



ABSTRACTS



3rd International Elm Conference
The elms after 100 years of Dutch elm disease
Florence, 9-11 October 2013

THE ELMS AFTER 100 YEARS OF DUTCH ELM DISEASE
3rd International Elm Conference
Florence (Italy) 9-11 October 2013

Abstracts

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INTRODUCTION

The purpose of this conference is to advance the progress on knowledge on elms one hundred years after the arrival of Dutch elm disease, allowing the collaboration between the scientific and management world. Updates on current status of advances on disease and its management, other biotic and abiotic constrain, conservation and characterization of genetic resources as well as possible use of elms in urban and landscape will be presented and discussed.

The last Elm Conference took place in Spain ten years ago. Since then the research and research tools have made significant progress and different problems and issues have arisen in the meanwhile. At the same time important results have been achieved, giving hope to a possible future use of this magnificent tree for urban, forest and landscape aims.

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The elm, tree of milk and wine

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Abstract:

Elm has played an important role in European culture for thousands of years, in many roles, with regional variation. In material culture, its wood has assisted in hunting and warfare for over seven thousand years; but more importantly, its leaves and bark were semi-indispensable for the production of milk and meat, and served as an emergency food for humans. In the Mediterranean, elm was the main tool for the production of a good quality wine by providing support for the grapevine, and it helped feeding the cattle. These functions sometimes found an echo in the non-material culture. The fact that in Germanic genesis stories the first woman was created out of an elm (the man out of an ash), as well as a severe local taboo on the use of elm wood for skis, threatening the offender with a place in hell, seem both connected to the superior feeding value of this tree. In England and in parts of continental Europe most sacred trees were elms, sometimes performing female functions such as the production of babies. In the Mediterranean, however, the elm was seen as the male partner in the “marriage of the vine to the elm”, which was the celebrated system of viticulture. That image has been used by poets and politicians over the ages to praise the effects of human marriage, cooperation and interdependence. It even forms the core of the apocryphal Bible book “The Shepherd”, where it is seen as a symbol and example for a kind of symbiosis between the rich and the poor. – In conclusion, the ultimate origin of the English elm or ‘Atinia’, as well as its discovery is discussed; that appears to be a question of milk and wine.

Come-back of the elm as street tree in the Netherlands

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Abstract:

Its unique combination of characteristics makes the elm an almost ideal street tree and landscape tree in the Netherlands. As a result, the elm became the dominant tree in large parts of the coastal provinces and in the cities in the Western and Northern part of the country in the 19th and early 20th century until Dutch Elm Disease (DED) started to spread. The subsequent epidemics caused by *O. ulmi* and *O. novo-ulmi* during the 20th century killed millions of elm trees. Removal schemes and replacement of dead and diseased trees led to high costs and the elm got a negative image. Although from the seventies on new varieties with increased resistance to DED were released, the use of elms as street tree and in the landscape decreased dramatically. Whereas the genus *Ulmus* was the dominant tree in Dutch tree nursery industry for a long time, it even was not among the top-20 list of nursery trees any more around 1990.

However, in the past decades, as a result of joint efforts by managers of urban green, tree nursery industry and elm researchers the elm is on the way back. An increasing number of urban green managers is ready to give elms a chance again and, although still at a substantially lower level than before, the numbers of elms being planted are increasing steadily.

In this paper several initiatives that contributed to this come-back will be reviewed, including the results of a nationwide survey into present position and future of the elm in the Netherlands completed in 2005; strict sanitation and replanting programmes as carried out by the city of Amsterdam and the Iepenwacht initiative in the northern provinces; and a programme for resistance testing and demonstration of their use as street tree of all recently released cultivars available in the Dutch tree nurseries. The resistance testing has been completed in 2012 (Buiteveld et al, this conference). The testing of the use of these new (resistant) varieties as street tree is continued in a network of demonstration plantings organised together with 8 participating cities. The approach and first results of this network will also be presented.

Key words: *Ulmus* varieties, DED resistance, sanitation, street tree testing

Amsterdam city of elms

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Abstract:

Four hundred years ago, Amsterdam began constructing its now world-famous canal ring. Soon after the first canals had been completed, trees were planted along the water according to a set pattern, spaced a distance of two 'Amsterdam rods' (7.36 metres) apart. The policy of systematically making the city greener with trees is still visible today. This systematic tree planting was mentioned explicitly when Amsterdam's canal ring was designated a UNESCO World Heritage site in 2010. The first trees planted along the canals were lime trees, which were later replaced by the stronger elm trees.

Over 20% of the 350,000 street trees currently growing in Amsterdam are elms (75,000). In the nearly 100-year history of Dutch elm disease, a great deal of expertise has been gained in controlling the disease. This has been done successfully, and many other municipal authorities are adopting Amsterdam's approach. The most important measures for this are identifying all elm trees (on public and private property) and inspecting for signs of the disease at least twice a year. Diseased trees are removed in the proper manner within ten working days (subject to fines of up to €480 per trunk) and replaced with more resistant elms. When diseased or healthy elm trees are removed, the trees are uprooted, debarked and/or chipped (very important) on site. The clearing of trees with Dutch elm disease in small private gardens is funded by the city. All of this is laid down in a municipal by-law. A monitoring system of beetle traps alerts the authorities when the limiting value is exceeded. A search will almost certainly lead to the culprit (a breeding ground in the form of a dead or diseased elm tree).

In 2010, an elm arboretum was planted in the windiest part of Amsterdam, representing all 32 elm species occurring in Amsterdam. The number of species has since been expanded considerably with Italian, French and American elms.

In 2011, activities commenced to make the natural scattering of elm seeds, nicknamed 'Springsnow', a unique harbinger of spring for Amsterdam, similar to the Cherry Blossom festivals in Japan, Washington and China.

The return of elm in Florence

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Abstract:

A number of trials for re-introducing elms in the city of Florence started around the year 2000. The first aim of the project was to evaluate the adaptability to urban conditions of several elm clones resistant to DED, which were obtained at the Institute of Plant Protection of CNR by recurrently crossing Asian and native elm species.

Elm plantations were grown in various urban environments, such as flowerbeds, roundabouts, squares, gardens, marginal areas, escarpments and historical parks, as well as in highly frequented areas.

We measured various performances of the resistant clones. Besides their aesthetical and functional value, their need for maintenance and management were evaluated. Altogether, the valuation was certainly positive. These garden plantations lasting many years confirmed the good qualities of elms as city trees. The clones resulted able to adapt to difficult environments, easy to root and establish, undemanding in terms of nutrients and water supply, generally easy to cultivate and very fast growing. These qualities together with other typical traits of elms, such as nice crown shape, small fruits, small leaves, early leaf unfolding and late leaf senescence, make elms ideal trees for urban environments.

Besides the 5 elm clones first patented, i.e. 'Arno', 'Fiorente', 'Morfeo', 'Plinio' and 'San Zanobi', a number of new clones, which seemed highly adaptable in preliminary trials, are currently evaluated in order to ensure that they have no major problems in a urban environment before releasing them to the general market.

The only one defect found in the clone with the greatest growth rate was its tendency to develop a rooting system consisting in an unique and spiral main root, a character that dramatically decreased the resistance to wind in young trees up to 4-5 meters tall.

The experimentation here described, that lasted more than ten years, gave a significant impulse to tree heritage in Florence, especially in the city district Quartiere 4, where most of the trials were established. Here, 1,200 elms were planted out of a total of 13,000 trees.

Nowadays there is indeed a great and renewed interest in elms in Florence. New tree species are also requested, since other trees that were traditionally used for urban plantations, such as plane

tree (*Platanus hybrida* Brot.) or Horsechestnut (*Aesculus hippocastanum* L.), are now subjected to different diseases, therefore DED resistant elms could become a choice worth to be considered.

Comparison of commercial elm varieties and promising new Dutch clones for resistance to DED in a field test

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Abstract:

Since the early 20th century, elm breeding for resistance to DED has resulted in the release of several more or less resistant varieties to the European market. Especially in the Netherlands and the USA large and long-term research- and selection programs were running resulting in a number of hybrid clones with moderate till very good resistance. Also more recently, in Italy and France several new varieties with good resistance have been released. Today a wide range of clones of different DED resistance and different parentage is available on the market. However, the use of these new elm varieties in the Netherlands is still limited. Apparently the lasting problems with DED in old varieties has led to a lack of confidence in the resistance of these newly released varieties among managers of landscape and urban green.

