

Ali Reza Kharazipour, Christian Schöpfer,  
Cora Müller and Markus Euring (Eds.)

## Review of Forests, Wood Products and Wood Biotechnology of Iran and Germany – Part III



Universitätsdrucke Göttingen



Ali Reza Kharazipour, Christian Schöpfer, Cora Müller, Markus Euring (Eds.)  
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of Iran and Germany - Part III

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## Foreword

In the year 2008 our research activities in the project “Deutsch-Arabisch/Iranischer Hochschuldialog” were successfully continued. Now, like in the two years before, the present book gives again an overview of the common research themes in which the Iranian and German young scientists and scientists of both countries works are summarised. Thereby the high variety of the both countries research studies is made clear, which is transferred into continuative cooperations between science and industry in Iran and Germany.

The project was financed by the Department of Foreign Affairs in the context of the „Europäisch/Islamischen Kulturdialog“ (EIK). My special thanks go to the German Academic Exchange Service (DAAD). Without its engagement and organisation this project would not have been realisable.

We are much obliged to all involved people in Iran and Germany who made and make this joined project and the continuative cooperation possible.

In particular my thanks go to Dr. Assareh, Dr. Farahpour as well as the partnership authorised person in Iran Mr. M. Sc. Hossein Hosseinkhani from the Research Institute for Forests and Rangelands, Teheran for their indefatigable engagement to continue this project. Thereby also the third year of our intention has contributed to the achievement of the commonly verbalised aims.

Beyond I want to thank all colleagues from the University of Mazandaran, Sari for their cooperation.

For the support and the organisation of the conducted events and conferences I would like to thank Dr. M. Euring, Dr. C. Schöpfer as well as Dr. C. Müller from the Büsgen-Institute, Department of Molecular Wood Biotechnology and Technical Mycology, University of Göttingen.

For the participation of different events in Germany I thank all institutes from the Faculty of Forest Sciences and Forest Ecology and our industry partners. Special thanks go to, Prof. U. Kües, dean of the Faculty of Forest Sciences and Forest Ecology and leader of the department of Molecular Wood Biotechnology and Technical Mycology and to Prof. A. Polle, leader of the department Forest Botany for their support and the good cooperation.

The project leader thanks all who have contributed to this project with their speeches and/or attendance. I wish that many readers are interested in this book and that the good German-Iranian cooperation in the past will be continued in the future.

Göttingen, January 2009

Project director Prof. Dr. Alireza Kharazipour

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# **Development of three- layered popcorn based particleboards by a combination of maize and wood**

*Nina Ritter, Alireza Kharazipour*

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## 1. Initial situation and problem

In Germany timber belongs to one of the most important renewable primary products since about one third of its area is covered with forest. The wood production is next to its environment- and protection function one of the most important attributes of the forest. Germany shows with 3,3 Mrd. m<sup>3</sup> of all European countries the highest stock of wood, in which only ca. 60 % of the wood stock used in terms of a sustainable utilization (VHI, 2007).

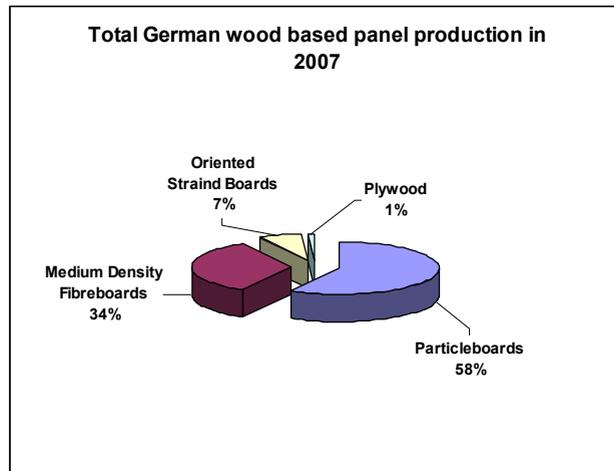
The derived timber product industry adds for stability of forest as in terms of a sustainable wood utilisation as soon as an intensive wood care inferior thinning material will be taken from and the increment support will be advanced. As a result of the increasing demand for derived timber products raw wood masses can be substituted and imports of overseas can be reduced by using there assortments (Schöpfer, 2006). The clearing of the tropical rain forest considerably decreased in Europe through the particleboard and medium density fibreboard- industry.

Derived timber products are very environment friendly, because no rubbish is accumulated by the production of these products and after the usage the products become are recycled.

## 2. Outline of wood composites

Over last years derived timber products have become generals accepted. The concept of derived timber products involves different products, which are produced from mechanically or thermo-mechanically opened wood (veneer, particles, fibres), which was added again under application of binder as soon as compression and heat. The importance wood based panels are, depending on production technique und dimension of utilised woodparticles, particleboards, medium density fibreboards (MDF), oriented strand boards (OSB) as soon as plywood and ply shell moulds (Marutzky et. al, 2005).

The wood based panel industry in Germany is with 29,6 % the second-important wood disposer behind the saw industry with 57,7 %. The year 2005 showed a production capacity of 16,3 mio. m<sup>3</sup> for particle boards, fibreboards and oriented strand boards as soon as core- and veneer boards (VHI, 2006).



**Figure 1: Total German wood based panel production in 2007 (VHI, 2008)**

Figure 1 shows the distribution of output figures of the primary wood based panel production in Germany in 2007. Particleboards account for 58 % of the production, followed by medium density fibreboards with 34 % (VHI, 2006). Compared with the year 2002, the group of particle boards (67,7 % in 2002) shows a decrease while the field of medium density fibreboards (23,5 % in 2002) and oriented strand boards (4,3 % in 2002) have increased (Marutzky, 2003).

### 3. Problem

Due to the heavy risen energy and material utilisation of low quality wood assortment, there are no adequate assortments in sufficient quantity for the wood based panel industry available. Therefore, the wood based panel industry is forced to apply expensive wood assortments. However, this implicates an obvious increase in the price of the manufactured material. The heavy, high quality wood assortments are mainly used in the field of shelf wood, construction wood and timber wood. A solution for compensating these high quality assortments is the applications of maize in the form of popcorn granulate.

### 4. Aims of the project

This doctoral thesis project is supposed to contribute to the utilisation of local, renewable raw materials. Hence, one main topic of the project is thereby the development of innovative and low emission particle boards. These boards are composed of maize and wood with a decreasing raw density. This implies a

climate- friendly and resource-efficient utilisation of these raw materials with the aim of the production of eco-friendly products. The CO<sub>2</sub>- neutrality of the developed products as well as the protection of fossil resources shall accomplish a positive contribution to climate protection.

The project combines local renewable raw materials as wood and maize from agriculture and forestry. The intended utilisation policy is supposed to lead to marketable products from renewable resources and allow new applications by substituting environmentally harmful of products or products of finite resources.

The combination of renewable raw materials out from agriculture and forestry, like wood and maize, allows a new generation of light particleboards consisting partially out of botanical component. Hereby low emission products are created, which bind the contained formaldehyde by the addition of maize and the final products` emission can be reduced as well. The health aspect results from the lower formaldehyde emission, because the popcorn granulates absorbs the formaldehyde better. By this the realisation of the formaldehyde- four-star-standard can be achieved, without a lost in quality and the application possibility of these composites.

