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The history of elm breeding

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Abstract

Breeding elms resistant to Dutch elm disease (DED) started in the Netherlands in the year 1928 on the initiative of a group of women scientists. They were active until 1954, when Hans Heybroek took over at the Dorschkamp Research Institute and carried on until his retirement in 1992. Two more programmes were initiated in Europe, in Italy and Spain, in 1978 and 1993 respectively, under the impulse of Dutch breeding activities. Elm breeding in America began in 1937 in the USDA-Agricultural Research Service Laboratories and is still being pursued under the leadership of Alden Townsend. Another programme was set up at the University of Wisconsin in 1958, led by Eugene Smalley and was closed after his retirement and death in 2002. A third programme found birth at the Morton Arboretum, Chicago, in 1972 where activities are still carried out by George Ware since his retirement. The number of resistant elm clones released on the market and the scientific progress fostered by breeding activities indicate that the long work needed to carry them on is a positive one. Among the key points considered are: elm germplasm collection, elm species crossability, inoculation system and disease evaluation, building up of resistance, and the possible consequences from introducing foreign species and hybrids to native elms. Because of shortage of funding long-term research and the perception that biotechnology will provide rapid solutions to long-term problems, traditional elm breeding activities seem now to be in difficulty. In this context, it seems wise to take all possible steps to avoid a loss in the precious gene resources so far collected and not to give up on traditional elm breeding activities, which so far has been found to be the sole means in providing tangible results for controlling DED.

Key words: Dutch elm disease, elm pathology, hybridization, inoculation methods.

Resumen

Historia de la mejora del olmo

La mejora genética de olmos resistentes a la grafiosis se inició en los Países Bajos en 1928 debido a la iniciativa de un grupo de investigadoras que estuvieron activas hasta 1954, año en que Hans Heybroek se hizo cargo del Dorschkamp Research Institute, donde prosiguió con los trabajos hasta su jubilación en 1992. Otros dos programas se iniciaron en Europa, en Italia y España, en 1978 y 1993 respectivamente, contando con el apoyo inicial de las actividades holandesas de mejora. La mejora genética del olmo en Norteamérica comenzó en 1937 en los USDA-Agricultural Research Service Laboratories, y está actualmente dirigida por Alden Townsend. Otro programa, establecido en la Universidad de Wisconsin en 1958, fue liderado por Eugene Smalley hasta que se clausuró tras su jubilación y muerte en 2002. Un tercer programa surgió en el Morton Arboretum, Chicago, en 1972, donde las actividades siguen dirigidas por George Ware desde su jubilación. El número de olmos resistentes comercializados y el progreso científico obtenido gracias a las actividades de mejora indican que el largo periodo de trabajo necesario para desarrollarlas merece la pena. Entre los elementos clave a considerar destacan: la recolección de germoplasma, la posibilidad de realización de cruzamientos, los sistemas de inoculación y de evaluación de la enfermedad, la obtención de resistencia, y las posibles consecuencias para los olmos nativos de la introducción de híbridos y especies alóctonas. Debido a la escasez de fondos para trabajos a largo plazo y a la percepción de que la biotecnología podría proporcionar en el futuro soluciones rápidas a los problemas, la mejora tradicional del olmo parece estar actualmente en dificultades. En este contexto, parece prudente dar todos los pasos necesarios para evitar la perdida de los preciosos recursos genéticos acumulados hasta el momento y no abandonar las actividades tradicionales de mejora que hasta el momento han sido las únicas que han proporcionado resultados tangibles para el control de la grafiosis.

Palabras clave: grafiosis, patología del olmo, hibridación, métodos de inoculación.

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Introduction

The activities of elm breeding for resistance to Dutch Elm Disease (DED) date back quite some time, and most of its initiators have either passed away or have retired, as is the case of one of the authors. Furthermore, two of the programmes which can be defined as historic have suspended their activities. However, other research institutes have collected the evidence and are still continuing the activity. This long march has been possible because the elm is 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 used to be an important and characteristic component of the cities' tree-lined roads and of the rural landscape in several European countries and also in North America. Starting from around 1910 in northwest Europe two destructive pandemics of DED caused by the introduction of two very different fungal pathogens, *Ophiostoma ulmi* (Buisman) and *O. novo-ulmi* (Brasier) afflicted the elms in Europe and, after 20 years, in North America (Brasier, 2000). The gravity and impressiveness of the damages caused by the disease stirred up the interest of researchers and the public opinion, such as to necessitate a solution to the problem. This is why, rightly, we can speak of the history of elm breeding, which began in an extraordinary manner, namely through the initiative of a group of women scientists.

The idea of searching for resistance to DED in elm species and elm populations, associated with the hope of enhancing resistance through breeding, arose early at the Willie Commelin Scholten Phytopathological Laboratorium in Baarn (The Netherlands), in the course of studies on the etiology of the disease that had been killing elms in western Europe since the end of the First World War. The causal fungus was first isolated by Dina Spierenburg of the Plant Protection Service, then described and named by Marie B. Schwarz, and this was followed by the development of a reliable inoculation method by Christine Buisman. 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 programme (Holmes, 1993).

The Dutch programme

In 1928 Christine Buisman and director Johanna Westerdijk began gathering elms from every available source and making selections among them by means of inoculation. Upon the premature death of Christine Buisman in 1936, the intense activity of collecting elm resources and the far-sighted improvement programme were left to Johanna C. Went, who, in 1936, started crossing elms and creating hybrid progenies. She took over the programme of elm improvement during the dangerous and difficult occupation years of World War II, and worked throughout these years. Even during the hunger winter of 1945 crossing work was not halted! She left in 1953; her successor Hans M. Heybroek headed the programme until his retirement in 1992. Between 1956 and 1965 the programme was gradually transferred to the Dorschkamp Research Institute in Wageningen (Heybroek, 1993).

