Ulmus Species
Recent Genetic Research

Gösta Eriksson and David Clapham

Ulmus Species
Recent Genetic Research

Gösta Eriksson and David Clapham

Cover photo: A fallen giant wych elm in Dalby Söderskog National Park in southern Sweden. This national park was once an elm forest, which by August 2023 had been devastated by Dutch elm disease. During the decline of the elms, ashes (*Fraxinus excelsior*) gradually replaced them, but the ashes are now suffering severely from ash shoot dieback. Photo: Barbro Ekberg.

Preface

Trees of the genus *Ulmus* are known as elms in English. Almost 50 species are distributed over north temperate regions and mountains in tropical Asia. They are taxonomically difficult because there are many introduced local types, sometimes given species names, that are connected by intermediates; some of these intermediates are probably hybrids. *Ulmus glabra*, the Wych Elm, is the only certainly natural species widely distributed in Britain and Sweden. It prefers nutrient-rich, non-acid soils. Other notable species are *U. americana, U. laevis, U. minor,* and *U. pumil*a.
Elms have some economic importance. The valuable wood of e.g. Wych Elm is tough and heavy, can be cleft, and is used for furniture and coffins; and garden varieties are produced. Elms have frequently been used in viticulture as supports for grapes. Farm animals such as cattle and sheep like feeding on elms, which explains the introduction of certain elm species into England, from perhaps as early as Roman times. In parts of USA the American elm was a much appreciated tree characterizing urban areas.
Approximately 100 years ago the fungus *Ophiostoma ulmi* caused a setback to elm cultivation in Europe and North-America. This disease was named Dutch Elm Disease (DED). After some successful breeding for resistance/tolerance against this fungus, a more aggressive fungus *Ophiostoma novo-ulmi* appeared during the 1940s. Both fungi are spread by *Scolytus* beetles. Mature trees, apparently healthy at the beginning of summer, have been losing nearly all their leaves in July and August ultimately leading to tree death.
Trees of the English Elm *Ulmus procera* were killed by DED in southern England, though the root systems have often survived. This allowed the development from the roots of adventitious shoots of *Ulmus procera*, but these are still susceptible to DED. This raises questions as to how to obtain genetically resistant elms, and the feasibility of conserving the various elm species.

As with previous reviews of tree genetics studies we have tried to summarize published reports on recent (2000 and onwards) elm genetics research. In contrast to other reviews we concentrate our summary on graphic illustrations of presented results. It should be noted that none of the illustrations were taken from the original papers. There are several reports which cover research in the different chapters. They are presented in one chapter only. The species names used in the quoted publications appear in this review.
As in previous books, papers written in languages that are not understood by the scientific society are with one exception not treated in our summary. An apology for missing relevant literature in our search for elm genetic investigations

Various kinds of review were published before but none with detailed presentation of individual papers. Santini and Faccoli (2015) thoroughly discussed the interaction between *Scolytus* beetles, the vector of DED, and elm trees. The focus was on resistance due to different phenologies of elms and of *Scolytus* beetles. They listed the following topics that remain to be solved:

- Beetle taxonomy based on molecular tools
- Selection of European elm clones based on avoidance of exposure to the pathogen
- Consequences of global warming for the interaction elm – beetle – fungus
- Accidental introduction of new vectors or new pathogen species following increased international trade.

They stated that a holistic approach to tackle the problem (= DED) is highly recommended.
Martin et al. (2019) presented a review with a thorough discussion of elm ecology and genetics with emphasis on Dutch elm disease.
Bernier et al. (2015) stated that there was a decline in the studies of elm genetics during the first decade of the current millennium in spite of the devastating effects of DED in America and Europe. They suggested it may be time for a large-scale joint international cooperation on DED genetics.

Finally, our sincere thanks to Christian Divander and Les Paul for their willingness to swiftly solve any computer problems.

Uppsala 2023

Gösta Eriksson and David Clapham

1 Differentiation among populations and among elm species

1.1 Metric traits

1.1.1 American studies

In a series of papers (Miller and Ware 2001a - 2001b, Miller and Ware 2002, Miller et al. 2003, Buso and Wagner 2007) the susceptibility of elm species and cultivars to the elm leaf beetle (ELB, *Xanthogaleruca luteola*) and spring and autumn cankerworms (*Paleacrita vernata* and *Alsophila pometaria*) was studied. The papers are mostly reports on routine investigations of susceptibility to different pests without testing of scientific hypotheses. They are of great value for identification of pest tolerant taxa.

In the paper by Miller and Ware (2001b) leaves from 12 taxa were used in a no-choice study. Leaves from trees in the Morton arboretum, Lisle, Illinois were placed in petri dishes and the mean number of eggs, percentage of females ovipositing, preovipositional period, and longevity of females and males were recorded. One unfed adult female and one male beetle were placed in each of ten Petri dishes.
In Fig. 1-1 we have illustrated the number of eggs laid and the percentage of ovipositing females for some taxa in common with Miller and Ware (2001c). In the four taxa with the highest frequencies of these traits the number of eggs laid was 1.6–2.7 times higher than the mean value. The corresponding figures for percentage of ovipositing females were 1.3–1.9 times higher. Two East Asian species, *U. elongata* and *U. propinqua*, did not have any eggs laid and no ovipositing. For both traits there were significant differences. The three other traits did not differ much. *U. elongata* and *U. propinqua* might be used in areas in which ELB occur in high frequency.

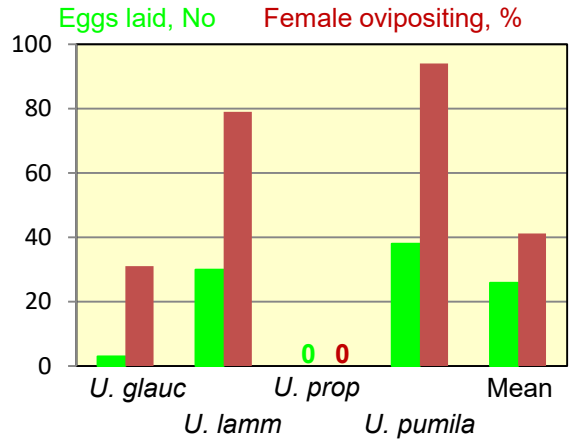

Figure 1-1. Number of eggs laid and percentage of ovipositing females in a no-choice feeding experiment with elm leaf beetles with 12 elm taxa. glauc = U. glaucescens, U. lamm = U. lammelosa, prop = U. propinqua. *Miller and Ware 2001b*

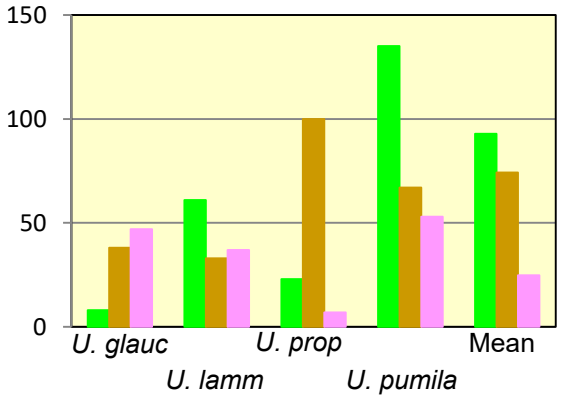

Figure 1-2. Number of eggs laid (green) and percentage of ovipositing females (brown) in a no-choice feeding experiment with elm leaf beetles in an experiment with 21 elm taxa. Lilac = percentage of emerging elm leaf beetles in 21 taxa. U. glauc = U. glaucescens, U. lamm = U. lammelosa, U. prop = U. propinqua. *Miller and Ware 2001c.*

In Miller and Ware (2001c) unfed 1-day-old larvae were put on leaves of 28 elm taxa; species, interspecific hybrids and cultivars and the emergence percentage were determined. The development time from larva to adult and percentage pupation were also recorded. A bioassay designed in the same ways as in the previous paper was carried out with 21 elm taxa. Some of the taxa analyzed were the same as in Miller and Ware (2001b).
The mean percentage of emerged adults was estimated at 24.6% and the differences among taxa were significant (Fig. 1-2). No adults emerged in six taxa while the maximum percentage 53 was noted for four taxa. There were significant differences among taxa for development time from larva to adult and percentage pupation. The longevity of both females and males varied significantly among the taxa.

A no-choice and a multiple-choice study research report was also published in 2001 (Miller and Ware 2001d), in which seven species were studied. Only females of *U. pumila* and *U. wallichiana* laid eggs. *U. pumila* had the highest number per female, 48 and 39, in the two years recorded. The corresponding figures for *U. wallichiana* were 31 and 25. The percentage of ovipositing females was also higher in *U. pumila* than in *U. wallichiana*. The multiple-choice experiment supported the results from the no-choice study, i.e. except for *U. pumila* and *U. wallichiana* there was more or less no leaf tissue removed in the other five species.

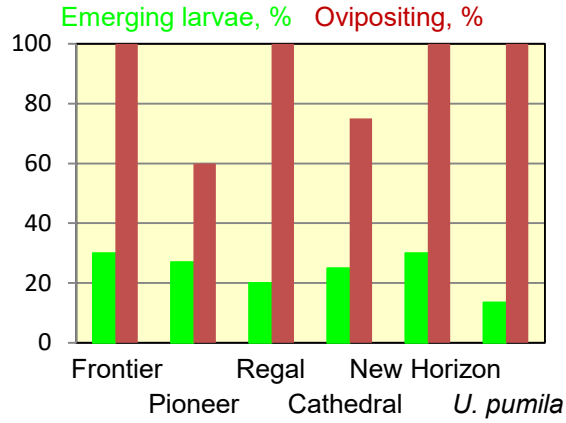

Figure 1-3. Emerging adults in a no-choice larval bioassay and percentage of ovipositing females from the larval bioassay. Five elm cultivars and U. pumila *were tested.* Miller and Ware 2002

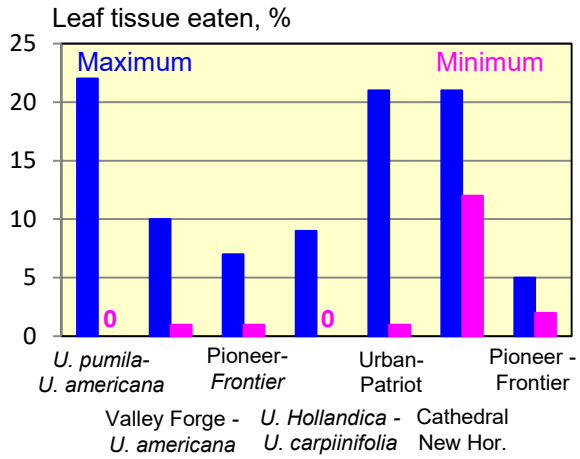

Figure 1-5. Maximum and minimum percentage of tissue eaten in 7 multiple-choice experiments with 3–5 taxa included in each experiment. Miller and Ware 2002.

In Miller and Ware (2002) the results from one no-choice larval suitability laboratory bioassay, one no-choice adult suitability laboratory assay, and one multiple-choice adult bioassay were reported. The larvae were 24 h old when the bioassay was initiated. The adults in the no-choice adult bioassay were second generation unfed adult elm leaf beetles. Newly emerged unfed female and male beetles were exposed to leaf sections from 3–5 taxa in 7 multiple-choice experiments. The same traits as in the above papers were studied.

For the larval suitability experiment we have illustrated the percentage of emerging adults for the 6 taxa, for which the ovipositing percentage of the emerging females was recorded (Fig.1-3). There was a significant difference in the percentage of emerging adults among the 18 different taxa tested. The highest percentage, 27, was noted for Frontier while no adults emerged in the 'Urban' elm. There were significant differences for larval survival, development time from larvae to adult, pupation, and pupal weight. Noteworthy are the high percentages of ovipositing females in this experiment and the high number of eggs laid per female on the cultivar Regal, 135.

In the 3 no-choice experiments Regal again had high number of eggs laid per female, 75, which was outnumbered by *U. pumila* with 102 eggs per female (Fig. 1-4). For 3 cultivars no eggs were laid. Significant differences were noted for eggs per female, ovipositing, and female and male longevity.

We have tried to extract the essence of the multiple-choice experiment in Fig. 1-5, in which the highest and lowest percentage tissue eaten by ELB are illustrated for the 7 experiments conducted. It should be noted that comparisons between the 7 experiments are not straightforward since a common taxon in the experiments is missing. In the first experiment 3 American elm taxa had no eaten tissue while the fourth had 3% eaten tissue. It is evident that *U. pumila* is a preferred taxon of the ELB. The cultivar Frontier was less attracted by ELB as seen from experiments 3 and 7. However, no significant differences were observed in these 2 experiments.

Based on the results presented in this paper the following cultivars were recommended for localities with high pressure from ELB: Homestead, Jefferson, Patriot, Prospector, and Valley Forge. *U. pumila* and interspecific hybrids with *U. pumila* should be avoided in such areas.

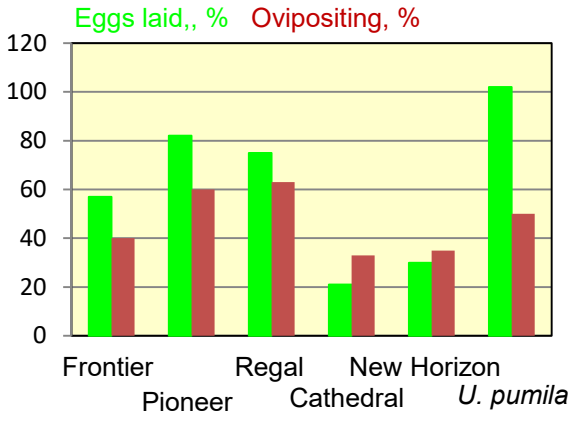

Figure 1-4. Percentage of eggs laid and ovipositing of adult females in a bioasay, in which five cultivars and U. pumila *were tested. Miller and Ware 2002*

Still another study focusing on eggs laid per female and ovipositing was presented in 2003 (Miller et al. 2003). The same traits as in previous studies were recorded in one no-choice bioassay with 14 taxa, half of them being interspecific hybrids. Four multiple-choice bioassays were conducted, each with 3 taxa.

The highest number of eggs laid per family was noted for *U. pumila* as seen from Fig. 1-6, in which the 4 taxa with the highest numbers of eggs per female are illustrated together with the mean value. Four taxa did not have any eggs laid, three of them having at least one American elm genome, Strongly significant differences were noted for

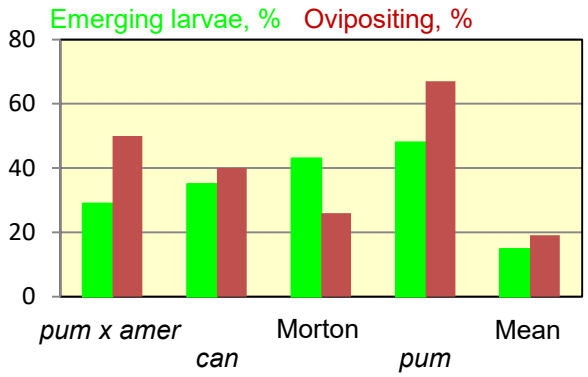

Figure 1-6. Eggs laid per female and percentage of ovipositing females in a no-choice experiment with 14 elm taxa. amer = U. americana, pum = U. pumila, can = U. canescens, *Morton = Morton Stalwart.* Miller et al. 2003.

eggs laid per female, percentage of ovipositing females, and female and male longevity.

There were significant differences in 3 of the multiple-choice bioassays (Fig. 1-7). Also in this experiment *U. pumila* appeared to be the most affected by ELBs. In the third experiment the cross *U. japonica* x *U. americana* was the only entry that showed any effect of ELBs while the two parental species were not touched by the beetles.

The experiments presented in Miller and Ware (2001a) is an applied research report. Eleven newly acquired elm taxa plus *U pumila* were included in this no-choice bioassay experiment. No eggs were laid on *U. elongate, U. propinqua*, and *U. szechuanica.* The percentage of ovipositing females varied in the range 0–94 with the highest percentage for *U. pumila*.

In Miller et al. (2001) the susceptibility to spring and autumn cankerworms was studied in 38 taxa. Both no-choice and 6 multiple choice experiments were conducted.

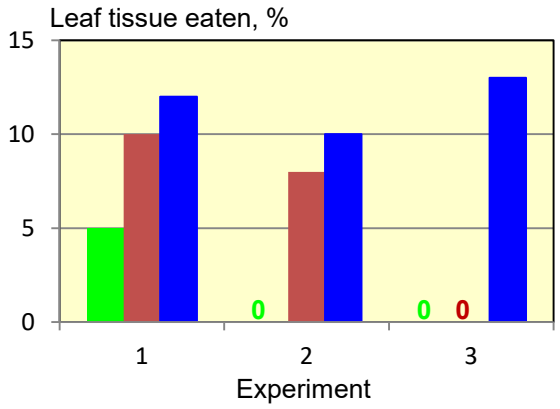

Figure 1-7. Percentage leaf tissue eaten in 3 multiple–choice bioassays, each with 3 taxa. Exp. 1 contained Accolae, Stalwart, and Accolade x U. japonica-wilsonia–pumila, *Exp. 2 contained* U. americana *Moline,* U. pumila x U. americana *Moline, and* U. pumila, *Exp. 3 contained* U. americana, U. japonica, *and* U. japonica x U. americana. *Miller et al. 2003.*

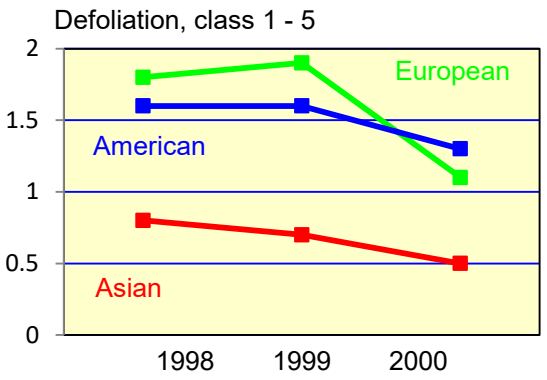

Figure 1-8. Mean defoliation by spring and autumn cankerworm feeding in five classes of 7 American, 25 Asian, and 10 European elm taxa growing in Morton arboretum, Illinois, USA. Class 1 = 0–10%, 2 = 11–20%, 3 = 21–30%, 4 = 31–50%, and 5 = > 50% defoliation. Miller et a. 2001.

The latter contained 5–8 taxa. Defoliation in the field was recorded in five percentage classes during three consecutive years in 42 taxa growing at the Morton arboretum, Illinois, USA. The following traits were studied in the no-choice experiment; significances are indicated:

Larval longevity	***
Larval development time	***
Percentage pupation	***
Fungal fresh weight	***
Fecal pellet weight	***

As regards pupation, no pupation took place in 23 of the 38 taxa while six taxa had percentages of 40 or higher. The taxa without pupation were either Asian species or interspecific hybrids with one Asian parent.

Fig. 1-8 reveals that on average the Asian taxa were less damaged by spring and autumn cankerworms than American and European taxa. Within each region there were significant differences among the taxa. No feeding at all was noted for 4, 5, and 3 Asian taxa in years 1998, 1999, and 2000, respectively.

This investigation shows that there is a higher probability of finding taxa resistant to the two cankerworms among Asian than American and European taxa.

In conclusion, Miller and coworkers' investigations suggest that Asian elm species are the most susceptible to ELB attacks while they are the most resistant to spring and autumn cankerworms. Particularly, *U. pumila* was shown to be susceptible to ELB in several experiments.

Another investigation related to ELB susceptibility of elm species was presented by Bosu and Wagner (2007). They studied the impact of water stress on leaf surface trichomes and mineral nutrients. Seedlings of 2- and 4-year-old *U. americana, U. parvifolia,* and *U.pumila* were planted in 5 gallon pots and exposed to standard nursery practice. Three watering intervals were used, 2.5, 7, and 14 days between watering. Ten seedlings per species and watering

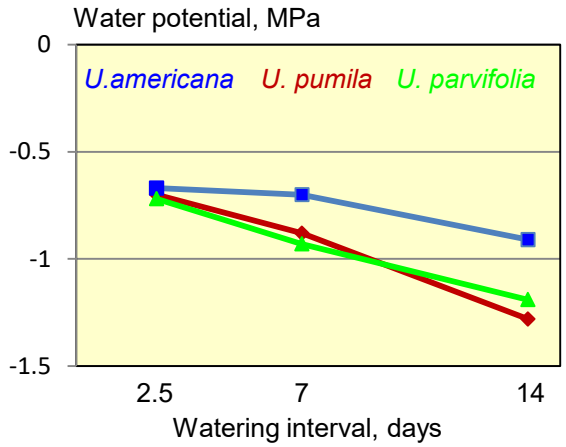

Figure 1-9. Water potential in three elm species at 14 weeks after start of watering treatment at intervals of 2.5. 7, and 14 days. Bosu and Wagner. 2007.

regime were tested. Water potentials were measured at 4, 8, and 14 weeks after the start of the experiment. At the termination of the experiment after 14 weeks, leaves were collected for scanning electron microscopy and for analyses of foliar nutrient components: nitrogen, phosphorus, potassium, calcium, magnesium, iron, and manganese. Plants can form trichomes on leaf surfaces and their density may increase or decrease under stressful conditions. Trichome density might have an impact on susceptibility to pests, and was therefore analyzed by the authors.

There was a strong effect on the water potential of the 3 watering intervals (Fig. 1-9) and a similarity in response of the two Asian elm species. As regards the American elm there was a slight but non-significant treatment effect on water potential at 4 weeks after the start of the treatment. Significant differences of the water potential among species were noted. In the joint ANOVA there was

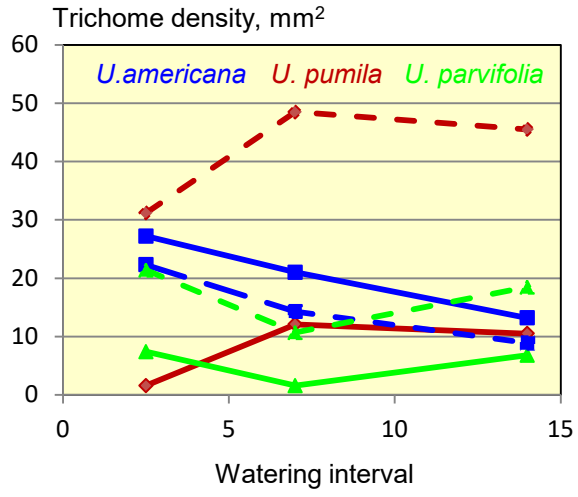

Figure 1-10. Total simple (continuous line) and bulbous (dashed line) trichome density, mm², in three elm species at 14 weeks after start of watering treatment at intervals of 2.5, 7, and 14 days, Bosu and Wagner. 2007.

no significance difference for watering level at the end of the experiment, which must be attributed to the strong species x treatment interaction at this stage.

As regards type of trichomes, American elms showed an opposite pattern to that of the Asian elms in having larger simple trichomes and decreasing trichome density with increasing water stress (Fig. 1-10). The two Asian species were characterized by low simple trichome density. As seen from Fig. 1-10 *U. pumila* differed from the two other species by having the largest bulbous trichome densities in all treatments. It was speculated that this high density would reduce the attraction of ELBs. Unfortunately, the crash of ELBs in 2002 made it impossible to carry out any bioassay studies with ELBs that year.

High iron and magnesium contents were noted in *U. pumila* for the 14-day watering treatment. All other treatments in the 3 species led to much lower levels of these metals. This resulted in strong significant differences for the species and species x treatment interaction for these elements. Significant species effects were also noted for calcium, manganese, nitrogen, and phosphorus.

The objective of an investigation by Elowsky et al (2013) was to identify and describe morphological traits of hybrids between *U. rubra* and *U. pumila* in populations growing in Nebraska, USA. Each species was represented by 32 trees and putative hybrids by 50 trees. Leaves were sampled from mature shoots on the sunny side of the tree. The 12 first traits listed in Table 1-1 are quantitative while texture of adaxial surface, distribution of trichomes on bud scales, color of trichomes on scales, color of trichomes on persistent calyx, and trichomes percentage overage are qualitative. The measurements applied were illustrated in figures in an instructive way. Fresh leaves were scanned microscopically to detect differences in trichome density and size.

There were significant differences between the two pure species for all traits assessed (Table 1-1) and significant differences for most comparisons. This means that there are good possibilities to distinguish hybrids between *U. rubra* and *U. pumila*. Since the 2 species can freely hybridize and grow together, there is a great potential for matings between these two species. It was speculated that the species hybrids constitute the first generation of hybrids since *U. pumila* is relatively new to Nebraska. The possibility that *U. pumila* hybridizes with other elm species was discussed and it was stated that *U. thomasii*, which grows not too far from Nebraska, might hybridize with *U. pumila,* but flowering phenology is quite different in *U. thomasii*.

Twigs of the three taxa were collected and forced into flowering, which resulted in flowering in the following sequence and hours after collection: *U. pumila* (18 h) – hybrid (30 h) – *U. rubra.*(72 h). In contrast with many other species hybrids no signs of reduced fertility of the species hybrid were noted.

Table 1-1. Morphological traits studied in populations with U. rubra, U, pumila *and their hybrid in Nebraska. The first 12 traits were assessed in a quantitative way while the remaining traits are qualitative.* r = rubra, p = pumila*, h = their hybrid.* Elowsky et al (2013).

Character	*Hybrid performance*	*Significances*		
		r - p	r - h	p - h
Lamina length	Intermediate ***	***	***	***
Lamina width	Intermediate ***	***	***	***
Length/width ratio	Intermediate	***	ns	***
Petiole length	As *U. rubra*	***	ns	***
Primary teeth/cm	As *U. rubra*	***	ns	***
Secondary teeth/cm	> both pure species	***	*	***
Secondary/primary teeth ratio	As *U. rubra*	***	ns	***
Pollen width		***	***	ns
Fruit length	> both pure species	ns	***	***
Fruit width	> both pure species	***	?	***
Fruit length/width ratio	> both pure species	ns	***	***
Stamen/ floret	Intermediate	***	***	***
Texture of adaxial surface	Either condition	***	***	***
Distribution of trichomes on bud scales	Either condition	***	***	***
Color of trichomes on scales	Either condition	***	***	***
Color of trichomes on persistent calyx	Either condition	***	***	***
Trichome percentage over seed	Closer to *U. pumila*	***	***	***

Considering the laborious assessments of the morphological characters it might be less laborious and probably more precise to use microsatellite markers for identification of species hybrids.

The morphologies of elm species do not differ much, which does not allow safe discrimination among species or a reliable phylogeny of elms. Therefore, restriction site associated DNA sequencing (RAD-seq) was used in a phylogenetic study of elm species by Whittemore et al. (2021). Some differences from the phylogeny based on cpDNA were noted. Subgenus *Ulmus* with 4 sections *Foliaceae*, *Trichocarpus, Microptelea, Ulmus* has the largest number of species, 17, with a domination of Asian species. Subgenus *Oreoptelea* has 9 species and 2 sections *Chaetoptelea* and *Blepharocarpus*, mainly American species. The Himalayan *U. villosa* was put in its own subgenus, *Indoptelea*. The American *U. rubra* belongs to the section *Ulmus.* The European *U. laevis* and the *Asian U. elongata* belong to the subspecies *Oreoptelea* and subsection *Blepharocarpus*.

!.1.2 Asian studies

Bud flushing and leaf fall in 46 *U. pumila* populations from the temperate zone in China were followed over 20 years (1986–2005) by Chen and Xu (2012). The objective was to estimate the effect of climatic warming on these two phenological traits. As far as we can understand the populations were growing adjacent to meteorological stations. The date when a few leaves were fully unfolded was recorded as start of flushing. The end of the growth period was the date when almost all leaves had fallen..
Instead of using temperature sums for attaining bud flushing and growth cessation, complex derivations of these dates were applied. It would have been useful to compare the results obtained with traditional temperature sum requirements for attaining bud flushing and growth cessation.
It was estimated that flushing took place 4 days earlier per decade during the period 1986–2005. The corresponding estimate for change of growth cessation was 2.2 days' postponement. It was stated that date of flushing was triggered by temperature while triggering of growth cessation was dependent on continuous low temperatures, photoperiod, and precipitation.

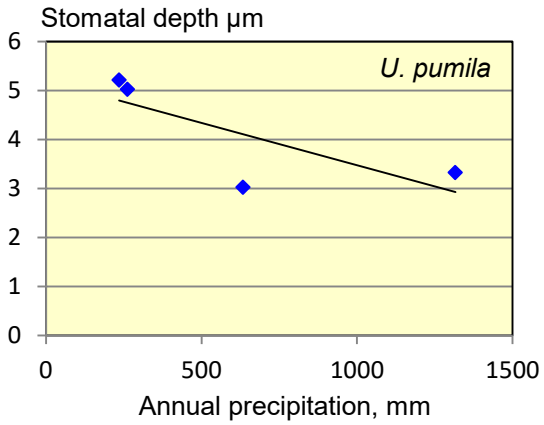

Figure 1-11. The relationship between annual precipitation at population origin and depth of stomata in 4 East-Asian populations of U. pumila*. Park et al. 2013.*

Stomatal depths were studied in four *U. pumila* populations from four localities with varying precipitation by Park et al. (2013). The hypothesis tested was that stomatal depth is larger in populations from localities with low precipitation than from wetter localities. Various types of microscopy were used to analyze stomatal depths.
Fig. 1-11 shows that there is support for the hypothesis even if four populations are too few for a reliable test.

1.1.3 European studies

The objectives of the thesis by Rachel Whiteley (2004) were to estimate the variation among and within populations of fragmentedly distributed *Ulmus laevis* with respect to juvenile growth, growth rhythm, and frost tolerance in 5 populations covering a geographically large part of Europe (see Fig. 1-12). Each population was represented by 19–20 open-pollinated families. The study

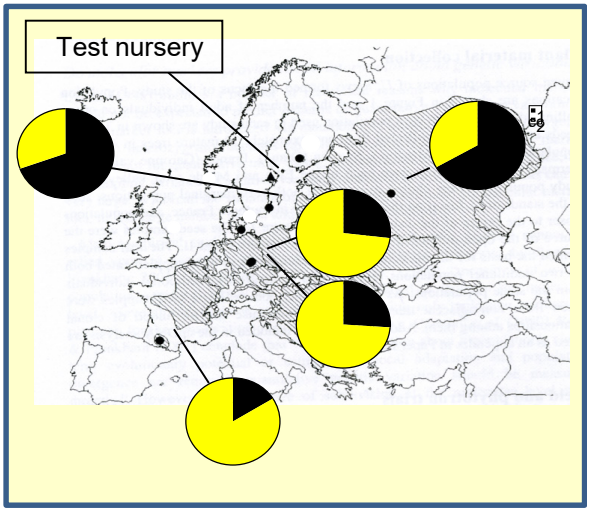

Figure 1-12. Budset in five U. laevis *populations studied in a nursery in Uppsala, Sweden. The dark blue circle sector indicates the number of hours for budset, a filled circle = 12 hours. Whiteley et al. 2003.*

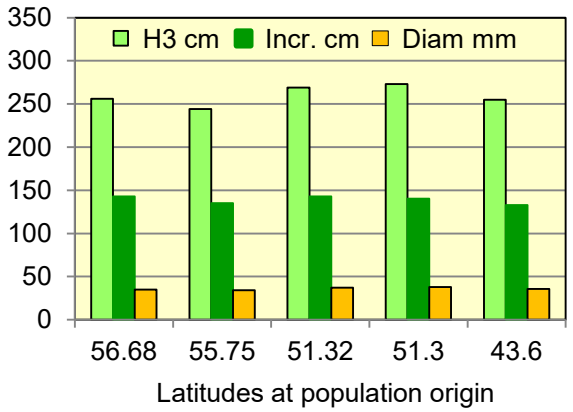

Figure 1-13. Tree height at age 3 (H3), Height increment between ages 2 and 3 (Incr.), Diameter at age 3 at 5–10 cm above ground (Diam.) in five U. laevis *populations from different latitudes. The study was carried out close to Uppsala at latitude 59.80°N and 15 masl.Whiteley et al. 2003.*

was carried out in a nursery at latitude 59.80°N, longitude 17.65°E and 15 masl. Single-tree plots in six fully randomized complete blocks were established. Bud flushing and budset were recorded five times in 7 and 4 classes, respectively. Leaf fall at ages 2 and 3 was recorded in 11 and 6 classes, respectively, while 6 classes were used for assessment of frost damage.
It was noted that most of the plants lost their apical buds during the first winter after plantation, which might have an impact on the growth traits. Observed results for some of the studied traits are shown in Figs. 1 - 12 - 1-4 . As seen from these figures the differences among the populations as regards growth traits were limited while considerable differences were noted for frost damage and most phenology traits. The observed results are reflected in the Q_{ST} estimates shown in Fig.1-15. In particular, leaf fall at ages 2 and 3 and frost damage differed between the two northerly populations, latitudes 55.75 and 56.68°N, and the other three populations. The greater frost damage in the latter three populations was attributed to frost exposure before complete building up of frost resistance during autumn. The limited frost damage in the two northerly populations evidently avoided frost damage owing to early growth cessation. Late growth cessation usually means a longer growth period and as a corollary of this, larger trees than those in populations with shorter growth periods. This is true as long as frost damage hampers growth. The southernmost population did not benefit from its longer growth period. This result was attributed to inbreeding in this marginal population or lower fitness value of good growth in its ambient conditions. The two German populations from latitude 51° differed significantly with respect to height at age 2 but not at age 3. The age 2 difference was attributed to microclimatic differences. However, most of the traits did not show any significant differences between these two populations.

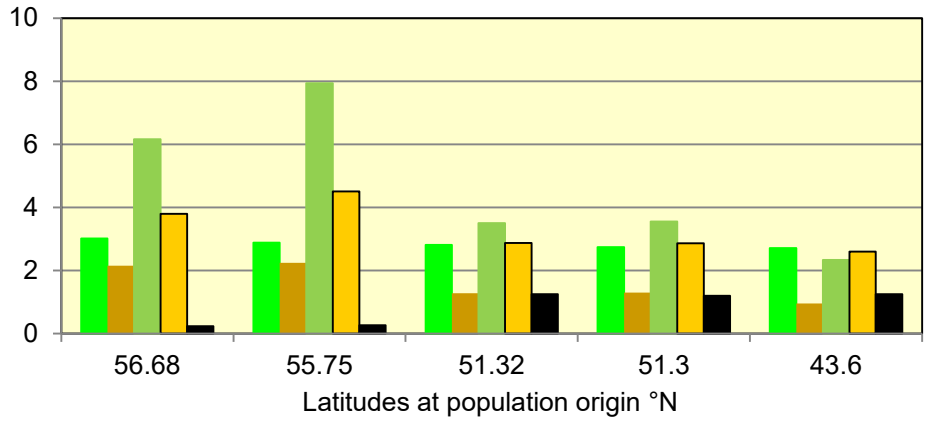

Figure 1-14. From left to right in each group: bud flushing (7 classes), budset (4 classes), Leaf fall ages 2 and 3 (11 classes), and frost damage (6 classes) in five populations from different latitudes. The study was carried out close to Uppsala at latitude 59.80°N and 15 masl. Whiteley et al. 2003.

Additive variance was presented only for the metric traits since it is problematic to estimate this variance for ´classes' traits. Therefore, we chose to illustrate heritabilities for a few of the traits analyzed in Fig. 1-16. The authors commented that they assumed that the family variance component estimated ¼ of the additive variance, which might lead to overestimation of heritabilities if inbreeding occurred in the population. The estimates for the excluded traits did not differ much from the heritabilities of their related traits. Some of the traits did not vary in some populations. It is obvious that the low percentages of frost damage in the two northerly populations (Fig. 1-14) will not lead to any high heritability in these populations. The 51.32° population seemed to have the largest within-population genetic differentiation with all heritability estimates above 0.20. The high variability in the Swedish 56.68° population was attributed to its scattered distribution, which might have resulted in matings predominantly within small cohorts with accompanying inbreeding. The low variability in bud flushing might be the result of late spring at the study site compared to the original locations of the populations.

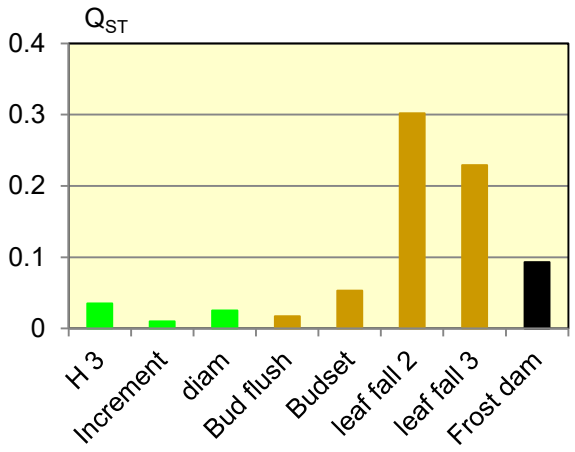

Figure 1-15. Q_{ST} for various traits from a study of five U. laevis *populations from France Germany, Russia, and Sweden, each population represented by 19 or 20 open pollinated families. 2 and 3 indicate years of age at recording. Whiteley et al. 2003.*

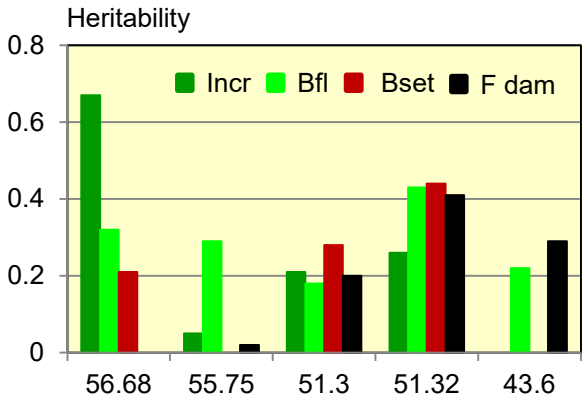

Figure 1-16. Heritability estimates for height increment between ages 2 and 3, bud flushing (Bfl), bud set (Bset), frost damage (F dam) in 5 U. laevis *populations from different latitudes. Missing columns = zero heritability. The study was carried out close to Uppsala at latitude 59.80°N and 15 masl. Whiteley et al. 2003.*

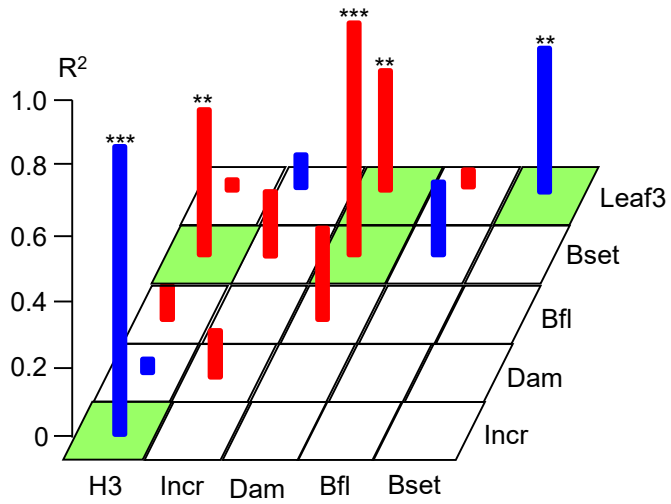

Figure 1-17. Genetic correlations between six traits in an investigation of 5 U. laevis *populations from Sweden, Russia (2), and France studied at Uppsala at latitude 59.80°N and 15 masl.*
H3 = height at age 3, Incr = increment between ages 2 and 3, Dam = frost damage, Bfl = bud flushing, Bset = budset, Leaf 3 = leaf fall at age 3. Red columns refer to negative relationships. Significant correlations are indicated. Whiteley et al. 2003.

A representative selection of calculated genetic correlations is illustrated in Fig. 1-17. Really strong correlations were noted only for two of them: *height* with *height increment* and *budset* with *frost damage*. The former is logical since increment is part of tree height. A late budset means a high risk for exposure to damaging early fall frosts.
In conclusion, significant differences among populations for several fitness-promoting traits were noted. High heritability estimates were found in individual populations for growth and phenology traits as well as for frost damage.

Leaf morphology in five Norwegian *U. glabra* populations was studied by Myking and Yakovlev (2008). Chloroplast haplotypes were studied in an additional 11 populations. The populations covered the distribution area of *U. glabra* in Norway. A trial in a nursery was established with single-tree plots with 40 plants per population. One or two lateral lobes were recorded one year after the establishment of the trial. Leaf tapering was recorded in three classes, long, intermediate, and short. Length and width of fully expanded leaves at the top of plants were measured. Chloroplast DNA was studied by PCR-RFLP.
The relationship between population latitudinal origin and the ratio leaf length/width is illustrated in Fig. 1-18, which shows a smooth clinal variation for the four populations from the maritime climate. Significant differences among families within populations were noted for this ratio. The percentage of leaves with acute lateral lobes and short tapering leaves showed a fairly similar pattern as leaf length/leaf width ratio (Fig. 1-19). These patterns suggest that adaptation to the ambient conditions at the growth localities had taken place. In the maritime parts of

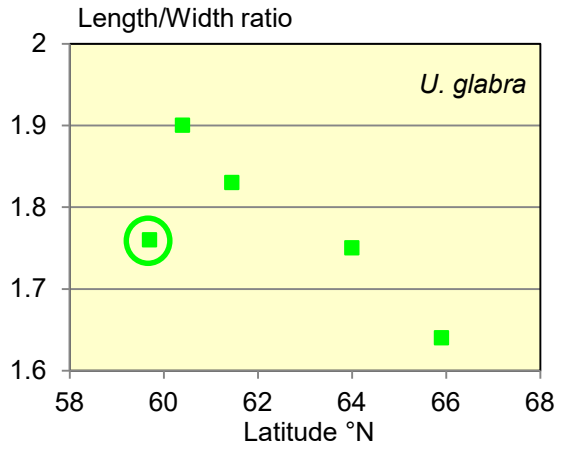

Figure 1-18. Leaf length/width ratio in 5 Norwegian U. glabra *populations from different latitudes. The encircled population originates from eastern Norway and the four other populations originate from localities with maritime climate. Myking and Yakovlev 2008.*

Norway the temperature climate gradually changes with latitude. It should be noted that the southeastern population originates from a less maritime climate.
All 16 populations were monomorphic with respect to haploplast genotype. Fourteen of the populations carried haploplast 1 and the remaining 2 contained haploplast 2. One southwestern and the northernmost population contained this "southern" haplotype, which is the most common haplotype in Sweden and Eastern Europe. The southern haplotype in one southwestern population was interpreted as random spreading of seeds. The northernmost population was characterized as the subspecies *montana* with respect to leaf shape (leaves are relatively long, with long tapering, and absence of acute lobes). Haplotype 1 is dominating in Western Europe and the western

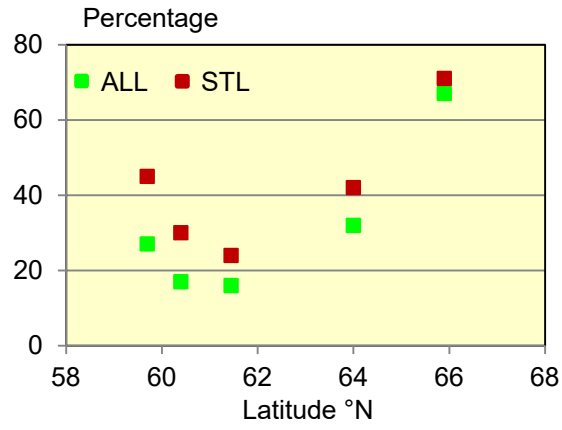

Figure 1-19. Percentage of leaves with acute lateral lobes (ALL, green) and short tapering leaves (STL, brown) in 5 Norwegian U. glabra *populations. All populations, except for the southernmost, originate from localities with maritime climate. Myking and Yakovlev 2008.*

population's leaf shape was of *glabra* type. The simultaneous presence of a specific haplotype and leaf shape was interpreted as the effect of *the same evolutionary forces*. However, it is hard to believe that there was any selection for haplotypes. It is more likely that the haplotypes reflect the migration routes after the latest glaciation.

The objectives of a study by Petrokas and Baliuckas (2012) were to identify morphological traits that distinguish elm taxa and estimate hybridization in contact zones between taxa. Five to 7 leaves from mature shoots were collected from 58 elms in 11 localities in Lithuania. Three localities with mixed populations of two or more elm species were included in this collection. Nineteen summer leaf traits were analyzed in the WinFOLIA 2004a program. In addition 3 tree descriptors were recorded. SAS cluster and SAS tree procedure were applied for clustering of the trees.
The clustering analysis resulted in a fairly good separation of four taxa:
U. glabra, U. minor, U. laevis, and *U. hollandica.*
It should be noted that leaf morphology differences between *U. glabra* and *U. minor* are limited with almost continuous variation. It was stated that a discontinuity of characters that separate taxa could not be identified. Natural hybrids occurred at low frequency, 2%, Such a low percentage was not discussed in spite of the coexistence of two species in some populations. It was suggested that molecular markers should be used for studies of hybridization in mixed stands with elm species. That would certainly be more efficient than recording numerous morphological traits.

The aim of a study by van der Mijnsbrugge et al.(2016) was to estimate the variation in leaf morphology among relict populations of *U. laevis* in Flanders, Belgium. Leaves from 2–3 healthy shoots per tree were sampled in a single-tree plot trial with cuttings. All 11 existing populations of *U. laevis* in Flanders were included in this trial. The number of trees representing the 11 populations varied in the range 1–16. The following six leaf morphology traits were studied:

Leaf margin toothing	MT
Split secondary veins	SSV
Lower side pubescence on mesophyll	LPM
Lower side pubescence on secondary vein	LPV
Upper side pubescence on mesophyll	UPM
Lower side pubescence on secondary vein	LPV
Upper side pubescence on secondary vein	UPV
Lamina length	LL
Lamina width	LW

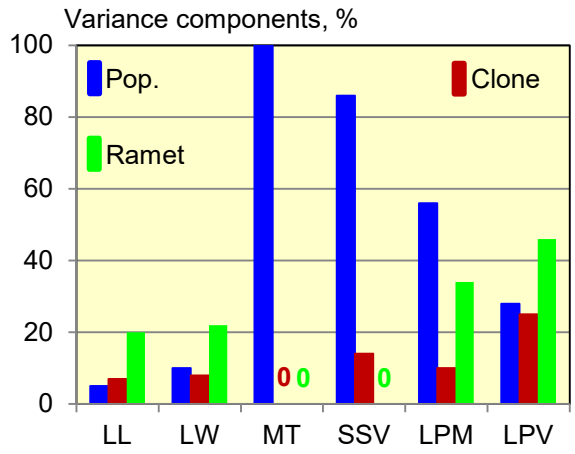

Figure 1-20. The partitioning of variance for six morphological leaf traits in a study of 11 relict U. laevis *populations in a field trial in Flanders, Belgium. LL = leaf length, LW = leaf width, MT = leaf margin toothing, SSV = split secondary veins, LPM= lower side pubescence on mesophyll, LPV = lower side pubescence on secondary vein. It should be mentioned that residuals could not be estimated for MT – LPV traits. van der Mijnsbrugge et al. 2016.*

ANOVA and principle component analysis (PCA) were applied.
The first three axes of PCA explained 23.2, 19.1, and 12.2% of the variation in leaf shape traits. Two populations deviated from the rest mainly attributed to MT and SSV traits. This differentiation was attributed to genetic drift, which is likely since these two populations consist of only four and five trees. Furthermore, the environmental conditions in Flanders do not differ much. The partitioning of the variance was presented for six traits (Fig. 1-20). It should be noted that residuals could not be estimated for traits MT – LPV, which means that a direct comparison with LL and LW cannot be made. However, it is evident that there was a strong population effect of MT and SSV in agreement with the PCA.
The ramet effect, which is attributed to random events, was larger than the two genetic effects for LL and LW. This was expected for traits that are easily influenced by the microenvironment around individual trees. Finally, the relationships between the morphological traits were weak even if some significant correlations were noted. The degree of explanation of the relationships was in all cases below 20%.

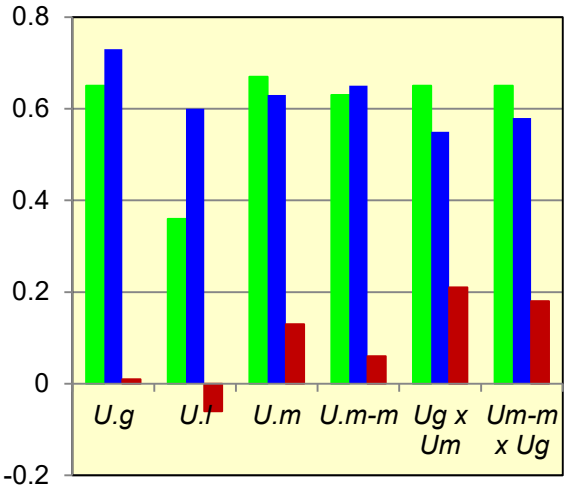

Figure 1-21. Allelic richness (green), observed heterozygosity (blue), and inbreeding coefficient (brown). Ug = glabra, Ul = U. laevis, Um = U, minor, U.m-m = U minor *ssp*. minor. *Tamošaitis et al. 2021.*

The objectives of a Lithuanian investigation were to study the diversity and differentiation among elm populations as well as hybridization of elms (Tamošaitis et al. 2021). The ultimate objective might be to find traits reliable for discrimination of elm taxa. They analyzed 31 leaf morphology traits and 8 microsatellite loci in 26 populations from a wide distribution in Lithuania. Six taxa were included in the sampled material, *U. glabra, U. laevis, U. minor, U. minor* ssp. *minor, U. glabra x U. minor,* and *U. glabra x U. minor* ssp. *minor*. One-way ANOVA was used to estimate the effect of taxon on the morphology traits. AMOVA was used to analyze the microsatellite data. For grouping of the taxa STRUCTURE 2.3.4, New Hybrids beta v.1, and principle component analysis were run.
Significant taxon effects were noted for almost all of the morphological traits. The genetic diversity for some of the population genetics parameters tested for the microsatellite data is illustrated in Fig. 1-21. Five of the six taxa showed an excess of homozygotes, in some cases rather high estimates of the inbreeding coefficient, >0.10. Despite the comparatively low allelic richness in *U. laevis* it cannot be attributed to inbreeding since this species was the only one with heterozygotic excess, although not high, 0.06. Both for morphology traits and markers *U. laevis* was singled out as most different from the other taxa. It had the second highest number of private alleles with *U. glabra* having the highest number. No interspecific hybrids with *U. laevis* as one parent were detected. The discriminant analysis of principal components (DAPC) of the other five taxa separated three major groups:

U. glabra

U. minor x U. glabra

U. minor, U. minor ssp. *minor, U. glabra x U. minor* ssp. *minor*

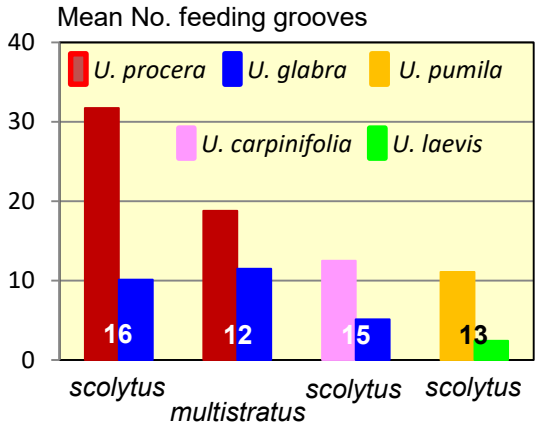

Figure 1-22. *Mean number of feeding grooves in 2-choice species experiment with 2 elm bark beetles,* Scolytus scolytus *and* Scolytis multistratus*, The experiment lasted 12–16 days. Webber 2004.*

It was stated that this analysis confirmed the genetic differentiation between *U. glabra* and the *U. minor* group. Further, leaf traits were shown to satisfactorily identify *U. minor x U. glabra* hybrids but not the reciprocal cross. With widely overlapping distribution and no crossing barriers between *U. glabra* and *U. minor* it is expected that interspecific hybrids occur. Moreover, if the overlapping distribution has lasted for generations, various types of backcrosses might have occurred resulting in a continuum of traits between the two parental species. The authors suggested that separate genetic resource populations should be selected for *U. glabra, U. laevis, U. glabra x U. minor* and the *U. minor* species complex, and that the selection should be based on leaf and stem morphology.

Webber (2004) reviewed the knowledge on factors influencing the selection of breeding ground for *Scolytus* beetles. The preferences of elm species in 4 two-choice experiments were presented (Fig. 1-22). As seen from this figure the number of feeding grooves for the two beetles *Scolytus scolytus* and *Scolytus multistriatus* was much higher in *U. procera* (English elm) than in *U. glabra*. The low number for *U. laevis* is noteworthy. The lower incidence of DED infection in this species must be attributed to its low attraction for the elm beetles since inoculation experiments have shown the high susceptibility of this species. Studies have shown that certain trees are highly attractive and can entice many beetles once a groove was established in such a tree. It is thought that the attraction is more dependent on the physical character of the bark than on its chemical composition.
Environmental conditions such as temperature influence feeding of the beetles. Thus, increasing temperature within the interval 15–30°C stimulated the feeding of *Scolytus scolytus* on *U. procera*.
The number of conidia of the pathogen required for DED

Figure. 1-23. Feeding of bark beetles Scolytus laevis *in a no-choice and in a 2-choice experiment with 2 varieties of* U. minor *for 48 hours. Anderbran et al. 2000.*

infection was shown to differ by a factor of 10 between *Ophiostoma ulmi* and *Ophiostoma novo-ulmi.* The lower number was found for the more virulent of these two species, *Ophiostoma novo-ulmi*. In connection with these results the statement by Heybroek (2000) that DED resistance has a clear entomological component becomes relevant. This component is frequently overlooked.

In a brief report Anderbrant et al. (2017) described *Scolytus laevis* feeding on one susceptible and one DED-tolerant *U. minor* variety in a no-choice and 2-choice experiment. No significant difference was noted in feeding between the 2 varieties (Fig. *1-23*). The size of feeding per beetle was approximately the same in the no-choice and 2-choice experiments, 5.6 and 6.2 mm^2.

1.2 Markers

1.2.1 Isozymes

The isozyme profiles of more than 300 trees belonging to *U. minor, U. pumila*, or their hybrid *U. minor x U.pumila* were determined in order to see if isozyme pattern could be used to distinguish the two species and their hybrid (Cogolludo-Augustin et al. 2000). Forty-six of the 116 *U. pumila* trees originated from China. Nine isozyme loci were analyzed.
Species-specific alleles occurred at seven loci. Table 1-2 shows that seven alleles were unique for *U. minor* while ten alleles including a null allele were unique for *U. pumila.* The 2 species could be totally distinguished by alleles at 3 isozyme loci, *Pgd2, Prx2,* and *Mdh1*.There was a difference between the 2 origins of *U. pumila*. Thus, two alleles occurred only in Spanish *U. pumila*, *Idh-4* and *Mdh2-1*.

