The Italian Elm Breeding Program for Dutch Elm Disease Resistance

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Abstract

In the 20th century, elms across Europe and North America were devastated by two pandemics of Dutch elm disease (DED), caused by the introduction of two fungal pathogens: *Ophiostoma ulmi*, followed by *O. novo-ulmi*. At the end of 1920s, research into a resistance to DED began in Europe and then in the United States. No worthwhile resistance was ever found within the native European or American elms. The Dutch team resorted to hybridizing European elms, from which the first cultivars exhibiting a resistance were obtained during the 1930s, but these clones did not have sufficient resistance to the second pandemic. It was the use of Asian species, with their higher resistance to DED, which accelerated progress on both sides of the Atlantic. Eventually a number of second-generation resistant clones derived from hybrids of native and Asian species were released to commerce.

Many efforts were dedicated to control the disease, such as the elm breeding program started in Italy in 1975 by the Institute of Plant Protection (IPP). This program was born of a conviction that the Mediterranean environment would demand its own selections. In Italy, the favorable adaptation of species such as the Siberian elm (*U. pumila* L.), and the unsuitability of the Dutch selections to the more arid regions, encouraged the wider assessment of the Asian elms. The purpose was twofold: to examine more fully adaptability to the climate and to broaden the genetic base of the native species.

Selection of superior genotypes ostensibly reduces genetic variation in cultivated species. However, when breeding is designed for obtaining plants adapted to different environmental conditions and for different uses, the outcome can actually result in increased variation. The case of elm breeding for resistance to DED is paradigmatic. The uses of elm are in fact manifold. For this reason, breeding for resistance is not enough. Many other features are required, including fast growth, and aesthetic factors such as attractive shape and foliage. To satisfy all these needs, whilst maintaining enough genetic variability to buffer the effects of climate change and possible arrival of new strains of DED or other diseases, it was decided to undertake a fundamental broadening of genetic resources, or, as it will be called later, “incorporation.” A base of native elms with enough good characters to act as parents was hybridized with those disease-resistant Asian species able to acclimatize.

This program has produced DED-resistant elm varieties able to adapt to arid conditions, yet endowed with some remarkable ornamental characteristics. Five of these clones have already been patented and released to commerce: ‘San Zanobi,’ ‘Plinio,’ ‘Arno,’ ‘Fiorente,’ and, more recently, 'Morfeo.' 'Morfeo' is a robust, attractive tree that is extremely resistant to DED. It is also fast-growing and tolerant of both summer drought and winter floods, thus proving as well adapted to the climate of northwestern Europe as that of the Mediterranean. Indeed, following trials in England, ‘Morfeo’ is now considered potentially the most important cultivar in the conservation of several invertebrates there endangered by the consequences of DED.

Key words: Dutch elm disease, *Ophiostoma*, Italian elm breeding program

Introduction

In the 20th century, elms across Europe and North America were devastated by two pandemics of Dutch Elm Disease (DED), caused by the introduction of two fungal pathogens with very different aggressiveness*:* *Ophiostoma ulmi* circa 1910, followed 60 years later by the three times more lethal

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O. novo-ulmi (Brasier 2000). The gravity and impressiveness of the damages caused by the disease stirred up the interest of researchers and public opinion, such as to necessitate a solution to the problem. The elm is, in fact, a plant of great beauty, has significant historic and artistic value, and adapts exceptionally well to stress and to difficult sites, such as those occurring in cities, alongside roads. and in windswept coastal areas. Thanks to these features, the elm is an important and characteristic component of the cities' tree-lined roads and of the rural landscape in several European and North American countries.

The idea of searching for resistance to DED through breeding arose in 1928 at the Willie Commelin Scholten Phytopathological Laboratorium in Baarn (The Netherlands). There, the first studies were performed on the etiology of the disease that had been killing elms in western Europe since the end of World War I. The causal fungus was firstly isolated by Dina Spierenburg (Buisman 1921), then described and named by Marie B. Schwarz (Schwarz 1922); later followed by the development of a reliable inoculation method by Christine Buisman (Westerdijk et al. 1931). The path-breaking research conducted by the two latter scientists—both of whom were working at the aforesaid Laboratorium—constituted the fundamental requisite for starting up a breeding program (Holmes 1993).