A long-range breeding programme cannot be precisely designed from the beginning: rather, it is oriented to be re-considered at different stages on the basis of discoveries and of on-going developments. From this point of view, the Dutch programme is paradigmatic. At the beginning, research focussed 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 programme 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 *Ophiostoma novo-ulmi*, to which «Commelin» was particularly susceptible, inflicted a hard blow on the Dutch breeding programme.

At that time, the best and most resistant clones available were second-generation hybrids which contained one grandparent of Asian provenance, an individual of *U. wallichiana* Planch., which, when crossed with the

Exeter elm (*U. glabra* «Exoniensis») in 1938, yielded a hardy and quite resistant seedling. This selection became the mother of many outstanding clones. These were ready for release in 1970, but were held up for three years, in order to obtain additional information on their behaviour with regard to the new, more aggressive pathogen (*O. novo-ulmi)* (Gibbs *et al*., 1975). It was then ascertained that resistance to this fungus was under the control of many genes and could be accumulated through subsequent crossings. Therefore, the prolonged work carried out up until that moment had not been in vain. The decision was thus taken to release on the market three elm selections of similar parentage and shape which presented a set of good properties, even if the level of their resistance was not yet fully satisfactory. Thus, the «Lobel», «Dodoens» and «Plantyn» clones were released with certain recommendations in 1973. Ten years later, a fourth clone, «Clusius», was released which was related to the former three in parentage, shape and resistance level (Heybroek, 1983).

At the same time, Heybroek continued to broaden the basis of the breeding population in his programme by proceeding to further crossing generations. In order to collect additional genetic material, he engaged in a number of study trips in Europe and Asia (Melville and Heybroek, 1971; Heybroek, 1981a and 1993). Several other species were also taken into consideration by the Dutch programme: Japanese elm, the *U. japonica* (R.) Sarg., which is morphologically close to *U. minor* to the point that it is regarded as the representative of this species in the Far East; the Chinese *U. laciniata* Mayr, morphologically related to the European wych elm, *U. glabra* Huds.; and the Siberian elm, *U. pumila* L., although that species and many of its hybrids do not perform well in the Atlantic climate of the Netherlands. As the work progressed, abundant hybrid elm material with an interesting level of resistance was gradually accumulated in Wageningen. Heybroek gave a part of this material to colleagues who were setting up elm breeding programmes in America. The international significance of the programme was acknowledged by British Forestry Commission, which, in a rare move, funded the post of an additional technical assistant in the Dutch institute during four years to help development of resistant clones.

In 1978 the European Economic Community funded a research project on elm and DED, one of the goals of which was to test the adaptability of the Dutch hybrid selections (68 clones) to the various European environments (48 trials in 9 countries) (Heybroek, 1983). The Community project achieved some interesting results:

1. It demonstrated that the different species and hybrids do indeed interact with the various environments found in Europe, indicating that they can be used successfully in the appropriate areas.
2. It also showed that certain parasites which have a moderate or negligible impact on native elm species may turn out to be damaging to some hybrid selections built up with foreign species or with clones coming from a different climate.
3. The project furnished material and impulse for other breeding projects in Europe.

In 1989, encouraged by the favourable results gathered in the European Community adaptability test, Heybroek released the «Columella» clone (Figure 1 and 2), which was characterized by a high level of

Figure 1. Fastigiate shape of selection «Columella» built up by H.M. Heybroek by selfing the clone «Plantyn» (*U. minor, U. glabra and U. wallichiana* are among the parents).

Figure 2. The curiously twisted leaves of «Columella» are inherited as recessive character from *U. glabra* «Exoniensis»

DED-resistance and an elevated ornamental value and, more recently, the clone «Lutèce» released along with the French partner INRA (Institut National de la Recherche Agronomique). Since the collection and evaluation of data from the adaptability plots is still in progress, it is possible that other clones included in the European test may be named and released.

The long task of elm improvement carried out through advanced generations of breeding at Wageningen placed emphasis on the European elm species. Thus, the released clones have no more than one quarter Asian «blood», while other good and resistant clones are 100% European (Heybroek, *personal communication*). Crossings were made every year from 1936 to 1983, including even 1945. The best and most resistant seedlings of the early crosses were included in the crossing schemes. As a result, about 200,000 seedlings of the F1-F4 generations and 2,000 clones were tested for resistance to DED in repeated inoculations and for other traits (Heybroek, 1993). Several hybrid individuals built up in Wageningen entered various breeding programmes in Europe and in North America, in an international effort to prepare resistant elms for the market. With Heybroek's retirement in 1992, elm breeding activities at the Dorschkamp Institute were suspended.

The Italian programme

The second elm breeding programme in Europe was set up in the late 1970s in Florence, Italy, by the Institute for Forest Tree Pathology (now the Institute of Plant Protection) of the Italian National Research Council (CNR), when the second more destructive pandemic of DED caused by *O. novo-ulmi* was invading the country. The idea underlying this project was the conviction that the Mediterranean environment would need its own selections. This could be argued by the favourable adaptation of Siberian elm in Italy, whereas this species did not thrive in the Netherlands, and by the unsuitability of the Dutch selections to the hot and dry areas of central Italy. The Italian programme is indebted to the work of the Dutch researchers and to the European project 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 foreign 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 for example the realisation of pollination without having to lift the isolation sack, by blowing the pollen into the sack (Mittempergher and La Porta, 1991).

One of the interesting aspects of the Italian programme has been to point out that the introduction of non-native elm species from different continents may involve the risk of susceptibility to local parasites of minor importance concerning native species. For instance, a disease which until 1985 had been regarded as disease of American origin was found to be dangerous and even deadly for a number of Asian elm species resistant to DED and for their hybrids. This disease, named Elm yellows (EY), is caused by phytoplasmas. It kills the American elm (*U. americana* L.); therefore, its outcome in this case does not dif-

Figure 3. Symptoms of Elm yellows on *U. villosa* grown in Pisa, Italy.

fer from the results of DED. Yet in Europe, EY is tolerated by the populations of native elms, with only a few individuals showing symptoms of yellowing (Figure 3), witches' brooms (Figure 4), growth retardation, or a general decline (Mittempergher, 2000).