In order to compare the level of DED resistance of elm varieties recently introduced into the Netherlands a field test was established. The field test comprised of 18 varieties, one species and 10 selections from the Dutch Alterra breeding program. Varieties included were those developed in the USA and not yet tested under Dutch conditions, Dutch varieties and two references with known DED response (Commelin and Lobel). Two-year old plants derived from cuttings or graftings were planted in the field according to a complete randomised block design (9 blocks, 4 plants per clone per block). In total four inoculation treatments with *Ophiostoma novo-ulmi* were carried out in two consecutive years (9 plants per clone per treatment). Symptoms such as disease index, defoliation and crown dieback were assessed after 4 weeks, 8 weeks and one year after inoculation and were statistically analysed using Monte Carlo tests.

The severe inoculation method used enabled us to demonstrate clear differences in resistance level between the varieties, ranging from highly resistant to very susceptible. Additionally, it showed that the varieties differed in recovery ability. Especially, the varieties with a good resistance from the American DED research program recovered well after initially showing clear disease symptoms. The ranking of varieties for level of DED resistance confirms, with a few exceptions, earlier published resistance levels and experience from plantings in the Netherlands. It was concluded that an ample number of varieties with good resistance is available. The Alterra selections performed well compared to the released varieties and give good opportunities to further broaden the current range of varieties on the Dutch market.

Keywords: DED resistance, Elm varieties, *Ulmus*, inoculation test, *Ophiostoma novo-ulmi*.

Studies on wood characters of parents, genetic parameters and correlation of their progeny performance in *Ulmus villosa* Brandis at seedling stage

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Abstract:

The present study on *Ulmus villosa* was carried out in Himachal Pradesh to know the progenies performances and estimated genetic variability for growth and biomass of progenies at nursery stage. Seeds were collected from five mother trees each at six seed sources and progenies were raised. All the growth and biomass characters showed wide range of values indicating the extent of variation existing in the plants. Site S₄ (Jagoti/ District Shimla) exhibited outstanding performance for the growth and biomass characters. Progenies of trees Jadh-T₄, Jugahan-T₄, Jhidi-T₅, Jagoti-T₅, Katouch-T₁ and Andhra-T₃ were found to be better within sites for the growth and biomass characters. Site S₆ (Andhra/District Shimla) trees exhibited outstanding performance for wood characters. Wood density was found maximum (0.91 g/cc) for tree Andhra-T₂ and fiber length (0.86mm) for tree Katouch-T₂.

High heritability with moderate genetic gain was observed for diameter (H=0.77, GG=34.49%) followed by plant height (H=0.71, GG=28.43) among growth and biomass characters. Among wood characters high heritability with moderate genetic gain was recorded in wood density (H=0.80, GG=26.40). Fresh and dry shoot weight exhibited high genotypic coefficient of variability and moderate heritability coupled with high genetic gain. High heritability with moderate genetic revealed that traits were under strong genetic control suggesting that additive genetic effects are important in the determination of these characters and therefore, selection would be effective for these traits. The phenotypic coefficient of variation was higher than the genotypic coefficient of variation in all the characters which indicates that the traits were greatly influenced by the environment.

Highly significant and positive genotypic and significant phenotypic correlation were observed for height and diameter (G=0.961, P=0.563), fresh shoot weight and fresh root weight (G=0.922, P=0.664) and dry shoot weight and dry root weight (G=0.859, P=0.676). Therefore, selection for any of these traits would be a reliable measure for other traits.

Key words: Seed source, Genetic variability, Correlation, Progeny, Growth, biomass and wood traits, Phenotypic, Genotypic.

Origins, spread and continuing evolution via horizontal gene transfer of the Dutch elm disease pathogens

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Abstract:

The pathogen responsible for the first DED pandemic across Eurasia and North America from ca 1910 – 1930 was *Ophiostoma ulmi*. The current or second pandemic probably began in parts of Eurasia and North America as early as the 1940s and was caused by the more aggressive *Ophiostoma novo-ulmi*. The two DED pathogens, *O. ulmi* and *O. novo-ulmi*, are anciently divergent species.

O. novo-ulmi migrated across Europe and North America as a series of genetic clones, replacing the previous new arrival *O. ulmi*. During its migration across Europe a series of remarkable evolutionary events occurred. Deleterious RNA viruses, capable of preventing the pathogen from infecting healthy elms, spread in the *O. novo-ulmi* clones. These viruses appear to have been acquired from *O. ulmi*. *O. novo-ulmi* simultaneously acquired vegetative compatibility (*vic*) and mating type (*MAT- 1*) genes from *O. ulmi* via horizontal gene transfer. As a result *O. novo-ulmi* became genetically highly diverse and able to resist the spread of the viruses. Today, across Europe, *O. novo-ulmi* has little overt virus infection and now carries the *O. ulmi* *MAT- 1* gene and presumably also a suite of *O. ulmi* *vic* genes fixed in its genome. Without this unusual series of events the second DED pandemic may never have developed in Europe.

We also have evidence that, some forty years earlier, *O. ulmi* also spread across Europe as a single genetic clone and later diversified into multiple genotypes. Furthermore, there is evidence that, before the arrival of *O. ulmi* in Europe, *Ophiostoma quercus*, a non-pathogen, was the fungal associate of the elm bark beetles. Quite possibly, therefore, *O. ulmi* went through a similar series of horizontal gene transfer events to *O. novo-ulmi*, acquiring deleterious viruses and *vic* and *MAT* loci from *O. quercus*.

Yet another evolutionary scenario is currently occurring within European *O. novo-ulmi* populations. *O. novo-ulmi* spread across the Northern Hemisphere in the form of two phenotypically distinct subspecies, subsp. *novo ulmi* and subspecies *americana*. In many places in Europe the two subspecies now overlap and unrestricted hybridisation is occurring, resulting in fully recombinant hybrid swarms. An important question is what hybrid forms will survive under the influence of natural selection. Studies show that surviving hybrids tend to combine some of the characteristics of subspecies *americana* (colony type, faster growth rate, higher pathogenicity) with some of the characteristics of subspecies *novo-ulmi* (e.g. distinctive perithecial morphology). Indeed some of the current hybrids appear to be 'hopeful monsters,' as they produce giant perithecia with exceptionally large bowls and extraordinarily long necks, the latter with apparently indeterminate rather than normal determinate growth. *O. novo-ulmi* is therefore reinventing itself

in Europe and, unfortunately, the tests also show the new hybrids remain highly pathogenic to elm.

In the aftermath of the damage caused by *O. novo-ulmi* millions of small recruitment elms have grown up from root suckers and seeds across Europe and North America. In the UK for example, in the place of the ~30 million large elms lost in the initial phase of the epidemic there may now be some 100-300 million small recruitment elms. Of these an estimated 1% (1-3 million) are still being killed annually in UK by *O. novo-ulmi*. So far subsp *americana* is the only subspecies of *O. novo ulmi* introduced into the UK. The three original UK outbreak locations from the early 1970s were resampled in 2011 to see if any changes had occurred in the *O. novo ulmi* subsp *americana* population. These isolates have been compared for their pathogenicity on *Ulmus procera* (*U. minor*) with a batch of isolates sampled in 1983 from the same three outbreak locations. Unfortunately there is again no evidence for any attenuation in pathogen aggressiveness: the 2011 sample was found to be just as aggressive as that from 1983.

Unless something else changes to alter the dynamics of these elm- pathogen - vector systems, such as an increase in the resistance of the host, for the foreseeable future the small recruitment elms across Eurasia and North America are likely to remain under heavy attack from the pathogen and its beetle vectors. There is also a risk to the elm of importation of further DED pathogens. Two more DED pathogens have been found, both in Asia, both apparently endemic, both with their own bark beetle associates and both still localised. One is *O. himal-ulmi*, which occurs on elm in the Himalaya. The other is '*Ophiostoma taxon zelkovae*' which occurs on *Zelkova* in Japan. This, together with recent evidence indicating that *O. ulmi* probably came from Japan, also suggests that the two *O. novo-ulmi* subspecies have an (as yet unidentified) east Asian origin.