At the beginning, research focused on selecting resistant individuals within the native species. During this phase, a couple of clones were selected into the species of field elm (*Ulmus minor* Miller) and named 'Christine Buisman' (1936) and 'Bea Schwarz' (1947). However, these proved disappointing because of their slow growth, poor shape, and susceptibility to a branch canker caused by the *Nectria cinnabarina* fungus. In order to combine resistance mechanisms of different species and enhance the growth rate, Dutch researchers started crossing different elm species. In addition to resistance to DED, the long-term goals of the program were resistance to coral spot (caused by *N. cinnabarina*), to frost, and to wind. Fast growth, good form, decorative leaves, and valuable timber were also considered. The first two releases, the 'Commelin' (1960) and 'Groeneveld' (1963) clones, were first-generation hybrids between European elm species. Initially, they proved to be a great success. However, the arrival in the late 1960s of the new, more aggressive species, *O. novo-ulmi*, to which 'Commelin' was particularly susceptible, inflicted a hard blow on the Dutch breeding program. Although decades of breeding have shown that it was possible to slowly accumulate resistance in second or third generation clones of purely European elms (Heybroek, personal communication), complete DED resistance was not found in European nor in American native elms, but individuals highly resistant to DED have nevertheless been identified (Townsend et al. 2005). At that time, it was noticed that the most resistant clones were second generation hybrids which contained one grandparent of Asian provenance.

Therefore, since then, Asian DED-resistant elm species have generally been crossed to native elms to speed up the selection of resistant trees. A base of native elms with desirable characteristics was bred with Asian elms species that, besides a fair level of DED resistance, showed the ability to adapt to a range of climatic conditions and environments. This artificial base broadening of the genetic resources, or "incorporation" (Simmonds 1993), was planned in order to satisfy all traditional elm uses, so taking into account not only DED resistance, but also fast growth, tree silhouette, leaf and bark color, leaf shape, and overall tree size. The resulting progeny were expected to combine the DED resistance of Asian species with the superior growth, ornamental value, and environmental adaptability of European elms.

The risk of adopting this kind of strategy was to go toward a genetic bottle-neck, since selection of superior genotypes reduces genetic variation (Simmonds 1993, Tanksley and McCouch 1997). However, when breeding, as in this case, is designed to obtain plants adapted to different environmental conditions and for different uses, the outcome could result in an increase of variability (Cox and Wood 1999).

The case of elm breeding for resistance to DED is, therefore, paradigmatic. The uses of elm are manifold: from wood to fodder, from medicine to urban silviculture, and many others. For this reason, breeding for resistance is not enough; many other features are requested as tree silhouette, fast growth, leaf and bark color, and leaf shape and dimensions. In order to satisfy all these needs, maintaining at the same time enough genetic variability to buffer the rising of possible new stresses, such as new forms of the disease or other diseases and climate change, we decided to initiate a base broadening of the genetic resources, or, as it will be called later, “incorporation” (Simmonds 1993).

The Italian Program

A second elm breeding program in Europe was initiated in the late 1970s in Florence by the Institute of Plant Protection (IPP), part of the Italian National Research Council (C.N.R.), when the second more destructive DED pandemic incited by *O. novo-ulmi* reached Italy. The goal of the Italian elm breeding program was the selection of elm cultivars resistant to DED, but suited to the Mediterranean climate, unlike the Dutch selections intended for northern Europe. European elm species and selections with desirable morphological and physiological characteristics were hybridized with DED-resistant Asian species (Smalley and Guries 1993) that had thrived in the Mediterranean climate, broadening genetic resources (Simmonds 1993). The breeding strategy program is reported in figure 1.