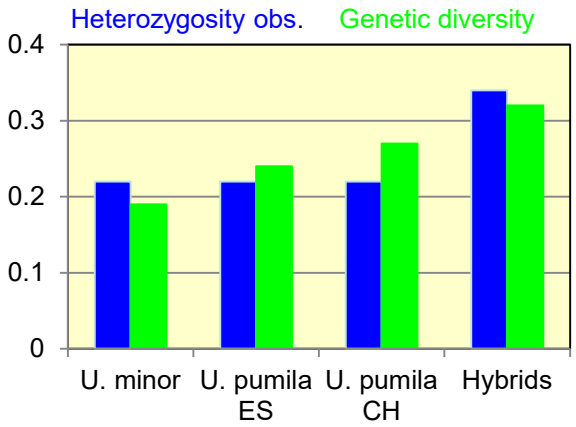

Figure. 1-24. The observed heterozygosity (blue) and Nei's (1973) genetic diversity (green) in U. minor, U. pumila *from Spain and China as well as Spanish* U.minor x U. pumila *hybrids in Spain. Cogulludo-Augustin et al. 2000.*

Two groups of hybrids were identified, those that had unique alleles of both species, 77.1%, and those that had unique *U. pumila*, 20.5%,. The remaining 2.4% could not be classified. An asymmetric pattern in hybridization in the hybrids was noted with closer association to *U. pumila* than *U. minor*. The high proportion of vegetative reproduction in *U. minor* is probably one explanation for the preferential backcrossing to *U. pumila.* The spatial distribution of the trees of the two species might also contribute to the asymmetry.
Fig. 1-24 shows that the observed heterozygosity and genotypic diversity were as expected larger in the hybrids than in the pure species.
This study shows that the pure species, *U. minor* and *U. pumila,* as well as their hybrids can easily be identified thanks to unique isozyme alleles in these two elm species.

Table 1-2. Alleles unique to U. minor *and* U. pumila *in Spanish populations*. Cogulludo-Augustin et al. 2000.

Locus	*U. minor*	*U. pumila*
Acph		2
Prx2	1, 2	null,3,4,5
Pgi2	1	
6Pgd2	1	2
Mdh1	3	1,2
Mdh2		1,3
Aat	1	

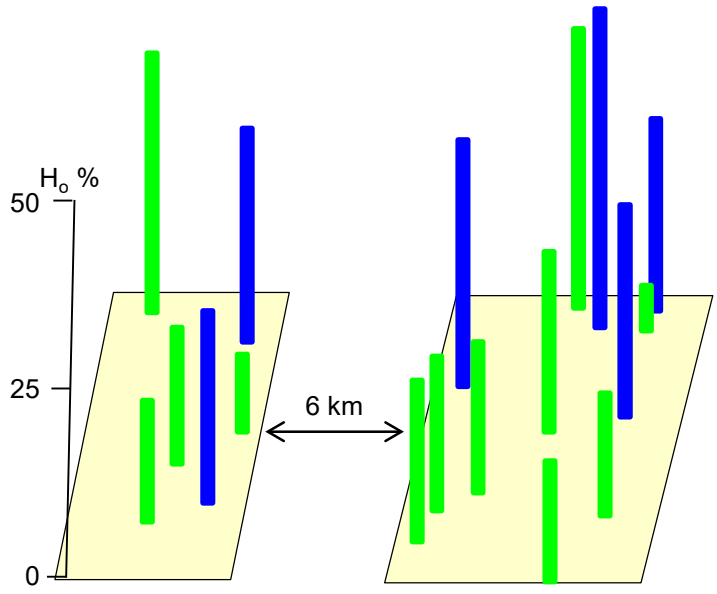

Figure 1-25. Schematic illustration of the observed heterozygosity in percentage of German U. laevis *(blue) and* U. minor *(green) from two localities along the Elbe River in central Germany. One locality had 6 and the other locality had 12 isolated groups of elms. Six isozyme loci were studied. Gehle and Krabel 2002.*

The objective of an investigation by Gehle and Krabel (2002) was to present a *preliminary characterization of field elm and white elm* in Germany. Two localities along the Elbe River approximately 6 km apart were selected for this study. Eight and 4 spatially isolated groups of field elm (*U. minor*) at these localities were sampled. As regards white elm (*U. laevis*) 3 natural populations and 3 planted populations of ages 20 to 40 years were studied. Six isozymes from juvenile leaves of 109 field elms and 238 white elms were characterized.

There was a large variation in observed heterozygosity in both species and at both localities as is schematically illustrated in Fig. 1-25. The population differentiation according to Gregorius (1978) was slightly higher in *U. minor* than in *U. laevis*, 35.5 versus 32.8. There was a tendency to more similarity in allele frequencies across species at each locality than between the same species at the two localities. If the isozyme loci analyzed would enable reliable discrimination between the two species it is required that one allele in one species occurs at moderate to high frequency and is absent in the other species. We have selected a few examples with the highest allele frequency in one species and the frequency of its counterpart in the other species in Fig. 1-26. There were loci with quite different allele frequencies (*Pgm-B* and *Pgm-A*) while other loci showed great similarity (*Idh-A*). About 20 years after this publication most geneticists would agree that species discrimination should be done with microsatellites rather than isozymes.

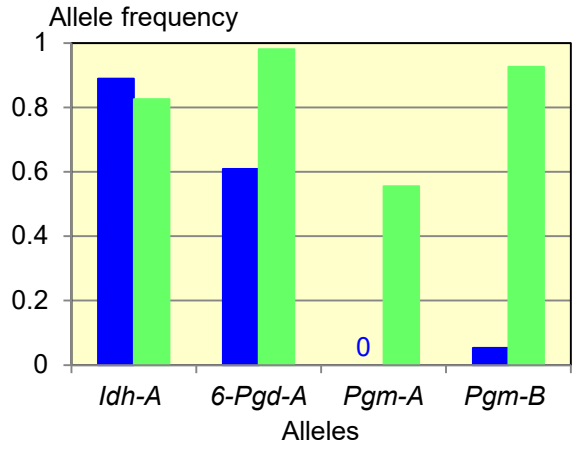

*Figure 1-26. Examples of similarity and differences in isozyme allele frequencies (*Idh-A-1, 6-Pgd-A-3, Pgm-A-3, Pgm-B-3*) of* U. laevis *(blue) and* U minor *(green) growing at two localities along the Elbe River in Central Germany. Gehle and Krabel 2002.*

Genetic diversity and differentiation of 13 Finnish and one Estonian marginal population of *U. laevis* were studied by Vakkari et al. (2009). Five of the Finnish populations are located on the shores of a lake and were designated as core populations while two populations were located at 90 and 100 km outside the main area for *U. laevis*. Twenty isozyme loci were used in this study and 8 of them were polymorphic in at least one population.

The mean number of polymorphic loci was 0.24. The within-population diversity was fairly limited. There was a strong relationship between expected and observed heterozygosities (Fig. 1-27). The F_{IS} estimates were negative in 9 Finnish populations, three of them significantly different from 0.

The most conspicuous result in this investigation was the high F_{ST} estimate, 0.290. This is remarkable since there is a limited distance among these wind-pollinated populations. Also after exclusion of the two isolated populations the F_{ST} remained high, 0.295. The F_{ST} between the two geographically isolated populations was 0.104. The relationship between F_{ST} and geographic distances between populations was estimated for the core populations and all Finnish populations. Only for the five core area populations was there a significant relationship, r = 0.83.

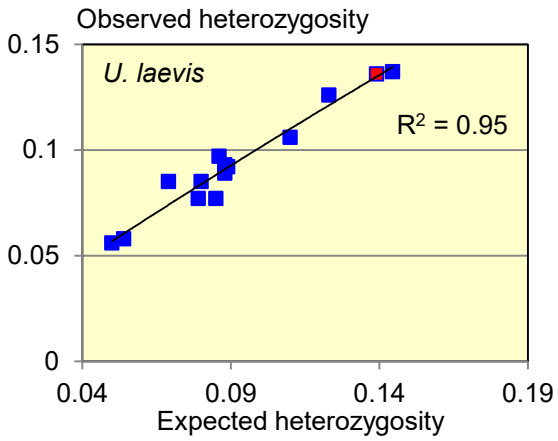

Figure 1-27. The relationship between expected and observed heterozygosity in 13 Finnish and one Estonian (red square) U. laevis *populations based on 20 isozyme loci. Vakkari et al. 2009.*

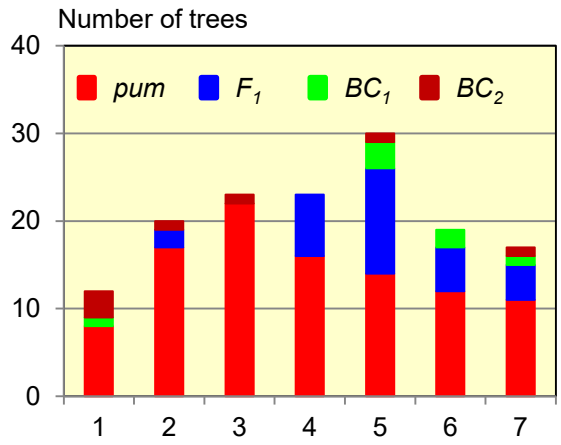

Figure 1-28. The number of trees of pure species U. pumila *(*pum*), F_1* U. pumila x U. rubra*, backcrosses* (*BC*) *to* U. pumila*, and second generation backcrosses to* U. pumila *in 7 naturalized populations of* U. pumila *in USA.* Zalapa et al. (2010).

The strong differentiation among the populations was attributed to genetic drift and supports the hypothesis that marginal populations have less within population diversity and more among-population differentiation.

1.3 Molecular markers

1.3.1 American studies

In a series of papers the genetic diversity of *Ulmus rubra* and its hybridization with *U. pumila* was treated, partly with the same populations (Zalapa et al. 2008a and b, Zalapa et al. 2009, Zalapa et al 2010, Brunet et al 2013, Brunet et al. 2016).

Estimates of the genetic diversity and differentiation among 53 *U. pumila* accessions from China growing in Wisconsin were presented by Zalapa et al. (2008a). Fifteen polymorphic microsatellite loci (90 alleles) were used in this study comprising one tree per accession. Forty-seven of the 53 accessions were lumped together in nine geographic groups to have replications at regional levels. No region had less than three accessions and ten microsatellite loci in Hardy-Weinberg equilibrium were used in this study.
The following ranges of some variables for individual polymorphic loci were obtained:

Expected heterozygosity	0.08 – 0.88
Observed heterozygosity	0.04 – 0,79
Polymorphic information content	0.08 – 0.85
Nei's diversity	0.08 – 0.87

The analysis of molecular variance (AMOVA) revealed that most of the variation, 95%, was within regions and only 5% among regions. There was no relationship between geographic and genetic distances.
We miss a discussion of how representative the sampling is with just one tree per locality.

Zalapa et al. (2010) studied the genetic diversity in several populations of the exotic species *U. pumila* in USA. Four groups of material were used:
Countrywide collection of 37 trees with *U. pumila* characteristics
Wisconsin collection of 20 trees + 52 herbarium individuals of *U. pumila*
Eight naturalized populations of *U. pumila* in USA represented by 12–30 trees each. Six populations from Wisconsin and one each from Illinois and South Dakota
U. rubra herbarium material
Ninety-nine alleles were detected, 32 were specific to *U. pumila* and 67 were specific to *U. rubra*.
Our focus will be on the eight naturalized populations. Genotyping of the eight naturalized populations took place with 13 microsatellites. Although all trees sampled had the leaf traits characteristic for *U. pumila* the genotyping revealed that a little more than 25% were not pure *U. pumila* (Fig. 1-28). Even some second generation backcrosses (7) were identified, which suggests that hybridization with *U. rubra* took place long ago. It is evident that hybridization between *U. pumila* and *U. rubra* is common. No backcrosses with *U. rubra* were detected.
The damage caused by DED on this species was probably contributing to the absence of such backcrosses.

Table 1-3. The range of population genetics variables in a study of 8 naturalized U. pumila *populations in USA. Zalapa et al 2010..*

Variable	Range	Range including hybrids
No private alleles	0–2	0–14
No alleles > 0.05	2.46–3.23	2.46–4.23
No effective alleles	2.00–2.53	2.02–3.52
Expected heterozygosity	0.36–0.41	0.36–0.62
Observed heterozygosity	0.34–0.44	0.35–0.70
Fixation index	–0.06–+0.09	–0.14–+0.08

The range of several variables in the naturalized populations is compiled in Table 1-3, which reveals fairly limited differences among the populations. The estimates are in most cases somewhat larger after inclusion of hybrids in the estimations. There was limited inbreeding in all populations with an excess of heterozygotes in five of the eight populations.

The AMOVAs for pure species and hybrids included showed that 6 and 7% of the variance were attributed to among-population differences. Thus, limited population differentiation. The mean F_{ST} was estimated at 0.07 and the individual population means varied between 0.033 and 0.093 (Fig. 1-29). Noteworthy is the low mean of the geographically most distant population from South Dakota.

The difference between Chinese *U. pumila* and US populations of this species was limited. This suggests that the ambient growth conditions are rather similar in the two continents.

Considering the strong susceptibility to DED of *U. rubra* and the asymmetry in backcrossing in favor of *U. pumila* it is likely that *U. rubra* will become extinct in a long-time perspective and be substituted by *U. rubra x U. pumila* hybrids unless measures are taken to conserve *U. rubra*.

To avoid inclusion of *U. rubra x U. pumila* hybrids, 12 markers were used, 66 specific to *U.rubra* and 43 to *U. pumila*. Of the 220 trees included in this study 32 turned out to be F_1 *U. rubra x U. pumila* and 28 were backcrosses with *U pumila.*

In a contact zone between the native *U. rubra* and the Asian *U.pumila* in Wisconsin 92 trees were collected for genotyping with 9 microsatellites to study hybridization between the two species (Zalapa et al. 2009). Genotyping of 53 *U. pumila* accessions across China took place to obtain a reference material for this species. Trees from 5 pure *U. rubra* populations were also genotyped for the same purpose. To be classified as first generation hybrid, heterozygosity for species-specific alleles should be present in all loci. To be classified as backcrossed tree, homozygosity for at least one species-specific allele should

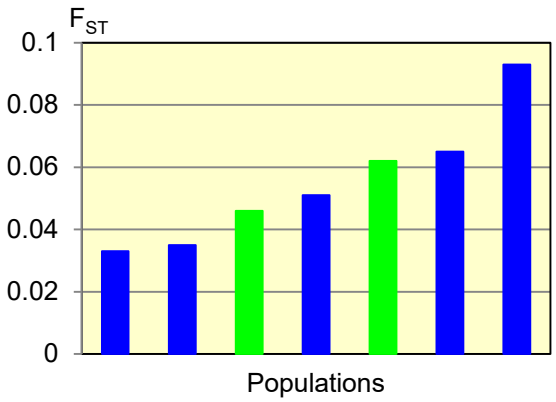

Figure 1-29. Mean F_{ST}s of 7 naturalized U. pumila *populations in northern USA. Blue indicates populations from Wisconsin. The other two populations (green) originated from South Dacota and Illinoise. Thirteen microsatellites were used. Zalapa et al 2010.*

exist. A principle coordinate analysis (PCoA) was conducted using the program AIEx version 6, Program Structure.

Ninety-nine alleles were detected in the 9 microsatellite loci; 67 alleles were specific for *U. rubra* while 32 were specific for *U. pumila*. Of the 92 trees, nine were identified as pure *U. rubra*, 32 were classified as *U. pumila*, and the remaining 51 trees belonged to various types of hybrids. Also the PCoA clearly separated the two species from the hybrids, with the first principle coordinates accounting for 87% of the variance. The large proportion of hybrids suggests that *U. rubra x U. pumila* hybrids are frequently formed in nature. Thirty-five of the hybrids were classified as first generation hybrids. F_2 trees would be homozygous for species-specific alleles from both parental species. Since no hybrid trees fulfilled this requirement it was concluded that the 16 hybrids constituted backcrosses, 14 being backcrosses to *U. pumila* while two were backcrosses with *U. rubra*. The susceptibility to DED of *U. rubra* was suggested as a reason for the asymmetry in backcrossing

We have preferred to illustrate a few diversity characteristics of the 92 trees from the contact zone in Fig. 1-30. Despite three times more *U. pumila* trees than *U. rubra* trees the diversity is lower in *U. pumila* for all traits illustrated. Largest diversity was found in the F_1 hybrids and backcrosses. The similar analyses of the reference materials showed similar results. It is somewhat surprising that *U. pumila* was characterized by less diversity in spite of its wide distribution in China.

The difference between the four categories of populations from the contact zone revealed a large F_{ST} between the two pure species. The limited differentiation between *U. pumila* and the backcross population is noteworthy. It is a reflection of the asymmetric backcrossing in favor of *U. pumila*. This is also observed in the F_{ST}s between *U. rubra* and F_1 and backcrosses, which are higher than the corresponding ones for *U. pumila*.

There is a general discussion of the effect of hybridiza-

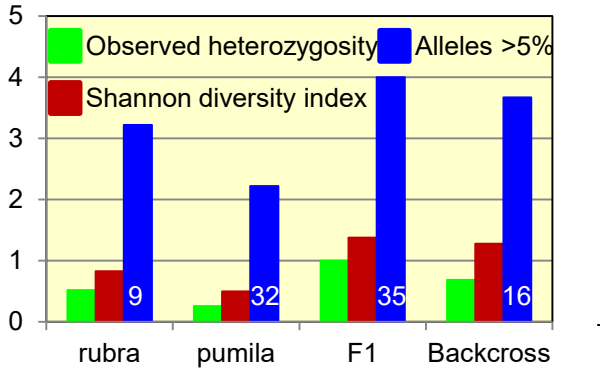

Figure 1-30. Observed heterozygsity, Shannon index, and mean number of alleles in frequencies above 5% in U. rubra, U. pumila, *their F_1 hybrid and backcrosses. The trees originated from a contact zone in Wisconsin, USA. The number of trees in each category is indicated. Zalapa et al. 2009.*

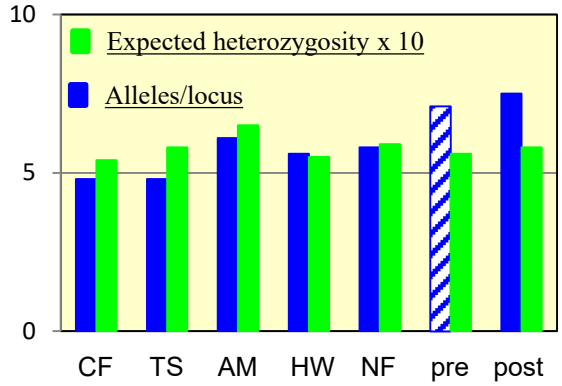

Figure 1-31. Alleles per locus and expected heterozygosity x 10 in 5 U. rubra *populations from southern Wisconsin and in herbarium material collected before (pre) and after (post) arrival of DED in 1961 to Wisconsin. Analysis comprised 13 microsatellite loci. Brunet and Guries 2016.*

tion with exotic species on the domestic American elms in Brunet and Guries (2016). The long-time use of the DED-tolerant *U. pumila* even before the arrival of DED to North America has opened up the possibilities/risks for spontaneous hybridization with American elms. Moreover, results indicated that backcrosses with *U. pumila* of the *U. rubra* x *U. pumila* hybrids were more frequent than the backcross with *U. rubra*. This means that there may be an erosion of genetic diversity in *U. rubra.* However, one study of five wild populations of *U. rubra* and herbarium specimens of this species before and after occurrence of DED indicated a difference in numbers of alleles per locus (Fig. 1-31) but this did not affect the expected heterozygosity: 0.51–0.60 in the wild populations and 0.57–0.58 in the herbarium populations. The lack of reduction of the genetic diversity in native *U. rubra* populations in spite of the appearance of DED was attributed to wind-pollination in *U. rubra*.

U. serotina is probably the elm species with most limited distribution among American elms and it is expected to be the most differentiated elm species in USA. Also *U alata, U. crassifolia,* and *U. thomasi* all have more limited distribution than *U. americana* and *U. rubra*. The wind-pollination of these elm species is expected to result in limited among-population variation. It was anticipated that most of the genetic variation might be found in a single or a few populations.

Selfing has not been studied to any great extent but the results so far indicate limited selfing.

The incompatibility between *U. americana* and *U.pumila* has been attributed to the tetraploidy of *U. americana*. After the detection of diploid *U. americana* the incompatibility between these 2 species might not be complete. The authors concluded that despite the strong impact of DED, large numbers of elms survive and reach reproductive maturity. Since wind-pollination with gene flow over large distances occurs it was expected that DED has not eroded the genetic variability to critical levels. On the one hand there is a long-term risk that hybridization and backcrosses to the exotic species would reduce the genetic diversity of the American domestic species. On the other hand interspecific hybridization would lead to increased genetic variability, which might be valuable for adaptation under fast changes of the climate.

1.3.2 Asian studies

The impact of the Great Wall of China on gene flow between populations on each side of the wall (one population north of and one population south of the wall) was the objective of a study of six species with different ecological characteristics (Su et al. 2003). *U. pumila* was one of these species. One population from each side of the wall was sampled north and south of the wall at 40.22°N and 116.07°E. A control site with the same topography as along the wall was selected with a separating path of the two control populations. Unfortunately, *U. pumila* did not occur at this locality, so *U. macrocarpa* was selected as a comparison species. Over 80 polymorphic RAPD bands were used for the *U. pumila* populations and over 50 bands for *U. macrocarpa*.

The variance component for the difference between the two wall populations was estimated at 13.2%. The corresponding percentage for the control *U. macrocarpa* populations was 7.6%. A neighbour-joining analysis revealed that two main clusters were obtained with two trees in the northern population clustered together with the southern population in this analysis. The two observed between-population estimates of differentiation must be regarded as exceptionally high for populations growing adjacent to each other, especially since in elm species both the pollen and the samara (winged fruit) are spread by the wind. It might be speculated that the topography along the wall, which runs on top of mountain edges in combination with predominating wind in east - west direction might explain the high between-population estimates.

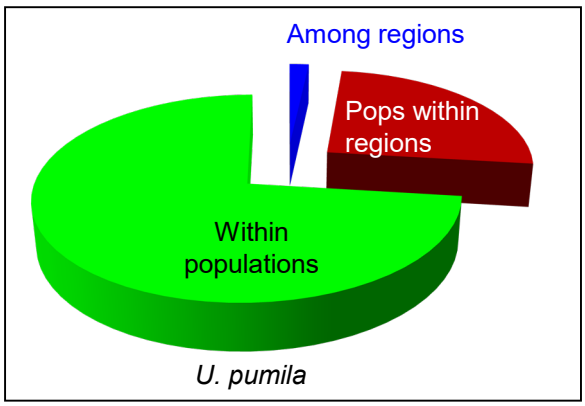

Figure 1-32. Partitioning of the percentage variance components among regions, among populations within regions, and within populations. Wesche et al. 2011.

With the objectives of estimating the genetic diversity and differentiation of 6 Mongolian *U. pumila* populations, RAPDs were analyzed by Wesche et al. (2011). Flowering, seed germination, as well as asexual recruitment were also studied. Four of the populations originated from different river banks in the central and southern Gobi desert and two originated from northern Mongolia. We shall limit this survey to the genetic part of the study. In all, 159 trees were sampled and 155 RAPD phenotypes were recorded. A limited number of clones occurred among the 159 trees. The range of distances between the Gobi populations was 13–187 km and the two northern populations grew at a distance of 54 km. Fifteen randomly chosen trees (height >3 m) per population were included in a more intensive study. Samples around these core trees were also taken in order to detect any clonal material.

The average gene diversity based on AMOVA calculated by Arlequin 2,000 varied in the range 0.132–0.192. There was no relationship between census number of trees per population and gene diversity of the populations. The Φ_{ST} estimate was relatively high, 0.269, which is not surprising with the large geographic distances among the populations as well as the isolated character of distribution of the populations. The individual Φ_{ST}s varied in the range 0.17–0.38. There was no relationship between Φ_{ST} and geographic distance. The partitioning of the variance is illustrated in Fig. 1-32, which shows that within-population variance was dominating while the variance for the four regions was less than 2%. It is surprising that the variance component for populations within regions is so much larger than the regional variance component. It would have been useful to have the mean Φ_{ST}s for individual populations to better understand the low regional variance component. It was speculated that large differentiation among populations within river valleys might be attributed to limited pollen flow despite *U. pumila* being a wind-pollinated species. Maybe the microclimatic conditions contribute to strong within-valley differentiation.

Leaf morphology and eleven SRAP (sequence-related amplified polymorphism) primer pairs were used in a study of 16 populations of the endangered *U. lamellosa* in China by Liu et al. (2016). ANOVAs were run for the 14 leaf morphology traits and the coefficient of variation (CV) and phenotypic differentiation coefficient V_{ST}, which is the percentage of variance among populations divided by the sum of among and within population percentage variances. The molecular data analysis comprised the standard population genetics variables.

A high variability was noted for the morphology traits. The range of the coefficient of variation (CV) for the morphology traits was 11.2 – 44.2 (vein angle and length/width ratio of the petiole), The mean V_{ST} was estimated at 27.3%. The Shannon-Wiener estimates for the morphology traits varied in the range 4.76 – 7.03 with a mean value of 6.68. The low value for leaf thickness, 4.76, might be regarded as an outlier trait. The CVs of individual populations varied between 21.74 and 35.95. These two populations are growing in the same region. The morphological cluster analysis identified three main groups containing 3, 9 and 4 populations. This grouping did not reveal a clear geographic separation of the groups.

The highest molecular genetic diversity was noted for the population represented with only seven trees while the lowest diversity was found for an isolated population. The AMOVA revealed that 66% of the variation was attributed to within-population variation.The differentiation among the populations was strong, G_{ST} being 0.37, which agreed with the AMOVA run for this material.

The large genetic and morphological variability both among and within populations was attributed to the wide geographic distribution and the wind-pollination of this species. The occurrence of the species mainly in mountain valleys may prevent gene flow among populations, which may promote population differentiation, especially if the population consists of a limited number of mating trees. Finally, there was no agreement between morphological data and genetic data.

It was suggested that all 16 populations should be included in *in situ* genetic conservation as well as preservation of the habitats in which *U. lamellosa* is growing. Population size should be increased in populations with low numbers of trees. However, mixes of populations should be avoided. It was also suggested that *ex situ* gene banks should be established.

Nuclear ribosomal internal transcribed spacer regions (ITS) and the single-copy gene (Aat) were used by Hou et al. (2020) to study genetic differentiation and evolution of the endemic elm, *U. lamellosa,* in China. Fourteen populations from three regions in China were sampled: Yaiihang Mountains, Yanshan mountains, and Yinshan Mountains. Each population was represented by 11–22 trees.

As regards ITS, 13 of the 14 populations were polymorphic and had 18 different haplotypes. Haplotype H1

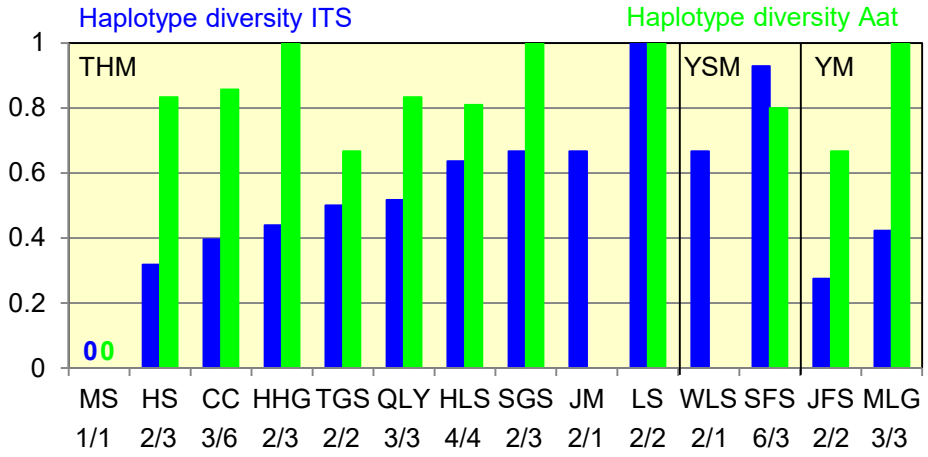

Figure 1-33. Haplotype diversity estimated by nuclear ribosomal internal transcribed sequence (ITS) or single-copy gene (Aat) in 14 U. lamellosa *populations from three regions in China, THM = Taihang Mountains, YSM = Yanshan Mountains, YM = Yinshan Mountains. The number of ITS/Aat haplotypes in each population is shown. Hou et al. 2020.*

seemed to be the most ancestral haplotype and the others could be traced from H1. A still higher number of haplotypes was noted for Aat, 23. Nine of the populations showed high Aat haplotype diversity (> 0.80 Fig. 1-33). The YSM region was regarded as being the most ancestral from which the two other regions had developed. The two YSM populations had high ITS haploid and nucleotide diversites but no Aat haploid and nucleotide diversities for one of the YSM populations.

In the principle component analysis three clusters corresponding to the three geographic regions were identified.

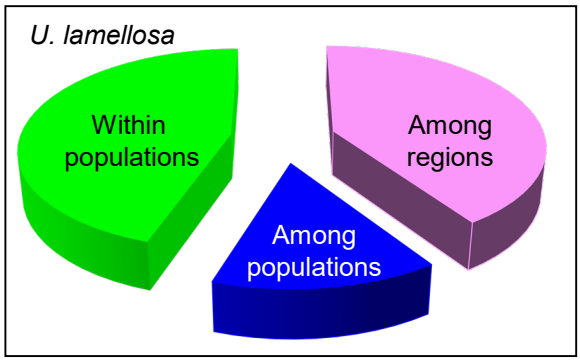

Figure 1-34. The partitioning of the variance among the 3 regions of U. lammelosa *in China*:
Among regions
Among the populations within regions
Within populations
Based on AMOVA of 14 U. lamellosa *populations from three regions in China, THM = Taihang Mountains, YSM = Yanshan Mountains, YM = Yinshan Mountains. Hou et al. 2020.*

The differentiation among populations estimated as G_{ST} was 0.117 for ITS and 0.192 for Aat, indicating a fairly strong differentiation among the 14 populations, which is expected considering the constraint by the mountain chains on pollen flow between the three regions. The AMOVA revealed that the differentiation among the regions is substantial, 40.6%, and almost as large as the within-population variation, 45% (Fig. 1-34). Positive relationships between geographic and genetic distances were noted for ITS, r = 0.59, and for Aat, r = 0.39. However, the degree of explanation did not exceed 35%.

The impact of the Tertiary geological/and or climatic events on the diversity of *U. lamellosa* was thoroughly discussed.

The substantial difference among the populations means that genetic conservation of *U. lamellosa* should encompass at least these 14 populations.

With the objective of studying the genetic diversity of seven eastern Chinese populations of *U. parvifolia,* specific locus amplified fragment sequencing, SLAF-seq, was used by Lyu et al. (2020). Traditional population genetics parameters were estimated. In addition, possible genes related to adaptedness were searched for. Each population was represented by 13–18 trees. The range for altitude of the populations was 10–180 masl. The annual precipitations varied in the range 802–2,395 mm. Traditional population genetics parameters were estimated. Also, possible genes related to adaptedness were searched for.

No fewer than 457,888 SNPs from the 107 trees were used for estimates of genetic diversity and population differentiation.

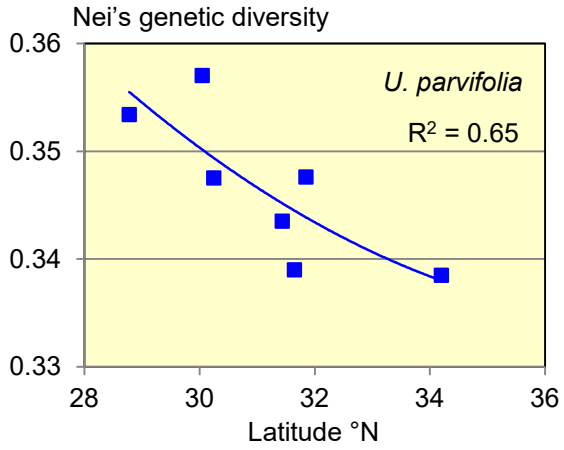

Figure 1-35. The relationship between population latitudinal origin and Nei's genetic diversity (Nei 1979) for 7 U. parvifolia *populations from eastern China. Specific locus amplified fragment sequences (SLAF-seq) were studied. Lyu et al 2020.*

The mean observed heterozygosity for the seven populations was significantly lower than the expected heterozygosity, 0.1599 versus 0.3315. The polymorphism information content varied in the range 0.2632 – 0.2761. Shannon index did not vary much among the populations; range 0.4948–0.5171. Except for one population (F_{IS} = -0.038), there was a slight deficit of heterozygotes (F_{IS} range 0.013–0.068). We found one moderately strong relationship between latitude and Nei's genetic diversity (Nei 1979, Fig. 1-35).

The AMOVA showed that more than 92% of the variation occurred among trees while only 4% was attributed to among-population variation. The estimates of mean F_{ST}s reflect these results as illustrated for the mean F_{ST}s of individual populations (Fig. 1-36). The two southernmost and the northernmost populations had the highest mean estimates of F_{ST} > 0.050 while the four central populations were less differentiated. No or very weak relationships were noted for annual precipitation and altitude. It was stated that differentiation among the populations was weak. However, F_{ST} estimates larger than 0.50 can hardly be regarded as low differentiation.

The Bayenv2 analysis (Coop et al.2010) for identification of relationships with environmental traits revealed that 8, 10, and 25 markers were significantly related to altitude, annual precipitation, and annual average temperature, respectively. The LFMM method (Frichot et al. 2013) with the same objective showed that 16, 10, and 4 markers were associated with annual temperature, altitude, and annual precipitation, respectively.

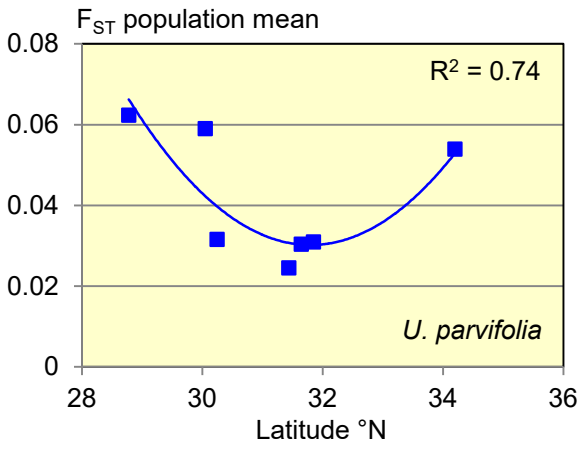

Figure 1-36. Mean F_{ST} based on SLAF-seq markers for 7 eastern Chinese U. parvifolia *populations plotted against their latitudinal origin. Lyu et al 2020.*

Five of the correlated SNP markers could be identified with known genes:

Altitude correlated	DEAD-box helicase
"	V-type proton ATPase
Annual precipitation	UDP-glycosyltransferase
"	Peroxisome biogenesis protein
Annual average temperature	Cysteine-rich receptor-like protein kinase

The identification of associations with known genes can lead to better understanding of factors contributing to the adaptedness of populations growing under varying environmental conditions.

An Indian study of the vulnerable Himalayan elm, *U. wallichiana*, was presented by Singh et al. (2021), in which microsatellites were identified and used. Two populations with 8 and 10 trees each were included in this study. The populations are growing at 32.85°N, 76.16°E and 33.43°N, 74.32°E. Fifty-seven loci were investigated, 28 being polymorphic and 29 monomorphic.

The observed and expected heterozygosities were 0.77 and 0.55, which suggests that inbreeding had been of minor importance. The number of effective alleles did not differ significantly between the two populations, 2.52 versus 2.54. Private alleles were found but numbers were not given. An AMOVA test showed that 27% of the variation could be attributed to population differences. It was stressed that markers are required for future studies to guide applied conservation of this species. We should like to state that variation in adaptive traits is more important than markers.

Samples from 27 *Ulmus villosa* trees of this rare and endangered species were collected in Hazara, Pakistan, for

Table 1-4. The number of the U. villosa *groups in the neighbour-joining trees based on analysis of four DNA barcode markers. Entries with adjacent relationship are indicated. Khan et al. 2022.*

Barcode marker	No. of groups	Neighbours according to neighbour-joining tree
rbcL	3	UV43 UV33 – UV29 UV40 – UV22
matK XF+5R	1	UV22-UV29-UV40-UV33
matK 390F+ 1326R	2	UV 43 UV29-UV33-UV40
trmH-psbAIn	6	UV 24 UV23-UV32 UV43 UV31 UV14-UV29-UV10- UV15-UV33 UV22-UV40

analysis of genetic differentiation using 4 DNA barcode markers (Khan et al. 2022). Samples from other species were also analysed. DNA sequences known to vary much, and thus useful for phylogenetic relationships, were studied. The regions used and number of sequences from *U. villosa* were.

rbcL,	6 sequences
matK XF + 5R	4 sequences
matK 390F+ 1326R	4 sequences
trnH-psbAIn	14 sequences

In addition to these sequences a large number of sequences from data bases could be utilized.

In spite of the title stating that population structure of *U. villosa* was the objective of this investigation, the main focus was on elm species relationships. The neighbour-joining (NJ) model was used for estimation of phylogenetic relationships.

In Table 1-4 we have compiled the results as regards *U. villosa* for the four DNA barcode markers used in this investigation. There was no information as to what RFXX stands for, but we assume that these are the identities of the 27 sampled trees. RF29 and RF33 occur together for all 4 markers. Markers RF22 and RF40 occur together for 3 of the markers. RF43 occurs alone for 3 of the markers. There is some consistency of the results from one marker to another marker. Since there was no information on geographic position of the sampled trees it is impossible to compare the grouping of neighbours with geographic location.

It was stated that the information gained should be used for genetic conservation of this species. However, no suggestion or discussion on this matter was presented. The most important conservation undertaking is to increase the present size of 283 trees to a much larger number.

Seven ISSR primers were used for a study of seven South Korean populations of *U. davidiana* var. *japonica* by Ahn et al. (2013, Abstract in English). They reported limited genetic differentiation among populations, F_{ST} = 0.042. No significant differences among fixation indices were found.

1.3.3 European studies

Molecular markers are useful for sorting out the relationships of elm species. The taxonomic classification of British elms by RAPDs was reported by Coleman et al. (2000). An endemic taxon *U. plotii* was particularly examined. Samples were collected according to morphological traits as follows:

19 *U. plotii,*
10 *U. pseudoplotii*
14 *U. minor*
16 *U. glabra*
12 *U. hollandica*
11 *U. plotii x U. minor*

The taxonomic classification was problematic in some cases. Accession 43, *U. hollandica,* one of the most planted elms in England, belonged to this category. With the 10 RAPD primers 77 polymorphic ones were available for analysis. Principle component analysis was performed.

In all, 61 genotypes were detected. Moreover, all 61 genotypes could be detected by use of three primers only. The PCO (principle coordinate) analysis revealed a clear separation of the *U. glabra* group of trees from the other taxa. *U. hollandica* was intermediate between *U. glabra* and the rest of the taxa studied. This means that no clear separation between *U, plotti, U. pseudoplotti, U. plotii x U. minor,* and *U. minor* could be identified. The most frequent genotype occurred in all *U. plotii* trees and in 3 samples tentatively classified as *U. plotii*. The most probable explanation for this uniformity is that these sampled trees belong to one clone. Most likely, spreading of cuttings by man was responsible for the spreading of this clone to distant places. Natural spreading by suckering would be too slow to match the existing distribution of one clone. It was strongly stressed that *it is necessary to*

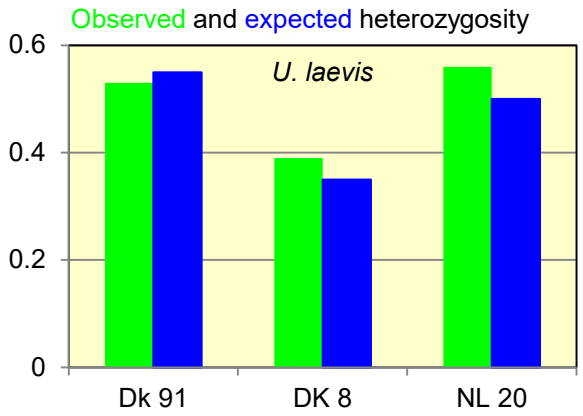

Figure 1-37. Observed and expected heterozygosities in 2 Danish and one Dutch U. laevis *population. The number of trees in the three populations are indicated. Nielsen and Kjær 2010a.*

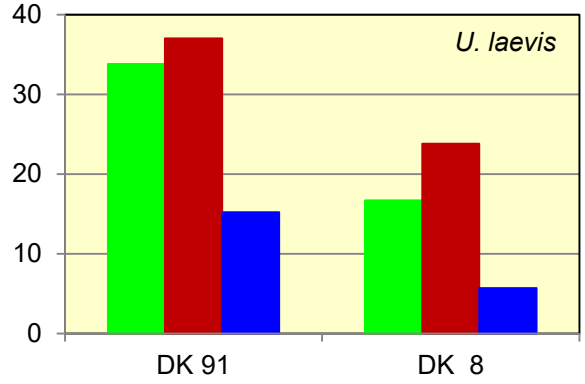

Figure 1-38. The effective pollen movement distances in meters assuming an exponential pollen distribution curve (green) and average pollen distances based on paternity analysis (brown). The blue columns refer to the effective number of pollen donors. This study was carried out in 2 Danish populations with 91 and 8 trees. Nielsen and Kjær 2010a.

employ rigorous identification procedures based on the full set of diagnostic characters. Since the characteristics of the clone *U. plotii* fall within the characteristics of *U. minor,* it does not deserve priority in genetic conservation according to the authors.

Two Danish and one Dutch population of *U. laevis* were studied with respect to genetic structure, mating pattern, and spatial genetic structure by Nielsen and Kjær (2010a). The Danish populations consisted of 91 and 8 trees and were growing more than one km apart. Twenty trees from a marginal population of *U. laevis* in the Netherlands were genotyped. Seven polymorphic microsatellites showing simple Mendelian inheritance were used for genotyping. Allelic richness, observed and expected heterozygosities, were estimated. Recent bottlenecks were tested. Paternity analysis was used to estimate average pollination distance in the two Danish populations. The average pollen movement was also estimated by paternity analysis and by the TWOGENER option in the program POLDISP (Robledo-Amuncio 2007). The effective number of pollen donors was also estimated.
Especially, the Danish 8-tree population showed limited genetic diversity (Fig. 1-37) but the effective number of alleles per locus did not vary much between the two Danish populations, 2.75 and 2.57 respectively.
U. laevis seems to be characterized by less variability than other comparable species. The estimates of outcrossing were close to 1.00 in both Danish populations. It is likely that there are self-incompatibility genes in *U. laevis*.
The number of pollen donors per tree did not differ much, 11.4 and 10.1. There was a clear negative relationship between relatedness and distance. In the pooled Danish material individual pairs of trees up to a distance of 70 meters were more closely related than randomly chosen trees. The large Danish population showed a week spatial genetic structure. Even if the seeds are winged their dispersal was limited. The situation might be different if seeds are dropped into streaming water. The effective pollen movement distances in meters assuming an exponential pollen distribution curve and average pollen distances based on paternity analysis gave fairly similar results. The pollen movement in the 91-tree population was twice as large as in the 8-tree population (Fig. 1-38). One offspring plant had been pollinated by a tree growing as far away as 1,165 meters from the mother tree. The number of effective pollen donors was for obvious reasons much larger in the 91-tree population than in the 8-tree population.
There were no signs of bottlenecks in the Danish populations while bottlenecks might have occurred in the Dutch population.
The almost 100% outbreeding and limited seed dispersal were striking observations in this investigation.

Genetic diversity, gene flow, and mating pattern (cf Table 1-5) in a Danish deciduous tree stand with 181 *U. glabra* trees were compared with the corresponding variables in seven isolated *U. glabra* elm trees (Nielsen and Kjær (2010b). This stand is located on Zealand at 55.37°N and 11.58°E and the isolated trees are distributed over Denmark. Four polymorphic microsatellites were used in this study.
We have listed the major results of this investigation in Table 1-5. As is evident from this table there were minor differences between the two types of population as regards the variables studied. For obvious reasons pollen dispersal among isolated trees is longer than in the stand. The outcrossing rates in both populations were close to 1.0, which indicates that independently of population structure, isolated or stand, outcrossing is totally dominating in wych elm. The ability of pollen movement over long distances in farmland landscapes compensates for the low number of neighbours to the isolated trees. The risk for genetic isolation of individual trees scattered in the landscape seems to be marginal. The absence of differences between the offspring in the two types of po-

Table 1-5. Variables studied in a U.glabra *forest (f) and in a distributed group of 6 isolated trees (it) and the difference in performance between these two groups. Nielsen and Kjær 2010b.*

Variables	*Performance of the 2 groups*
Allele frequencies in parental and offspring generations	no difference
Alleles in offspring from isolated trees and forest	no difference
Allele frequencies in offspring from isolated trees and forest	no difference
Number of alleles corrected for sample size	4.9 forest 5.2 isolated trees
Multilocus outcrossing rates	0.999 (f) 0.985 (it)
Correlation of paternity estimate, r_p	0.032 (f) 0.029 (it)
Fathers for the given proportion of paternity	31.3 (f) and 34.5 (it)
Pollen dispersal distances	104.4 m (f) longer (it)
Effective density of pollen donors per hectare	2.9 trees/ha
Effective pollen donors per mother tree N_{ep}	2 estimates 27.1 and 31.3 (F) 34.48 (it)
Multilocus minimum estimate of the number of contributing pollen donors	4 - >6 (F) 3 - >6 (it)

pulation supports this statement. The strong outbreeding means that selfing did not occur in any of the populations. Only one of the isolated trees showed signs of biparental inbreeding.
The average distance of successful pollination in the stand was estimated at 104 meters. According to the authors the low sample size and genetic structure reduces the precision of this estimate.
It was speculated that young trees, which are not attacked by *Scolytus* beetles, and do not suffer from DED, have started flowering and contributed to remote pollination. An evolution towards an early flowering in wych elm might have started.

The objective of a study by Cox et al. (2012) was to investigate whether or not existing gene conservation efforts in Flanders had been successful in sampling the existent genetic diversity of European elms in this province. Since it is likely that cultivars have hybridized with autochthonous elms, identification of pure species, species hybrids, and backcrosses was another objective of this investigation. Cox et al. (2012) studied the genetic diversity in and differentiation of 54 samples of *U. laevis* from Belgium, France, and the Netherlands using AFLP. In addition one sample from each of Germany and Kazakhstan were included as reference material. Another part of this investigation concerned the *U.glabra-U.minor* complex. In this case 59 samples and 19 cultivars were studied. Each sample analyzed contained fewer than 10 trees with the exception of one *U. laevis* sample of 18 trees. Almost all sampling took place at localities with just a low number of trees, which explains the low numbers of each studied sample.
U.laevis. Nei's genetic diversity (Nei 1979) was calculated for the 34 populations with more than one tree/sample. The diversity had a range of 0.034 – 0.266, which was based on the assumption that no inbreeding occurred; $F_{IS} = 0$. With the assumption of $F_{IS} = 0.10$ the range of diversity was 0.150 – 0.276. Thus, both methods resulted in low diversities, which is expected with so low numbers of trees/sample.
The F_{ST}s estimated for the two fixation indices 0.0 and 0.1 gave different results, 0.146 for the no inbreeding case and 0.0036 for the second case. An AMOVA test showed that 13% of the variation was attributed to among-population variation. The 0.1 inbreeding F_{ST} estimate seems very unlikely. One seed orchard was planned for the Flanders region, in which clones from surrounding regions might be included to increase the genetic variability.
U. glabra and U. minor and their hybrids and backcrosses. Of the 106 samples 29 turned out to be pure *U. glabra* and another 29 were found to be pure *U. minor*. The remaining 48 samples were either species hybrids or backcrosses to the two species. Nei's genetic diversity in the Belgian populations was low: 0.032 – 0.216. This was expected since the number of trees per sample was low, 2 – 8 trees. A very high F_{ST} estimate was noted, 0.25, which is not surprising since the samples consist of different taxa. It was speculated that such a high estimate might be attributed to DED leading to bottlenecks in many elm populations. Another estimate of differentiation, ϕ_{ST}, was obtained from AMOVA and was still higher, 0.33.
In addition, there was some focus on the taxonomy of the material in the *U. glabra-U. minor* complex. The identification of F_1 hybrids and backcrosses to the two parental species is difficult based on morphological traits. With AFLP technique the proper status could be certified in several cases.
The low number of trees/sample means that the estimates of diversity and differentiation are imprecise. In addition, the human transfer of material over the centuries has probably affected the present diversity and differentiation. Thus, the existing diversity and differentiation might be different from a situation without any human interference with Mother Nature.

The human impact on the genetic structure of indigenous Flemish elm populations was studied by Cox et al. (2014). Three sets of material were used:

Trees from a gene bank with *U. glabra, U. minor*, and their hybrids

Reference sample of elm clones

Cultivars mainly from the Netherlands

In all, 385 polymorphic AFLP loci were identified, of which seven samples had low quality and had to be excluded from further analysis. The number of polymorphic markers was 197 for *U. glabra*, 172 for *U. minor*, and 222 for their hybrid..

Interspecific hybridization was estimated by use of the Bayesian analysis of population structure (BAPS) version 5.4. Another Bayesian method, New Hybrids version 1.1b3, was also used for detection of hybrids.

Of the 106 trees from Flanders, 28 and 29 were *U. glabra* and *U. minor*, and 49 were hybrids based on the AFLP analysis. No fewer than 38 of these 49 trees were morphologically classified as pure species. The high frequency of hybrids suggests the absence of any strong crossing barriers between these two species. It was suggested that habitat disturbance by human activities might have facilitated interspecific hybridization. Moreover, full-sibs were often found at different localities and this was true for ramets of individual clones. Ramets of the same clone were found at distances in the range 4–60 km. This was most common in *U. minor* but it also occurred in *U. glabra* but to a lesser extent. In some cases backcrosses to both parental species occurred while no F_2 individuals were detected. This was attributed to reduced fitness of homozygous plants/trees.

Three cultivars, Klemmer, Belgica and Major, were potential parents of several sampled trees. It was stated that these three cultivars *have influenced the natural elm populations, either through planting and possibly clonal reproduction or through hybridization*.

The consequences of the obtained results for genetic conservation were discussed. A high relatedness of the trees was frequently noted, which might be attributed to matings within populations of limited size. Dutch elm disease and habitat loss have probably caused loss of genetic variation in remaining populations with low efficient population size. It was suggested that conservation in an *ex situ* field gene bank of the remaining elms ought to be carried out. Since the interspecific hybrids occupy the same ecological habitats as the pure species it was suggested that also hybrids should be considered in the conservation of elms. It was stated that introgression might increase the genetic variance in the progeny, which might promote adaptation.

The genetic structure in two small *U. laevis* populations 52 km apart (Qu and Va) in central Spain was studied by Venturas et al. (2013). Owing to human activities no regeneration takes place in these two localities. Nine microsatellites and two chloroplast haplotypes were used to estimate several population genetics variables. The spatial genetic structures as well as previous bottlenecks were also estimated.

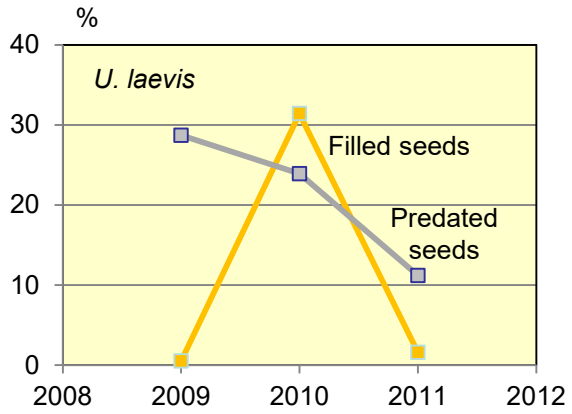

Figure 1-39. The percentages of filled seeds and predated seeds in years 2009 – 2011 in one Spanish U. laevis *population. Venturas et al. 2014.*

The gene diversities were 0.43 and 0.45, which agree with other estimates for *U. laevis*. The fixation indices were significantly different from 0 for the Qu population, -0.07. The overall F_{IS} of -0.02 was not significantly different from 0. In spite of the small population sizes inbreeding did not occur. As regards cpDNA, all trees in VA showed haplotype A while 46 of the 57 trees in Qu carried haplotype B.

The number of clusters based on the admixture model was 2; one cluster contained 52 trees from Va + 4 trees from Qu while the other cluster consisted of 53 trees from Qu and 1 tree from Va. A fairly high F_{ST}, 0.17, for a wind-pollinated tree species was estimated.

Neighbourhood size was estimated at a range of 32–44 trees and the parent offspring distance was 19–41 metres. The highest estimates were obtained for a density of 15 trees/hectare and the lowest for 100 trees/ ha.

M statistics according to Garza and Williamson (2001) was applied to test for bottlenecks. The estimates for Qu and Va were 0.56 and 0.48, suggesting long-lasting bottlenecks in both populations.

It was concluded that habitat transformation, which prevents regeneration, is of much greater importance for conservation of these populations than anything else. This is a situation shared with many riparian European species.

Seed production and seed release in the Va stand was studied over three years, 2009–2011, by Venturas et al. (2014). Seed traps were used to estimate filled seeds, empty seeds, and predated seeds. Seed collection took place every 2–3 days. Seed dispersal was calculated by multiplying the fecundity (seeds per year) and dispersal kernel under the assumption that fecundity of a tree is proportional to its basal area. Thus, dispersal was not estimated by genetic markers of trees and seeds.

The main pollen dispersal occurred during two weeks in 2010, which was a mast year, while it was extended to 1-1½ months in 2009 and 2011.The seed production varied in the range 700 thousand to 17 million seeds.

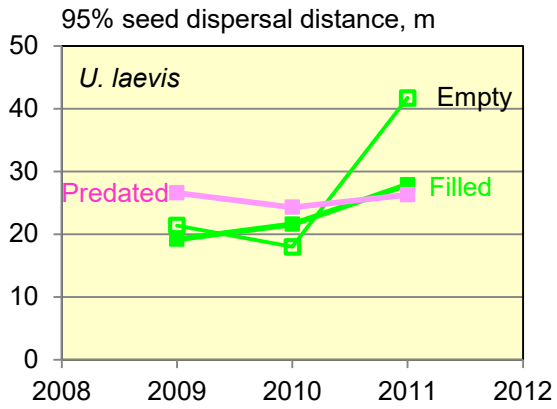

Figure 1-40. The distances within which 95% of the filled, empty and predated seeds were found during years 2009–2011 in a Central Spanish U. laevis *stand. Venturas et al. 2014.*

Low percentages for filled seeds were noted for years 2009 and 2011 (Fig. 1-39). Even if the percentages are low there would be 3,600 filled seeds in 2009 and more than 70,000 filled seeds in 2011. Such large numbers of filled seeds would certainly guarantee a long-term survival of this population unless the habitat destruction is of such a character that *U. laevis* germination is prevented. This figure also reveals that seed predation is considerable. Mice, voles, and bird species are the main predators. With one exception, 95% of the seeds were found within 30 meters from the tree (Fig. 1-40). Empty seeds in 2011 constituted the exception, 41.7 meters. It is evident that seed dispersal is limited in this species in spite of the low seed weight. Wind did not have a great impact on seed dispersal. Long-distance seed dispersal may occur from populations growing close to streaming water.
Also from this paper: loss of habitats for *U. laevis* is a greater threat than loss of genetic diversity.

