COST E42 Growing Valuable Broadleaved Tree Species“
Report of Italian partner
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WG3 responsibles: Francesco Ferrini (Università di Firenze)

The activity of the Italian Group fits with the items of the E42 Actions. Scientist developed researches in the different fields in order to enlarge the knowledge on the analysis and management of Valuable Broadleves Species for wood Production.
The report of the Italian Group has been articulated in the framework of the WG 1, WG 2 and WG 3 of the Action E42

WORKING GROUP 1

Partner - DIVAPRA – Plant Genetics and Breeding University of Turin,

Fraxinus excelsior

Genetic variability and differentiation within the Italian range of the species have been analysed by means of molecular markers (nuclear and chloroplastic microsatellites). Genetic data have been joined with ecological characteristics of the sampled sites, in order to define Regions of Provenance, which are a basic aspect for a rational management of activities linked with forest trees propagation, including afforestation and *in situ* genetic preservation.
*(Ferrazzini D., Monteleone I., Belletti P., 2006. Genetic variability and divergence among Italian populations of common ash (*Fraxinus excelsior *L.). Annals of Forest Science 64: 159-168)*

Acer pseudoplatanus

Genetic diversity and differentiation of 12 native populations of sycamore from north-western Italy have been investigated by means of variation detected in polymorphic isozyme systems. The trees showed relatively large genetic variability but small interpopulational variation, suggesting the presence of a single gene pool. There was a positive correlation between the age of the individuals sampled and heterozygosity, suggesting the occurrence of natural selection against homozygotes. No significant correlation was detected between genetic and geographic distances of populations, confirming the lack of any barriers to gene flow.
*(Belletti P., Monteleone I., Ferrazzini D., 2007. Genetic variability at allozyme markers in sycamore (*Acer pseudoplatanus*) populations from north-western Italy. Canadian Journal of Forest Research 37: 395-403)*

Sorbus torminalis

Genetic diversity and differentiation of 22 populations from north-western Italy have been investigated by means of variation scored at 53 polymorphic RAPD loci. Populations largely differed in terms of their internal genetic variation. Most of the genetic variation was found within populations, with a significant proportion of variance attributable to genetic differences between regions and between populations. A significant positive correlation was detected between genetic

and geographical distances, suggesting that isolation by distance is an ongoing process in the set of populations that were sampled.
*(Belletti P., Monteleone I., Ferrazzini D. A population genetic study in a scattered forest species [*Sorbus torminalis *(L.) Crantz] using RAPD markers. European Journal of Forest Research, in press)*

Prunus avium

Genetic variability detected at morphological, biochemical and molecular markers is analysed within and among populations from Italy. Aims of the research are the identification of Regions of Provenance as well as the correlations among the different markers which are tested.
A supplementary aim of the research is the analysis of seed chain and the evaluation of the effect of ordinary nursery activity on genetic variability of the processed material. The study involves 4 steps of seed production chain: a natural population, two seed orchards made up with different numbers of clones, nursery plantlets and two artificial stands established using plants produced in the nursery. Genetic variability is estimated through the variation scored at nuclear microsatellite loci.

Partner CNR IBAF (Porano)

Juglans spp.

In the framework of the National Project RI.SELV.ITALIA, "Area 1-Risorse Genetiche Forestali", sottoprogetto 1.1. ,"Biodiversità e produzione di materiale vegetale da propagazione", a population including *J.regia* e *J.nigra* plants and natural interspecific hybrids (7 diploids individuals and a triploid trees) was analysed. The general objective of the project is the characterization of the natural interspecific hybrids to be used for the genetic improvement of walnut and forest plantation for quality wood.
The following research activities have been carried out:

- **1.1.** Identification of two mothers J. nigra giving rise to hybrid progenies
- **1.2.** Collection of free pollination progenies of 7 putative *J. nigra* mother trees
- **1.3.** Collection of 48 putative fathers *J. regia* grooving into and around the population
- **1.4.** fingerprinting of the collected germplasm by nuclear microsatellites
- **1.5.** Maternity analysis in the half-sib families to test the correct attribution of each mother
- **1.6.** Paternity analysis to estimate the reproductive success of mails and to identify the super-father
- **1.7.** Study of the fertility of the triploid plant by cytological and molecular analysis.
- **1.8.** Fingherprintig of the progeny of the triloid plant

Research development

Material: in season 2004 the progenies of 7 mother plants J. nigra (N3, N4, N17, N18, N22, N23, N24) and of the triploid plant N21, obtained for free impollinazione have been collected. From the germination of the seeds 461 plants, subdivided in the 8 families, are been born like follow: family-N3 118 individuals, family-N4 26 individuals, family-N17 88 individuals, family-N18 24 individuals, family-N22 15 individuals, family-N23 59 individuals, family-N24 114 individuals, family-N21 17 individuals.
In such plants, in the first half of september 2005, approximately 5 fresh leaves for each plant have been collected. The leaves have been immediately frozen in liquid nitrogen and therefore conserved to -80°C. Such families added to the material previously analyzed, 69 *J. nigra* plants, 48 *J. regia* and 7 interspecific hybrids, give a total of 600 samples.

Molecular analysis

The sampled leaves have been used for the extraction of the genomic DNA on which it has been carried out the analysis of fingerprinting. The extraction method has been partially automated thanks to the use of the Mixer Mill 300 (QIAGEN) for the powdering of the frozen tissue, and of the 96 DNeasy Plant Kit (QIAGEN) for the fast purification of the genomic DNA. This methodology has concurred to try 192 samples simultaneously and to extract pure genomic DNA in not more than 5-6 h.

The genomic DNA of every plant has been analyzed by means of 10 nuclear microsatellirati markers (SSR): WGA1, WGA4, WGA9, WGA69, WGA89, WGA118, WGA202, WGA321, WGA331. The fragments have been amplifyed by means of GENEAmp 9700 Thermocycler and visualized, after appropriate optimization of the method, in the 16 capillary automatic sequencer ABI Prism 3100.

Statistical analysis: The comparison between the collected genotypes, for the identification of the interspecific hybrids, has been carried out for each pair of samples calculating the Simple Match Coefficient (SM). On the correspondent matrix it has been lead the Analysis of the Principal Coordinates. Such analysis, concurs to reconstruct the relative position of the samples considering their matrix of genetic similarity and to order them in a space to two and/or three dimensions. The interspecific hybrids place between the two groups of parental genotipes. The test of allocation of frequentistic type (Paetkau 2004) is used to confirm, using a genetic model, the results of the clusters. The analysis of maternity and paternity of the half-sib families has been executed by means of the approach "Most likely" (software CERVUS ver. 2.0). This approach is possible only with polimorfic and codominant markers like the SSR.

Preliminary results

By means of the ten selected microsatellites 129 alleli have been amplifies (in average 12,9 alleli for primer), of which 39 *J. regia* - specific, 80 *J. nigra* – specific, and 10 in common between the two parental specie. The Principal Coordinates analysis lead on the SM coefficient reveals a clear subdivision of the total germplasm in four groups; the first one is constituted from the plants belonging to the *J. regia* species, the second and the third from the plants of the *J. nigra* species, the quarter, in middle position between the cluster *J nigra* and that one of the *J. regia plants*, groupes the hybrids. The allocation test confirms the identification of 198 diploid interspecific hybrids *J. regia* x *J. nigra*. In addition two hybrids showing a particular genetic profile different from the others has been identified.

