

# Lights and Shadows of a Possible Strategy to Cope with Alien and Destructive Forest Pathogens: the Example of Breeding for Resistance to Dutch Elm Disease in Italy

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Pecori, F., Ghelardini, L., Luchi, N., Pepori, A.L. and Santini, A. 2017. Lights and Shadows of a Possible Strategy to Cope with Alien and Destructive Forest Pathogens: the Example of Breeding for Resistance to Dutch Elm Disease in Italy. *Baltic Forestry* 23(1): 255-263.

## Abstract

Since the onset of the 20th century, two pandemics of Dutch elm disease (DED) destroyed native elms throughout Europe and North America. The disease is caused by two invasive fungi, *Ophiostoma ulmi* (Buisman) Nannf. and *Ophiostoma novo-ulmi* Brasier, which appeared one after another and were new to science. From the late 1920s, in Europe and in the US, researchers strove to find natural resistance to DED in native elms, but their efforts yielded ephemeral success. The resistant cultivars obtained in the 1930s by hybridizing European elm genotypes, were defeated by the second pandemic. The inclusion of Asian resistant species in breeding programs finally produced resistant second-generation hybrids. In Italy a program to breed resistant clones for the Mediterranean climate, was started in the mid 1970s. The successful use of Asian species in the country encouraged an in-depth assessment of their adaptability to local climates for broadening the genetic base of breeding. Selection of superior genotypes reduces genetic variation. However, when breeding is designed to obtain multiple genotypes for diverse conditions and uses, variation can be maintained. The case of elm is paradigmatic. Since elms have many uses, and an important one is as ornamentals, breeding included the selection of genotypes with fast growth, and attractive crown shape and foliage. To meet all needs and provide genetically variable cultivars to deal with climate change and new diseases, genetic resources were broadened. Native elms with good aesthetic qualities were crossed with DED-resistant and adaptable Asian genotypes. The program produced resistant clones adapted to summer drought and winter floods, yet endowed with notable ornamental features. Five of these clones were patented. A similar strategy including both the crossing of European *Fraxinus* species and of native with non-native resistant genotypes, may be successful against *Hymenoscyphus fraxineus*, the invasive agent of the European ash dieback epidemic.

**Keywords:** Breeding for disease resistance, invasive alien pathogens, Dutch elm disease, *Ophiostoma novo-ulmi*, biodiversity conservation, ash dieback, *Hymenoscyphus fraxineus*

## Introduction

Since the beginning of the 20th century, elms throughout Europe and North America have been devastated by two pandemics of Dutch Elm Disease (DED),

caused by the introduction of two alien and invasive fungal pathogens with different aggressiveness: *Ophiostoma ulmi* (Buisman) Nannf., probably introduced in the early 1910s, followed in the 1970s by *O. novo-ulmi* Brasier, a three times more deadly pathogen of elms (Brasier 2000). In

Europe the current DED epidemic is caused by two subspecies of *O. novo-ulmi*, the ssp. *novo-ulmi*, which was introduced to Europe from the Moldova-Ukraine basin and moved westward, and the ssp. *americana*, which was introduced, through infected rock elm wood, in the UK, whence it reached continental Europe spreading both west and eastward. *O. novo-ulmi* subspecies differ in many morphological and physiological characters, such as for instance colony morphology, growth rate, and pathogenicity (Brasier 1986, Brasier and Kirk 2001). Since the 1980s, hybrid individuals of the two subspecies have been found in several European countries, and in some areas their distribution ranges seem to have overlapped for quite a long time (Brasier 1979).

The elm was at the time an important and characteristic component of the cities' tree-lined roads and of the rural landscape in several European and North American countries. The epidemic had so widespread, severe and impressive effects that it stirred up the interest of researchers and public opinion, such as to necessitate a solution to the problem.

Research efforts to find a source of DED resistance in native elm species and to accumulate it in hybrid clones through breeding were initiated in 1928 at the Willie Commelin Scholten Phytopathological Laboratory in Baarn (The Netherlands). The etiology of the disease was studied and the causal agent was finally isolated by Dina Spierenburg (Spierenburg 1921, 1922) and short afterwards described and named by Marie B. Schwarz (Schwarz 1922). Christine Buisman developed a reliable inoculation method (Westerdijk et al. 1931). The studies by Spierenburg and Buisman laid down the foundations for building a breeding program (Holmes 1993).