A study of hybridization between the Asian *U. pumila* and the native *U. minor* in Italy was presented by Brunet et al. (2013). Ninety-six elms were included in this study. According to their morphology the following number of trees was obtained:

U. minor	41
U. pumila	11
Hybrids	42

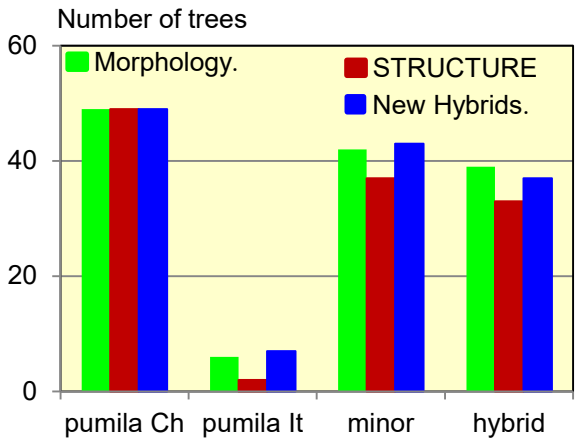

Figure 1-41. The number of trees according to morphological classification and grouping according to the Bayesian clustering method in STRUCTURE or the Bayesian algorithms in STRUCTURE. Brunet et al. 2013.

In addition, 49 *U. pumila* trees from China were used as reference material. Genotyping took place with 10 microsatellite loci. Three types of analysis were used for genetic classification of the 96 trees as pure species or hybrids:

The Bayesian clustering method in Structure
The Bayesian algorithms in Structure, New Hybrids
The Principal Coordinate Analysis (PCoA)

The absence of species-specific alleles and the relatively low number of microsatellite loci makes the estimates somewhat uncertain according to the authors. Fig. 1-41 reveals that only two of the six morphologically classified *U. pumila* trees were classified as pure *U. pumila* by method 1 while seven trees were classified as *U. pumila* by method 2. Generally, there was a better agreement between method 2 and the morphological classification than the corresponding relationship for method 1.
The results from methods 1 and 2 differed as regards the hybrids. Thus, 35 of the 37 hybrids were classified as F_2 by method 2 while method 1 resulted in 21 F_1, six backcrosses with *U. minor* and 12 backcrosses with *U. pumila*.
The differentiation among the four groups was analyzed by use of the AMOVA method of GENAIEx with 9999 bootstrap iterations. This analysis comprising the four groups resulted in an among-group genetic diversity of 34%. The observed heterozygosity was larger in the hybrids and *U. minor* than in the two *U. pumila* groups.

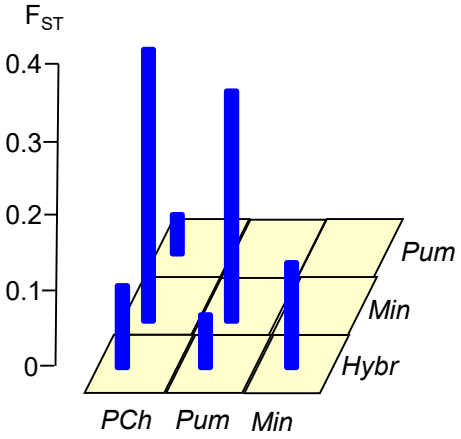

Figure 1-42. F_{ST}s between 4 groups of populations:
PCh = U. pumila *from China*
PI = U. pumila *from Italy*
Min = U. minor
Hybr = Hybrids between U. minor *and* U. pumila. *Brunet et al. 2013.*

There was limited variation between the two *U. pumila* groups, F_{ST} = 0.05 (Fig. 1-42). As expected the F_{ST}s between the two groups of *U. pumila* and *U. minor* were largest. The F_{ST} between hybrids and *U. pumila* was low, 0.08, reflecting the large contribution of *U. pumila* to the hybrids. In a long-time perspective there is a risk that hybrids will outcompete *U. minor*, especially with DED damage contributing to such a process.

According to the title of the paper by Brunet et al. (2016) conservation of *U. rubra* should be treated. However, conservation is more or less limited to the last sentence in the Conclusion without any discussion of how conservation should be implemented. The paper once more deals with genetic diversity of 77 herbarium specimens and five *U. rubra* populations from southern Wisconsin. Each population was represented by 20 trees. Separate analyses of herbarium specimens collected before and after arrival of DED to Wisconsin in 1961 were carried out.
The two traits shown in Fig. 1-43, alleles per locus and expected heterozygosity, were used to exemplify the genetic diversity in the analyzed materials. Noteworthy is the limited difference in expected heterozygosity in the herbarium populations. The number of private alleles was higher in the herbarium populations than in the five natural populations; means 9 and 2.6, respectively. Six of the F_{IS} estimates were negative, range –0.12 to –0.003. The mean F_{ST}s for the natural populations varied in the range 0.034 – 0.051 and it was concluded that this range of F_{ST}s is in agreement with other wind-pollinated species. Considering wind-pollination and the limited geographic range sampled (≈200 km) the F_{ST}s might even be regarded as large. No bottleneck was found in any of the populations.

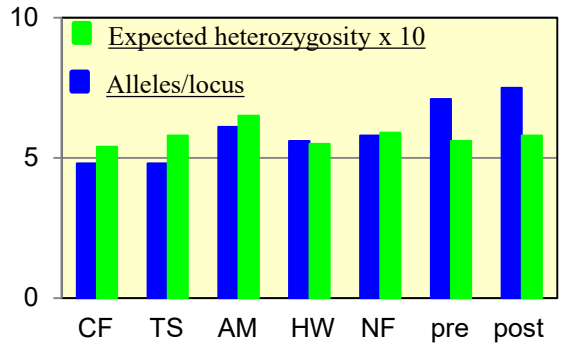

Figure 1-43. Alleles per locus and expected heterozygosity x 10 in 5 U. rubra *populations from southern Wisconsin and herbarium material collected before (pre) and after (post) arrival of DED in 1961 to Wisconsin. Thirteen microsatellite loci were analyzed. Brunet et al. 2016.*

A genetic contribution to the discussion whether Balearic populations of *U. minor* are introductions to the islands or indigenous populations was reported by Fuentes-Utrilla et al. (2014). They studied three populations from each of Majorca, Minorca, and mainland Spain. *U. minor* grows along rivers and the populations are growing along three different river banks. Two of the mainland Spanish populations originate from Catalonia and the third from the Beatic Mountains. Except for the three Majorca populations each population was represented by 30 trees; Majorca populations contained 21, 28 and 29 trees. Nine microsatellites were used in this investigation with a total of 97 alleles. There was a great focus on clonal structure since a high frequency of clones support the hypothesis that *U. minor* is an indigenous species in the Balearic Islands. According to Spanish law indigenous species must be included in genetic conservation.
The number of observed multilocus genotypes (MLGs) was assessed. The number of private MLGs, repeated MLGs, unique MLGs, and locally private MLGs was determined. Simpson's diversity index (D) was calculated. $D = 1 - \Sigma[n_i(1 - n_i)/N(1 - 9)]$, in which n_i is the number of trees with genotype i, N = number of trees analyzed.
The modified index of genotypic richness (R) was also determined. $R = (G - 1)/ (N - 1)$.
Clustering was studied by principle component analysis, Bayesian approach, and by STRUCTURE.
The allelic richness was twice as large in the mainland populations as in the Majorca and Minorca populations. The genotypic richness in the Balearic populations varied between 0 and 0.11 while the corresponding range for the mainland populations was 0.86–0.97. In Fig. 1-44 we have illustrated the number of multilocus genotypes and unique multilocus genotypes. There is a dramatic difference for both parameters, with extremely low numbers in the Balearic populations. It should be noted that Major-

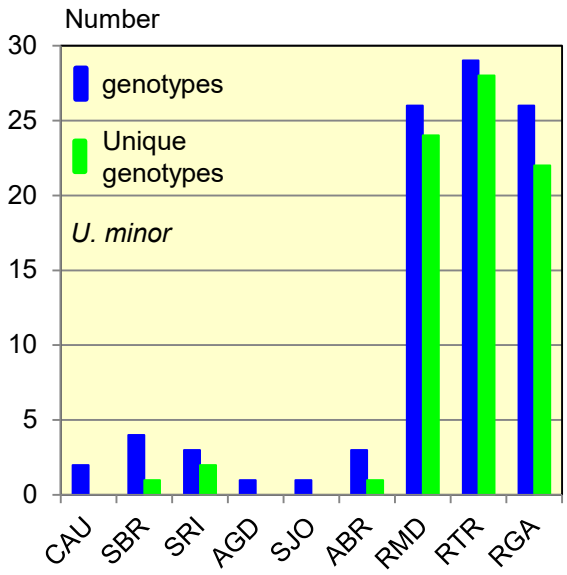

Figure 1-44. Number of multilocus genotypes and unique multilocus genotypes in populations from Majorca (CAU–SRI), Minorca (AGD–ABR), Beatic Mountains,(RMD), and Catalonia (RTR and RGA). The number of trees per population was 21–30. Nine microsatellites were used in the analysis. Fuentes-Utrilla et al. 2014.

ca population CAU was represented by a lower number of trees than in all other populations: 21 versus 29–30. It is likely that vegetative propagation played a great role for the Balearic populations. The adverse conditions for regeneration in the Balearic populations were suggested as the reason for the high clonality in these populations.

As a corollary of this the Simpson diversity indices were much larger in the mainland populations (Fig. 1-45). Despite the lower number of genotypes in the CAU population, its diversity index was larger than the index for the SRI population with one more genotype: 3 versus 2. This was caused by a more even distribution of the two genotypes in CAU than the distribution in SRI, in which 27 of the 29 trees had the same genotype.

The first three axes of the principle component analysis

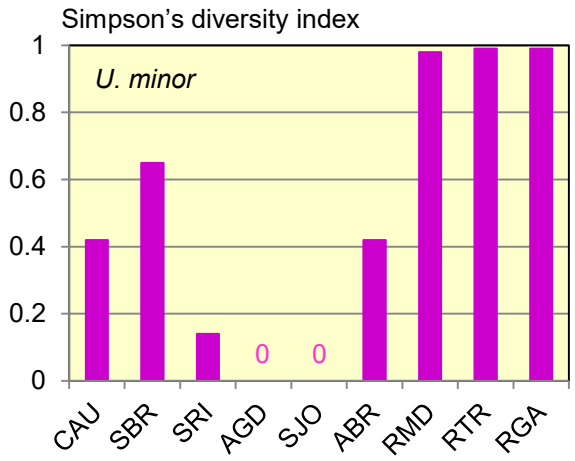

Figure 1-45. Simpson's diversity index in populations from Majorca (CAU – SRI), Minorca (AGD – ABR), Beatic Mountains,(RMD), and Catalonia (RTR and RGA). Nine microsatellites were used in the analysis. The number of trees per population was 21–30 Fuentes-Utrilla et al. 2014.

explained 38.3, 18.9, and 12.5% of the variation and agreed with geographic location. Axis 2 mainly separated Majorca and Minorca populations while axis 3 separated the 2 Catalonian populations. This clustering was corroborated by the Bayesian analysis, which indicated five clusters. Two or 3 clusters were noted for the STRUCTURE-derived clustering analysis. In this analysis the Catalonian populations belonged to one cluster and all other populations to a second cluster. In the case of 3 clusters, the Beatic Mountain and Balearic Islands were separated. It was stated that the more or less absence of sharing of alleles between the Balearic and the mainland populations suggest independent origin of the Balearic populations. The clustering of the Balearic populations independently from the mainland populations lends further support to such an independent origin.

It should be added that regeneration of *U. minor* under the adverse conditions on the Balearic islands was thoroughly discussed.

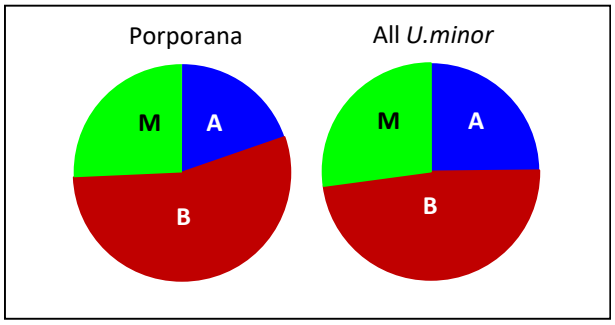

Figure 1-46. The percentages of U. minor *trees in three elm clusters (A, B. and M) from the Porporana stand in the Po valley as well as additional 17 U. minor trees in its surroundings within a radius of 5 km. Six microsatellites were used in this study. Bertolasi et al. 2015.*

The genetic structure and the mating pattern in the last stand (Porporana) of elms in the Po valley, Italy, were studied by Bertolasi et al. (2015). This stand is surrounded by farmland with scattered elms. All 350 elm trees in the Porporana stand as well as 89 elms within a circle of radius 5 km were morphologically classified either as *U. minor* or *U. pumila* or as their hybrid. Based on their morphology all 350 trees in Porporana were classified as *U. minor*. Pollen dispersal from 20 and 28 trees in 2007 and 2008 was studied. Genotyping with six microsatellites took place. The program CLUSTER was run to detect any grouping of the material.
The number of alleles was estimated at 10.8 and the expected heterozygosity 0.69, indicating a highly polymorphic population.

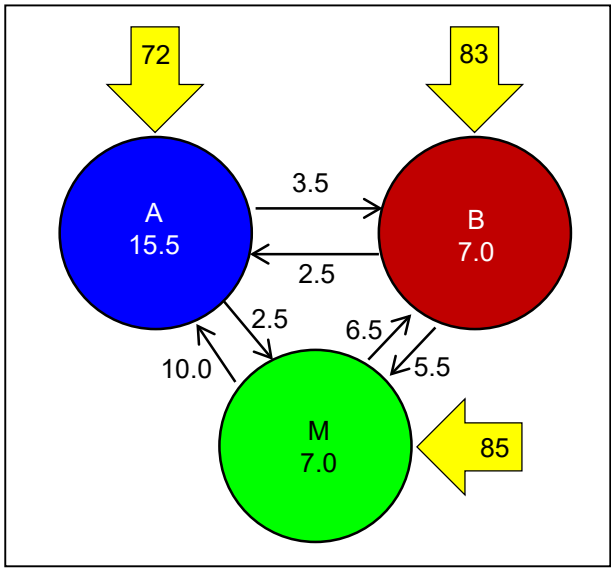

Figure 1-47 The mean percentage of pollinations within and between three elm clusters (A, B. and M) from a stand in the Po valley and its surroundings within a radius of 5 km. The numbers in the yellow arrows show the estimated percentage of pollinations from trees outside the study area. Means for two years are presented. Bertolasi et al. 2015.

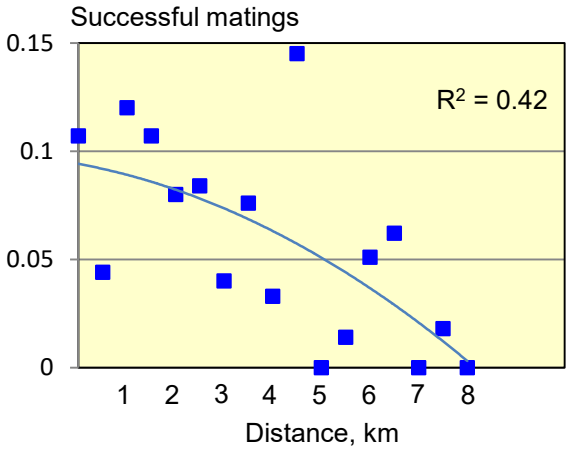

Figure 1-48. The relationship between distance and pollination success in a U. minor *stand in the Po valley and its surroundings within a radius of 5 km. Six microsatellites were used in this study. Bertolasi et al. 2015.*

Two distinct groups (A and B) were identified in the cluster analysis, A with 101 trees and B with 195 individuals. The remaining 110 trees were pooled into a cluster denoted M (Fig. 1-46). The trees in the 3 clusters were not geographically separated. The correspondence between morphological and genetic classification was weak according to Fisher's exact test ($p<0.001$). It is surprising that the strong genetic structuring did not correspond to separate areas of the clusters in the Porporana stand. Fig. 1-46 shows that cluster B dominated among the trees. Seventeen of the 22 morphologically classified *U. pumila* trees belonged to cluster A. Few individuals were classified as interspecific hybrids.
The paternity analysis revealed that pollinations from outside sources dominated while pollinations between clusters were low and never exceeding 10% (Fig. 1-47). Similarly, the pollinations within clusters were limited and highest in cluster A, 15.5%. A limited number of trees participated in the pollination process: 22 trees in 2007 and 36 trees in 2008. In Fig. 1-48 the relationship between pollination distance and the fraction of successful matings is illustrated for data from 2008 ($R^2 = 0.42$), in which the longest distance for successful mating was recorded. The tree(s) at a distance of 4.5 km were exceptionally successful. If this value is excluded the relationship became a little stronger, $R^2 = 0.58$. Mean pollination distances were long, 2.86 and 2.93 km in 2007 and 2008. Noteworthy is the success of remote pollen: up to 8 km. Selfing was low and estimated at 0.6 in 2007 and 0.8 in 2008. Species hybridization did not occur to any great extent.
This investigation clearly shows that even under fragmented distribution, effective pollination of *U. minor* may take place, reducing the risk for genetic drift.

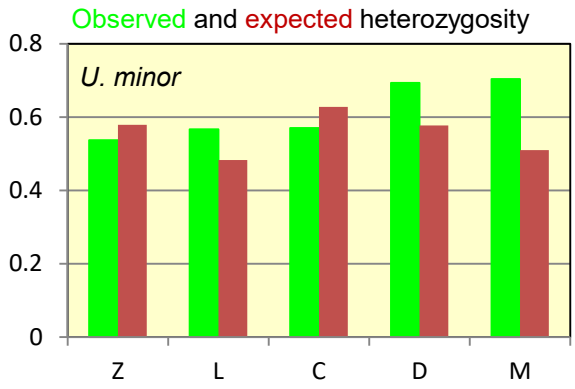

Figure 1-49. The observed (green) and expected (brown) heterozygosities in 5 Dutch populations of U. minor. *Ten microsatellites were used in this study. Buiteveld et al. 2015.*

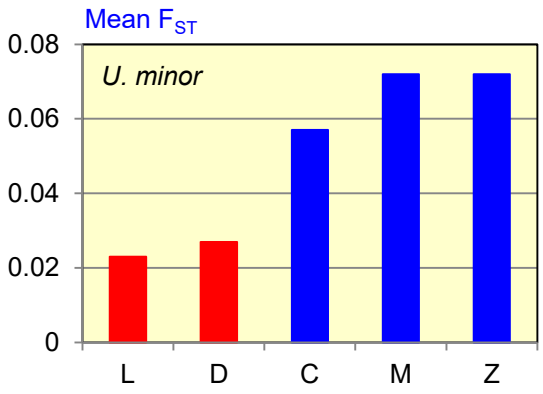

Figure 1-50. The mean F_{ST} for five Dutch populations studied with ten microsatellite loci. Red columns denote F_{ST} for the 2 populations with largest distances to the other 3 populations. Buiteveld et al. 2016.

The genetic diversity of six widely distributed Dutch *U. minor* populations was studied by Buiteveld et al. (2016). The number of trees varied in the range 17–41. In addition 2 reference collections from Belgium and France were included. Ten polymorphic microsatellites were used in this study. Occurrence of clones was a first task, which was done by calculating the probability that two or more individuals with the same multilocus genotype were derived via asexual reproduction given the allele frequencies in the population under study. After exclusion of one of the populations, which consisted of 12 species hybrids, clonal diversity and genetic structure were estimated.
The number of distinct multilocus genotypes among the 159 genotyped trees was estimated at 66, of which 61 occurred in one population only. Three reasons for occurrence of clones in the Dutch populations were given:

Root suckering and sprouting

Layering propagation and transplantation of root suckers

Cultivar plantation

Root suckering was thought to be the most important of these factors. The five populations showed a low to moderate level of genetic diversity. The genotypic richness varied considerably among the populations, 0.06–0.96. The Simpson index, which is another estimate of clonal diversity, was high in all populations except for the one with species hybrids. The allelic richness corrected for population size did not differ much, 2.4–2.9. The percentage of private alleles was fairly high, 24%. There was some discrepancy between expected and observed heterozygosity in the five populations (Fig. 1-49). Especially, the M population had much larger observed heterozygosity than was expected. In agreement with this, population M also had a strongly significant fixation index, –0.367. Populations C and Z also had significant fixation indices but in these cases there was an excess of homozygotes.
The mean F_{ST}s for five of the populations are illustrated in Fig. 1-50. There was no relationship between genetic and geographic distance with lowest mean F_{ST}s for the two populations with largest distances to the other three populations. It was pointed out that human impact has had a great impact on the genetic structure of *U. minor* in the Netherlands.
The Belgian and French populations showed great similarities with the Dutch populations.

The objective of a Croatian study was to estimate the genetic diversity and differentiation of *U. minor* in Croatia (Zebec et al. 2016). They studied two populations from the interior part of Croatia and three populations closer to the Mediterranean Sea. In all, 96 trees were sampled and five microsatellites were analyzed. Standard population genetics parameters were estimated, BOTTLENECK 1.2.02 being used to identify any bottleneck in any of the populations.
Fig. 1-51 reveals that the observed heterozygosity was above 0.50 in all five populations, which means that the diversity is moderate in these Croatian populations. It should be noted that the range of the estimates for individual loci was large. For observed heterozygosity it

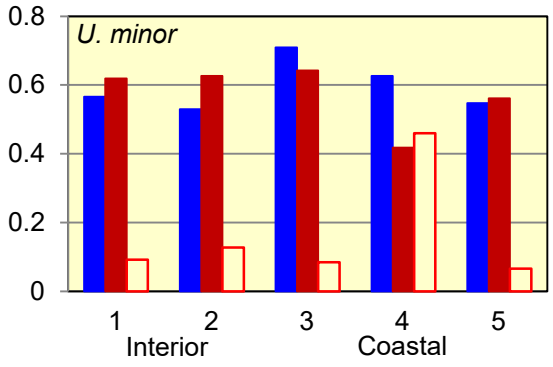

Figure 1-51. Observed (blue) and expected (brown) heterozygosity as well as inbreeding coefficient (red) in five Croatian U. minor *populations. Empty red columns = negative F_{IS} estimates. Five microsatellite loci were used. Zebec et al. 2016.*

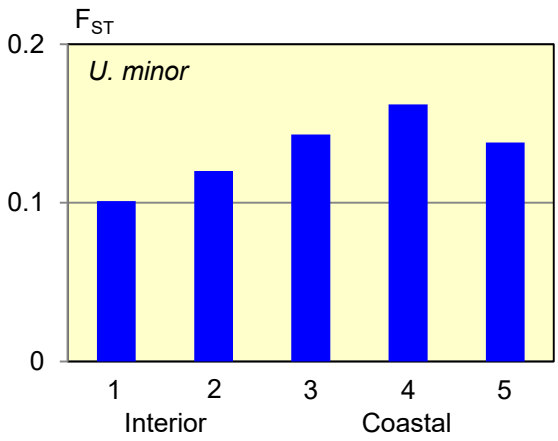

Fig 1-52. Mean pairwise F_{ST} estimates among 2 interior and 3 coastal Croatian U. minor *populations. Zebec et al. 2016.*

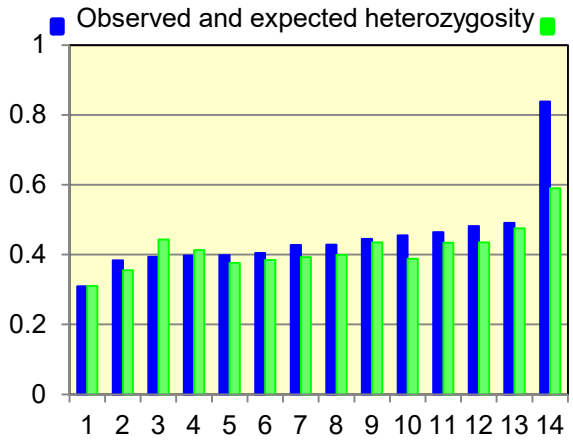

Figure MartinDP17-1. Observed and expected heterozygosity in 14 relict and fragmented U. glabra *populations from Central Spain analyzed by 11 microsatellite loci. All populations had 10 or more trees. Martin del Puerto et al. 2017.*

was 0.47–0.78, and for polymorphic information content it was still wider, 0.47–0.86. In the "coastal" population No 4 the difference between observed and expected heterozygosity for individual loci was large, 0.20. The inbreeding coefficient varied considerably. One extreme estimate was noted, –0.46. For population 3 the range was extremely large, –0.596–+0.211. It would have been useful to have a discussion of such large differences among loci.

A bottleneck was found only in population No 3. This population is growing in a former pure elm forest, in which extensive land conversion to farm land has taken place with a great loss of elm trees.

The differentiation among the populations was large considering the limited geographic area included in this study (Fig. 1-52). Except for the difference between populations 1 and 2, all pairwise F_{ST}s were significant. There was no complete agreement between the F_{ST} estimates and principle correspondence analysis, in which population 3 showed the clearest differentiation from the other four populations.

It was concluded that a satisfactory genetic diversity exists in the *U. minor* populations studied despite high incidence of DED in Croatia.

The main objective of a study by Martin del Puerto et al. (2017) of relict and fragmented populations of *U. glabra* was to estimate their genetic diversity. A total of 427 trees from 22 populations in Central Spain were genotyped by 11 microsatellite loci. The GenAlEx version 6.5 was used for calculation of standard population genetics parameters. The relationships between the 19 populations containing six or more trees were calculated by NTSYS-pc version 2.2 to construct UPGMA diagrams. Two methods were used for detection of bottlenecks, T2 statistics in BOTTLENECK version 1.2 and the M-ratio calculated by Arlequin software version 3.5. The existence of bottlenecks was estimated in 15 populations with 10 or more trees. STRUCTURE software version 2.3.4 was used to identify relationships of *U. glabra* populations with *U. minor* trees as well as groups of *U. glabra* populations.

The percentage of heterozygous loci varied in the range 46–100%. Only one tree was growing in the 46% population. Neither observed nor expected heterozygosities showed any strong relationships with the investigated number of analyzed trees per population, R^2 = 0.11 and 0.34, respectively. Approximately 7% of the 360 observed genotypes were common to two or more individuals. In Fig, 1-53 we have illustrated observed and expected heterozygosities for populations with 10 or more trees. With one exception, population 14, there was a fairly strong relationship between and observed and expected heterozygosities. All 46 trees in this population were analyzed. Private alleles were found in seven of the 22 populations, 3 of them occurring at high frequency.

The STRUCTURE analysis resulted in two main groups of populations with 17 and 2 populations, respectively. The relatively high number of putative species hybrids in the latter two populations might be the explanation for their separation from the other 17 populations. The variation among populations was strongly significant and amounted to 24% of the total variation, and 76% was attributed to variation within populations. Such a high variation among populations (F_{ST} = 0.239) for a wind-pollinated species from a relatively limited geographic area must be attributed to the small number of trees in most populations leading to an impact of genetic drift in several populations.

The effective population size was estimated for 10 populations with more than 10 trees per population. Fig. 1-54 reveals that one population had an extremely high N_e (668 and 16.6) for the two ways of estimating this size. Only 2 or 3 populations had N_e above 20 independently of the calculation method.

M-ratios below 0.68 indicate bottlenecks. Since these ratios varied in the range 0.21–0.37, ancestral and extended

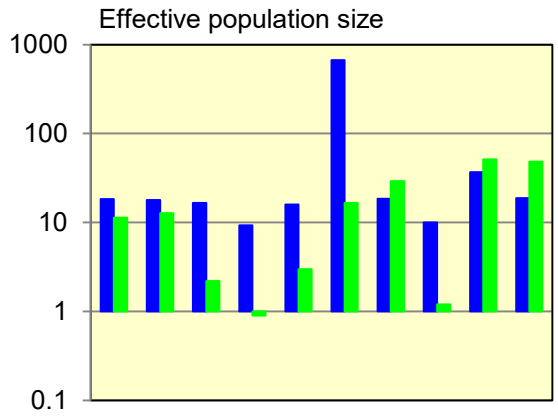

Figure 1-54. Effective population size was estimated in two ways: approximate Bayesian computation (ONeSAMP v. 1.1 blue) and linkage–disequilibrium information (LDNe v. 1.31 green) for the 10 U. glabra *populations with at least 20 trees. Martin del Puerto et al. 2017.*

bottlenecks have occurred in the past in all populations analyzed. The T2 statistics showed a significant excess of heterozygotes in four populations, which suggests that recent bottlenecks had occurred in these populations. It cannot be excluded that sampling might have included ramets of the same clone, which could lead to exaggeration of occurrence of bottlenecks. It should also be noted that forestry operations in many populations have had a great impact on the present genetic set-up.

Data from nine microsatellite loci, which were amplified in *U. glabra* and in *U. minor,* were used to analyze hybrids between these two species. Nineteen alleles detected in *U. minor* were common to *U. glabra*. Two of them occurred at high frequencies. Moreover, all *U. glabra* samples shared at least two alleles with the *U. minor* trees. In all, 55 of the 427 sampled *U. glabra* trees turned out to be interspecific hybrids. There are evidently no reproductive barriers between these species, which means that interspecific hybridization may easily occur in mixed populations of these two species.

Nine microsatellite loci were analyzed by Chudzińska et al. (2018 English abstract) to estimate the genetic differentiation of 17 Polish *U. glabra* populations. The latitudinal range was 40–55°N and the longitudinal range being 15–23°E. The number of trees per population varied between 10 and 51.

Fig. 1-55 shows that the mean observed heterozygosity was close to 0.60. The mean expected heterozygosity was almost identical wih the observed. The mean inbreeding coefficient, F_{IS}, was 0.031. Six populations had negative estimates, two of them very close to zero: populations 4 and 13. There was no relationship between tree number per population and F_{IS}. A moderately large F_{ST}, 0.089, was noted, which was attributed to decline of *U. glabra* in Poland owing to Dutch elm disease.

The objective of a study of 18 Bavarian *U. laevis* populations was to estimate genetic diversity and differentiation for guiding genetic conservation of *U. laevis* in Bavaria (Kavaliauskas et al. 2022). In addition to the 824 *U. laevis* trees 24 trees from each of *U. glabra* and *U. minor* were included in this investigation. Twelve polymorphic microsatellites were used in this investigation. Traditional population genetics parameters were estimated. Population differentiation was estimated by AMOVA (GenAlEx 6.5 (Peakall and Smouse 2012), pairwise differentiation according to Nei (1972), genetic distances between all pairs of populations according to Jost (2008), and Bayesian clustering using STRUCTURE 2.3.3.

Species differentiation. An analysis by STRUCTURE 2.3.3 of the three elm species resulted in two clear groups, *U. laevis* and *U. glabra* + *U. minor*. Differentiation according to Nei (1972) and the discriminant analysis of principle components corroborated the 2-group differentiation. The AMOVA showed that 20% of the variation was attributed to among population differentiation.

U. laevis populations. Below the ranges for some genetic parameters are listed:

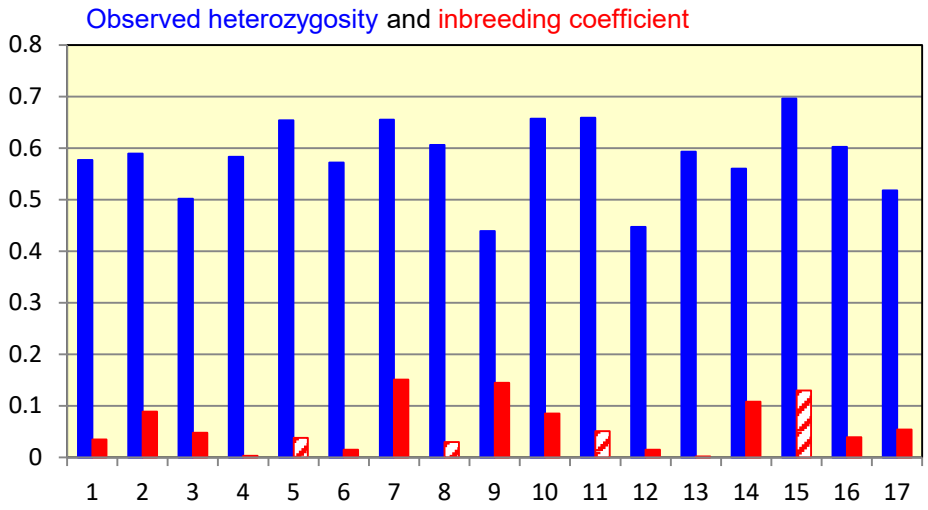

Figure 1-55. Observed heterozygosity (blue) and inbreeding coefficient (red) in 17 Polish U. glabra *populations analyzed with 9 microsatellite loci. Striped red columns refer to negative estimates of F_{IS}. Chudzińska et al. 2018.*

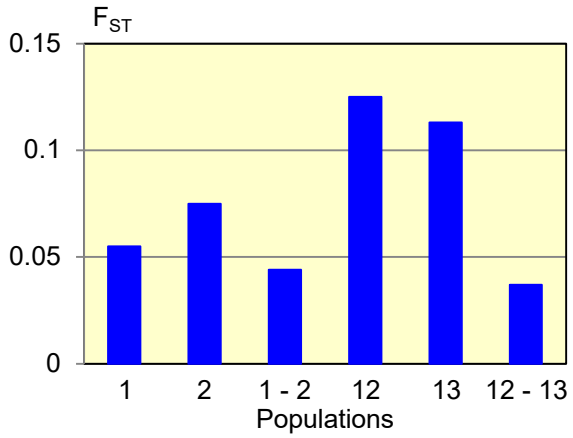

Figure 1-56. Mean F_{ST}s according to Nei (1972) for populations 1, 2, 12, and 13 as well as the F_{ST}s for comparisons between populations 1 and 2 and between 12 and 13. The populations withtin each pair are growing adjacent to each other. Kavaliauskas et al. 2022.

Number of alleles	2.00–3.83
Number of effective alleles	1.60–2.11
Allelic richness	1.94–3.54
Expected heterozygosity	0.30–0.41
Observed heterozygosity	0.29–0.46
Inbreeding coefficient	-0.25–0.11

As seen from the compilation above, the genetic diversity was not substantial. The scattered occurrence of the populations might have contributed to the relatively low level of diversity. However, the negative inbreeding coefficients in 15 of the populations suggest that genetic drift was not significant in most of the populations.

The number of private alleles in the entire material was 15. Two populations had 3 and 4 private alleles, another 2 populations had 2 private alleles. The remaining 4 occurred in each of 4 populations. Ten populations did not have

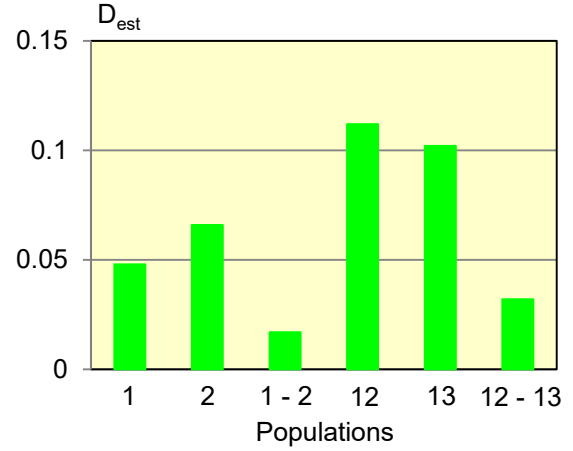

Figure 1-57. Mean D_{est} according to Jost (2008) for populations 1, 2, 12, and 13 as well as D_{est}s for comparisons between populations 1 and 2 and between 12 and 13. The populations withtin each pair are growing adjacent to each other. Kavaliauskas et al. 2022.

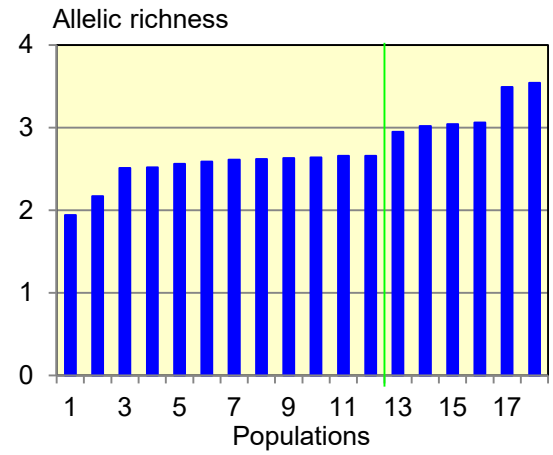

Figure 1-58. Allelic richness in 18 Bavarian U. laevis *populations based on 12 polymophic microsatellite loci. Columns to the right of the green line have estimates higher than the mean, 2.72. Kavaliauskas et al. 2022.*

any private alleles. The observed heterozygosity was higher than the expected heterozygosity in 15 populations.

The result based on the AMOVA showed that the differentiation among populations was 11% and the variation among-trees within populations was 89%. An extremely large F_{ST} estimate between two of the populations was noted, 0.20, while the mean F_{ST} was 0.11. The STRUCTURE analysis resulted in two main groups, one for 14 Danube basin populations and the other for Main river basin with 4 populations. In the former, 2 additional subgroups were identified. As regards the Danube cluster, all populations contained trees belonging to different subclusters. Further support for the isolation of populations owing to their scattered occurrence was obtained in the gene flow estimates. Two adjacent populations in the Danube basin (No 12 and 13) had no gene flow with the other 16 populations. These 2 populations had the highest mean F_{ST}s of all populations (Fig 1-56). These 2 populations are located at the margin of the *U. laevis* distribution in Bavaria, which most likely explains their differentiation from the other populations. Two adjacent populations from Main basin (No 1 and 2) with peripheral location showed a different F_{ST} pattern, with their mean F_{ST}s being higher than their mutual F_{ST}, but the difference was not of the same magnitude as for populations 12 and 13 (Fig 1-56). The estimation of D_{est} according to Jost (1978) resulted in a similar pattern (Fig 1-57). The data in these two figures were correlated, R^2 = 0.82. In conclusion, there was a good agreement between the methods for estimation of population differentiation. The high estimates of among-population differentiation for a geographically limited area must be attributed to the scattered distribution of the species.

The genetic conservation was discussed in some detail and it was suggested that *ex situ* genetic conservation via various types of genetic plantations should be implemented. Selection of one population from each of the four clusters identified would probably be a sensible step for genetic conservation. It was strongly stressed that allelic

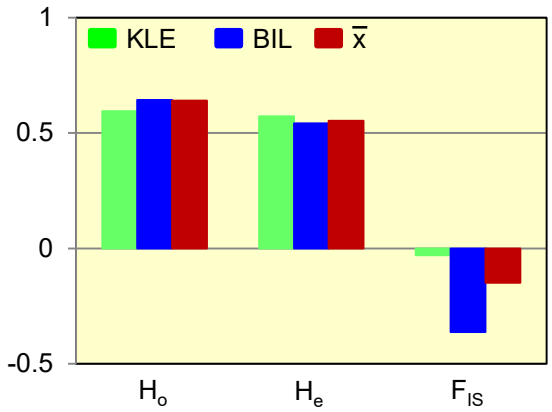

Figure 1-59. Observed (H_o) and expected (H_e) heterozygosity in 2 Polish U. laevis *populations (KLE and BIL) with largest and snallest heterozygosity excess (F_{IS}). This study comprised 41 populations covering the distribution of* U. laevis *in Poland. Six microsatellite loci were used. Litcowiec et al. 2022.*

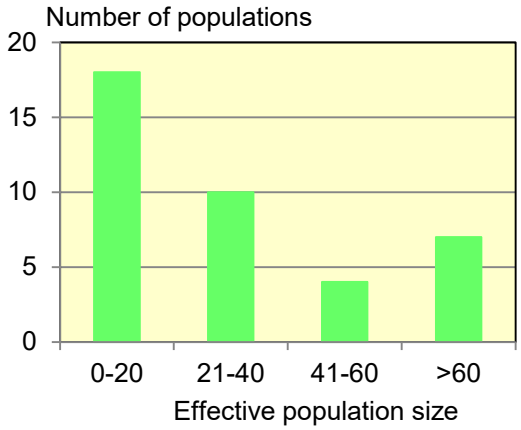

Figure 1-60. The number of populations in different classes of effective population size in a study of 39 Polish U. laevis *populations. Six microsatellite loci were studied. Litkowiec et al. 2022. Litcowiec et al. 2022.*

richness should be considered in the selection of genetic resource populations (Fig 1-58).
Three steps in the selection of seed tree stands for collection of seeds were presented:

A minimum number of 30 seed trees

The seed trees should be well distributed in the seed tree stand

High genetic diversity is required, which may require additional laboratory work

It was noted that 17 of the studied populations fulfilled the requirements for being classified as seed tree stands.
There was no mention of differentiation in adaptive traits for guidance of selection of genetic resource populations.

Litkowiec et al. (2022) studied 41 *U. laevis* populations using six microsatellite loci. The populations covered the distribution of the species in Poland. Sampling comprised 11–50 trees per population. Traditional genetic parameters in population genetic studies were estimated in this study. Pairwise F_{ST}s as well as grand F_{ST} were estimated. Population bottlenecks were estimated by the ratio of number of microsatellite loci (n) over overall range in allele size (r) and by the Wilcoxon test for heterozygosity excess (Cornuet and Luikart 1996).
Only 59 alleles were found at the six loci included and 14 of them were private alleles. One population contained four private alleles while most populations did not contain any private alleles. The mean number of alleles was 4.2 with a range of 3.7–5.7. The corresponding estimates for allelic richness were 4.0 and 3.0–3.9. In Fig 1-59 we have illustrated observed and expected heterozygosities of the two most extreme populations with respect to fixation index. The large difference between expected and observed heterozygosities is reflected in a large excess of heterozygotes in the BIL population, while observed and expected heterzygosities in the KLE population did not differ much. The mean heterozygosities were lower than for other wind-pollinated tree species. All populations had low estimates of F_{IS}. There was no relationship between the studied population size and F_{IS} ($R^2 = 0.006$). Most of the populations had low estimates of effective population size (Fig 1-60) and only eight populations had an estimate above 50, and the mean for all populations was 16.8. The low effective population sizes would lead to an impact of genetic drift and thereby a high F_{ST} estimate. The individual population estimates varied dramatically, 0.0033–0.277. Unfortunately there was no information on the population sizes of these two populations. One would expect more genetic drift in populations with low number of trees leading to random fixation of alleles. The data obtained suggest that there was a relatively large gene flow among these Polish populations. There was no indication of a relationship between geographic and genetic distances.
The bottleneck test suggested that there was a genetic decline during post-glaciation recolonization. The loss of genetic diversity owing to DED was discussed and it was suggested that the loss of diversity had not been substantial.

Table 1-6. The absence of certain triterpenes in elm species or elm species hybrids. Martin-Benito et al. 2005.

Triterpene	*Taxon*
Alnulin	*U. minor, U. pumila, U. minor x U. glabra, U. minor x U. pumila*
Lupenon	*U.pumila*
Stigmastenon	*U. glabra, U. minor x U. pumila*
Moretenol	*U. pumila*
Ilexol	*U. minor, U. minor x U. glabra*
Epifriedelinol	*U. minor*
Methyl betulinate	*U.laevis*

The triterpene composition in the 3 European elm species, *U. pumila* and interspecific hybrids was studied by Martin-Benito et al. (2005). Principle component analyses were used to separate the different taxa.
Certain of the triterpenes were missing in some taxa (Table 1-6) and might be used for taxonomic purposes as complements to morphological identification. The total triterpene content was 2 to 3 times higher in *U. laevis* and *U. glabra* than in *U. pumila*. It was speculated that this made them less attractive to the *Scolytus* beetles. Moreover, the same effect might be attributed to alnulin, which occurred only in *U. glabra* and *U. laevis*.

1.4 Development of markers

Whiteley et al. (2003a) extracted DNA from 2 *U. laevis* trees from the Swedish latitude 56.68°N population for development of microsatellite markers. Primers could be designed for 19 microsatellite sequences, of which six showed clear polymorphic amplification. These six loci were cross-amplified to four other species: *U. Americana, U. glabra, U. minor,* and *U. pumila*. Polymorphism was noted for five of the six loci but never for all four elm species for any of the five loci.

Based on an AFLP study of five elms from each of six populations, five microsatellites were developed for *U. minor* by Collada et al. (2004). The number of alleles per locus varied in the range 3–8. Cross-amplification with *U. glabra* and *U. laevis* was successful for three of the microsatellites. In the case of *U. laevis* only 2 were polymorphic.

Zalapa et al. (2008b) developed 11 microsatellite loci for the American elm, *U. rubra*, and tested their cross-species amplification with the Siberian elm, *U. pumila*. In both species eight primer pairs were amplified in both species with 2–8 polymorphic loci. Species-specific alleles were found for all loci. The expected heterozygosity varied in the range 0.19–0.77.

The complete chloroplast genome of the Chinese rare and endangered *U. chenmoui* was studied by Zhang et al. (2019). The genome contains 121 genes, including 79 protein-coding genes, 30 tRNA genes, and 8 rRNA genes. Based on the cpDNA this species was separated from 5 other Asian elm species.

The complete chloroplast genome of *Ulmus americana* was presented by Ebrahimi et al. (2021). They also compared this genome with other elm species. The genome contains 85 protein-coding genes, 34 tRNA genes, and 8 rRNA genes. The *petB, petD, psbL, trnK,* and *rps16* genes in American elm do not occur in Asian elms.

Liu et al. (2022) presented the complete genome structure of cpDNA from four elm species:
U. castaneifolia
U, lamellosa
U. parvifolia
U.pumila
All four species had 86 protein coding genes, 37 tRNA genes, and 8 rRNA genes. No among-population differences were studied. The relatedness of these four species and 11 other elm species were presented. These cpDNA markers are valuable for tracing the phylogeny of elms.

1.5 Summary

1.5.1 Species differences

Elm subgenera contained mainly elm species from one continent. The American *U. rubra* and the European *U. laevis* constitute exceptions to this. The Himalayan *U. villosa* is the only elm species in its own subgenus. In several investsigations it was shown that *U. laevis* clearly deviates from the two other European elm species. Unique isozyme alleles for the 2 species U. *minor* and *U. pumila* were found, which should enable identification of interspecific hybrids between these two species. Contrary to this, one investigation claimed that absence of species-specific alleles contributed to difficulties in proper taxonomic identity.
Hybridization occurs frequently in contact zones with two or more species, an exception being tetraploid *U. amercana*.
Backcrosses to *U. pumila* of the hybrid *U. minor x U. pumila* was much more frequent than the reciprocal cross.

Two German localities 6 km apart both contained *U. laevis* and *U. minor.* There was a tendency to more similarty in allele frequencies across species than between the same species at the 2 localities.
A fairly good separation of *U. glabra, U. minor, U. laevis,* and *U. hollandica* based on leaf morphology traits was noted in a study of 58 elms from 11 localities in Lithuania.
Analysis of 31 leaf morphology traits and 8 microsatellite loci in 26 populations from a wide distribution in Lithuania was carried out to study diversity and differentiation among elm populations. Six taxa were included in the sampled material, *U. glabra, U. laevis, U. minor, U. minor* ssp. *minor, U. glabra x U. minor,* and *U. glabra x U. minor* ssp. *minor*. *U. laevis* was the only species with excess of heterozygotes and this species differed most from the other species, which in turn were clustered in three groups 1. *U. glabra, 2. U. minor x U. glabra,* and *3. U. minor, U. minor* ssp. *minor, U. glabra x U. minor* ssp. *minor.*

1.5.2 Population differences

!.5.2.1 Markers

Moderate to large differentiation was noted for *U. laevis, U. lamellosa, U. minor, U. parviflia and U. pumila.*
The diversities in herbarium specimens collected before the arrival of DED and in now existing populations of *U. rubra,* USA, were rather similar.
Limited genetic differentiation was observed between *U. pumila* from China and naturalized *U. pumila* in Wisconsin, USA.
Almost 100% outbreeding and limited seed dispersal were noted in two Danish *U. laevis* populations with 8 and 91 trees, respectively. The observed and expected heterozygosity was higher in the 91-tree population. Up to a distance of 70 meters trees were more closely related than beyond this distance.
Almost 100% outbreeding and limited seed dispersal were noted in one Danish *U. glabra* population with 181 trees and in seven widely scattered trees. There were limited differences between the two populations as regards several genetic parameters. The pollination distances were different for obvious reasons.
Unexpectedly large differentiation was observed between two *U. pumila* populations growing on each side (south and north) of the Great Wall in China. The predominating east – west wind direction might explain the limited north – south exchange of pollen and samaras

1.5.2.2 Metric traits

The differences in 17 morphological traits in *P. rubra, P. pumila* and their hybrid were studied. Almost all traits were significantly different between the two pure species while 12 and 16 differed between the hybrid and *U. rubra* and *U. pumila*, respectively.
Leaf length/width ratio, acute lateral leaf lobes %, and short tapering leaves % in 4 maritime Norwegian *U. glabra* populations showed a latitudinal cline while an interior population deviated from this pattern. Sixteen populations were monomorphic with respect to chloroplast haplotypes, 14 of them with the same haplotype, which is common in Western Europe.
Leaf fall in 5 *U. laevis* populations of wide origin varied significantly with high Q_{ST} in a nursery study while bud flushing and growth varied less. Frost damage took an intermediate position. Significant differences among populations for several fitness-promoting traits were noted. High heritability estimates were found in individual populations for growth and phenology traits as well as for frost damage.
Bud flushing and leaf fall in 46 *U. pumila* populations were followed over 20 years to estimate the effect of global warming. It was estimated that flushing took place 4 days earlier per decade during the period 1986–2005. The corresponding estimate for change of growth cessation was 2.2 days' postponement.
The effect of water stress on two types of trichomes was studied. *U. pumila* was found to produce more bulbous trichomes than *U. americana* and *U. parvifolia* following water stress. Large numbers of trichomes might be related to the low susceptibility to Dutch elm disease (DED) of *U. pumila*. Significant differences were noted.
Support for the hypothesis that stomatal depth is related to precipitation at *U. pumila* population origins was noted in one investigation with 4 populations.

1.5.3 Small populations

Strong differentiation among small Finnish *U. laevis* populations was observed and attributed to genetic drift, which supports the hypothesis that marginal populations have large among-population differentiation and less within-population diversity. In spite of the small size of the populations, many inbreeding coefficients were negative.
Relict and fragmented *U. glabra* populations in Spain contained moderate levels of diversity with high global F_{ST} estimates, 0.24. The effective population size was mostly below 20. No crossing barrier seems to exist between *U. glabra* and *U. minor*.

Two of 11 relict *U. laevis* populations from Flanders deviated strongly from the 9 other populations with respect to leaf margin toothing and split secondary veins. The observed strong population differentiation was attributed to strong impact of genetic drift.
Inbreeding seemed to be absent in two small Spanish *U. laevis* populations. F_{ST} between the two populations was relatively high, 0.17. Habitat transformation is the greatest threat to sustained genetic diversity. Seed production varied strongly among years and largest dispersal of filled seeds occurred within 30 meters from the trees.
One Italian investigation showed that even under fragmented distribution, effective pollination may take place in *U. minor,* reducing the risk for genetic drift.
Great impact of human activities on genetic diversity was stated in several papers.

1.5.4 Pests

With a focus on horticulture application the susceptibility of species and cultivars to elm leaf beetle, and spring and autumn cankerworms, were studied in certain projects. Bioassays as no-choice and 2 and 3-choice experiments were carried out.
The susceptibility of species and cultivars to elm leaf beetle, *Xanthogaleruca luteola*, was studied in a series of papers. Two East Asian species, *U. elongata* and *U. propinqua*, did not have any eggs laid and no ovipositing. The Siberian elm (*U. pumila*) was found to be most attractive to the beetles based on no-choice and multiple-choice experiments.
On average the Asian taxa were less damaged by spring and autumn canker worms than American and European taxa but with significant differences within continents.
Two-choice feeding experiments showed that *U. procera* was preferred to *U. glabra* by *Scolytus scolytus* and *S. multistriatus,* and that *U. pumila* was preferred to *U. laevis*.

2. Progeny testing

2.1 Dutch elm disease, DED

A Spanish study aimed at estimating variance components for DED resistance and growth factors was published by Solla et al. (2014). Ten parents in a partial diallele mating design were studied after inoculation with *Ophiostoma novo-ulmi*. Eight of the parents were *Ulmus pumila* while the other two were interspecific *U. minor x U. pumila* hybrids. The design was unbalanced owing to absence of seeds from three *U. minor* clones, so that these clones could only be used as males. Leaf wilting was recorded at 60 days after inoculation.

The mean leaf wilting percentages of the progenies from individual parents are illustrated in Fig. 2-1 together with results from offspring in open-pollinated families. The parent with the lowest mean percentage, 12.4%, was represented in only two full-sib families and is therefore less precise than the other estimates. The other parents had a fairly narrow range of wilting percentage. It is further seen that the agreement between the percentages following open pollination and the partial diallel matings is poor. This suggests that open pollination testing is less reliable in this material. The mean values for the two full-sib families with interspecific hybrid parents do not deviate in a significant way from the rest of the mean values. The narrow-sense heritability for leaf wilting was estimated at 0.14 (Fig. 2-2). It is possible that the heritability would have been somewhat higher if the global mean for the entire material had been closer to 50% than the observed wilting of 37%. Since family selection will probably be used in applied breeding, it was useful to estimate the family heritability, which turned out to be high. Height increment had the highest h^2, 0.21, which does not deviate in a significant way from estimates in other tree species.

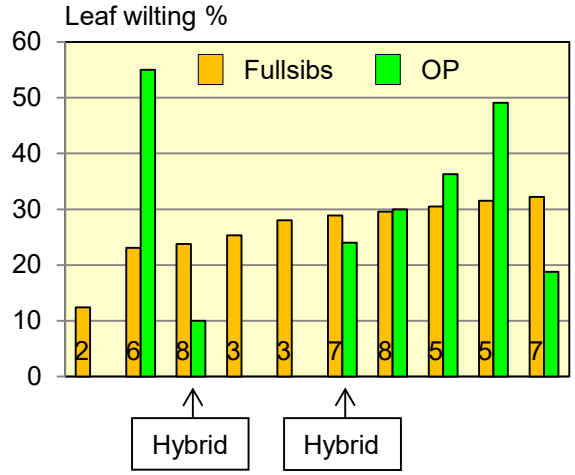

Figure 2-1. Percent leaf wilting of fullsibs (orange) and open-pollinated families (green) of Ulmus minor *and interspecific* Ulmus minor x U. pumila *females. Wilting was recorded 60 days after inoculation with* Ophiostoma novo-ulmi. *The number of fullsib families for the individual parents is presented. No open-pollinated progenies were obtained from 3 females. Solla et al. 2015.*

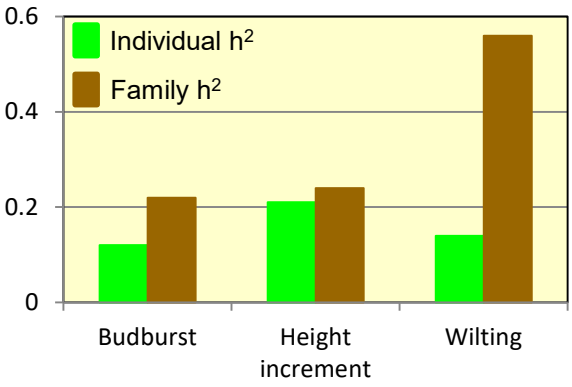

Figure 2-2. Narrow-sense heritabilities and family heritabilities (repeatabilities) for bud flushing, height increment, and leaf wilting percentage in an investigation with ten parents crossed according to a partial diallele mating design. Eight Ulmus minor *clones and 2* U. minor x U. pumila *clones were included in this mating design. Solla et al 2015.*

The relationship between leaf wilting and height growth is plotted in Fig. 2-3. As seen from this figure, there is no relationship between these traits. This means that selection for DED resistance will not lead to any strong decrease in height growth. Fig. 2-3 also shows that the two *U. minor x U. pumila* parents were ranked as second and third parent with respect to height increment.