The maternity analysis in each half-sib family has evidenced a series of sampling errors and has determined a new classification of the families: family-N3 41 individuals, family-N4 29 individuals, family-N17 97 individuals, family-N18 15 individuals, family-N22 73 individuals, family-N23 75 individuals, family-N24 114 individuals, family-N21 18 individuals. Such analysis also reveals that the triploid hybrid plant N21 is not sterile. The fingerprinting analysis demonstrates that the plants N3, N4, N18, N22, do not produce hybrids but only J. nigra individuals, while the plants N17, N23 and N24 give rise respectively to 68, 17 and 100 interspecific hybrids; the relative percentage of hybrids in each progeny is 70%, 22,7%, and 87.8%, respectively. The triploid N21 generates 13 diploid hybrids, 3 *J. nigra* plants and 2 triploids hybrids (a locus only) due perhaps to chromosomic aberration. The paternity analysis on the hybrids has revealed a different reproductive success of the *J. regia* putative fathers and an elevated percentage of external pollination.

New objectives and perspecives

- Cytological analysis of the progeny of the triploid plant on apical root
- Study of cromosomic segregation during the meiotic division
- Comparison of the genetic data of the collected genotypes with morphological characters (shape leaf, number of branches, inclination of branches, apical dominance, stem shape), growing parameters (survival rate, diameter of the stem, total height) and resistance to the bark cancer.
- Selection of the hybrid plants of greater interest from the arboriculture for wood production
- Setting up of the techniques for the identification of functional molecular markers closely associated the genes-R (Resistant) or genes RAGs (Resistant-analogs), useful for the early identification of resistant plants to the bark cancer.
- First attempt of setting up of the NBS-profiling approach on the two species parental species *J. nigra, J.regia* and their hybrids.

In addition SSR fragments were sequenced to test the "**Cross – amplification and sequence evolution of ten microsatellite loci in *Juglans nigra* and *Juglans regia* species" This reaserch was carried out in cooperation with other foreign scientists:**, Keith Woeste (U.S.D.A. Forest Service, Hardwood Tree Improvement and Regeneration Center, Department of Forestry and Natural Resources, Purdue University, 715 West State Street, West Lafayette IN 47907-2061) and Agnes Major (Molecular Tassonomy Laboratory, Hungarian Natural History Museum, Baross u. 13, 1088 Budapest, Hungary). The paper is submitted to Euphytica for evaluation. Below the aims and the resuls of the research is briefly summarized:

Abstract: *J. nigra* (Eastern black walnut) and *J. regia* (common or Persian walnut) are economically important species in Europe, Asia and North America. Natural hybrids between the two species, known as *Juglans* x *intermedia* (Carr), and they are valued for timber production. We tested a sub-set of ten nuclear microsatellite markers developed in *J. nigra* by Woeste et al. (2002) to investigate their transferability to Persian walnut in order to: (1) measure the inter- and intra specific genetic diversity in the genus *Juglans* (2) use this diversity to characterise *J. nigra* and *J. regia* and their interspecific hybrids (3) assess sequence variation in the flanking and repeat region of SSR fragments within and between species. The objective was both to confirm the cross amplification of microsatellites and to determine whether interspecific differences between alleles are due to simple changes in the number of repeats or to deletions / insertions in the flanking regions. All ten microsatellites amplified in both species, producing fragments of variable size. Out 112 total alleles, eight (7.14 %) were common to black and Persian walnut, 68 (60.7 %) amplifying in *J. nigra* and 36 (32.1 %) in *J. regia* only (private alleles). Indices of genetic variability (Na, Ne, Ho, H_E, F_{st}, R_{st}) revealed high level of genetic variability in the tested samples.

Other activity including in the WG1 is the COST-STSM fellow of Paola Pollegioni in the University of Wageninghen. She spent a months to acquire the NBS profiling technique useful to the early screening of resistance in Forest plants to biotic stresses that are very dangerous for the plant growing and development.

Below is attached the report of the activity developed by Dr. Pollegioni in Wageningen:

ACTION COST NUMBER E42; STSM Fellowship 2007

Beneficiary: Paola Pollegioni
Reference code: COST-STSM-E42-02811
Scientific Report

Application of the NBS profiling technique in *Juglans* spp.

Introduction

J. nigra (Eastern black walnut) and *J. regia* (common or Persian walnut) are both major economically important species in Europe, Asia and North America. The Persian walnut, an indigenous species in Eurasia from the Balkans to southwest China, is cultivated throughout the temperate regions of the world for its high quality wood and edible nuts. Black walnut, native to the Eastern part of North America, is a fast growing species with less valuable wood in comparison to *J. regia*. Since the 17th century, *J. nigra* has been imported from the Eastern and Central hardwood forests of the United States to the European continent for ornamental purposes and subsequently for its rapid growth.

Both common and black walnut are sensitive to soil conditions, developing best on deep, well-drained, moist and fertile soils (Williams, 1990), but they differ in response to pathogenic infection (Weber et al.1980). *J. regia* is susceptible to the damage caused by pests, abiotic factors (flooding, drought) and biotic stresses (e.g. anthracnose, bacteriosis)(McGranahan and Leslie, 1990). Particularly the anthracnose caused by *Gnomonia leptostyla* (Fr.) Ces. is one of the most important diseases of walnut for timber production; symptoms develop on leaves, stem and fruit as irregular

necrotic areas that are often surrounded by small chlorotic halos. In severe cases, these lesions may cause premature defoliation , fruit drop or poorly filled nuts (Funk et al. 1980; Woeste et al. 2001).
Although phylogenetic analysis based on nuclear RFLP, *mat*K and ITS sequence has demonstrated that black walnut and Persian walnut belong to different sections of genus *Juglans, Rhysocaryon* and *Dioscaryon* respectively (Fjellstrom and Parfitt, 1955; Stanford et al. 2000) hybridization between them is possible. *Juglans x intermedia* (Carr) is a natural hybrid between *J.nigra* and *J.regia*. Compared to the parental species, most *J*. x *intermedia* hybrids show increased vegetative vigour, distinct disease resistance, good wood quality, and greater winter-hardiness than *J. regia* (Mapelli et al. 1997; Fady et al 2003). For these reasons there is a great demand for *J. x intermedia* for forestry, especially in Northern Europe. Investigation on the resistance to anthracnose infections of *J. regia*, *J. nigra* and inter-specific hybrids (*J. nigra x J. regia*) plants proved that *J. nigra* is resistant, while the hybrids showed an intermediate behaviour toward *Gnomonia leptostyla* infection (Anselmi et al 2005).
Most resistance to pathogens in plants is based on gene-for-gene model: an R-gene product recognises a pathogen gene product in the host, conferring full or partial resistance (Flor et al, 1971). Generally two different classes of molecular markers are applied for the characterisation of genetic diversity: neutral markers and functional markers. Neutral markers, such as AFLP and microsatellite markers, are generally not under selective pressure. They are useful for a lot of applications, including the evaluation of gene flow, population differentiation and inbreeding but correlation to functional traits (ex : resistance disease) occurs by chance. Instead functional markers are targeted to specific, and display the diversity or are correlated with diversity in genes that may affect the trait of interest. Functional markers can be amongst others generated by methods that target a gene or group of genes; One of these methods is the NBS-profiling approach (Van der Linden et al 2004).
During the past decade an increasing number of plant disease (R) genes from different species have been identified, cloned and sequenced. These genes fall into five mainly subclasses (Baker et all. 1997), but the vast majority of them are members of the cytoplasmic nucleotide binding site - leucine rich repeat (NBS-LRR)-containing R-gene family. The NBS region of R genes contain several highly conserved motifs. These include P-loop (ATP and GTP binding proteins domain), kinase-2 motif (this domain coordinates the metal ion binding necessary for phosphate-transfer reactions) GLPL motif (also known as hydrophobic domain, a putative membrane-spanning domain) (Saraste et al. 1990; Meyers et al. 1999). The NBS-LRR proteins seem to be involved in recognizing pathogens and activating signal transduction pathways to induce the hypersensitive defence responses in plant.
Van der Linden (2004) proposed a new strategy to amplify a large collection of R-gene and R-gene Analogues (RGA) fragments to samples genetic variation in these genes. The NBS-profiling approach is based on PCR amplification using an adapter primer for an adapter matching a restriction enzyme site and a degenerate primer targeting the conserved domains present in the NBS region. In this way it is possible to obtain DNA profiles with markers mainly present in R-genes and RGAs. The NBS profiling method is a technique that has proven to be useful in a large number of crops including apple (Calenge et al. 2005), lettuce, potato, barley, tomato (Van der Linden et al. 2004; Malosetti et al 2007) and wheat (Mantovani et al. 2004). It is a technique that requires optimal DNA quality and optimal components for each step in the procedure. NBS profiling was developed at Plant Research International, Wageningen, Netherlands, and is used routinely in the Business Unit Biodiversity and Breeding in this institute.