A further basic key activity is the exploration of new, lower composites with reduced densities, with the DIN /EN standards requiring the mechanical-technological qualities. Appropriate as well as adequate attributes of the raw materials should implement a raw density range from 200 kg/m<sup>3</sup> to 500 kg/m<sup>3</sup>. By comparison to industrial common densities of 650 kg/m<sup>3</sup> to 700 kg/m<sup>3</sup>, over half of the material usage of wood and adhesive agent could be saved. Hence, on the one hand could be reduced the cost of production of the composites on the other hand would allowed this the development of innovative, eco- friendly materials. Therefore in particleboards adopted the renewable raw material maize in form of popcorn granulates.

## **5. Consideration of ecological relevance and problems**

At the production of particleboards with renewable raw materials it is supposed to avoid harmful emissions or to reduce it significant. In this project it is supposed to fix the formaldehyde through the insert of maize in the particleboard production about the popcorn. Hence, the health risk for the human is supposed to reduce considerably in product process, in the product utilisation as well as the further material recycling.

The decrease in weight of the produced composites constitutes a further important aspect. In the wood based panel production is it to be due by the addition of popcorn a significant benefit in the reduction of the density of the boards, because the popcorn have a little apparent weight (ca. 35 kg/m<sup>3</sup>) adverse the wood (ca. 160 kg/m<sup>3</sup>). Out of it shows revised transport conditions because mayor board amounts can carry on streets and rails and so it can achieve a fuel

economisation. Further through the usage of maize the needed wood can be reduced till 50 % and the needed amount of binder till 30 %.

## 6. Material and methods

For production of three- layered maize based particleboards in laboratory scale with low densities we were using wood particles from our industry partner and popcorn granulate, which we were producing in our pilot plant station.

### 6.1 Maize

Maize (*Zea mays* L. *subsp. mays*) is a crop from the family of *Poaceae* and is next to wheat and rice one of the most important globally cultivated plant species. Already the evolution of the today's maize plant has been heavily affected by its previous domestication in the Indian high crops (Messner, 2005). Today maize is cultivated globally and is a staple food in many countries. In Europe and North America maize is used primarily as an animal fodder and also in the food industry, as maize starch and maize meal for example.

### 6.2 Production of popcorn granulates

For the production of popcorn granulates we used popcorn, paper bags and a microwave. We put the popcorn into bags and heated these for 2 minutes by 2000 W in the microwave. Afterwards the popcorn will be milling into 5 mm particles in a Retsch- mill. According to requirements of middle- layer or in the surface the particles would be separate in different fractions.



**Figure 2: Different fractions of popcorn garnulate after sieving**

### 6.3 Production of three-layered maize based particleboards with reduced densities

The main objective in this research study is to produce particleboards with lower densities with a substitute for wood, in this case maize, we want to realise densities of  $400 \text{ kg/m}^3$  and lower. Further in this project we want to achieve a reduction of the formaldehyde emissions of the wood based panels.



**Figure 3: Strewing of particleboards pressing**



**Figure 4: Particleboard before**

Table 1 presents the production parameters for the three-layered maize based particleboards in laboratory scale with densities of 450 kg/m<sup>3</sup> and 550 kg/m<sup>3</sup>.

**Table 1: production parameters**

<b><i>Surface layer ratio %</i></b>	2 x 20
Particle material	Wood particles
Urea formaldehyde resin, dry on dry particle (%)	10
Ammonium sulphate (33 %), dry on dry particle (%)	0,5
Hydrophobic substance, dry on dry particle (%)	1
<b><i>Middle layer ratio %</i></b>	
Particle material	Wood and popcorn
Urea formaldehyde resin, dry on dry particle (%)	8,5
Ammonium sulphate (33 %), dry on dry particle (%)	1
Hydrophobic substance, dry on dry particle (%)	1
<b><i>Dimension</i></b>	
Length (mm) x breath (mm)	800 x 400
Density (kg/m <sup>3</sup> )	450 kg/m <sup>3</sup> und 550 kg/m <sup>3</sup>
Thickness (mm)	20
<b><i>Press factors</i></b>	
Temperature (°C)	195
Pressure (bar)	220
Press time (sec/mm)	12

## 7. Results

The following chapter show the results of some mechanical- technological properties of the three-layered particleboards in laboratory scale.

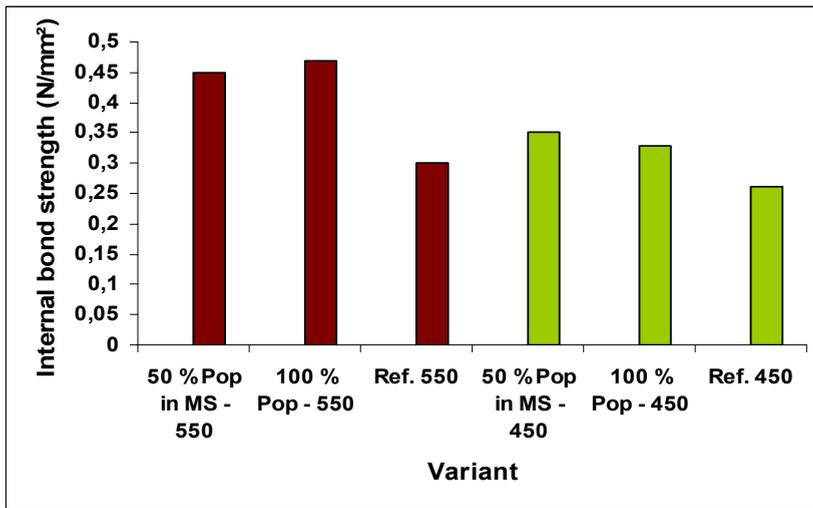
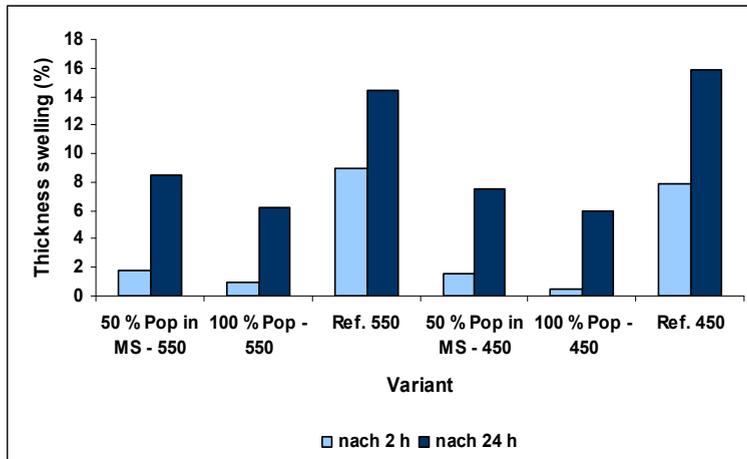


Figure 5: Internal bond strength (N/mm<sup>2</sup>) of the different variants

Figure 2 represent the results of the internal bond strength in dependance of the raw density. The achieved results show that is possible to produce particleboards with light raw densities. In comparisons with reference boards (0,3 N/mm<sup>2</sup>) out of industrial material the popcorn based boards fulfill with 0,47 N/mm<sup>2</sup> (100 % popcorn) and 0,45 N/mm<sup>2</sup> (50 % popcorn in the middle layer) the actual DIN and European Norms. This achievement appears for both raw densities.