Genomics of the Dutch elm disease pathosystem: are we there yet ?

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Abstract:

Due to its dramatic impact on elm populations worldwide, Dutch elm disease (DED) has been studied for nearly 100 years. Over the years, researchers have applied state-of-the-art approaches and techniques for elucidating the intricacies of this complex host-pathogen-vector interaction and designing ways to fight the disease. During the last decades, molecular analyses and omics technologies have been incorporated into the arsenal of tools available for DED researchers. In this presentation, I review how some of these approaches are being used for advancing our knowledge of DED in general, and of its causal agents in particular.

Genetic and molecular analyses have led to the identification, cloning and characterization of a few genes that contribute to parasitic fitness (including pathogenicity) in the DED fungi *Ophiostoma ulmi* and *O. novo-ulmi*. Quantitative PCR and high-throughput methods, such as expressed sequence tag (EST) analysis, have been used for measuring gene expression and identifying subsets of genes that were differentially expressed in DED fungi grown under defined experimental conditions. Quantitative PCR and EST analysis have also helped identify elm genes that are differentially expressed in the presence of *O. novo-ulmi*. Until recently, however, functional analysis of the DED fungi was hampered by the lack of protocols for efficient gene knockout and by the unavailability of a full genome sequence. While the selective inactivation of genes in *O. ulmi* and *O. novo-ulmi* by gene disruption remains a challenge, an alternative approach based on RNA interference is now available for downregulating the expression of targeted genes. In 2013, two research groups publicly released the genome sequences of *O. ulmi* and *O. novo-ulmi*. In the case of *O. novo-ulmi*, annotation of the 8521 protein-encoding genes predicted *in silico* led us to identify 763 genes potentially involved in pathogen-host interaction, in addition to 276 genes encoding carbohydrate-active enzymes and 48 genes encoding cytochrome P450s. Ongoing functional analysis of some of these genes will be discussed in the context of pathogenicity, yeast-mycelium transition, and interaction with elm bark beetles.

Seven Iberian *Ulmus minor* clones resistant to DED registered for their use as forest reproductive material

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Abstract:

Following the introduction of DED fungi in Spain during the mid-20th century, and the devastating impact caused on natural elm grooves and urban areas, the Spanish Elm Breeding Program started in 1986. At its origin, the Program focused on increasing the resistance of the native field elm (*Ulmus minor*) by crossing it with resistant Asian elms. Although very few *U. minor* individuals were tolerant to *Ophiostoma novo-ulmi*, in the last decade the Program gave priority to selection and breeding of native elms. After 27 years of work and being several thousands of elm genotypes screened for resistance, seven selected resistant elms from different locations of Spain have been registered by the Spanish Environmental Administration according to Directive 1999/105/EC. For the first time in Spain, their use and marketing as forest reproductive material will be allowed. The morphology, ornamental value, phenology and genetic background of the seven clones have been assessed. In order to help preventing that any eventual emerging pathogen race could overcome the resistance of selected material, nowadays the Program aims to obtain a wider range of resistant elms by new selections and crossings.

The Italian elm breeding program for Dutch Elm Disease resistance.

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Abstract:

Starting from around 1910 two destructive pandemics of Dutch Elm Disease (DED) caused by the introduction of two very different fungal pathogens, *Ophiostoma ulmi* (Buisman) and *O. novo-ulmi* (Brasier) afflicted the elms in Europe and in North America.

The idea of searching for resistance to DED in the European elm species and populations, arose in The Netherlands, where a wide collection of elms was constituted and the first resistant cultivars were obtained within the European species. Elm clones developed in Holland in the '30s did not show sufficient resistance to the second pandemic and no complete resistance was ever found in European and American native elm species. But the use of Asian species with their higher original resistance would speed up the process. Further, several 2nd generation Dutch and also American resistant clones, simple or complex hybrid between native and Asian species, were developed and delivered on the market.

In Italy an elm breeding program started in 1975 and is still going on in Florence by the Institute of Plant Protection of C.N.R. The idea underlying this project was the conviction that the Mediterranean environment would need its own selections. The favorable adaptation of Asian species in Italy, like Siberian elm (*U. pumila* L.) and others, and the unsuitability of the Dutch selections to the hot and dry areas of central Italy encouraged to test the Asian species both in order to assess more in depth their adaptability to the Mediterranean climate and to broaden the genetic base of the native species.

Selection of superior genotypes reduces genetic variation in cultivated species. However, when breeding is designed for obtaining plants adapted to different environmental conditions and for different uses, the outcome could result in an increase of variability. The case of elm breeding for resistance to DED is paradigmatic. The uses of elm are in fact manifold. For this reason breeding for resistance is not enough: many other features are requested as tree silhouette, fast growth, leaf and bark color, leaf shape and dimensions. In order to satisfy all these needs, maintaining at the same time enough genetic variability to buffer the rising of possible new stresses, such as new forms of the disease or other diseases and climate change, we decided to operate a base broadening of the genetic resources, or, as it will be called later, "incorporation". A base of native elms with enough good characters to act as parents was bred with different Asian elm species that showed ability of acclimatization to the different climates in which elms have to be planted as well as a fair level of disease resistance.

The results of the 30-year programme on elm breeding built up with the aim of obtaining DED resistant elm varieties that accomplished the remarkable aesthetic ornamental characters with adaptability to different conditions were a wide range of elm clones originated by very different

crossings. Four of these resistant hybrid elm clones are already patented and released on the market: 'S. Zanobi', 'Plinio', 'Arno' and 'Fiorente', and 'Morfeo'. 'Morfeo' is extremely resistant to DED and has an attractive form and foliage. It is also fast-growing, tolerant of drought and soils waterlogged in winter, therefore proving well adapted to the climates of both north-western Europe and the Mediterranean.

Heritability estimates for vegetative budburst, height growth and resistance to *Ophiostoma novo-ulmi* in *U. minor* x *U. pumila* hybrids

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Abstract:

Progenies obtained by crossing *Ulmus minor* and *U. minor* x *U. pumila* hybrids in a breeding population were assessed for vegetative budburst, growth and resistance to *Ophiostoma novo-ulmi*. Complementary, a study of flowering and elm bark beetle incidence was carried out. Most progenies had traits similar to that of their parents, but some of them presented heterosis in their annual growth or in their resistance to *O. novo-ulmi*. Disease resistance, height growth and vegetative budburst were different when *U. minor* x *U. pumila* was used as male or as female parent. Leaf wilting was significantly lower in progenies with *U. minor* x *U. pumila* as female parent than in progenies with *U. minor* as female parent ($P < 0.05$). The heritability estimates reported for *O. novo-ulmi* resistance indicate a large degree of additive genetic control and point out that selection for DED resistance is effective.

Epidemiology and combat of Dutch elm disease in Gotland Island of Sweden

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Abstract:

The population of elm (*Ulmus minor*) in Gotland is unique in its origin, history, development, and ecology. Elm can be found in all parts of the island, where it grows in forest stands, and also in parks, orchards, pastures, cemeteries, alleys and pollard meadows. According to rough estimate, there are at least one million elms on the island (Östbrant et al. 2009). Under 19th century there was about 35 000 ha of pollard meadow in Gotland, comprising about 10% territory of the whole island, with ash, elm and oak as the most common and characteristic tree species. Despite presently remaining 300 – 350 ha, Gotland represents the richest region of Sweden in terms of pollard meadow landscape. Besides that, characteristic for Gotland is also so-called noble hardwood dominated land, of which elm, together with ash and oak, constitutes a crucial component. Over the Gotland, such ecosystems currently comprise about 4600 ha (Mebus 2006). During at least 1500 years they have been intensely used for pollarding (hamling), hay harvesting and pasture (Hultengren et al. 2006), thus consequently elm as a tree species for Gotland is of exceptional cultural and historical value, especially in context of currently ongoing in the island large-scale decline of ash.