Numerous insects are also known to damage European elms. Among these, the elm leaf beetle (*Xantogalerucha luteola* Müller) (Figure 5) and the goat moth (*Cossus cossus* L.) have a very high ranking. The various Asian elm species used in breeding programmes 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 Institute in Flo-

Figure 4. Witches' broom caused by natural infection of Elm yellows on *U.* «Lobel» in the Apennines, Italy.

Figure 5. Dried up leaves eaten by the elm leaf beetle.

Figure 6. The fast growing clone «San Zanobi», bred in Florence, Italy, by crossing the Dutch clone «Plantyn» with *U. pumila*, gives hope to be grown also for timber production.

Table 1. Rating of some elm species in decreasing order for susceptibility to EY (Elm yellows) from natural infection, to DED (Dutch elm disease), and to ELB (elm leaf beetle) based on the Italian breeding work (from Mittempergher, 2000)

Species	Susceptibility to EY	Susceptibility to DED	Susceptibility to ELB
Ulmus americana	+++a	+++	++
Ulmus villosa	++	+	+
Ulmus chenmoui	++(?)	–	–
Ulmus laciniata	++(?)	+	++
Ulmus wallichiana	++(?)	+	++
Ulmus japonica	++ / +	+	+
Ulmus parvifolia	++ / +	–	–
Ulmus laevis	+	+++	–
Ulmus carpinifolia	+	++	++
Ulmus wilsoniana	+	–	+
Ulmus pumila	+	–	++
Ulmus glabra	+	+++	++
Ulmus macrocarpa	– (?)	+(?)	–(?)

[a] Level of susceptibility: highest (+++), lowest (–), ranking unclear because of the low number of the tested individuals (?).

rence has thus set up a rating programme to assess the extent to which the commonly used Asian species may be susceptible to EY and to the elm leaf beetle. The susceptibility ratings are taken into consideration when preparing the crossing project (Table 1). It is very conceivable that the Mediterranean climate may be more favourable than the Atlantic climate of the Netherlands to the build-up of insect populations and to phytoplasma infection, which is vectored by some species of insects belonging to phloem-feeding hemipters.

The first results of the Italian programme are the «San Zanobi» and «Plinio» clones (Figure 5), which were obtained by crossing «Plantyn» with two different individuals of Siberian elm (Santini *et al.*, 2002). More than 50,000 hybrid seedlings have been raised and tested, of which 80 individuals totalled a very high score. Other clones with different parentage are due to be released in the near future.

The Spanish programme

Recently, another elm breeding program was started in Spain by Luis Gil and his co-workers at the Unidad de Anatomia, Fisiologia y Mejora Genetica Forestal of the Universidad Politecnica of Madrid. Starting of the programme was prepared by an extended literature review, an inventory of the host on the Spanish territory, as well as a characterization of the pathogen, and a study of the vector (Gil, 1990). The Spanish programme has likewise greatly profited from participation in the European project which materialised later, during the course of the second contract. It, too, relies mainly on the Siberian elm (whose presence in Spain precedes DED appearance) as a source of DED-resistance, and aims to introduce this resistance into the native *U. minor* species. The first breeding cycles were started in 1993, on a number of genotypes chosen for their resistance, ornamental value, reproduction success, and growth. These researchers followed the standard procedure set up in the Netherlands and Italy, and have reached the concluding stages of the selection process for resistance of the first hybrid generation. Preparations have now begun for the second generation crossing (F2) and for evaluation of clones to be released (Solla, 2000). In order to speed up the process of selection for resistance to the disease, some early selection techniques were studied, including the use of culture filtrates of the fungus and measurements of anatomical and physiological parameters in elm seedlings (Solla *et al.*, 2000; Gil *et al.*, 2003).

Other European programmes

In order to complete the European panorama on activities designed to select elms resistant to DED, mention should also be made of the work carried out at Volgograd and at other research institutes in southern Russia. Siberian elm is a species which thrives well in the arid steppe, and is a valuable tree for shelter-belt and farmstead plantings, as well as a source of fodder (Heybroek, 1981b). A selection for disease tolerance and adaptability to the local environment was carried out, and the best genotypes have been planted in an orchard for seed production (Mattis and Mukhaev, 1979; Uvarov and Butorina, 1986). However, the literature on this project is not easily available.

Mention should also be made of the activity of testing Dutch and other elm clones for disease resistance carried out in a few laboratories in Europe, particularly in France by Jean Pinon. This has already led to the release of the clone «Lutèce», while other clones may follow.

The American programmes

The arrival of the DED epidemic in America was not unexpected. Therefore, 3 years after the first report of the disease in the state of Ohio, an attempt at eradicating DED having failed, the collection and testing of the germplasm of American elm for resistance to DED was begun in 1933 within the framework of a cooperative programme between Cornell University and the Boyce Thompson Institute (Sinclair *et al.*, 1974). The results of this experiment were then transferred to longer-lasting programmes. There are two very important programmes as far as the effort of facilities and the duration of the research efforts are concerned. Subsequently, a third breeding programme was added. As of today, only the programme carried out at the research institutes of the Agricultural Research Service of the US Department of Agriculture (USDA-ARS) continues its activity at full capacity.