The thickness swelling after 2 and 24 hours is showing in figure 3. The swelling should not cross 15 % after 24 hours water storage.



**Figure 6: Thickness swelling after 2 h and 24 h of the different variants**

The popcorn based particleboards with raw density of  $550 \text{ kg/m}^3$  have values from 9 % (50 % popcorn in the middle layer) and 7,5 % (100 % popcorn) after 24 hours and the boards with raw densities of  $450 \text{ kg/m}^3$  shows thickness swelling of 7,5 % (50% popcorn in the middle layer) and 6 % (100 % popcorn). The reference boards offer 15,2 % ( $550 \text{ kg/m}^3$ ) and 16 % ( $450 \text{ kg/m}^3$ ) after 24 hours water storage.

## 8. Conclusions

At these project is it to be matter of ecofriendly, innovative and future oriented research based on renewable raw materials from agriculture and forestry. It accrue lower composites with reduced densities, which have good mechanical-technological properties and so they abide the given standards. So the project bring ecological and economic advantages for the wood based panel producers and the consumer.

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# **Pilot plant particle boards production with modified binding agents**

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## Introduction

The use of natural based binding systems is known and used since centuries. But the developments of synthetic binding agents such as urea- phenol- or melamine-formaldehyde binding agents have decreased the application of the natural based binders. Although in the past the research activities on the field of the development of natural binders and there application in the industries has been reinforced for there use as basic binder as well as in combination with commonly used petrochemical based binders. [DUNKY, M.; NIEMZ, P. 2002]

The aims to use natural based binders instead of commonly used binders are different. Due to the fact that natural based binders contain no or very low amounts of formaldehyde, one aim is to reduce the formaldehyde emission of the produced wood based panels and to fulfil the tightening emission-laws for the panels. Especially the Company IKEA leads the way by demanding a perforator restriction value of 4 mg/100g<sub>atro</sub> from there particle board supplier such as EGGER, Pfleiderer, Gluntz and Kronotex, witch is nearly a bisection of the EN 13985 restrictions for the so called “E1-boards”. Also the Californian Air Resources Board (CARB) implemented a new restriction called Airborne Toxic Control Measure (ATCM) to reduce formaldehyde emissions from Composite Wood Products in two steps beginning at 1st of January 2009 from 0.18 ppm to 0.09 ppm based on the ASTM E-1333-96 test protocol. [HOLZZENTRALBLATT, 2008; MALIN, N.] Beside that the oil shortage leads to raising oil prices and there derivatives. To find alternatives for the commonly used petrochemical formaldehyde binders is another aim. Marketing reasons also led to the rediscovery of the natural based binders, because the customers demand for ecological and biological materials has been highly developed.

Several sources for natural based binders are known such as tannin, lignin, starch, pulp and cellulose as well as protein. The protein based natural binders can be divided into animal protein based and plant protein based. Different applications of plant proteins such as soy, maize and especially wheat proteins as binding agents or filler has been investigated and partly established in the industry. [SCHOEPPE, 2006; MÜLLER, 2005; KLÖSER, 2007]

Silanes coupling agents are basically used to improve the adhesion between organic and inorganic materials. [HAN et al., 1998, 2001] There are at least two functional groups in there molecule: One is the organofunctional group witch can be an amino, epoxy, or mercapto group. The silane can react with organic material over that group. Another function group is the silicafunctional group or alkoxy group such as methoxyl or ethoxyl. With that group the Silane can react with inorganic substrates or with other silica compounds. [JONES et al., 2000]

To use wheat protein as substitute for UF resin for the production of particle boards and to reduce the disadvantages of this natural binder, such as the high

affinity for water, the reduced reactivity or the reduced bending strength, by adding silane in low amounts is the object of that study.

## **Material and Methods**

### Wood particles

The used wood particles were obtained from the particle board plant Gütersloh belonging to the company Pfleiderer AG. The material consists of different wood assortments such as the industrial junk timber like thinnings and, the forest industrial timber like trunk wood and also the recycling timber like pallets or in-house rejections. It has to be noticed that the proportion of the different timber assortments in the produced particle material is varying.

### Wheat protein

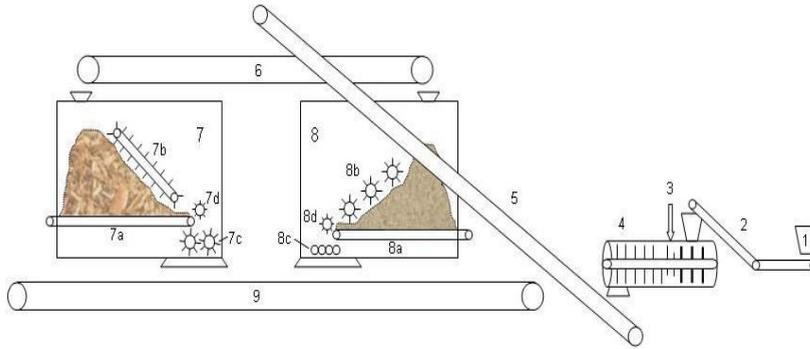
The used wheat protein was provided by the company Cerestar, Krefeld. With a solid content of 50 % and a pH value of about 3.4 the protein is characterised.

### Silane

The Silane used to modify different bonding agents was provided by the company Evonic Degussa GmbH, Rheinfelden. For the following study we used the silane DYNASILAN HS 1151 which is a modified amino/alkylsilane with bis-aminofunctions. It has a solid content of 21 % and a pH-value of 10.5 %

## Pilot plant particle board production

In the Figure 1 the schematic drawing of the particle board pilot plant of the University of Göttingen is shown and will be explained.



**Figure 1: scheme of the pilot plant for particle board production**

The particles are transported from the hopper (1) to the conveyer belt (2) going to the blender (3). The blender consists of a tubular body and a rotating spindle with different kinds of paddles on it, which divide the blender into three sections. In the first section of the blender, a constant amount of particles is transported to the blending zone which is the second sector. There the blender is injected. The paddles transport the binder-coated particles into the last sector. There the chips grinding on each other to create a homogeneous binder coat on the surface of the particles. From there binder coated particles are transported with another conveyer belt (5) to a distribution belt (6). The particle fractions for surface and middle layer have to be glued separately. After the gluing the surface layer particles were transported in the surface layer storing bunker (7) and the middle layer particles in the middle layer storing bunker (8). The bunkers contain also a conveyer belt (7a/8a) to transport the particles to the scatter units (7c/8c). To guarantee a constant output of particles to the scatter head, riddles (7b/8b) are installed over these conveyer belts to level the filling capacity of the belts. To control the amount of particles per area and time, the speed of the scatter belt (9), located under the storing bunkers, is adjustable, so that a constant output of particles per time from the scatter head is strewn on the strewing belt with a specific speed depending on the designated raw density for the board. After the particle fleece is strewn in the three layer system the fleece will be pressed in a platen hot press. Then the produced particle boards are cooled down and stored in piles. At the end the mechanical-technological properties such as tensile, internal and bending strength, as well as swelling and formaldehyde-emission will be analysed.