As compared with other areas of Sweden and Europe, Dutch Elm Disease (DED) has arrived only recently to Gotland Island, and for the first time was observed there only in year 2005. Already the same year Swedish Forestry Agency started to implement control measures. Data on DED-diseased and destroyed trees and trees, attacked by *Scolytus multistriatus* will be presented. The data show that number of DED-diseased and *S. multistriatus*-attacked elms in Gotland has increased alarmingly during the years 2005 – 2008, but that since the year 2009 the incidence of DED has stabilized. This can be explained by shift in the applied control strategy. Thus, until 2009 only all DED-diseased trees have been destroyed each year. However, numerous secondary infections seemingly occurred from root systems of their stumps to roots of adjacent healthy trees, resulting in the observed rise of DED incidence (without bark beetle attack). Then, in the beginning of 2009, new combat strategy was adopted, when not only DED-diseased elms were felled, but also visually healthy elms growing in their vicinity, thus in root contact with DED-diseased ones. Stumps of all felled trees were then treated with the herbicide aiming to kill root systems and to prevent further spread of the disease via roots and sprouting.

Since 2011, two research projects, entitled “Biology and control of Dutch elm disease in Gotland”, were acquired from two private Swedish foundations *Stiftelsen Oscar och Lilli Lamms Minne* and *Carl Tryggers Stiftelse för Vetenskaplig Forskning*. Moreover, in 2013 a large *LIFE+ Nature* project “Saving wooded Natura 2000 habitats from alien invasive fungi on the Island of Gotland, Sweden” was granted by European Commission, and is focused mainly on DED. Currently, those projects form the basis for implementation of control measures, monitoring and research on DED in Gotland Island. Main objectives of the past, ongoing and future work: i) to identify DED pathogens and to determine their genetic population structure; ii) to monitor their distribution and spread; and finally and the most importantly, iii) to continuously minimize number of DED-infested trees and bark beetles. Preliminary results of this work will be presented.

Fungal endophytes in Dutch elm disease complex

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Abstract:

Fungal endophytes receive nutrition and shelter from their host plants, but generally do not cause visible symptoms. Evidence is accumulating for the role of these ubiquitous fungi in plant resistance against biotic and abiotic stressors. Due to their effects on breeding of the vector insects, endophytes have also been linked to Dutch elm disease biocontrol. Here, we summarize results from studies where we characterized endophyte frequency and diversity in leaves, bark and xylem of selected elm trees differing in the degree of susceptibility to DED, and investigated the relation between phenolic defensive metabolites, DED susceptibility and endophyte frequency and diversity. Finally, we evaluated the ability of selected endophyte isolates to suppress *O. novo-ulmi* using greenhouse studies and laboratory tests. We found that the frequency and diversity of xylem associated fungal endophytes may covary with the degree of DED susceptibility in elms. We also identified endophytic fungi that in *in vitro* tests showed chemical antagonism against an aggressive *Ophiostoma novo-ulmi* strain. The carbon substrate utilization profile of these endophytes overlapped with that of *O. novo-ulmi*, indicating that the endophytes may compete with the pathogen for a nutritional niche. In summary, our results imply that endophytes may show direct antagonism against the Dutch elm disease pathogen. Preventive treatments of elm trees with certain endophytes slightly reduced DED symptoms. The potential of endophytes and their products in biological control of Dutch elm disease is discussed.

Measurement of sap flow in elms inoculated by *Ophiostoma novo-ulmi* (short presentation)

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Abstract:

In a field experiment, the sap flow of four year old wych elm (*Ulmus glabra*) was measured in response to inoculation with *Ophiostoma novo-ulmi*. Three trees were inoculated with three different strains of *O. novo-ulmi* (reference strain H327 – *O. novo-ulmi*, ssp. *novo-ulmi*, and two strains of czech origin collected in 2007 and 2008 – one hybrid of *O. novo-ulmi* subspecies and one *O. novo-ulmi* ssp. *novo-ulmi*). Defoliation of the trees was estimated after 4, 10, 16 weeks and one year after inoculation. Sap flow of those trees was measured by trunk heat balance method with external heating and constant temperature difference between the heated and reference point of 4°C (EMS 62, Czech republic).

The defoliation started very quickly after inoculation. Just after two weeks leaves became very sear and after next two weeks the leaves of inoculated branches were nearly dead. Also in the rest of the tree the defoliation was very serious and during the summer elm inoculated by H327 had no living leaves. One year after inoculation the situation was partially recovered. Although the inoculated branches were completely dead, the rests of the crowns resprouted, but showed defoliation of the new leaves 20 – 60%.

Our experiment confirmed ability of *O. novo-ulmi* to quickly kill young elm trees. After five days, sap flow of infested trees began to steadily decline, so that first statistically significant difference between the group of infested trees and control group appeared ten days after inoculation. Finally, no sap flow through xylem of infested branches 16 days after inoculation.

To our best knowledge, this is the first sap flow measurements on trees inoculated by *O. novo-ulmi*. Sap flow measurements by heat balance technique provided a non-invasive tool for continuous quantitative monitoring of progression of the vascular disease.

Keywords: Dutch elm disease, *Ulmus glabra*, heat balance method, defoliation

Geosmithia fungi – a widespread and intimate associates of elm bark beetles

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Abstract:

Numerous studies have addressed phytopathogenic ophiostomatoid fungi associated with elm bark beetles. However, fungi involved in this symbiosis are more taxonomically diverse, and cycloheximide sensitive non-ophiostomatoid fungi are often neglected, which limits the understanding of beetle-related ecosystem processes. Among them, *Geosmithia* spp. (Ascomycota: Hypocreales) belong to regular associates of elm bark beetles, and were found in all studied gallery systems. Fungi from the genus *Geosmithia* are associated with a broad spectrum of bark beetles, though little is known about their ecology. Several species were proven as primary nutritional symbionts of ambrosia beetles, *G. morbida* is a known pathogen of walnut in the USA and many species possess allelopathic effect against bacteria, entomopathogenic fungi, penicillia and ophiostomatoid fungi. There are known over fifty species of *Geosmithia* and nine of them are associated with elm-bark beetles. The community structure of *Geosmithia* spp. associated with elm bark beetles is similar across geographically distant locations, suggesting a stable association. *Geosmithia* sp. 13 seems to be an elm specialist, known from Europe and USA, what could be a consequence of a long term association between *Geosmithia* and elm bark beetles. *Geosmithia* produces class II hydrophobin named as GEO1, but also ceratoulmin (identical with those of *O. novo-ulmi*) and its role in the beetle/*Ophiostoma*/tree symbiotic complex should be further studied.

Molecular characterization of *Geosmithia* populations on elm

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Abstract:

The species of the genus *Geosmithia* (Ascomycota: Hypocreales) have been found to be regularly associated to many insect species, in particular bark beetles (*Coleoptera: Curculionidae, Scolytinae*) that invade the phloem or sapwood of various tree genera. On *Ulmus* spp., bark beetles also function as vectors of *Ophiostoma ulmi* s.l., the agent of Dutch elm disease (DED), one of the most widely-known diseases, affecting elms world-wide.

In this paper we collected 47 elm branch samples all over Czech Republic and 13 in some locations in Italy. From this samples, we isolated up to 200 *Geosmithia* spp. strains. Colonies were grouped into morphological taxonomic units, based on colony characteristics and morphology. Genomic DNA was extracted from monosporic strains, belonging to all morphological taxonomic units. The genomic DNA was used as template in the amplification reactions of the ITS region, β -tubulin gene and elongation factor 1- α . A phylogenetic analysis for each gene and A complete joining sequence of the three genes was designed by using the software MEGA 5.

The morphological and molecular analysis of wild strains of *Geosmithia* spp. collected from dying elm trees, showed the presence of the species *G. pallida* and *G. langdonii*, and some phylogenetically related previously undescribed taxonomic entities.

These entities differ for morphological and molecular profiles. The area of distribution of one of these entities is Mediterranean, while the others central European.

More research will focus on whether exist a relation between these species and *Ophiostoma novo-ulmi*.

Widespread horizontal transfer of the cerato-ulmin gene between *Ophiostoma novo-ulmi* and *Geosmithia* spp.