At first, researchers tried to select DED-resistant individuals within native species. Two *U. minor* clones were indeed selected and named 'Christine Buisman' (1936) and 'Bea Schwarz' (1947). These genotypes however turned out to have slow growth and poor shape, and to be in addition susceptible to branch canker by *Nectria cinnabarina* (Tode) Fr.. In order to possibly combine different resistance mechanisms and improve growth, Dutch scientists started to cross genotypes from different elm species. In addition to DED-resistance, the breeding program aimed to select clones resistant to coral spot by *N. cinnabarina*, frost, and wind. Fast growth, good crown shape, decorative leaves, and valuable timber were also sought. The first two clones launched onto the market, 'Commelin' (1960) and 'Groeneveld' (1963), were first-generation hybrids between individuals belonging to European elm species, and seemed to be a successful completion of research efforts. Unfortunately, in the late 1960s the new and more aggressive pathogen *O. novo-ulmi* was introduced to which 'Commelin' was especially susceptible. Decades of breeding have shown that, although

slowly, it is possible to accumulate resistance through subsequent crossings and back crossings in second or third generation clones of purely European elms (Heybroek, personal communication). Complete resistance to DED has never been found in native European or American elms, but highly resistant individuals have nevertheless been identified (Townsend et al. 2005).

When the second DED epidemic was spreading in Europe, it was noticed that the clones still surviving the new pathogen were second-generation hybrids with a grandparent of Asian origin. Since then, DED-resistant Asian elms have been crossed to native elms to accelerate selection of resistant trees. A group of genotypes belonging to native elm species and bearing desirable morphological features was bred with genotypes of Asian elm species that were fairly resistant to DED and had shown the ability to adapt to a range of climatic conditions and environments. This way the genetic resources involved in selection were artificially broadened, a process so called "incorporation" (Simmonds 1993), in order to obtain DED-resistant clones suitable at the same time for all traditional uses of elms, showing also fast growth, nice tree silhouette, decorative leaf and bark colour, and leaf shape.

The risk inherent in selecting superior genotypes is to reduce genetic variation and move toward a genetic bottleneck (Simmonds 1993, Tanksley and McCouch 1997). In the case of elm breeding, the involved risks were, however, contained by designing breeding as such to obtain numerous genotypes with different genetic background and suitable to different environments and uses, possibly resulting even in increased variability (Cox and Wood 1999).

#### *The Italian Program for breeding DED resistant elms*

In Europe the second program for breeding DED resistant elms was started in Florence by the Institute of Sustainable Plant Protection of the Italian National Research Council (IPSP-C.N.R.) in the late 1970s, when the arrival of *O. novo-ulmi* was causing a new DED epidemic in Italy. The goal of this program was the selection of DED-resistant elm cultivars adapted to the Mediterranean climate. A group of clones of European origin and bearing desirable morphological and physiological traits were hybridized with individuals of DED-resistant Asian species (Smalley and Guries 1993), which had shown good adaptation to the Mediterranean climate, broadening that way the genetic resources involved in the program (Simmonds 1993).

The Italian program owes to Dutch researchers the breeding strategy (Fig. 1) and many of the clones used in crossings. Additional plants were collected from wild populations of native elm species and from plantations of Siberian elm, or received from American colleagues and

research Institutes worldwide (Tab. 1). The techniques used in Florence for inoculation and crossing were also borrowed from the breeding program carried out in The Netherlands, with few technical improvements, such as for instance pollination by blowing the pollen without the need of lifting the isolation sack (Mittempergher and La Porta 1991).

## Materials and Methods

### Collection of plant material

In the late 1970s, individuals of elm species and provenances from all around the world were collected with

a preference for Asian species, which are generally more resistant to DED (Smalley and Guries 1993), and established in a clone collection in order to check the ability of these plants to adapt to environmental conditions in the Mediterranean area, including biotic and abiotic damage agents that might affect introduced species, and their hybrid progenies, and to use them for crossing.