The USDA programme

The USDA-ARS has been involved in the development of genetically improved elms since shortly after the arrival of the disease in America. Initially, in the year 1937, attention was concentrated on the objective of saving from destruction the American white elm (*Ulmus americana* L.) that characterized the lines of trees of North America. More than 35,000 seedlings were screened for DED susceptibility upon inoculation of the disease at the USDA Laboratories (Smucker, 1944; Clapper, 1952). It was soon quite clear that susceptibility of this species to the disease was extremely high (out of the original population, only two individuals were selected as being resistant), and that, on the other hand, the introduction of resistance through hybridization met with enormous difficulty in the barrier to hybridisation with the donor species. This barrier (Ager and Guries, 1982) was not due solely to the fact that the American white elm was the only tetraploid within the genus, because the creation of haploid American elms (Lester,1970) or tetraploid Eurasian elms (Derman and May, 1966) failed to improve crossability. Even methods based on protoplast production and fusion of protoplasts were not successful when American elm was involved (Redenbaugh *et al.*, 1981; Townsend and Masters, 1984). In passing, we wish to note that the late Frank S. Santamour Jr. (who used to work in the U.S. National Arboretum in Washington, D.C.), in trying to modify the ploidy of the American elm or of the diploid species that are carriers of resistance to DED in order to obtain hybrids, was the first researcher at the beginning of the 1970s to consider the Chinese elm, the *U. parvifolia* Jacq. Despite its great variability, Chinese elm has acquired considerable importance in the American improvement programmes (Santamour, 1973). It has a high level of resistance to the DED fungus, a form of vase-shaped tree behaviour which approaches that of the American elm, as well as high resistance to the elm leaf beetle, *Xanthogalerucha luteola*, which in America —differently from Holland— occasionally represents a problem.

During that same period, due to the difficulty of working with *U. americana*, at the Delaware, Ohio, Laboratory, breeding work was concentrated on determining hybridization potential among diploid elm species and hybrids and on screening hundreds of seedlings of the progenies. Barriers to gene exchange were generally not present among most diploid species, although the success of many crosses depended on which species was used as the female and which was used as the male. In addition, differences between reciprocal progenies were evident as to height, diameter growth, and time of flushing, which suggested that these differences were attributable to some extra-chromosomal inheritance (Townsend, 1975, 1979). By also using many hybrid clones produced in Holland, more than 60 combinations of hybrid elms were created and various types of knowledge on the transmission of resistance were gathered.

In 1980, the group of researchers headed by Alden Townsend initiated advanced generational breeding, using a factorial design in which they made many combinations of crosses among their best selections or cultivars (Townsend and Santamour, 1993). The progenies were evaluated for their resistance to the aggressive species of DED fungus and to the elm leaf beetle. During this phase of the work, it was shown that

Figure 7. The clone «Frontier» (hybrid between *U. minor* and *U. parvifolia*) is among the several selections released by the USDA programme (photo by A. Townsend).

the male and female parents, and their interactions influenced the expression of the disease symptoms (Townsend and Douglass, 1996), and that clones from within the same full-sib family differed significantly in their resistance to DED, as an effect of the heterozygosis of the parents and also of the existence of specific combining, or non additive, gene action. The work of hybridizing and selecting among the progeny for disease and insect resistance continues at present with the non-American elms, with emphasis on the production of sterile lines by crossing spring-flowering elm with selections of Chinese (or lacebark) elm, *U. parvifolia* (Figure 7). Prevention of the introgression of genes into the native elms and of seed production are reasons for the development of sterile lines.

In spite of the rather poor preliminary results, the work of American elm breeding, i.e. selection and intra-specific crossing, was carried out by the USDA Laboratories on materials coming from previous already mentioned experiments. Evaluation for DED resistance of the promising American elm clones was carried out at Delaware, Ohio, from the 1960s through the 1980s, and at Glenn Dale, Maryland, during the 1980s and 1990s. This led to the release of two clones with a high level of disease tolerance: «Valley Forge» and «New Harmony». Controlled crosses were again made among selected American elm clones in the 1980s and 1990s. As a result of the work carried out with this material and with survivors in areas hit hard by DED epidemics, another comprehensive test was prepared with 20 American elm clones, including Eugene Smalley's «Independence» elm and Jim Sherald's «Jefferson» elm. From this test, in which inoculations took place in May 2002, Townsend's research group hopes to achieve better results in the levels of resistance than those of existing selections. A programme of American elm breeding is also being conducted at the Laboratory in Delaware, Ohio, by J. Slavicek and S. Eshita of the Forest Service of the USDA in collaboration with D. Townsend. Townsend's group recently joined in collaboration with Dr. Wayne Sinclair of Cornell University, in order to determine the relative susceptibility to EY of the American and non-American cultivars and selections. So far, 11 elm clones resistant to DED have been named and released on the market.

The Wisconsin programme

The development of an elm-breeding program at the University of Wisconsin, in Madison, has depended primarily upon satisfying an educational/basic-research function while responding to a mandate from the Wisconsin State Legislature to «solve the problem of Dutch elm disease». The most important product of this programme was the training of graduate students and the discovery of basic facts about elm biology and the nature of host-pathogen interactions. The production of improved elms alone could not justify the continued involvement of the University in selecting and testing elms. At the beginning of the activities in 1958, the practical goals were two-fold: a) to develop a DED-resistant American elm; b) to develop hardy, pest-resistant, and ornamentally-useful Eurasian elms.

Thousands of seedlings of 87 accessions collected throughout the whole growth area of *U. americana* were assessed for resistance to DED. Controlled crosses among selected survivors as well as resistant individuals from the New York and USDA-ARS programmes began in 1969. The repeated selection effected on the progenies resulted in producing the named «American Liberty Multiclone», consisting of 6 individuals, one of which was later patented as the «Independence» elm. Overall, the parents of «American Liberty» elms are the survivors of over 60,000 inoculated American elm seedlings collected from many locations over the northern range of American elm. In fact they included superior survivors from the Wisconsin screening programme, as well as resistant individuals from the New York and the USDA programmes (Smalley *et al.*, 1993). Beginning in 1985, a second generation breeding was started, using as parents the few survivors of the F1 parents, the most vigorous of the survivors of the F1 progenies, and a few previously unselected parents.