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Abstract:

Preliminary work had shown the presence of a gene encoding class II hydrophobin cerato-ulmin (*cu*) from the phytopathogenic fungus *Ophiostoma novo-ulmi* in the unrelated species *Geosmithia pallida*. As the two species coexist inside elm trees occupying the same habitat but different ecological niches, a horizontal gene transfer event was proposed. We used PCR amplification to search for the *cu* gene in 70 *Geosmithia* spp. strains from different european locations (Czech Republic, Hungary, Spain, Italy) and host plants: 46 strains were derived from insect vectors infesting elm trees or from decaying elm trees and 24 strains were from insect vectors on plants other than elms. The gene was present in 52,1% of the strains from elm trees, while none of those isolated from non-elms possessed it. The presence of *cu* mRNA was assessed by real time PCR in the reference strain *G. pallida* IVV7 grown in liquid shaken culture for 4, 8, 12, 16 and 20 days. *cu* mRNA was present in *G. pallida*, even if in very low amount, reaching its maximum after 8 days of growth. The same time-point was used to test for *cu* expression in 7 isolates of different species, where *cu* mRNA was found in amounts comparable to IVV7. Finally, the induction of *cu* gene expression was tested in IVV7 grown on elm sawdust and in dual culture with *O. novo-ulmi*. In both conditions the expression level was increased, however the amount of *cu* mRNA remained extremely low thus raising the question of its functional significance.

Infection of *Ulmus laevis* (Pall.) with an unknown putative viral agent

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Abstract:

Plant viruses occur in herbaceous and woody plants worldwide such as in trees of forests and urban green and are known to cause severe yield losses. Visible symptoms of a virus infection comprise from chlorotic ringspots, mottling, necroses, deformation of leaves and sprouts. As a result plants suffer from a reduction of photosynthesis capacity, growth depression, dieback and a strong susceptibility to other stress factors. Due to the lack of curative measures, one of the most important management tools is to provide healthy plants and seeds for planting and prevent virus transmission within the stand. Prerequisite for understanding the spread of infectious viruses and for epidemiologic prognoses is the characterization and identification of the pathogen. Elms are known to be affected by viruses such as *Elm mosaic virus* (EMV), *Elm mottle mosaic virus* (EMoV), *Cherry leaf roll virus* (CLRV), *Tomato bushy stunt virus* (TBSV) and *Tomato ringspot virus* (ToRSV) based on visual and serological studies.

Within the study a 150 years old population of European white elm (*Ulmus laevis* Pall.) of 30 trees in the park of Caputh near Berlin with assumed virus symptoms were frequently monitored over 13 years. 15 elms were selected developing distinct leaf symptoms such as chlorotic ringspots, necroses and dieback, suggesting a virus as causal agent. Investigations on characterization and identification of a putative viral pathogen in these trees are initiated. Leave samples were tested towards so far known viruses, none could be detected. The mechanical transmission of the agent was confirmed by transmission experiments with *Chenopodium quinoa* as well as by grafting tests. Applying molecular biology methods such as reverse transcriptase polymerase chain reaction (RT-PCR), analysis of double stranded RNA (dsRNA) and virus purification sequence information of the pathogen shall lead to identification of the causal agent. The putative viral sequence will be confirmed by comparison with virus sequences available in the NCBI (National Center for Biotechnology Information) database.

Elm Losses and their Causes over a 20 Year Period –A long-term Study of Ulmus in Saxony, Germany

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Abstract:

A major study of Ulmus in the German state of Saxony was started in the spring of 1994. Elm populations and individuals were recorded in the floodplains of three rivers (Elbe, Mulde, Spree). In a total of 134 plots 559 elms were found: 378 *Ulmus laevis*, 143 *U. glabra*, 34 *U. minor* and 4 *U. x hollandica*. By extrapolation we concluded that between 110,000 and 140,000 individual elms still exist in the state, the majority of them being *U. laevis*. The findings were documented in a series of papers and were presented at elm conferences in both Chicago and Segovia.

Within the framework of this comprehensive study, we started a monitoring program involving a total of 52 elms in 15 separate locations across Saxony. The individually monitored trees were visited six times from spring 1994 until autumn 2013, thus covering a span of 20 growing seasons. During this space of time 27 trees were lost. Between 1994 and 2000 three elms died, 22 more died between 2001 and 2008, and another two in 2013. Nearly one half of the losses were attributed to Dutch Elm Disease (13 cases). Four elms were lost due to natural factors, such as storm damage and diseases other than DED. But one third of the losses were due to human influence, mainly construction works (10 cases).

A closer look at the history of two floodplain landscapes in Saxony (Dresden and Leipzig) reveals that human activities are a major factor in the destruction of elm habitats and individual elms. In the Dresden Basin, the primeval floodplain forest (elm, ash, oak) was almost entirely lost to urbanisation, industrialisation and infrastructure. In the Leipzig area, a forest still exists, but the original floodplain hardwood forest has degraded to a dry variant of oak-hornbeam woodland. This development was caused by large-scale river regulations in the region.

Over the centuries, human impact may have destroyed 90% of original elm habitats in the two Saxon floodplain landscapes. DED accounted for another 90% of the remaining population, so that today we see just 1% of what once were wide floodplain landscapes dominated by elm.

Elm Yellows: another threat to elm conservation.

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Abstract:

Phytoplasma are an emerging phytosanitary problem both in crops and wild plants. Among phytoplasma, Elm Yellows (EY) are currently spreading possibly because of increased cultivation of DED resistant elm clones.

Generally, resistant hybrids are obtained by crossing native to Asian elms, which are resistant to DED, but susceptible to EY.

The co-occurrence of several favourable conditions, such as increasing frequency of DED resistant hybrids sensitive to EY, presence of host plants, as for instance *Rubus* spp., *Vitis vinifera*, that serve as reservoirs of phytoplasma inoculum, and large populations of the insect vectors, has recently produced such a visible outbreak of symptoms to draw the general attention on the disease. European elms also are susceptible to EY, and symptomatic plants are nowadays often found in city parks and gardens.

Aim of this study was to investigate the pattern of natural infection by EY in a field-planted clonal collection of elms, containing various species and provenances. The clonal garden is part of the "European Clonal Collection" and includes Italian clones (4 ramets per clone), DED resistant European x Asian hybrids, and clones originating from Spain, France, Greece and Malta. In recent years, infections by EY have dramatically increased in the collection, with sometimes severe symptoms and cases of plant death. We applied ANOVA to test the effect of *species*, *geographical origin* and *position within the collection*, on expression of EY symptoms (susceptibility). Symptoms were significantly more severe in clones originating from Spain, suggesting these clones to be genetically more susceptible to EY or more attractive for insect vectors.

Figure 1.

At least 1:4 clone yellowing – at least 1:4 clone with yellowing and reduce size of the leaf – at least 1:4 clone with yellowing, reduce size of the leaf and dieback.

In order to protect the European Clonal Collection, we are propagating all the clones by cuttings treated with hot water to eliminate the phytoplasma according to Budon-Padieu et al. (2004) protocol.

Implementing elm dynamic conservation in Europe: case studies and perspectives

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Abstract:

Many European countries have undertaken the static preservation of native elm genotypes in clonal collections maintained *ex situ*. Less development has yet been devoted to the dynamic conservation of elm populations *in situ*. Dynamic conservation methods were promoted by the European Forest Genetic Resources (EUFORGEN) programme through leaflets, strategies, a Technical Guideline and the geo-referenced database EUFGIS^(*) on Dynamic Conservation Units (DCUs). Dynamic conservation case studies are presented here as support for discussion on methods used at country level and perspectives on a pan-European scale.

The numbers of DCUs entered by the National Focal Points in the EUFGIS database as of 9 September 2013, are 27 for *Ulmus glabra*, 17 for *U. laevis* and only 2 for *U. minor*; the numbers of country involved are respectively 7, 6 and 2.

The creation and management of the French DCUs show that, in the case of *U. laevis* in fragmented riparian forests, habitat protection oriented management was compatible with forest genetic resources dynamic conservation, and that the species can still be conserved *in situ* in spite of Dutch Elm Disease (DED). Two DCUs were selected in two different climatic zones of France. A strong collaboration with habitat conservationists enabled the monitoring of losses to DED and the assessment of within-population diversity for flowering phenology. Restoration plantations were successfully carried out in a sector invaded by exotic species. Collaboration with forest geneticists revealed that the diversity of the Garonne population was low but that it contained rare DNA variants and adaptive traits.

On a pan-European scale, the network of dynamic conservation units needs to be representative of the partitioning of adaptive diversity across the species distribution range. For this reason, EUFORGEN has developed a GIS-aided approach to identify conservation gaps, i.e. comparing existing DCUs entered in EUFGIS and the environmental zones defined by Metzger 2013. In the case of *U. laevis*, a revision of the distribution range is needed to add the marginal populations recently discovered in Spain, Italy and south-east France.