The observation that the non-additive variance for bud flushing and height increment was three times larger than the additive variance is worth mentioning. At least phenology traits usually show strong additive variance. The large presence of non-additive variance also explains the fairly low heritability for budburst.

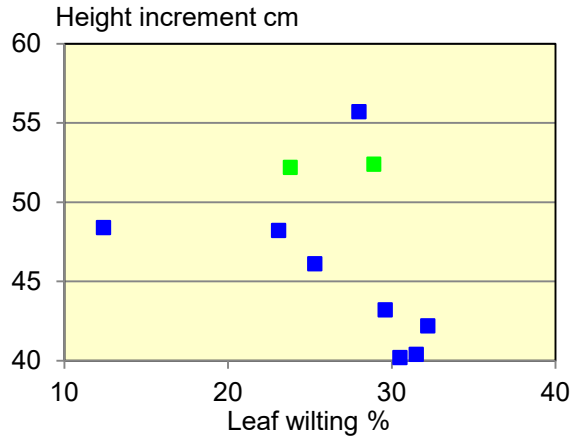

Figure Solla 2-3. The relationship between mean increment and leaf wilting percentage for progenies of 10 parents. The mating design was partial diallele with 8 Ulmus minor *clones (blue) and 2* U. minor x U. pumila *clones (green). Solla et al. 2015.*

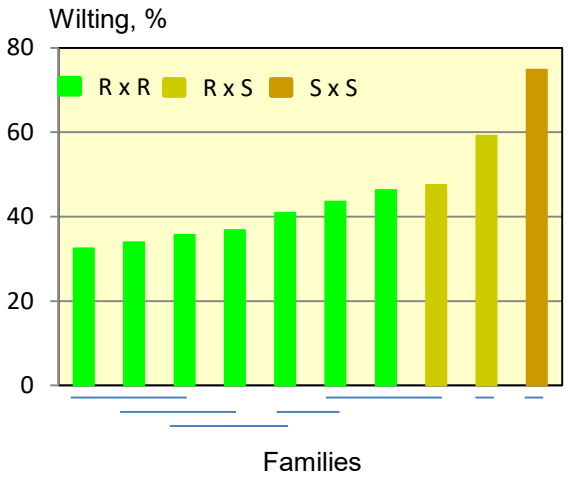

Figure 2-4. Percentage wilting (least square means) after Ophiostoma novo-ulmi *inoculation of 3 types of family, resistant x resistant, resistant x susceptible, and susceptible x suseptible* U. minor *parents. Non-significant differences are indicated. Venturas et al. 2014a.*

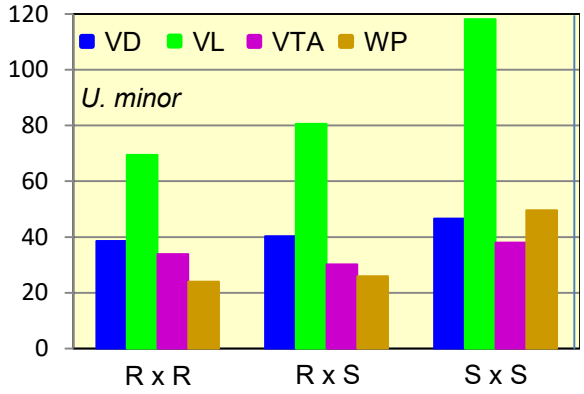

Figure 2-5. Performance of three types of families, resistant x resistant, resistant x susceptible, and susceptible x susceptible in an experiment with Ophiostoma novo-ulmi *inoculations of Spanish* U. minor *seedlings at age 3. VD = Vessel diameter μm, VL = vessel length, mm, VTA = vessel transectional area %, WP = wilting percentage. Venturas et al. 2014a.*

.

The objectives of a study by Venturas et al. (2014a) were to estimate the heritability for resistance to DED in *U. minor* and to compare the xylem anatomy in resistant and susceptible elms. They inoculated 7 resistant x resistant, 2 resistant x susceptible, and one susceptible x susceptible Spanish *U. minor* families with *Ohiostoma novo-ulmi*. Four blocks with groups of 3 potted seedlings from each family were randomly located in each block. Inoculations took place three years later when the mean tree height was 2.8 meters. In all, 19 anatomical and physiological traits of the xylem were determined and 8 of them showed significant differences between the type of cross. The heritability for DED resistance was high and estimated at 0.54. It should be noted that this estimate is based on a non-random selection of parents with extremely differing DED-values. This might cause an exaggerated heritability estimate. However, with such a high estimate as 0.54 an exaggeration of 30% means that the heritability still is high. The least square means for the individual crosses are illustrated in Fig 2-4. This figure shows that the two families with the highest susceptibility to DED were significantly different from all other families while the most resistant R x S was not significantly different from the two least resistant R x R families. As expected wilting was highest in the S x S family.

Significant differences between the three groups of family were noted for the following traits:

- Vessel length
- Vessel diameter
- Vessel transectional area
- Relative theoretical hydraulic conductance
- Vessel frequency
- Resistance to implosion
- Percentage of grouped vessels

The results as regards a few of the anatomical traits showing significant differences among the three groups of trees are illustrated in Fig 2-5. For vessel diameter the hybrids took a position between the 2 parental families. Since the two water potential estimates did not differ among the groups it was argued that xylem resistance to water stress cavitation was not related to resistance to DED in *U. minor*. In contrast, vessel size showed greatest significance for DED susceptibility. Trees with longer and wider vessels were associated with DED susceptibility. It was speculated that this would lead to a more rapid upward movement of the fungus in the xylem. Conductivity is another trait that can influence susceptibility: high conductivity was associated with high susceptibility.

The objective of an investigation by Martin et al. (2005a) was to study if biomarkers could be identified by use of Fourier transform-infrared (FT-IR) spectroscopy. Seeds were collected from 40 *U. minor* trees growing at 31 localities in Spain. At age 4, progenies from these trees were inoculated with a spore suspension of *Ophiostoma novo-ulmi* with a cell density of 104 per ml. This density was selected to get a broad range of tolerance/susceptibility and to avoid a high mortality. A wound at 20–25 cm above ground was made for the inoculations. Distilled water was used as a control. Wilting was recorded 60 days after inoculation. Histological analyses were carried out to ascertain that infection by the fungus had taken place. Fourier transform-infrared (FT-IR) spectroscopy was carried out on material two days before inoculation and 40 days after inoculation. FT-IR spectroscopy is viewed as a fingerprint of the metabolic composition of a tissue. Samples from trees harvested before inoculation did not show any considerable formation of lignin or suberin. The same was true for control material harvested after inoculation. In contrast to this, material harvested after inoculation showed strong tylose formation. In the resistant seedlings the radial growth increment range was

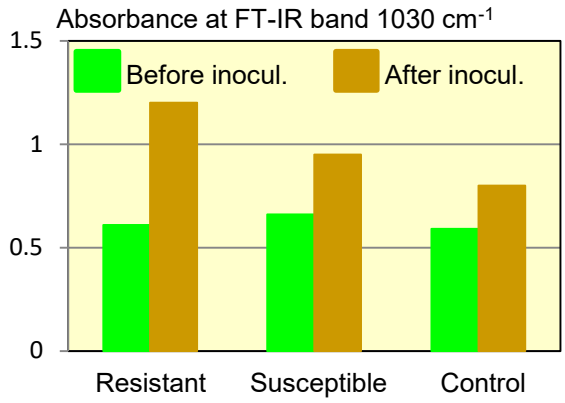

Figure 2-6. The absorbance at FT-IR band 1,030 cm^{-1} before and after inoculation with Ophiostoma novo-ulmi *of resistant, susceptible, and control progenies of* U. minor *from Spain. Control trees were inoculated with water. Martin et al. 2005a.*

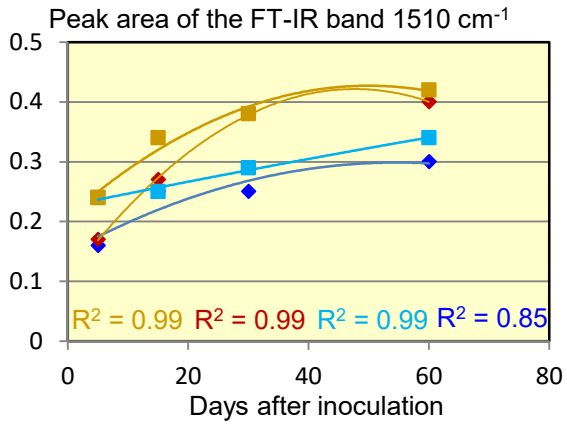

Figure 2-7. Peak area of lignin-associated 1510cm^{-1} FT-IR band of U. minor (*diamonds*) U. minor x U. pumila *(squares) inoculated with* Ophiostoma novo-ulmi. *Brown = inoculated, blue = control,. Martin et al. 2007.*

300–1,000 µm while the corresponding range for susceptible seedlings was 50–400 µm.

The absorbance of the FT-IR band spectrum between 800 and 1,800 cm^{-1} for all genetic entries (resistant, susceptible, and control) collected before inoculation was significantly lower than for the material collected 40 days after inoculation. The highest absorbance was noted for band 1,030 cm^{-1}. Fig. 2-6 shows that there is a considerable difference in absorbance at this wavelength between the resistant and susceptible progenies after inoculation while the differences before inoculation were minor. The 1,510 cm^{-1} band was regarded as a key band for diagnostic purposes of DED effects on xylem tissues. Therefore, peaks at other wavelengths were related to peaks of this band (Table 2-1). The chemical binding of the bands in the numerator in these ratios are indicated. It was pointed out that the 1,460/1,510 ratio is representative for the syringyl/guaiacyl ratio in lignin. Since this ratio was higher in the resistant seedlings than in the susceptible seedlings it suggests that formation of syringyl was induced by the fungus. All results obtained suggest that *Ophiostoma novo-ulmi* induced chemical defence reactions in the inoculated material and to a larger extent in resistant than in susceptible seedlings.

Another investigation using FT-IR spectroscopy for estimation of changes in xylem after inoculation with *Ophiostoma ulmi* in comparatively low density of the spore suspension, 103 conidia ml^{-1}, was presented by Martin et al. (2007). Five progenies from *U. minor* and 5 progenies from *U. minor x U. pumila* trees were included in this experiment. Each tree was represented by 8 seedlings. The spore solution was applied on 2 twigs per seedling. Sampling of material from inoculated and control plants took place at 5, 15, 30, and 60 days after inoculation. Peak areas for the aromatic skeletal vibration lignin-associated FT-IR band at wave length 1,510 cm^{-1} were recorded. The ratios between data for the 1,510 cm^{-1} band and carbohydrate reference peaks at 1,158 cm^{-1} and 1,738 cm^{-1} were calculated.

No wilting was observed during the 60-day period after inoculation, which was attributed to the low density of the inoculation solution. Fig. 2-7 reveals that that there is a difference in FT-IR 1,510 band absorbance between control and inoculated progenies both for the interspecific hybrids and the pure species from day 30 after inoculation. The results suggest that the hybrids responded faster to the inoculation than the pure species.

Table 2-1. Peaks at various wavelengths in Fourier transform-infrared (FT-IR) spectroscopy of U. minor *were related to peaks of the 1510 band. Martin et al 2005a.*

Ratios and chemical bonds	Observations
1,230/1,510 methoxyphenolic substitutions in aromatic units of lignin	Resistant after and Control after > > resistant before and control before
1,460/1,510 C-H bending in alkyl groups	Resistant after > All other entries
1,738/1,510 C=O groups	Resistant after > All other entries, control after > control before
3,400/1,510 OH groups	Resistant after and Control after >> the 4 other entries

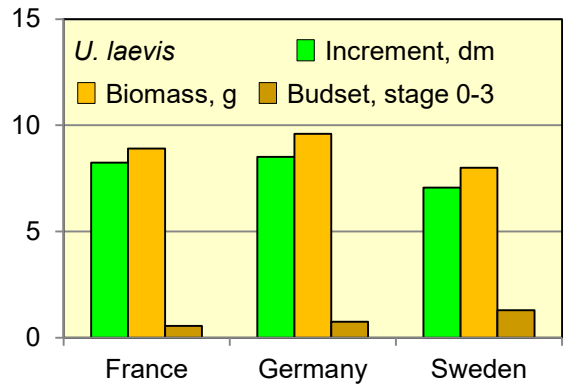

Figure 2-8. Population mean values for increment, biomass, and budset in three U. laevis *populations in control and one drought treatment = 40% of water availability for 11 weeks. Incr = final height in dm minus height at start of the experiment, Biom = total biomass in grams. Black-Samuelsson et al. 2003*

Fairly similar relationships were observed for the two ratios A1,520/A1,738 and A1,520/A1,188 with the most pronounced difference between control and inoculated progenies for the latter ratio. It was concluded that this rapid and sensitive technique might be useful for discrimination between susceptible and DED-tolerant genetic entries.

2.2 Growth and phenology

Black-Samuelsson et al. (2003) studied the effect of drought treatment on within- and among-population variation for growth, budset and leaf morphology traits in three *U. laevis* populations from France, Germany and Sweden. Each population was represented by 8 open-pollinated families. The seedlings were grown in growth chambers under photoperiods simulating conditions in southern Sweden. The drought treatment lasted for 11 weeks during which watering took place when the weights of the trucks with their 15 plants were reduced to 40% of the original weights. The control watering took place when the truck weight was reduced to 80%. There was no intention to determine LT_{50}. The experimental design was single-tree plots in 40 blocks with 24 plants per block. Height after treatment during five weeks and height increment at termination of the experiment minus

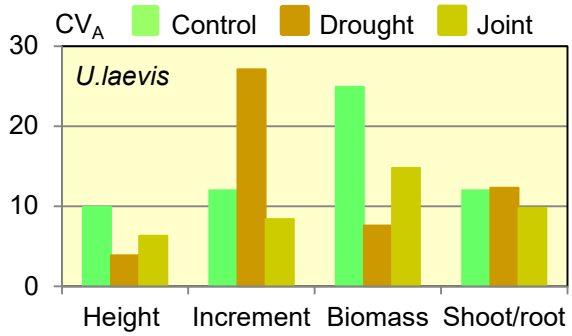

Figure 2-9. Additive coefficient of variance for four traits in an experiment with one drought treatment = 40% water availability. Height is height at five weeks after onset of treatment. Increment is the final height minus the height at start of the experiment in grams. Black-Samuelsson et al. 2003.

height at start of treatment were presented. Biomass of shoots and roots were determined as well as budset on a 4-degree scale. Finally, leaf form was examined. Leaf size was determined, since small leaf size might contribute to fitness under dry conditions through reduction of leaf water vapour conductance. The coefficient for additive genetic variance was calculated for growth traits but not for traits with low mean values since this coefficient might be overestimated in such a case.

The increment was largest in the German population and lowest in the Swedish population, which had the earliest budset (Fig. 2-8). The French population finished growth latest. This is a confirmation of the hypothesis that growth cessation and budset in northern populations are triggered by shorter night lengths than southern populations. This leads to a shorter growth period of the northern populations and less growth than in southern populations when studied under common growth regimes.The poor growth of the French population was attributed to inbreeding in this small and marginal population.

Below the level of 40% water availability the plants started wilting. In spite of such a strong effect of the drought treatment, only two of the traits showed significant effects for treatment, budset and leaf size (Table 2-2). Budset was earlier in the control than in drought treatment. These two traits both showed strongly significant population effects. Noteworthy is the absence of significances for

Table 2-2. Significances for the effects of treatment, population, population x treatment, family, and family x treatment for growth traits, budset, and leaf size in an experiment with drought treatment of young seedlings of U. laevis *populations and open-pollinated families in growth chambers. Three populations from France, Germany and Sweden, each represented by eight open-pollinated families were included. Black-Samuelsson et al. 2003*

	Treatment (T)	Population(P)	P x T	Family (F)	F x T
Increment			*	*	**
Total biomass					*
Shoot/root biomass		***	*	*	**
Budset	**	***		**	
Leaf size	***	***	*	*	**

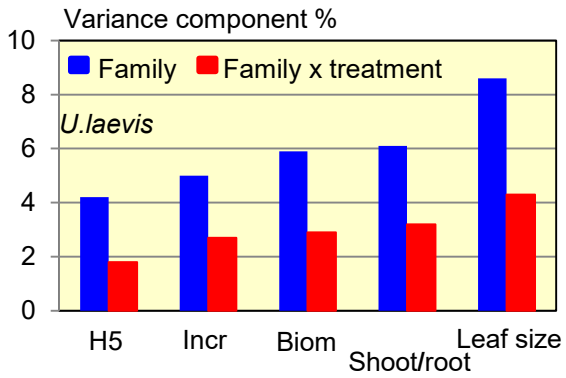

Figure 2-10. Variance components for family and family x treatment interaction in an experiment with 3 U. laevis *populations, each represented by 8 open-pollinated families and 2 treatments for 11 weeks: control and drought. H5 = height 5 weeks after start of treatment, Incr = final height minus height at start of the experiment, Biom = total biomass. Shoot/root = biomass ratio. Black-Samuelsson et al. 2003.*

population x treatment and family x treatment effects for budset. These estimates suggest that budset is genetically strongly regulated.

The leaf size was larger, narrower, and more lanceolate in the control than with drought treatment. This might reflect a buffering capability to withstand the adverse conditions under drought. It was suggested that the shifting water availability at the population localities in France and Germany had favoured genotypes performing well under such growth conditions.

As regards family variation, large coefficients of additive variance (>10%) were noted for increment, biomass, and shoot/root biomass ratio in some of the treatments (Fig. 2-9). Especially high percentages were obtained for increment in drought treatment and biomass in the control material. The family variance components were approximately twice as large as the family x treatment interaction (Fig. 2-10) suggesting a fairly strong genetic stability.

In conclusion, strong additive genetic variation was noted for the traits studied at this juvenile stage. The absence of treatment effects for the growth traits studied is another conspicuous result. Finally, the authors cautioned for too far-reaching conclusions based on this study of juvenile *U. laevis* seedlings.

An investigation of growth and phenology variation among and within Norwegian populations of *U. glabra* was reported by Myking and Skrøppa (2007). The populations originated from the latitudinal range 59.70 – 65.90 °N. The northernmost population and one of the southern populations were coastal populations. Owing to seed shortage the northernmost population was represented by seven open-pollinated families while the 4 other populations had 14–15 open-pollinated families. A field trial was established at lat. 60.25°N with 40 single-tree plots.

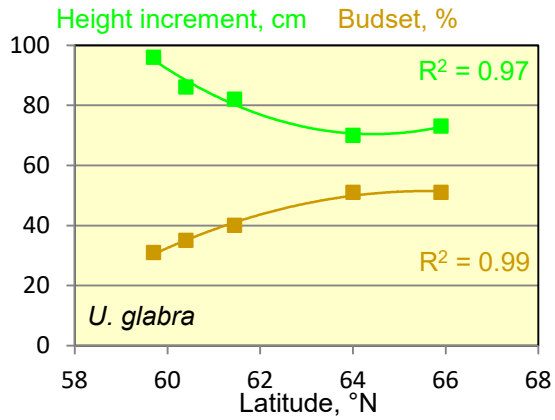

Figure 2-11. The relationship between population original latitude and percentage budset or height in five Norwegian U. glabra *populations studied in a trial at lat. 60.25°N and long.5.34°E. Myking and Skröppa 2007.*

Bud burst was assessed once at age 4 and twice at age 5 in six classes. Growth cessation had only two classes: apical shoot growing and or winter bud formed. Also in this case one and 2 recordings were carried out at ages 4 and 5, respectively. Height increment is the difference between tree heights at ages 3 and 1 year after trial establishment. The two relationships between latitude and budset and height increment shown in Fig. 2-11 reveal the strong dependence between these two traits and latitude. The two curves in this figure are reflected images of each other. It is an extremely good fit to the two second degree polynomial functions, R^2 = 0.97 and 0.99. It is evident that night length is a strong triggering factor for growth cessation. Moreover, the impact of growth cessation on tree height is substantial. The authors reported an r = –0.94 for this relationship. In contrast, there is no relationship between latitude and bud burst (Fig. 2-12). This figure reveals that the two coastal populations are characterized by late bud burst. It was assumed that the coastal populations had developed a more extensive dormancy. Since bud burst is mainly regulated by temperature the

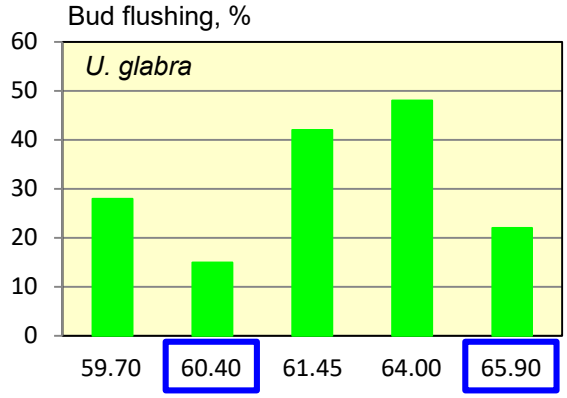

Figure 2-12. Bud flushing % in a Norwegian trial with five U. glabra *populations. Assessments from age 5. Original latitudes, °N, are shown; blue-framed latitude indicate a coastal locality. Myking and Skröppa 2007.*

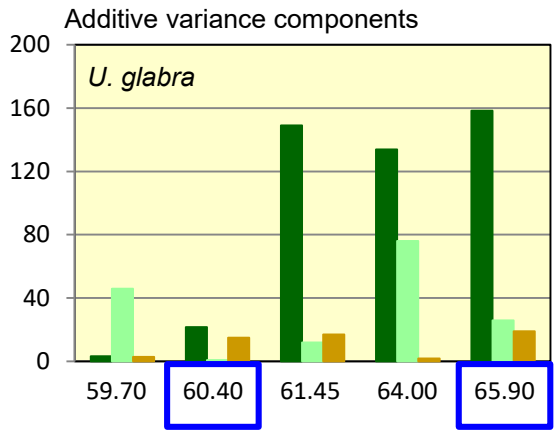

Figure 2-13. Additive variance coefficients for height increment (dark green), bud burst (light green x 10^3), and growth cessation (brown 10^3) in a Norwegian trial with 5 U. glabra *populations represented by 7–15 open-pollinated families. Assessments from age 5. Original latitudes, °N, are shown; blue-framed latitude indicates an Atlantic coastal locality. Myking and Skröppa 2007.*

cooler spring experienced by the coastal populations close to the Atlantic Ocean leads to a later bud burst than in the interior Norwegian populations originating from a less maritime climate. This might be another contributing factor to the results obtained. However, the limited difference in bud burst between the two coastal populations originating 5½ degrees from each other is surprising.

Except for family differentiation of growth cessation all other effects of population and family were strongly significant for the 3 traits studied in the joint analysis of all populations. The family effect for growth cessation was significant at the 5% level. The weaker differentiation for growth cessation was attributed to too few recordings of this trait. Just 2 classes might also have contributed to the result. Fig. 2-13 shows that family differentiation within the five populations was strongest for height increment and significant for the three northern populations. Only in the 61.45°N population was significance for growth cessation noted. Again, recording of growth cessation just twice might not have been enough to identify any possible family differences for this trait. The estimated heritabilities were:

Increment	0.12 ±0.06
Bud burst	0.31 ±0.08
Growth cessation	0.08 ±0.04

It is regrettable that the recordings of the phenology traits comprised so few recordings. However, the strong relationships between latitude and the two traits, increment and growth cessation at population level, are spectacular.

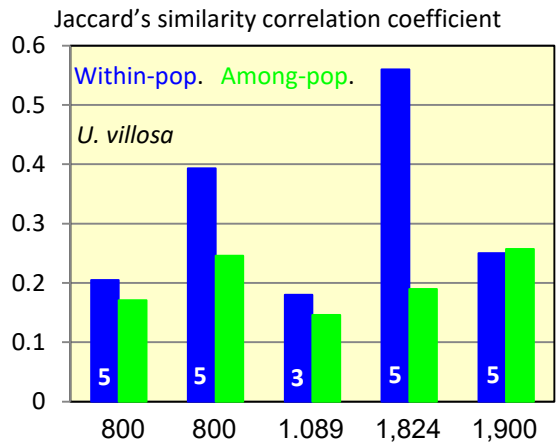

Figure 2-14. Jaccard's similarity correlation coefficients for 3–5 clones for 10 RAPD loci in 5 populations of U. villosa *from different elevations in Himachal Pradesh in India. Coefficients are shown for within- and among - population similarity. The figures refer to the number of progenies in the populations. Thakur et al. 2014.*

2.3 Markers

Seeds were collected from 5 *U. villosa* trees in each of six populations in Himachal Pradesh in India by Thakur et al. (2014) for RAPD analysis. The stand covered an elevation range of 800–2,200 masl. The 23 "best performing" progenies were selected for the RAPD analysis. Ten primers were used resulting in 57 polymorphic bands without any monomorphic bands. Jaccard similarity correlation coefficients were calculated.

The cluster analysis resulted in the following number of trees in the four clusters obtained: 1, 12, 6, and 4 trees. The largest cluster included trees from all 5 populations represented among the 23 selected trees. The single tree in the first cluster belonged to one of the populations from 1,089 masl. The Jaccard similarity coefficient was 0 in its comparison with 10 of the 20 trees from the other populations. In 4 of the 5 populations the Jaccard index was lower in the comparisons with trees from other populations with the most pronounced difference for the 1,824 population (Fig. 2-14). Without any description of the populations it is hard to get a reliable interpretation of the deviating pattern for this population. Increased inbreeding in its passed history might partly explain the high similarity in the 1,089 population. The low similarity in three of the populations might be attributed to genetic drift with an accompanying random fixation of alleles.

2.4 Summary

A partial diallele mating design with 10 parents, 8 *U. minor* and 2 *U. minor x U. pumila* hybrids, was used in a study of heritabilities of DED susceptibility and budburst. Wilting heritability was estimated at 0.11 while height increment had a heritability of 0.21. There was no relationship between height increment and wilting. The non-additive variance for bud flushing and height increment was three times larger than the additive variance, which was somewhat unexpected.

An investigation with *U. minor* progenies from 3 types of crosses, resistant x resistant, resistant x susceptible and susceptible x susceptible, were studied with respect to several anatomical traits and wilting. Significant differences between the 3 types of cross were noted for 7 vessel traits. A high heritability, 0.54, was noted for DED susceptibility, which might be partly attributed to the selection of extremely different DED susceptibilities of the parents in the crosses.

Fourier tramsform-infrared (FT-IR) spectroscopy was studied to identify biomarkers for use in DED breeding. Since the 1,510 cm^{-1} band was regarded as a key band for diagnostic purposes, peaks at other bands were related to this band. Treatment with *Ophiostoma novo-ulmi* resulted in different responses between treatment and control for 4 band ratios 1,230/1,510, 1,460/1,510, 1,738/1,510, and 3400/1510. In conclusion, *Ophiostoma novo-ulmi* induced chemical defence reactions in the inoculated material and to a larger extent in resistant than in susceptible seedlings.

Another FT-IR study after inooculation with a low-density spore solution of *O. novo-ulmi* was reported. No wilting was noted until 60 days after inoculation. The relationship between time after inoculation and the peak area of the FT-IR band at 1,510 cm^{-1} differed between control and inoculated material both for open-pollinated progenies from 5 *U. minor x U. pumila* families and one *U. minor* family. It was claimed that this rapid and sensitive technique might be useful for discrimination between susceptible and DED-tolerant genetic entries.

Strong additive genetic variation was noted for juvenile growth traits in a growth chamber investigation of drought tolerance in open-pollinated *U. laevis* families in one population from each of France, Germany, and Sweden. The absence of treatment effects for the studied growth traits was another conspicuous result. This suggests a strong genetic control of the growth traits.

Extremely strong relationships between population original latitude and height increment or budset were noted in a study of 5 Norwegian *U. glabra* populations. Each population was represented by 7–15 open-pollinated families. A high heritability, 0.31, for budburst was noted while growth cessation had heritabilities around 0.10. The low number of recordings might be responsible for such a low heritability.

3. Clone testing

3.1 Dutch elm disease DED

3.1.1 Basic studies

The DED resistance in four transgenic lines of *U. americana* containing a synthetic antimicrobial peptide EFS39A was studied by Newhouse et al. (2006). The peptide was transferred by *Agrobacterium tumefaciens* strain EHA105. Transformants were confirmed by GUS assay. These transformants as well as those from an earlier experiment were inoculated with *Ophiostoma novo-ulmi.* Six young trees from each material were inoculated. *Ophiostoma novo-ulmi* growth in varying concentrations of the peptide EFS22B, 0–100 µmol, were tested. EFS22b constitutes the core of EFS39A.
Reverse-transcriptase PCR with primers ESF39F and ESF39R confirmed that transformation had taken place. Moreover, the transformants contained single copies of the peptide gene.
The percentages of remaining leaves 11 and 13 weeks after inoculation were much larger in the transformants than in the control material (Fig. 3-1). However, it was claimed that there was no clonal difference, which is unexpected looking at the non-overlapping of standard errors of the three materials (Fig. 5a in the paper). Staining of the sapwood below the point of inoculation was measured 14 weeks after inoculation to estimate spreading of the *Ophiostoma novo-ulmi.* Limited staining was noted for one of the transformants of this study (≈2 cm). In contrast, there was a non-significant difference between the control and the other transformant but staining at larger distances from the point of inoculation (≈14 and 9.5 cm). No effect in sapwood was noted for concentrations below 42 µmol of EFS22B.

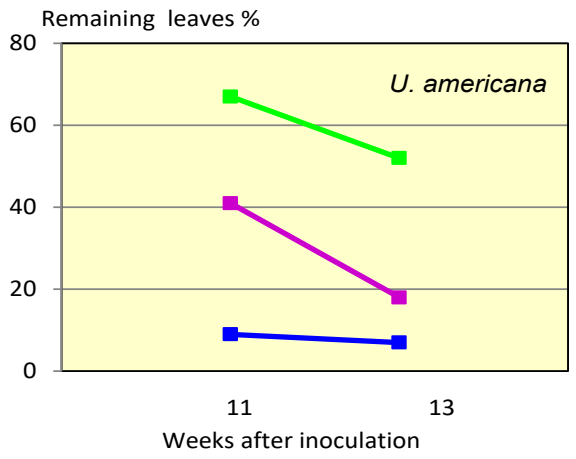

Figure 3-1. Remaining leaves 11 and 13 weeks after inoculation with Ophiostoma novo-ulmi *of two transformants (green and lilac) containing the antimicrobal peptide EFS39A and control plants (blue). Newhouse et al. 2007.*

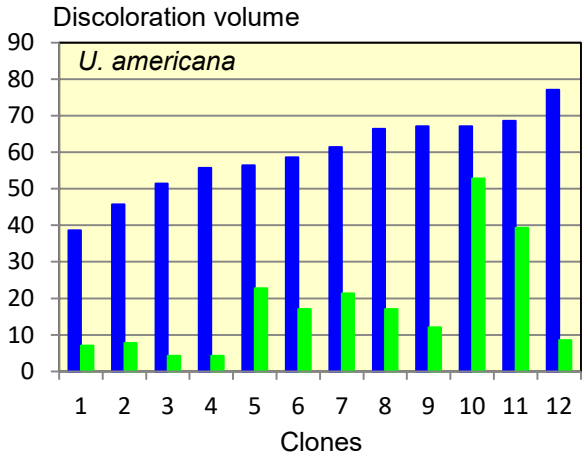

Figure 3-2. Discoloration volume (height x cross-sectional area) following inoculation with Ophiostoma novo-ulmi *+ water (blue) or with* Verticillium albo-atrum *+* O. novo-ulmi *(green) of 12 two-year-old* U. americana *clones. Sheffer et al. 2008.*

The transformants had mycorrhiza, which shows that transformation does not have any negative impact on mycorrhiza associations
The authors warned for too far-reaching conclusions from this study with young single stem trees since young trees are less susceptible to DED than old trees.

Sheffer et al. (2008) reported on an experiment with injection of *Verticillium albo-atrum* 2 weeks before inoculation with *Ophiostoma novo-ulmi* of 12 two-year-old *U. americana* clones. The volume of discoloration of the wood was measured.
The pretreatment with *Verticillium albo-atrum* resulted in a reduction of the effect of *Ophiostoma novo-ulmi* treatment compared to the effect of *Ophiostoma novo-ulmi* without pretreatment with *Verticillium albo-atrum* (Fig. 3-2). It was suggested that this fungus triggers a defence reaction against DED in the trees. The response to treatment with *V. albo-atrum* varies among the elms. The treatment with *V. albo-atrum* must be repeated every year since this fungus only survives for a short period of time in the trees. Injections with *V. albo-atrum* or water resulted in negligible discoloration.
Large scale application of *V. albo-atrum* was started in 1992 in the Netherlands and up to 32,800 trees were treated. Losses of 1% of trees were noted for the period 1992-2006. Half of the losses were attributed to root grafting. In areas without treatment the range of losses was 4–14%. Thus, a substantial improvement thanks to the injections. Successful results from injections were also reported for The Hague, the Netherlands, and Denver in Colorado, USA.

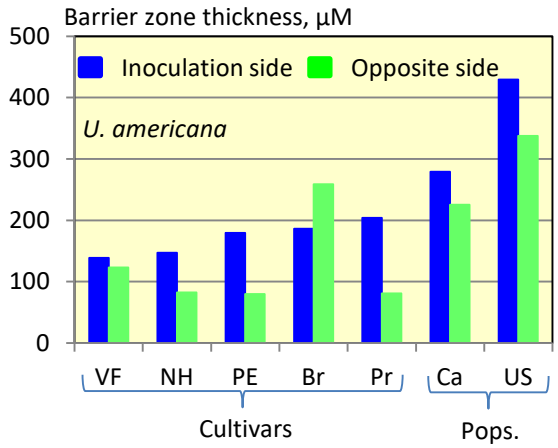

Figure 3-3. Barrier zone thickness on inoculation side and on opposite side of the trunk at 90 days after inoculation with Ophiostoma novo-ulmi. *The age of the trees was 4 years. Five American cultivars and 2 populations, one from USA (US) and the other from Canada (Ca), were included in this study. Beier and Blanchette 2018.*

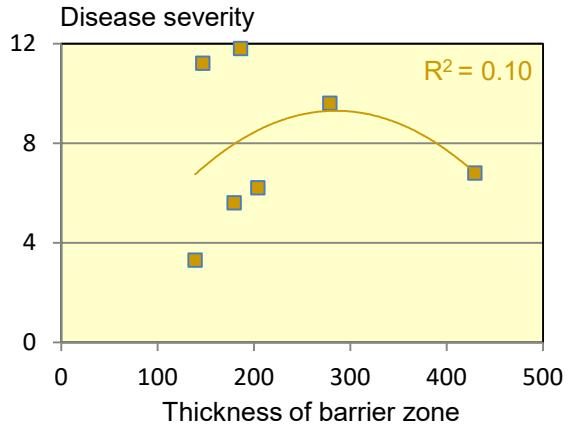

Figure 3-4. The relationships between disease severity at 90 days after inoculation with Ophiostoma novo-ulmi *or water and thicknessof barrier zone. Disease severity had 12 classes 1–12; 1 = no visible DED symptoms, 12 = dead tree. Beier and Blanchette 2018.*

The formation of barrier zones and the timing of their appearance in xylem as well as the xylem conductivity might have an impact on DED susceptibility. In their study including five American cultivars and two populations Beier and Blancette (2018) addressed these matters. Barrier cells are flatter than typical fiber and axillary parenchyma cells. They are mostly somewhat darker than other cells probably owing to the concentration of defence substances such as phenols. Inoculations with *Ophiostoma novo-ulmi* were carried out at ages 3 and 4. Measures were taken to guarantee that the holes drilled for inoculation were exactly of the same depth, 4 mm. Examination of wilting was carried out 5–90 days after inoculation. A 12-degree scale was used with 1 = 0 wilting and 12 = 100% wilting. Classes 2–11 represented 10 percentage units. Samples from 3–6 individuals (age 3) per entry were collected at 5, 10, 15, 20, 40, and 90 days after inoculation for anatomical examination. Control inoculations (sterile water) were sampled at the same times. For inoculations at age 4, samples were taken 90 days after inoculation. Transverse sections collected at 1–12 cm above the inoculation sites were used for anatomical analyses.

Since accumulation of lignin and suberin might indicate defence responses to inoculation, tissues were stained with phloroglucinol-HCl and Sudan black C to localize these compounds by their autofluorescence under blue light.

The low number of trees per entry and sampling date means that it is hard to prove differences among the genetic entries. It should be remembered that this is a laborious study. It would be better to have more trees per entry and sampling date and fewer sampling dates. For these reasons we shall not be too detailed about the huge amount of data presented in this paper. There was a large variation in barrier zone thickness among the entries in this study (Fig. 3-3). Mostly the barrier zone was thicker on the inoculation side of the trunk.

We are surprised that no relationships between data in this study and observed disease severity were presented. In Figs. 3-4–3-5 we have illustrated such relationships. Obviously there is no relationship between thickness of barrier zone and disease severity. In contrast, there was a fairly strong relationship between autofluorescence and disease severity in the inoculated material. The cultivar Valley Forge with a disease severity index of 3.3 had a strong impact on the relationship in Fig. 3-5. The control did not show any relationship between severity index and autofluorescence.

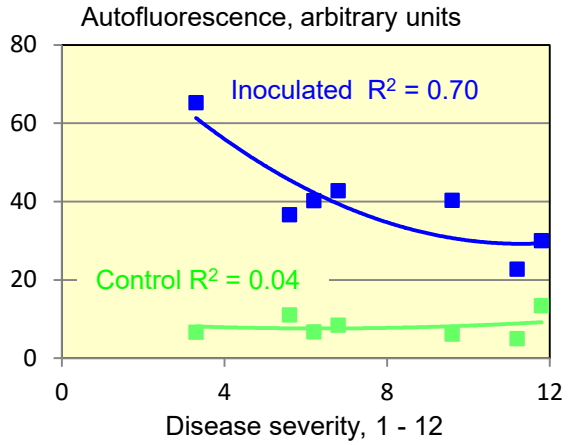

Figure Beier18-3. The relationships between disease severity at 90 days after inoculation with Ophiostoma novo-ulmi *or water and autofluorescence in arbitrary units. Disease severity had 12 classes 1–12; 1 = no visible DED symptoms, 12 = dead tree. Beier and Blanchette 2018.*

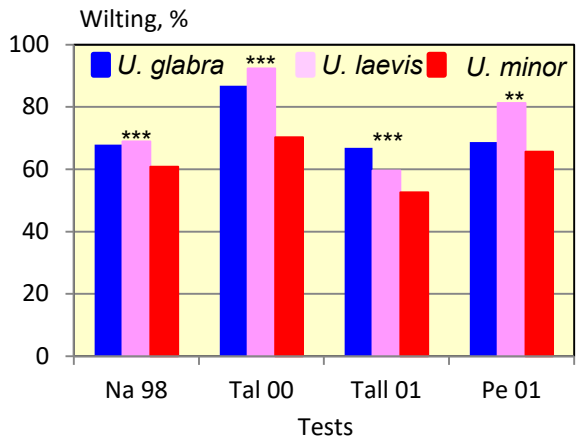

Figure 3-6. Wilting at the last assessment in the year of inoculation with Ophiostoma novo-ulmi *of French clones of* U. glabra, U. laevis, *and* U. minor. U. glabra *contains hybrids between* U. glabra *and* U. minor *with morphology close to* U. glabra. U. minor *contains hybrids between* U. glabra *and* U. minor *with morphology close to* U. minor. *Significant species differences are indicated. Pinon et al. 2005.*

Pinon et al (2005) inoculated 222 French elm clones, comprising several taxa and reference clones, with *Ophiostoma novo-ulmi*. The largest number of clones were *U. minor* while *U. glabra* was represented by few clones. To enable species comparisons with enough clones per species, the *U. minor* x *U. glabra* were classified as resembling *glabra* or *minor*. The former were referred to *U. glabra sensu lato* and the latter to *U. minor sensu lato* in the following comparisons. Field trials, mostly with single tree plots, were established and assessments of wilting in four trials were presented. Several assessments were

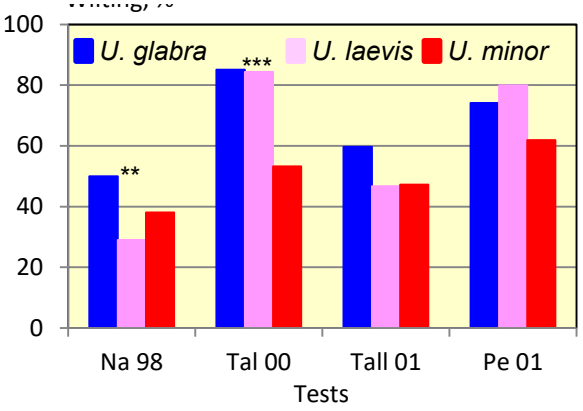

Figure 3-7. Percentage wilting the second year after inoculation with Ophiostoma novo-ulmi *of French* U. glabra, U. laevis, *and* U. minor *clones*. U. glabra *contains hybrids between* U. glabra *and* U. minor *with morphology close to* U. glabra. U. minor *contains hybrids between* U. glabra *and* U. minor *with morphology close to* U. minor. *Significant species differences are indicated. Pinon et al. 2005.*

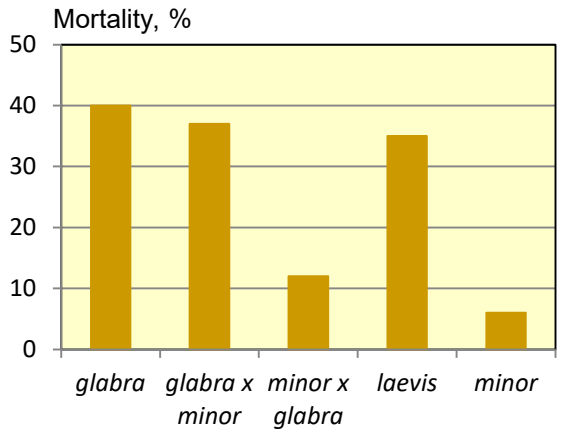

Figure 3-8. Mortality at the end of year 3 following inoculation of several elm taxa with Ophiostoma novo-ulmi. *Hybrids between* U. glabra *and* U. minor *with morphology close to* U. glabra *are denoted* U. glabra. *Hybrids resembling* U. minor *are denoted* U. minor. *Pinon et al 2005.*

carried out during the year of inoculation as well as the following year. Wilting was estimated in slightly different ways the different years but in essence it was an assessment of percentage of affected crowns.

There was a large variation in susceptibility among the clones and also a large within-clone variation of some clones. We have illustrated the percentage of wilting at two occasions following inoculation:

Final recording during the year of inoculation (Fig. 3-6)

Final recording the second year after inoculation (Fig. 3-7)

These figures reveal that wilting percentages were higher during the year of inoculation than the following year. This means that recovery had occurred. Differences among species were non-significant in two of the trials year 2. *U. minor* showed least susceptibility in six of the eight comparisons. Surprisingly, *U. laevis* showed as high susceptibility as *U. glabra*. It was stated that there might be some bias in this investigation since the number of *U. minor* trees was much higher than in the two other species.

In spite of the substantial susceptibility the mortality was fairly low except for the Tal 00 trial. Also with respect to mortality the difference between *U. laevis* and *U. glabra* was limited but considerably higher than in *U. minor* (Fig. 3-8). Three reasons for the limited mortality were presented:

The trees are young and have good ability to resprout

Overestimation of mortality in nature

Unconscious selection of trees with good resistance in this study

Finally, it was stated that superior French clones were found in all taxa, and 16 clones that had shown total recovery were listed.

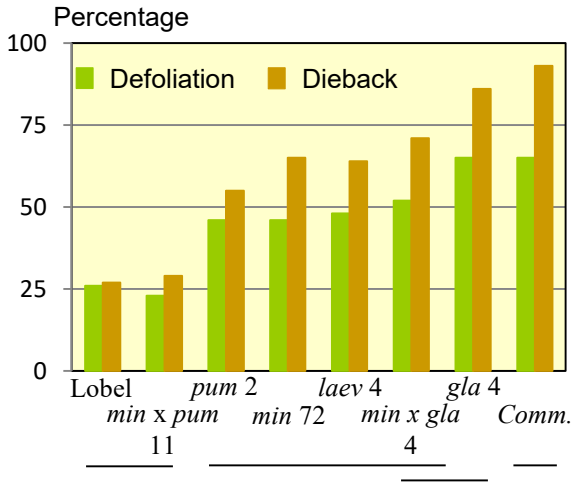

Figure 3-9. Mean percentages of foliation and dieback 30 days and 14 months after inoculation with Ophiostoma novo-ulmi *of various elm taxa;* min = U. minor, pum = U.pumila, laev = U. laevis, gla = U. glabra*, Lobel is a DED-resistant clone and Commelin is a DED-susceptible clone. The number of clones of the individual taxa are presented. The lines below indicate the four groups obtained according to a Tukey test. Santini et al. 2005.*

Inoculations with *Ophiostoma novo-ulmi* on several taxa and two reference clones, one classified as resistant (Lobel) and the other as susceptible (Commelin) was carried out by Santini et al. (2005). The inoculations were carried out on 3-year old cuttings in mid-May, at a time when the susceptibility against the fungus is highest in Toscana, Italy. A single knife-cut into the xylem with spores on the knife was used. Defoliation was recorded at three occasions the first summer, June 19, July 17, and September 18. The following year dieback was registered on April 30 and July 18. At age three bud flushing was recorded in five classes.

Fig. 3-9 reveals that the two reference clones performed as expected with respect to DED symptoms. It is also evident that the *U. glabra* genome brings high susceptibility. The two *U. pumila* clones had an unexpected high susceptibility. It was stated that other studies had shown large variation in susceptibility to DED of *U. pumila* clones. Furthermore, the origin of these two clones was unknown. There was an extreme variation in susceptibility among the 72 *U. minor* clones: 15–75% defoliation on June 19. There was a strong and positive relationship between clonal origin latitude and defoliation at June 19. French clones were most susceptible and southern Italian clones were least susceptible. It was speculated that the southern Italian clones had passed the most sensitive stage for *Ophiostoma novo-ulmi* infection at the date of inoculation.

At the early assessments of defoliation there was a relatively strong and significant relationship between date of bud flushing and defoliation, r = 0.74 and 0.59 (Fig. 3-10). At the latest recording the second year, July 18, this relationship had almost disappeared.

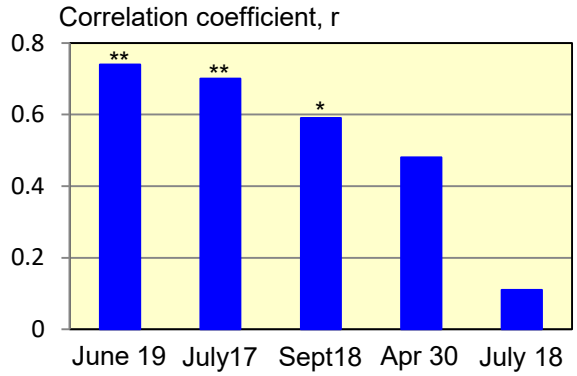

Figure 3-10. The relationship between bud flushing and defoliation at certain dates after inoculation with Ophiostoma novo-ulmi, *which took place on May 17. Significances are indicated. Santini et al. 2005.*

The hybrids *U. minor x U. pumila* showed the best growth both for height and diameter. A large variation in tree height among the 72 *U. minor* clones was noted. Clones from southern Italy had larger diameters than clones from other parts of Italy. The French clones had the poorest growth. There were negative and significant relationships between taxa means for defoliation and height (r = 0.74*) or diameter (r = 0.71*). However, these relationships within *U. minor* were all weak and did not explain much of the variability in susceptibility.

One take home message from this investigation is the large variation in susceptibility among the *U. minor* clones. Clones from southern Italy had larger diameters than clones from other parts of Italy. The authors stressed that it is not satisfactory to rely on first year results of inoculations. Studies ought to continue for another year. Finally, the 11 *U. minor x U. pumila* clones were the most resistant clones among the taxa studied.

A detailed study of the impact of vessel size and resistance to *Ophiostoma novo-ulmi* was carried out by Solla et al. (2005b) in a trial with four clones from each of *U. minor* and *U. pumila.* The vessel development was followed monthly over one year in lateral branches. The concepts of VTA and THC were defined by the authors as follows: *Interior vessel diameter was measured and the vessel transectional area (VTA) was calculated. The relative theoretical hydraulic conductance (THC) was also calculated. VTA was obtained by dividing the area occupied by the vessel in a sector (wall excluded) by the total area of the sector, then multiplying by 100. The THC, predicted by the Hagen-Poiseulle equation, was determined by dividing the sum of the fourth power of internal radii (Σrn^4) by the sector area. Vessels less than 20 µm were ignored.* They also studied bud flushing and presence of *Scolytus* beetles by the use of traps 300 meters from the trial.

Cambial activity did not occur before March 15 in any of the species. There was a pronounced peak of *Scolytus* beetles in late May and early June, which coincided with a maximum in radial growth in both species. There were

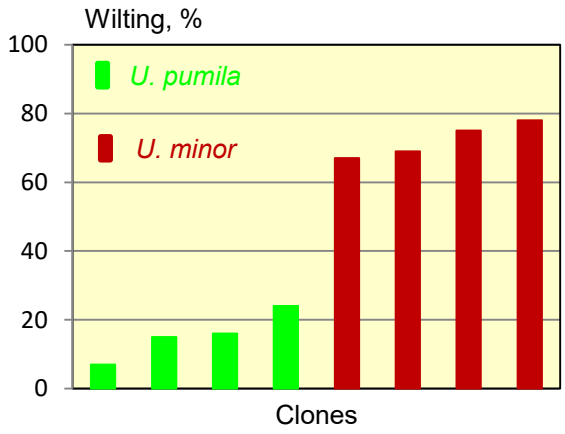

Figure 3-11. The wilting percentage four months after inoculation with Ophiostoma novo-ulmi *in four 4-year old* U. minor *and 4* U. pumila *clones. Solla et al. 2005b.*

only minor differences in radial growth among clones within the two species

The wilting four months after inoculation was much lower in the four *U. pumila* clones than in the 4 *U. minor* clones (Fig 3-11). Despite the selection of the *U. minor* clones to represent varying DED resistance, the wilting did not show a broad variability, 67–78%. A couple of differences between *U. minor* clones and *U. pumila* clones were noted:

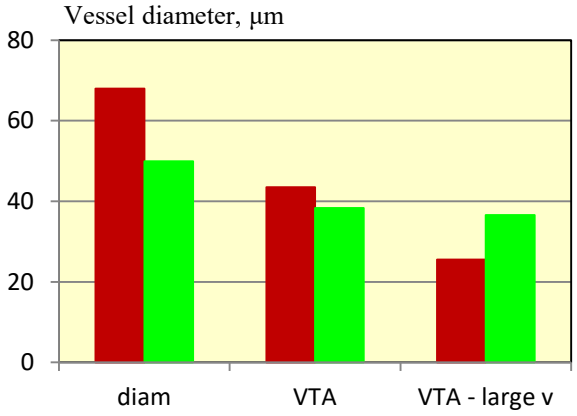

Figure 3-12. The mean vessel diameter in µm, VTA including all vessels, and VTA excluding large vessels, > 160 µm 4 months after inoculation with Ophiostoma novo-ulmi *in 4 four-year old* U. minor *(brown) and 4* U. pumila *(green) clones. Solla et al. 2005b.*

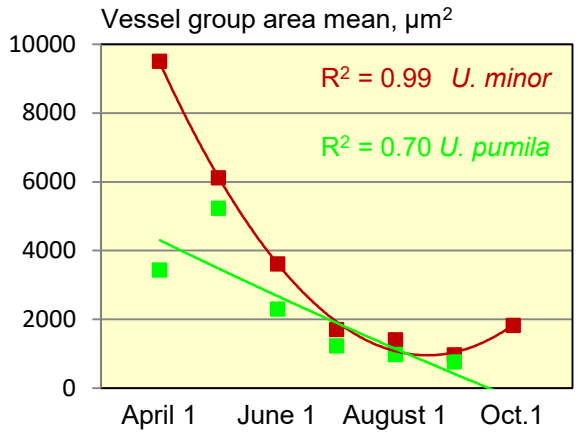

Figure 3-13. The relationship between date and vessel group area. Mean values for 4 four-year old U. minor and 4 U. pumila *clones. Solla et al. 2005b.*

Bud flushing occurred almost two weeks earlier in *U. pumila* than in *U. minor*.

At peak of growth in early June, the apical and radial growth were both around 1.6 times larger in *U. minor* than in *U. pumila*.

At peak of vessel diameter at April 15, mean vessel diameter as well as VTA were larger in *U. minor* than in *U. pumila* (Fig. 3-12). This was also the case for WTA when large (>160 µm) or large + medium (80–160 µm) vessels were excluded in the estimates of WTA.

Radial growth lasted until November 15 in *U. minor* while it ended one month earlier in *U. pumila*..

At April 15 THC was 2.8 times higher in *U. minor* than in *U.pumila*.

Exclusion of the largest vessel in the calculation of THC led to a reduction of THC by 10.4% in *U.pumila* and 68.6% in *U. minor*.

U. pumila had a higher vessel density than *U. minor,* 46.3 and 30.5 vessels per mm², respectively. The development over time of the vessel area is visualized in Fig. 3-13.

Based on the results in this investigation as well as literature the authors concluded that the time for large vessel formation is the most critical for DED disease infection. Moreover, the authors stated that *Vessel density and vessel size group are thought to constitute a more significant unit than measurements of individual vessels.* To allow comparison of different inoculation tests, inoculations should be carried out at defined stages in vessel formation.

