceae* and *O. quercus*. Recent results from various interspecific pairings carried out in the author's laboratory support this conclusion, with the caveat that the number of isolates subjected to interspecific pairings was relatively modest. However, one pairing between *O. himal-ulmi* and *O. quercus* yielded large numbers of viable offspring (Bernier, unpublished results). The ability of *O. himal-ulmi* and *O. quercus* to mate is not that surprising given their relative phylogenetic proximity (Fig. 1). Although results from laboratory pairings must be interpreted with caution, the above results suggest an additional way for DED fungi to acquire new traits through interspecific hybridization. Upcoming analyses of *O. himal-ulmi* × *O. quercus* cross will shed light on the parasitic fitness of the F_1 progeny and potential emergence of highly fit individuals within the progeny.

The saprobe *O. quercus* probably deserves renewed attention. Del Sorbo et al. (2000) reported that they had recovered individuals that were pathogenic to elms among a collection of *O. quercus* mutants transformed with the *O. novo-ulmi* CU gene. The authors' conclusion was that CU was a pathogenicity factor, thus contradicting conclusions to the contrary by Bowden et al. (1996), Brasier et al. (1995), and Temple et al. (1997). However, not all *O. quercus* CU-producing transformants were pathogenic to elms. An alternative explanation for the occurrence of pathogenic *O. quercus* individuals could be that random integration of the CU gene in their genome had inactivated effector genes encoding molecules that trigger elm resistance against wild-type *O. quercus*. In any case, the results obtained by Del Sorbo and colleagues (2000) suggest that a very thin line separates *O. quercus* from becoming a pathogen of elms and reinforce previous speculation that the DED fungi might have originated from a phylogenetically close sapstaining species of *Ophiostoma* (Brasier 1990).

As mentioned previously, results from inoculations to nonhost species have confirmed that *O. novo-ulmi* can successfully attack at least one species (*Prunus pensylvanica*) not related to the genus *Ulmus* (Rioux and Ouellette 1989). This suggests that the specificity of the DED fungi toward elms observed in nature results in good part from their association with elm bark beetles. Therefore, if *O. novo-ulmi* were acquired by an insect vector that could inoculate the fungus to *P. pensylvanica*, this could result in a successful host jump for the pathogen, as already proposed by Brasier (1990) who pointed out that *S. scolytus, S. laevis,* and *S. pigmaeus* did not feed only on elms. One may therefore wonder if new diseases of elms could also occur if another ophiostomatoid (e.g., the oak wilt fungus *Ceratocystis fagacearum*) or non-ophiostomatoid pathogen were acquired by elm bark beetles through feeding or breeding on a different host. To the author's knowledge, few beetle-associated fungal species outside of the DED fungi have been tested on North American elms in controlled inoculation trials, with the exception of the sapstaining species *Ceratocystis* (now *Endoconidiophora*) *resinifera* (Morin et al. 2007). Therefore, it would seem a good idea to carry out controlled inoculations of *U. americana* with selected pathogens that are currently thought to be specific to other host species.

Genome-wide Investigations of Parasitic Fitness in the DED Fungi

Our knowledge of the biology and evolution of the DED fungi stems mostly from small-scale studies of specific biological traits and, in some cases, of their genetic and molecular determinants. These studies have been facilitated by the relative ease with which the DED fungi can be grown and manipulated in the laboratory. More recently, post-Sanger sequencing technologies have been used to explore the genomes and transcriptomes of *O. ulmi* strain W9 (Khoshraftar et al. 2013) and *O. novo-ulmi* subsp. *novo-ulmi* strain H327 (Comeau et al. 2015, Forgetta et al. 2013, Hintz et al. 2011, Jacobi et al. 2010, Nigg and Bernier 2016, Nigg et al. 2015).