### Hybridization Studies

A large survey was carried out under Mediterranean climatic conditions to assess the crossability among elm species, which included European elms and several Asian

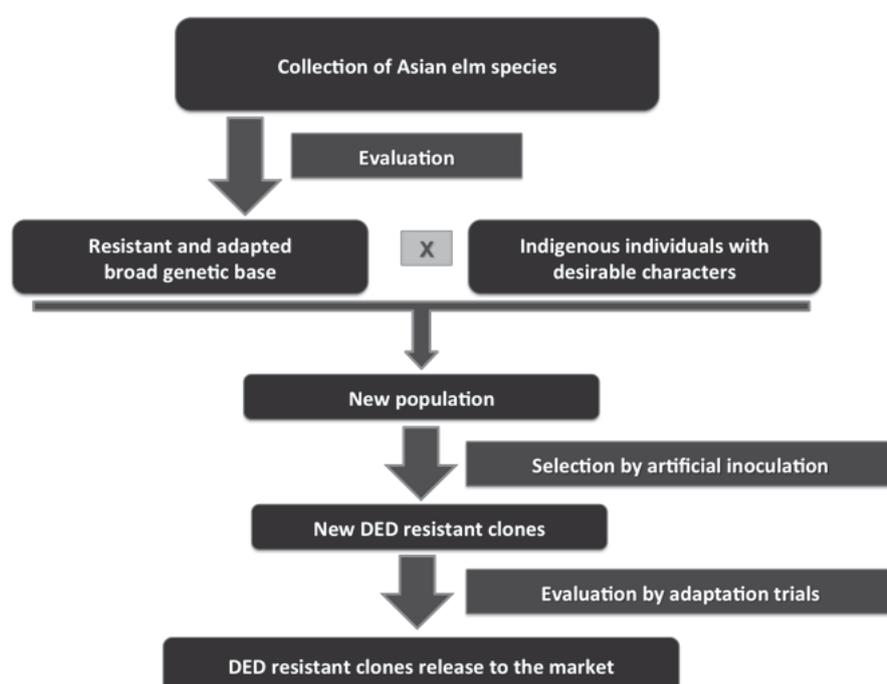


Figure 1. Scheme of the strategy applied in the Italian program for elm breeding

Table 1. Genotypes most frequently used in the Italian breeding program for selection of DED-resistant elms. DED resistance scores: - = non resistant; + = resistant; ++ highly resistant

Species	Common name	Origin of parent trees	DED Resistance
<i>U. laevis</i> Pall.	European white elm	France	-
<i>U. minor</i> Mill.	European field elm	Italy	-
<i>U. glabra</i> Huds.	Wych elm	Italy	-
<i>U. pumila</i> L.	Siberian elm	Turkestan, W Siberia	++
<i>U. japonica</i> Sarg.	Japan elm	Japan	+
<i>U. wilsoniana</i> Schn.	Wilson elm	China	++
<i>U. elliptica</i> Koch.	Armenian elm	Caucasus	-
<i>U. x hollandica</i> Mill.	Dutch elm	The Netherlands	-
<i>U. parvifolia</i> Jacq.	Lacebark elm	Korea, Japan	++
<i>U. chenmoui</i> Cheng	Chenmoui elm	NE China	+
<i>U. villosa</i> Brandis	Cherrybark elm	Himachal Pradesh	+

\* - = non resistant; + = resistant; ++ highly resistant

species belonging to different taxonomic sections (Mitterpergher and La Porta 1991). Pollen was obtained from cut branchlets held in vases with water during the pollen dispersal phase. Different species and individuals were kept in separate rooms of a greenhouse to avoid contamination. The pollen was dehydrated to 10 percent relative humidity (RH) and conserved at 3° to 4°C for use within few days to weeks. Dehydrated pollen was conserved at -20 °C when it had to be stored for 6 months (Asian species that flower in autumn), or for about 1 year (to cross the later pollen donor with the earlier spring flowering species). Pollen vitality was checked before pollination by using the Fluoro-chromatic Reaction technique (Heslop-Harrison and Heslop-Harrison 1970, Heslop-Harrison et al. 1984).