The improvement work with Eurasian elms also started in 1958, with the grafting of 13 clones from the Dutch breeding programme and the introduction of seeds from various parts of the world. Except for Japan, the collection of elm genetic resources in Asia was found to be difficult for political and logistical reasons. Around the year 1980 Eugene Smalley, the leader of the programme, engaged in a couple of adventurous trips in China searching for native elm material. *U. pumila* and *U. japonica* were the most reliable sources of resistance during the first years of work, along with clones coming from the various Arboreta and from the Dutch and USDA breeding programmes. *U. minor* seed accessions contained resistant individuals with a frequency of about 13%, essentially the same as the best *U. americana* accessions coming from the north-eastern USA, while Hungarian and Polish accessions tended to be better than average. The situation was the same for *U. glabra*, with a special performance of accession from the Ural Mountains that showed 39% of resistant individuals. In all the Eurasian species, variation among provenances was obvious, thus making the choice of accessions for breeding an important consideration. High variation was found in particular among accessions of *U. pumila* and *U. parvifolia* (Smalley and Guries, 1993).

At the beginning of the hybridisation work with diploid species in 1968, three species, *U. pumila*, *U. japonica*, and *U. rubra* Muhl., were designated to be combined in order to replace *U. americana*. Later on, the last species, reputed to contribute vigour and ornamental value, was dropped because of its extreme susceptibility to DED. *U. pumila, U. japonica, U. parvifolia* and *U. wilsoniana* were the major sources of resistant genes for elm cultivar release in America up until the early 1990s. Starting from 1984, the Wisconsin breeding programme relied more and more on *U. parvifolia* because of its resistance to DED, to black leaf spot (*Stegophora ulmea*), and to the elm leaf beetle to which *U. pumila* was very susceptible. This was also because of the possibility of obtaining a certain number of hybrids with *U. americana* when the Chinese species was used as the female parent. Hundreds of *U. parvifolia* X *U. americana* hybrid seedlings were obtained with the prospect that vigorous resistant elms liable to preserve the structural characteristics of the American elm could be glimpsed. Nine resistant clones of Asian origin, of which «Sapporo Autumn Gold» (Figure 8), a natural hybrid between *U. japonica* and *U. pumila*, is the file-leader, have been patented and are sold also in Europe (Smalley and Guries, 1993). In addition, in 1993, Smalley and Guries wrote that, at that time, they had sufficient material under test to provide new cultivar releases for several decades. At present, following the retirement in 1994 and subsequent death in 2002 of Eugene Smalley, elm breeding activities have been closed, and there is concern as to how to preserve and utilise the abundant genetic material, which he accumulated

Figure 8. «Sapporo Autumn Gold», a natural hybrid between *U. japonica* and *U. pumila*, is the file-leader of nine selections patented by the Wisconsin University and sold also in Europe.

The Morton arboretum programme

The third structured elm breeding programme in North America originated at the Morton Arboretum in Chicago, Illinois. Here, the largest elm collection in the US has been built up, due to the interest in trees that tolerate the adversities of urban tree-planting sites. 23 Asian species, 6 American species, and the European species and subspecies or varieties appeared in a recent list of elm material at the Arboretum. Completion of this elm collection was made possible, thanks to the increased availability in recent years of elm seeds from China. Among these species, several are little known. Generally speaking, the elms from China are found to be resistant to DED, leaf beetles and, in some cases, also EY; in addition, they show great variation in growing habits, ornamental characteristics, environmental adaptability, and pollution abatement. Therefore, they offer great opportunities for increasing the diversity of tree species within the urban landscape and for avoiding the reconstitution of the monoculture of American elm which proved to be so vulnerable. The elms from China represent a precious resource for providing answers to the most varied requirements. In this way, they expand the place that is traditionally reserved for the elm among city plants (Ware, 1995).

The breeding programme began in 1972 (Ware, 1992; Ware, 2000) with the finding of an outstanding tree resembling the American elm on the grounds of the Arboretum near the Thornhill Educational Center. The Thornhill elm was identified as a natural hybrid between *U. japonica* and *U. wilsoniana*, and was named «Accolade» (Figure 9). It became a parent of many of the resistant elm selections built up in the hybridisation activity of the Arboretum. Five hybrid clones have been named and released containing *U. pumila* and *U. minor* in their parentage in addition to the named *U. japonica* and *U. wilsoniana*. New crossings have been carried out in recent years involving seldom-used Chinese species, e.g. *U. szechuanica* Fang., *U. macrocarpa* Hance and *U. propinqua*. Breeding activity is still carried out on a reduced scale by the leader of the project, Dr. George Ware, who has retired.

Figure 9. «Accolade», a natural hybrid between *U. japonica* and *U. wilsoniana*, is the first of several releases of the Morton Arboretum (photo by G. Ware).

As for North America, in the USA and Canada there are also other, less important elm breeding programmes. These have had a shorter life, and in general have been limited to making selections within the framework of the species. Several of these resistant selections have been named and released on the market, as for example «Assumption», a selection of *U. americana* obtained after X-rays mutation treatment (Ouellet and Pomerlau, 1965); «Discovery», «Jacan» and «Thompson», selections of *U. japonica*; and «Across Central Park» and «Prairie Shade», selections of *U. parvifolia* (Santamour and Bentz, 1995).

A few highlights of the activity

Collection of native and foreign elm genetic resources

The first attempt at elm improvement for disease resistance was from within indigenous species selections, both in Europe and in America, where the operation was promoted with more determination, on a greater amount of material and for a longer time. In Europe, the collection and testing of indigenous elms was resumed recently, essentially for conservation purposes, and is illustrated by Eric Collin in this book. In this regard, however, we must report here the continued sparse knowledge and lack of material relative to the species indigenous to the eastern part of Europe, from where several highly-resistant clones collected in America originated (Smalley and Guries, 1993).