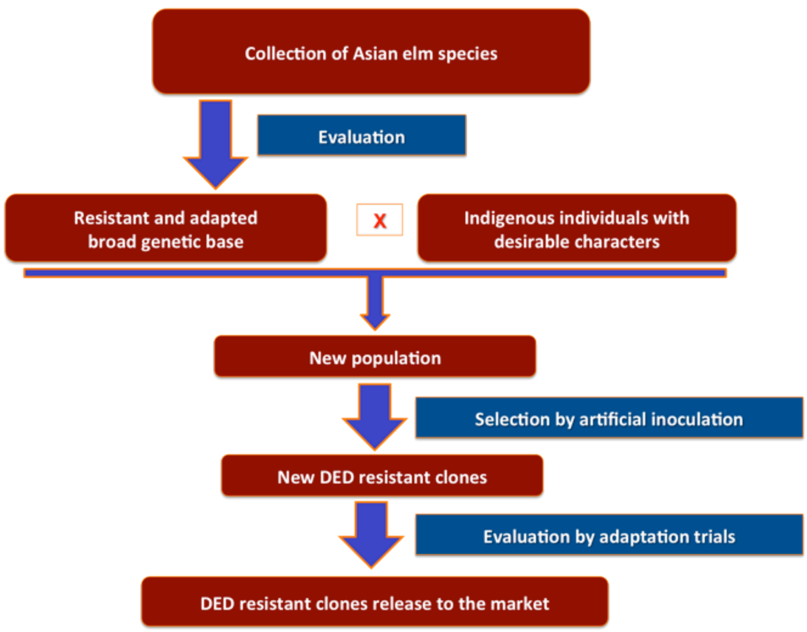

Figure 1—Italian elm breeding strategy program.

The Italian program is indebted to the work of the Dutch researchers for the breeding strategy and for much of the material used in the crossings. Other materials came from native species and from extant plantations of Siberian elm, as well as through exchanges with Americans and other research Institutes. The inoculation and crossing techniques used in Florence were also derived from the Dutch experience, with the introduction of a few improvements, such as the realization of pollination without having to lift the isolation sack, by blowing the pollen into the sack (Mittempergher and La Porta 1991).

Material and Methods

Collection of Material

In late 1970s, a wide collection of elm species and provenances from all over the world with a particular preference for Asian species, generally more resistant to DED (Smalley and Guries 1993), was set up. Collections were established with two aims:

1) to check adaptation to a new environment, constituted by the Mediterranean climate and by all the biotic and abiotic damage agents that may affect introduced species under these conditions, and, as a consequence the new hybrid clones; and

2) to obtain adult plants for breeding work.

Hybridization Studies

Crossability among elm species which involved the use of several European and Asian species of different taxonomic sections was checked under Mediterranean climatic conditions (Mittempergher and La Porta 1991). Pollen of the desired species 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 collected pollens were conserved at 3° to 4°C and dehydrated at 10 percent relative humidity (RH) when used within a short period of time (few days or weeks). When pollen had to be stored for 6 months (Asian Autumn flowering species), or for about 1 year (to cross the later pollen donor with the earlier spring flowering species), it was conserved at -20 °C and 10 percent RH. 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 by injecting pollen into the pollination bags with forced air. At least three bags were used for each single cross, and, at least three control bags on each mother tree did not receive foreign pollen in order to check the selfing for each mother tree. Matured seeds were harvested and sown in open air nursery beds, monitoring germination. The seedlings were checked morphologically during the first and second growing season in order to ascertain their hybrid nature. The percent of viable seedlings from full seeds sown was scored at the end of the first growing season.

Screening Disease Resistance

The seedlings were evaluated for DED resistance. Selection for resistance was operated following a two-step protocol:

1) Mass inoculation. Three-year-old elm seedlings obtained by controlled crosses were grown in the IPP nursery and planted in the field. The following year, during the third week of May, which is the time of beetle flight in the area of Florence, trees were inoculated. Inoculation was performed with a single wound per plant, using a knife blade carrying two drops of a 0.2 ml of a $1x10^6$ ml^{-1} yeast phase cells, consisting of two tester isolates of the subsp. *novo-ulmi* and subsp. *americana* of *O. novo-ulmi* (Brasier and Kirk 2001), so that the inoculum would be absorbed by the tree's rising sap. Symptoms of disease (percent of defoliation and percent of dieback) were observed after 4 weeks and at 3 and 8 months from inoculation date and assessed by three independent assessors.