Flower pollination was carried out in triplicates by injecting pollen into pollination bags with forced air. In order to test for occurrence of self-pollination, foreign pollen was not injected in at least three control bags on each mother tree. Matured seeds were sown in open-air nursery beds where germination was monitored. Morphological traits of taxonomical relevance were assessed in the seedlings for two years in order to ascertain their hybrid nature. Viability at the end of the first growing season was recorded.

#### Screening Disease Resistance

DED resistance was assessed through mass inoculation, which was followed by selection of resistant genotypes

Three-year-old elm seedlings from controlled crosses were grown in the nursery and planted in the field. In the third week of May of the following year, at the time of peak of flight activity of the beetles species (*Scolytus* spp.) that vector the disease in the area of study, seedlings were inoculated. Inoculation was performed with a single wound per plant, using a knife blade carrying two 0.2 ml drops of a  $1 \times 10^6 \text{ ml}^{-1}$  suspension of yeast-phase cells of *O. novo-ulmi* so that the inoculum got sucked into the vertical sap flux (Santini et al. 2008). A blend of two tester isolates, one of subsp. *novo-ulmi* and the other of subsp. *americana*, was used to account in the selection process also for differences in virulence and other relevant traits between subspecies of the fungus (Brasier 1986). Resistant elm genotypes were selected according to standard protocols, which set inoculum type and quantity, inoculation method, and assessment of disease symptoms guaranteeing efficacy and reproducibility of the process.

Symptoms of disease (percent defoliation and percent dieback) were assessed 4 weeks, 3 months and 8 months after inoculation by three independent observers. Seedlings showing less than 10 percent dieback were propagated by hardwood cuttings and after a year were planted in the field using a randomized complete block

design. Twelve rooted cuttings per genotype and three blocks were used. Seedlings showing less than 25 percent dieback were considered resistant, and were evaluated for additional traits. Two years after planting, inoculation and disease symptoms assessment were repeated as described above, and symptoms were compared with those expressed by reference clones with known response to DED, i.e. the Dutch clones ‘Commelin’ and ‘Lobel’, which are defined as ‘highly susceptible’ and ‘intermediately resistant’, respectively.

#### Adaptation trials

In order to assess phenotypic plasticity and to determine the optimal environmental condition for growth of each selected genotype, a phenotypic assessment of the clones at several traits including DED-resistance was repeated in field trials at multiple sites under different ecological conditions. Clones were planted in a randomized complete block design with three blocks and four ramets per block and clone. Traits were measured once per year at all sites and a final assessment was done at the end of the trial, 6 to 10 years after planting.

Clones defined as DED-resistant on the basis of defoliation and dieback after repeated artificial inoculations were evaluated for the following additional traits: 1) Leaf shape, including length, breadth, and slenderness. The shape defined as preferable was that of *U. minor* leaves, which are generally rounder than the leaves of Asian elm species. 2) Leaf colour. Dark green, which is the colour of the leaves of the native field elm, was considered preferable. 3) Shape of the crown. The favourite shape was columnar with a monocormic straight trunk and slender branches. 4) Tree growth in height and diameter.

Three independent observers attributed to each genotype a score on a 5-step scale that synthesises the phenotype at morphological traits, growth and DED-resistance. The scale goes from ‘no marks = not an eligible clone’, to ‘four marks = clone that accomplishes all the requested characteristics: resistance, adaptation (growth), and leaf colour, trunk and crown shape’.

## Results and Discussion

More than 50,000 hybrid seedlings were grown and tested, about 80 of which received a very high score. A quite numerous group of resistant elm clones obtained by crossing very diverse parents and showing valuable traits are in the process of being placed on the market. Availability of a large number of resistant genotypes with different genetic background and adapted to different environmental conditions should reduce the risk of being defeated by new and possibly more aggressive strains of the pathogen, as it occurred in the 1970s when the *O. novo-ulmi* appeared in Europe, or by other unforecasted

environmental changes (Santini et al. 2008).