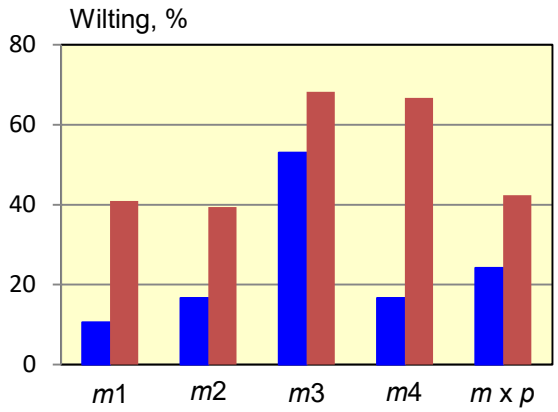

Figure 3-14. The wilting percentage at 60 days after inoculation in trees of ages 3 (blue) and 4 (brown) at inoculation with Ophiostoma novo-ulmi *in 4* U. minor *clones (m1–m4) and one* U. minor x U. pumila *clone (m x p). Solla et al. 2005c.*

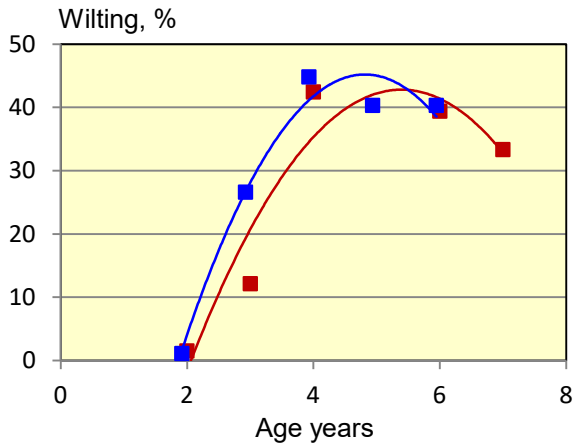

Figure 3-15. The wilting percentage at 60 days after inoculation in trees of ages 2–7 at inoculation with Ophiostoma novo-ulmi *in one* U. minor *clone (brown, no data for age 5) and one* U. minor x U. pumila *clone (blue, no data for age 7). Solla et al. 2005c.*

To get a standardization of DED resistance tests Solla et al. (2005c) carried out a study with inoculations of the same material at different ages, 2–7 years. All clones were not represented each year. They established a trial with rooted cuttings of 4 *U. minor* clones and one *U. minor x U. pumila* clone. The design was 2 blocks with 3 ramets-plots. Inoculations at 8–10 cm above ground took place in mid-May 2000 with a highly virulent strain of *Ophiostoma novo-ulmi* subsp. *americana*. Leaf wilting was recorded 15–120 days after inoculation. At the last date crown dieback was assessed. VTA and THC were calculated in agreement with the above paper. Xylem development was studied microscopically on material collected in January 2001. Inoculations were missing for some clones some years.

Fig. 3-14 reveals that the age of the inoculated material caused different ranking of these 2 clones with respect to wilting, confirming the authors' hypothesis that age at inoculation may influence the DED resistance. In Fig. 3-15 we have illustrated the relationship between ages at inoculation and wilting at 60 days after inoculation of two clones with data from most years. For both clones there was a maximum at ages 4–5 and the authors suggested that tests should be carried out at age 4–5 to have relevant and comparable ranking of DED resistance. As seen from this figure it is self-evident that there were significant differences depending on age at inoculation.

The vessel area varied also depending on age, but to a somewhat lower extent. There were also significant differences for VTA and THC. There were extremely strong relationships between vessel diameter and wilting as seen in Fig. 3-16. The relationships among the histological traits and their relationships to tree vessel diameter or vigour before inoculation are visualized in Fig. 3-17. The strong relationships between vigour and xylem vessel diameter was expected (r = 0.81). The strongest correlation was noted for the relationship between vessel diameter and THC, 0.88.

The authors presented 2 causes for the age effect on DED resistance:

Changes in physiology with age leading to differences in compounds at different ages such as toxins for fungi

Variation in anatomical constraints for fungal growth, especially the ability to develop tyloses

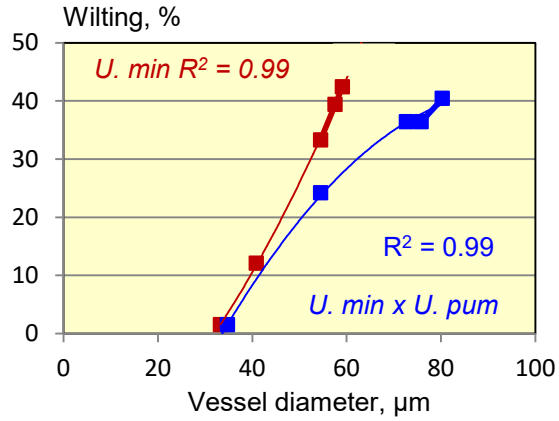

Figure 3-16. The relationships between earlywood vessel diameter and wilting at 60 days after inoculation with Ophiostoma novo-ulmi *of trees from one* U. minor *clone and one* U. minor x U. pumila *clone inoculation at different ages 2–7 years. Solla et al. 2005c.*

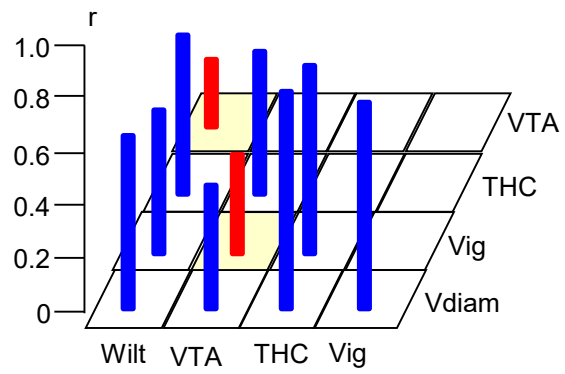

Figure 3-17. The correlation coefficients for the relationships between five traits in a study of inoculation with Ophiostoma novo-ulmi *of 4* U. minor *clones and one* U. minor x U. pumila *clone inoculated at different ages, 2–7 years. Wilt = wilting at 60 days after inoculation, VTA = vessel transectional area, THC = relative theoretical hydraulic conductance, Vig = growth in the year before inoculation, Vdiam = vessel diameter. Red columns indicate negative relationships.* Solla et al. 2005c.

The strong relationship between wilting and vessel diameter (Fig. 3-17) suggests a strong role of this trait regarding DED.

Results from studies of inoculation with *Ophiostoma novo-ulmi* of 324 elm clones in five European countries were presented by Solla et al (2005a). Six taxa were represented in this study: *U. glabra, U. laevis, U. minor, U. pumila, U. minor x U. glabra* and *U. minor x U. pumila*. Seven control clones with known susceptibility to DED were also included. The clones were selected based on their superior sanitary status in the respective populations or they were selected for desired ornamental characteristics. The number of clones in the six taxa differed considerably from one to 64, which means that some estimates are imprecise. All taxa were not included at each test plot, which further reduces the possibility for accurate comparisons over sites. The inoculations took place at age 3. Percentages in 5% units of wilting or death of foliage were recorded 4, 10, 16 weeks, and one year after inoculation. Height before inoculation was measured and correlated with damage after inoculation.

In Figs 3-18–3-19 we have illustrated the range of dieback percentage for *U. glabra, U. laevis,* and *U. minor* at test localities having five or more clones. These figures illustrate that the ranges are wide in almost all cases and that high percentages of dieback occur in the majority of cases. The hybrid *U. minor x U. glabra* was tested 2 years in France and had dieback % ranges of 0–45 and 19–70% while the range for the hybrid *U. minor x U. pumila* tested in Italy was 0–65%.

Most of the correlations between wilting ten weeks after inoculation and dieback one year after inoculation were

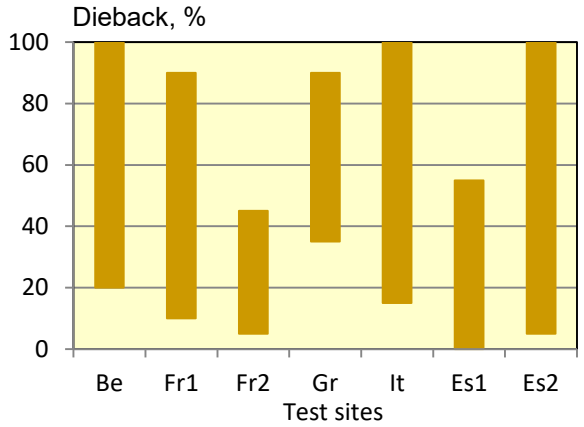

Figure 3-18. Ranges of dieback in U. minor *clones one year after inoculation with* Ophiostoma novo-ulmi *at five localities in Europe. In France (Fr) and Spain (Es) inoculations were carried out during two consecutive years.* Solla et al. 2005a.

significant. The range of the correlation coefficients varied between –0.30 and +0.80 as far as we can understand. More than half of the correlations between pre-inoculation heights and damage at ten weeks after inoculation were non-significant. It is hard to interpret the correlation results without estimates of correlation coefficients. Significances can be obtained without a large degree of explanation of the relationship.

Despite the high recorded susceptibilities the authors expressed optimism as regards breeding for DED resistance and presented a selection of 13 *U. minor* and *6 U. minor x U. pumila* clones.

It is regrettable that such a European initiative was carried out without the possibility for joint evaluation of the results based on a satisfactorily large number of clones per taxa at several test localities.

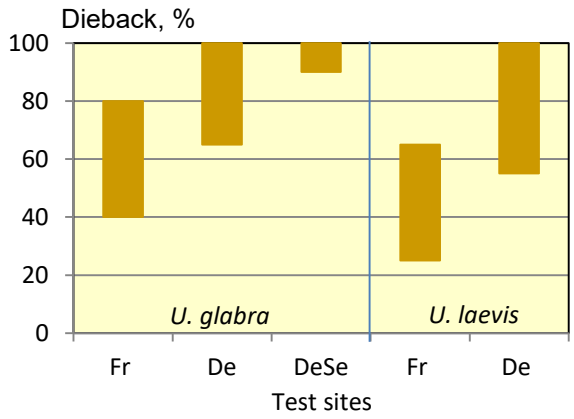

Figure 3-19. Dieback in U. glabra *and* U. laevis *clones one year after inoculation with* Ophiostoma novo-ulmi *at two localities in Europe. GeSe stands for separate data for Swedish clones tested in Germany.* Solla at al. 2005a.

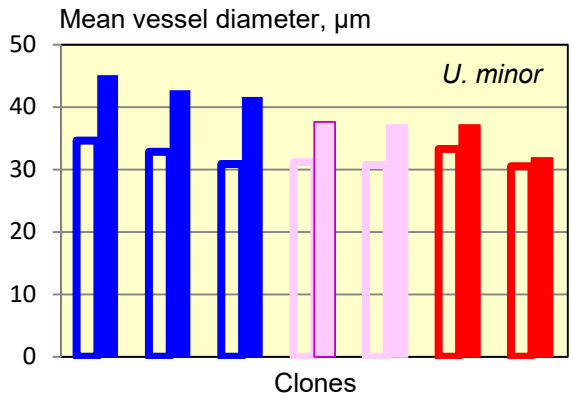

Figure 3-20. The mean vessel diameter in the second and third rings in µm of seven elm clones classified as high (blue), medium (lilac), and low susceptibility (red) to DED. Open columns refer to the second ring and filled columns refer to the third ring. Solla and Gil 2002b.

Solla and Gil (2002b) studied the anatomical structure of vessels in seven clones with varying susceptibility to DED. In 1993 three-year-old cuttings were inoculated. After 60 days, symptoms of DED were recorded. Three percentages of wilting were used to classify the clones as high, 67–100%, medium, 34–66%, or low susceptibility, 0–33%. The anatomical studies were carried out on rings formed 1996 and 1997 in branches sampled approximately three and a half years after inoculation. The percentage of vessel transectional area (VTA) and the relative theoretical hydraulic conductance (THC; µm^2) were calculated. Low vessel diameter, 20–39 µm, dominated in the third ring without any difference among susceptibility groups. Larger rings occurred in lower frequency, and difference between susceptibility groups occurred in the diameter groups above 100 µm. Fig. 3-20 reveals that there is limited difference in mean vessel diameter between the three categories of susceptibility in the second ring while the most susceptible group had the largest vessel diameter in the third ring. Vessel diameter index defined as *vessels greater than 100 µm with respect to the total* differed among the three groups of susceptibility (Fig. 3-21). Similarly, the relative hydraulic conductance differed among the three groups. It was assumed that xylem vessel diameter and distribution influence the dispersal of the *Ophiostoma novo-ulmi*. It is of importance to understand the mechanisms behind susceptibility but analyses of vessel diameters do not seem to be any quick fix for identification of DED susceptibility.

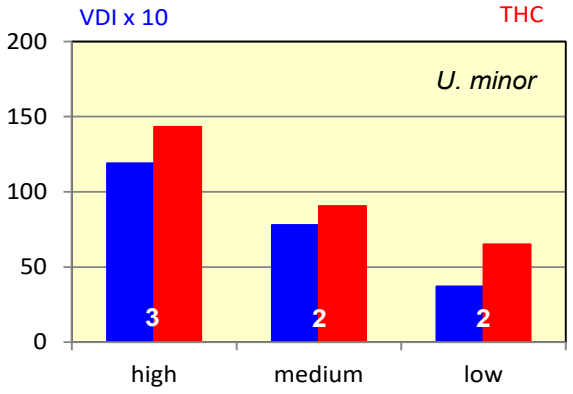

Figure 3-21. The mean vessel diameter index, VDI, greater than 100 µm with respect to total multiplied by 10 and relative theoretical hydraulic conductance in clones with high, medium and low DED susceptibility. The number of clones in each category is indicated. Solla and Gil 2002b.

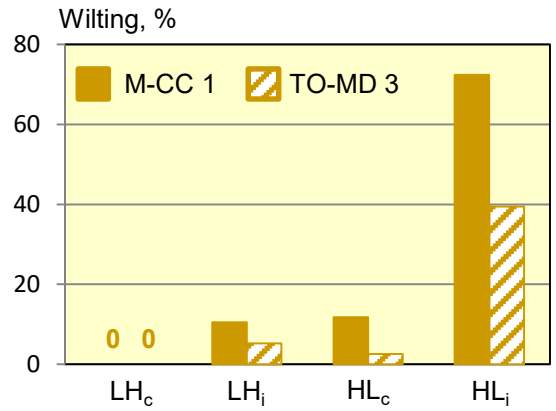

Figure 3-22. Percentage wilting at day 120 after inoculation in two Spanish U. minor *clones (M-CC-1 and TO-MD 3) exposed to high watering or restricted watering before inoculation with* Ophiostoma novo-ulmi. *Fourteen days after inoculation the richly watered trees were exposed to restricted watering and vice versa. Indices i and c stand for inoculated and control treatment.Solla and Gil 2002a.*

Solla and Gil (2002a) studied the effect of water stress on DED after inoculations with *Ophiostoma novo-ulmi* in two Spanish *U. minor* clones. Eighteen potted rooted cuttings were included in this investigation. Two watering regimes were used, denoted as high watering followed by limited watering (HL treatment) and vice versa, limited watering followed by high watering (LH). In the HL treatment watering to field capacity took place twice a week with start on April 5, while restricted watering to field capacity took place once a week. On May 14 inoculation was carried out. On May 29 HL plants were watered once a week and the LH trees were watered twice a week. Following a heat wave, watering was increased with one additional watering in each treatment. On May 13 leaf water potential and stomatal conductance were measured. The measurements were repeated on June 14. Xylem elements of the control plants were measured in December the following year.

In both clones a high level of wilting was noted from day 20 after inoculation. After this day the wilting remained more or less at the same level in the HL treatment until termination of the experiment at day 120, approximately 50 and 70%. Wilting in controls and LH treatments had

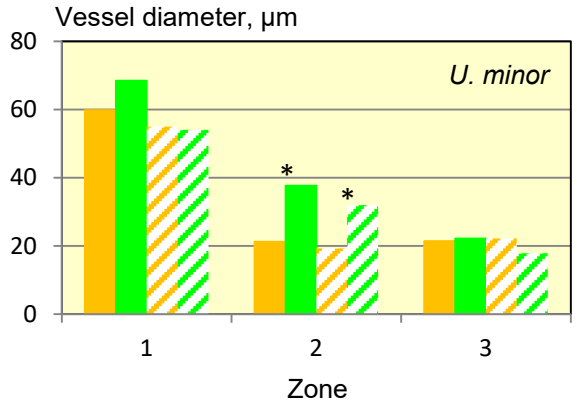

Figure 3-23. Average vessel diameter in three zones of the zylem in control treatments. Filled columns refer to U. minor *clones M-CC 2. Striped columns refer to clone TO-MD 3. Orange columns refer to the LH treatment and green to HL treatment. (For treatments see text). Significant differences within zones are indicated. Solla and Gil 2002a.*

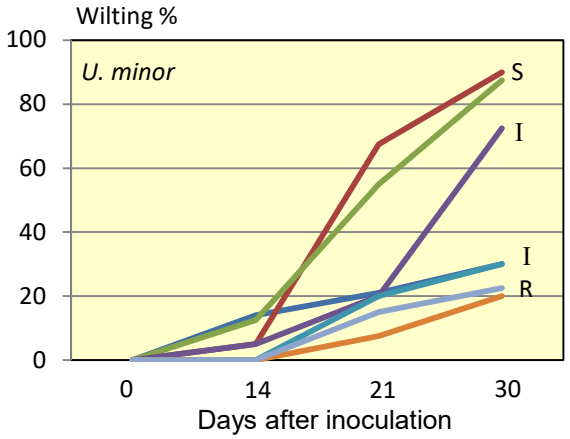

Figure 3-24. Development of wilting after inoculation with Ophiostoma novo-ulmi *of two susceptible (S) clones, two clones with intermediate (I) susceptibility, and two resistant (R) clones of* U. minor. *Li et al. 2016.*

low percentages of wilting all the time (Fig, 3-22). The mean dieback showed the same pattern with 56.7 % in the HL treatment and 7.9% in the LH treatment. This result shows that water stress causes increased susceptibility to DED infection.

Three zones in the xylem were observed. It was suggested that zone 2 corresponded to the start of the limited watering period and that zone 3 corresponded to the increased watering in this treatment. Heavy initial watering resulted in significantly larger vessel diameter in zone 2 in both clones (Fig, 3-23). The rapid development of latewood in the LH treatment probably reduced the risk for DED development. It was pointed out that artificial inoculation in breeding should avoid recently transplanted trees since their xylem vessel development will be hampered, thus giving a false resistance to DED.

There was a dramatic difference in average stomatal conductance in the control material between the two measurements (May 13 and June 14) in the HL treatment, 72 versus 3 $mMm^{-2}s^{-1}$. The higher vertical transition area and theoretical hydraulic conductance in plants from the HL treatment favored the infection rate by *Ophiostoma novo-ulmi*.

Li et al. (2016) studied the effect on water metabolism (housekeeping of water availability) after inoculation of 6 Spanish *U. minor* clones with different susceptibility to *Ophiostoma novo-ulmi*. Inoculation took place at ages 5 or 6 in early May with the strain *americana* Z–BU1. The effect of treatment was recorded at 7, 14, 21 and 30 days after inoculation. Besides wilting, several physiology traits were studied (see Table 3-1).

The development of wilting of the six clones is illustrated in Fig 3-24, which shows a pairwise similar pattern of the two susceptible and the two resistant clones with a dramatic difference between these two pairs. One of the intermediate clones showed a high percentage of wilting at day 30 and approached the susceptible clones while the other intermediate clone did not differ much from the two resistant clones.

In the resistant clones there was no difference in water conductivity between the control and inoculated materials, in contrast to the susceptible clones. From 14 days after inoculation there was a drop in water conductance. This drop was most pronounced in the susceptible clones at 30 days after inoculation. Consequently, the stomatal conductance and net photosynthesis rates were lowest

Table 3-1. Mean performance of wilting and physiology traits 30 days after inoculation with Ophiostoma novo-ulmi *of two resistant, two intermediate, and two susceptible U. minor clones at ages 5 or 6. Li et al. 2016.*

	Resistant	Intermediate	Susceptible
Wilting %	21.2	51.2	88.8
Water potential p_d	–0.45	–1.10	–1.34
Water potential m_d	–2.22	–2.43	–3.43
Stomatal conductance of water vapour g_s	2.09	1.04	0.12
Net photosynthesis rate P_n	124	94	11
Dark respiration rate, R_d	14.4	15.3	18.2
Initial hydraulic conductivity K_i	0.26	0.22	0.18
Maximum hydraulic conductivity K_{max}	7.60	7.06	5.88
Percentage loss of conductivity, PLC	50.0	51.5	82.4

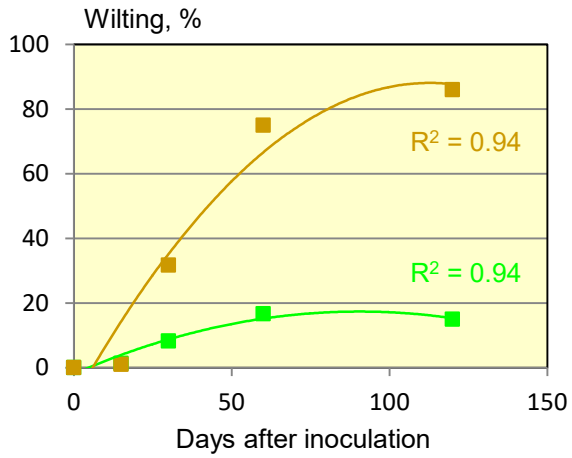

Figure 3-25. The development of mean wilting over time in 3 susceptible (brown) and 3 resistant (green) U. minor *clones following inoculations with* Ophiostoma novo-ulmi. *Martin et al. 2005b-1.*

in the susceptible clones, which indicates that the host trees responded differently to the pathogen. This was attributed to embolism or direct vessel blockage.Table 3-1 further shows that maximum hydraulic conductivity and percentage loss of conductivity were most affected in the susceptible clones. It is evident that water metabolism is differently affected in the clones with different susceptibilities to *Ophiostoma novo-ulmi* infection.

Before the inoculation, susceptible clones differed chemically from the resistant clones. The IR spectroscopy indicated higher content of carbohydrates, suberin, fatty acids, cellulose, hemicellulose, and phenolic compounds. Especially the latter compounds are known to participate in the defence against pathogens.

In conclusion, the results suggest that differences in water availability are responsible for the difference in susceptibility to DED between susceptible and resistant clones. Differences in phenolic compounds can also contribute to susceptibility differences.

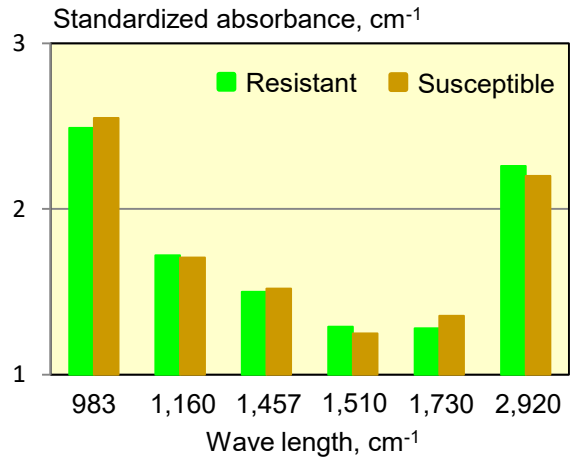

Figure 3-26. The mean standardized absorbance in FT-IR spectroscopy at 6 wavelengths of 3 resistant and 3 susceptible clones of U. minor *from Spain exposed to* Ophiostoma novo-ulmi *at age 10. The absorbance was recorded 60 days after inoculation of 5-year-old branches in 10-year-old trees. Martin et al 2005b.*

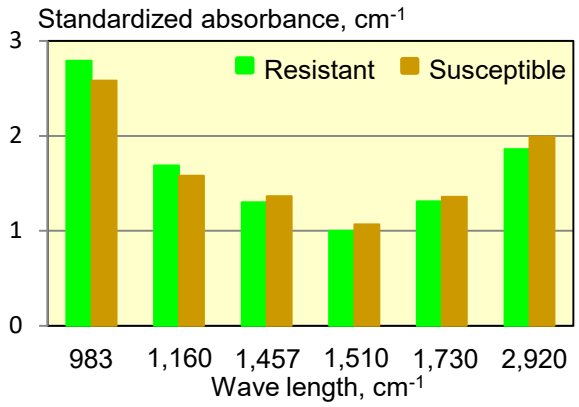

Figure 3-27. The mean standardized absorbance in FT-IR spectroscopy at 6 wave lengths of 3 resistant and 3 susceptible clones of U. minor *from Spain in the control material in an experiment with* Ophiostoma novo-ulmi *inoculations at age 10. The absorbance was recorded 60 days after start of the experiment with 5-year-old branches in 10-year-old trees.Martin et al 2005b.*

Fourier Transform-Infrared (FT-IR) spectroscopy was used by Martin et al. (2005b) to get insight into the metabolism in xylem tissue following inoculation with *Ophiostoma novo-ulmi*. At age 4, 8 cuttings from each of 6 clones, 3 susceptible and 3 resistant to DED, were inoculated with *Ophiostoma novo-ulmi* to test the susceptibility of the clones. Wilting was recorded at 15–120 days following inoculation. The FT-IR spectroscopy was made on samples from 3-year old twigs from trees with an age of 10 years. Branches 5 years old had been inoculated. Powder of the outermost ring of the xylem was used in this experiment, which comprised the wave length window, 1,800–800 cm^{-1}, which covers major components of the xylem tissue.

The development of mean wilting for susceptible and resistant clones is illustrated in Fig. 3-25. It should be noted that one of the susceptible clones should be designated as moderately susceptible since wilting was only 60% at 60 days. Even so, there is a dramatic difference between the 2 classes.

The FT-IR spectroscopy revealed several positive and negative peaks. The associations of these peaks with substances or bindings are presented in Table 3-2. Since the effects of the inoculations were substantial at 60 days after inoculation we have preferred to illustrate the absorbances for some key bands for susceptible and resistant clones in Fig. 3-26. For 3 bands (1,457, 1,510, and 2,920 cm^{-1}) the differences between inoculated and control materials were more pronounced than between inoculated susceptible and inoculated resistant materials. Nor were there any pronounced differences in the control material between susceptible and resistant clones (Fig. 3-27).

Table 3-2. Observed positive and negative peaks in the FT-IR spectroscopy of 6 U. minor clones *following inoculations with* Ophiostoma novo-ulmi. *Martin et al. 2005b.*

	Associated substances	
896	β-linkage in carbohydrate region	Positive
983	Pectin ester groups and starch	Positive
1,045	Xylan	Positive
1,100	Pectin	Positive
1,160	Glycosidic linkage	Positive
1,215	C-O-H deformation and C-O stretching of phenolic compounds	Negative
1,260		Negative
1,457	Alkyl bending	Negative
1,510	Aromatic rings in lignin	Negative
1,594		Negative
1,730	Pectin ester groups and starch	Positive

It was pointed out that the levels of polysaccharides were lower in inoculated materials but they contained higher levels of phenols. It was suggested that fungal enzymes were responsible for degradation of carbohydrates and that phenols were produced as a defence against fungal growth. The accumulation of aliphatic compounds in inoculated materials as indicated by the peaks of bands 1,457 and 2,990 was also attributed to response to the infection. Aliphatic monomers are thought to lead to suberization of cell walls, which prevents fungal degradation.

One of the goals with the FT-IR spectroscopy study, although not explicitly stated, was to investigate whether or not this technique could be used for an early identification of resistant elm genetic entries. It seems as if the differences in tolerance to DED, both following inoculations and in control material, are too limited to enable an application of this technique in elm breeding. However, this investigation has broadened the knowledge of metabolic steps in defence to *Ophiostoma novo-ulm*i attacks.

The same technique was used by Martin et al. (2008) in a study of clones with different DED susceptibility in an attempt to develop early testing for DED susceptibility. Four *U. pumila* and 5 *U. minor* clones of low DED susceptibility, as well as 5 *U. minor* clones with high susceptibility to DED, were inoculated at age 4 with one strain of *Ophiostoma novo-ulmi* in a trial close to Madrid, Spain. Samples for FT-IR analysis were taken at five occasions from May 1 to September 1. FT-IR spectra in the range 1,800 to 800 cm^{-1} from the outermost ring of the xylem were determined. *The spectra were auto-scaled (divided by the standard deviation and centered).*

Except for data from May 1 the principle component analysis of the obtained spectra data did not give a clear distinction among the 3 groups of material. After application of discrimination functions of the obtained spectra data, a clear distinction among the 3 groups was observed. However, there was overlapping of the data among the groups. We have compiled the significances obtained between and among clonal groups at certain bands in Table 3-3. This table reveals that significant discrimination between *U. pumila* and the pooled data of the two *U. minor* groups was noted at four of the five sampling occasions. Significant discrimination among the 3 groups was noted at two sampling dates, May 1 and July 1.

Table 3-3. Comparison of FT-IR spectra between and among three groups of elms, U. pumila, *DED-resistant* U. minor, *and DED-susceptible* U. minor *at different bands. Martin et al. 2008.*

Comparison	*Band* cm^{-1}	*Significant differences at sampling dates*
U. pumila – pooled *U.minor*	*1,027* and *1,310*	May 1, June 1, July 1, Sept. 1
Resistant – susceptible *U. minor*	*930* and *989*	May 1, June 1, July 1
U.pumila – resistant *U.minor* – susceptible *U. minor*	*1,078* and *1,560*	May 1, July 1

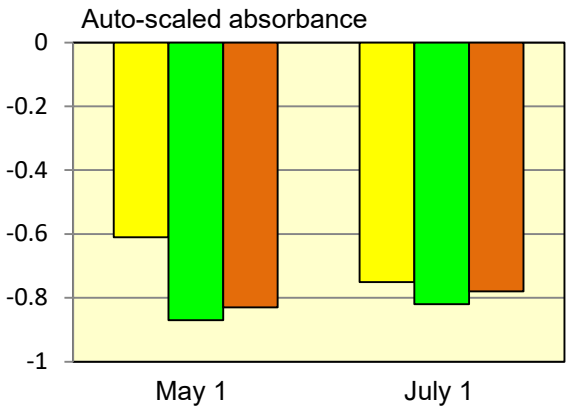

Figure 3-28. The auto-scaled absorbance following Fourier transform-infrared spectroscopy in band 1,560 cm^{-1} of U. pumila *(yellow), resistant* U. minor *(green), and susceptible* U.minor *(brown) at two occasions, May 1 and July 1. Martin et al, 2008.*

In Fig 3-28 we have illustrated the auto-scaled absorbances for two sampling dates with significant difference among the three groups. At May 1 there was a clear separation between the two elm species. *A priori* one would expect that there would be agreement between *U. pumila* spectra and resistant *U. minor* spectra. However, the difference in spectra is largest between these two groups while the susceptible clonal group took an intermediate position.

The compounds associated with different absorbance peaks and their impacts on DED resistance were thoroughly discussed.

Besides the graphic illustrations of the performance of individual clones, variation within groups was not discussed. With the title containing the word fingerprinting, it was anticipated that data on individual variation would be presented.

Two hypotheses were put forward in a study by Martin et al. (2009):

DED-resistant elms have narrower pit openings and fewer pit contacts than susceptible elms

Resistant elms have smaller medullary rays than susceptible elms

This study comprised 4 low-susceptible *U. pumila* clones, 5 low-susceptible *U. minor* clones, and 5 highly susceptible *U. minor* clones. The *U. minor* clones were selected among 92 clones in order to get clones with different susceptibility. Three resistant and 3 susceptible clones were inoculated with a strongly virulent *Ophiostoma novo-ulmi* OR-VR1 strain at the base of five-year old branches. Three weeks after inoculation, samples 4 cm long were collected from three-year-old twigs. Half of the material was used for scanning electron microscopy and the other half was used for light microscopy. Wood density and 22 xylem traits were assessed: 8 border pit, 10 vessel and 4 vascular ray traits were measured. Only four of the 22 traits did not differ significantly among the three groups.

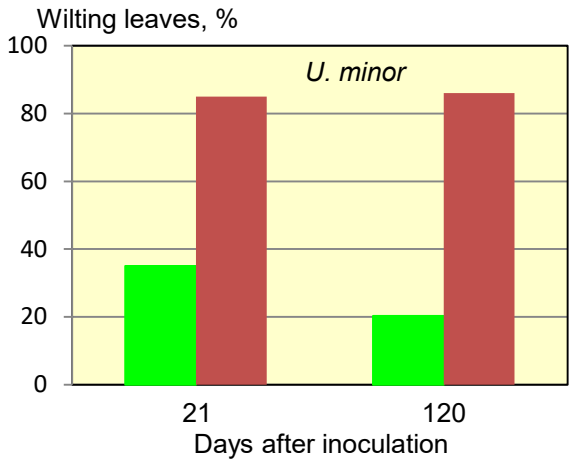

Figure 3-29. Mean percentage of wilting leaves 21 days after inoculation of 3 resistant (green) and 3 susceptible (brown) U. minor *clones with* Ophiostoma novo-ulmi. *Ditto for wilting of entire crowns at 120 days after inoculation. Martin et al. 2009.*

Wilting after inoculation was assessed 21 and 120 days after inoculation and is shown in Fig. 3-29. The group of resistant and the group of susceptible *U. minor* clones differed substantially.

Five traits that did not differ between the resistant *U. minor* and the *U.pumila* clones while they both differed significantly from the susceptible *U. minor* clones are illustrated in Fig. 3-30 together with wood density and the

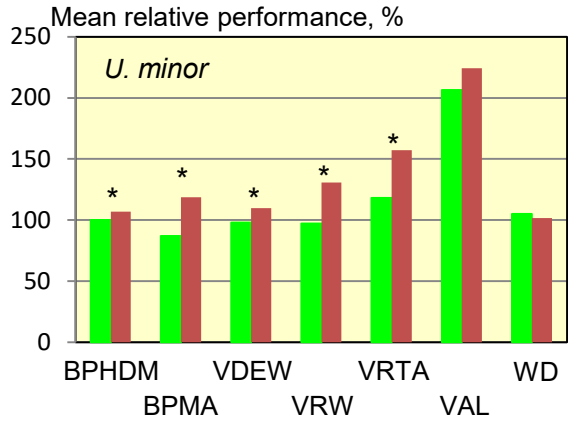

Figure 3-30. The mean relative performance in % of 8 xylem traits in 5 resistant (green) and 5 susceptible (brown) U. minor *clones compared to 4 DED-resistant* U. pumila *clones. * = susceptible* U.minor *clones that are significantly different from resistant* U.minor *and* U. pumila *clones. The difference between the latter two was non-significant.*
BPHDM = bordered pit diameter of membrane
BPMA =bordered pit membrane abundance
VDEW = vessel diameter of early wood
VRW = vascular rays width
VRTA = vascular rays tangential area
VAL = vascular rays abundance
WD = wood density
Martin et al. 2009.

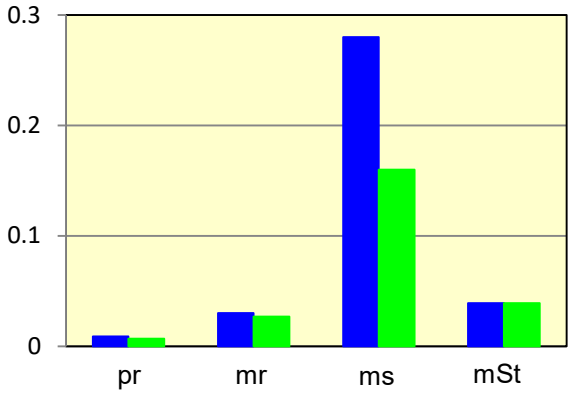

Figure 3-31. Endophyte frequency (blue) and diversity (green) in xylem for resistant U. pumila *(pr), resistant* U. minor *(mr) and susceptible* U. minor *(ms) in a trial, and in a stand (mSt). Martin et al. 2013.*

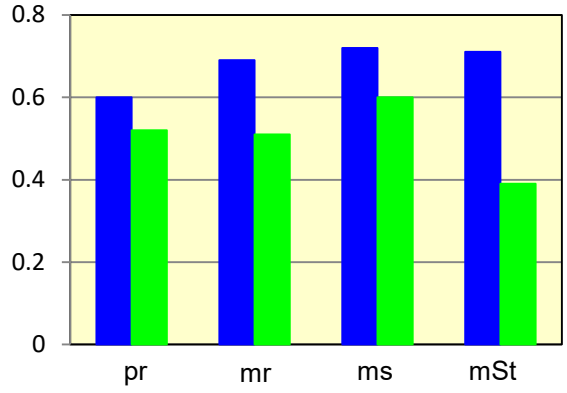

Figure 3-32. Endophyte frequency (blue) and diversity (green) in bark for resistant U. pumila *(pr) , resistant* U. minor *(mr) and susceptable* U. minor *(ms) in a trial, and in a stand (mSt). Martin et al. 2013.*

abundance of vascular rays. The ratios between the two groups of *U. minor* clones and the *U. pumila* clones are illustrated in this figure. Therefore, these 5 traits are in our opinion the most likely ones that participate in the regulation of DED tolerance.
The authors concluded that *The interaction between vessel size and pit conductivity may provide a better explanation for host colonization by the pathogen and the host resistance.*

The hypothesis of low frequency and diversity of endophytes in DED-resistant elms was the motivation for an investigation by Martin et al, (2013). Such a difference could be attributed to strong defence against fungi in DED-resistant elms. The occurrence of endophytic fungi in leaves, bark, and xylem of elms with different susceptibility to *Ophiostoma novo-ulmi* was studied. *Endophyte diversity of each tree group was estimated as the average of the number of different morphotaxa observed in each Petri dish divided by the number of tissue samples placed in the dish.* Occurrence of chemical compounds was also investigated. The following 3 groups of material growing at the tree breeding center in Madrid, Spain were included in this investigation:

1. 4 DED-susceptible *U. minor* clones; age 14
2. 4 DED-resistant *U. minor* clones; age 14
3. 2 DED-resistant *U. pumila* clones; age 14

In addition, a fourth group consisting of 4 DED-susceptible *U. minor* trees with an age of 65–75 years from a stand 30 km away from the tree breeding center were included.
Sampling of terminal shoots at 2–3 m above ground took place in 2008. Sixteen 2-year-old segments (4 cm) of the twigs were used for analysis of endophytes in bark and xylem. Four samples were taken from each aspect (south, west, north, and east). Four samples of approximately the same size were put on Petri dishes with malt agar. The frequency of endophytes was *calculated as the average of the number of endophytes colonies in each Petri dish divided by the total number of tissue samples placed in the dish*. The endophytes were identified in the microscope and via molecular markers. Among the 274 isolations of the 816 plant samples 16 taxa were identified. *Pyronochaeta.cava, Monographella nivalis* and *Aureobasidium pallulans* were most frequently found. *M. nivalis* causes serious damage to cereals and other grasses.
The frequencies and diversity of endophytes in xylem tissue and bark for the 4 groups of material are shown in Figs. 3-31–3-32. We shall start the presentation of the situation as regards the xylem study since this tissue is of greatest significance for DED. There was a strong difference between the two susceptibility groups. Two reasons might explain this difference. First, there was a difference in age and second, the ambient conditions differed between these two groups. Fig. 3-32 shows that the differences among the 4 groups as regards endophyte frequency and diversity in bark were not dramatic. As regards endophyte diversity, only the mSt group differed significantly from the other three groups. The results support the hypothesis outlined above, which suggests that resistance to DED affects endophyte frequency and diversity in xylem negatively. It was concluded that the tree genotype plays a major role for its endophyte frequency and diversity in xylem. In other tissues such as bark and leaf such strong influence of genotype does not exist. It was pointed out that these results are valid for endophytes and might not be representative for other fungi.
It was speculated that the endophyte flora might influence the chemical quality of the trees. The HPLC analysis showed that a rosmarinic derivative was the most discriminating compound among the four groups of material. The amount in the two DED-resistant groups was 3 times less than in the DED-susceptible group and still lower than in the elm stand group.

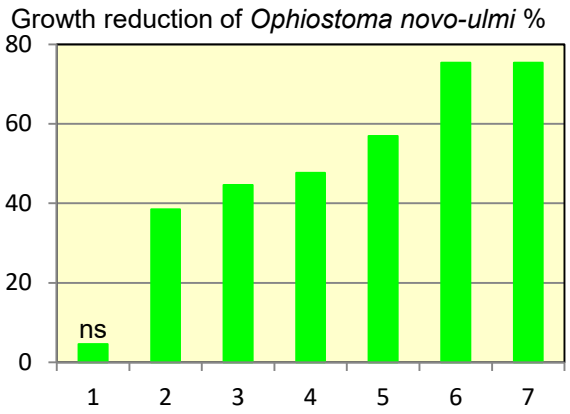

Figure 3-33. The growth reduction of Ophiostoma novo-ulmi *in* in vitro *dual cultures with*
1 = Fusarium *sp.*
2 = Aureobasidium pullutans
3 = Penicillium crustosum
4 = Monographella nivalis
5 = Alternaria tenuissima
6 = Neofusicoccum luteum
7 = Sordaria *sp.*
The only non-significant difference in growth reducton is indicated. Martin et al 2015.

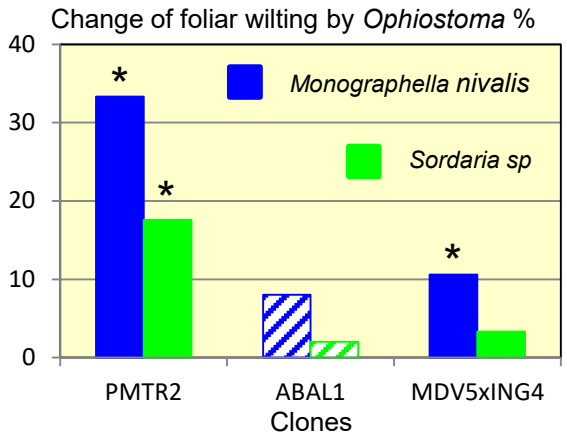

Figure 3-34. The change of foliar wilting in three elm clones by Ophiostoma novo-ulmi *conditioned with* Monographella nivalis *or* Sordaria *sp. in a field trial. Filled columns = reduced wilting, striped columns = increased wilting. Significant differences are indicated. Martin et al 2015a.*

The antagonism between *Ophiostoma novo-ulmi* and endophytic fungi in xylem tissues was studied *in vitro* and *in vivo* with 4 *U. minor* and one *U. minor x U. pumila* clone by Martin et al. (2015a). Three mature trees without DED symptoms were selected in populations severely affected by DED. Several endophytes were isolated and used in this report: *Alternaria tenuissima, Aureobasidium pillufans, Monographella nivalis, Penicillium crustosum,* and *Sordoria* sp. *In vitro* dual cultures in Petri dishes with *Ophiostoma novo-ulmi* and several endophytic fungi were carried out to guide the *in vivo* experiments.
Conditioning of three clones with *Monographella nivalis* and *Sordoria* sp. was carried out in late April and was followed two weeks later by *Ophiostoma novo-ulmi* inoculation in 2011. The percentage change of foliar wilting was followed for 120 days. In 2012 one clone was treated, and in 2013, 2 clones.
Fig. 3-33 reveals a large variation in response to the antagonism of the endophytic fungi tested, from 5 to 95% growth reduction. It was found that the reduction of growth was inhibited by antibiosis after conditioning with *M. nivalis* or *P. crustosum,* while competition for the substrate caused the inhibition by *N. luteum* or *Sordaria* sp. The strongest effect was noted for *M. nivalis*, which formed a thick barrier in the pathogen.
In the 2013 study there was no effect of the treatment while in 2011 there was significant treatment effect but with a considerable difference of the response of the 3 clones (Fig. 3-34). There was a strong reduction of wilting in clone PM-TR2 while the wilting was larger, although not significantly, in clone AB-AL1 after conditioning with *M. nivella* or *Sordaria* sp. It should be noted that different clones were tested in 2011 and 2013. It was speculated that the weather conditions might have had an impact on the protective effect of the endophytes. It was also discussed if the inoculated endophytes had the ability to survive and spread in the host plant. The present study did not address these matters.
The ambiguous results call for further research along present lines.

An RFLP study of cpDNA of English elm, *U. procera*, was undertaken by Gil et al (2004) to trace the origin of this elm. Of the 4 cpDNA lineages existing, A–D lineage-C haplotypes occur in Britain, Italy, and Spain. Lineage-C haplotypes do not occur in Spanish *U. glabra*, in contrast to the situation in Italy. This was taken as an indication that the C-lineage has an Italian origin. Eighteen *U. minor* from Britain, Italy, and Spain and 5 Lineage-D individuals were analyzed using seven nuclear microsatellites and 2 AFL loci. A widely distributed lineage-C clone was detected in 5 Spanish and three British elms. These elms do not produce seeds but they produce pollen and are easily propagated vegetatively. These features together with historic documents support the hypothesis that “English elm” is a 3,000-year-old Roman clone.

Perdiguero et al. (2015) formulated their objective in the following way: *the aim of this work was to obtain the first transcriptome from* U. minor *in response to abiotic and biotic stress response emphasizing on DED*. Three clones with varying susceptibility to DED were inoculated with *Ophiostoma ulmi, Ophiostoma novo-ulmi,* and the endophytic *Daldinia concentrica*. The cuttings were exposed to 2 watering regimes: one or 7 days without water, in order to induce drought stress. The intention was not to study transcriptomes of individual clones, but rather to identify as many transcriptomes as possible.

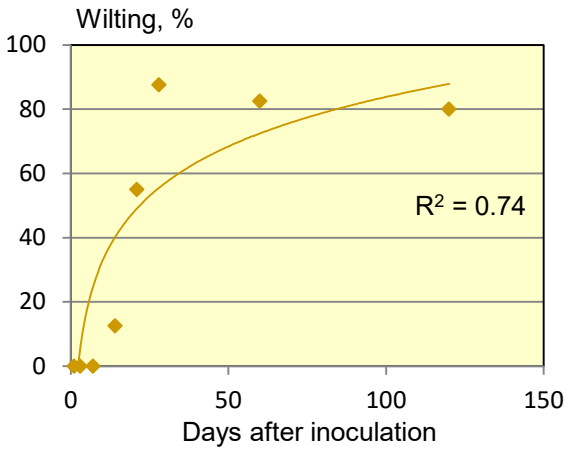

Figure 3-35. The development of wilting over time following inoculation of the Atinium *clone of* U. minor. *A logarithmic curve is fitted to the observed data. Perdiguero et al. 2017.*

The transcriptomes of *U. minor* were compared with GenBank RefSeq protein database number of unique transcripts and amounted to 47,640. No fewer than 32,192 of the 47,640 transcripts were associated with different proteins. Similarities of transcriptomes in lower numbers were also found with *Ophiostoma ulmi* (141), *Ophiostoms novo-ulmi* (6), and *Daldinia eschscholzii* (29). The Blast2GOo gene ontology term was used to assign unique transcripts to biological processes (58%), cellular component (25%), and molecular function (17%). The number of polymorphic SNPs amounted to 27,359.
It was concluded that this investigation has paved the way for future molecular studies of DED susceptibility.

Gene expression of a high number of genes following *Ophiostoma novo-ulmi* inoculation of cuttings of one *U. minor* clone denoted *Atinium* was studied by Perdiguero et al. (2017).The cuttings were 6 years old at inoculations, which took place at the base of the trunks with an aggressive strain of the fungus. Sections of 3-year-old branches at an approximate height of 2 metres were sampled for anatomical investigation of material at 1–21 days following inoculation. Total RNA from each sampling day was extracted and included in a microarray analysis. Three replicates of inoculated and control samples were analyzed. Wilting was followed until 4 months post inoculation.

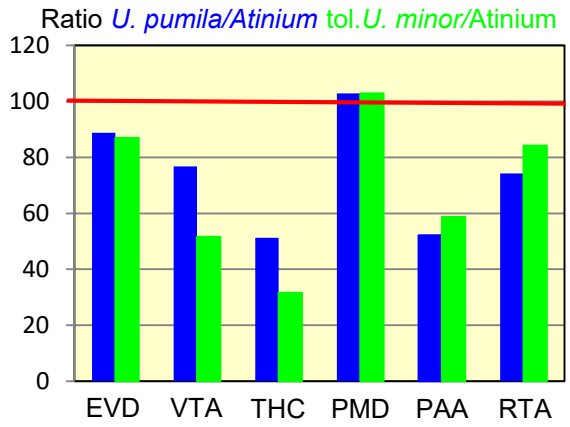

Figure 3-36. The ratio U. pumila/Atinium U. minor *control and treatment/*Atinium *control for 6 vessel-asociated traits. EVD = Earlywood vessel diameter, VTA = vessel tangential area, THA = relative theoretical hydraulic conductance. PMD = Pit membrane diameter, PAA = Pit aperture area, RTA = Ray tangential area. Perdiguero et al. 2017.*

Fig. 3-35 illustrates that already three weeks after inoculation more than 50% wilting was recorded.
All anatomical traits except for pit membrane diameter were smaller in *U. pumila* and the DED-tolerant *U. minor*. It was stated that the anatomical structure of *Atinium* elm is perfectly adapted to maximize hydraulic conductance in early spring. Fig. 3-36 reveals the large difference between the two DED-resistant entries (*U. pumila* and the DED-tolerant *U. minor*) on the one hand and the *Atinium* elm on the other hand for THC (theoretical hydraulic conductance) and PAA (pit aperture area). High values for both these traits are assumed to facilitate the infection of *Ophiostoma novo-ulmi*. Similarly, the probability for blocking of water transport by production of exudates increases with increasing RTA (ray tangential area).
No fewer than 1,696 isotigs showed significant differences in transcription levels following inoculations. These differentially expressed genes (DEGs) could be grouped into 12 clusters. Half of them showed upregulation and the other half showed downregulation. The top 25 of the upregulated DEGs varied in the range 52–350 times higher expression values than in control trees.

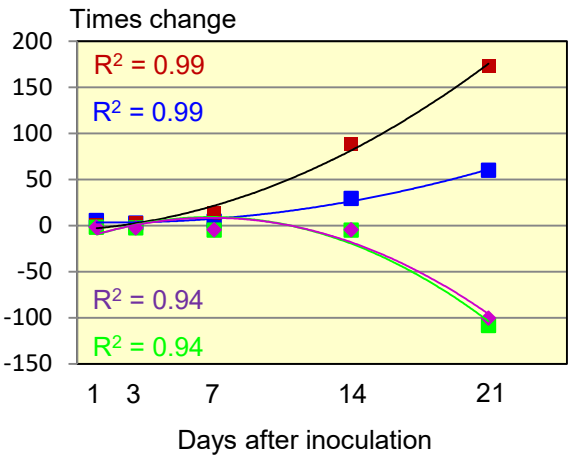

Figure 3-37. The ratio treatment/control of upregulated and downregulated gene expression of 4 genes in U. minor *inoculated with an aggressive strain of* Ophiostoma novo-ulmi. *Brown = Early nodulin-55-2-like, blue = phenylalanine ammonia-lysase-like, lilac = fasciclin-like AGP 15 family protein, green = fasciclin-like AGP 15 family protein. Perdiguero et al. 2017.*

The corresponding range for downregulated DEGs was 30–170 times lower. The development over time, 1–21 days after inoculation, for 2 upregulated and 2 downregulated DEGs with observed differences during this period are illustrated in Fig. 3-37. It is noteworthy that already one day after inoculation responses were noted in the sampled material approximately 2 metres from the inoculation wound.

It is beyond the scope of this survey to discuss all the physiologically complex interactions noted following inoculations. Briefly, DEGs observed in this project were compared with known genes in data bases, which revealed that there were genes involved in:

Perception
Signal transduction
Defence
Transduction factors

This investigation is an important contribution for the understanding of molecular mechanisms in elm responses to DED infection, which has led to presentation of a model for processes initiated by inoculation of *Ophiostoma novo-ulmi*.

The previous assignment of 23 elms on the Isle of Man as English elms was revised following morphological and molecular studies (Coleman et al. 2015). Rather they were classified as *U. hollandica.* The slow spread of DED on the island was attributed to unfavorable conditions for the *Scolytus* beetles.

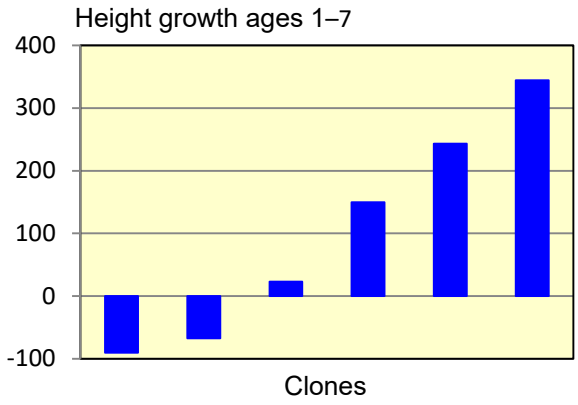

Figure 3-38. Height growth increment over ages 1–7 following inoculations with Ophiostoma novo-ulmi *of 6 clones. The highest increments were noted for the cultivar Prospector (*U. wilsoniana*) and Valley Forge (*U. americana*). The negative growth of two American elm clones was attributed to sustained crown dieback. Townsend and Douglas 2001.*

Two susceptible *U. minor* clones were included in a study of the effect of phenol treatment on DED by Martin et al. (2008b). Four treatments were applied:

Control
Phenol-treated
Ophiostoma novo-ulmi inoculation
Phenol-treated + *Ophiostoma novo-ulmi* inoculation

Since no significant differences between the 2 clones were noted we shall only mention that wilting was significantly higher in the control material than in the three other treatments. The inoculated treatments with phenols had significantly lower wilting percentages than the treatment without phenol.

3.1.2 Studies oriented towards breeding

With the objective of identification of new American elm clones for the market a field trial with 8 American elm clones and 2 non-American clones (*U. wilsoniana* and a hybrid *U. carpinifolia x U. parvifolia*) was established in Maryland USA (Townsend and Douglas (2001). The trial was designated as *randomized block, split-plot design with seven blocks* with four trees per block as long as ramets were available. Inoculations with *Ophiostoma novo-ulmi* of half of the ramets took place on May 18 and the other half on May 27. Dieback, survival, and height increment were followed up to age seven.

There were strongly significant clonal differences every year. Height increment was reported for years 1 to 7 and also for this trait strongly significant clonal differen-

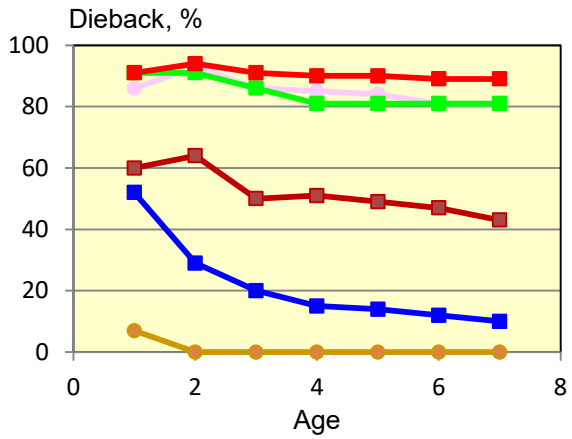

Figure 3-39. Percentage dieback in six clones after inoculation with Ophiostoma novo-ulmi. *Orange-brown circles = cultivar Prospector. Blue squares = cultivar Princeton. Other symbols =* U. americana *selections. Townsend and Douglas 2001.*

ces were noted (Fig.3-38). The negative growth of two American selections was attributed to repeated dieback throughout the test period. In Fig. 3-39 we have illustrated the development over time for six clones, three of them being highly susceptible, two intermediate, and the *U. wilsoniana* clone with good resistance. The authors stressed the importance of studies over several years since some clones might recover, which is particularly expressed by the Princeton cultivar (blue squares). The ability to recover was evidently dependent on the clone.
The relationship between individual tree height before inoculation and dieback years 1–7 showed significances in 23 of the 100 relationships. For the American Liberty clone, significances were noted for all 7 years. However, none of the 23 correlations explained more than 50% of the co-variation.
The take-home message from this investigation is that tests for resistance to *Ophiostoma novo-ulmi* should be followed over longer periods than one year.