Although the value of foreign species (in particular Asian ones) as a source of resistance to DED was recognized very quickly and although some of them have been used since the very start of the Dutch programme, their utilisation in a more intense manner began only around the beginning of the 1980s, with the opening up of China to scholarly interaction and communication. Indeed, the adequate use of these species involves the introduction of seeds and, thus, of populations having different geographic origins. Only in this manner is it possible to evaluate —and thus to make appropriate use of— the great variability present in the species vegetational and physiological characteristics, therein including resistance to various diseases (DED, black leaf spot disease; EY; elm leaf beetles; the Japanese beetle) (Miller, 2000). Unquestionably, *U. pumila*, *U. japonica* and *U. wallichiana* are the best known and most used species in the breeding programmes. Many other species with Chinese origins have been imported over the past 20 years at the initiative of the University of Wisconsin and the Morton Arboretum. The material introduced has been evaluated only partially; it therefore represents an interesting potential for the work of breeding (Smalley and Guries, 2000).

Species crossability and self-sterility

Several elm breeding programmes have explored crossability patterns among elm species, because this was an opportunity to produce a wide spectrum of hybrid progenies on which to make a selection (Heybroek, 1968; Santamour, 1972; Townsend, 1975; Hans, 1981; Mittempergher and La Porta, 1991) (Figure 10). From these tests it was found that crossability barriers among elm species are generally weak or are absent, but that the success of several combinations depends on favourable male-female interactions. This result may well explain the variable data reported in other trials. Strong barriers to hybridization were found with *U. americana* and *U. laevis* Pallas, which belong to the Blepharocarpus section which seems genetically isolated from the other sections of the genus (Heybroek, 1968, 1993; Townsend, 1975). Nevertheless, hundreds of hybrid seedlings were successfully obtained by Smalley and Guries (2000) from *U. parvifolia* used as the female parent and *U. americana* as the male parent. A rare case of post-zygotic sterility was noted by Mittempergher and La Porta (1991) in crossing *U. laevis* used as the female parent with *U. villosa* Brandis as the male parent: thousands of seedlings, some of which even showed hybrid vigour, started dying at the age of 2-3 months, and none survived the following year. On the contrary, the frequency of spontaneous crossing in nature is so high between *U. pumila* and *U. minor* in Italy and Spain and between *U. pumila* and *U. rubra* in the northern USA, that it is highly probable that an introgression of the species will occur.

Self-sterility is a common event in elms, a fact that makes it possible to effect crossings without recurring to emasculation, i.e. in the presence of self-pollination. But the phenomenon is not always complete in the different species, and varies from year to year and

Figure 10. Insufflation of preserved pollen for controlled crossing.

in different genotypes. For this reason, in every crossing experiment some pollination bags are kept to check self-fertility. However, in cross-pollinated families a number of seedlings were suspected —for morphological reasons— of coming from self-pollination, while the control bags yielded few or no seedlings. The explanation is that the presence of foreign pollen acted as «mentor pollen», thus facilitating self-fertilisation (Heybroek, 1993).

Inoculation system

The availability of a reliable inoculation system that incites disease in a large number of plants is fundamental to proceeding quickly and surely in the long procedure of forest tree breeding. The Dutch inoculation method (Figure 11), which introduces the inocu-

Figure 11. The Dutch slit inoculation technique.

lum directly into the large vessels of the lower trunk by cutting into the new sapwood and letting two drops of the conidium suspension, placed on the knife blade, be sucked into the rising sap, guarantees approximately 100% infection of the treated trees. For this reason, even it is an unnatural method remote to the infectional courts made by the bark beetle vectors, it has been adopted by European breeders and by the Forestry Commission in Great Britain. The latter has promoted fundamental studies on the genetics of the fungus.

At the beginning of the activity of selection for resistance, Fransen and Buisman (Heybroek, 2000) tried a complicated inoculation procedure in which the inoculation was performed by bark beetles artificially loaded with fungus propagula, in order to compare this «more natural» inoculation with the easier and more uniform «slit» method. Because of its complications, the method did not have success among breeders. In any case, it must be stressed that an inoculation technique that bypasses the work of the vectors disregards the possibility of disease escape being shown in species and in individual elms that are not preferred by the bark beetles while they are searching for a place in which to dig their feeding groves (Webber and Kirby, 1983). The lack of attractiveness may be so strong as to confer a rather successful protection from infection on elms which otherwise might be very susceptible to the fungus, as occurs in Italy and other parts of Europe with the European white elm (*U. laevis*) (Sacchetti *et al*., 1980; Heybroek, 2000).

We should add that the slit method devised in Holland has been considered to be overly strict, and thus has been modified over the past 20 years, by both the pathologists and European breeders, by raising the inoculation point on the trunk to one-third of the way down the crown, and then introducing the inoculum into smaller vessels. Even in this case, the method succeeds in causing disease in almost all cases, so that even genotypes with a reasonable resistance level may show some leaf symptoms before making a rapid recovery. It makes it possible to distinguish differences in the resistance level of the host and in the degree of virulence of the pathogen.

The Dutch method was judged to be too severe in America, where the principal vector of the disease is *Scolytus multistriatus*, and the more aggressive and efficient major scolitid, *S. scolytus*, is not present. Thus, for the USDA-ARS project during the 1970s, the Townsend group made a chisel wound in the trunk and introduced the conidium suspension into the wound with a syringe. Beginning in 1992, they developed a new inoculation technique that mimicked the bark beetle transmission: the conidium suspension was introduced into a 2.4 mm slanting hole excavated by a battery powered drill and located in the bottom one-third of the main trunk (Townsend *et al*., 1995). The Wisconsin technique is similar to the latter , but is even smoother because the holes are drilled in branches approximately the size upon which natural twig-crotch feeding occurs. But Wisconsin researchers found that field results almost always overestimated the genetic resistance of the host. They therefore developed a procedure for inoculation and for evaluation in a controlled environment, in the greenhouse or plant growth chambers (Green *et al*., 1984; Smalley *et al*.,1993). The procedure provided for raising strictly standardised plants, and for evaluating the disease mainly through the volume of xylem discoloration occurrence. Even if Heybroek (1993) found that the relation between the internal and external symptoms could vary (with the extreme case of *Hemiptelea davidii*, which does not wilt upon inoculation while the twigs grow almost black internally), the researchers in Wisconsin claimed that the ranking of the clones, families, and accessions were in substantial agreement when screened in a controlled environment or in the field.