2) Selection. Seedlings with less than 10 percent dieback were vegetatively propagated by hardwood cuttings and planted out the following year in a randomized complete block design. Clones showing less than 25 percent dieback were considered resistant, and were evaluated for other characters. Twelve rooted cuttings per clone, divided into three blocks, were used. Inoculations and disease evaluations were performed 2 years after, as described above, and the symptoms were compared with those expressed by clones having known DED responses acting as controls, generally the Dutch clones 'Commelin' and 'Lobel', highly susceptible and intermediately resistant, respectively.

Adaptation Trials

In order to check the possible effect of phenotypic plasticity and to determine the best environmental condition for growth of each, a series of traits were evaluated in different ecological conditions. Several field trials were planted in different climates in Italy. In each trial, a randomized complete block design was used with generally three blocks of four ramets for each clone. Traits were measured each year and a final evaluation was performed at the end of the trial, generally after 6 to10 years.

Clones showing DED resistance were also evaluated for:

1) leaf shape: length, breadth, and slenderness, with the favorite shape being that of *U. minor* leaves which are rounder than Asian elms.
2) leaf color: dark green was considered preferable for being similar to the native field elm.
3) shape of the crown: columnar with a monocormic straight trunk and slender branches is the favorite shape.
4) height and diameter growth.

Clones were ranked for these traits and resistance according to a 5-step scale by three independent observers. This datum is reported together with the clone code as a variable number of marks. The scale goes from no marks = not an eligible clone, to four marks = clone that accomplishes all the requested characteristics: resistance, adaptation, leaf color, trunk and crown shape.

Results and Discussion

More than 50,000 hybrid seedlings have been raised and tested, of which circa 80 individuals totaled a very high score. A wide range of resistant elm clones with different parentage and valuable characters are going to be released. This should help further safeguard against the appearance of new and even more aggressive strains of the pathogen, as occurred in the 1970s when the *O. novo-ulmi* appeared in Europe, or against different unpredictable risks (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), both obtained by a controlled crossing of the Dutch hybrid ‘Plantijn’ with two different individuals of *U. pumila*, were released in 1997. The resistance levels of the clones were significantly higher than the resistance levels of ‘Lobel’ and other reference clones.

‘San Zanobi’ is monocormic and shows exceptionally rapid growth on fertile soils and in temperate climates, suggesting that it could be used also for production of construction timber (Santini et al. 2002, 2010). Its habit is cone-shaped with pronounced apical dominance, resulting in limited lateral branching on the developing shoots of the current season’s growth (fig. 2). The crown is therefore narrow and columnar. Apical dominance is so marked that seedlings rarely need pruning or training. The wood characteristics of this clone are not different from those known for European field elm (*Ulmus minor* Mill.) (Santini et al. 2004).

‘Plinio’ grows rapidly, although slightly slower than ‘San Zanobi,’ roughly similar to that of fast-growing benchmark ‘Lobel.’ ‘Plinio’ can be used as an ornamental shade tree (fig. 2). The crown is vase shaped. In isolated trees, the width of the crown can be up to 70 percent of its height. The trunk is straight, at times slightly sinuous and short.

Ulmus 'San Zanobi' *Ulmus* 'Plinio'

Figure 2—Characteristics of two Dutch elm disease-resistant clones.

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 a *U. pumila* with a *U. minor* that originated in Italy. The resistance levels of the clones are comparable to that of 'Lobel.'