Another investigation with the same objective included 19 selected clones, 6 cultivars, American elm seedlings, and two non-American elm selections, *U. carpinifolia* and a hybrid [*U. glabra x (U. wallichiana x U. carpinifolia)*] in a field trial, which was established in Maryland, USA in April 1993 by Townsend et al. (2005). The design of the trial was 7 blocks with four trees per clone in each block. Tree height was measured after nine years in the field before inoculation with a mixture of 2 strains of each of *Ophiostoma ulmi* and *O. novo-ulmi.* Wilting was assessed at four weeks after inoculation and dieback was determined one and two years after inoculation.
There were highly significant differences in symptoms among the American clones. The range of dieback at one year after inoculation was 0–58%. There was some

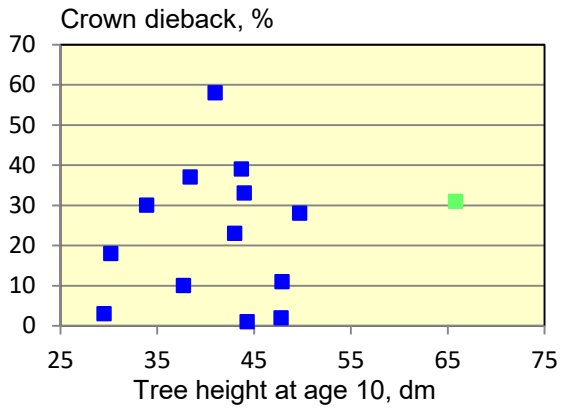

Figure 3-40. Tree height at age 9 in the field and dieback at age 11 after inoculation with a mixed inoculum of two strains from each of Ophiostoma novo-ulmi *and* O. ulmi *of 19* U. americana *clones (blue) and* U. americana *seedlings (green) studied in a field trial in Maryland, USA. Townsend et al. 2005.*

recovery between years one and two and the range was 0–42%. The relationship between tree height before inoculation and dieback at age one is illustrated in Fig. 3-40. There was no relationship between these two traits. Nor was there any relationship between flushing and dieback. There were four clones with 10% or less dieback, which means that these clones might be marketed and included in further breeding. It should be added that superior cultivars also performed well in this trial. So did the two non-American elm selections, no dieback at all.

The taxonomic state of 43 elm accessions (clones) was assessed by AFLP analysis by Pooler and Townsend (2005). Of particular interest was to identify the parents of one clone 3487, which was selected among 600 elm trees planted in the mid-1930s in Washington, DC for its good resistance to DED and was marketed as Jefferson. Also, *U. americana, U. laevis, U. parvifolia, U. carpinifolia, U. rubra, U. pumila, U. bergmanniana,* and *U. szechunicana* as well as some interspecific hybrids were represented in this investigation.
Five AFLP selective primer pairs were used with 135 polymorphic markers. The UPGMA dendrogram indicated that *U. americana* and *U. laevis* constituted one group separated from the other species and species hybrids. It was noted that the American elm group was very variable, which at least partly could be attributed to the tetraploidy of American elms. The analysis showed that clone 3487 was an American elm clone and not any interspecific hybrid. There were no unique markers in 3487, not occurring in other American elm clones.
Finally, it was stated that AFLP analysis is a useful technique for identification of the taxa of elm trees.

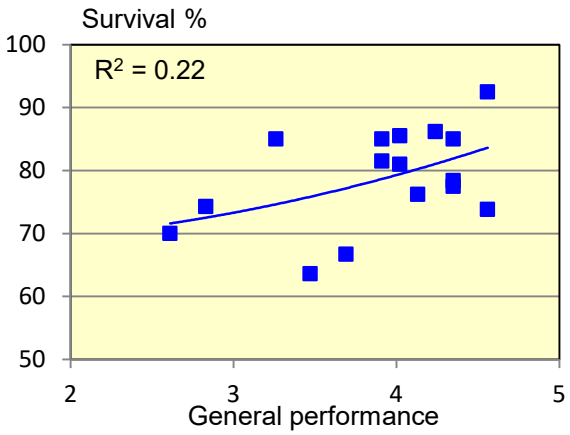

Figure 3-41. The relationship between cultivar general performance (1–5) and mean survival at 16 test localities in USA of DED-resistant cultivars. Griffin et al 2017.

A national elm trial in USA was initiated by Griffin et al. (2017) to demonstrate the performance of 16 DED-resistant elm cultivars at 16 widely distributed test localities. The experimental design was 5 blocks with 1 tree per cultivar in each block. Growth traits, survival, pests, diseases, and horticultural values were evaluated at age 10 (general performance). Mostly, only mean values based on the performance at all localities were presented. With such a wide distribution of test localities, clone x site interaction is highly likely, which means that grand means are of less value.

We have preferred to present the relationship between two important traits, general performance and survival, in Fig. 3-41. Cultivar Morton Red Tip was the best performing with respect to these two traits. It also showed good growth. In addition to the cultivars shown in Fig. 3-41, three clones with low survival (25.7–55.0%) were included in the national elm trial. The low number of trees per test locality means some imprecision in the results.

Within the elm breeding program in Tuscany, Italy, the performance of 24 selected clones was studied in three Italian field trials with strongly different ambient conditions (Santini et al. 2010). There was focus on identification of clones that could be patented and marketed. Two-year-old rooted cuttings were planted in year 2000 at the following localities:

Feudozzo (F)	41.75°N, 14.42°E, 960 masl
Marsiliana (M)	43.02°N, 10.80°E, 300 masl
Castellaccio (C)	42.97°N, 12.60°E, 192 masl

Each clone was represented by 12 cuttings per trial. The design was complete randomization. Tree height and DBH (1 m above ground) were measured during 2001–2009. Annual height growth was calculated for years 2005–2008.

Ecovalence (w^2) according to Wricke (1962) was calculated. A low ecovalence means a stable performance across sites. In addition, rank-based stability measures were also calculated.

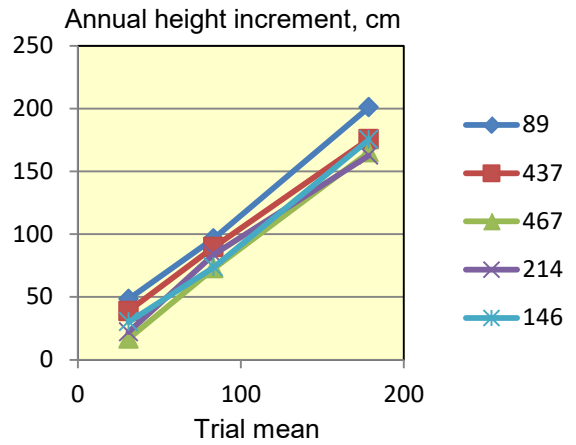

Figure 3-42. Finlay-Wilkinson diagram for height increment in the 5 hybrid elm clones with lowest ecovalences based on studies at three Italian localities with strongly varying ambient conditions. Santini et al. 2010.

No DED infections were observed until 2010. The mean annual height increment in the three trials varied between 40 cm in the F trial and 178 cm in the C trial. The corresponding values for diameter increment were 0.4 cm and 2.6 cm. These results are a reflection of the different environmental conditions at the trials.

We have illustrated the annual height increments for the five clones with the lowest and highest ecovalence in Finlay-Wilkinson diagrams (Figs. 3-42–3-43). There is a considerable difference in performance of the two groups of clones. In Fig. 3-42 almost straight lines were obtained for the relationships. In contrast, some of the relationships for the other group of clones deviate from straight lines (Fig. 3-43). This figure reveals that the growth at the M trial contributed most to the high ecovalences. The corresponding relationships for diameter growth for the same ten clones are presented in Figs. 3-44–3-45. The low ecovalence clones showed less straightness than for height increment but substantial deviation from straight lines did not occur. The high ecovalence clones showed lar-

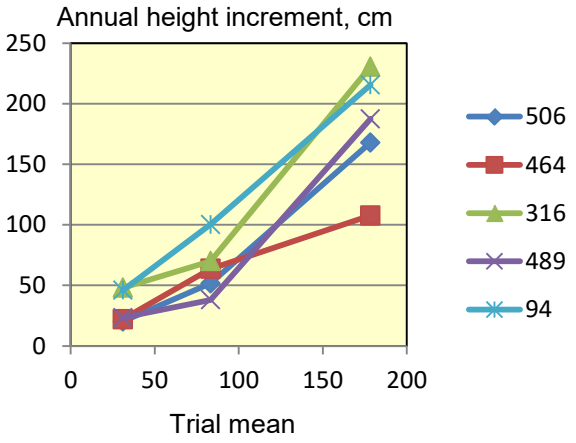

Figure 3-43. Finlay-Wilkinson diagram for height increment in the 5 hybrid elm clones with highest ecovalences based on studies at three Italian localities with strongly varying ambient conditions. Santini et al. 2010.

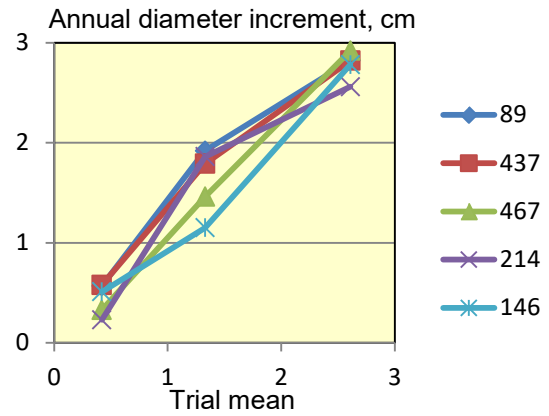

Figure 3-44. Finlay-Wilkinson diagram for annual diameter increment in the 5 hybrid elm clones with lowest ecovalences based on studies at three Italian localities with strongly varying ambient conditions. Santini et al. 2010.

ger deviations from straight lines, particularly clones 489 and 506. As judged from Fig. 3-43, clone 94 had an astonishingly high ecovalence estimate for height increment. There was a negative relationship between diameter increment and the coefficient of variation, explaining approximately 33% of the variation. There were significant relationships between ecovalence estimates and rank-based stability estimates (height and diameter increment). However, the degree of explanation for these relationships was less than 20%.

From a commercial point of view the ideal clone should have a low ecovalence and good growth, i.e. a generalist with good performance independent of site conditions. Clones 89 and 94 match these requirements best. Clone 316 had one of the lowest ecovalence estimates as regards diameter increment and would be attractive for marketing.

Several well performing clones resistant to DED were identified, which could be used for wood production as well as for ornamental purposes.

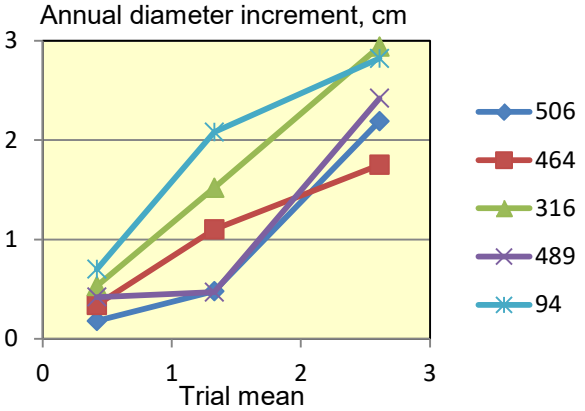

Figure 3-45. Finlay-Wilkinson diagram for annual diameter increment in the 5 hybrid elm clones with highest ecovalences ina study at three Italian localities with strongly varying ambient conditions. Santini et al. 2010.

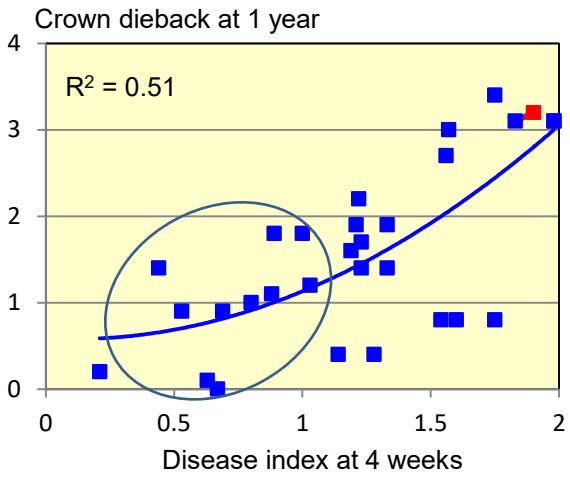

Figure 3-40. The relationship between disease index 4 weeks after inoculation and crown dieback one year after inoculation of 28 clones in a Dutch clone trial. The encircled clones are unreleased. Red square is U. laevis *seedlings, This figure is based on data in Table S1 in the paper. Buiteveld et al. 2016.*

Buiteveld et al. (2016) reported on the effects of DED inoculations of 18 cultivars and 10 new unreleased selections. All 28 clones are hybrids. In addition, 2 reference clones with known susceptibility were included. Seedlings of *U. laevis* were also included in this experiment. A trial with nine blocks with four-tree plots was established. Four inocculations were carried out with one ramet per block at each time. One inoculation failed. Evaluations of defoliation and disease index took place 4 and 8 weeks after inocculation as well as after one year. Nine classes (0–4) were used for disease index considering the extent of wilting and twig dieback of the trees. Recovery from disease was also recorded. Since many ambient conditions might influence the results of inoculations it was pointed out that it was important to establish one trial with many cultivars and clones to eliminate uncertainties from independent studies with a limited number of entries.

There was a large variation in disease index at 4 weeks after inoculation and crown dieback one year after inoculation (Fig. 3-40). The average percentage of defoliation at 4 weeks after inoculation varied in the range 0–60.4%. The corresponding range for defoliation one year after inoculation was 5–90.8%. Fig. 3-40. shows that two of the unreleased clones recovered from the initial damage. The other five clones that recovered were hybrids with American origin.

It was pointed out that the severe inoculation in this study was intended to enable a high resolution among the clones tested and not to predict field resistance. The high susceptibility of *U. laevis* seedlings compared to low field susceptibility was attributed to the low attraction of this species to *Scolytus* beetles under field conditions.

It was concluded that many clones showed a good to excellent resistance to DED to the benefit for planting of elms in The Netherlands.

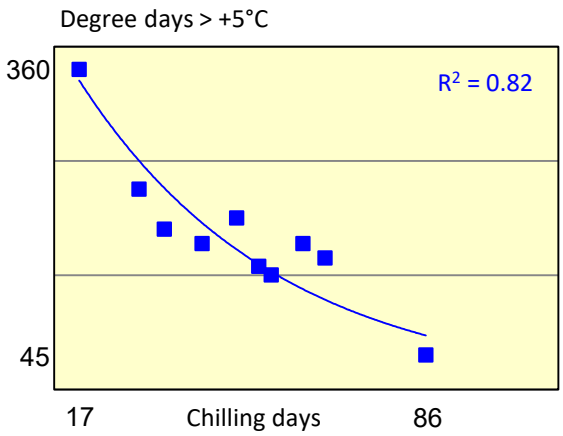

Figure 3-47. The relationship between chilling days and degree days to bud flushing of U. glabra *clones studied in six trials in Belgium, France (2), Germany, Italy, and Spain. Threshold temperature +5°C. The coordinates were read from Fig. 4 in the paper. Santini et al. 2004b.*

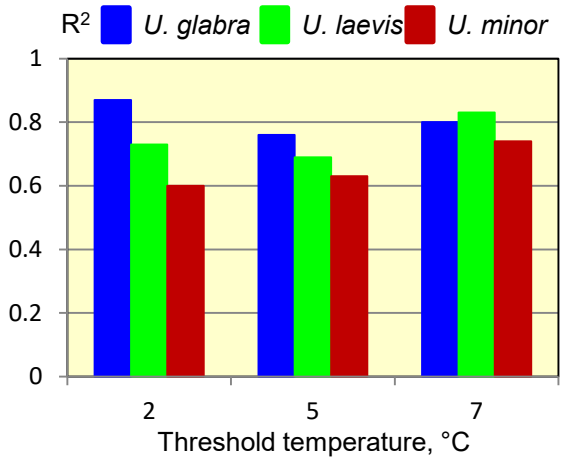

Figure 3-48. The degree of explanation for the fit to inverse exponential relationships between bud flushing and chilling plus thermal time with different threshold temperatures, 2, 5, and 7°C. The study was carried out in six trials in Belgium, France (2), Germany, Italy, and Spain. Santini et al. 2004b.

3.2 Phenology and growth

3.2.1 Basic studies

Santini et al (2004b) presented data on bud flushing of *U. glabra, U. laevis* and *U. minor* recorded in six trials in Belgium, France (2), Germany, Italy, and Spain. A 5-degree scale was used for the weekly recordings of bud flushing. Stage 3 = bud scales open and the first leaves are just visible. When half of the buds had reached this stage it was defined as bud flushing. There was no strong relationship between thermal time and bud flushing for any of the three species. Therefore, chilling to break dormancy had to be considered besides thermal time. Since efforts have been made to describe bud flushing by an inverse exponential function including thermal time and chilling (Cannell and Smith 1983) this function was tested:

$$DD = a + b.e^{rCD}$$

in which DD, degree days, is the number of days >+5°C, CD is the number of chill days <5°C, and a, b, and r are constants. The fit of the data obtained to this function was tested using 2, 5, and 7°C as threshold temperatures. Separate tests were used with hourly and daily temperatures. A wide variation among clones with respect to bud flushing at each test locality and year was noted, which was not surprising with the wide origin of the clones within the individual species:

U. glabra	23–39 days
U. laevis	10–32 days
U. minor	15–52 days

It was noted that the lowest ranges were found for clones from the same regions. To get an idea about the relationship between chilling days and degree days to bud flushing we read the coordinates for *U. glabra* in Fig. 4 in the paper. The inverse exponential relationship is clearly seen in Fig. 3-47.

In Fig. 3-48 the explained variance for the relationship for different species and threshold temperatures is illustrated. An especially high percentage is shown for *U. glabra* at a threshold temperature of +2°C, $R^2 = 0.87$. Using hourly instead of daily temperature recordings was also tested, which did not result in any large difference from the functions using daily temperature recordings. At low level of chilling *U. minor* clones flushed at lower temperature sums than *U. glabra* and *U. laevis* clones.

The summation to degree days is linear; as an example the increase from 2 to 3°C has the same value as the increase from 9 to 10°C. If there is an exponential increase of the effect by increasing temperatures, the summation of degree days might not capture the real effect of temperature on bud flushing. However, until a non-linear temperature effect is proven the summation is probably the best proxy for this type of study.

In conclusion, the inverse exponential function including thermal time and chilling for bud flushing described the bud flushing well for *U. glabra* and *U. laevis*.

Gheraldini et al (2006) recorded bud flushing in U. minor clones in three trials, two in France, and one in Italy, with the objective to study variation in bud flushing of this species in Europe. Details about the trials are shown below:

Nogent-sur-Vernisson, France; lat. 47.85°N, long. 2.75°E, 130 masl, 118 clones from a large part of Europe.

Guémené-Penfao, France; lat. 47.54°N, long. 1.84°W, 15 masl, 4 clones from each of northern and southern France.

Antella, Italy; lat.43.72°N, long. 11°E, 170 masl. Year 2000 and 2001, 65 clones from Italy and France; in year 2003 and 2004, 272 clones from Italy, France and Spain.

Recording of bud flushing was carried out weekly with a 3-degree scale during February–May. When 50% of the buds of a tree had open bud scales it was defined as bud

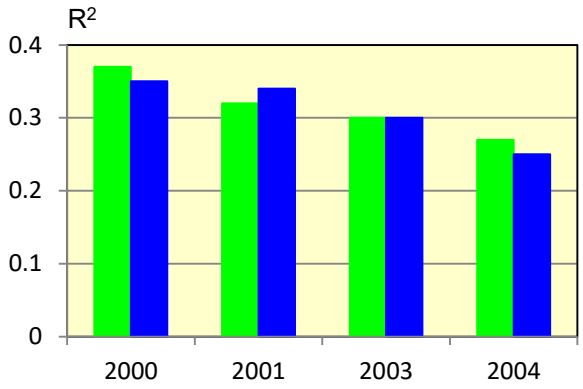

Figure 3-49. Degree of explanation, R^2, obtained from multiple regressions involving days (green) and temperature sum (blue) for bud flushing of U. minor clones *related to latitude at clone origin. All relationships are positive. Recordings were carried out over four years in an Italian trial. Gheraldini et al. 2006.*

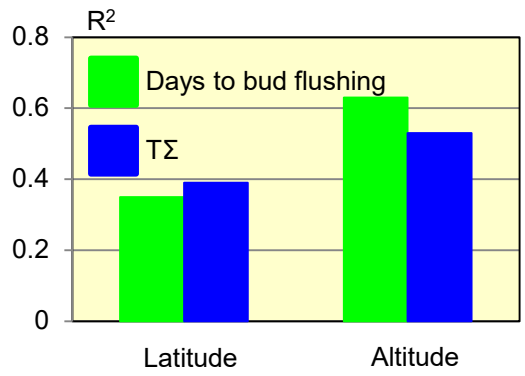

Figure 3-50. Degree of explanation, R^2, obtained from multiple regressions involving days to bud flushing (green) and temperature sum (blue) for bud flushing of U, minor clones *related to latitude or altitude at clone origin. All relationships are positive. Recordings were carried out during four years in a French trial. Gheraldini et al. 2006.*

flushing. Chilling temperature was calculated from November 1st until flushing with temperatures below +5°C. The degree days calculation for temperature required for bud flushing was started on February 1 with +5°C as threshold temperature. The clones in the Italian trial were separated into four groups:

Northern France
Southern France
Northern Italy
Southern Italy

The obtained results were related to latitude. Based on the French 47.85°N trial a relationship with altitude was estimated for 11 clones from the same region. They were selected to overcome the problem of no high-elevation clones at certain latitudes. ANOVAs were run in which groups of clones were pooled according to geographic origin.

Table 3-4. The significances for different effects on bud flushing determined by temperature sum, TΣ, in degree days (>+5°C) or total days to bud flushing. Gheraldini et al. 2006.

	French trials	*Italian trial*
TΣ		
Group ***G***	***	***
Chilling duration ***D***	***	***
G x D	*ns*	***
Total days to bud flushing		
Group ***G***	***	***
Year ***Y***	***	***
G x Y	*ns*	***

The statistical evaluation of the results is summarized in Table 3-4, which shows strongly significant differentiation among the groups both for total days to bud flushing and temperature sums. The interactions group x chilling days and group x year were significant in the Italian trial while no significances for these two interactions were found for the French trials. Nor was there any significance for the 2 French trials including site in the interactions. The strongly significant effect ($p<0.001$) of year on bud flushing date suggest that temperature conditions influence the bud flushing more than photoperiod.

There were significant relationships between latitude and total days to bud flushing or temperature sum for bud flushing during the four years of recordings in the Italian trial (Fig. 3-49). However, the R^2 did not exceed 0.40 in any year. The only R^2 >0.50 was noted for the relationship between altitude and total days to bud flushing for the French 47.85°N trial (Fig. 3-50). The low R^2s must be attributed to large variation among clones within latitudes. Since the relationship with altitude comprised clones from a limited geographic region, these two relationships were based on more homogeneous conditions with respect to macroclimatic conditions, thus avoiding disturbing environmental conditions.

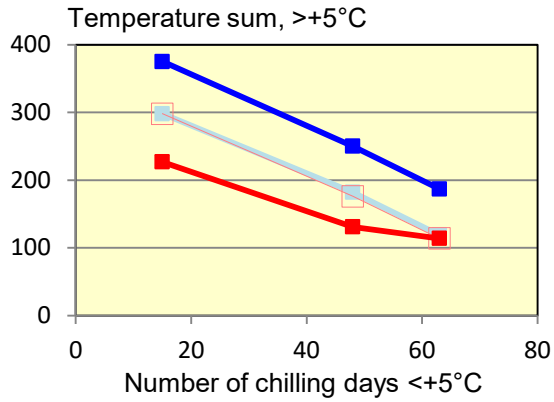

Figure 3-51. The relationship between chilling days and temperature sum in degree days at bud flushing (+5°C as threshold temperature) for 4 groups of U. minor *clones studied in an Italian trial.*
Blue = northern France
Light blue = southern France
Red = southern Italy and light red = northern Italy.
The data for southern France and northern Italy are almost identical. Gheraldini et al. 2006.

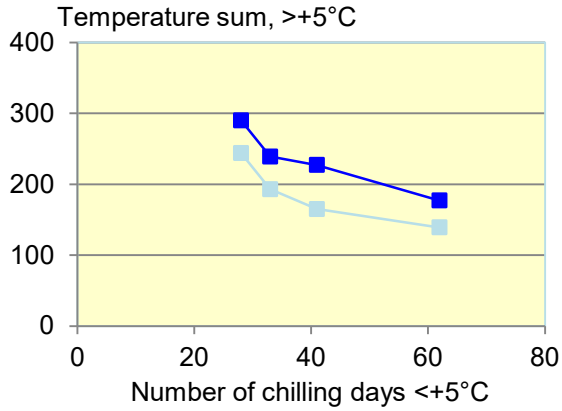

Figure 3-52. The relationship between chilling days and temperature sum in degree days at bud flushing (+5°C as threshold temperature) for two groups of U. minor *clones studied in 2 French trials.*
Blue = northern France
Light blue = southern France
Gheraldini et al. 2006.

The bud flushing was dependent on the number of chilling days (Fig. 3-51). The southern French clones and the northern Italian clones originate from the same latitudes and their curves are almost identical (Fig. 3-51). With high number of chilling days only the northern French clones differ from the other three groups of clones. In year 2004 the same difference between the northern French group of clones and the three other groups was noted for total days to bud flushing. Generally, there was a strong relationship between total days to bud flushing and temperature sum to bud flushing. The same difference between the two French groups was observed in the French 47.85°N trial (Fig. 3-52). These figures also show that the longer the chilling, the earlier the bud flushing. It seems as if temperature to some degree compensates for a limited chilling.

In conclusion, high latitude and high altitude *U. minor* populations have a later bud flushing than southern and low elevation populations. Two explanations were given for these results:

The chilling requirement for breaking of dormancy is greater in high latitude and high elevation populations and this requirement is rarely reached at southern latitudes for high latitude clones.

At southern latitudes there is an advantage for southern populations to start the growth early, before summer drought appears, which is an adaptation to the Mediterranean climate. These results agree with the early bud flushing in *Castanea sativa* populations from southern Spain with its Mediterranean climate of dry summers (Fernández-López et al. 2005a).

An extension of the above study was presented by Ghelardini and Santini (2009), in which modeling of bud flushing was treated, partly based on published data. Several results were presented in earlier papers. The questions particularly raised in this report were:

Is there a relationship between spring phenology and DED susceptibility?

Can phenology differences be related to temperature?

Is there a relationship between bud flushing in *U. minor* and geographic origin?

Rooted cuttings of *U. glabra, U. laevis, U. minor, U. pumila, U. minor x U. glabra,* and *U. minor x U. pumila* were included in this study together with one resistant clone, Lobel, and one susceptible clone, Commelin. The study comprised 6 test localities in Germany, Belgium, France (2), Spain, and Italy.

A large variation in susceptibility to DED was observed. Susceptible clones showed continuous increase of symptoms. During the second year recurrence of disease symptoms were frequent in susceptible clones. Flushing of dormant secondary buds explained the relative resistance against DED in some clones. It was found that maximum susceptibility in European elms starts around 40–50 days after bud flushing. The duration of this maximum varies according to genotypes and ambient conditions. It was speculated that the avoidance of DED infection may partly be attributed to an earlier transition from spring wood to late wood.

For the Italian trial at Antella (lat.43.72°N, long 11.37°E, 170 masl) the dependence of mean defoliation on mean clonal latitude or total days to bud flushing were both positive and strongly significant: R^2 0.99 and 0.74, respectively. However, the input of data about these relationships was not explicitly presented. In the latitudinal relationship there were 6 clonal mean latitudes included while there were 14 days included in the second relationship.

Efforts have been made to describe bud flushing by an inverse exponential function including thermal time and chilling (Cannell and Smith 1983):

$DD = a + b.e^{rCD}$

in which DD is degree days>+5°C, CD is the number of chill days <5°C, and a, b, and r are constants. The data obtained were fitted into this function including 2°C and 7°C as critical temperatures. It turned out that 2°C gave the best fit for *U. glabra* while 7°C gave the best fit for *U. laevis* and *U. minor.* The degrees of explanation by the model for the individual species were:

U. glabra	89.0 %
U. laevis	83.2 %
U. minor	62.9 %

Thus, good fit to the function for *U. glabra* and *U. laevis*. This function also suggested that bud flushing will take place earlier with global warming even in the southern parts of elm distribution, in which chilling will be harder to reach. This condition will be compensated for by the increase of spring temperatures.

There was limited difference in bud flushing among years within sites. Besides the impact of chilling and thermal time on bud flushing, this limited difference was interpreted as a role of photoperiod on bud flushing as well.

It was pointed out that different critical temperatures should be used for different species. It was concluded that European elms have relatively low chilling requirements for dormancy release, their terminal time requirement to bud burst becoming steady when chilling exceeds 100 chill days. Bud flushing before the peak activity of the *Scolytus* beetle might be one remedy to avoid DED infections.

Gheraldini et al. (2010) studied dormancy release and bud burst in one clone from each of *U. glabra, U. macrocarpa, U. minor, U. parvifolia, U. pumila,* and *U. villosa*. This study was carried out in growth chambers. Since results from one clone per species cannot lead to far-reaching conclusions about species differences we shall only briefly report the main results:

Photoperiod had no effect on bud burst date

Dormancy was most pronounced in *U. minor* and least pronounced in *U. pumila*

The variation in temperature sum for bud burst *was efficiently explained by the inverse function of the number of chill days <5C received outdoors during autumn and winter* (cf Santini et al. 2004 above).

The objective of a Chinese study of eight *U. pumila* clones and one hybrid clone by Mu et al. (2016) was to estimate their variability in salt tolerance and the mechanisms of the regulation of salt tolerance. An ultimate goal was to find material for salt-affected areas. The kind of hybrid was not presented but it was stated that the 9 clones were of *U. pumila* (p 188). These clones were selected among 56 clones with proven insect and disease resistance as well as good growth in long-time testing in Shandong, China. The clones were selected based on low mortality in preliminary tests with 0.7% NaCl. The plantlets in this study were obtained from repeated (5–6 x) tissue culture propagation. A salt mix of NaCl, $CaCl_2$, $K2SO_4$, $MgSO_4$, $NaHCO_3$ in proportions 60/15/15/7/3 was used in one experiment, and with NaCl in another experiment. The mixed treatment should mimic the sea water salt concentration. In both experiments the salt concentrations varied in the range 0–0.9%. The salt treatment lasted for two weeks. Shoot growth and branching were recorded. In addition chlorophyll, proline, sugars, and protein were assessed. The activities of superoxide dismutase (SOD), peroxidase (POD), malondialdehyde (MDA), and catalase (CAT) were determined.

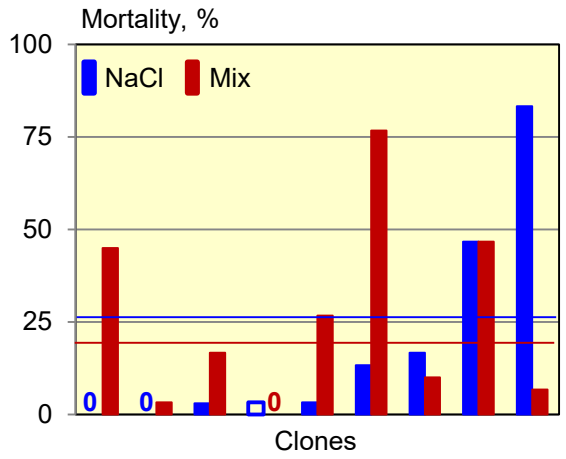

Fig 3-53. Mortality percentage after 2 weeks treatment with a salt mix and NaCl at 0.8% concentration of 8 U. pumila *clones and one hybrid clone (empty column). Mean values for the two treatments are indicated. For the components of salt mix see text.* Mu et al. 2016.

To get a general picture of salt tolerance a so-called membership function was calculated. This function was described in the following way: *The membership function of a fuzzy set is a generalization of the indicator function in classical sets; it represents the degree of truth as an extension of valuation (Chen et al. 2012; Mi et al. 2013).*

Mortality was first noted at 0.5% in the mixed salt treatment and at 0.7% in the NaCl treatment. The best resolution among the clones is at 50% mortality, which was noted for both treatments at 0.9 salt concentrations (50.3 and 50%). Fig 3-53 shows a substantial difference in plantlet mortality in both treatments and a considerable clone x treatment interaction. The hybrid clone had the lowest mortality in both treatments. This clone had the highest branching rates in the 0.8 and 0.9% mixed salt treatment. A large variation in mortality differentiation was also noted at the 0.8% concentration in both salt treatments.

All clones had longer shoots in the 0.3% NaCl treatment than in the control, suggesting growth stimulation from this treatment. Three clones had longer shoots in the 0.3% mixed salt treatment than in the control, two of them being significantly longer. The difference in response to the two salt treatments was attributed to $NaHCO_3$ in the mixed salt treatment.

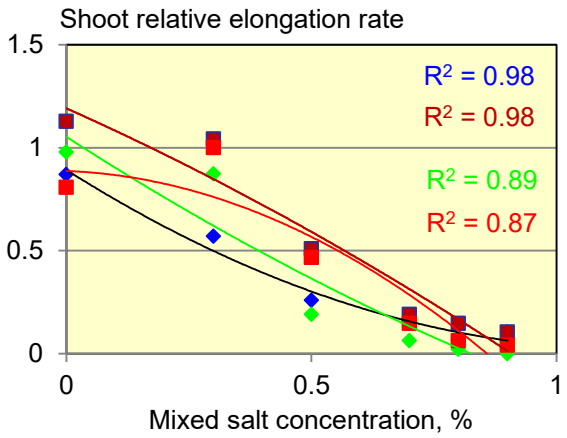

Fig 3-54. The relationship between mixed salt (see text for information) concentration in percentage and shoot relative elongation rate in 4 U. pumila *clones treated over 2 weeks. Mu et al. 2016.*

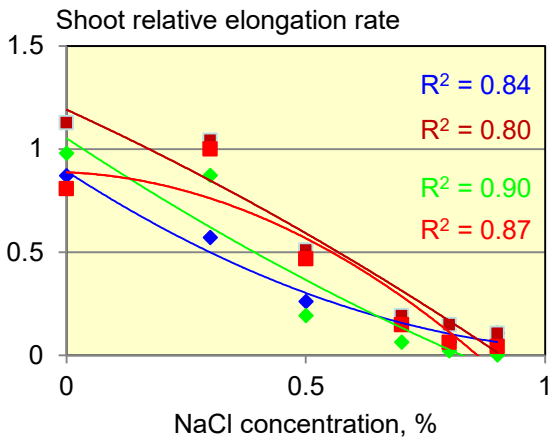

Fig 3-55. The relationship between NaCl concentration in percent and shoot relative elongation rate in 4 U. pumila *clones treated over two weeks. Mu et al. 2016.*

We have preferred to illustrate the relationship between treatment and shoot length for one clone with good growth and one clone with poor growth and 2 clones with intermediate growth in Figs 3-54–3-55. These figures show good fit to the second degree polynomial curves for all 4 clones in both salt treatments.

Based on dose response relationships for the various compounds illustrated in the paper, we have tried to synthesize the main findings in Table 3-5. Mainly there was agreement between the two salt treatments. With respect to the increased maladialdehyde response two groups of clones were noted in the NaCl treatment while no grouping was observed in the mixed salt treatment. The authors added that the plantlets probably responded to increased salt by increasing the production of proline, which acts as osmotic regulator and scavenger of free radicals as well as a stabilizer of sub-cellular structures. As regards chlorophyll there was a decline with increased salt concentration in both treatments but with varying strength of the decline among clones. The decline was less in the most salt tolerant clones. There was a trend of decline in soluble protein in the mixed salt treatment but there was much up and down in most of the relationships. In the NaCl treatment there was a regular decline for soluble proteins. In the NaCl treatment the hybrid clone had a much higher maximum content of sugar than the other clones. It was speculated that sugar had a similar role as proline as an osmotic regulator. In the mixed salt treatment the hybrid clone shared a separate maximum with another clone. No dramatic differences between the two treatments were noted for free proline. The relationships for the three enzymes showed pronounced difference as regards the peak of the relationships, with 1–3 clones peaking later than the other clones. The hybrid clone was among the deviating clones.

Table 3-5. Functions of a number of compounds studied and the effects of them in NaCl and mixed salt treatment in a study including 8 U. pumila *clones and one interspecific hybrid clone. Mu et al. 2016,*

Compound	*Function*	*NaCl treatment*	*Mixed salt treatment*
Maladialdehyde	State of membarane stability	Increase with salt concentration	
Chlorophyll	Photosynthesis	Decrease with salt concentration	
Soluble protein		Decrease with salt concentration	Erratic pattern
Sugar	Osmotic regulation	Flat maximum 0.3–0.7%	Flat but variable maximum
Free proline	Osmotic regulation	Maxmum at 0.7%–0.8% salt concntration, sharp drop to 0.9%	
Superoxide dismutase SOD	Antioxidant enzyme, defence against oxidative stress	Maximum at 0.7%, at 0.8% for the hybrid clone	
Peroxidase POD	Antioxidant enzyme, defence against oxidative stress	Maximum at 0.7%, at 0.8% for 2 clones	
Catalase CAT	Antioxidant enzyme, defence against oxidative stress	Maximum at 0.7%, at 0.8% for 2 clones	Maximum at 0.7%, at 0.8% for 3 clones

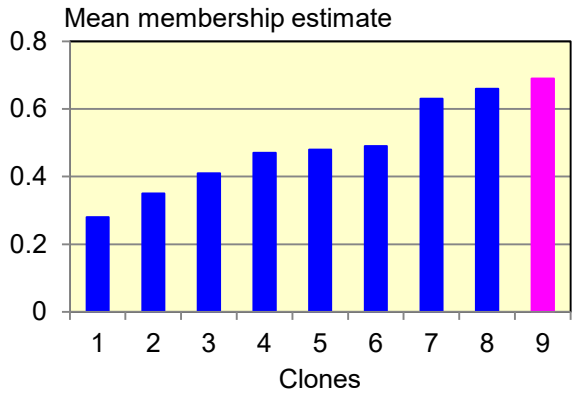

Fig 3-56. Mean membership estimates (0-1) of 8 U. pumila *clones and one hybrid clone tested in 2 salt treatments, NaCl and mixed salt, with 0–0.9% concentrations. The estimates are based on 3 growth traits and the 8 traits in Table 3-5. No 9 is the hybrid clone. Mu et al. 2016.*

The variation in the membership estimation is visualized in Fig. 3-56, which shows that three clones passed 0.60. The highest estimate (0.69) was noted for the hybrid clone, No 9, in the figure. Its superiority emanates from its performance in the two highest salt concentration treatments as can be seen in Fig. 3-57. If this superior performance lasts over the rotation time it would be an excellent clone for salt-contaminated ground. It was stated that the study had developed a method for *in vitro* selection for application in breeding salt-tolerant Siberian

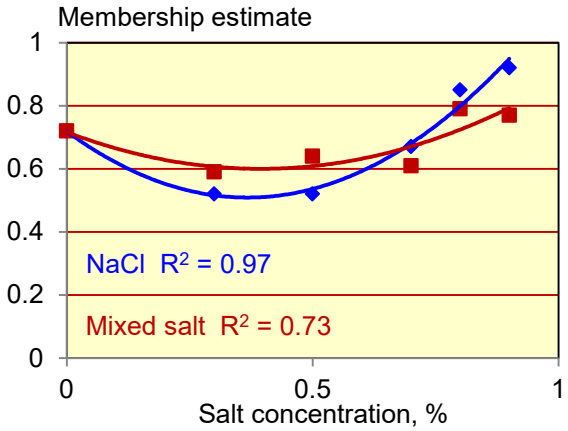

Fig 3-57. The relationship between salt concentration in two treatments, NaCl and mixed salt, and membership estimates (0-1) for the hybrid clone. Mu et al. 2016.

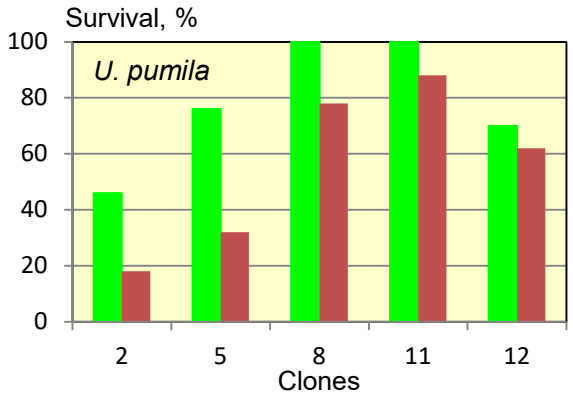

Figure 3-58. Survival of 5 U. pumila *cultivars cultivated on 150 (green) and 200 (brown) mMol NaCl for 6 months. Chen et al. 2021.*

elm. This statement must be regarded as premature until we know more about long-time performance of the earlier selected clones.

In conclusion, low levels of NaCl stimulated plantlet growth while the same level of mixed salt stimulated growth in just a few clones. The most salt-tolerant clones had a better osmoregulation and higher antioxidant enzyme activities than salt-sensitive clones.

Chen. et al (2021) studied salt (NaCl) tolerance in five Chinese *U. pumila* cultivars as well as the underlying physiological mechanisms leading to tolerance. A 6-month-long greenhouse experiment with three replications of four treatments, 0, 100, 150, and 200 mM NaCl solutions, was carried out. The salt solution of 1.5 liters was applied every 15 days. Each cultivar was represented by 50 plants in each treatment. A detailed physiological genetics study was carried out with the most salt-tolerant cultivar (No 11) after treatment with 150 mM NaCl solution. The variables studied are listed in Table 3-6, in which the performance of the cultivars during the first three and last three months of treatment is summarized.

All cultivars had 100% survival at the end of the experiment at six months after start of the experiment in control and 100 mM NaCl solution. At the two highest salt treatments, 150 and 200 mM NaCl solution, the survival of the cultivars varied significantly (Fig.3-58). The growth rate of biomass peaked in August four months after onset of treatment in control and 100 mM NaCl. There was a drop in growth from July and from the beginning of the experiment in treatments 150 mM and 200 mM, respectively.

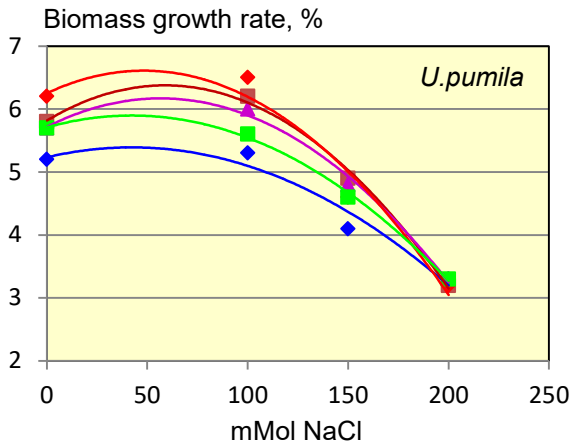

Figure 3-59. The relationship between NaCl solution treatment and biomass growth rate at 4 months (August) after onset of treatment for 5 U. pumila *cultivars. R^2 varied in the range 0.95–0.99 for the 5 relationships. Chen et al. 2021.*

We have illustrated the relationship between treatment and biomass growth rate at four months in Fig. 3-59, which shows exceptionally good fits to the second degree polynomial curves. Cultivar No 11 (red) shows the best growth rate in control and in the 100 mM treatments. There was a slight indication that low salinity might promote growth. Four cultivars had slightly higher biomass growth rate in the 100 mM treatment than in the control. It was suggested that accumulation of Cl^- ions might explain the reduction in growth during the latter part of the experiment. For several of the physiology variables studied in cultivar No 11 at 150 mM there was a shift in performance after three months. Therefore, the response during the first 3 months and the last 3 months is separated in Table 3-6.
Soluble sugar, soluble proteins, and proline are good osmolytes, and for this reason they occur in higher amounts in the salt treatment. The higher amount of malondialdehyde indicates that peroxidation of lipid membrane takes place.
It was noted that salt stress caused an increase of light saturation point and light compensation point during the initial months of salt treatment. This indicates that an increase of both these traits leads to increased use of light. The higher dark respiration rates caused stress-resistant energy consumption. The increased photosynthesis rate in May–July was partly attributed to the observed higher apparent quantum yield of photosynthesis during this period. During August–October the light utilization capacity and efficiency dropped markedly.
The water use efficiency was higher in the salt treatment during the entire growth period, while the transpiration rate showed an opposite trend.
There were large numbers of differentially expressed unigenes (DEG) in the salt treatment and a substantial variation of this number dependent on the sampling time during the day; with a maximum of DEGs at 15.00. Generally the identified DEGs are related to starch production (photosynthesis) and carbon metabolism. This paper has a detailed and deep discussion of plant physiological processes, which is beyond the scope of this presentation but it opens up for a deeper understanding of the role of various compounds and processes regulating growth under stressful conditions.
It is obvious that the large number of DEGs expressed under saline conditions verify the hypothesis that growth and tolerance against adverse ambient conditions are polygenically inherited traits.

3.3 Summary

3.3.1 DED basic studies

Some transformed American elms containing an antimicrobial peptide had more remaining leaves than the *Ophiostoma novo-ulmi*-inoculated control trees. The spreading of the fungus in one transformant was much less than in the control, 2 versus 14 cm.
The discolouration volume after injection of *Verticillium albo-atrum* into 12-year-old American elm clones revealed large differences among the 12 clones tested. Much lower discoloration was noted in the *Verticillium albo-atrum* treated trees. Such injections have been applied in Denver, USA, and the Netherlands.
Barrier zone in the xylem was studied in American elm cultivars and one population from Canada and one from USA. In most taxa the barrier zone was larger in the inoculation side of the tree than in the opposite side of the tree. No relationship between barrier zone thickness and disease severity was found. In contrast, there was a fairly strong relationship between disease severity of inoculated material and autofluorescence, $R^2 = 0.70$.
Comparisons of the 3 European elm species in four French trials including more than 400 clones revealed significant differences among the species in all trials the year of inoculation with *Ophiostoma novo-ulmi*. At the second year after inoculation there was significance in 2 trials only, which was attributed to recovery from damage. The mortality at year 3 was much lower in *U. minor* (5%) while it was above 30% in *U. glabra* and *U. laevis,* confirming the results from years 1 and 2 that *U. minor* has lower susceptibility than the two other European elms.
A large variation in defoliation of *U. minor* clones was noted in an Italian investigation. French clones were most susceptible and southern Italian clones were least susceptible. *U. minor* clones from southern Italy were taller and had larger diameters than clones from other parts of Europe. The relationship between clonal origin latitude and defoliation assessed on June 19 was strong while late assessments did not show any strong relationships. It was stressed that DED symptoms ought to be followed over several years after inoculation.
A study of 4 *U. minor* and *4 U. pumila* clones revealed large differences in wilting between these 2 species, ≈70% versus ≈15% The latter had lower vessel diameter and vessel area at the beginning of the growth period. It

Table 3-6. The performance of 18 traits in control and 150 mM treatment over months April – October of 2-year-old seedlings of one U. pumila *cultivar. Chen et al. 2021.*

Trait	*May–July*	*August–October*
Soluble sugar	Higher in NaCl	
Soluble protein	Higher in NaCl	
Malondialdehyde	Higher in NaCl	
Free proline	Higher in NaCl	
Photosynthesis rate	Limited difference	
Intercellular CO_2 concentration	No difference	
Stomatal conductance	Lower in NaCl	
Apparant mesophyll conductance	Higher in NaCl	No difference
Transpiration rate	Lower in NaCl	
Water use efficiency	Higher in NaCl	
Light saturation point	Higher in NaCl	No difference
Apparant quantum yield of photosynthesis	Higher in NaCl	Lower in NaCl
Light compensation point	Higher in NaCl	No difference September–October
Dark respiration rate	Higher in NaCl	
CO_2 saturation point	Limited difference	Lower in NaCl
CO_2 compensation point	Lower in NaCl (–August)	No difference September–October
CO_2 carboxylation efficiency	Higher in NaCl	Lower in NaCl
Light respiration rate	Slightly higher in NaCl	Lower in NaCl

was concluded that to allow comparison of different inoculation tests, inoculations should be carried out at defined stages in vessel formation.

A joint European study comprising 5 countries and more than 300 elm clones of 6 taxa was presented in 2005. Inoculations were carried out at age 3. There was a large variation in dieback at all test localities. For most localities there were significant relationships between wilting diameter and vessel area at the beginning of the growth period. It was concluded that to allow comparison of different inoculation tests, inoculations should be carried out at defined stages in vessel formation.

A joint European study comprising 5 countries and more than 300 elm clones of 6 taxa was presented in 2005. Inoculations were carried out at age 3. There was a large variation in dieback in all test localities. For most localities there were significant relationships between wilting 10 weeks after inoculation and dieback one year after inoculation. No estimates of clone x test locality interaction were presented.

The mean vessel diameter and theoretical hydraulic conductance of *U. minor* clones decreased in the sequence susceptible – intermediate – resistant. Xylem vessel diameters and the proportion of large vessels were correlated with the susceptibility of *U. minor* to DED.

The effect of water stress on susceptibility to *Ophiostoma novo-ulmi* inoculation was studied under controlled conditions. Wilting in controls and LH (low watering followed by high watering) treatments had low percentages of wilting until termination of the experiment at day 120 after inoculation. The opposite HL treatment had 40 and 70% wilting in 2 clones. The higher vertical transition area of vessels and theoretical hydraulic conductance in plants from the HL treatment favored the infection rate by *Ophiostoma novo-ulmi*.

Fourier-Transform-Infrared (FTIR) spectroscopy was used following inoculations with *Ophiostoma novo-ulmi* of 3 DED-susceptible and 3 DED-resistant *U. minor* clones. Positive peaks and negative minima were found in the spectrum 800–1,800 cm^{-1} wave length and the corresponding metabolic function was reported. The differences between inoculated and control materials were more pronounced than between inoculated susceptible and inoculated resistant materials. It was assumed that fungal enzymes degraded carbohydrates and that phenols were produced as defence against *O. novo-ulmi* growth.

Development of early testing for DED resistance was the objective of another FTIR spectroscopy study. Three types of material were included: resistant and susceptible *U. minor* clones as well as resistant *U. pumila* clones. It was hypothesized that there should be similarity between resistant *U. minor* clones and *U. pumila* clones. However, the autoscanned absorbance in band 1,560 cm^{-1} did not support this hypothesis. Variation among clones was not discussed.

The same clones as used in the previous study were analyzed with respect to 22 xylem-related traits after inoculation with *Ophiostoma novo-ulmi*. Five of the 22 traits showed significant differences between the susceptible *U. minor* clones and the two resistant groups of clones, which in turn did not differ significantly. These 5 traits might have a crucial role for DED susceptibility.
Occurrence and frequency of endophytic fungi were studied in *U. pumila*, and resistant and susceptible *U. minor* clones. It was concluded that the genotype of the trees plays a major role for its endophyte frequency and diversity in xylem. The lowest frequencies were found in *U. pumila* and resistant *U. minor*. In other tissues such as bark and leaf the genotype did not have such strong influence.
In vitro cultures with endophytic fungi and *Ophiostoma novo-ulmi* revealed a large variation in growth reduction of *O. novo-ulmi.* The largest reduction was noted for *Alternaria tenuissima* and *Neofusicoccum luteum.* The response to conditioning with *Monographella nivalis* or *Sordaria* sp. of 3 field-growing clones before inoculation with *Ophiostoma novo-ulmi* varied strongly, In one clone wilting was reduced by 15 and 32% for *Monographella nivalis* and *Sordaria* sp,, respectively.
Molecular genetic approaches to understand processes leading to DED symptoms were carried out. More than 25,000 SNPs related to DED were found. The ratio of DED-tolerant elms to the susceptible elms varied in the range 0.31–1.03 for 6 xylem traits with values below 0.60 for pit aperture area and theoretical hydraulic conductance. Large values for these two traits are assumed to facilitate infection by *Ophiostoma novo-ulmi*. Based on known genes in data bases it was found that differentially expressed genes were related to perception, signal transduction, transduction factors, and defence.

3.3.2 DED studies oriented towards breeding

Large differences in tree growth and DED susceptibility were observed for American elm clones. It was stressed that juvenile data may be misleading. Therefore, final evaluation should take place at higher ages.
As expected, large clone x test locality interaction was observed in an American study including 16 test localities in USA. Some clones showed high survival and good performance.
Ecovalence estimates were obtained for growth traits in 3 Italian trials from widely differing altitudes. Clones with low as well as high estimates of ecovalence were identified. Based on the results, clones for different climate zones could be recommended.
One Dutch clone trial with 28 interspecific hybrids showed large variation in disease index at 4 weeks after inoculation with *Ophiostoma novo-ulmi* as well as large variation in dieback one year after inoculation. Some clones had recovered from damage between these two occasions.

3.3.3 Growth and phenology

Italian studies have focused on bud flushing phenology and its relation to DED.
Based on 6 clone trials in various European countries it was concluded that an inverse exponential thermal function (page 64) including thermal time and chilling for bud flushing described the bud flushing well for *U. glabra* and *U. laevis* but less well for *U. minor*.
It was shown that high latitude and high altitude *U minor* populations have a later bud flushing than southern and low elevation populations. Two explanations were given for these results:
The chilling requirement for breaking of dormancy is larger in high latitude and high elevation populations and this requirement is rarely reached at southern latitudes for high latitude clones.
At southern latitudes there is an advantage for southern populations to start the growth early before summer drought appears as an adaptation to the Mediterranean climate.
It was concluded that European elms have relatively low chilling requirements for dormancy release, their terminal time requirement to bud burst becoming steady when chilling exceeds 100 chill days.
Temperature conditions influence the bud flushing more than photoperiod. Such a relationship does not exist if localities with extreme oceanic climate are included in the relationship (cf Myking and Skrøppa 2007).
Low levels of NaCl stimulated plantlet growth while the same level of mixed salt stimulated growth in just a few clones. High concentrations of NaCl or mixed salt led to reduced growth of *U. pumila* cultivars, with strong relationships between salt concentration and growth of the individual cultivars. The most salt-tolerant clones had a better osmoregulation and higher antioxidant enzyme activities than salt-sensitive clones. The impact of the osmoregulators sugar and proline did not vary much between the 2 salt treatments with a maximum of the dose relationship and a drop at the highest salt concentration.
U. pumila cultivars exposed to various levels of salt solutions, 100, 150 and 200 mM, seemed to be somewhat stimulated by the 100 mM solution but showed reduced growth at the 2 highest salt solutions. Detailed studies of gene expression in one cultivar grown on 150 mM-NaCl-solution and control revealed large numbers of differentially expressed genes in the two treatments. These results lead to a better understanding of the processes under stress conditions.

4. Breeding

To mitigate the problem with DED, hybridization with Asian elm species was undertaken to obtain elms combining DED resistance with satisfactory morphology and adaptedness to European conditions.

In two papers, Santini et al. (2002) and Santini et al. (2004a), partly the same results were presented. Two clones San Zanobi and Plinio are two examples of successful hybridizations for the Italian market. San Zanobi is an offspring from the cross [(*U. glabra* Exoniensis *x U. Wallichiana* p39*) x* (*U. minor1 x U. minor*28)] *x U. minor* 15. Plinio was obtained from the cross between Plantyn [(*U. "Exoniensis"* × *U. wallichiana)[1] x* "302"(*U. minor* "1" × *U. minor* '28'*) x U. pumila* S2].