## Modification of binder systems

One aim of the work is to substitute petrochemical based binders with binders from renewable sources such as wheat proteins. For that purpose different rates of urea formaldehyde were substituted by the protein.

To adjust the occurring problems by the use of natural binders such as reduced binding properties and decreased water affinity, silanes were used as an additive in small amounts. All the constituents were mixed together mechanically to a binding system.

The following table shows the produced variants with different degrees of protein substitution and different additions of silane.

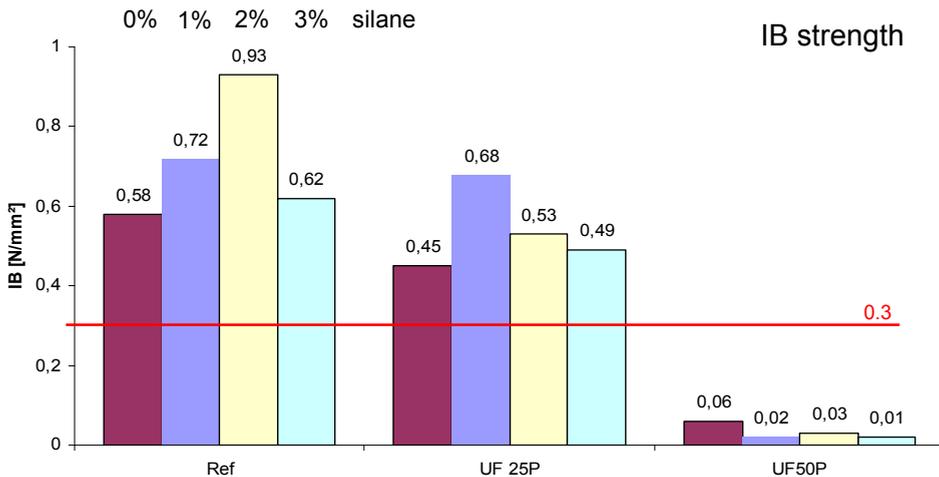
**Table 1: composition of the binder systems**

type of modification	specification
100 % UF	unmodified UF-binder
100 % UF + 1 % silane 1151	UF-binder + 1 % 1151 silane additiv
100 % UF + 2 % silane 1151	UF-binder + 2 % 1151 silane additiv
100 % UF + 3 % silane 1151	UF-binder + 3 % 1151 silane additiv
75 % UF – 25 %P	25 % protein – substituted UF binder
75 % UF – 25 %P + 1 % silane 1151	25 % protein – substituted UF binder + 1 % 1151 additiv
75 % UF – 25 %P + 2 % silane 1151	25 % protein – substituted UF binder + 2 % 1151 additiv
75 % UF – 25 %P + 3 % silane 1151	25 % protein – substituted UF binder + 3 % 1151 additiv
50 % UF – 50 %P	50 % protein – substituted UF binder
50 % UF – 50 %P + 1 % silane 1151	50 % protein – substituted UF binder + 1 % 1151 additiv

## Results and Discussion

Mechanical technological properties

The results for the testing of the internal bonding strength are shown in figure 2.

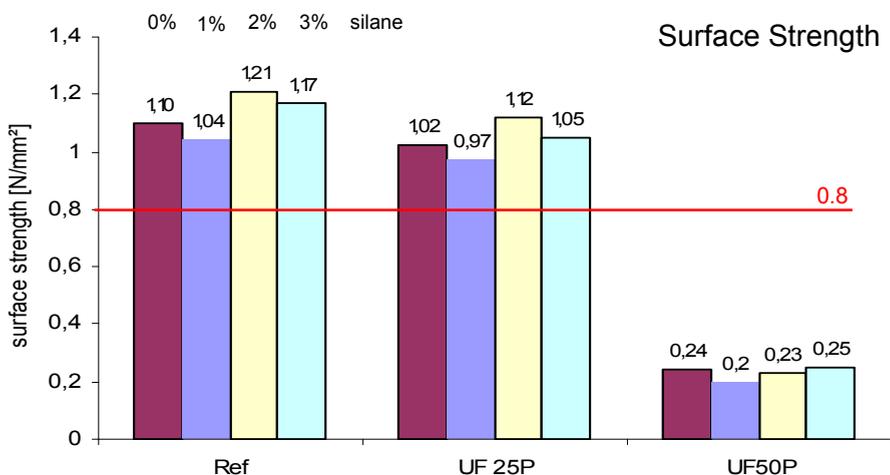


**Figure 2: Mechanical technological properties of particle boards produced with modified binding agents – 20 mm particle boards; 200 °C; 3 min; 700 kg/m<sup>3</sup>; glue ratio: 11 % / 8.5 % - Internal Bonding Strength**

Three main variants are displayed, which are characterized by the ratio of protein substitution of 0 %, 25 % and 50 %. Additionally, these three variants are modified with Silane as an additive in low amounts of 1 %, 2 % and 3 %. The results have shown that it is possible to substitute urea formaldehyde with wheat protein up to a level of 25 % to fulfill the requirements of the EU standard EN 312 concerning the internal bonding properties. The boards produced with a binder substituted with 50 % protein do not fulfill the restrictions of the EN 312.

The additional use of Silane leads to increased bonding properties. Up to an addition level of 2 % silane for the unsubstituted variant the internal bonding strength was increased from 0.58 N/mm<sup>2</sup> to 0.93 N/mm<sup>2</sup>. By increasing the amount of silane to a level of 3 % the strength properties are reduced to 0.62 N/mm<sup>2</sup>. For the 25 % protein substituted variant it was shown that only the addition of 1 % silane results in an increased strength of 0.68 N/mm<sup>2</sup>. The enhancement of the silane addition to 2 % and 3 % does not lead to better results with bonding strength of 0.53 N/mm<sup>2</sup> and 0.49 N/mm<sup>2</sup>.

The results for the testing of the surface strength properties are shown in figure 3.

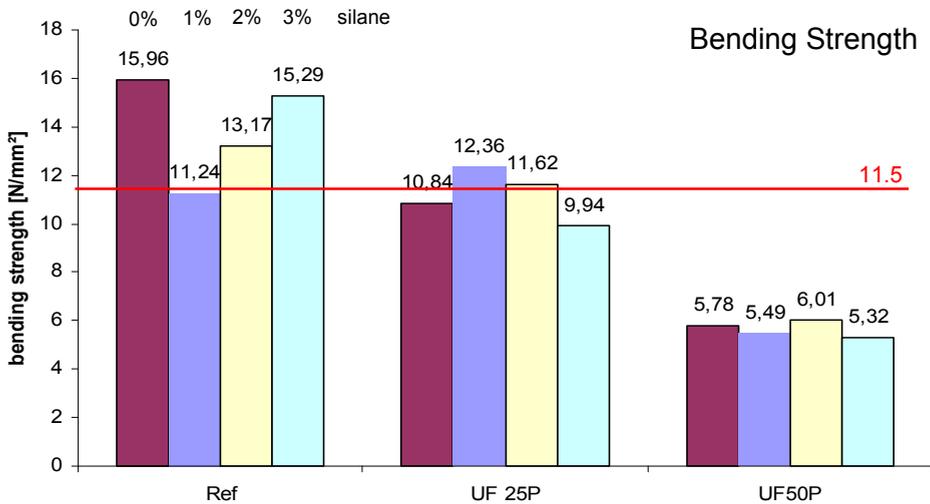


**Figure 3: Mechanical technological properties of particle boards produced with modified binding agents – 20 mm particle boards; 200 °C; 3 min; 700 kg/m<sup>3</sup>; glue ratio: 11 % / 8.5 % - Surface Strength**

Clearly the influence of the rate of urea formaldehyde substitution with wheat protein is displayed. While the substitution rate of 25 % decreases the surface strength slightly the substitution rate of 50 % significantly decreases the surface strength down to a level under the requirements of the EN 312.