Elmscape restoration with *U. minor* can be viewed as a very dynamic way of conserving the genetic resources of the species. Because it requires the plantation of clones or seedlings selected on a regional scale rather than locally, it is a form of *ex situ* conservation. It is also a very clear form of dynamic conservation if the ultimate goal of such plantations is to reinforce the local gene pool with trees able to exchange pollen with elms in the neighbourhood, and hence contribute new genotypes that will be submitted to natural selection and provide fuel for ongoing adaptation process.

Session 4 : Elm taxonomy and conservation

Since 1987, hedge restoration plantings have been carried out in France with elm clones selected in the national collection and tested for their susceptibility to the agent of DED in artificial inoculation tests. Native European material was never found strongly resistant to the pathogen but clones showing some recovery ability in inoculation tests were selected for experimental plantations in field hedges reconstruction projects. Elms were planted among other tree and shrub species, with at least five ramets/clone in randomized single tree plots. First results on natural contamination and mortality have been recorded but more time is needed to assess the elm survival percentage at sexual maturity age.

The development of elm dynamic conservation at a national level is very difficult without financial incentives from the EU and strong support from stakeholders. The latter may be found with habitat conservationists and hedge re-constructors. EU support could be applied for in proposals for a COST action on *in situ* conservation and/or a GENRES multispecies project for diversity monitoring in DCUs.

(*) Establishment of a European information system on forest genetic resources

Metzger MJ, Bunce RGH, Jongman RHG, Sayre R, Trabucco A, Zomer R, 2013. A high resolution bioclimate map of the world: a unifying framework for global biodiversity research. Manuscript submitted to *Global Ecology and Biogeography*, 22: 630-638

Conservation status of red elm (*Ulmus rubra*) in the north central United States

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Abstract:

Dutch elm disease (DED) devastated native elm (*Ulmus* spp.) populations in the US beginning in the 1930s. The numbers of elms lost to DED are unknown but estimates range into the hundreds of millions. Such enormous declines pose the threat that genetic diversity in species such as red elm (*Ulmus rubra*) could be lost due to 'genetic bottlenecks' or restructured due to 'genetic drift'. The introduction of Siberian elm (*U. pumila*) to the US more than a century ago poses an additional threat to the genetic integrity of red elm. Siberian elm escapes from cultivation and readily hybridizes with red elm where the two co-occur. How concerned should we be about these threats to red elm?

We used 13 microsatellite loci to characterize *U. pumila* accessions from the People's Republic of China (PRC) and native populations of red elm from Wisconsin. We collected samples from six Wisconsin elm populations in which some trees had phenotypic characteristics intermediate between *U. pumila* and *U. rubra*. Finally, we examined 85 *U. rubra* herbarium specimens collected between 1890 and 2004 to represent a broad sample of red elm's original diversity. We detected 6 *U. pumila* X *rubra* hybrids among the herbarium specimens, but none in the wild, putatively pure red elm populations. We analyzed data from the native red elm populations to assess their levels of genetic diversity and possible changes in 'genetic structure', and for evidence of recent 'genetic bottlenecks'. We analyzed data from the putative hybrid populations for evidence of hybridization and introgression between Siberian elm and red elm.

The native red elm populations possessed relatively high levels of genetic diversity and a significant number of rare alleles, comparable to that of other long-lived forest trees that have not experienced recent population declines. Despite enormous mortality, we found no evidence of reduced genetic diversity in any wild *U. rubra* populations when compared to our herbarium samples. We also found little or no evidence of 'genetic bottlenecks' in any population, nor did we detect evidence for inbreeding resulting from population declines. We identified 9 *U. rubra*, 32 *U. pumila*, and 51 hybrid individuals in our hybrid populations. Of the 51 hybrids, 35 were classified as first-generation hybrids and 16 as backcrosses. The majority of the backcrosses (88%) were introgressed toward *U. pumila*, but it is not clear whether this pattern relates to greater DED tolerance or other factors. We observed greater genetic diversity and new combinations of alleles in the hybrids. Our study points to the potential for widespread hybridization between *U. pumila* and *U. rubra* and an asymmetric pattern of introgression toward *U. pumila*. It seems possible that introgressed hybrids will eventually replace the native red elm in many portions of the native range. Hybrid swarms and introgression towards Siberian elm impact red elm in Wisconsin and pose a growing threat to the genetic integrity of the species.

Is hybridization a necessary condition for the evolution of invasiveness in non-native Siberian elm?

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Abstract:

Siberian elm, *Ulmus pumila*, was introduced in the United States and Italy partly as a response to the Dutch elm Disease which was decimating native populations of red elm, *U. rubra*, in the United States and Field elm, *U. minor*, in Italy. Siberian elm was also introduced in Spain and has quickly naturalized in all these countries and been shown to hybridize with native elm species. In the midwestern United States, hybridization between *U. pumila* and native *U. rubra* occurs frequently and between 0-50% hybrids have been detected in populations previously identified as *U. pumila* based on phenotypic traits. In Italy and Spain, *U. pumila* hybridizes with the native *U. minor*. Hybridization increases the level of genetic diversity of *U. pumila* populations, can facilitate the expression of novel genotypes especially in the F₂ generation, and may influence the evolution of invasiveness in *U. pumila*. Second-generation hybrids (F₂) have been detected to date between *U. minor* and *U. pumila* in Italy. While hybridization increases the level of genetic diversity, which in turn can facilitate the evolution of invasiveness, it is not clear whether hybridization is a necessary condition for the high genetic diversity detected in non-native *U. pumila* populations.

To address this question, we quantified the level of genetic diversity and looked for the presence of hybrids in areas where *U. pumila* was planted but where no native elm species were known to occur. These areas included the western part of the United States and Argentina. Trees of *U. pumila* from 30 populations from the western United States and 11 populations from Argentina were genotyped using microsatellite markers. Because *U. minor* trees were also introduced in Argentina, we tested for hybrids between *U. pumila* and *U. minor* in Argentina. We also tested for hybrids between *U. pumila* and *U. rubra* in Argentina because *U. pumila* trees were introduced from the United States. The presence of hybrids in the western United States would suggest that hybrids between *U. pumila* and likely *U. rubra* trees were planted in these areas. We used reference samples of *U. pumila*, *U. rubra* and/or *U. minor* to detect the presence of interspecific hybridization. To compare the levels of genetic diversity between native *U. pumila* populations and non-native *U. pumila* populations, we quantified genetic diversity in the 30 populations from

the western United States, the 11 Argentinian populations and compared it to the levels observed in 14 native Asian *U. pumila* populations. We also compared the level of genetic diversity to non-native *U. pumila* populations from the midwestern United States where the percentage of hybridization with *U. rubra* within a population had previously been quantified.

In *U. pumila* populations in the western United States and Argentina, we detected little hybridization with either *U. rubra* or *U. minor*. The level of genetic diversity remained high, however, in these non-native populations and comparable to the level of genetic diversity observed in known hybrid populations in the midwestern United States. The average level of genetic diversity in non-native populations was greater than in native populations. We will discuss some of the factors, besides hybridization, that could help maintain a high level of genetic diversity in the non-native populations. Hybridization is not a necessary condition for the maintenance of high genetic diversity in non-native *U. pumila* populations. Moreover, because *U. pumila* is invasive in Argentina and the western United States, hybridization is not a prerequisite for the potential evolution of invasiveness in Siberian elm.