Following inoculations with an aggressive strain of *Ophiostoma novo-ulmi*, seedlings with less than 10% dieback were selected and vegetatively propagated and established in clone trials together with 2 strains with low and high susceptibility towards the fungus. The defoliation at 4 weeks and dieback were recorded 3 and 8 months after inoculation. The newly selected clones had lower percentages of defoliation and dieback than the low-susceptibility clone Lobel (Fig. 4-1) and did not show any symptoms of elm yellow infection

San Zanobi and Plinio showed improved height growth compared to the DED-resistant Lobel clone, with 64 and 32%, respectively. San Zanobi also had an improved diameter of 20% while Plinio had more or less the same diameter increment as Lobel. San Zanobi is characterized by pronounced apical dominance and a straight stem. Flowering starts around an age of five years. The flowering occurs simultaneously with *U. minor* or slightly later. Plinio is performing well under northern Italian mountainous climatic conditions. The width of the crown may reach 70% of the height and this clone has a fairly straight stem. Abundant flowering starts already at an age of three years.

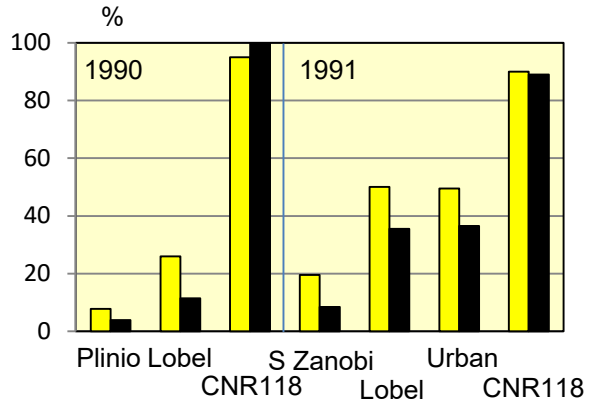

Figure 4-1. Percentages of defoliation (yellow) 4 weeks after inoculation with Ophiostoma novo-ulmi *and dieback 8 months (black) after inoculation in Italian clones of* U. minor *in years 1990 and 1991. For comparison CNR118 was selected as a strongly susceptible clone. Santini et al. 2002 and 2004.*

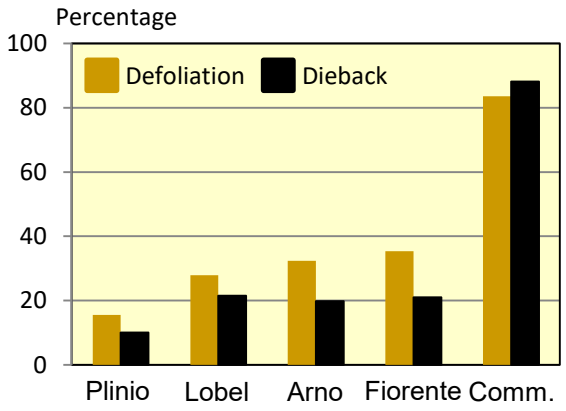

Figure 4-2. Percentage defoliation and dieback in 5 clones after inoculation with an aggressive strain of Ophiostoma novo-ulmi *carried out in 1990 in an Italian trial. The clones Arno and Fiorente were newly selected clones that were compared with the DED-resistant clone Lobel, the susceptible clone Commelin and one previously selected clone, Plinio. Santini et al. 2002*

In the paper from 2004 information on several wood quality traits was presented. Wood density did not differ much among San Zanobi, FL 090, and *U. minor* while the percentage of heartwood and modulus of elasticity were lower in *U. minor*.

Santini et al. (2007) reported on 2 other released clones, Fiorente and Arno. Fiorente is a species hybrid, *U.pumila* S10 *x U. minor* C.02. Arno is obtained from the same cross as Plinio, *i.e.* Plantyn [(*U. "Exoniensis"* × *U. wallichiana*)[1] x "302"(*U. minor* "1" × *U. minor* '28') x *U. pumila* S2]. The two new clones emanate from the same selection as presented in the above report. Field trials were established at two northern Appenine localities with different elevation, 312 and 599 masl.

The defoliation at 4 weeks after inoculation and dieback at eight months after inoculation in 1990 is shown in Fig. 4-2. The percentages for Plinio are somewhat higher than the figures in the above paper with the same material. The defoliation in Arno and Fiorente is somewhat higher than in the low-susceptibility clone Lobel but dieback percentages were of the same size. It was reported that the low susceptibility towards DED of the 2 clones remained for an additional 10 years.

Flowering in Arno takes place at an age of 5 years while it takes place somewhat earlier in Fiorente. This clone is monocormic and had the largest diameter and tallest trees at both test localities, while Arno's height and diameter growths were comparable with the other clones at the two test localities. Arno is also monocormic and its flowering coincides with the local *U. minor*.

Table 4-1. Species hybrids of seedlings obtained from full seeds in percentages >5%. In each group of hybrids some seedlings had male parent morphological characteristics. vi = villosa. *Santini et al. 2008.*

Female	*Obtained hybrids with male characteristic percentages*
ch*enmoui*	*Wil 8.0*
el*liptica*	*ch 14.1; Ja 9.7;*
gl*abra*	*Ja 31.8; ch 19.6*
Ho*llandica*	*Ja 16.0; ch 8.6;*
Ja*ponica*	*pa 14.0; gl 12.6; ch 10.8; pu 10.4; vi 10.1*
la*evis*	*vi 6.9*
mi*nor*	*gl 18.9; vi 11.3; ch 10.8; Wil 7.3*
pu*mila*	*gl 42.9; vi 5.6*
Wil*sonia*	
pa*rvifolia*	*Ja 11.1; pu 8.8; gl 6.8;*

In 2008, 4 resistant clones have been identified, one species hybrid and 3 from hybrid crosses Santini et al. (2008). To broaden the breeding population in Italy a wide collection of species, mainly from Asia, was established. The prime objective was to test the resistance to DED of exotic populations as well as their adaptedness to Italian ambient conditions. The collection was a starting material for species hybridization. A full diallele with eleven species was aimed at, with the species listed in Table 4-1. For various reasons, only 85 of the planned 121 crosses could be accomplished. To verify that the seedlings were true hybrids their morphology was checked during the two first growth periods.

At age 4 the seedlings were inoculated on May 17, which was assumed to be at peak time for infection via *Scolytus* beetles. A single wound by a knife blade covered with two tester isolates of *Ophiostoma novo-ulmi* was applied. Assessments of damage were carried out 30 days, and 3 and 8 months after inoculation. Percentages of defoliation and dieback were recorded. Seedlings with less than 10% dieback were vegetatively propagated and 12 cuttings per clone were planted in three blocks in two trials. One trial was located at lat. 41.75°N and 960 masl, and the other was located at lat. 43.02°N and 300 masl within a typical Mediterranean climate. In addition to DED damage, leaf shape, leaf color, crown shape, height, and diameter were assessed.

A great variation in the success of the matings was reported. *U. villosa* as female did not result in any seeds while other crosses resulted in 1,500 seeds. Table 4-1. shows the hybrids with with more than 5% seedlings obtained from the full seeds in each species hybrid. This table shows that *U. glabra, U. japonica,* and *U. chenmoui* had the highest percentages of "successful" hybridizations as males. Noteworthy are the high percentages for the *U.glabra x U. japonica* and *U.glabra x U. chenmoui* hybrids, which suggests that transfer of DED resistance to *U. glabra* is a possible option. This table also shows that unilateral crossing barriers exist. Thus, *U.minor x U. glabra* was successful but the reciprocal cross was unsuccessful.

Based on the two steps of inoculation and the following evaluation, 60 clones were selected (Table 4-2), 2 of them being open-pollinated offspring from *U. japonica* and *U. parvifolia*. This table reveals that hybrids with *U. pumila* dominate this selection. Even a few hybrids between the two subgenera *Ulmus* and *Microptelia* were selected.

The mean annual height assessed at the low-elevation trial was 85.5 cm, and 24.5 cm at the high-elevation trial. The diameter was three times larger at the low-elevation trial than at the high-elevation trial.

Table 4-2. The number of clones selected after 2 inoculations of species hybrids in an Italian investigation. Santini et al. 2008.

Female	*Ulmus*					*Microptelia*	
	Japonica	*laevis*	*minor*	*pumila*	*Wilsonia*	*chenmoui*	*parvifolia*
elliptica				1			
glabra							
Hollandica				14	3	1	
Japonica				15			
laevis							
minor					1	1	
pumila	1		4		3		1
Wilsonia			3	5			
chenmoui							
parvifolia		1		3	1		
villosa							

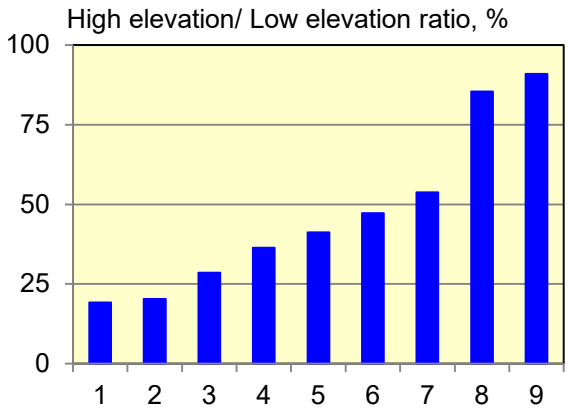

Figure 4-3. The percentage height increment of 9 selected clones at an Italian mountainous trial at 960 masl compared to the increment in a trial at 300 masl. Santini et al. 2008.

There was no strong relationship between the increments at the 2 trials of individual clones as seen in Fig. 4-3 for the 9 clones presented in the paper. This suggests that different breeding zones must be delineated.
Unfortunately, the elm yellow disease was found in some Asian parents and their hybrids. Also, damage caused by elm leaf beetle was noted. These findings demonstrate that attacks by pests and diseases that are not harmful to the domestic species might be harmful to species hybrids and must be considered in the breeding programs.
The risk of introducing species hybrids for the domestic ecosystems was presented but no solutions as to how to tackle such a threat.
In conclusion, some promising elm clones for Italy were identified and delineation of breeding zones seems to be needed.

In a paper from 2012 a review of the recent Italian elm breeding program was briefly outlined (Santini et al. 2012). A first step in this program was the collection and selection of Asian elm species growing in Italy in order to identify exotic trees well adapted to the Italian conditions. Thus selected trees were used in crosses with domestic *U. minor* trees with desirable characteristics. More than 80,000 hybrid seedlings were obtained and tested. The morphology of the seedlings was checked during the first and second growing season to verify the hybrid character of the seedlings. The seedlings were inoculated with *Ophiostoma novo-ulmi* during the third week of May when there is a peak of the *Scolytus* beetles in Tuscany. The effect of the inoculations was evaluated at 4 weeks and 3 and 8 months after inoculation. Seedlings with less than 10% dieback were propagated by hardwood cuttings. Clones having less than 25% dieback were evaluated for other characters. Field trials with 12 cuttings per clone were established in different climatic zones. Each trial contained clones with known susceptibility to DED as reference material. Evaluation was performed two years after establishment of the trials. Besides DED resistance additional traits were considered:

1. Leaf shape
2. Leaf color
3. Crown shape
4. Growth: height and diameter

Usually, a 5-degree score was used in these examinations. Out of the more than 50,000 seedlings, 80 seedlings with high scores were selected. In 2012 five elm clones had been patented and released to the market. They are described in separate papers.
The risk that pests and diseases that are not harmful for the domestic elm might be harmful for Asian elm species was discussed. Elm yellow caused by phytoplasma and elm leaf beetle were two major potential threats to elm species hybrids. Therefore, a rating program was used to assess whether the Asian elms were exposed to serious threats from these two harmful organisms.
It was stated that selected clones might be used for wood production as well as for ornamental plantations.
It was concluded that the Italian program has a broad genetic base in the hybrid breeding population. Any long-term breeding strategy was not discussed.

Martin et al. (2013) described the Spanish breeding program for DED resistance, which started in 1986. The 7 clones selected so far were also presented. The prime selection criterion was healthy trees in areas affected by DED. Ornamental value was also considered. In all, 4 trials with 164 selected clones were established. One DED-resistant and one DED-susceptible clone were included in the trials. One trial was established at lat. 40.74°N long. 3.55°E, 685 masl, the others were planted at lat. 40.45°N, long 3.75°E, 600 masl. The design was two blocks with 3 – 4 ramets per clone in each block. Local strains of *Ophiostoma novo-ulmi* were inoculated at peak sensitivity for infection, *i.e.* 15–30 days after full leaf development. A spore suspension of 0.1 ml containing 106 spores per ml was inserted after incision by a razor blade at base of the trunk. Data for wilting 30, 60 and 120 days after inoculation in 4 tests were presented. Tree height before inoculation was measured and around 15 morphology characteristics of the selected clones were presented.

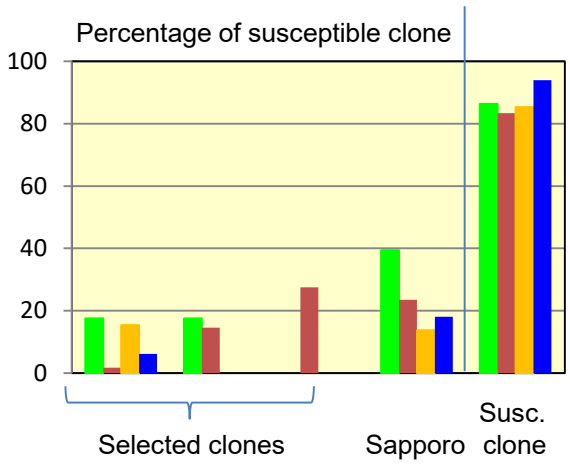

Figure 4-4. The mean percentage of wilting compared with one susceptible clone in 4 tests indicated by different colours. The mean values originate from recordings 30, 60 and 120 days after inoculation. Sapporo is a DED-resistant clone. Martin et al. 2015.

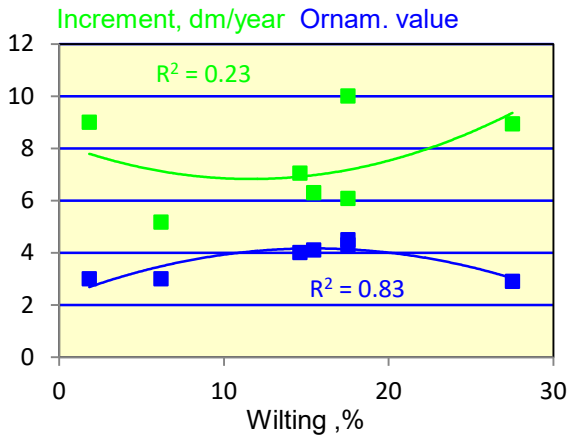

Figure 4-5. The relationship between wilting in % of the reference cultivar Sapporo autumn and height increment or ornamental value in seven selected U. minor *clones. Martin et al. 2015.*

In Fig. 4-4 we have illustrated the mean wilting percentages of the 7 selected clones based on assessments 30, 60, and 120 days after inoculation. The 4 colors refer to the 4 trials. The wilting percentages of the susceptible clone are in all four trials above 80%, which means that clones with low susceptibility in these trials are good candidates for approval. Two clones, one in the brown and one in the blue trial, showed exceptionally low wilting percentages. The annual height increment of the seven clones varied in the range 50–100 cm. The ornamental values varied in the range 2.9–4.5. Fig. 4-5 reveals that there is evidently no strong relationship between these two traits and wilting. The expectation that increased wilting would reduce growth was thus not fulfilled.

Five classes were used to estimate differences among the clones with respect to bud burst. However, information as to how frequently recordings were carried out was not given. Only weekly differences in bud burst were reported, which means that resolution of this trait suffers from imprecision. Therefore, relationships with other traits become troublesome.

Marketing within the European Union requires that material to be released must be unequivocally identified. Twelve nuclear microsatellites were used to fulfil this requirement.

It was stated that the defence mechanism differs among resistant elm genotypes. This means that crosses among resistant clones would give rise to some progeny plants combining two types of resistance mechanisms with improved resistance to DED.

A study of regeneration of transformed plantlets of *U. procera* was presented by Gartland et al. (2000). This investigation did not include regeneration of different elm genotypes but is briefly referred to, since vegetative propagation might be applied in future elm breeding. Stem sections 1 cm long from shoot tip cultures were submerged in a suspension of *Agrobacterium tumefaciens* C58pMP90p35SGUSINT. Two different cultivation media were used. In cocultivation with kanamycin, selected surviving plantlets were isolated and propagated. The success of transformation was studied by GUS histochemical staining or GUS fluorimetric assay.

The transformation efficiencies were estimated at 0.9% and at 7% in the two media. It was shown that the technique used for transformation was successful and that morphologically normal plants were obtained.

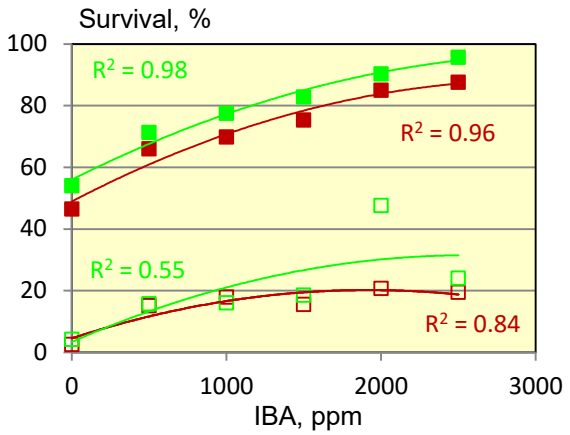

Figure 4-6. Percentage survival in sand (brown) and in coconut:vermiculite:perlite 2:1:1 (green) substrates of cuttings of U. villosa *(filled squares) and* U. wallichiana *(open squares) exposed to various concentrations of indole butyric acid, IBA. Nazir et al. 2021.*

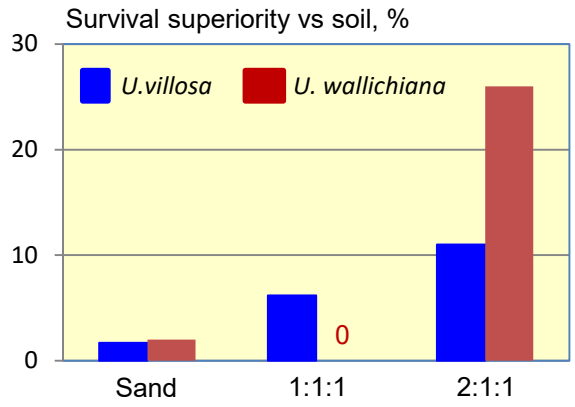

Figure 4-7. Survival superiority in % compared to soil as substrate. 1:1:1 and 2:1:1 stand for the proportions of the mixture of cocopeat;vermiculite:perlite. Data from the 2,500 ppm treatment of IBA. Nazir et al. 2021.

Poor regeneration of the Himalayan elm species *U. villosa* and *U. wallichiana* prompted actions for production of planting material. A study of production of cuttings of these two species was therefore carried out by Nazir et al. (2021). They took 5 cm leaf basal portions of young seedlings. After treatment with fungicide and 1% sucrose they were treated with 500–2,500 ppm of indole-butyric acid and put into soil, sand, or cocopeat:vermiculite:perlite in two proportions 2:1:1 and 1:1:1. The experimental design was 4 blocks with 10 plants per block. After 13 weeks sprouting, the rooting, survival, shoot length, root length, and leaf area were recorded.

In Fig, 4-6 we have illustrated the relationship between treatment and the most important trait, survival, for two media, sand and the 2:1:1 cocopeat:vermiculite:perlite substrate. The success rate is several times higher in *U. villosa* than in *U. wallichiana*. For both species there was a positive effect of the IBA treatment.

Fig, 4-7 shows that there is a clear superiority for the 2:1:1 proportion of cocopeat/vermiculite/perlite as regards survival at the highest dose of IBA. It should be noted that the mean survival was several times lower for *U. wallichiana* (20.7%) than for *U. villosa*. This means that a similar deviation from the mean in this species results in a higher percentage for *U. wallichiana*.

There were similar responses of the five other traits studied. It was speculated that the porosity of the 2:1:1 cocopeat mixture was favorable for the superiority of performance in this substrate.

Huang et al. (2022) suggested that a TDSTI statistical test method based on the Monte Carlo idea might be used in elm breeding. After a detailed treatment of this technique it was applied to leaf shape and 13 microsatellite markers in *U. pumila*. Neither the number of leaf shape traits nor the number of individuals in the analysis were given. The average value, standard deviation, coefficient of variation, and repeatability of each leaf shape trait were determined. Cluster analyses and ANOVAs of the obtained data were carried out. Correlation analyses were performed between distances for leaf shape traits obtained in the cluster analysis and the microsatellite distance data. It resulted in a correlation coefficient of 0.48. It was reported that 10 microsatellites were strongly associated with leaf length traits. The absence of details as to number of traits analyzed and number of individuals tested makes it hard to evaluate the potential of this statistical technique for elm breeding.

4.1 Summary

A review of the recent Italian elm breeding program was briefly outlined. A first step in this program was the collection and selection of Asian elm species growing in Italy in order to identify exotic trees well adapted to the Italian conditions. Thus selected trees were used in crosses among 12 elm species. All interspecific hybridizations were not realized. Sixty trees were selected based on two steps of inoculation and morphological evaluation. A majority of the 60 selected trees contained *U. pumila* as one parent.

The performances of selected and patented clones were described.

The Spanish elm breeding program was described in 2015. The prime selection criterion was healthy trees in DED-affected areas. In all, 4 trials with 164 clones were established. The selected clones were much less affected by DED than susceptible control clones. Based on the 4 trials 7 clones were selected with satisfactory tolerance against DED, i.e. <25% wilting after inoculation with virulent strains of *Ophiostoma novo-ulmi.* They also showed good annual height increment, 5–10 dm. Four of the clones had the next highest scores for ornamental value.

Measures were taken to produce reforestation material of the rarely occuring *U. villosa* and *U. wallichiana* for applied planting. Greatest success was noted for *U. villosa.*

5. Genetic conservation

5.1 Theoretical and applied conservation

In our summary of the activities carried out in the EUFORGEN (European Forest Genetic Resources) network ´Noble hardwoods´ I discussed the principles for forest tree genetic conservation and identified three corner stones of forest tree genetic conservation (Eriksson 2001):

Objectives
Genetic information
Methods

Safeguarding the potential for adaptation was identified as the prime objective for genetic conservation within this network. Especially in times of rapid change of the environmental conditions it becomes extra important for conservation that the species to be conserved can respond genetically and rapidly to environmental changes.

Preferably, the among-population differentiation in adaptive traits should guide selection of genetic resource populations. For many tree species such knowledge is missing and we have to rely on genetic marker differentiation or educated guesses about adaptive differentiation.

The **M**ultiple **P**opulation **B**reeding **S**ystem (MPBS) first elaborated by Namkoong (1984) was suggested as a method for genetic conservation by the network. The MPBS is schematically illustrated in Figure 5-1. The conservation population is split into around 20 subpopulations in such a way that they cover the genetic differentiation of the species concerned. Each subpopulation should have an effective population size (N_e) of 50. With an N_e of 50 the loss of additive variance and increase of inbreeding are in both cases 1% per generation. This means that the possibility for adaptation in each subpopulation is not considerably constrained. Over generations the differentiation among the subpopulations will increase. The merit of MPBS is that it combines the capture of the total existing genetic variation with a satisfactory variation within each subpopulation. Further, it allows the target population to adapt to the prevailing environmental conditions of each of the subpopulations. MPBS is a dynamic genetic conservation method in contrast to *ex situ* methods which are static preservations of existing genetic variation.

Another group within EUFORGEN (de Vries et al. 2015) suggested the same method for genetic conservation but did not use the term MPBS; and the subpopulations were called ‘core populations’. This term will appear in some papers below.

There was a special treatment of the European elms. As regards *U. minor*, which easily forms root suckers and thereby creates small clonal populations, *ex situ* conservation was suggested. Conservation of *U. minor* should be separated from localities with Siberian elm to avoid interspecific hybridization with this species.

Owing to the high incidence of DED in *U. glabra*, *in situ* genetic resource populations were not judged as a feasible solution for its conservation. Rather, low pruned clone hedges were suggested since such trees are not attacked by the *Scolytus* beetles.

In situ populations of *U. laevis* were suggested for conservation of this species. *U. laevis* is also susceptible to DED but less affected since the *Scolytus* beetles are less attracted by this species. The loss of habitats for this species is a great concern and supplemental plantations should take place to increase the N_e.

Whenever possible, joint genetic conservation with other tree species might take place. Management to improve flowering and seed production should also take place.

In essence the above text covers the content of our EUFORGEN summary in the paper by Collin et al. (2000).

Collin et al. (2004) presented the European state of the art for elm conservation initiated within EUFORGEN. In this paper basic concepts of forest tree genetic conservation were presented and discussed. A questionnaire about the elm genetic resources was sent to the 30 countries in-

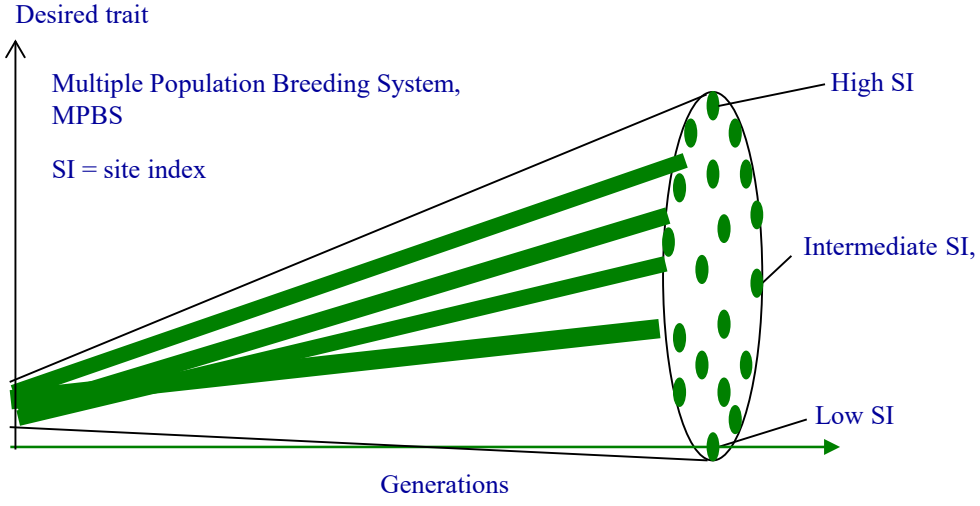

Figure 5-1. A schematic illustration of the essence of The Multiple Population Breeding System. The gene conservation population is split into 20 subpoplations, which diverge over generations. Eriksson 2001.

volved in EUFORGEN. DED was identified as a main threat to elms. Loss of habitats for elms and hybridization with exotic elm species were other concerns for elm conservation. It was stressed that *Dynamic conservation must be implemented in a network of natural conservation populations covering the ecological range of the species*. Brief status reports were presented for Hungary and Finland. Owing to the limited sizes of elms in Hungary, *ex situ* conservation had to be applied. In addition, elms will be conserved together with other tree species in mixed deciduous tree stands.
The Finnish program for conservation of *U. laevis* was presented. In Finland this species grows close to its northern limit and is considered as an endangered species. Natural stands are appointed as protection areas. As in Hungary, conservation of elms takes place jointly in mixed stands of deciduous trees. At this point of time 83 clones from 19 populations were grafted for establishment of an *ex situ* clone archive that will serve both for seed production and conservation. More data on the Finnish *U. laevis* populations are given below.

In a meeting presentation Collin (2006) reported on the completion of six tasks of the European elm conservation project, GENRES 78 EU:

Establishment of a common database

Molecular characterization of a large sub-sample of the total clone collection

Evaluation of important traits in the clone collection

Selection of priority clones for a core collection

Securing of a long-time conservation of elm clones

Dissemination of information obtained in the European project.

Genotyping of 535 clones by molecular markers in established European *ex situ* gene resources clone banks was used to identify the pure species and hybrids between elm species (Godall-Copestake et al. 2005). The following taxa occurred among the 535 clones in western European clone collections:

U. glabra	151
U. glabra x U. minor	34
U. minor x U. glabra	42
U. minor	211
U. minor x U. pumila	8
U. pumila	10
U. laevis	59

Eight markers were used in the genotyping: 5 RAPDs and 3 microsatellites.
The principle component (PCO) analysis clearly separated *U. laevis* from the other species and hybrids and three clusters for *U. glabra, U. minor,* and *U. pumila* were found. The boundaries between these clusters were diffuse owing to the species hybrids. The first PCO plots were used for a reexamination of the material. This resulted in change of taxon for 74 clones.
AMOVA was run for partitioning of the variation among

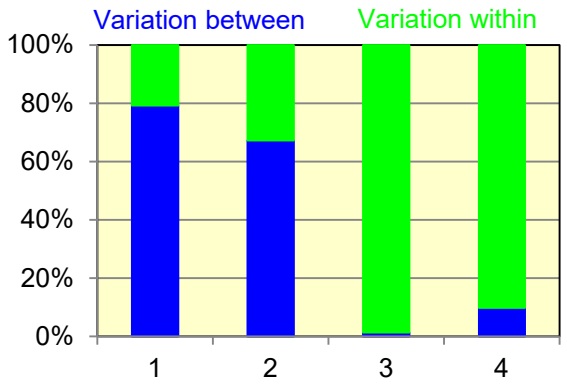

Figure 5-2. The partitioning of the variation among taxa (blue) and within taxa (green) or among regions within species (blue) and within regions (green). (1 = U. glabra, U. laevis, U. minor*; 2* = U. glabra U. minor*, 3* = U. glabra, *4* = U. minor*) Five RAPDs and three ISSRs were analyzed. Goodall-Copestake et al 2005.*

and within taxa. Fig. 5-2 reveals that variation among taxa was larger than within taxa while the variation among regions was much smaller than within-region variation for *U. glabra* (3) and *U. minor* (4). No strong correlations between genetic and geographic distance were observed. The limited genetic variation among regions is somewhat surprising since clones originated from widely differing climatic conditions: from Sweden to Italy. This suggests that sampling anywhere in Western Europe would be enough to capture the existing genetic variation in this region. Moreover, unless misclassification is substantial among the 151 existing *U. glabra* clones, the genetic conservation of this species would be satisfactory as regards Western Europe. As long as we do not know how representative the existing clones are for their populations it would be desirable to have clones from all climatic zones in the distribution area of *U. glabra*.

Collin and Bozzano (2015) presented the genetic conservation developed by EUFORGEN. Europe is divided into climatic zones. In each zone one core unit (CU) should be identified as a part of the genetic conservation of the species in question. An important issue is to identify gaps, *i.e.* climatic zones that lack an identified core unit. The French program for conservation of *U. laevis* was described as an example of an *in situ* dynamic genetic conservation of a tree species. The conservation was into 2 western hydrographic areas, Loire and Garonne River. Autochthonous forests were not a criterion since it is hard to prove whether or not a population is autochthonous, and besides, the populations at the margin of the species distribution might have specific adaptedness to the ambient conditions at the margin of the distribution of the species. The Garonne dynamic conservation unit comprises 118 hectares 6 km along the Garonne River and 700 trees with dbh > 5 cm. Scattered *U. laevis* popula-

tions are growing upstream and might contribute to the pollination of the CU. This population was included in the thesis by Rachel Whiteley (2004) and its unique characteristics support its appointment as a CU. A number of large trees were labelled in year 2000 to enable a study of DED impact on this CU. An inventory in 2012 revealed limited mortality in the CU.
The Loire CU is 200 ha of riparian forests and stretches over 20 km along a tributary to the Loire River. It contains 540 *U. laevis* trees, most of them less than 30 years old. Flowering phenology as well as abundance were recorded in 98 of these trees in 2004–2006. The percentage of flowering trees varied in the range 90–100% while full seed varied in the range 49–71%. The occurrence of DED was recorded in 2014 and the loss of trees amounted to 27%; and the incidence of DED was disturbingly strong in some sectors of this CU.
Since loss of habitat is a major threat to *U. laevis*, dynamic restoration of habitats has become an important means for conservation of this species as well as other riparian species. In cases of such restoration it was stressed that regeneration should be done with local material.
U. minor reintroduction experiments were carried out in France. Five ramets of DED-tolerant clones from the hedged clone conservation archives were planted among other trees and shrubs. They are planted in single-tree plots, which enables an evaluation of field tolerance of the clones. It is also expected that crosses among the clones may take place, which most likely will increase the genetic diversity of the filial generation.

Collin et al. (2020) presented the French program for conservation of elms. Collection of scions free from DED-symptom-free elms in Lower Normandy was initiated in 1985-1986. The grafts were planted in hedges in clone archives, in which the grafts were kept at a height of approximately 2 meters to avoid attacks by the bark beetles carrying the disease fungus in European countries. Low grafts were not attacked by the bark beetles. In all, there were 205 *U. minor,* 107 *U. x hollandica*, 100 *U. laevis*, and 29 *U. glabra* trees. Later on 181 elm clones from other European countries were included in the clone collections. The clones were characterized with respect to various exterior traits but also genotyped by molecular markers. The DED inoculation tests revealed the low resistance in many of the clones but a large variability among the clones. No geographic structuring was found. The management costs were high, which called for revision of the strategy. A core collection of 195 clones was established based on "*multiple criteria of diversity (geography, environment, taxonomy, molecular markers, resistance to DED, ornamental or patrimonial value)*. Establishment of seed orchards was also considered. For *U. laevis,* loss of its habitats is a greater threat than DED. Therefore, two *in situ* genetic resource populations were identified within the frame of European conservation of *U. laevis*. Both gene resource populations are located in south-western France. *In situ* conservation of *U. glabra* was not considered feasible owing to severe DED attacks. In their study of marginal *U. laevis* populations in Finland (Vakkari et al. 2009), the Finnish program for genetic conservation of *U. laevis* was presented. The 13 populations in this report were appointed as genetic resource populations. The differences noted among these 13 populations might partly be attributed to adaptation to different site conditions. The low F_{IS} speaks in favor of adaptive differences. Besides these 13 units, material from 19 populations are growing in an *ex situ* conservation collection with 2–10 clones per population. The total number of clones in this collection is 121. Several grafts per clone were planted but when seed production has started only one graft per clone should remain. This clone collection is also seen as a seed orchard that should produce seeds with high genetic diversity. It was stressed that it is of greatest significance that suitable habitats for regeneration of *U. laevis* are available. To reduce the risk of DED, three measures should be taken:
1. Establishment of a duplicate population further north
2. Hedging of the grafts
3. Cryopreservation

In the article on ecology and conservation of *U. laevis,* Venturas et al. (2015) concluded: *Long term conservation of U. laevis requires restoration of hydrological regimes and re-establishment of ecological processes where they have been destroyed by humans (Howe and Miriti 2004), taking in consideration landscape ecology concepts(Lafortezza et al. 2013)*. Even if habitat destruction is a greater threat for *U. laevis* conservation than genetic erosion, it is still important to study the genetic diversity in existing *U. laevis* populations in Spain. As a first step an inventory of the occurrence of the species in Spain was carried out. Whenever possible, *in situ* conservation of populations should be implemented. The sites should be managed to improve the possibilities for regeneration in the genetic resource populations. In some cases seeds were collected for establishment of genetic resource populations or seed orchards with 65 open-pollinated families. Cryopreservation of seeds was also considered.

A study of small and scattered *U. laevis* populations in Spain was carried out by Fuentes-Utrilla et al (2013) to determine whether this species is native to Spain or originates from human introductions. As a native species, actions to guarantee its genetic conservation have to be taken. Eleven Spanish populations were included in this investigation. The number of trees per population varied in the range 6–25. In addition, 9 western European populations (Belgium, France, and Germany) were included with the range of trees per population 2–13. Three regions of the chloroplast genome were studied by PCR-RFLP-analysis. Only one of these regions proved to be polymorphic. Five nuclear microsatellites were also used. STRUCTURE v2.1 (Pritchard et al. 2000) and BAPS v4.0 (Corander et al. 2008) were used to assess population structure from microsatellite data. The occurrence of

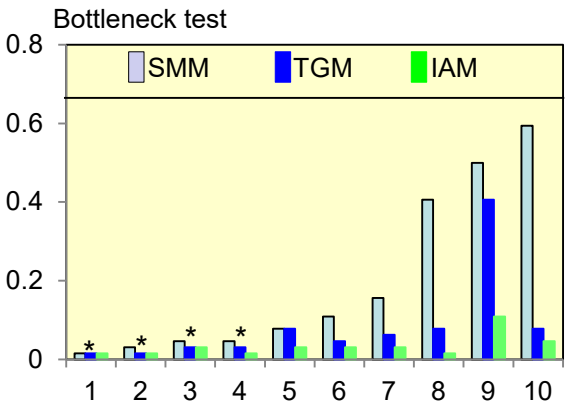

Fig. 5-3. Bottleneck test in 10 Iberian U. laevis *populations with > 11 trees in each population. Three methods were used: SMM stepwise mutation model, TGM two-phase model with 40% mutations following SMM, and IAM infinite–alleles model. Significances according to all three methods are indicated. Fuentes-Utrilla et al. 2013.*

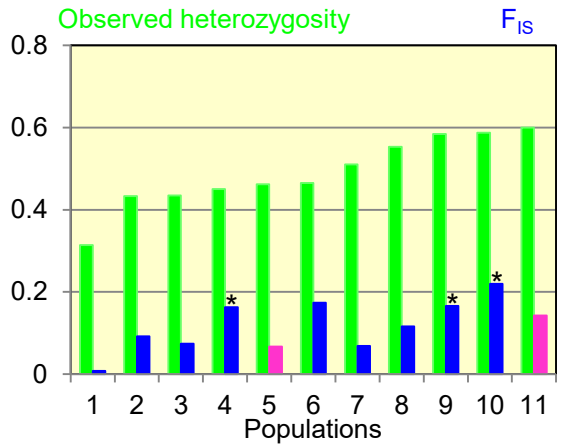

Fig. 5-4. Observed heterozygosity and inbreeding coefficients in 11 Iberian populations of Ulmus laevis. *Green columns show observed heterozygosity, blue columns show negative estimates of the inbreeding coefficient; red columns = positive inbreeding coefficients. Significances are indicated. Fuentes-Utrilla et al. 2013.*

bottle necks in populations with >11 trees was estimated using the M-value according to Garza and Williamson (2001). M-value is the mean ratio of the number of alleles to the range in allele size (Fuentes-Utrilla et al. 2013). An M-value <0.68 is indicative of a bottleneck. It was stated that a bottleneck signature in allele size distribution is more likely to be associated with remnant native populations than a recent introduction.

The cpDNA data identified two main clusters with a possible subdivision of cluster No 2. The first cluster contained 5 western and southern Spanish populations and one southern French population. Five Spanish and one French population belonged to subcluster 2.1. A high G_{ST} estimate was noted, which must be attributed to the fixation of one haplotype in most populations. An AMOVA for the Iberian populations showed a partitioning of the genetic variation as follows:

Among clusters	52%
Among populations within clusters	35%
Within clusters	13%.

The microsatellite data indicated 5 clusters with a clear geographic pattern. Two clusters, northwestern and northeastern Spain, contained just one population each. The genetic variation in many populations was low. The observed heterozygosity was below 0.50 in half of the Iberian populations. The mean allelic richness per sample in these populations was low, 1.91. Only two populations of the Iberian populations had private alleles.

The results of the bottleneck analysis are presented in Fig. 5-3, which shows that all estimates of bottleneck were below 0.68. Four populations showed that all three methods for estimation of bottleneck occurrence were significant. One population showed significance for two methods while five populations showed significant bottlenecks for one method (all IAM), and the remaining population did not show any significance.

A recent introduction of *U. laevis* into Spain requires that the Iberian populations show a simple subset of genetic diversity as is present in Central Europe, which was not the case in this investigation. A rapid mutation rate in the Iberian populations would be an alternative explanation for the present results. However, the cpDNA data do not support such an explanation. The small population sizes would be reflected in excess of homozygosity owing to genetic drift. However, this was not the case as seen from Fig. 5-4. Most populations had an excess of heterozygotes.

It was concluded that the bottleneck signatures of Iberian populations reflect natural population fragmentation associated with Holocene expansion and not recent introductions. The conservation efforts should concentrate on increase of the population sizes and new establishments in riparian sites suitable for *U. laevis.* Creation of connectivity among existing populations is desirable. Finally, the genetic variation within populations was limited.

In Germany *U. laevis* was the tree of the year 2019. In connection with this appointment Kätzel et al. (2019) presented a survey of this species distribution in Germany. An inventory was carried out during 2005–2017. *U. laevis* is most frequent in an eastern part of Germany in the basins of the Elbe and Saale rivers. In all, 825 localities with at least 5 trees were found. As in many other cases, loss of habitats is the greatest threat to this species. In 40% of the localities no regeneration was observed. Preferably genetic conservation should be done *in situ*. Stands with a large number of vital, flowering trees with satisfactory regeneration could be classified as genetic resource populations. It is also required that there is a long-term guarantee that these stands can remain as genetic resource populations in the foreseeable future. In all, 219 stands fulfilled the requirements for genetic resource populations. In addition 36 established stands and two clone ar-

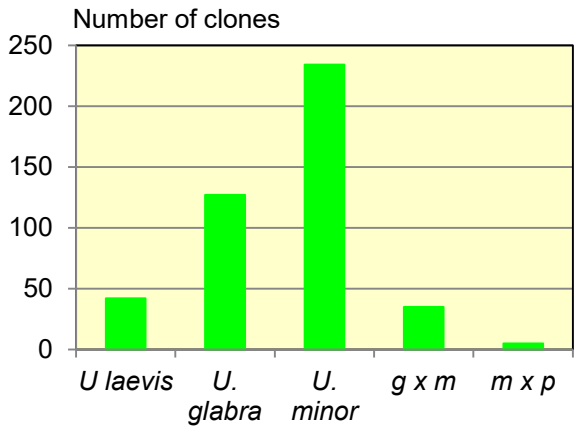

Fig. 5-5. Number of clones of different elm taxa included in cryopreservation; g x m = glabra x minor, m x p = minor x pumila. *Harvengt et al 2004.*

chives with 70 clones constitute *ex situ* genetic resource populations. The implementation of this program would well serve for the genetic conservation of *U. laevis* in Germany.

5.2 Cryopreservation

Harvengt et al. (2004) reported on a European cryopreservation project of elms. Since this a genetic review we shall not report on cryopreservation techniques but on tests of plant production following cryopreservation. The number of clones of different taxa is illustrated in Fig. 5-5. In total 444 clones from nine European countries are cryopreserved in two organizations, AFOCEL (Association Foret Cellulose) in France and NFV (Niedersächsische Forstliche Versuchsanstalt) in Germany.
The ability to produce plants from a random sample of 26 cryopreserved clones was studied. Direct regrowth occurred in 65 and 42% of *U. laevis* and *U. minor,* respectively. *U. glabra* had to be micrografted to obtain regrowth, which amounted to 76%. *In vitro* cultures of cryopreserved and fresh winter buds were compared with respect to shoot regrowth after eight weeks, and multiplication after 14 months. Fig. 5-6 reveals that there were limited differences between the two types of material. But there were distressingly low percentages of multiplication success at 14 months in both types of material. In spite of the limited success in the last-mentioned experiment, there is "cryopreserved" material growing in field plantations.

Välimäki et al (2021) studied shoot production and rooting of cryopreserved material of *U. laevis* and *U. glabra* from Finnish genetic resource populations of these two species. The total numbers of clones of *U. laevis* and *U. glabra* included in various experiments were 36 and 13. Several experiments were carried out. Only general procedures will be presented. Twigs 5–20 cm long were collected after inwintering had taken place in October–Janu-

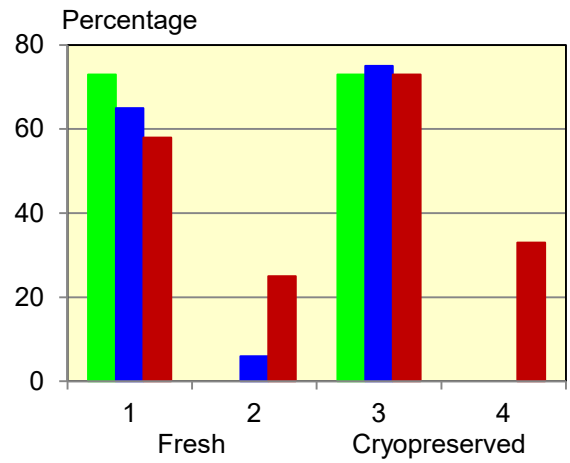

Fig. 5-6. Shoot regrowth after 8 weeks (1 and 3) in fresh winter buds, and in cryopreserved material. 2 and 4 = multiplication after 14 months. Green = U. glabra, *blue* = U. laevis, *brown* = U. minor, *Harvengt et al. 2004.*

ary. Buds were cut from the twigs and placed in 1.8 ml cryo tubes. The tubes were frozen the following day at a speed of 0.17°C/min until they reached –38°C, when the tubes were submerged into liquid nitrogen. Tubes were thawed in a water bath at +38°C for 2 minutes and then placed on ice for at least 2 minutes. Sterilization took place with 70% ethanol for 10 or 20 minutes. Sterilization with 10% H_2O_2 for 3 hours followed by 10 minutes in 70% ethanol was also applied in some experiments.
Before initiation started, outer bud scales were excised and the stems were shaped into small wedges. The buds were placed in sterilized de Wit tubes containing 7 ml modified Murashige and Skoog (MS) media or modified woody plant media (WPM) with growth hormones, gibberellic acid 4 + 7 or 6-benzylaminopurine or thidiazuron. In some cases sucrose in concentrations of 30 g/L was used. According to Välimäki et al. (2021): *Media were supplemented with varying concentrations ... of myo-inositol, glycine, nicotinic acid, pyridoxine-HCl, and thiamine-HCl. All media were adjusted to a pH of 5.8 prior to autoclaving for 20 min at 121°C. GA 4 + 7, TDZ, and meta-topolin (mT) (Ducheva Biochemie) (mT) were added through filter sterilisation after autoclaving.*
After initiation the tubes were put into growth chambers with a temperature of 25°C and covered to reduce the light intensity for a few days or for the entire test period. After the start of growth the shoots were placed in Magenta glass jars on multiplication media without GA 4 + 7. Material was multiplied to enable rooting tests.
Rooting *in vitro*, in semi-solid media, was tried out with material originating from Punkaharju and grown on Driver and Kuniyuki walnut (DKW) media. Rooting was in half-strength DKW media, either with 0.5 mg/L indole-3-butyric (IBA), no plant growth regulators, or with 3 days induction with 3 mg/L IBA (Sigma Aldrich), after which the shoots were transferred onto hormone-free me-

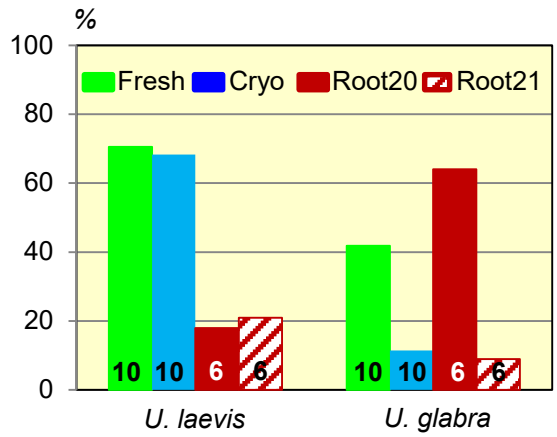

Fig. 5-7. Mean percentage of shoot production in fresh and cryopreserved material of U.laevis *and* U. glabra *as well as rooting percentage at day 41 after initiation of cryopreserved material. The number of clones tested is indicated. 20 and 21 stands for experiments in 2020 and 2021. Välimäki et al. 2021.*

dia.

Based on the first experiment it was decided to use MS medium with 0.1 mg/L GA4+7 and 0.5 mg/L BA in coming experiments. A third of the initial light intensity improved the shoot formation both in fresh and cryopreserved buds.

The mean percentages of shoot formation were higher in *U. laevis* than in *U. glabra* (Fig. 5-7). In *U. laevis* there was no difference in shoot formation between fresh and cryopreserved buds. As regards *U. glabra* 7 of the ten clones tested did not have any shoot formation in the cryopreserved buds while all 10 clones in *U. laevis* had shoot formation in the cryopreserved buds; the range being 18–100%. The corresponding range for the 3 *U. glabra* clones with shoot formation was 30–60%. Fresh buds from all *U. glabra* clones produced shoots. It was noted that the *U. glabra* buds were larger than the *U. laevis* buds. It was speculated that this condition might lead to different temperature influence on cryopreserved buds and tissue damage. Contamination, mainly by fungi, was a large problem. Different methods for sterilization were tested but none of them were better than the standard method with 70% ethanol.

Rooting percentage was much larger in *U. glabra* (64, range 59–100) than in *U. laevis* (18, range 0–29) in the 2020 experiment but dropped considerably to 9% for *U. glabra* in the 2021 experiment. IBA treatment improved the rooting in *U. laevis* from 7% in hormone-free media to 67%.

It was pointed out that contamination is the main obstacle for a successful production of plants following cryopreservation, and particularly so for *U. glabra*.

An attempt to improve *U. glabra* bud regeneration following cryopreservation by means of dehydration was presented by Välimäki et al. (2022). Five clones were included in this attempt. Cryopreservation was carried out as in the previous paper. Twigs were dehydrated on trays kept for 19 days in a cold room at –5°C. The moisture content had dropped to 32% from the initial 52%. Surface sterilization was carried out by sodium dichloroisocyanurate (NaDCC) at a concentration of 20g/L for one hour followed by 15 minutes in 70% ethanol.

The percentage of shoot sprouting following dehydration treatment varied in the range 11–83% (mean = 43%) while the corresponding percentages for the control material were 0–55% (mean = 11%). The percentage of contamination in the control material was larger in all clones and in most clones much larger. It is evident that clones differ in their response to dehydration, which means that development of a general method for cryopreservation has failed so far. In spite of the shortcomings, dehydration will be applied in future cryopreservation of *U. glabra.*

It deserves mention that several papers presenting the techniques for cryopreservation of elms were published but without different genetic materials included.

5.3 Summary

Within the Noble Hardwoods network within EUFORGEN the principles for genetic conservation were discussed. Three cornerstones of genetic conservation were identified:

Objectives
Genetic knowledge
Methods

Safeguarding the potential for adaptation was identified as the prime objective of genetic conservation of the species belonging to this network. It was stated that knowledge of genetic variation of adaptive traits is a prerequisite for a solid genetic selection of populations to be included in the genetic conservation population. For many tree species such knowledge is missing and we have to rely on genetic marker differentiation or educated guesses about adaptive differentiation. The so called Multiple Population Breeding System is suitable for matching the prime objective of genetic conservation. The conservation population is split into around 20 subpopulations in such a way that they cover the genetic differentiation of the species concerned. Each subpopulation should have an effective population size (N_e) of 50. With an N_e of 50 the loss of additive variance and increase of inbreeding are in both cases 1% per generation. The subpopulation may diverge over the coming generations.

There was much focus on the genetic conservation of *U. laevis* since it is an endangered species in several countries. Several reports stated that loss of habitats for this species was the greatest threat for its conservation. Many populations of this species were selected as genetic resource populations. In addition *ex situ* clone archives were established as an insurance and they would serve for seed production as well. Much information on among-population differentiation was presented in the papers dealing with genetic conservation of *U. laevis.*

Dynamic conservation of *U. glabra* was ruled out as conservation method owing to its susceptibility to Dutch Elm Disease (DED) caused by the fungus *Ophiostoma novo-ulmi*. Clone archives with *U. glabra* and *U. minor* were established in hedges. Trees of a size of 2 metres in pruned hedges are not attracted by the *Scolytus* beetles, which are the vectors of DED.

Existing programs for genetic conservation of elms in a few countries were described.

Cryopreservation as a means for genetic conservation was presented and discussed in some papers. However, most papers deal with this technique without any aspects of genetic differences in success of this method.

No difference in shoot growth at 8 weeks was noted between cryopreserved and fresh material of *U. glabra, U. laevis,* or *U. minor*. The success rates were around 70%. However, no multiplication after 14 months was noted for *U. glabra* and *U. laevis* while 33% was noted for *U. minor*.

In another study, rooting was much larger in *U. glabra* than in *U. laevis* in one year (64 and 18%, respectively) while low percentages (8 and 21%) were obtained another year. Contamination of the cultures critically affected the success of cryopreservation.

6. Miscellaneous

6.1 Polyploidy

In Washington DC, USA, two triploid *U. americana* trees are growing: they most probably originate from a cross between a diploid elm and a tetraploid American elm. To get an idea about the possible occurrence of diploid American elms, the ploidy level of 81 American elms from the entire distribution area in North America was analyzed in a flow cytometry study by Whittermore and Olsen (2011).
They reported that 17 of the 81 trees were diploid based on their DNA content (Fig. 6-1). As seen from this figure, the DNA content in the triploids is approximately half-way between the tetraploids and the diploids. The two triploid trees did not flower. Diploid trees were only observed south of latitude 40°N. In some cases diploids and tetraploids were growing not too far away from each other, which opens up a possibility for successful matings between diploids and tetraploids

The expression of genes regulating phenylalanine ammonia-lyase (PAL), chitinase (CHT), and polygalacturonase-inhibiting protein (PGIP) was studied in susceptible *U. americana* seedlings following inoculation with a virulent strain of *Ophiostoma novo-ulmi* (Nasmith et al. 2008). Stress-induced PAL gene expression has been shown in other disease systems.
RNA from leaf midribs were analyzed 2, 7, 11, 14 and 21 days after inoculation. This study was an attempt to develop a method to reveal gene expression during DED disease development.

Table 6-1. Gene expressions at certain days after inoculation with Ophiostoma novo-ulmi *of susceptible* U. americana *seedlings. PAL is phenylalanine ammonia-lyase, CHT is chitinase, and PGIP is polygalacturonase inhibiting protein. Nasmith et al. 2008.*

Gene	0	2	7	10	14	21
PAL	+	+	+	+	+	+
CHT			+	+	+	
PGIP		+	+	+		

The main results are summarized in Table 6-1, which shows that CHT and PGIP expressions were limited to certain times following inoculation, while PAL was expressed at all sampling dates during the 3-week test period. No expression of CHT and PGIP was found in the control material while weak expression was noted for PAL at all sampling dates. The fungus was re-isolated at all sampling dates. No fungus was detected until day 7 in the inoculated material and it was absent from the control seedlings.