Five DED-resistant elm clones have been patented and released to the market. 'San Zanobi' (Pat. n. RM97NV0006) and 'Plinio' (Pat. n. RM97NV0005) (Santini et al. 2002), significantly more resistant than 'Lobel' and other reference clones, were obtained by crossing the Dutch hybrid 'Plantijn' with two genotypes of *U. pumila* and were launched on the market in 1997. In 2006, 'Arno' (Community Plant Variety Right n. 27598) and 'Fiorente' (Community Plant Variety Right n. 27599) (Santini et al. 2007) were released. The first is a full sib of 'Plinio,' while the second is a first generation hybrid between *U. pumila* and a genotype of *U. minor* native to Italy. The DED-resistance of these clones is similar to that of 'Lobel.' In 2010, the Italian elm breeding program produced a new variety obtained by crossing a specimen of *U. chenmoui* W. C. Cheng with the Dutch hybrid clone '405.' This new release, named 'Morfeo' (Community Plant Variety n. 2011/0223) (Santini et al. 2012), is extremely resistant to DED, has attractive crown shape and foliage, is fast-growing, able to stand without a support stick at a very early age, and tolerates drought and soil waterlog in winter (fig. 2). 'Morfeo' seems therefore able to adapt to maritime climates with wet and mild winters, such as those found in north-western Europe and in some parts of the Mediterranean region. The results of growth trials performed in England indicate that 'Morfeo' might help in the conservation of several invertebrates endangered as a consequence of elm disappearance due to DED.

#### ***Evaluating the risk of damage by other pests***

Introduction of elm species from other continents was one of the key-points of many programs for breeding DED-resistant elm clones. Introduction of non-native species involves the risk that local parasites, which cause minor damage to native species, might seriously attack new and naive introduced species. For instance, a disease named 'Elm Yellows' (EY), caused by phytoplasma (*Candidatus Phytoplasma ulmi*, Lee et al. 2004) was found to be harmful and even deadly for a number of Asian elms resistant to DED and for their hybrids (Mittempergher 2000). This disease was known in North America since the 1930s (Sinclair 2000) where it commonly kills the American elm (*U. americana* L.). In Europe instead EY was well tolerated by the populations of native elms, with only a few individuals showing typical symptoms, such as witches' brooms, growth retardation and general decline (Mittempergher 2000). The disease is commonly vectored and locally spread by some species of phloem-feeding Hemiptera (Carraro et al. 2004). Nowadays, EY has become common because of the co-occurrence of several factors: large spreading of leafhopper vectors, presence of host-plants that act as a reservoir for phytoplasma, and an increasing number of susceptible elm clones. The result is

that even *U. minor* is seriously attacked by EY, especially in mixed plantations such as clonal banks (Pecori et al., 2013).

Numerous insects are also known to damage European elms, including the elm leaf beetle (*Xanthogaleruca luteola* Müller) and the goat moth (*Cossus cossus* L.). Asian elm species used in breeding programs show variable susceptibility to these insects. For example, the Chinese species *U. laciniata* (Trautv.) Mayr is susceptible to the elm leaf beetle to such a high degree that the tree can be hardly grown in central Italy without chemical control. *U. parvifolia* Jacq. and *U. wilsoniana* Schneid. are instead scarcely damaged by the insect. The Institute for Sustainable Plant Protection (IPSP-CNR) in Italy has thus established a research program to assess the susceptibility to Elm Yellows and to the elm leaf beetle of the most commonly used elm species of Asian origin. Resistance to multiple pests is scored and evaluated in adaptation trials planted in field conditions by scoring the susceptibility to natural infection or infestation.



**Figure 2.** The Dutch elm disease-resistant clone 'Fiorente'

### ***Environmental risks associated to the introduction of non-native species***

In Italy, the native field elm (*U. minor* Mill.) has been commonly employed for various uses since ancient times, for instance as living support for grapevine, as fodder for cattle, timber for construction, and as firewood. Field elm was also important as shadow tree in pastures and as an ornamental tree along city avenues and in parks (Goidanich 1936). Given its widespread use, the disappearance of field elm owing to the first DED epidemic was disastrous, and stimulated private nurseries and academic researchers to try to find suitable tree species for substituting dead elms (Sibilia 1932, Ansaloni 1934, Passavalli 1935). The introduction of *U. pumila* as a barrier against DED was strongly encouraged by local authorities during the 1930s (Passavalli 1935). The effects of the DED epidemic on field elm populations were obviously disastrous, while the impact of hybridization with *U. pumila* is more difficult to evaluate. Recent studies indicate that hybridization and introgression between field elm and Siberian elm are causing irreversible changes in the genetic structure of the European species (Brunet et al. 2013, Zalapa et al. 2009).