Objective

The aim of this work was to set up all necessary steps for a preliminary NBS-profiling application in *J. regia, J.nigra* and hybrids. Once the optimal primer-enzyme combinations were identified, NBS profiling would be applied on several walnut genotypes in order to provide molecular markers tightly linked to R-gene and RGAs involved in anthracnose resistance.

Research activity

Material

In spring 2007 young leaves were sampled directly from the crown of 14 plants conserved in the experimental field of the CRA-Unità di Ricerca Forestale, Poplar Research Institute, Rome (Italy) and stored at -20°C until molecular analysis. According to morphological observations, 7 samples were classified as *J. nigra* (352JN, 355JN, 378JN, 371JN, 369JN, 361JN, 356JN) and 2 samples as interspecific hybrid (HT18; HU18). The others leaves were picked from 5 *J. regia* trees (RA3, RA21, RA5, RA1.3, RA22.5). In RI.SELV.ITALIA, project,"Area 1-Risorse Genetiche Forestali", sottoprogetto 1.1. ,"Biodiversità e produzione di materiale vegetale da propagazione", Prof. Anselmi proved that these seven *J. nigra* genotypes are resistant to anthracnose infection. On the contrary the sampled *J. regia* and interspecific hybrid plants resulted susceptible (except for RA1.3 that is tolerant) and tolerant toward *Gnomonia leptostyla* infection respectively.

DNA extraction

Genomic DNA was extracted from leaf tissue using the DNeasy Plant Mini Kit (QIAGEN), suspended in 100 µL of Buffer AE (Qiagen) and stored at -20°C. DNA presence was monitored by subjecting sample to 1% agarose gel electrophoresis in 0.5 x TBE buffer. The amount of DNA was spectrophotometrically determined and was brought to a working concentration of 20 ng / µL.

NBS profiling application (Van der Linden et al. 2004; PRI protocol)

1. Restriction digestion and adaptor ligation

Restriction digestion and adaptor ligation were performed in a single reaction. Two hundred nanograms of DNA was digested with either MseI or RsaI restriction enzyme in the appropriate buffer for 3 h at 37°C. An adapter was ligated to the restriction fragments using high concentration of ligase (1U for MseI-sticky enzyme and 5U for RsaI- blunt enzyme). The reaction was stopped by heat inactivation (15 minutes at 65°C). The adapter consists of a long arm and a short arm, with the short arm blocked for elongation by an amino group at the 3' end and a P-group at the 5'-end to facilitate ligation to blunt-end fragments.

Adapter sequence:

5'-ACTCGATTCTCAACCCGAAAGTATAGATCCCA-3'….long arm

| | | | | | | | | | | | | | |

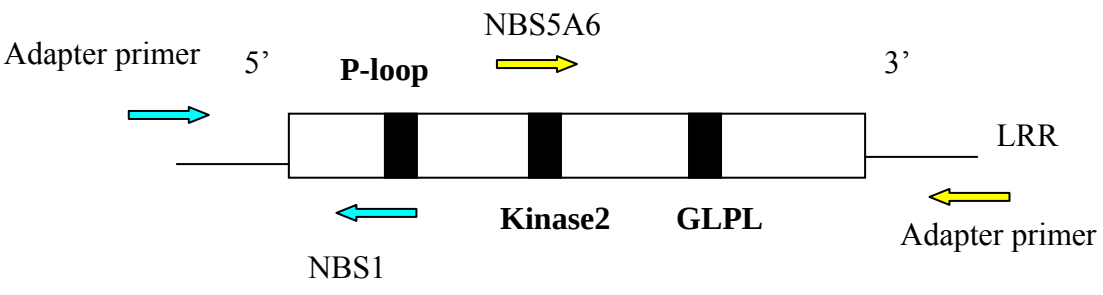
3'-NH_2TTCATATCTAGGGT -5'P short arm

Adapter primer sequence: 5'-ACTCGATTCTCAACCCGAAAG-3'

In the short arm the amino-group blocks the elongation of the 3' end by Taq polymerase. In this way at the start of PCR amplification, the adapter primer can not anneal to the DNA template; but only after the specific NBS primer is annealed elongated, an annealing template is available for the adapter primer. The annealing of adapter primer relies therefore on the selective motif-specific primer to anneal and to be elongated into a complementary strand. This prevents the amplification of adapter-adapter fragments.

2. First Amplification

Two different NBS-specific primers, NBS1 and NBS5A6 (NBS 5A combined with NBS6) were used in a total of 4 primer-enzyme combinations. These primers were designed from a part of the conserved P-loop motif for NBS1 and of kinase-2 for NBS5A6; NBS1 primer amplified DNA towards the 5'end of the targeted genes, outsite the NBS region, and NBS5A6 towards the 3' end, inside the NBS region. Amplification of NBS-specific fragments was performed in a single polymerase chain reaction with NBS-primer and adapter primer rather than two-step PCR procedur in the original protocol (Van der Linden et al. 2004, see also Mantovani et al, 2007).



Polymerase chain reaction (PCR) was done in 25 μL of reaction volume adding 5μL of restriction-ligation template (diluted two times), 2.5 μL of HotStartTaq PCR buffer 10 X, 200μM dNTPs, 20 pmol for each primer and 0.4U of HotStartTaq polymerase (Qiagen, Germany). Reactions were performed in a GENEAmp 9700 Thermocycler according to the following procedure: 15 min at 95°C (to activate HotStartTaq polymerase) followed by 30 cycles of 30 sec at 95°C, 1.40 min at 55°C, and 2 min at 72°C; then a final extension step at 72°C for 20 min. The amount and estimated size of the amplified fragment was checked testing 15 μL aliquot of the amplified reaction by electrophoresis on 1% agarose gel in 0.5x TBE buffer, and stained with ethidium bromide.
All 4 combinations (MseI-NBS1; MseI-NBS5A6; RsaI-NBS1; RsaI-NBS5A6) amplified in *J. regia*, *J. nigra* and hybrid genotypes. A smear with several distinct bands in the size range of 100-1000 bp was observed in the agarose gel (1%) for each combination.

3. Labelling PCR

The PCR products were re-amplified using labelled adapter primer (fluorescence, IRD-700). Polymerase chain reaction (PCR) was done in 10 μL of reaction volume adding 5μL of 10 X diluted PCR mixture, 1μL of PCR buffer 10X, 200μM dNTPs, 3 pmol of NBS primer, 0.6 pmol of IRD-labelled adapter primer and 0.2 U of SuperTaq DNA polymerase (Qiagen, Germany). Reactions were performed in a GENEAmp 9700 Thermocycler according to the following procedure: 3 min at 95°C followed by 35 cycles of 30 sec at 95°C, 1.40 min at 55°C, and 2 min at 72°C; then a final extension step at 72°C for 20 min. The labelled PCR products are mixed with an equal volume (10μL) of formamide-loading buffer (98% formamide, 10mM EDTA pH 8.0 and 0.1% Bromo Phenol Blue) and an aliquot (0.3μL) was analyzed in the LI-COR 4300 DNA Analysis System (Li-COR Biosciences). The 4300 System is an instrument based on LI-COR's highly sensitive infrared fluorescence detection technology. The labelled PCR products were separated on a 6% polyacrylamide gel, and the individual fragments visualized by fluorescence detection.