The nuclear genomes of *O. ulmi* and *O. novo-ulmi* are similar in size (*ca* 32Mb) and gene content (8639 and 8640 predicted genes, respectively). Not surprisingly, these genomes contain a wide repertoire of genes that encode putative pathogenicity and virulence factors, enzymes known to degrade plant cell wall constituents, oxidative enzymes, and secondary metabolites (Comeau et al. 2015, Khoshraftar et al. 2013). When the genome of *O. novo-ulmi* is compared with those of other Ascomycete species, however, interesting trends appear (Table 1). The genome of *O. novo-ulmi* is smaller and contains fewer genes. In the case of genes encoding cytochrome P450 oxydases (CYP450s), which may be involved in the synthesis of toxins and detoxification of plant resistance molecules, it is noteworthy that *O. novo-ulmi* possesses a smaller and less diversified repertoire than other vascular plant pathogens such as *Fusarium oxysporum*, *Verticillium dahlia,* and *V. albo-atrum* (Moktali 2013). In their genomic analysis of *V. dahliae* and *V. albo-atrum*, Klosterman et al. (2011) identified the expansion in genes encoding carbohydrate-active enzymes (CAZymes) as a feature of vascular wilt fungi. Once again, the genome of *O. novo-ulmi* does not fit this model, as it contains a relatively modest number of CAZyme-encoding genes. Comeau et al. (2015) suggested this may reflect the fact that the association of DED fungi with elm bark beetles gives them direct access to the elm vascular system, whereas *F. oxysporum* and *Verticillium* spp. have to penetrate outer layers of protective plant tissue. Further validation of this hypothesis must await the public release and comparison of annotated genomes from more fungal pathogens, including species that are associated with vectors and species that are not.

Once a genome has been sequenced, the next step is to validate the actual function and contribution of candidate genes identified *in silico*. Genome-wide gene profiling by RNA-Seq is a powerful tool for this purpose and has been used successfully in ongoing investigations of yeast-mycelium dimorphism in *O. novo-ulmi*. Based on RNA-Seq data, it is clear that a substantial (10 percent) portion of *O. novo-ulmi* genes are differentially expressed between the two growth phases (Nigg et al. 2015) and that significant changes in the transcriptome occur as early as 2 hours after the switch from yeast to mycelium under defined laboratory conditions (Nigg and Bernier 2016). Since yeast-mycelium dimorphism in the DED fungi can be induced by a variety of physical and chemical stimuli, transcriptomic analyses run under different sets of conditions may provide insight into different metabolic pathways used by the pathogen to transition between the two growth forms. Other biological traits of the DED fungi are amenable to RNA-Seq analyses, both *in vitro* and *in planta*. Although the recovery of fungal transcriptomes from inoculated elms is technically challenging due to the overrepresentation of plant messenger RNA (Aoun et al. 2010, Perdiguero et al. 2015), it can be achieved, as reported by Sherif and colleagues during the 2016 Elm restoration workshop. This is an important development for the eventual identification of fungal transcripts (and ultimately molecules) that are produced when the pathogen interacts with its host.

Table 1.—Partial genomic comparisons between *Ophiostoma novo-ulmi* and other pathogens and saprobes belonging to the Sordariomycetes (Ascomyceta). Compiled from data from Comeau et al. (2015), Klosterman et al. (2011), Lah et al. (2013), and Moktali (2013).

	Order							
	Ophiostomatales[a]		Hypocreales	*Incertae sedis*	Magnaporthales	Sordariales		
	Ophiostoma novo-ulmi	*Grosmannia clavigera*	*Fusarium oxysporum f.sp. lycopersici*	*Verticillium dahliae*	*Magnaporthe oryzae*	*Chaetomium globosum*	*Neurospora crassa*	*Podospora anserine*
Lifestyle	Pathogen	Pathogen	Pathogen, saprobe	Pathogen	Pathogen	Saprobe	Saprobe	Saprobe
Genome size (Mb)	31.8	29.8	61.5	33.8	41.0	34.9	41.0	33.5
Total nb genes	8,640	8,314	17,735	10,535	11,074	11,124	9,734	10,545
CYP450[b]	48	54	169	69	135	92	43	115
Nb families	39	44	96	55	77	69	39	72
CAZymes[c]								
GH	165	146	367	265	262	233	172	205
PL	2	4	27	36	5	15	4	7
CE	20	7	50	50	52	39	21	39
CBM	35	26	91	82	120	87	43	96
GT	77	82	126	97	102	93	76	87
AA	12	4	33	29	30	45	17	38
Total CAZyme	**311**	**269**	**694**	**559**	**571**	**512**	**333**	**472**
% CAZyme[d]	3.60	3.24	3.91	5.31	5.16	4.60	3.42	4.48