The Influence of the additiv use of silane with the quantities of 1%, 2 % and 3 % is very slight. Up to the addition rate of 2 % the strength properties can be increased to 1.21 N/mm<sup>2</sup> for urea formaldehyde binder and 1.12 N/mm<sup>2</sup> for the 25 % protein substituted variant. But by adding 3 % silane the strength properties are decreased again to 1.17 N/mm<sup>2</sup> for the urea formaldehyde binder system respectively 1.05 N/mm<sup>2</sup> for the 25 % protein substituted binder system.

Figure 4 shows the test results for the bending strength properties.

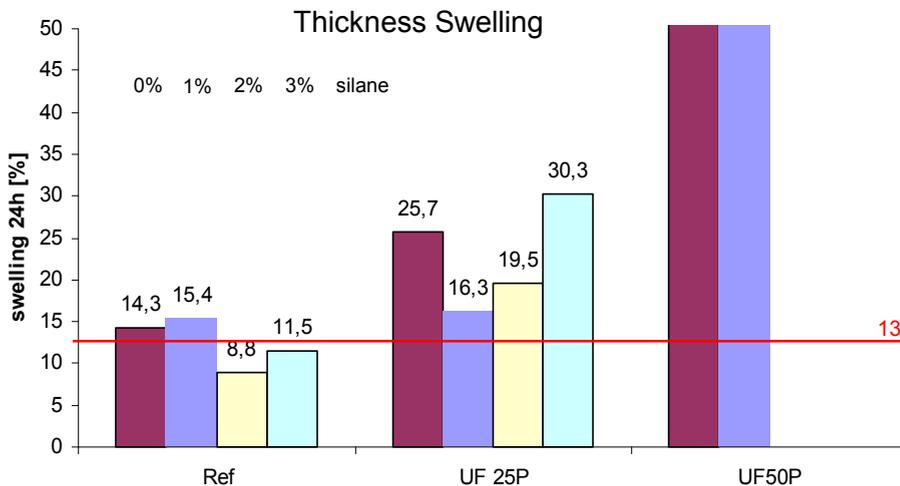


**Figure 4: Mechanical technological properties of particle boards produced with modified binding agents – 20 mm particle boards; 200 °C; 3 min; 700 kg/m<sup>3</sup>; glue ratio: 11 % / 8.5 % - *Bending Strength***

The results for the bending strength show a similar tendency like the other strength property testing. With the increase of the substitution rate of wheat protein the bending strength is decreasing. At the rate of 25 % with 10.84 N/mm<sup>2</sup> the values are in the range of the restrictions of the EN 312. Whereas with the increased substitution of 50 % the bending strength properties are decreased to a level of 5.78 N/mm<sup>2</sup> which is under the restrictions of the EN 312.

The additional use of silane affects the results just slightly. By adding 1 % to the urea formaldehyde the bending strength of the boards is decreased to 11.24 N/mm<sup>2</sup> and by adding 3 % of silane the level of the reference board without silane is almost reached with 15.29 N/mm<sup>2</sup>. For the 25 % protein substituted variant only 1 % silane additive leads to a better bending strength of 12.36 N/mm<sup>2</sup>.

The water affinity of the produced boards is tested with the thickness swelling test after 24 hours of water storage. The results are shown in figure 5.



**Figure 5: Mechanical technological properties of particle boards produced with modified binding agents – 20 mm particle boards; 200 °C; 3 min; 700 kg/m<sup>3</sup>; glue ratio: 11 % / 8.5 % - Thickness Swelling after 24h of water storage**

The results for the thickness swelling test show the high stake of the rate of urea formaldehyde substitution with protein on the observed thickness swelling. The higher the amount of wheat protein the higher is the water affinity of the boards. The unsubstituted urea formaldehyde binder shows a thickness swelling of 14.3 % whereas the boards produced with the 25 % protein substituted binder systems posses a thickness swelling of 25.7 %. With the 50 % protein substituted binder produced boards where totally destroyed during the water storage test.

By adding silane in low amounts it is possible to reduce the water affinity of the particle boards. For the unsubstituted urea formaldehyde boards it was possible to reduce the swelling to 8.8 % by adding 2 % of silane. For samples produced with the 25 % protein substituted urea formaldehyde binder the swelling was reduced from 25.7 % to a level of 16.3 % witch is close to the EN 312 restriction. But it was successful to increase the amount of silane to a level higher than 2 %

The 50 % protein substituted samples where totally destroyed during the water swelling test, witch can be explained with the very low mechanical properties of the variant.

## Conclusion

The executed research shows that the influence of the substitution of urea formaldehyde binder with wheat protein has a high effect on the mechanical properties of the produced particle boards. It also shows that it is possible to realize a grade of urea formaldehyde substitution with wheat protein of 25 % and still achieving the requirements of the EN 312 concerning the mechanical properties. An increased rate of substitution of 50 % leads to strongly decreased mechanical properties clearly missing the EN 312 standards.

The additive use of the silane partly leads to enhanced strength properties. The research shows that, only up to a certain grade of addition the strength properties are increased.

Concerning the internal bonding strength the rate of addition of 2 % to the urea formaldehyde shows the highest internal bonding strength properties. While the addition of only 1 % to the 25 % protein substituted variant shows the best internal bonding strength properties

For the surface strength the rate of silane addition of 2 % for both basic binding systems seems to be the optimal amount to increase the surface strength properties.

The bending strength of the boards produced with 1 % silane as an additive to the urea formaldehyde is significantly decreased compared to the variant with pure urea formaldehyde resin. With the stepwise increase of the silane addition it was only possible to reach the strength level of the reference board. With the additive amount of 1 % silane the bending strength of the 25 % protein substituted variant with a value of 12.36 N/mm<sup>2</sup> is increased, so that the EN 312 restriction is fulfilled. Above this addition level of 1 % silane the bending strength properties are decreased again.

For the thickness swelling the analysed specimens show very diverse results. The protein as a substitute for urea formaldehyde is very influential for the thickness swelling of the produced boards. With the 25 % protein substituted boards possess an increase of about 80 % from 14.3 % to 25.7 %. By adding only 1 % of silane the thickness swelling is reduced by 36 % from 25.7 % to 16.3 % within the range of the EN 312 requirements.

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# Development and production of innovative, formaldehyde-free middle density fibreboards (MDF)

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## Introduction

Derived timber products are products where the structure of the native wood is dissolved and a new structure with a variable dimension is being built. Solid wood is restrained to a limited application range. This is due to dimension, consistency, durability and dimensional stability. So a major advantage of derived timber products compared to solid wood is achieving a controlled homogenisation. This leads to a standardisation of important technical properties for relevant usage. The higher the grade of refining of wooden raw material, the higher the achievable homogenisation will be. Derived timber products based on fibres therefore have a much higher level of homogenisation.