4. Preliminary results

All fragments amplified in *J. nigra*, *J.regia* and hybrid plants for each enzyme-NBS primer combination and visualized by LI-COR 4300 DNA Analysis System were scored. For example figure 1 shows the NBS profiles of *J. nigra*, *J. regia* and hybrid genotypes with primer NBS5A6 and restriction enzyme MseI, visualized with LI-COR 4300 DNA Analysis System.
Table 1 summarizes the number of "common" and/or private NBS fragments detected with the four enzyme-NBS primer combinations. Out of the total (341 bands) , 89 fragments were common to black and Persian walnut and were labelled as "common". In addition, 254 fragments amplifying in *J. nigra* and 128 in *J. regia* only, were classified as species "private NBS-bands". No private bands were observed in the interspecific hybrids.

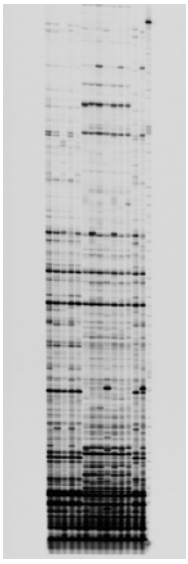


FIGURE 1.

LI-COR 4300 DNA Analysis System Gel for NBS5A6-MseI combination

Combination NBS Primer/Enzyme	Number of total fragments	Number of private NBS-bands		Number of common NBS-bands
		J. nigra	*J. regia*	
NBS1-RsaI	85	26	32	27
NBS5A6-RsaI	56	19	30	7
NBS1-MseI	84	26	22	36
NBS5A6-MseI	116	55	42	19
Total	**341**	**126**	**128**	**89**

TABLE 1. Summary of NBS- fragments amplified in *J. nigra*, *J. regia* genotypes in four different enzyme-NBS prime combination.

This is the starting point to find an association or "linkage disequilibrium" between the NBS markers amplified in walnut germplasm and the resistance for antracnose disease.

Future Collaboration

These initial results with some test samples of walnut species and hybrids showed a lot of promise. I am planning to visit again the Plant Research Institute (Wageningen) in September/December 2007 using my PhD fellowship in Forest Ecology (Tuscia University, Viterbo, and CNR-IBAF Porano, Italy). I could have the opportunity to extend my NBS profiling experience and to apply the technique on a larger set of walnut genotypes. This may enable to identify molecular markers linked to disease resistance (most notably anthracnose resistance) in walnut species.

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WORKING GROUP 2

- **WG2 activity**

the Italian WG2 (Pelleri, Pividori, Brunetti) have filled the wood quality table and the silviculture table for the main Italian valuable broadleaved species.

Partner Padova University

ANSWERS TO THE PRUNING OF SPECIES FORVALUABLE TIMBER. WALNUT AND WILD CHERRY (PRUNING FOR WOOD THIS DISOWNED)

The main causes of because many information are not available on the techniques of pruning on walnut and wild cherry for wood production are:
- Loss of the patrimony of past knowledge;
- Change of the techniques of cultivation (from two old to one attitude);
- Creation *ex novo* of a new plantation type

The rules of base for a correct pruning are:
- To maintain more then 50% of the crown;
- To never prune branches with a diameter greater then 3 cm;
- Don't prune branches where the stem is more then 10 cm of diameter;
- the pruning should be carried out in the phase of qualification (from the complete rooting of the plant until the attainment of the objective)

A mistaken pruning provokes:
- Reduction of the increments;
- Production of adventitious buds along the stem;
- Presence of defects in the wood;
- Loss of the cultural objectives.

During the experimentation it is proceeded in various step with the following objectives:
- I phase: verification of the effectiveness of the "replicativa" pruning on walnut
- II phase: verification of the effects of winter and summery pruning and of the role of brachyblasts on cherry
- III the phase: tests of pruning to "cassage" on walnut

The main obtained results can be reassumed as follow:
On the walnut the pruning technique which maintain free the top of the tree does not limit the apical increasing and maintains the apical dominance.
- The return pruning limits the diametrical increasing of the branches of first order.
- No reductions in the diametrical and longitudinal increase and low emissions of adventitious branches.

Between the cherry plants pruned in summer, in winter and without pruning, no differences in the diametrical increase were observed.
Referring to the heights, the pruned plants increase more the crown width then the height. The summery pruning reduces the emission of new branches of first level without any limitation for the diametrical increment.
The winter pruning strongly compensates the reduction of the crown with the production of brachyblasts.

The walnuts undergo "cassage" showed an increasing of the branches of the first order not significantly different in comparison to ordinary pruned branches.
Both are meaningfully inferior to those without any pruning.

- *Buresti Lattes E. and Pividori M, 2007- Le potature negli impianti di arboricoltura da legno: tecniche e risposte in specie di pregio (noce e ciliegio).*(Pruning in farm forestry: techniques and responses in valuable species (cherry and walnut). *Sherwood. Foreste e alberi oggi, 139, in press.*

Partner CRA-ISSEL, Firenze

The activity has been carried out in the framework of the National Project RI.SELV.ITALIA and of other regional projects.

Forestry

Thinning trials on Fraxinus excelsior and Acer pseudoplatanus secondary forests:

In Italy In the last 50 years ash-maple stands are spreading on former agricultural land. Actually, according to the National Forest Inventory (INFC 2007) there are about 154.000 hectares of ash-sycamore and ash-lime stands principally concentred in the north of Italy (Alpine and Prealpine region). Our working group is carrying out a research to improve the production of good quality timber in secondary ash-maple stands, grown in agriculture abandoned lands and actually unmanaged or used for fuel-wood. Considering the good potentiality of these species to produced good quality timber different thinning systems (traditional thinning from below and target tree systems around a restricted number of target tree per hectare) were tested in stands of different ages (from 15 to 30 years) using a thinning frequency of 4-6 years.

The results, after 10 years of experimentation, have shown good possibility to improve the saw-timber production using target tree system especially if thinnings start when the stands are 15-20 years old. At these ages constant diameter increment around 9 mm has been obtained; while worse results have been obtained in stand older (25-30 years) with irregular and lower diameter increment around 5-6 mm.

- *Giulietti V., Ferretti F., Pelleri F. 2007 - Prove di diradamento in acero-frassineti di neoformazione nella Comunità Montana Agno-Chiampo (VI): Risultati dopo il secondo intervento*.(Thinning trials in secondary growth ash-maple stands carried out in the Agno-Chiampo district (VI): results after second thinning). *Ann. Ist. Sper. Selv.,34, In press*

- ***Improvement of valuable broadleaved species in coppice stands*:**

A recent research activity on coppice stands rich of valuable broadleaved species has been developed. The research activity has been carried out both in coppice stands and in coppice converted in to high forest stands with the main aim to obtain not only fuel-wood but also saw-timber. The research was carried out in central and southern Italy

<u>*Central Italy (Tuscany)*</u>: In a young oak coppice stand (aged 7 yr.), some selected trees of *Sorbus* and *Pyrus* were favoured using different techniques to reduce oak's competition (girdling, breaking terminal shoot "cassage"). The growth of these young selected trees will be compared to other unmanaged young trees (control plot). A first paper will be presented to the next National SISEF Congress (Arezzo 25-27 September 2007).