A possible advantage of introgression from *U. pumila* toward *U. minor* would be the transmission of DED resistance genes, which would most probably increase *U. minor* survival in Italy. On the other hand, introgression toward *U. pumila* could facilitate the acquisition of useful genes from the native *U. minor* that would enhance the ability of *U. pumila* to invade the habitats originally occupied by *U. minor* and enhance its capacity to spread (Brunet et al. 2013).

### ***Conservation of native species***

One of the most successful strategies for conservation of European elms was *ex situ* conservation. In the EU RESGEN 78 project, which was devoted to characterization and conservation of the genetic resources of European elm species, hundreds of elms were collected in many European countries (Belgium, France, Germany, Greece, Italy, Spain and Sweden) and planted outdoors in clone collections at different sites.

In these collections, studies on morphological and phenological traits and genetic characterization of elms from different parts of Europe have been carried out, which has facilitated the selection of native genotypes displaying resistance to DED and desirable phenotypes at adaptive and ornamental traits (Collin et al. 2000).

In the past few years, several *U. minor* clones were selected for their resistance to DED in susceptibility tests in Spain and recommended also as reproductive material for employment in forestry (Martín et al. 2015).

An interesting result from studies on bud burst phenology and inoculation trials performed on elms in clone collections is that early flushing *U. minor* clones,

generally originating from southern regions of Europe, are less susceptible to DED than late flushing clones, when inoculated at the same date (Santini et al. 2005, Ghelardini et al. 2006). The hypothesis that DED susceptibility is related to spring phenology was investigated observing the relation between disease susceptibility and date of bud burst in European and hybrid elm clones. This result might be explained by a different physiological response of early flushing elm that early in the growing season, when the flight of beetles that vector the disease reaches a peak, are allocating carbohydrates to secondary metabolism, ensuring a better defence against DED (Herms and Mattson 1992, Ghelardini 2007, Ghelardini and Santini 2009). Moreover, at the time of beetle flight, early flushing elms are already producing summerwood with small and scattered vessels with thick cell walls, which may hinder the diffusion of fungal spores and hyphae in the vascular system (Solla et al. 2005).

The studies on phenotypic plasticity have shown that some DED-resistant elm clones have superior growth at all experimental sites, while other clones have stronger genotype x environment interaction and have superior growth only under specific environmental conditions (Santini et al. 2010). The clones that proved to be DED-resistant and adaptable to various sites may be recommended for use in a wide range of environmental conditions.

### ***May breeding for resistance be a suitable strategy against *Hymenoscyphus fraxineus*?***

Dutch elm disease was the first, with chestnut blight (*Cryphonectria parasitica* (Murrill) Barr.), and one of the most impressive tree diseases caused by introduction and spread of alien fungi, which have almost wiped out the populations of native host species from Europe and North America. The strategies adopted to conserve native elms and to cope with such an implacable disease might serve as an example for developing prompt and effective responses against recently introduced invasive fungi, which are currently menacing native tree species. Collection and screening of native populations for finding resistance genes, accumulation of resistance through recurrent breeding and selection, and breeding with non-native and resistant species as a last resort, may be suggested for protecting European ash species against *Hymenoscyphus fraxineus*, the fearsome agent of European ash dieback (Kowalski and Holdenrieder 2009, Baral et al. 2014).