Another critical point is the selection of isolates for the preparation of inoculum, owing to the extreme variability of the fungus which is present through the DED-infected area as different species, subspecies, hybrids and populations. At present, in one part of Europe (as, for example, in Italy), two sub-species of the so-called «aggressive strain» are present: *O. novo-ulmi* subsp. *novo-ulmi* and *O. novo-ulmi* subsp. *americana* [formerly known as Eurasian (EAN) and North American (NAN) races, respectively] (Brasier and

Kirk, 2001) and probable hybrids. In the Italian selection programme, therefore, two isolates belonging to the two sub-species are used. In America, where only the *americana* sub-species is present, a combination of aggressive and non-aggressive individuals is often used for preparing the inoculum. This procedure seems incorrect to us, because the presence of non-aggressive biotypes could induce resistance in elm clones that resist the non-aggressive species of the fungus (Scheffer *et al*., 1980). In fact, we know that the protective effect of induced resistance does indeed work in DED control, even if it is with several limitations (Hubbes and Jeng, 1981; Sutherland *et al*., 1995).

Disease evaluation

The evaluation of the amount of disease is the scoring instrument of the susceptibility/resistance of the host or of the virulence of the pathogen. For this, during field trials, the percentage of defoliation of the crown and the percentage of dieback are generally measured. Measurements are made during the year of inoculation and also during the following year, when it is important to assess the recurrence of the disease. As symptoms in the more sensitive genotypes usually increase during the inoculation year, it is important to measure them more than once during the period. The total symptoms are strongly influenced not only by host and pathogen genotypes, but also by the environment, understood as a whole: i.e. effect of soil type and soil moisture, the season, and age of the tree, declining when the plant becomes older (Smalley and Kais, 1966; Sutherland *et al*.,1997). Symptoms are also influenced by air temperature and hours of sunshine following inoculation (Sutherland *et al*., 1997). Resistance scores often differ from year to year, so that even the ranking of standard clones of similar resistance value, which ordinarily are kept as controls, may differ in different years (Heybroek, 2000). In fact, Smalley's research group found that the time of greatest susceptibility and the duration of the susceptibility varied greatly among elm species and even among seed sources in a given species. Thus, the favourable period for inducing the disease may be short in some species, beyond which a lack of symptoms may be mistaken for resistance. To obviate this shortcoming, if requirements of space and time are constringent factors, it is recommended to determine the peak of elm susceptibility for the location of study and to inoculate at that moment. Repeated inoculations may be needed over several years, in order to establish a true interaction between host and pathogen. The importance of establishing very detailed protocols which take into account all the variables and the necessity of always keeping several control trees which have known degrees of susceptibility, in every inoculation trial, is evident. Considering that the inoculation systems may be different, as well as the isolates used for inoculum preparation, it will be understood that evaluation of sensitivity to the disease obtained in the course of various programmes are not comparable: in fact contrasting evaluations are shown in published works for the same clone and for the same species. Several standardized clones are presently available which could be used as controls together with well characterized isolates of the fungus, in inoculation experiments, favouring means of comparisons for evaluating resistance to DED of the selections at stake. Sound, long overdue comparisons are of prime interest to potential users. A protocol for inoculation and symptom evaluation is going to be published as some of the results obtained as an outcome of the EU Res Gen CT96-78 Project on the «Conservation of elm genetic resources in Europe».

Building up resistance

In order to produce by breeding elms resistant to Dutch Elm Disease, the elite trees must possess a level of resistance, which in the progeny is not always the mean of that of the parents: in fact, some individuals in the offspring may have a higher resistance than either of the parents. In contrast, a resistant individual may give rise to a mainly susceptible offspring. It has already been reported that resistance to DED is polygenic in nature but with the presence of major genes. There is generally considerable variation in resistance within one species, so it is important to use properly chosen accessions and individuals. First-generation products are rarely satisfactory as regards the level of resistance and their vegetative traits, because Asian species often carry some negative traits. The possibility of gradually accumulating favourable characteristics in subsequent crossings suggests that breeding work should be carried on over several generations. As research proceeds to advanced generations, it is possible to broaden the genetic base by adding genes from diverse elm species, in addition to vegetative traits close to those of the native species, and thus to achieve a satisfactory level of di-

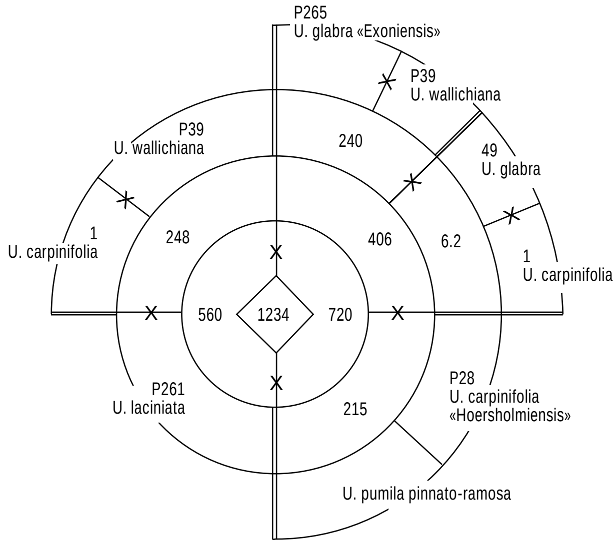

Figure 12. The genetic origin of the fourth-generation hybrid clone n° 1234 built up by H.M. Heybroek, in which 4 species are involved (from Heybroek, 1993).

sease resistance. This may be inferred by the graph in Figure 12, which shows the genetic origin of the fourth-generation hybrid clone N° 1234 produced by Heybroek, in which four species were used.