Always in Tuscany in a coppice stand converted in to high forest (aged 40 yr.) the second thinning was realised using different techniques (thinning from below and free thinning around 150 target trees) to improve the presence and the quality of valuable broadleaved species. These experimental plots have been just realised and for the moment is impossible to value the effect of the treatments.

A technical handbook "Silviculture of sporadic species in Toscany" has been recently published. The volume suggest a new type of forestry to improve sporadic species (especially valuable broadleaved species) in the forest of Tuscany Region. For 25 species, protected by Regional Law regional distribution, botanical, morphological, ecological, and forestry information are given.

- *Giulietti V., Pelleri F. 2007- Caratterizzazione di u n giovane ceduo di cerro ad elevata presenza di rosacee arboree*.(Characterization of a young Turkey oak coppice stand with high presence of Rosaceae trees) *VI Congresso Sisef. "La gestione delle foreste tra cambiamenti globali e azioni locali". Arezzo 25-27 Settembre 2007.*

- *Mori P., Bruschini S., Buresti E., Giulietti V., Grifoni F., Pelleri F., Ravagni S., Berti S., Crivellaro A.,* 2007 - *La selvicoltura delle specie sporadiche in Toscana*. (Silvicolture of sporadic species in Tuscany) *Supporti tecnici alla Legge Regionale Forestale della Toscana 3. ARSIA Firenze: pp. 355*

<u>*Southern Italy (Molise)*</u>: During this years in mixed coppice with reserve stands (turkey oak with beech and hornbeam) characterized by the presence also of Sorbus, Fraxinus and Acer a different density of reserve (60, 90, 120 per hectare), mainly characterized by valuable broadleaved species, has been tested. The aim is to convert progressively coppice system in to coppice with standard systems able to produce both fuel-wood and saw-timber from valuable broadleaved species. Next winter in a similar stand a conversion of coppice in to high forests will be realised using different methods: traditional system (thinning from below with the selection of the main shoot for stamp) and target tree system (free thinning around 300- 400 trees per hectares).

Farm Forestry:
The activity in the farm forestry plantations regard especially the definition of different typologies of mixed plantation using valuable broadleaved species, shrubs and nitrogen fixing trees and to set up the best cultivation models and techniques for pruning and thinning. Recently new mixed plantations have been tested using both species cultivated with short rotation (S.R.F. 3-5 years and traditional poplar cultivation 10 years.) and long rotations (noble hardwood 20 years and more).
The result of 30 years of research on mixed plantation in Italy was recently published.
- *Buresti, E., Mori, P., Pelleri, F. and Ravagni, S*. 2006 Enseignements de 30 années de recherche sur les plantations mélangées en Italie. (Knowledge of 30 years of research on mixed plantation in Italy) *Forêt-entreprise,* **170**, 51-55
- *Pelleri F., Piegai F., 2007 - Indagini sui lavori di diradamento in impianti di arboricoltura con specie di pregio.* (Thinning investigations on farm forestry plantations with valuable broadleaved species). *Sherwood. Foreste e alberi oggi, 139, in press.*
Ravagni S., *Buresti Lattes E. 2007- Inpianti policiclici per la produzione di legname di pregio.* (Polycicled farm forestry plantations for roundwood production). *VI Congresso Sisef. "La gestione delle foreste tra cambiamenti globali e azioni locali". Arezzo 25-27 Settembre 200*

Other Cost E42 activity on valuable broadleaved.

- **Species review**.

Francesco Pelleri together with Cristophe Mhoni (CH) and Gabriel E. Hemery are writing a paper on "The modern silviculture of Juglans regia L.: a literature review ". This paper will be published in to the web site of the Cost action E42 and than in to a journal.

- **STMS**

Valentina Giulietti, researcher with grant on valuable broadleaved, benefited by her COST-STSM (two weeks) in BOKU University (Vienna, Austria). She was analysing single tree oriented management held in Austrian eastern part (Weinviertel) in order to improve timber quality and value production. In the specific: coppice and coppice with standard oak and ash-maple forests.

Short Term Scientific Mission (STSM)
Scientific Report – COST E42

Valentina Giulietti
in Universität für Bodenkultur Wien- Institute of Silviculture (Vienna)
30th April-11th May 2007

- **Purpose of the visit**

Analysis of single tree oriented management held in Austrian eastern part (Weinviertel) in order to improve timber quality and value production. In the specific: coppice and coppice with standard oak and ash-maple forests. This experience will be compared to similar Italian studies.

- **Description of the work carried out during the visit**

The first phase of the STSM concerned indications about the management of broadleaved tree species in Austria, with information about research in BOKU University and related publications.

The STSM was focused on field surveys of experimental and demonstration plots, located in eastern Austria, dealing with different forest management systems:

Coppice with standards system

In dry Lower Austria forests (Weinviertel), annual precipitation (450-700 mm) and mean annual temperature (8-10 °C) characterize these sites. The coppice with standards system (CWS) and coppice system dominate. The STSM focused on the study of CWS. This silviculture system is characterized by an over wood devoted to the high-quality timber production and an under wood with coppice for firewood production. Several of these dry sites are suitable for oak and other species (maple, ash, cherry, wild service tree and service tree) timber production. The analysis of these areas has shown interesting results either in under wood or in over wood.

Figure 1 - Coppice with standards, coppice (hornbeam) 45 years old

- UNDER WOOD: in the Weinviertel, the most important agriculture production area in Austria, land owners need coppice for its important biomass sink. The over wood density cannot be too high (<66% of crown cover) to maintain a good coppice vitality. The rotation in association with the site-specific conditions should not exceed 20-40 years for the maintenance of coppice shoot potential. In one of the experimental plot visited (45 years old) hornbeam dominates the under storey and the other species are replaced (Fig.1).

- OVER WOOD: the origin of this population is both gamic and agamic. While the management aims to seed regeneration, in unfavourable years (dry season, pests, etc.) the under storey coppice is utilized to select individuals for the dominant layer. Since the over wood destination is timber production, the selection criteria are: species, stem and crown shape, spacing (if possible). A good explanation of this management is a mixed oak (*Quercus petraea* and *Quercus robur*) and wild service tree stand in Weinviertel region (Fig. 2-*left*). In order to produce valuable assortments (40 (50) cm and branch free bottom log of 5-7 m) a rotation of 100-120 years has been implemented. This goal is achieved with an age graduation of 4 different classes, that permits every 20-40 years the fells of the under storey coppice and the biggest classes (diameter classes) of the over storey. With special tending the valuable trees can be achieve a good development of the crowns and the periodical formation of water sprouts, after removal of the under wood, can be prevented. Additionally, frequent pruning (branches <3cm) was realized. The catastrophic infestation of oak-dieback (*Lorathus europaeus*) has led to the promotion of biodiversity: the more diverse is the forest, the less relevant is the economic loss in case of pest. For this reason in the demonstration plot an artificial enrichment of sycamore and cherry tree has been carried out (Fig. 2-*right*).

Figure 2 - ***Left*:** coppice with standards**.** *Sorbus* and *Quercus* in the over wood. ***Right***: enrichment of valuable

On the whole, the coppice with standards management in practice offers a broad setting for ecological and economic possibilities.

Target tree management system (in "high forest system character" stands)

In the Austrian broadleaved stands regeneration has been mainly carried out by artificial planting while natural regeneration has not played a major role (although the latest is favoured through silvicultural treatments). With the STSM both afforestations and natural forests were visited.