Recent studies have shown that susceptibility to the ash dieback pathogen varies within European ash populations (Cleary et al. 2014) and potentially resistant genotypes are found in stands of European ash trees under high infection pressure by *H. fraxineus* (Lenz et al. 2016, Enderle et al. 2015, Lobo et al. 2014, McKinney et al. 2011). Studies also suggest that healthier clones are able to limit the growth and spread of the fungus thereby

minimizing the occurrence of symptoms, and emphasize that high susceptibility is associated with low fitness (McKinney et al. 2012; Lobo et al. 2014). In Europe, besides the European ash *Fraxinus excelsior* L., the narrow-leafed ash has also been found susceptible (Kirisits et al. 2009) but nothing is known about variation in susceptibility in this ash species. The flowering ash (*Fraxinus ornus* L.) can be naturally infected by the pathogen when exposed to heavy disease pressure (Kirisits and Schwanda 2015). However, circumstantial evidence from lab tests on mycelial growth of the pathogen on media containing leaf extracts of different host species, suggest that *F. ornus* might be less susceptible to the disease than the other European ash species (Carrari et al. 2015). The discovery of additive genetic variation in susceptibility (Kjaer et al. 2012, McKinney et al. 2012, Enderle et al. 2015) encourages wider screening of native ash species and gives hope for selection and breeding of resistant clones of European origin (McKinney et al. 2014).

As in the case with elm susceptibility to DED, ash susceptibility to *H. fraxineus* seems to depend on host phenology and seasonal variation in growth rhythm. Ash genotypes less affected by *H. fraxineus* are more frequently found among trees that shed leaves earlier (McKinney et al. 2011). To explain this observation, McKinney and colleagues have hypothesized that rapid leaf senescence shortens the infection period and reduce the available time for hyphal growth in woody tissues. Whichever the mechanism behind, this source of resistance might be exploited for selection and breeding of resistant clones, possibly in combination with other types of resistance.

In order to obtain ash clones resistant to *H. fraxineus* to be used as ornamental trees or for plantation in artificial forests, and in general for aims other than reintroduction in natural forests, susceptible individuals of native ash species bearing valuable phenotypes at interesting traits might be crossed with genotypes from resistant ash species of foreign origin. Risks linked to introgression of non-locally adapted genetic variation at other traits that might decrease the mean fitness of native populations (Keller et al. 2000) should be considered. The pathogenicity of *H. fraxineus* to different ash species has been little investigated so far, but available studies indicate variable resistance between and within non-European ash species. Manchurian ash (*Fraxinus mandshurica* Rupr.) is generally reported to be asymptomatic in the native range in Asia (McKinney et al. 2014). In Europe, necrotic stem lesions have been observed at a very low incidence on naturally infected ornamental *F. mandshurica* var. *mandshurica* Rupr. trees (Drenkhan et al. 2014). The Central Asian ash species, *F. sogdiana* Bunge is susceptible to natural infections in Europe (Drenkhan et al.

2015). *F. mandshurica* var. *japonica* resulted susceptible when the pathogen was inoculated in the stem, suggesting that defense mechanisms against *H. fraxineus* act in leaves and/or before penetration (Gross and Holdenrieder 2015). Among American ash species, the black ash (*Fraxinus nigra* Marshall) seems to be highly susceptible, while the white ash (*Fraxinus americana* L.) shows only minor symptoms after infection (Drenkhan and Hanso 2010). The green ash *Fraxinus pennsylvanica* Marshall seems moderately susceptible and definitely less susceptible than *F. excelsior* (Drenkhan and Hanso 2010; Gross and Sieber 2016, Kowalski et al. 2015).

*F. excelsior* and *F. angustifolia* are interfertile, naturally hybridise in contact zones (Fernandez-Manjarres et al. 2006), and can be crossed with *F. mandshurica* that belongs to the same section of the genus, i.e. section *Fraxinus*. *F. pennsylvanica* and *F. ornus* belong instead to two different sections, i.e. *Melioides* and *Ornus*, respectively. Both a systematic assessment of resistance to *H. fraxineus* in the genus *Fraxinus* and a detailed survey of crossability between *Fraxinus* species remain to be done.

## Acknowledgments

*The authors wish to thank Lorenzo Mittempergher, who led for many years the Italian program for elm breeding. His memory will be with us always. We warmly thank Fabio Ferrini and Alberto Fagnani, without whom this program would not have achieved such brilliant results. We thank our late friend Abdellah Dahmani for his precious and enthusiastic contribute to the success of our work, sadly he will not be here to celebrate with us.*

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