'Arno' is single stemmed (monocormic), with erect habit, ascending branches, and an upright oval crown (fig. 3). Field trials indicate that 'Arno,' although slower than 'Fiorente,' is among the fastest growers ever tested. The trunk is straight, branching at a height of 3 m. The wood characteristics of this clone are not different from those known for field elm (Santini et al. 2004).

'Fiorente' is monocormic and shows exceptionally rapid growth, significantly greater than the other cultivars planted at the same site, suggesting that it could also be used for timber production. Its habit is conical with pronounced apical dominance, a result of the limited lateral branching on the developing shoots of the current season's growth (fig. 3). The crown is therefore slender and columnar. The trunk is straight and long.

Ulmus 'Arno' *Ulmus* 'Fiorente'

Figure 3—Characteristics of two Dutch elm disease-resistant clones.

In 2010, the Italian elm breeding program raised a new variety 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. 2011), is extremely resistant to DED and has an attractive form and foliage. It is also fast-growing, able to free stand at a very early age, and tolerant of drought and soils waterlogged in winter (fig. 4). Therefore, it is proving well adapted to the maritime, wet winter climate, where temperatures are moderated by the gulfstream of northwestern Europe, as well as the Mediterranean climate. Following trials in England, 'Morfeo' is considered of potential importance in the conservation of several invertebrates endangered by the consequences of DED.

Ulmus ‘Morfeo’

Figure 4—Characteristics of the Dutch elm disease resistant clone ‘Morfeo’.

Regarding genotype x environment interaction (GxE), results show that some DED-resistant elm clones showed superior growth at all experimental sites. These clones are likely suitable for successful planting in a range of different environments. In addition, several resistant clones showed a more marked GxE interaction having high-growth parameters at a particular site (Santini et al. 2010). Results deriving from biometric studies strongly support our belief that these elm clones can be successfully used for timber and biomass production.

The introduction of non-native elm species from different continents is one of the main features of the elm breeding program for DED resistance. It may involve the risk that local parasites of minor importance toward native species might cause some damage to new the hosts. For instance, a disease named ‘Elm Yellows’ caused by phytoplasmas, which until 1985 had been regarded of American origin, was found to be harmful and even deadly for a number of Asian elm species resistant to DED and for their hybrids (Mittempergher 2000). This disease commonly kills the American elm (*U. americana* L.); therefore, its outcome in this case does not differ from the results of DED. Yet in Europe, Elm Yellows is tolerated by the populations of native elms, with only a few individuals showing symptoms of yellowing, witches’ brooms, growth retardation, or a general decline (Mittempergher 2000) and sometimes death.

Numerous insects are also known to damage European elms. Among these, the elm leaf beetle (*Xantogalerucha luteola*) and the goat moth (*Cossus cossus*) have to be mentioned. The various Asian elm species used in breeding programs because of their resistance to DED show varying susceptibility to these insects. For example, in our experience, the Chinese species *U. laciniata* (Trautv.) Mayr is so susceptible to leaf beetle that it is very difficult to raise it in central Italy without chemical control, whereas *U. parvifolia* Jacq. and *U. wilsoniana* Schneid., are scarcely damaged. The IPP has thus set up a rating program to assess the extent to which the commonly used Asian species may be susceptible to Elm Yellows and to the elm leaf beetle.

Multiple resistances to all of these different threats is taken into account when the clones are in adaptation trial fields by scoring their natural susceptibility. It is very conceivable that the climate could play a primary role in the build-up of insect populations and to phytoplasma infection, which is vectored by some species of insects belonging to phloem-feeding Hemiptera.

Moreover, the strategy adopted for elm genetic improvement had produced a new population that has a high proportion of unique, exotic-derived alleles that indeed broaden the elm genetic base, so that variance will be enhanced (Simmonds 1993). The gain in genetic richness and variability should encompass the strong bottleneck that, for the use of exotic species, even if hybridized with endogenous germplasm, is represented by adaptation to new environments. In conclusion, these results open the possibility for elms to overcome the condition of shrubs to which DED seemed to have destined these magnificent trees, and to be cultivated again for providing us not only with beauty, but also with new and different possible uses.

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