The particular importance can be seen in the stable growth of production capacity. In 2007 MDF production reached 13.3 mio. m<sup>3</sup> in Europe of which 4.3 mio. m<sup>3</sup> were produced in Germany alone. The advantages of MDF compared to standard particle boards come into effect in the area of furniture manufacturing and interior fittings. Due to these advantages of MDF an increase in production numbers will be likely in the next years (KLOESER, 2006).

The wood based panel industry is one of the largest purchaser of binding agents. In the past glue was made of protein compounds based on plants and animals. Today binding agents on the basis of petrochemical substances are almost exclusively used by the the wood-based panel industry. These include in particular urea-formaldehyde resins (UF-resins), melamine reinforced urea-formaldehyde resins (MUF-resins) as well as phenol-formaldehyde resins (PF-resins) making up to more than 85 % of present production (KRUG et al., 2007). The advantages of these binding agents based on formaldehyde are the high mechanical and technical properties boards can reach, a high reactivity and a low price (BOEHME, 2000). The disadvantage is a finite supply of crude oil and natural gas but above all the danger to humans caused by formaldehyde.

Formaldehyde is a colourless substance which is gaseous at room temperature. It has a typical pungent odor and it can be perceived by humans in very low concentrations. Through holes and other uncoated furniture parts formaldehyde can escape into the air. It excites several allergenic reactions like skin-, eye- and airway irritations, headache as well as sickness (BfR, 2004). For this reason regulations concerning a formaldehyde emission already exist a very long time in Germany. For example in 1977 the German health authority made a recommendation regarding the restriction of formaldehyde content in compartment air to 0.1 ppm (0.124 mg/m<sup>3</sup> air). The directive for the formaldehyde content of MDF determined by the perforator method was 10 mg/100g of absolute dry board (DUNKY, 2002). In 1994 the German Institute for

Construction Engineering (DIBt) reduced this guideline again. This limiting value of 7 mg/100 g is still relevant today (DIBt, 1994).

Much more serious concerning formaldehyde is the fact that this substance was classified as carcinogen by the International Agency for Research on Cancer (IARC) in 2004. After this the Federal Institute for Risk Assessment in Germany took this new knowledge as a reason to revalue the cancer-causing risks of formaldehyde (BfR, 2004). By now the World Health Organisation (WHO) has classified formaldehyde in categorie 1 (demonstrably carcinogen) of the carcinogenic substances. It is therefore expected that the existing limiting- and guiding values for formaldehyde contents in derived timber products und secondary products will be regulated in the near future (EPH, 2008). In this year the federation of european wood-based panel manufacturers (European Panel Federation, EPF) already agreed on an new standard (EPF-S) with stringent guidelines concerning formaldehyde contents in panel boards. This new standard provides for a formaldehyde content of 4.0 mg/100 g board in MDF. Regarding marketing products with low formaldehyde contents the swedish furniture manufacturer IKEA leads the way. Since a short time IKEA demands the compliance of the new standards from its suppliers. So the five largest wood-based panel manufacturers in Europe produce in accordance with the new guidelines, which have not yet officially come into effect. This development will make other manufacturers produce in the same way. Therefore the new standards concerning formaldehyde can be expected to come into effect in the foreseeable future (HZB, 2008).

On the one hand the finite supply of petrochemical products but mainly the harmful character of formaldehyde have led to increased research on natural or near-natural binding agents from renewable resources in the last few years. So it is possible to use wheat protein as a natural and renewable raw material. The comparison between wood-based boards produced by several types of herbal proteins showed a significant advantage of wheat protein concerning technical and mechanical properties. The determined formaldehyde content was similar to native wood (SIRCH & KEHR, 1997). A detrimental effect of binding agents based on wheat protein is a very high thickness swelling after water storage. In addition, the technological and mechanical properties of wheat protein bonded boards are not as high as the properties of commonly bonded MDF. So the use of organofunctional silanes should lead to an improvement regarding dimensional stability and strength of wheat protein bonded boards. Silanes are silicon organic compounds based on silicon. The Silica sand is the mineral raw material of silicon. Because of its high degree of purity the silica sand is obtained from the Scandinavian countries. The properties of organofunctional silanes are due to their special molecular structure and reactions. The silicon as central unit of silane is combined with two different functional groups. The organofunctional group on the one side is strongly bound to the silicon via a stable unreactive carbon chain. The adhesion to a polymer occurs via this group. The siliconfunctional group on

the other side is attached to the silicon directly. It can react after its hydrolysis with active centres of inorganic substrates or condensation with other silicon compounds (JONES et al., 2000).

The aim of this project is the production of medium density fibreboards bonded by near natural formaldehyde-free binding agents which have similar mechanical and technological properties to commonly bonded MDF. On the one hand silane should be used as an additive, on the other hand it should substitute other binding agents.

## Material and Methods

### Fibre-material

For the production of the MDF-boards presented in this book chapter industrial fibre-material from the STEICO company was applied. This material consists of pine wood (*pinus sylvestris*). It was factory-made by the Asplund-process.

### Urea-formaldehyde resin (UF-resin)

The reference-boards were produced with the urea-formaldehyde resin K 465 from the BASF company. It has a solids content of 66 %. This resin is located in a pH-range of ca. 7.5.

### Wheatprotein-binding agent (WP-binding agent)

As a near-natural binding agent the wheatprotein 216 A 3 from CERESTAR company was used. The Solids content is approximately 50 %. The binding agent has as pH-value of 3.4.

### Silan

For the modification of near-natural binding agents by an addition of silane respectively by an exchange of binding agent components the 3-aminopropylsilane hydrozylate Dynasytan HS 1151 (EVONIK) was applied. It has a low solids content of approximately 27 %. The type of silane which was used was located in a ph-value of ca. 3.1.

### Water repellent

Depending on the ph-value of the applied binding agent two different water repellents in the form of liquid paraffin were used. The water repellent Hydrowax 46 was added to binding agents which were located in a acidic pH-range. Another water repellent, Hydrowax 730, was applied to binding agents with a neutral or low alkaline pH-value. Both types of liquid paraffin have a solids content of approximately 60 %. They are products of the SASOL company.

### Production of pilot scaled medium density fibreboards

For the production of all medium density fibreboards the full automatically MDF pilot plant station of the Büsgen-Institute at the Georg-August University was used. By using this pilot plant it is possible to produce MDF-boards under controlled and near-industrial conditions. After bulcking the compressed fibre-material the fibres and the bonding agent are mixed in a fast rotating ring-blender. After this mixing-process the fibres are dried in a dryer unit composed of a gas torch and a 50 meter long drying line. It is possible to use different temperatures in this drying-process to get a adequate moisture content of approx. 10 %. This procedure should avoid possible damages during the pressing-process depending on a too high moisture content of the fibres. Transported by a conveyer-belt the material is transferred into a fibre bunker, where the glued fibre-material is collected. From there the fibres are strewed by a strewing-hat to a non-woven fibre mat in different thicknesses depending on the later end-thickness and density of the boards. After a manual pre-pressing in a second step the fibres are pressed in a hot press to medium density fibreboards. The last steps after conditioning are grinding, formatting and testing the panelboards.