A major objective of this silvicultural system is the production of high-quality timber at the lowest cost. In order to achieve 50 (60) cm diameter of straight trunk and branch-free bottom logs, the system is based on the following phases:

- SELECTION OF PLUS TREES: individuals are selected using the same criteria of CWS method (over storey): market interest for the species, good development and vitality of the crown, good stem conformation and uniform spacing. Interesting experimental plots (thinning and control), in a natural stand, were examined. In both kind of plots oak, sycamore, cherry and ash (in order of Austrian market importance) were selected.
- EARLY INTERVENTIONS (THINNED PLOTS): the first thinning starts both when the natural pruning (due to high competition between individuals) produces free-branch bottom log (of 4-5 m) in plus trees and when their competitors have a negative crown development impact.
- THINNING: in thinned experimental plots (Fig.3-*left*) and all plants around plus trees with the radius of 1 m were cut in order to facilitate workers in their pruning activity. Additionally, variable distance between plus trees and its neighbours was experimented, cutting all competitors in dominant and co-dominant tree layer. With this system interventions are concentrated exclusively around plus trees, with therefore a consistent lowering of the silviculture management costs (Fig.3-*right*). In general, through frequent thinning (every 5-6 years) a well development of the crown and 10-14m final spacing can be reached.
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Figure 3. Target tree management system in a natural "high forest". *Left*: oak plus tree, in an experimental thinned plot, competitors-free around a radius of 2.5 m. *Right*: well developed crown of *Fraxinus excelsior*.

- PRUNING: in addition to natural pruning, plus trees need an artificial live branches pruning in order to reach a free-branch bottom logs of 6-7 m (for 60-75 trees/ha). Some experimental plots with artificial pruning of different species (oak, sycamore, ash, *sorbus* and *pyrus*) were visited. The more afforestation densitiy is the less is the artificial green-pruning intensity. In the aim of cost reduction, is quite common in Austria to plant trees with a low density with insufficient pruning interventions.

The Target Tree Management System play an important role to increase the value of forest production. More experimental information for each stand and each tree species about the potential sites and trees is however needed. Since the plots are only 10 years old, it is still too early to have some significant results.

Since the aim of both CWS and Target tree management system is the increase of high quality products, another aspect to be taken into account is the study of provenience of some valuable sporadic species.

In some Austrian afforestations with presence of *Sorbus domestica* and *S. torminalis*, considerable differences in terms of timber quality and yield among were observed depending on the provenience of the seedlings. These two species are interesting for timber production as the price of their good-quality wood is extremely high and its demand is increasing on the European market. Studies about Austrian provenience of *Sorbus sp.* and their behaviour need to be carried out as same as in Italy. The research activity must be focused on selection of best *Sorbus* individuals, collection of seeds, cultivation in controlled fields, monitoring and selection of best individuals in terms of growth performance, form and stem characteristics. Some information about this topic were given by Raphael Klumpp in BOKU University during the visit of the local nursery .

- **Description of the main results obtained**

Better knowledge of coppice with standards system for high quality-timber production

In the past, the CWS system in Italian Turkey oak stands has been used, even for firewood production, also for pasture and railroad sleepers production. Nowadays, these traditional products are no longer required. Furthermore, the high density of the overstorey depresses the coppice production. The Austrian experience may be applied to certain extends to the Italian situation.

In such way, may be possible to improve the cultivation and the quality of valuable broadleaves with CWS management system.

Better knowledge of target tree management system

This selviculture system is not applied in Italy being only few experimental researches. The Research Institute of Selviculture in Arezzo and Firenze took part to the redaction of a manual "Silviculture of sporadic species in Tuscany" in order to promote this management system for the improvement of quality timber production. The STSM gave the opportunity to visit interesting Austrian experimental plots where target tree management system is applied.

- **Future collaboration with host institution**

The Austrian research in CWS and target tree management system is recent and this could be a good opportunity for both countries to develop a collaboration during this early stage of experimentation. Furthermore, our research group is willing to find other European partners in order to share the experiences and create networks about these management systems.

PARTNER CNR IVALSA

- ***Increase the value of timber from thinnings (Cherry)***

Current activities

- characterisation of timber coming thinnings made on arboricultural plantations;
- roundwood grading;
- evaluation of the most suitable industrial transformation process;
- sawing trials to produce boards from a portable sawing machine,
- boards grading
- check of a possible fruitful industrial transformation of the produced boards.

Main results

- The 30 % of the roundwood was unable for sawing.
- The obtained assortments were all in the NC (Non Classifiable) grading class, due to their small dimensions, high sapwood percentage and/or big knots.
- Better results can be obtained by utilising the grading system according to the North American specification NHLA (*National Hardwood Lumber Association*), written for the furniture industry
- The industrial utilisation of such material is limited also by the small dimensions of the stems and by the high sapwood percentage
- Even if the quality of the boards from cherry bolts was quite low, nevertheless semi-finished composed parquet were produced.
- The chosen industrial transformation technique, was selected in order to reduce the negative effects produced by the combination of the characteristic defectiveness of this material: small dimensions and frequent presence of anomalies, such as small knots and discolorations. The result is a product having good technical and aesthetical characteristics.

Figure 1. Cherry board with knots

Figure 2. Semi-finished element made by gluing small cherry boards.

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Figure 3. Floor made with the produced prototypes

- Zanuttini R., Cremonini C., Brunetti M, Berti S., 2006 "Caratterizzazione del tondame di noce e ciliegio" Sherwood n° 120, Marzo 2006, pp. 7-13
- Brunetti M., Cremonini C., Ruffinatto F."Caratteristiche fisico-meccaniche del legno proveniente da impianti di arboricoltura da legno"in press.
- Berti S., Boetto G.,Brunetti M., Cremonini C., Macchioni N., Zanuttini R. "Valorizzazione del legname di noce e ciliegio proveniente da diradamenti .In "Nuove indicazioni per la realizzazione e la conduzione di piantagioni da legno a ciclo medio-lungo". 18 Febbraio 2005, Padova
- Brunetti M., Cremonini C., Ruffinatto F., 2006 "Caratteristiche fisico-meccaniche del legno proveniente da impianti di arboricoltura da legno" In "L'arboricoltura nella filiera del legno pregiato" 5-6 Ottobre 2006, Cornaredo – Milano
- Berti S. "Valorizzazione del legname proveniente dai diradamenti" In "RI.SELV.ITALIA. Le esperienze nel Centro-Sud Italia". 19-20-21 Giugno 2007, Potenza

- ***Effects of pruning on wood quality (European walnut)***

Current activities:

- detection of the best cutting tool among those commonly utilised (saws and shears);
- detection of the best pruning season, through the comparison between those done during the vegetative rest and those done during vegetating season;
- improvement of knowledge on the cicatrisation mechanism of the pruning wounds through an anatomical analysis on the cicatrisation tissue and on the wooden part.
- looking for the best techniques to be adopted for postponed pruning or for recovery pruning: the aim is to investigate on the mechanism that lead to the appearance of the discoloration (see pictures 1 and 2) and to identify the best pruning technique to limit the appearance of new branches after pruning.