It is relevant to recall that introduced exotic elms resistant to DED may become affected with problems of present minor importance for the native species, while other diseases, such as EY in America, can destroy the elms preserved from DED attack (Lanier *et al*., 1988). It is therefore primordial to select for resistance to other diseases and pests at the same as to DED, depending on the area in which the programme is being carried out.

The biology of elm reproduction and pollen conservation is well- known, as are the techniques of flower isolation, pollination without removing the isolation bag (Mittempergher and La Porta, 1991), and protection of the growing seed from insects. We can say that there are no major problems in this sector.

At present, the genetic bases and physiological responses that confer resistance to the host and virulence to the pathogen are not fully understood and remain a goal for the future. Such knowledge would improve and speed up the long work of breeding which still remains more an art than a science.

Concluding remarks

The balance of many years of work is positive, because up to now genetic improvement has given concrete results and found to be the sole means for controlling this serious disease Among these results figures the release of a moderate number of resistant cultivars. In 2000, 18 resistant elm clones were in production in the J. Frank Schmidt and Son Co. Nurseries in Oregon and a further 12 cultivars were being evaluated for commercial production (Warren, 2000). In addition, several clones are at present in an advanced state of testing and will certainly be released in coming years with the aid of still active breeding programmes. At Darmstadt, in Germany, the Conrad Appel nurseries list nine resistant elms in their catalogue, all coming from the Wisconsin programme. As a consequence of the breeding activity programmes, there is today a revival of interest in elm in the nursery industry. The introduction of Asiatic material has made elms available that also satisfy requests for ornamental trees which were not covered previously by our indigenous elms. For this reason, the space reserved for elms among the plants used in cities has potentially been enlarged.

A second advantage of the breeding activity is that of having favoured accumulating and made available a considerable wealth of knowledge relative to the biology of the elm and DED, as well as knowledge relative to susceptibility to DED and to other diseases and pests in a large number of elm species, which up to recently were unknown. This knowledge, together with the availability of precious genetic material which has been accumulated in several of the laboratories mentioned, represents a valuable treasure to safeguard, because it could be a reserve from which to obtain a large number of new cultivars. Developing new cultivars is warranted as an opportunity of creating new selections that are more suitable to local requirements and with a different genetic background. Genetic diversity is indeed the only route to build up defence against very variable pathogens and parasites, such as the DED pathogen, and against unpredictable risks. The large interaction between genotype and environment has already been mentioned. This open up on the need to evaluate how selections adapt to new environments, including of other continents, which may affect the level of resistance to DED and other parasites present locally. Another positive effect of the breeding activity has been an interdisciplinary interaction with other scientific fields. For example, the need to know the reaction of native elms to the disease has stimulated the *ex situ* conservation of these species during the assembly of core collections. This material and materials collected from abroad were at the onset not only of studies on breeding, but also of taxonomic, physiological, and molecular genetic studies. The need to authenticate the hybrid nature of the offspring has stimulated the study of genetic markers. The precise definition of both inoculation and selection techniques has brought about a more attentive examination of the aspects of the Host-Pathogen-Vector interaction with the environment.

Despite these positive results, the traditional breeder has to face several difficulties in pursuing his work. Firstly, the mentality has changed, as reflected in the lack of interest in and, therefore, in the shortage of available funding for long-term applied research projects. Secondly, there is a propensity by many to believe that biotechnology will provide a rapid solution to problems of the sort. In particular, genetic transformation is considered as an ideal alternative to conventional breeding, by introducing the desired resistance genes into locally well adapted elite elm clones, without disrupting their better genetic features. While the expectations of biotechnology are yet at a standstill, it is expedient not to abandon the traditional activity proven to provide concrete answers and the more so the results are reliable as the selection progress.

Finally, one of the concerns of foresters and conservationists is that resistant selections generated from exotic material may pollute the genetic sources of domestic elms, due to the well-known ease of elm interspecific hybridization. This concern may be mitigated considering the behaviour of complex hybrid selections of Dutch origin raised in the experimental plots planted in Italy within the framework of the European adaptability test. No seedlings were found growing naturally in the field, while plenty of seedlings of Chinese and Japanese elm are in fact growing in the same field where accessions of the two pure species have been planted. These observations, in need of being confirmed by appropriate trials, suggest that the complex hybrid nature of the Dutch selections involves a certain loss of fertility, in open air, which could nevertheless be partly overcome in pollination bags, where the particular environment and the presence of two pollen sources could give rise to a number of successful crosses.

The question of interfertility does not arise with sterile hybrids obtained with *U. parvifolia*, but is current and important for hybrids containing *U. pumila*, the Siberian elm, which crosses very easily with the field elm, *U. minor*, in southern Europe (Italy and Spain) (Figure 13) where it has been introduced abundantly,

Figure 13. A spontaneous hybrid between *U. pumila* and *U. minor* in northern Italy.

and with *U. rubra* in North America, giving rise to hybrid swarms that show morphological traits typical of the two species. Favouring the introgression of Siberian elm into the two aforementioned species, which otherwise is occurring in nature, would be an easy operation, that could foresee a solution to elms in forest stands as well, to which traditional breeding cannot respond. The negative aspect of the operation would be a loss of identity for the native species. The discussion about the pros and cons of the operation would take us too far away from the history of elm breeding. Nevertheless, we though it right to raise this matter as another possible solution in a moment in which traditional breeding meets with difficulties, biotechnology still has not produced concrete results, while DED prosecutes its destructive progress among the re-sprouted elm populations.

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