Estimation of genetic diversity in progenies of selected genotypes of *Ulmus villosa* Brandis using RAPD markers

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Abstract:

The present study was conducted on 23 promising progenies of *Ulmus villosa* Brandis for molecular characterization of *U. villosa* progenies which were selected on the basis of previous study in the department. Molecular diversity among the progenies was estimated using ten RAPD primers. A total of 57 markers were generated, which exhibited 100% polymorphism. All of the ten primers showed 100% polymorphism. Such a high level of polymorphism reflects the outcrossing nature of the species. UPGMA dendrogram based on RAPD markers resolved into four major clusters and the similarity coefficient among 23 progenies of *U. villosa* ranged from 0.04 to 0.70 indicating a broad genetic base of *U. villosa* progenies. Maximum similarity (70%) was observed between Jag-T₂ (Jagoti) and Jag-T₃ (Jagoti). Thus, these progenies showed maximum degree of similarity in their genetic makeup. However, the minimum values were observed between Ka-T₄ (Katouch) and Jag-T₂ (Jagoti) (0.04). It may be attributed to the fact that both belong to different areas with different origin. The progenies were grouped into four clusters. The cluster II consisted maximum of 12 progenies followed by cluster III (5 progenies), cluster IV (4 progenies) whereas cluster I consisted of single progeny. In fact, progeny Ju-T₃ (Jugahan) was found to be the most divergent so accordingly its mother tree can be used in further hybridization programme. RAPD analysis proved helpful for estimating the magnitude of genetic diversity at molecular level. On the basis of banding pattern, RAPD's were effectively used for molecular characterization of *U. villosa* progenies used in this study.

Key words: RAPD, *Ulmus villosa*, Variation, Correlation, Progenies

***Ulmus laevis* Pallas a native elm in the Iberian Peninsula: a multidisciplinary approach**

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Abstract:

European white elm (*Ulmus laevis* Pallas) populations are rare, small and fragmented in the Iberian Peninsula. Due to these characteristics their indigenous status in the region was questioned, and the species role in Iberian riparian forests unknown. Herein we use a multidisciplinary approach to further knowledge on the conservation status, distribution and ecology of this species in the Iberian Peninsula. A bibliographical search and field surveys conducted by the Spanish Elm Breeding and Conservation Program served to elaborate the present distribution map of the species. The combination of palaeogeographic, historic, climatic modelling and genetic data showed that Iberian Peninsula was a glacial refuge for *U. laevis*, and that several of these populations are relicts. Moreover, their genetic diversity is comparable to, or even higher than the observed in other European populations. Thus, its native status was confirmed. Later, *U. laevis* distribution in relation to soil nature and water availability was evaluated. *Ulmus laevis* only grows on slightly acid (siliceous) soils in Spain, meanwhile field elm (*Ulmus minor* Mill.) also grows on calcareous soils. We compared both species iron uptake efficiency, which showed that the lower proton extrusion capacity and ferric reductase activity of *U. laevis* could be responsible of its calcifuge nature. In relation to water availability, *U. laevis* xylem vessels are very susceptible to drought-stress cavitation, thus, water table depletion affects more negatively this species than field elm. Finally, the reproductive biology and seed production,

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predation and dispersal were assessed. Empty samara production and masting were shown to reduce pre- and post-dispersal seed predation. Recruitment is not source limited (number of seeds), at least in most years, but could be dispersal limited. Wind disperses *U. laevis* samaras at short distances (< 30 m) and hydrological control of rivers might affect negatively long distance seed dispersal by water. Recruitment may be limited by water table depletion and lack of floods that eliminate pre-existing vegetation and deposit mud. Human induced landscape transformation seems to be the main threat for the species.

About *Ulmus laevis* Pall. in Italy

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Abstract:

Ulmus laevis Pall. (European white elm) is widely cultivated in Italy for aesthetic aims in parks, gardens and roads and it is preferred over other elm species because of its capacity to avoid Dutch elm disease (DED) infections (Sacchetti *et al.*, 1990). Botanists agree in considering *U. laevis* as "exotic cultivated" in Italy.

In relatively remote areas of northwestern Italy, along rivers and streams, more than thirty small and scattered *U. laevis* populations were recently identified. Since there are no signs of human mediated plantations a native status has been suspected. A broad sampling on these populations has been carried out in order to ascertain the existence of genetic relations with white elm French natural populations. The genetic profiles obtained by amplification of nuclear and plastidial SSR markers obtained by Italian and French populations were compared.

The populations of European white elm in northwestern Italy appeared not to be related to the French native populations. The high heterozygosity suggests that these fragmented Italian populations may be relics of a wider population ranging the whole Po valley, before the dramatic man-mediated changes that have destroyed the natural ecosystems of this area, such as drainage and intensive agriculture practices.

Further studies comparing the Italian and French populations with samples obtained from Balkan peninsula (Courtesy of Prof. N. Keca) will probably clarify the origin and the post-glacial re-colonisation routes of European white elm.

Sacchetti P, Tiberi R, Mittempergher L. 1990. Preference of *Scolytus multistriatus* (Marsham) during the gonad maturation phase between two species of elm. *Redia* 73: 347-354.

Monitoring Wych elm population in southern Apennines

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Abstract:

In Central and Southern Italy, Wych elm (*U. glabra* L.) is a sporadic species. The natural distribution range is discontinuous and populations are isolated from one another preventing gene exchange. These populations are of great interest because Apennine Mountains are thought to have hosted glacial refugia of wych elm, from where the species re-colonised the entire European continent after the last glaciation. These populations are therefore rich of genetic diversity and variability, rare alleles, and worth to be conserved.

The recurrence of DED epidemics threatens these small fragmented populations with extinction. Elms killed by DED were found even at 1300 m of altitude.

In cooperation with the Italian Forest Service (CFS), a program to protect Wych elm has been started, which includes:

- *Monitoring*. GPS populations mapping.
- *Sampling*. To evaluate genetic diversity and variability.
- *Static conservation in situ*. Removal and destruction of diseased plants to reduce DED spreading.
- *Dynamic conservation*. Seeds harvesting or cutting propagation for new plantations.
- *Awareness*. Accurately inform and aware practitioners and foresters to avoid accidental felling of this species, a frequent occurrence because wych elm is not recognized or not considered of major concern

The Elm and the Butterfly

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Abstract:

The elm is host to a wide range of fauna in Europe, including over 200 species of invertebrate, 60 of them Lepidoptera; of these 18 rely exclusively on elm.

Some of these species have inevitably suffered greatly as a consequence of Dutch elm disease. A prime example is the White-letter Hairstreak butterfly, *Satyrrium w-album*, now classed as 'Endangered' in the UK, and accorded DEFRA BAP Priority status. The butterfly not only needs elm, but *sexually-mature* elm. The White-spotted Pinion moth *Cosmia diffinis* is likewise dependent on older trees.

Butterfly Conservation has been assessing disease-resistant hybrid elm cultivars for 14 years at four sites in southern Hampshire, to determine which, if any, could help conserve the White-letter Hairstreak. As all are of foreign origin, it was of primary importance to determine their adaptation to the winter-wet English climate, so heavily influenced by the gulfstream, and geology.

Of the 13 cultivars assessed, it is 'Morfeo' which appears to have the most potential to conserve the butterfly. The second stage of the project will begin in 2014, with the release of the butterfly at two of the trials sites to assess adaptation.

Bud dormancy in elm (*Ulmus* spp.) clones of Asian and European species - a case study of photoperiod and temperature responses

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Susceptibility to Dutch elm disease (DED) varies dramatically during the growing season. The period in which elms can be infected and express DED symptoms is restricted to several weeks after growth resumption. This phenomenon is not well understood, but few studies indicate that the seasonal variation in susceptibility depends on the host's rhythm of morphogenesis and growth. Susceptibility to DED is correlated to the bud burst date in *Ulmus minor*, suggesting that a differentiation in spring phenology may cause an asynchrony between host susceptibility and the time of disease transmission by the vector, possibly representing a disease escape mechanism.

Despite its putative importance for disease progression, little is known about the environmental control of dormancy and bud burst in elms, and additional information on the subject would be desirable for DED research and breeding purposes.

In this paper we studied the environmental control of bud dormancy in elm clones used in breeding against DED. This knowledge has at least two important applications: 1) To characterize and select interesting clones to be tested in more advanced studies of the dependence of DED susceptibility on phenology, in the prospect of exploiting such a disease escape mechanism to breed DED resistant elms; 2) to define the environmental factors to be considered in order to develop process-based models to predict bud phenology of elm clones under variable environmental conditions.