*Table 2: Manufacture parameters of MDF-boards*

Fibre material	Pine ( <i>pinus sylvestris</i> )
Raw density	700 kg/m <sup>3</sup>
Dimension	500 mm x 300 mm x 9 mm
Press temperature	200 °C
Pressure	220 bar
Press time factor	18 sec/mm

The Reference-boards glued with UF-resin as well as the wheat-protein bonded MDF have a gluing-ratio of 12 % (relating to the absolute dryweight of fibre-material). The additional amounts of silane were 1 %, 2 % and 3 %. The liquid paraffin was applicated in an amount of 1 %.

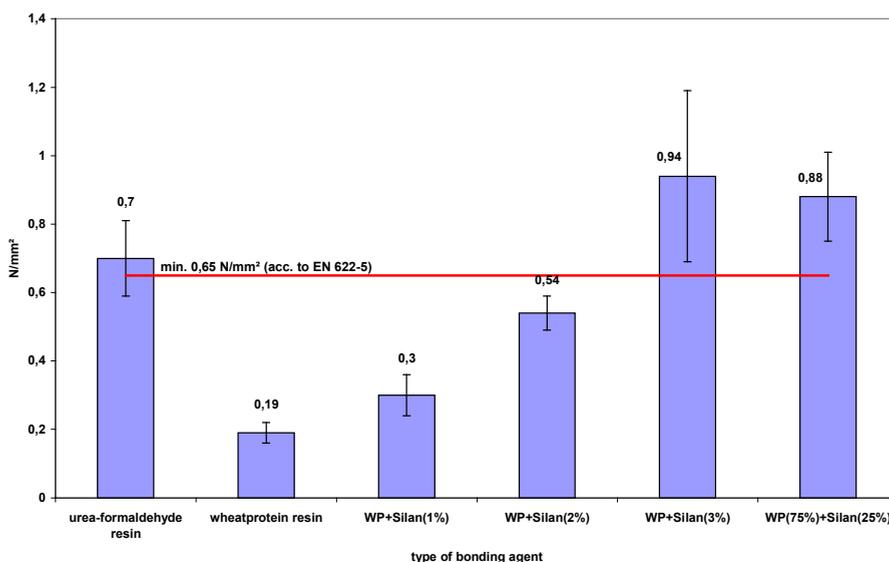
The following test methods and requirements according to the european standards are the most needed test procedures for quality inspection of MDF. The mechanical-technological properties of medium density fibreboards are looked into these test specifications:

*Table 3: Performed methods of testing*

EN 319	Internal bonding strength
EN 310	Bending strength
EN 317	Thickness swelling after water storage (24 h)
EN 120	Determination of formaldehyde content

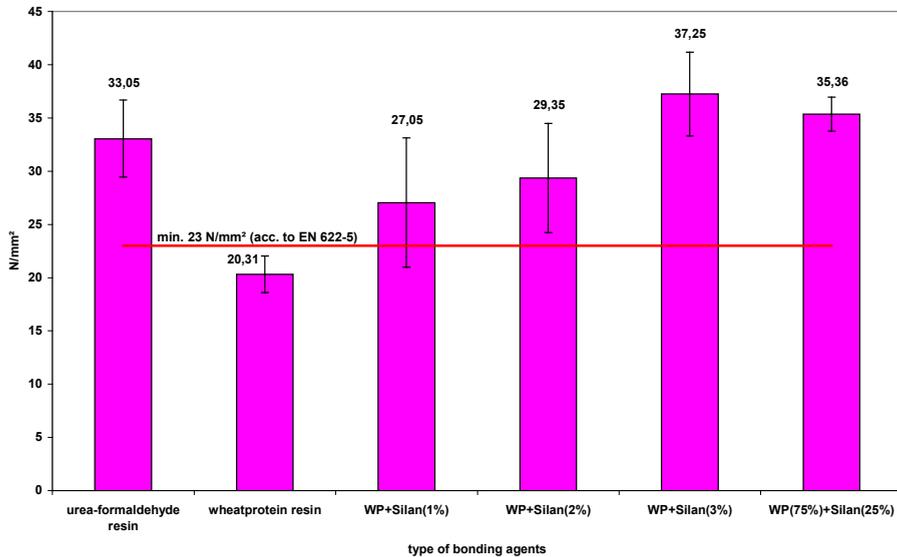
## Results

The following chapter presents the results of the mechanical-technological investigations of medium density fibreboards produced with UF-resin, common wheat protein or wheat protein modified with silane. The testing of the internal bonding strength, of the bending strength and of the thickness swelling after 24 h water storage as well as the determination of the formaldehyde content serve as a base for investigations on MDF.



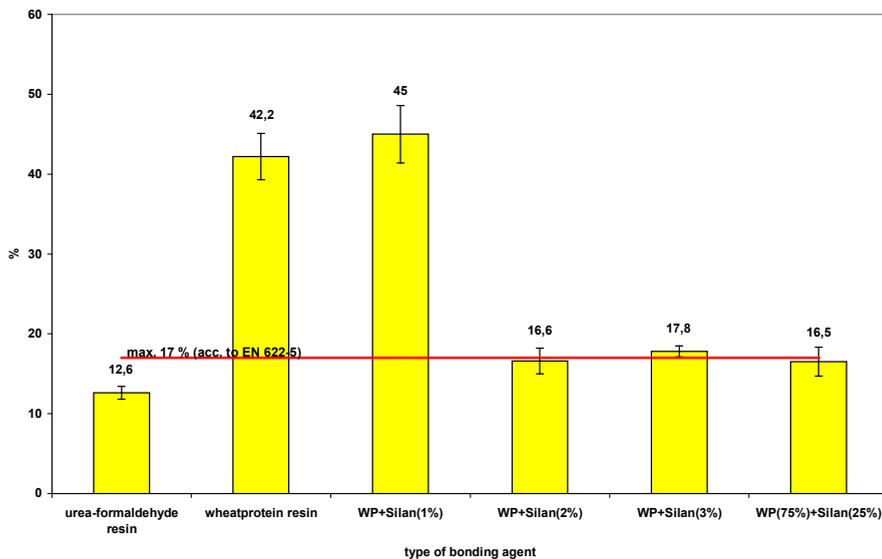
*figure % Internal bonding strength of reference boards and modified wheat protein bonded boards*

Figure 1 shows that the internal bonding strength of the UF-reference is clearly differed from the type of board bonded with pure wheat protein. The UF bonded type was able to achieve the limiting value of the European standards with a internal bonding strength of 0.7 N/mm<sup>2</sup>, the wheat protein-type has a very low IB (0.19 N/mm<sup>2</sup>) which is far below the guideline of 0.7 N/mm<sup>2</sup>. An addition of silane causes a significant improvement of the internal bonding strength. A modification of the wheat protein bonding agent with 1 % respectively 2 % of silane leads to a clear improvement of the IB but these types are still not able to achieve the limiting value of the European standard. In contrast to these boards a silane modified binding agent with a content of 3 % has a greater effect on the internal bonding strength. This modified wheat protein bonded MDF has an average internal bonding strength of 0.94 N/mm<sup>2</sup> and so they are significant above the limiting value as well as the values reached by the UF-references. A substitution of 25 % wheat protein by silane shows similar high values as the addition of 3 % of silane.