Figure 1 ***–A type discoloration, less extended and limited to the tissues of the pruned branch***

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Figure 2 ***B type discoloration, extended over the tissues of the pruned branch***

.Main results

- In order to limit the negative effects of pruning on wood quality, pruning must be done on small dimension branches (diameter lower than 3 cm), respecting the integrity of the branch ring (in order to limit the dimensions of the wound), operating during winter.
- Nevertheless the factor "pruning period" is subordinated to the dimension of the cut branch, so that to limit the negative effects is better to act on small dimension branches.
- It seems that there are no significant differences on the extension of the discoloration in the stump between the trees pruned during March and those pruned during July; the extension of the discoloration to the stump seems to be independent from cut branch dimension

- Brunetti M, Nocetti M., Zanuttini R., 2006 "Effetti delle potature sulla qualità del legno di noce" Sherwood n° 125, settembre 2006, pp. 5-9
- Brunetti M., Nocetti M. "Influenza delle pratiche colturali sulla qualità del legno" in press
- Brunetti M., Macchioni N." Caratteristiche del legno di noce sottoposto a potatura con diversi utensili e in differenti stagioni" In "Nuove indicazioni per la realizzazione e la conduzione di piantagioni da legno a ciclo medio-lungo". 18 Febbraio 2005, Padova
- Brunetti M., Nocetti M. 2006 "Influenza delle pratiche colturali sulla qualità del legno" In "L'arboricoltura nella filiera del legno pregiato" 5-6 Ottobre 2006, Cornaredo – Milano
- Brunetti M. "Effetti delle potature sulla qualità del legno" In RI.SELV.ITALIA. Le esperienze nel Centro-Sud Italia. 19-20-21 Giugno 2007, Potenza

PARTNER FLORENCE UNIVERSITY

SUSTAINABLE CULTIVATION TECHNIQUES TO IMPROVE GROWTH AND PHYSIOLOGY OF VALUABLE BROADLEAVED SPECIES

GROWTH AND PHYSIOLOGY OF FIELD GROWN ACER PSEUDOPLATANUS L. TREES AS INFLUENCED BY IRRIGATION AND FERTILIZATION

Alessio Fini[1], Francesco Ferrini[1], Piero Frangi[2], Gabriele Amoroso[2]

Irrigation and fertilization are commonly-used practices in the nurseries in order to obtain trees of adequate size and quality for sale within the least time. This study focused on the effects of slow release fertilizers and drip irrigation on physiology and growth of a widely-used field-grown shade tree species. Results confirmed that fertilization is a useful mean to improve growth, but may cause stress to the trees whether not coupled with adequate irrigation. On the other hand irrigation had only limited effect on growth but greatly increased leaf gas exchange and carbon assimilation.
The aim of this work is to investigate the effect of irrigation and fertilization, on growth and leaf gas exchange of sycamore maple trees grown in the field.

In spring 2005, 60 uniform (10-12 cm circumference) *Acer pseudoplatanus* trees were planted in the field. The experiment was located in the orchard of Fondazione Minoprio, located in Vertemate con Minoprio (Como, Italy; 45°44' N, 9°04' E). The experimental design 2x2 factorial, consisting in two irrigation and two fertilization treatments. 15 trees were irrigated (I), 15 were fertilized (F), 15 were both irrigated and fertilized (F+I). The remaining 15 trees © were selected as control and were neither watered or fertilized. Fertilization of the F and F+I trees was done at planting by incorporating in the planting hole XXg of a 8-9 months slow-release fertilizer Ficote® (15-8-12) (Scott International B.V., Geldermasel, The Netherlands). In spring 2006 F and F+I plants were re-fertilized with XXg/plant of Ficote®, used as topdressing placed near the stem. Irrigation was provided by drip irrigation only to I and F+I trees, which were irrigated ?twice per week? from the beginning of July till the end of August in 2005 and from the half of May till the end of July in 2006. Weed control was performed with a herbicide in the row, while turf was left between the rows and periodically cut.
Measured parameters were shoot elongation, stem diameter at breast height and plant height (measured in winter both in 2005 and 2006). Leaf gas exchange were investigated 11 times in 2005 and 4 times in 2006 with a portable infrared gas analyser (CIRAS-2, PP Systems, Hertfordshire, UK). Measured variables were instant net photosynthetic rate (A; □mol m-2 s-1) and transpiration rate (E; mmol m-2 s-1). Water Use Efficiency (WUE; □mol CO2 / mmol H20) was calculated as A to E ratio (Jifon and Syvertsen 2003). Measurements were taken between 8.00 and 12.00 h. Fifteen (3 leaves per 5 plants) fully expanded leaves per irrigation and fertilization treatment were checked for gas exchanges. In each measurement, different leaves were sampled from the outer part of the crown at different heights. Chlorophyll fluorescence was measured twice with a portable analyzer (Handy Pea, PP Systems, Hertfordshire, UK) during summer 2005. Chlorophyll fluorescence is a good indicator of drought stress in plants (Percival et al.2006).
Results and Discussion: in 2005 shoot elongation was increased in both irrigated and fertilized trees. The effect of fertilization was more evident (lover P-value) and went on also in 2006, while irrigation alone failed to increase shoot elongation in the second year of the experiment. Stem diameter was increased by irrigation both in 2005 and 2006, while fertilization increased secondary growth only in 2006 (Tab. 1). Plant height was unaffected by the considered factors. There is a clear interaction between irrigation and fertilization: plants that were both irrigated and fertilized developed a thicker stem and longer shoots than control plants or plants that were only irrigated or fertilized. This is also confirmed by a greater weight of the pruned material of the F+I treatment, if compared to the other thesis.
Irrigation significantly increased leaf gas exchange: well-watered plants were able of 9a greater carbon assimilation and transpired more water than un-watered trees both in 2005 and 2006. The adoption of a water-conservative strategy limiting transpiration by increased stomatal resistance is common in drought stressed plants. The lower stomatal conductance reduces net photosynthesis as well as transpiration and this may lead to lower carbohydrates to support future growth. Irrigation decreased water-use efficiency and the decline of WUE is typical of plants with a favourable water

status (Warren et al. 2001). Fertilization, whether not coupled with irrigation, significantly reduced carbon assimilation in the first year after transplant (2005). In 2005, fertilized, not irrigated plants showed lower carbon assimilation and transpiration if compared to irrigated plants and control plants. This made us hypothesize that, as reported by Lloyd et al.(2006), nutrient supply can decrease plant tolerance to drought. In 2006, carbon assimilation was no more affected by fertilization, which only decreased transpiration either in presence or absence irrigation.
Chlorophyll fluorescence was influenced by irrigation and fertilization treatments on 28th June, while no significant difference was found on 14th July. Percival et al. (2006) proposed that, in absence of stress, the ratio between variable fluorescence and maximum fluorescence (Fv/Fm) should remain above 0,75. On 28th June irrigated plants were not stressed, while some stress occurred in not-irrigated maples. Fertilization also decreased fluorescence on 28th June, and fertilized plants were more stressed than those which didn't receive nutrient supply. Significant interaction between factors showed that fertilization, whether not coupled with irrigation, leaded to a great stress in the period after planting. On the other hand, if coupled with irrigation, fertilization may help producing fast-growing, unstressed trees.
In conclusion, our results show that fertilization increased shoot growth of sycamore maple. Anyway, if fertilization is applied in absence of irrigation, it may lead to stomatal closure, reduced carbon assimilation and gas exchange and may increase tree predisposition to water stress. Thus, in fertilized, not irrigated stands, nutrient availability may increase shoot growth at expenses of root growth and reserves storage, at this may lead to transplant failing, especially in the first year (Walters and Reich 1989, Franco et al. 2006, Lloyd et al. 2006). Irrigation had a slight effect on shoot growth, but increased stem diameter, gas exchange and chlorophyll activity. As reported by other authors (Allen et al. 2005, Choi et al. 2005, Waterworth et al.2007), our results confirm that, in order to maximize stand productivity, irrigation shall be coupled with fertilization**.**

THE EFFECTS OF SOIL MANAGEMENT TECHNIQUES ON GROWTH AND PHYSIOLOGY OF SHADE TREE SPECIES: PRELIMINARY RESULTS

1F. Ferrini, 2A. Sæbø , 1A. Fini, 4Antonio Ferrante, 3G. Amoroso, 3P. Frangi

A key to success for new tree planting both in open-filed nursery and in the urban environment is the protection of young plants from non-crop plant species (including some hardwoods, shrubs, grasses, and forbs). These fast-growing plants often kill or greatly suppress desired trees by competing with them for light, water, and nutrients needed to grow. As a result, nurserymen, arborists and urban forester managers have used herbicides to suppress non-crop vegetation.