[a]*Ophiostoma ulmi* is not incuded in the table since its CAZYme genes have not been annotated. The other member of the Ophiostomatales listed in the table, *Grosmannia clavigera*, is associated with the current mountain pine beetle (*Dendroctonus ponderosae*) outbreak, which has killed millions of ponderosa (*Pinus ponderosae*) and lodgepole (*P.contorta*) pines in western North America.

[b]CYP450: genes encoding cytochromes P450 oxydases.

[c]CAZYmes: genes encoding carbohydrate-active Enzymes. The following CAZYme families are recognized: glycoside hydrolases (GH), polysaccharide lyases (PL), carbohydrate esterases (CE), carbohydrate-binding modules (CBM), glycosyltransferases (GT), auxiliary activities (AA).

[d] Percentage CAZyme: percentage of CAZyme-encoding genes in the genome.

Full validation of gene function, however, requires the recovery and analysis of null mutants in which targeted genes have been inactivated. Nowadays, this is achieved through insertional mutagenesis and the resulting null mutants are also known as knockout mutants. Mutants for genes encoding cerato-ulmin (Bowden et al. 1996), endopolygalacturonase (Temple et al. 2009) and cyclooxygenase cox1 (Naruzawa 2015) have been obtained from *O. novo-ulmi* but the overall efficiency of recovery by standard procedures remains extremely low. Systematic inactivation of genes and analysis of the resulting phenotypes in knockout mutants must await the development of more efficient procedures for the mutagenesis of targeted genes. In the meantime, gene knockdown by RNA interference remains the best alternative for the DED fungi (Carneiro et al. 2010, 2013).

Full-scale "omic" investigations of DED fungi (and their saprophytic relatives), however, require financial means that were not available for previous projects. Fortunately, a new project, BioSAFE, launched in late 2016 and funded by Genome Canada, Genome British Columbia, and Genome Quebec will provide financial resources for a more thorough investigation of the DED fungi. The project aims at using next-generation sequencing approaches for understanding the development of the two successive DED pandemics through genomic profiling of fungal populations, identifying genes and markers associated with fitness and outbreak-associated biological traits, and developing biosurveillance tools. It is expected that large-scale, genome-wide investigations will help reconstruct the evolutionary origin of the DED pathogens, understand the dynamics underlying the replacement of *O. ulmi* by *O. novo-ulmi*, and provide plausible scenarios for the continuing evolution of DED fungi. Tools for achieving these goals will include *de novo* sequencing and comparative analysis of many *Ophiostoma* genomes (including pathogens and saprobes), genomic and phenotypic analysis of progeny from controlled genetic crosses, as well as development of protocols for efficient induction and recovery of knockout mutants for targeted genes.

Conclusion

The face of DED research in North America has changed dramatically since the 1981 DED symposium and workshop held in Winnipeg (Kondo et al. 1982). Unfortunately, the last decades have seen a decline in the number of scientists and level of funding, and this decline has been particularly evident in government agencies such as the Canadian Forest Service. However, several programs have been maintained in academia and government agencies, and new research groups have become involved, both in the United States and Canada. Contrary to the notion that North American elm species might become extinct (or have already been eradicated, as one can sometimes read in the popular press), survivor elms have been identified and are being integrated into disease resistance breeding programs. As more survivor trees are added to the program, one can envision that a comprehensive catalogue of DED resistant North American elms will become available. The identification, by fungal geneticists, of molecular determinants of pathogenicity in DED fungi should, in turn, help tree geneticists target genes contributing to resistance. Although this is a long-term endeavor, combining desirable alleles from several genes into single elm genotypes would be expected to promote longer-term resistance to DED. It will also be important to keep monitoring the DED pathogens closely, and possibly other fungal species that do not currently cause problems but may evolve into pathogens of elms, in order to make sure that the current efforts to restore North American elms are not annihilated by a new pandemic.

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