Dormancy release as influenced by duration of outdoor winter chilling in Florence (Italy) was studied under different photoperiod and temperature treatments in twigs of two European (*Ulmus glabra* Huds. and *Ulmus minor* Mill.) and four Asian (*Ulmus pumila* L., *Ulmus parvifolia* Jacq., *Ulmus macrocarpa* Hance and *Ulmus villosa* Brandis) elm clones. Photoperiod had no effect on dormancy release, neither it influenced bud burst during quiescence. Thermal time to bud burst decreased in all the clones with increasing outdoor chilling. Dormancy was released earlier in the Asian than in the European clones and the clones could be ranked from *U. pumila* (very weak and short dormancy) to *U. minor* (stronger and longer dormancy), the other clones being intermediate. The decrement in thermal time to bud burst was explained as an inverse exponential function of the number of chill days ≤ 5 °C received outdoor in autumn and winter.

The effect of propagation method on the stability of elm trees

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Abstract:

In the Netherlands elms are generally known for their resistance to wind and wind throw. However from about the sixties on increasingly incidents have been reported of 15 to 25 years old elm trees that showed a remarkable degree of instability caused by an abnormally developed root system.

Gradually it became evident that a substantial part of the number of fallen down trees concerned clones that were propagated on rootstocks from *Ulmus glabra* (syn: *Ulmus montana*) seedlings.

To get more insight in the preferred way of propagating elm clones and to test two promising rootstock clones for this purpose, young plants of 10 commonly used elm clones were studied in a comparative test during the period 2008-2011 in a trial field on a medium heavy clay soil. The plants were propagated either by cuttings or on rootstocks of 'Belgica' or 'Stavast'. Observations were made on growth (height and stem diameter), root development and signs of incompatibility between graft and rootstock.

Although on the basis of the results no fully reliable predictions can be made on the rooting behaviour of trees at older age and rootability of cuttings, clones that can be easily propagated on their own roots offer favourable prospects, both for the nursery trade as well as from the point of view of stability in practice.

With regard to resistance to DED of the root stock propagation on 'Stavast' may be preferred above propagation on 'Belgica'. This refers only to those clones that are difficult to propagate by cuttings and therefor have to be propagated on rootstocks.

Keywords: *Ulmus*, elm varieties, graft rootstock incompatibility, stability

The role of genus elm (*Ulmus* sp.) in structure and long-term dynamics of natural (strictly protected) forest stands of the Białowieża National Park, NE Poland

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Abstract:

The Białowieża National Park (present area 10,500 ha), established in 1920s, is located in the central part of the Polish sector of the Białowieża Forest (NE Poland). The Park consists of mosaic of natural forest communities, built by a total of 26 different tree species. Among them, the most important are: hornbeam (*Carpinus betulus* L.), small-leaved lime (*Tilia cordata* Miller) and Norway spruce (*Picea abies* (L.) Karsten). The much larger group of minor tree species includes, among others, the genus elm (*Ulmus* sp.) represented by three species: Wych elm (*Ulmus glabra* Hudson) (occurring most frequently), fluttering elm (*Ulmus laevis* Pallas) and small-leaved elm (*Ulmus minor* Miller).

The goals of this study were: 1) to thoroughly analyze the site requirements as well as the most important features of the life-history strategy of elm, determining its role in the composition and structure of the natural Białowieża forest communities, 2) to investigate the impact of the Dutch disease, which arrived in the Białowieża Forest in 1960s, on a long-term dynamics of forest stands with elm as the co-building tree species, 3) to determine, on the basis of the long-term data from permanent sample plots, elm natural mortality rates, growth and regeneration patterns and to develop on this basis the theoretical model of a balanced diameter distribution for the elm population.

To achieve the study goals, data from three major sources were used: 1) a set of ca. 400 circular sample plots (4 are in size), established in 1999 and re-sampled in 2009, distributed in a regular grid over the whole area of the Strict Reserve of the Białowieża National Park; 2) five permanent sample plots (total area ca. 15 ha), established in 1936 and reinvestigated so far on six major occasions (the last time in 2011-2013); 3) 30 pairs of sample plots (fenced and unfenced) established to study the impact of ungulates on forest regeneration and recruitment processes. Diversity of data made it necessary the application of several methodological and statistical approaches, including the methodology of demographic equilibrium calculation, quantitative indices of stand structure and forest stand visualization tools.

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An analysis of elm occurrence along major gradients of water moisture and soil fertility existing in the Białowieża Forest revealed, that very high site requirements of elm are one of the most important natural constraints limiting its role as a potential component of natural forest

communities – in spite of his potentially high competitive ability. The long-term dynamics of elm population was heavily impacted by a Dutch disease, which caused sudden drop in the population density and strongly modified the overall shape of diameter structure of the elm population (by killing trees representing mostly the largest diameter classes). Nevertheless, the empirical model of demographic equilibrium, developed for elm on the basis of growth and mortality data, suggests that, in contrast to many other tree species of the Białowieża Forest, elm can potentially be able to maintain its current population density and diameter structure, assuming that growth and mortality patterns would not change significantly in the future. The results from enclosure experiments indicate that elm could even increase in number. However, its recruitment from seedling to sapling and mature stand stages is largely impeded by a very high pressure of wild ungulates.

Effects of waterlogging on two riparian elm species

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Abstract:

The ecophysiological response to experimental waterlogging may help in determining species sensitivity to this stress, explaining natural distribution of species, and selecting adequate species for riverbank protection. In this study we aimed at comparing the sensitivity of *Ulmus laevis* Pallas and *Ulmus minor* L. to waterlogging. We immersed pots with two-year-old seedlings of both species in a pond with running water over several weeks and compared them with non-immersed well watered control plants, by means of several ecophysiological parameters. We measured stem growth continuously with linear variable displacement transducers; leaf water potential and leaf gas exchange once or more times per week; and CO₂ efflux, hydraulic conductivity and anatomical features in stems and roots twice over the course of the experiment. Preliminary results indicate an initial increase in growth after waterlogging. Moreover, despite some differences between species, the results do not point to clearly distinct sensitivity to waterlogging of *U. laevis* and *U. minor*, even if the former is more restricted to grow near rivers and streams than *U. minor*. Relatively high waterlogging-tolerance of *U. minor* and the ability of this species to grow in areas temporary exposed to water table depletions and drought, points to higher plasticity to water availability than *U. laevis* at the seedling stage. The fact that *U. minor* does not grow as near to the river as other trees species do, could be not related to a higher waterlogging sensitivity of *U. minor*, but to its lower ability to compete with other species near the river bank.

Impact of thinning on irradiance and soil water supply in young elm stands

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Abstract:

Pandemic illness of Dutch elm disease has hit twice the elm species, which caused disasters for them in the whole northern hemisphere. There are limited tools to fight this disease. Furthermore, these tools are often not efficient enough or cannot be applied in forest stands. Therefore we can apply only preventive measures against this disease. One of such measures could be to keep trees under more favorable growing conditions, first of all by the artificial control of optimum irradiance and netto soil water supply from precipitation. The study was done in a floodplain forest in southern Moravia, Czech Republic. We particularly did the work at the experimental site, where *Ulmus laevis* Pall. occupied 70 % of the area. Other species were represented by *Carpinus betulus* L., *Fraxinus excelsior* L., *Tilia cordata* Mill. and *Prunus avium* (L.) L. Stand age was 15 years.

We estimated leaf area distribution by the classical destructive ("leaf cloud") method. Total leaf area index (LAI) and sunlit part of this index (SLAI) was measured. We also estimated relative irradiation of the canopy (IRc), which was based on measurement of leaf dry mass per area in different canopy layers together with LAI and took long-term mean interception (22%) in a similar forest. All the above data were included in the model, which was applied for optimization.

Thinning operations of different intensity were reflected by changes of stand irradiation. Low level of thinning (removal of 50 % trees, 3,0 LAI) caused increasing irradiance by 15 % when compared to original situation. Medium level thinning (removal of 55 % stems from the stand, 2,8 LAI) caused increasing irradiance (IRc) by 18 % and high level of thinning (removal of 60 % trees, 2,5 LAI) caused increasing irradiance by 21 %.

If interception was considered as proportional to leaf area, its percentage would decrease by 13, 12 and 11 % respectively and therefore soil watering (= netto precipitation) would increase by 9, 10 and 11 % respectively.

Our study suggests that better knowledge of ecophysiological parameters and characteristics of stand environment can improve field applicable modeling. By optimization of parameters related to canopy irradiance and netto precipitation control we can assure better utilization of stand leaf area due to increasing (1) its photosynthetic potential and (2) soil watering from precipitation, thus support its vitality.