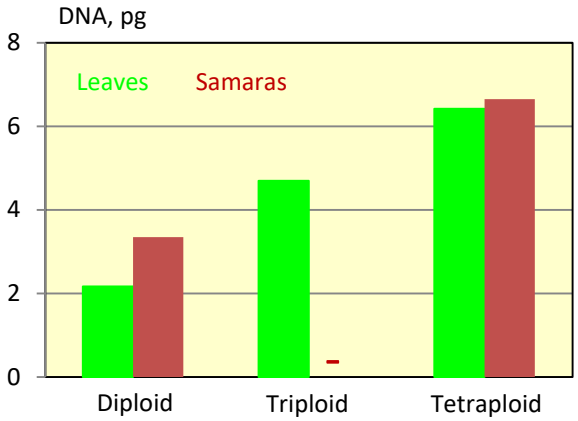

Figure 6-1. DNA content in picograms for leaves and samaras of diploid, triploid, and tetraploid U. amercana. *Whittermore anf Olsen 2011.*

6.2 Callus cultures

Two types of *Ulmus americana* cultures, friable and hard, were inoculated with *Ophiostoma novo-ulmi* for histologic and genetic studies (Aoun et al. 2009). The hard culture was obtained from buds and the friable from seedlings of susceptible *U. americana*. Small paper disks with the fungus strain H327 were applied at the center of the callus at inoculation. Pure water inoculations were used as controls. Samples were taken at 4, 24, 48, 72 and 144 hours after inoculation. Phenylalanine ammonia lyase (PAL) gene expression was studied at each sampling occasion. This substance is known to play a role in defence against pathogens. Callus reactions to the inoculation were studied by light microscopy (LM), transmission-

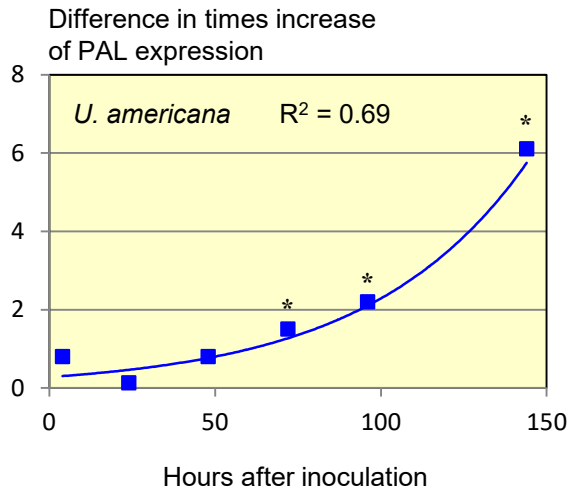

Figure 6-2. The difference in gene expression of the phenylalanine ammonia lyase (PAL) gene at certain hours after inoculation with Ophiostoma novo-ulmi *expressed as multiples of starting value in control material (0). Significant differences between control and inoculated material are indicated. Aoun et al. 2009.*

electron microscopy (TEM), and scanning-electron microscopy (SEM).

Fig. 6-2 reveals that the expression of the PAL gene was significantly higher in the inoculated material from 72 hours after inoculation and onwards. The expression of PAL in the control material did not exceed 1.5 times the expression at the start of the experiment while it was around 7 times higher in the inoculated material at 144 h. The pattern of the curve in Fig. 6-2 was taken as evidence for its induction by *Ophiostoma novo-ulmi*. At 72 h after inoculation fungal hyphae were found in the callus tissues. Already at 48 h after inoculation phenolic and starch deposition were found to be more abundant in the inoculated material than in the control.

Cell wall degradation was most pronounced in cells close to fungal hyphae. Suberization and lignification were most pronounced in the hard tissue cultures. This investigation showed that the presence of the *Ophiostoma novo-ulmi* fungus triggered the activity of the PAL gene. Whether the same reactions occur after inoculation of seedlings or trees *in vivo* remains to be tested.

The objective of another study by Aoun et al. (2010) was to identify genes upregulated during the interaction between *Ophiostoma novo-ulmi* and *U. americana*. Hard callus cultures of *Ulmus americana* were inoculated with *Ophiostoma novo-ulmi* for analysis of transcripts. The inoculations were carried out in the same way as in the previous paper. In addition mycelium of the fungus was grown on solid medium. Pure water inoculations were used as controls. Samples were taken at 4, 24, 48, 72, 96 and 144 hours after inoculation.

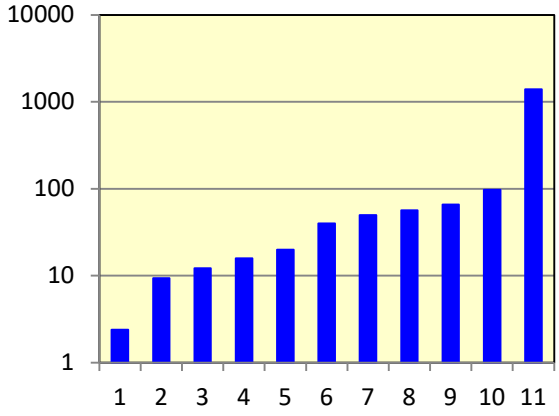

Figure 6-3. The difference in gene expression of 11 U. americana *unisequences after inoculation with* Ophiostoma novo-ulmi *expressed as multiples of starting value in control material. Clone No 7 is related to formation of lignans, No 9 to isoflavanoids, and 11 to anthocyanins. Note the logarithmic scale. Aoun et al. 2010.*

To fulfil the objective of this study the following steps were taken:

1. An interactive *U. americana – Ophiostoma novo-ulmi* cDNA library was constructed based on material collected 72 hours after inoculation, at which time the fungus had germinated and spread over the entire callus tissue.
2. To obtain expressed sequence tags (ESTs) cDNA ESTs were assembled into unisequences
3. Differential screening to identify unisequences upregulated during the interaction between host and fungus
4. Validation by quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) of a subset of American elm unisequences

We shall concentrate on the last point. Constitutive expression was noted for six cDNA clones and another 12 clones showed upregulation in the material analyzed at 72 h after inoculation. Their development over the timespan 0–144 h after inoculation was studied in detail by RT-qPCR. Four of the six constitutive clones were stable over time while 11 of the upregulated 12 cDNA clones showed increased expression from 48 h after inoculation. Fig. 6-3 reveals a large variation of the difference in expression between the control and inoculated material with the range 2.4–1,326 times. The highest value was noted for O-methyltransferase while the lowest was noted for ripening-induced protein. It was noted that genes for enzymes leading to accumulation of phytoalexins occurred among the upregulated genes following inoculation. Also genes involved in cell wall formation occurred among the upregulated genes.

The results in this investigation show that defence reactions in *U. americana* tissue cultures do not occur until germination and spread of the pathogen has taken place at 48 h after inoculation.

In all, 638 cDNA clones were found in the interaction cDNA library. Differential screening of the 638 clones resulted in 178 clones that hybridized more strongly with the unsubtracted tester probe.

The interaction between *Ophiostoma novo-ulmi* and American elm at molecular level was studied by Sherif et al. (2014). A DED reporter system with *cerato ulmin* (CU) promotor was used. *In vitro*-grown plantlets and callus tissues were inoculated by a transformed isolate denoted MH75. Three different transformants of this strain with three reporter proteins, GFP, GUS, and LUC, were prepared. The spores and hyphae of transformants could be traced in the host and even quantified. The results were summarized in the following way: *These reporters can be easily detected and quantified allowing for deeper investigations and reliable experimental setups to study the induction kinetics of CU under different conditions.* It was also pointed out that this system might be used for early testing for DED susceptibility. If a reliable early testing method along these lines could be developed it would be a great achievement for DED-resistance elm breeding.

Another study of the interaction at the gene level between *Ophiostoma* and American elm was presented by Sherif et al. (2016). This study focused on jasmonic acid (JA) and salicylic acid (SA), which are believed to be defence-response elicitors. Inoculations took place at several points on the stems of commercial and susceptible American elm seedlings. The development of JA and SA was followed until 144 hours after inoculation.
Inoculations in the study of the defence mechanisms of SA and JA took place on the main stem at a point 20–25 cm above ground. Vascular colouration 80 cm above the inoculation point was assessed 60 days after inoculation. RNA extraction and gene expression were analyzed.
The levels of JA, SA and ferulic acid were determined.
The expression of the fungal *On.cerato ulmin* and *On.Actin* genes was analyzed following inoculations by three transgenic fungal strains.
The presence of the fungus in inoculated material at one cm above the inoculation point was examined by scanning electron microscopy at four occasions 0, 48, 96 and 144 h after inoculation.
No visible DED symptoms were noted during the 144 hours after inoculation. The scanning electron microscopy revealed that many hyphae and conidia were found inside or among xylem elements, especially in the susceptible material.
The expression of 15 defence-responsive genes was reported. The development over time for nine of them was presented in the paper and six of them in the supplementary document. In Table 6-2 we have indicated how many times stronger the expression was in the tolerant clone than in the DED-susceptible material. Twice there was a stronger expression in the susceptible material and once significantly stronger. In both cases the peak was noted at the termination of the experiment, 144 h. For six of the expressed genes there were significant differences, which suggest that these genes play a role in DED-sensibility. Four of the six genes reached the peak at 96 hours while one each reached the peak at 122 h and 144 h. Two peaks were noted for pseudo-hevein and S-norcoclourine synthase II. In both cases there was a drop in expression at 122 h followed by an increase at 144 h. It was concluded that DED-susceptibility is not a question of presence or absence of disease-responsive genes; rather it is a matter of timing of the expression of such genes.
Based on inoculation of calli it was hypothesized that a group of genes involved in the phenylpropanoid pathway are differently expressed in susceptible and tolerant American elms. Therefore, the expression of phenylpropanoid lyase (PAL) was studied after inoculation. A pronounced peak of PAL in the susceptible elms was noted at 24 h. At later sampling occasions 48–144 h there was no significant difference between the two types of mate-

Table 6-2. Differential expression times of disease-responsive genes at peak difference between one tolerant American elm clone and one susceptible American elm followed for 144 hours after inoculation with Ophiostoma novo-ulmi.*Sherif et al. 2016.*

	Peak difference at sampling after inoculation, hours	*Material*	*Times difference*	*Presence of 2 peaks*
Pseudo-hevein	96	tolerant	3.0	+
Glycocide hydrolase	144	susceptible	4.4	
pr3a	144	susceptible	1.8 ns	
pr4	96	tolerant	2.4	
pr5b	122	tolerant	29.0	
Proteinase inhibitor	122	tolerant	1.7 ns	
Kunitz inhibitor	144	tolerant	1.5	
S-norcoclourine synthase II	96	tolerant	3.4	+
E-class P450	63	tolerant	4.6	

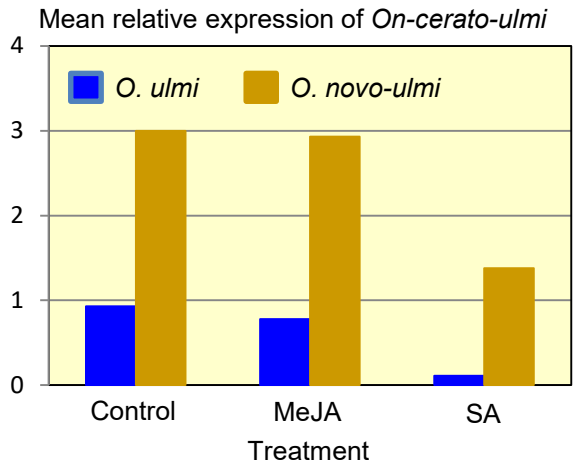

Figure 6-4. The mean relative expression of the fungal gene On-Cerato-ulmi *following inoculations of American elm seedlings with three strains of* O. ulmi *and 3 strains of* O. novo-ulmi. *Besides the control two treatments were applied: 50 M methyl-jasmonate and 2mM salicylic acid. Sherif et al. 2016.*

rial. Ferulic acid, which is another compound that may be involved in DED-sensitivity, did not show any significant difference between susceptible and tolerant materials up to 144 h after inoculation. These *in vivo* results differ from results obtained after inoculation of callus tissues, which suggests different response to inoculation *in vivo* and *in vitro*.

The levels of salicylic acid (SA) and jasmonic acid (JA) in inoculated elms were studied since these compounds probably constitute the backbone of plant defence systems. A significantly higher amount of SA was noted at 144 h for the susceptible material. A pronounced peak of JA at 96 h in the tolerant clone was found while the level of JA in the susceptible material remained low during the experiment time, 0–144 h. These results suggest that JA plays a major role for tolerance to DED.

Whether defence elicitors such as SA and methyl-jasmonate (MeJA) were expressed following spraying with two concentrations of SA (2 and 4 mM) and MeJA (50 and 100 µM) were tested. Disease-responsive genes were induced significantly more by MeJA treatment and most so at the highest concentration of MeJA.

It was stated that application of defence elicitors enhances the field tolerance to DED. However, the results following treatments with SA, MeJA, or a combination of these two compounds, did not result in any significant difference in disease incidence between treated and control materials. Actually, the highest disease incidence was noted for the SA treatment. In contrast with these results, the fungal *On-Actin* gene was significantly repressed by SA and SA + MeJA treatment. The fungal fresh weight was substantially reduced (3.5 x) in the treatment with 2 or 4 mM SA.

The variation in expression of the fungal *On-cerato ulmin* gene among three strains in each of *O. ulmi* and *O. novo-ulmi* was studied following treatment with 50 µM MeJA and mM SA. The expression was much less in the three *O. ulmi strains* than in the *O. novo-ulmi* strains (Fig 6-4). Moreover, in the three *O. ulmi* strains the expression was significantly lower in the SA treatment than in the control. The variation in expression among the three *O. novo-ulmi* strains was substantial, with SA treatment having the lowest expression.

This multi-faceted investigation has considerably improved our knowledge of important mechanisms regulating DED susceptibility.

6.3 Female sterility

The significance of female sterility was the objective of a study in one natural (Natpop) and one artificial population (Artpop) of *U. minor* in Spain by López-Alamansa et al. (2003). Both populations are growing in central Spain. The Artpop consists of clones collected from all over Spain and was studied two consecutive years. The mean number of ovules in 10 inflorescences in each of 25 (Natpop) and 40 trees (Artpop) in the two populations was calculated. Fruit per flower and seed per flower was determined in 71 trees of Natpop and 40 trees in the Artpop. Prospective femaleness was calculated as:

$$\text{Femaleness} = g/[g + a/(\Sigma g/\Sigma a)],$$

in which g = number of seeds per flower and a = the average number of stamen-bearing flowers.

In both populations there was a bimodal distribution of prospective femaleness with a higher proportion of male trees in the Natpop than in the Artpop, 0.71 versus 0.44. Gynoecial malformation, gynoecial necrosis, and seed abortion were attributed to low seed set in both populations. Two categories of trees were observed: trees with substantial seed and pollen production constituted one group and the other contained more or less female sterile trees with normal pollen production. In the Artpop, which was studied two years, there was a significant relationship between gender the two years with the degree of explanation for this relationship ≈52%.

There were large variations among trees for all traits studied, which probably are genetic, but which cannot be proven in this study.

6.4 Summary

The occurrence of 2 triploid *U. ameicana* trees called for an analysis of the ploidy level of American elm trees in USA. In a sample of 81 trees, 17 were diploid. They all occurred south of latitude 40ºN.

The expression of genes regulating phenylalanine ammonia-lyase (PAL), chitinase (CHT), and polygalacturonase inhibiting protein (PGIP) was followed after inoculations with *Ophiostoma novo-ulmi.*The stress-related PAL gene was found at all sampling days after inoculation, 2–21 days.

The relationship between time after inoculation and PAL activity was observed in callus cultures. From 72 hours after inoculation and onwards, PAL activity was significantly higher than at the start of the experiment, which indicates that PAL was triggered by *Ophiostoma novo-ulmi* inoculation.

Another callus culture study with susceptible and DED-resistant materaial revealed that defence reactions in *U. americana* tissue cultures do not occur until germination and spread of the pathogen have taken place at 48 h after inoculation.

One study focused on jasmonic acid (JA) and salicylic acid (SA), which are believed to be defence-response elicitors. Disease-responsive genes were induced significantly more by methyl-jasmonate (MeJA) treatment. A significantly higher amount of SA was noted at 144 h in DED-susceptible material. A pronounced peak of JA at 96 h in DED-tolerant material was found while the level of JA in the susceptible material remained low during the experiment time, 0–144 h.

Female sterility was studied in 2 Spanish populations, one natural and the other planted with clonal material. Gynoecial malformation, gynoecial necrosis, and seed abortion contributed to low seed set in both populations. Two categories of trees were observed: trees with substantial seed and pollen production constituted one group and the other contained more or less female-sterile trees with normal pollen production.

7. References

Ahn, J.Y., Hong, K.N., Lee, J.W., and Yang, B-H. 2013. Population genetic variation of *Ulmus davidiana* var. *japonica* in South Korea based on ISSR markers. J. Korean For. Sci. 102:560-565.

Anderbrant, O., Yuvaraj, J., Martin, J., Gil, L. and Witzell,.J. 2017. Feeding by *Scolytus* bark beetles to test for differently susceptible elm varieties. J. Appl. Entom. 141:417-420. doi: 10.1111/jen.12354.

Aoun, M., Rioux, D., Simard, M., and Bernier, L. 2009. Fungal colonization and host defense reactions in *Ulmus americana* callus cultures inoculated with *Ophiostoma novo-ulmi*. Phytopathology 99:642-650. DOI:10.1094/Phyto-99-6-0642.

Aoun, M., Volker, V., Boyle, B., and Bernier, L. 2010. Identification and monitoring of *Ulmus americana* transcripts during *in vitro* interactions with the Dutch elm disease pathogen *Ophiostoma novo-ulmi*. Phys. Mol. Plant Path. 74:254-266. doi:10.1016/j.pmpp.2010.04.003

Beier, G.L. and Blanchette, R.A. 2018. Defense responses in the xylem of *Ulmus americana* after inoculation with *Ophiostoma novo-ulmi*. For. Path. 48e12453. 18pp. doi.org/10.1111/efp.12453.

Bernier L., Aoun, M., Bouvet, G.F., Comeau, A., Dufour, J., Naruzawa, E.S., Nigg, M., and Plourde, K.V. 2015. Genomics of the Dutch elm disease pathosystem; are we there yet? iForest8:149-157. http://www.sisef.it/iForest/contents/?id=iFor1211-00.

Bertolasi, B., Leonarduzzi, C., Piotti, A., Leonardi, S., Zago, L., Gui, L., Goian, F., Vanetti, I., and Binelli, G. 2015. A last stand in the Po valley: genetic structure and gene flow patterns in *Ulmus minor* and *U. pumila*. Ann. Bot. 115:683-692. doi:10.1093/aob/mcu256

Black-Samuelsson, S,, Whiteley, R.E., and Jansson, G. 2003. Growth and leaf morphology response to drought stress in the riparian broadleaved tree, *Ulmus laevis* (Pall.). Silvae Genet. 52:292-299.

Bosu, P.P. and Wagner, M.R. 2007. Effects of induced stress on leaf trichome density and foliar nutrients of three elm (*Ulmus*) species. Implications for resistance to elm leaf beetle. Env. Entom. 36:595-601.

Brunet, J. and Guries, R.P. 2016. Elm genetic diversity and hybridization in the presence of Dutch elm disease. Proc. Amer. Elm Restor. Workshop 2016. 99-107.

Brunet, J., Zalapa, J.E., Pecori, F., and Santini, A. 2013. Hybridization and introgression between the exotic Siberian elm, *Ulmus pumila*¸ and the native field elm, *U. minor*, in Italy. Biol. Invasions 15:2717-2730. DOI 10.1007/s10530-013-0486-z.

Brunet, J., Zalapa, J.E., and Guries, R. 2016. Conservation of genetic diversity in slippery elm (*Ulmus rubra*) in Wisconsin despite the devastating impact of Dutch elm disease. Cons. Genet. 17:1001-1010. DOI 10.1007/s10592-016-0838-1.

Buiteveld, J., Van der Werf, B., and Hemstra, J.A. 2015. Comparison of commercial elm cultivars and promising unreleased Dutch clones for resistance to *Ophiostoma novo-ulmi*. iForest - Biogeosciences and Forestry 8(2):158-164. DOI:10.3832/iFor1209-008

Buiteveld, J., Van den Broeck, A., Cox, K., and Collin, E. 2016. Human impact on the genetic diversity of Dutch field elm (*Ulmus minor*) populations in the Netherlands: implications for conservation. Plant Ecol. Evol. 149:165-176.

Cannell, M.G.R., and Smith, R.I. 1983. Thermal time, chill days and prediction of bud burst in *Picea sitchensis*. J. Appl. Ecol. 20: 951- 963. Doi:10.2307/2403139.

Chen, P., Liu, P., Zhang, Q., Bu, C., Lu, C., Srivastava, S., Zhang, D., and Song, Y. 2021 Gene coexpression network analysis indicates that hub genes related to photosynthesis and starch synthesis modulate salt stress tolerance in *Ulmus pumila*. Int. J. Mol. Sci. 2021, 22, 4410. https://doi.org/10.3390/ijms22094410

Chen, X., Min, D., Yasir, T.A., and Hu, Y-G. 2012. Evaluation of 14 morphological, yield-related and physiological traits as indicators of drought tolerance in Chinese winter bread wheat revealed by analysis of the membership function value of drought tolerance (MFVD). Field Crop Res. 137:195–201

Chen, X. and Xu, L. 2012. Phenological responses of *Ulmus pumila* (Siberian elm) to climate change in the temperate zone of China. Int. J. Biometeorrologi 56:695-706. Doi;10.1007/ss00484-011-0471-0.

Chudzińska, M., Pałucka, M., Pasławska, A., Litkowiec, M., Lewandowski, and A., Kozioł, C. 2018. Wyniki wstęp− nych badań nad zmiennością genetyczną oraz zróżnicowaniem genetycznym między populacjami wiązu górskiego (*Ulmus glabra* Huds.) w Polsce. Sylwan 162 (9): 727−736.

Cogolludo-Agustin, M.A., Aguândezà, D., and Gil, L. 2000. Identification of native and hybrid elms in Spain using isozyme gene markers. Heredity 85:157-166.

Coleman, M., A'Hara, S.W., Tomlinson, P.R., and Davey, P.I. 2016. Elm clone identification the conundrum of the slow spread of Dutch elm disease on the Isle of Man. New J. Bot. 6:79-89.

Coleman, M., Hollingsworth, M.L., and Hollingsworth, P.M. 2000. Applications of RAPDs to the critical taxonomy of the English endemic elm, *Ulmus plotii* Druce. Bot. J. Linn. Soc.133: 241-262. Doi: 10.1006/bojl.1999.0331.

Collada, C., Fuentes-Utrilla, P., Gil, L., and Cervera, M.T. 2004. Characterization of microsatellite loci in *Ulmus minor* Miller and cross-amplification in *U. glabra* Hudson and *U. laevis* Pall. Mol. Ecol. Not. ¤:731-732.

Collin, E., Bilger, I., Eriksson, G., and Turok, J. 2000. The conservation of elm genetic resources in Europe. In: The Elms: Breeding, Conservation and Disease Management. Ed. C.P. Dunn. Kluwer Acad. Publ. Boston, pp 281-293.

Collin, E., Rusanen, M., Ackzell, L. Bohnens, J., de Aguiar, A., Diamandis, S., Franke, A., Gil, L,. Harvengt, L, Hollingsworth, P., Jenkins, G. Meier-Dinkel, A., Mittempergher, L, Musch, B., Nagy, L., Pâques, L., Pinon, J., Piou, D., Rotach, P., Santini, A., Van den Broeck, A., and Wolf, H. 2004. Methods and progress in the conservation of elm genetic resources in Europe. Invest. Agrar: Sist. Recur. For. 13:261-272.

Collin, E. 2006. Conservation and utilization of genetic resources of European elm species. Workshop on genetics Leuven, Belgium April 18-22 2006. 9pp.

Collin, E, and Bozzano, M. 2015. Implementing the dynamic conservation of elm genetic resources in Europe: case studies and perspectives. IForest 8:143-148. DOI 10.3832/iFor1206-008.

Collin, E., Rondouin, M., Joyeau, C., Matz, S., Raimbault, P., Harvengt, L., Bilger, I., and Gulbert, M. 2020. Conservation and use of elm genetic resources in France: results and perspectives. IForest 13:41-47.

Coop, G., Witonsky, D., Di Rienzo, A., and Pritchard, J.K. 2010. Using environmental correlations to identify loci underlying local adaptation. Genetics 185:1411–1423.

Corander, J., Sirén, J., and Arjas, E. 2008. Bayesian spatial modeling of genetic population structure. Computation Stat. 23: 111–129.

Cornuet, J.M and Luikart, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001-2014.

Cox, K., Van den Broek, A., and Mijnsbrugge, K. 2012. Genetic variation in European elms. Genetic characterization of populations of *Ulmus laevis* and of the *U. minor-U. glabra* complex. Rapp. Inst. Natur. Bosonderzoek 2012. 44pp.

Cox, K., Van den Broeck, A., Van der Mijnsbrugge, K., Buiteveld, J., Collin, E., Heybroek, H.M., and Mergeay, J. 2014. Interspecific hybridisation and interaction with cultivars affect the genetic variation of *Ulmus minor* and *Ulmus glabra* in Flanders. Tree Gen. Genom. 10:813-826. DOI 10.1007/s11295-014-0722-4

Ebrahimi, A., Antonides, J.D., Pinchot. C.C., Slavicek, J.M.,. Flower, C.E., and Woeste, C.E. 2021. The complete chloroplast genome sequence of American elm (*Ulmus americana*) and comparative genomics of related species. Tree Gen. Genom, 17:5 doi.org/10.1007/s11296-020-01487-3

Elowsky, C.G., Jordon-Thaden, I.E., Kaul, R.B. 2013. A morphological analysis a hybrid swarm of native *Ulmus rubra* and introduced *U. pumila* (*Ulmaceae*) in southeastern Nebraska. Phytoneuron 44:1-23.

Eriksson, G. 2001. Conservation of noble hardwoods in Europe. Can. J. For. Res. 31:577-587.

Frichot, E., Schoville, S.D., Bouchard, G., and François, O. 2013. Testing for associations between loci and environmental gradients using latent factor mixed models. Mol. Biol. Evol. 30, 1687–1699.

Fuentes-Utrilla, P., Venturas, M., Hollingworth, P.M., Squirrell, J., Collada, C., Stone, G.N., and Gil, L. 2013. Extending glacial refugia for a European tree: genetic markers show that Iberian populations of white elm are native relicts and not introductions. Heredity (2013): 1-9.

Fuentes-Utrilla, P., M Valbuena-Carabaña, M., Ennos, R., and Gil, L. 2014. Population clustering and clonal structure evidence the relict state of *Ulmus minor* Mill. in the Balearic Islands. Heredity 113:21-31. doi:10.1038/hdy.2014.21

Gartland, J.S., McHugh, A.T., Brasier, C.M., Irvine, R.J., Fenning, T.M., and Gartland, K.M.A. 2000. Regeneration of phenotypically normal English elm (*Ulmus procera*) plantlets following transformation with an *Agrobacterium tumefaciens* binary vector. Tree Phys. 20:901-907.

Garza J.C, and Williamson E.G. (2001) Detection of reduction in population size using data from microsatellite loci. Mol Ecol 10:305–318

Gehle, T. and Krabel, D. 2002. Genetic differentiation of elm (*Ulmus minor* Mill., *Ulmus laevis* Pallas) in mixed stands from the Elbe flood-plains. For. Gen. 9:39-46.

Gheraldini, L. Falusi, M., and Santini, A. 2006. Variation in timing of bud-burst of *Ulmus minor* clones from different geographical origins. Can. J. For. Res. 36:1982-1991. Doi:10.1139/X06-092.

Gheraldini, L and Santini, A. 2009. Avoidance by early flushing: a new perspective on Dutch elm disease. IForest 2:143-153. Doi:10:3832/iFor0508-002

Gheraldini, L and Santini, A., Black-Samuelsson, S., Myking, T., and Falusi, M. 2010. Bud dormancy release in elm (*Ulmus* spp.) clones – a case study of photoperiod and temperature response. Tree Phys. 30:264-274.

Gil, L., Fuentes-Utrilla, P., Soto, A., Cervera, T.M., and Collada, C. 2004. English elm is a 2,000-year-old Roman clone. Nature 43:1053.

Goodall-Copestoke, W.P., Hollingsworth, M.L., Hollingsworth, P.M., Jenkins, G.I., and Collin, E. 2005. Molecular markers and *ex situ* conservation of the European elms (*Ulmus* spp). Biol. Conserv. 122:537-546-

Gregorius, H.-R. 1978. The concept of diversity and its formal relationship to heterozygosity and distance. Math. Biosci. 41:253-271.

Griffin, J.J., Jacobi, W.R., McPherson, E.G., Sadof, C.S., McKenna, J.R., Gleason, M.L., Gautier, N.W., Potter, D.A., Smitley, D.R., Adams, G.C., Gould, A.B., Cash, C.R., Walla, J.A.. Starrett, M.C., Chastagner, G., Sibley, J.L., Krischik, V.A., and Newby, A.F. 2017. Ten-year performance of the United States national elm trial. Arbo. Urban For. 43:107-120.

Harvengt, L., Meier-Dinkel, A., Dumas, E., and Collin, E. 2004. Establishment of a cryopreserved gene bank of European elms. Can. J. For. Res. 34:43-55.

Heybroek, H.M. 2000. Notes on elm breeding and genetics. In The Elms: Breeding, Conservation, and Disease Management, Christopher P. Dunn Ed., Kluwer Academic Publishers, Boston, pp 249-258.

Heybroek, H. 2015. The elm, tree of milk and wine. iForest – Biogeosciences and Forestry 8:181-186. doi: 10.3832/iFor1244-008

Hou, H., Ye, H., Wang, Z Wu, J., Gao, Y., Han, W., Na, D., Sun, G, and Wang, Y. 2020. Demographic history and genetic differentiation of an endemic and endangered *Ulmus lamellosa* (*Ulmaceae*). BMC Plant Biol.20:526. Doi:.org/10.1186/s12870-020-02723-7

Howe HF, Miriti MN (2004). When seed dispersal matters. BioScience 54: 651-660. - doi: 10.1641/0006-3568(2004)054[0651:WSDM]2.0.CO;2

Huang,H., Yang, R., Hao, L., and Zhang, G. 2022. The practical application of agricultural genetic breeding technology in elm cultivation based on big data analysis. Hindawi Mobile Info. Syst. Volume 2022, Article ID 2441292, 9 pages. https://doi.org/10.1155/2022/2441292

Jost L (2008). G_{ST} and its relatives do not measure differentiation. Mol. Ecol. 17(18):4015–4026. /doi. org/10.1111/j.1365-294X. 2008.03887.x

Kätzel, R., Liesebach, M., Haverkamp, M., Tröber. U., and Wolf, H. 2019. Genetische Ressourcen der Flatterulme erhalten and nutzen. AFZ, der Wald: Allgemeine Forstzeitschrift für Waldwirtschaft und Umweltvorsorge 74:17–21.

Kavaliauskas, D., Fussi, B., Rau, B., and Seho, M. 2022. Assessing genetic diversity of European white elm (*Ulmus laevis* Pallas) in Bavaria as an indicator for *in situ* conservation and sustainable use of the species genetic resources. Eur. J. For. Res. 18pp. doi.org/10.1007/ s10342-022-01509-w.

Khan, R.U., Ali, N., Rahman, S.U., Rahman, I.U., Hashem, A., Almutairi, K.F., Abd¬Allah, E.F., Harosonowati, W., Khan, M.A., Rahim, F., Khan, F.U., and ul Haq, N. 2022. Genetic diversity and population structure of the endangered *Ulmus villosa* in Pakistan revealed by DNA barcode markers. Appl. Sci. 2022, 12, 9293. doi. org/10.3390/app12189293.

Lafortezza R, Sanesi G, Chen J (2013). Largescale effects of forest management in Mediterranean landscapes of Europe. iForest 6: 331-335. doi: 10.3832/iFor0941-006

Li, M., Lopez, R., Ventura, M., Martin, A., Dominguez, J., Gordaliza, G.G., Gil, L., and Rodriguez-Cacerrada, J. 2016. Physiological and biochemical differences among *Ulmus minor* genotypes showing a gradient of resistance to Dutch elm disease. For. Path. 46215-228.

Litkowiec, M., Chudzinsssssska, M., Paslawska, A., Palucka, M., Koziol, C., and Lewandowski, A. 2022. Population history, genetic variation, and conservation status of European white elm (*Ulmus laevis*) in Poland. Ann. For. Sci. Open Access. 13pp.

Liu, L.. , Chen, W., , Zheng, X., Li, J., Yan, D-T., Liu, L., Liu, X., and Wang, Y-L. 2016. Genetic diversity of *Ulmus lamellosa* by morphological traits and sequence-related amplified polymorphism (SRAP) markers. Biochem. Syst. Ecol. 66:272-280.

Liu, L., Li, Y., Feng, S., Yan, S., Wang, J., Huang, Y. , and Yang, M. 2022. Complete chloroplast genome structure of four *Ulmus* species and *Hemipetelea davidii* and comparative analysis within *Ulmaceae* species. Natureportfolio. 16pp. doi.org/10.1038/s41598-022-20184-w

Lopez-Almansa, J.C., Pannell, J.R., and Gil, L. 2003. Female sterility in *Ulmus minor* (*Ulmaceae*): A hypothesis invoking the cost of sex in a clonal plant. Amer. J. Bot. 90:603-609-

Lyu , Y-Z., Dong, X-Y., Huang, L-B., Zheng,J-W., He, X-D., Sun, H-N., and Jiang, Z-P. 2020. SLAF-seq uncovers the genetic diversity and adaptation of Chinese elm (*Ulmus parvifolia*) in Eastern China. Forests 11,80; DOI:10.3390/f11010080.

Martin, J.A., Solla, A., Woodward, S., and Gil, L.. 2005a. Fourier transform-infrared spectroscopy as a new method for evaluating host resistance in the Dutch elm disease complex. Tree Physiol. 25:1331-1336

Martin, J.A., Solla, A., Coimbra, M.A., and Gil. 2005b. Metabolic distinction of *Ulmus minor* xylem tissues after inoculation with *Ophiostoma novo-ulmi*. Phytochem. 66:2458-2467. Doi: 10.1016/j.phytochem.2005.08.004

Martin, J.A., Solla, A., Woodward, S., and Gil. 2007. Detection of differential changes in lignin composition of elm xylem tissue inoculated with *Ophiostoma novo-ulmi* using Fourier transform-infrared spectroscopy. For. Path. 37:187-191.

Martin, J.A., Solla, A., Coimbra, M.A., and Gil, L. 2008a. Metabolic fingerprinting allows discrimination between *Ulmus pumila* and *U. minor*, and between *U. minor* clones of different susceptibility to Dutch elm disease. For. Path. 38:244-256. Doi: 101111/j1439-0329.2007.00542.x

Martin, A.J., Solla, A., Domingues, M.R., Coimbra, M.A., and Gil, L. 2008b. Exogenous phenol increase resistance of *Ulmus minor* to Dutch elm dsease through formation of suberin-like compounds on xylem tissue. Envir. Exper. Bot. 64:97-104.

Martin, J.A., Solla, A., Esteban, L.G., de Palacios, P., and Gil, L. 2009. Bordered pit and ray morphology involvement in elm resistance to *Ophiostoma novo-ulmi.* Can. J. For. Res. 39:420-429. Doi:10.1139/X08-183.

Martin, J.A., Witzell, J., Blumenstein, K., Rozpedowska, E., Helander, M., Sieber, T.N., and Gil, L. 2013. Resistance to Dutch elm disease reduces presence of xylem endophytic fungi in elms (*Ulmaceae* sp). PLoS ONE 8(2): e56987 Doi: 10.1371/journal.pone.0056987

Martin, J.A,.Maccaya-Sanz, D., Witzell, J., Blumenstein, K., and Gil, L. 2015a. Strong *in vitro* antagonism by elm xylem endophytes is not accompanied by temporally stable *in planta* protection against a vascular pathogen under field conditions. Eur. J. Plant Pathol. 142:185-196. DOI 10.1007/s10658-015-0602-2.

Martín, J.A., Solla A., Venturas, M.,, Collada, C., Domínguez, J., Miranda, E., Fuentes P., Burón, M., Iglesias, S., and Gil, L. 2015b. Seven *Ulmus minor* clones tolerant to *Ophiostoma novo-ulmi* registered as forest reproductive material in Spain. iForest – Biogeosciences and Forestry 8:172-180. doi: 10.3832/iFor1224-008.

Martin, J.A., Fuentes-Utrilla, P., Gil, L., and Wizell, J. 2019. Ecological factors in Dutch elm disease complex in Europe. Ecol. Bull. 53:209-224.

Martin-Benito, D., Garcia-Vallejo, M.C., Pajares, J.A.; and López. D. 2005. Triterpenes in elms in Spain. Can. J. For. Res. 35:199-205.

Martín del Puerto M., García, F.M., Mohanty, A., and Martín, J.P. 2017. Genetic diversity in elict and fragmented populations of *Ulmus glabra* Hudson in the central system of the Iberian peninsula. Forests 8, 143, 20pp. doi:10.3390/f8050143.

Mi, Y.F., Ma, X.W., and Chen, S.C. 2013. Resistant evaluation of kiwifruit rootstocks to root zone hypoxia stress. Am. J. Plant Sci. 4:945–954.

Miller, F., Malmquist, K., and Ware, G. 2001. Evaluation of Asian, European, and North American elm (*Ulmus* spp.) biotypes to feeding by spring and fall cankerworms. 2001. J. Environ. Hort. 19:216-221.

Miller, F., and Ware, G. 2001a. Evaluation of eleven newly acquired Asian elms for their suitability to adult elm leaf beetle (*Coleoptera*: *Chrysomelidae*). J. Environ. Hort. 19:96-99.

Miller, F., and Ware, G. 2001b. Feeding and ovipositional responses of adult elm leaf beetle (*Coleoptera: Chrysomelidae*) to simple and complex Asian elm hybrid selections. J. Environ. Hort. 10:18-131.

Miller, F., and Ware, G. 2001c. Host suitability of Asiatic elm species and hybrids for larvae and adults of the elm beetle (*Coleoptera: Chrysomelidae*). J. Arboric. 27:118-123.

Miller, F. and Ware, G. 2001d. Resistance of temperate Chinese elms (*Ulmus* spp) to feeding by the adult elm beetle (*Coleoptera: Chrysomelidae*). J. Econ. Entomol. 92:162-166.

Miller, F. and Ware, G. 2002. Suitability and feeding preference of selected north American, European and Asian elm (*Ulmus* spp) biotypes to elm leaf beetle (*Coleopteran:Chrysomelidae*). J. Envr. Hort. 20:148-154.

Miller, F., Malmquist, K., and Ware, G. 2003. Resistance of *Ulmaceae* to feeding by the adult elm leaf beetle (*Coleopteran:Chrysomelidae*). J. Arboricult. 29:98-103.

Vander Mijnsbrugge, K., LeClercq, R., and Michiels, B. 2016. Dissection of leaf morphological traits from isolated and relict populations of *Ulmus laevis* reveals putative random ecotype evolution. Plant Syst. Evol. 302:219-229' DOl 10.1007/00606-015-1255-5.

Mittembergher, L. and Santini, A. 2004. The history of elm breeding. Invest. Agrar: Sist Recur. For. 13:161-177.

Mu, D., Zwiazek, J.J., Li, Z., and Zhang, W. 2016. Genotypic variation in salt tolerance of *Ulmus pumila* plants obtained by shoot micropropagation 38:188. 16pp. DOI 10.1007/s11738-016-2189-4

Myking, T. and Skrøppa, T. 2007. Variation in phenology and height increment of northern *Ulmus glabra* populations: Implications for conservation. Scand. J. For. Res. 22:369-374.

Myking, T. and Yakovlev, I. 2008. Variation in leaf morphology and chloroplast DNA in *Ulmus glabra* in the northern suture zone: Effects of distinct glacial refugia. Scand. J. For. Res. 21:99-107.

Namkoong, G., 1984. Strategies for gene conservation in forest tree breeding. In: C.W. Yeatman, D. Kafton and G. Wilkes (Editors), Plant Genetic Resources: A Conservation Imperative, AAAS Selected Symposium 87. Westview Press, Boulder, CO, pp. 93-109.

Nasmith, C., Jeng, R., and Hubbes, M. 2008. A comparison of *in vivo* targeted gene expression during fungal colonization of DED-susceptible *Ulmus americana*. For. Path. 38:104-112.

Nazir, I., Dutt, V., Raj, A., Bath, G.M., Bath, B.A., and Wani, A.A. 2021. Vegetative propagation of *Ulmus villosa* Brandis and *U. wallichiana* Planchon: optimizing plant growth regulators and growing media on root formation in hardwood stem cuttings. Current Sci.121:691-696.

Nei M (1972) Genetic distance between populations. Am. Nat. 106:283–292. doi.org/10.1086/282771

Nei, M. 1973. Analysis of genetic diversity in subdivided populations. Proc. Natl. Acad. Si.70:3321-3323.

Newhouse, A.E., Schrodt, F., Liang, H., Maynard, C.A., and Powell, W.A. 2007. Transgenic American elm shows reduced Dutch elm disease symptoms and normal mycorrhizal colonization. Plant Cell Rep. 26:977-987. DOI 10.1007/s00299-007-0313-z.

Nielsen, R.L. and Kjær, E.D. 2010a. Fine-scale gene flow and genetic structure in a relic *Ulmus laevis* population at its northern range. Tree Gen. Genom. 6:643-649. DOI 10.1007/s11295-010-0280-3

Nielsen, R.L. and Kjær, E.D. 2010b. Geneflow and mating patterns in individuals of wych elm (*Ulmus glabra*) in forest and open land after the influence of Dutch elm disease. Conserv Genetics 11:257-268. DOI 10.1007/s10592-009-0028-5.

Park, G.E., Kim, K.W., Lee, D.K., Hyun, J.O. 2013. Adaptive phenotypic plasticity of Siberian elm in response to drought stress: increased stomatal pore depth. Microsc. Microanal. 19:178-181. Doi: 10.1917/s1431027613012610

Peakall, R. and Smouse, P.E. 2012. GenAlEx Tutorials-Part 2: Genetic distance and analysis of molecular variance (AMOVA). Bioinformatics 28:2537–2539

Perdiguero, P, Venturas, M, Cervera, MT, Gil, L and Collada, C. 2015. Massive sequencing of *Ulmus minor*'s transcriptome provides new molecular tools for a genus under the constant threat of Dutch elm disease. Front. Plant Sci. 6:541. doi: 10.3389/fpls.2015.00541

Perdiguero, P., Sobrino-Plata, J., Venturas, M., Martin, J.A., Gil, L., and Collada, C. 2017. Gene expression trade-offs between defence and growth in English elm induced by *Ophiostoma novo-ulmi*. Plannt Cell Envir. 41:198-214.

Petrokas, R. and Baliuckas, V. 2012. Natural hybridization within elms (*Ulmus* L.) in Lithuania. Balt. For. 18:237-246.

Pinon, J., Husson, C., and Collin, E. 2005. Susceptibility of native French elm clones to *Ophiostoma novo-ulmi*. Ann. For. Sci. 62:689-696.

Pooler, M.R. and Townsend, A.M. 2005. DNA fingerprinting of clones and hybrids of American elm and other elm species with AFLP Markers. J. Environ. Hort. 23:113-117.

Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

Robledo-Arnuncio, J.J., Austerlitz, F., Smouse, P.E. 2007. POLDISP: a software package for indirect estimation of contemporary pollen dispersal. Mol Ecol Notes 7:763–766

Santini, A., Fagnani, A., Ferrini, F., and Mittempergher, L. 2002. 'San Zanobi' and 'Plinio' elm trees. Hort. Science 37:1139–1141.

Santini, A., Fagnani, A., Ferrini, F., Mittempergher. L., Brunetti, M., Crivellaro, A., and Macchioni, N. 2004a. Elm breeding for DED resistance, the Italian clones and their properties. Inv. Agr. Sist. Recur. For. 13:179-184.

Santini, A., Ghelardini, L., Falusi, M., Bohn, J., Buron, M., Collin, E., Solla, A., and Van den Broeck A. 2004b. Vegetative bud-burst variability of European elms. Investigatión Agraria: Sistemas y Recursos Forestales 13: 37-45.

Santini, A., Fagnani, A., Ferrini, F., Ghelardini, L., and Mittempergher, L. 2005. Variation among Italian and French elm clones in their response to *Ophiostoma novo-ulmi* inoculation. For. Path. 35:183-193.

Santini, A., Fagnani, A., Ferrini, F., Ghelardini, L., and Mittempergher, L. 2007.'Fiorente' and 'Arno' elm trees. HortScience 42:712–714.

Santini, A., La Porta. N, Gheraldini, L., and Mittempergher, L. 2008. Breeding against Dutch elm disease adapted to the Mediterranean climate. Euphytica 163:45-56.

Santini, A., Pecori, F. Pecori, A.L., Ferrini, F., and Gheraldini, L. 2010. Genotype x environment interaction and growth stability of several elm clones resistant to Dutch elm disease. For. Ecol. Manag. 260:1017-1025. DOI.10.1016/jforeco.2010.06.025.

Santini, A., Pecori, F. Pecori, A.L., and Gheraldini, L. 2012. The Italian elm program for Dutch elm disease resistance. Proc. 4th Intern. Worksh. Gen Host-prasite Ineraction For. 326-335.

Santini, A. and Faccoli, 2015. Dutch elm disease and elm bark beetles: a century of association. IForest 8: 126-134 doi.org/10.3832/iFor1231-008

Sheffer, R.J., Vorten, J.G.W.F., and Guries, R.P. 2008. Biological control of Dutch elm disease. Plant Disease 92:192-200.

Sherif, S.M., Jones, A:M.P., Shukla, M.R., and Saxena, P.K. 2014. Establishment of invasive and non-invasive reporter systems to investigate American elm–*Ophiostoma novo-ulmi* interactions. Fung. Gen. Biol. 71:32-41. http://dx.doi.org/10.1016/j.fgb.2014.08.005

Sherif S.M., Shukla M.R., Murch S.J., Bernier L., and Saxena P.K. 2016. Simultaneous induction of jasmonic acid and disease-responsive genes signifies tolerance of American elm to Dutch elm disease. Sci. Reports, Open. 15pp. DOI:10.1038/srrep21934.

Singh, A., Majeed, A., and Bhardwaj, P. 2021. Transcriptome characterization and generation of marker resource for Hamalayan vulnerable species, *Ulmus wallichiana*. Mol. Biol. Rep 48:721-729. Doi:org/10.1007/s11033-021-06138-x.

Solla, A. and Gil, L. 2002a. Influence of water stress on Dutch elm disease symptoms in *U. minor*. Can. J. Bot. 80:810-817.

Solla, A. and Gil, L. 2002b. Xylem vessel diameter as a factor in resistance of *U. minor* to *Ophiostoma novo-ulmi*. For. Path. 32:123-134.

Solla, A, Bohnens, J, Collin, E, Diamandis, S, Franke, A, Gil, L, Burón, M, Santini, A, Mittempergher, L, Pinon, J, Vanden Broek, A (2005a). Screening European elms for resistance to *Ophiostoma novo-ulmi*. Forest Science 51: 134-141.

Solla, A., Martin, J.A., Corral, P., and Gil, L. 2005b. Seasonal changes in wood formation of *Ulmus pumila* and *U. minor* and its relation with Dutch elm disease. New Phyt. 166:1025-1034. Doi: 10.1111/j.1469-8137.2005.01384.x

Solla, A., Martin, J.A., Oullette, G.B., and Gil, L. 2005c. Influence of plant age on symptom development in *Ulmus minor* following inoculation by *Ophiostoma novo-ulmi*. Plant Disease 89:1035-1040.

Solla, A., López-Almansa, J.C., Martin, J.A., and Gil, L. 2014. Genetic variation and heritability estimates of *Ulmus minor* and *Ulmus pumila* hybrids for budburst, growth and tolerance to *Ophiostoma novo-ulmi*. iForest 8: 422-430 [online 2014-12-14] URL:http://www.sisef.it/iForest/contents/?id=iFor1227-007

Su, H., Qu, l-J., He, K., Zhang, Z., Wang, J., Chen, Z., and Gu, H. 2003. The Great Wall of China: a physical barrier to gene flow? Heredity 90:212-216.

Tamošaitis, S., Jurkšiené , G., Petrokas, R., Jurata Buchovska, J., Kavaliauskiené, I., Danusevïcius, D, and Baliuckas, V. 2021. Dissecting taxonomic variants within *Ulmus* spp. complex in natural forests with the aid of microsatellite and morphometric markers. Forests 12, 653. 19 pp. doi.org/10.3390/f12060653

Thakur, S., Thakur, I.K., Singh, N.B., Sharma, J.P., and Sankanur, M. 2014. Estimation of genetic diversity in progenies of selected genotypes of *Ulmus villosa* Brandis using RAPD markers. Ind. For. 140:1221-1229.

Townsend, A.M. and Douglas, L.W. 2001. Variation among American elm clones in long-term dieback, growth, and survival following *Ophiostoma* inoculation. J. Environ. Hort. 19:100-103.

Townsend, A.M., Bentz, S.E., and Douglas, L.W. 2005. Evaluation of 19 American elm clones for tolerance to Dutch elm disease. J. Envir. Hort. 23:21-24.

Vakkari, P., Rusanan, M., and Kärkkäinen, K. 2009. High genetic differentiation in marginal populations of European white elm (*Ulmus laevis*). Silva Fenn.43:185-196.

Välimäki, S., Rusanen, M., Pečínková, D., Tikkinen, M., Aronen, T. 2021. Cryopreservation and micropropagation methods for conservation of genetic resources of *Ulmus laevis* and *Ulmus glabra*. Forests 2021, 12pp, 1121. https://doi.org/10.3390/f12081121

Välimäki, S., Rusanen, M., Pečínková, D., Tikkinen, M., Aronen, T. 2022. Dehydration treatment improves *Ulmus glabra* dormant bud regeneration from cryostorage. Forests 2022. 6pp. 1923. Doi:org/103390./f13111923

Venturas, M., Fuentes-Utrilla, P., Ennos, R., Collada, C., and Gil, L. 2013. Human-induced changes on fine-scale genetic structure in *Ulmus laevis* Pallas wetland forests at its SW distribution limit. Plant Ecol. 214:317-327.

Venturas, M., Lopez, R., Martin, J.A., Gasco, A., and Gil, L. 2014a. Heritability of *Ulmus minor* resistance to Dutch elm disease and its relationship to vessel size, but not to xylem vulnerability to drought. Plant Path. 63:500-509. Doi: 10.1111/ppa.12115

Venturas, M., Nanos, N., and Gil, L. 2014b. The reproductive ecology of *Ulmus laevis* Pallas in a transformed habitat. For. Ecol. Manag. 312:170-178.

Venturas, M., Fuentes-Utrilla, P., Lopez, R., Perea, R., Fernández, V., Gasco, A., Guzmán, P., Li, M., Rodriguez-Calcerrada, J., Miranda, E., Dominguez, J., González-Gordaliza, G., Zafra, E., Fajardo-Alcántara, M., Martin, J.A., Ennos, R., Nanos, N., Lucena, J.J., Iglesias, S., Collada, C., and Gil. L. 2015. *Ulmus laevis* in the Iberian peninsula: a review of its ecology and conservation. 135-142 [online 2014-08-07] URL: http://www.sisef.it/iForest/contents/?id=iFor1201-008

DeVries, S.,M.G., Alan; M., Bozzano, M., Buriánek, V., Collin, E., Cottrell, J., Ivankovic, M. Colin, E. Kelleher, C. Koskela, J., Rotach, P., Vietto, L., and Yrjänä, L. 2015. Pan-European strategy for genetic conservation of forest trees and establishment of a core network of dynamic conservation units. (EUFORGEN), Bioversity International, Rom, Italien. 40pp.

Webber, J.J. 2004. Experimental studies on factors influencing the transmission of Dutch elm disease. Invest. Agr. Sist. Res. For. 13:197-205.

Wesche, K., Wather, D., von Wehrden, H., and Hensen, I. 2011.Trees in the desert: Reproduction and genetic structure of fragmented *Ulmus pumila* forests in Mongolian drylands. Flora 206:91-99. Doi:10.1016/j.flora.2010.01012.

Whiteley, R. 2004. Quantitative and Molecular Genetic Variation in *Ulmus laevis* Pall. Doctoral thesis Swedish University of Agricultural Sciences Uppsala 2004. 33pp.

Whiteley, R.E., Black-Samuelsson, S. and Clapham, D. 2003a. Development of microsatellite markers for the European white elm (*Ulmus laevis* Pall.) and cross-species amplification within the genus *Ulmus*. Mol. Ecol. Notes 3:598-600.

Whiteley R.E., Black-Samuelsson S. and Jansson G. 2003b. Within and between population variation in adaptive traits in *Ulmus laevis* Pall. the European white elm. Forest Genetics 10:313-323.

Whittermore, A.T. and Olsen, R.T. 2011. *Ulmus americana* (*Ulmaceae*) is a polyploidy complex. Amer. J. Bot. 98:754-760. DOI:org/10.37327ajb.1000372

Whittemore, A.T., Fuller, R.S., Brown, B.H., Hahn, M., Gog, L., Weber, J.A., and Hipp, A. L. 2021. Phylogeny, biogeography, and classification of the elms (*Ulmus*). Syst. Bot. 46: 711-727. DOI 10.1600/036364421X1631 2068417039

Wricke, G. 1962. Über eine Methode zur Erfassung der ökologischen Streubreite in Feldversuchen. Z. Pflanzenzuecht 47:92-96

Zalapa, J.E., Brunet, J., and Guries, R.P. 2008a. Genetic diversity and relationships among Dutch elm disease tolerant *Ulmus pumila* L. accessions from China. Genome 51:492-500. Doi:10.1139/G08-034

Zalapa, J.E., Brunet, J., and Guries, R.P. 2008b. Isolation and characterization of microsatellite markers for red elm (*Ulmus rubra* Muhl.) and cross-species amplification with Siberean elm (*Ulmus pumila* L.). Mol. Ecol. Resources 8:109-112. Doi: 10.1111/j.1471-8286.2007.01805.x

Zalapa, J.E., Brunet, J., and Guries, R.P. 2009. Patterns of hybridization and introgression between invasive *Ulmus pumila* (*Ulmaceae*) and native *U. rubra*. Amer. J. Bot. 96:1116-1128. DOI:10.3732/ajb.0800334.

Zalapa, J.E., Brunet, J., and Guries, R.P. 2010. The extent of hybridization and its impact on the genetic diversity and population structure of an invasive tree, *Ulmus pumila* (*Ulmaceae*). Evol. Appl. 3:157-168. Doi:10.1111/j.1752-4571.2009.00106x

Zebec, M., Idžojtić1, M., Šatović, Z., Igor Poljak, I., and Liber, Z. 2016. Alive and kicking, or, living on borrowed time? – Microsatellite diversity in natural populations of the endangered *Ulmus minor* Mill. sensu latissimo from Croatia. Acta Bot. Croat. 75 (1), 53–59, CODEN: ABCRA 25 DOI: 10.1515/botcro-2016-0007

Zhang, H., Zhang, Q., Fan, W., Chen, X., Wang, J., Li, Q., and Zhao, G. 2019. The complete chloroplast genome of a rare and endangered elm (*Ulmus chenmoui*) endemic to East China. Cons. Gen. Res. 11:169-172. Tech. Note. doi.org/10.1007/s12686-018-0991-6

A trunk of *Ulmus pumila* in Kiviks Esperöd arboretum in Southern Sweden. *U. pumila* was frequently used in species hybridizations to confer Dutch elm disease resistance to American and European elms. Photograph Barbro Ekberg.

BioCenter
Department of Plant Biology, SLU
Box 7070, 750 07 Uppsala
Sverige