However the EU's Fifth Environmental Action Programme (5EAP) set out a series of targets for the year 2000 including 'the significant reduction in pesticide use per unit of land under production, and the conversion to methods of integrated pest control, at least in areas of importance for nature conservation'.

As a consequence, to protect young trees, environmentally sound, effective, cost-efficient, and socially acceptable techniques for managing non-crop vegetation are needed . Research on the development of non-chemical alternatives and better herbicide application technologies will be central to this effort.

In this project we focus on the effects of in-row cultivation techniques on growth and physiology of newly planted Tilia x europaea trees.

Preliminary results of this a long-term project have shown that the cultivation techniques applied can have some effects on tree physiology, but results can be variable according to the species.

Materials and methods

Plant material. In 2004 Before bud-break, uniform, 2.5-3 m tall, 10-12 cm in circumference (measured at 1.3 m height), balled and burlapped (B&B), Tilia x europaea trees were planted in an experimental plot located at the Fondazione Minoprio (Como). Planting holes were two times the

width and one-and-a-half times the depth of the root ball. All trees with almost identical size characteristics were obtained from the same nursery and planted at the same time.

Trees have been planted following the randomised block design with 3 blocks and four plants per block. The treatment are mulching with pine bark, mulching with green compost, weed control by herbicide (Figure 1).

In 2004 no data was collected with the exception of plant height at the end of the growing season. In 2005 the following parameters were measured:

a) plant height at the beginning and at the end of the season
b) bud-break date
c) shoot elongation
d) leaf gas exchange (Instantaneous net photosynthesis (Pn), evaporation transpiration rate (E), water use efficiency (WUE, calculated by dividing Pn by E)
e) chlorophyll fluorescence

In conclusion, soil management techniques have shown some effects on tree growth and leaf gas exchanges during the first year of measurement. Mulching with compost was effective in increasing leaf gas exchange and shoot elongation. Although we found some differences, results are limited to one year and only to some parameters and need to be confirmed in the next years.

AN ASSESSMENT OF DROUGHT TOLERANCE OF WIDELY GROWN SHADE TREE SPECIES AND THEIR RESPONSE TO IRRIGATION IN THE FIELD (PRELIMINARY RESULT).

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The selection of drought-tolerant species or cultivars suitable for urban forestry can be greatly beneficial to improve growth and survival rate of urban trees. The aim of this work was to investigate the drought tolerance of different *Tilia* species and of different cultivars of *Acer platanoides* grown in the field and to evaluate irrigation effect on their growth and physiology.
Previous experiments carried out in container with the same species have shown significant differences among the genera and between the species.
In Spring 2004 168 trees (8-10 cm circumference) of *Tilia platyphyllos*, *T. cordata*, *T. x europaea*, *T. tomentosa*, *Acer platanoides* 'Summershade', *A. platanoides* 'Deborah' and *A. platanoides* 'Emerald Queen' were planted in the field. 84 plants were irrigated and 84 were not. In experiment 2 leaf gas exchange, chlorophyll content and activity, shoot length, plant height and stem diameter were measured. Preliminary results indicate that *Acer* leaf gas exchange were influenced either by cultivar and irrigation. Irrigation increased net photosynthesis, transpiration but decreased water use efficiency in all cultivars. Despite of irrigation treatment, *A. platanoides* 'Deborah' had the highest leaf gas exchange, chlorophyll content and shoot elongation and exhibited the greatest drought tolerance. Irrigation increased gas exchange and shoot growth in *Tilia*, but didn't affect chlorophyll content and activity, plant height and trunk diameter, which were only influenced by the specie. *T. cordata* and *T. tomentosa* appeared to be the more drought tolerant species because they were able to carry on efficiently physiological processes without being stressed even in absence of irrigation.

WORKING GROUP 3

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TREE IN THE RURAL LANDSCAPE: HISTORICAL, CULTURAL AND TECHNICAL ASPECTS OF POLLARDING IN ITALY

Pollarding has a long tradition in Italy: since the most ancient times it has allowed multiple uses of trees located on farmland or in pastures. Indeed, one of the main uses of pollarded trees was to provide grazing for domestic animals and, at the same time, pollarded stands constantly supplied stovewood for domestic consumption or wood for carving which, however, required a longer

pruning cycle. For forage production, trees were cut back to allow them to produce new sprouts, which were then cut and the foliage and bark stripped by animals. The woody remains were used as firewood. The size of cuttings allowed even children with hatchets to gather wood for home fires. Woody materials were also woven into yard fences and outbuilding walls. Sometimes pollarded trees of those species that could not be used as food for livestock were used for the production of litter material. Coarse baskets and twine-making materials were also taken from the cuttings. Oak material was used to extract tannins for curing leather (Anonymous, 2006).
Therefore, a steady production of stovewood, forage and/or litter material through pollarding was maintained at an equilibrium level that lasted for centuries.
Especially in the northern plain regions of Italy, country hedges were cultivated and utilized as source of firewood. In many cases these were formed by elms (*Ulmus campestris* L.), salix (*Salix viminalis* L.), or mulberry (*Morus alba* L.) which had originated from suckers and were thus placed closely but, often, at irregular intervals.
In addition, linear plantings which developed along channels, roads, and along the borders of the so called "capezzagne" (the rough roads at the edge of cultivated fields), formed a net of tree hedges that, together with cultivated crops, contributed to the income of the peasants and provided other important services like the consolidation of the channel banks, the formation of shelter spots for wild fauna and rendered the rural landscape more attractive and variable. In addition, pollards were used (and sometimes they still are) as semipermanent boundary markers. In this case they were not regularly on a short rotation but only when the need arose.
After World War II, agricultural mechanization and the introduction of fossil fuels for heating, caused the gradual and, nowadays, almost complete elimination of these traditional plantings. However, at present, the restoration of these plantings to meet environmental needs is being considered and some rational models of multifunctional linear plantings are proposed (Agostinetto, 2004).
As a matter of fact, at the beginning of the XVIII century the use of pollarded trees in Italy was widely used for silk production. For this purpose, mulberry *(Morus alba* L.) was used; in commercial practice, trees were periodically (i.e. annually) pruned in order to permit harvesting at a convenient height, thereby inducing a shrubby form (Suzuki and Kohno, 1987).
In the second half of the XIX century this cultivation became so common on the flatlands and in the hills of Northern Italy that it still characterizes the agricultural landscape of some relic areas. At the same time, grapevine cultivation was mainly done using trees as a support.
The traditional viticultural system was the so called "*vite maritata"* a tradition introduced in this area by Etruscans and it was common in all the other areas where these ancient people lived. The system consisted in having the vine climbing a *live* support which usually was a hedge maple tree (*Acer campestre* L.), though sometimes also native elm (*Ulmus campestris* L.), poplar (*Populus spp*.), fraxinus (*Fraxinus ornus* L.) or mulberry (*Morus alba* L.) were used. For this reason, pollarded trees have an important part in our history and some great European landscape painters of earlier centuries clearly represented the pollarded trees of pastures, canal edges, and farmsteads. Probably the most famous painting is the "Field with Pollard Trees and Mountains" painted by Vincent Van Gogh in 1889, who also realized other paintings representing pollarded trees in the landscape.
Also some famous writers have mentioned pollard trees in their books, like Leo Tolstoj in "War and Peace", underlining their diffusion in the cultivated land and their importance as a typical feature of the countryside.