*figure & Bending strength of reference boards and modified wheat protein bonded boards*

The results for the bending strength shown in figure 2 are nearly identical. But there is not such a high difference in strength between both reference types like in the testing method before. The wheat protein bonded MDF which are not modified have an average bending strength of 20.31 N/mm<sup>2</sup> and so they do not correspond to the European standard (23 N/mm<sup>2</sup>). An addition of 1 % of silane causes an increased strength which meets the standards Further increases of the quantity of silane leads to a significant increase in strength. An addition of 3 % of silane as well as a substitution of wheat protein by 25 % of HS 1151 causes an improvement of strength which is higher than the bending strength of the reference-type bonded with UF.



*figure ' : Thickness swelling after 24 h water storage of reference boards and modified wheat protein bonded boards*

Thickness swelling after water storage is a big problem for wheat protein bonded medium density fibreboards. The swelling results of this type shown in figure 3 are much higher in comparison to the UF bonded reference. A thickness swelling value of 42.2 % is far above the maximal swelling properties that MDF may have (17 %). An addition of 1 % of silane does not cause any effects neither. But a significant improvement of swelling properties can be reached by higher additional amounts of HS 1151. So the modification of wheat protein with 2 % leads to a decreasing thickness swelling under the allowed limiting value of the EN. Further additional increasing of the quantity of silane has no improving impact on swelling properties. A substitution of silane also does not have any decreasing effect on the swelling properties of MDF.

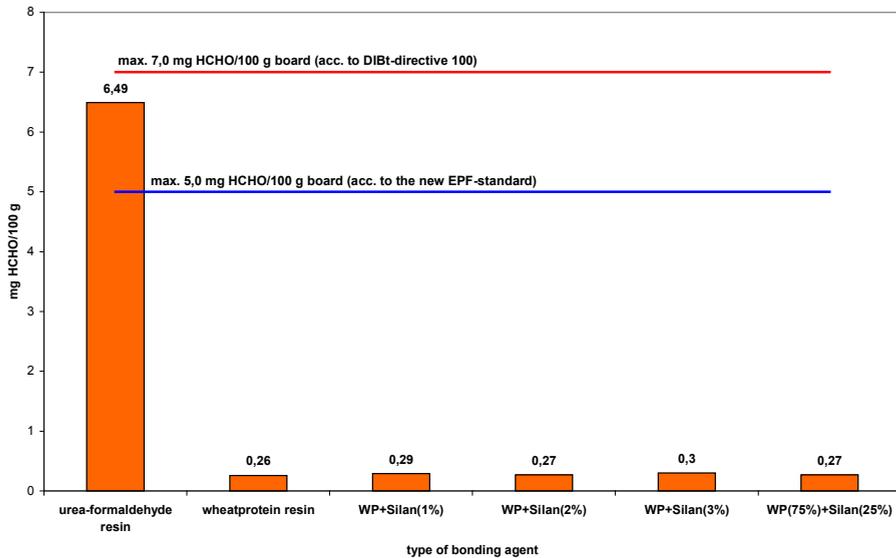


figure 4: Formaldehyde content of reference boards and modified wheat protein bonded boards

But the main problem that commonly produced UF bonded boards have (see Fig. 4) is the current content of formaldehyde. At present UF bonded boards achieve the guidelines of the European standard concerning the formaldehyde content but if a new limiting value comes into effect in the near future these panelboards will not observe the new requirements. The pure wheat protein bonded boards as well as the modified types have very low formaldehyde contents comparable with natural wood which are hardly detectable.

## Conclusion

On the one hand the results reached by pure wheat protein bonded boards show the disadvantages of near natural binding agents in comparison to common UF resin. All properties do not fulfil the required properties of the European standard. The mechanical and technological properties of medium density fibreboards bonded with natural wheat protein were much lower than the properties of UF bonded boards and in most cases they could not fulfil the limiting values by far

On the other hand an addition of silane in near natural binding agents mostly causes a significant improvement of properties. In several cases it was necessary to apply a higher amount of this substance to achieve the guidelines because of very low property values in some testing disciplines reached by non-modified boards. An addition of 3 % as well as a substitution of wheat protein by silane leads to excellent results in all testing disciplines. In this case these boards do not only achieve the limiting values but they also clearly surpass the values of the UF bonded boards.

The conclusion regarding the formaldehyde content is that silane is a formaldehyde-free substance. The low formaldehyde contents of natural or modified wheat protein boards show that it is possible to produce nearly formaldehyde-free MDF because wheat protein also does not contain any formaldehyde. The barely measurable formaldehyde content results exclusively from natural wood. The final result is that it is possible to produce medium density fibreboards with similar mechanical and technological properties like commonly bonded fibreboards. Against the background of problems with cancer caused by formaldehyde these modified near natural binding systems are an alternative to currently produced fibreboards.

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# **Microarrays – a tool for analyzing salt tolerance in trees**

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## Introduction

At the end of the last century, 76.3 million ha of land were affected by salinization through human-induced soil degradation (Oldeman *et al.* 1991). In the report of the Intergovernmental Panel on Climate Change (IPCC) for 2007, several reasons for increasing salinization in the future are given: In areas with decreasing precipitation, groundwater salinization may occur due to a reduction in groundwater recharge. In arid areas, this effect might even be increased at the surface level by irrigation. Furthermore, in coastal areas, sea level rise might lead to the intrusion of salt water into fresh groundwater (Kundzewicz *et al.* 2007). Thus, the availability of salt tolerant economic plant species will become increasingly important, and in areas where salinization coincides with soil erosion, salt tolerant tree species might solve many problems.

Research on mechanisms that allow plants to tolerate increased salt levels in the soil has been going on for several decades now, but since the beginning of the last decade, new powerful tools have become available in the form of microarrays, also known as DNA chips or gene arrays. With these tools, it has become possible to simultaneously investigate the regulation of thousands of genes in plants under different conditions. The first organisms for which this technique became available were important model organisms and crop plants such as *Arabidopsis thaliana* and rice, but with progress in sequencing techniques, whole genomes of a variety of species were sequenced. With *Populus trichocarpa*, the first genome of a tree species was fully sequenced (Tuskan *et al.* 2006), thus allowing the construction of a poplar microarray that encompasses the whole genome, the Affymetrix GeneChip Poplar Genome Array.

In this review, we sum up the history of the development and describe the molecular background of microarrays. We further explain the previous, recent and future possibilities of this technology, with an emphasis on tree specific questions and salt stress research.

## A Short History of Microarrays

At the end of the 1970s, the first method to simultaneously examine multiple RNAs was developed (Alwine *et al.* 1977): The Northern blot, named after the Southern blot, a similar invention for DNA by E.M. Southern. Southern transferred single-stranded DNA to a nylon membrane and then covalently crosslinked the DNA to the membrane. In a second step, single-stranded probe DNA was labeled, either with radioactive elements or with fluorescent colors, and hybridized with the DNA on the membrane. The pattern of the hybridization could thus be determined after excess DNA was washed off. In northern blotting,

the same blotting method was applied to RNA instead of DNA. By hybridizing labeled RNA or cDNA to the crosslinked RNA and measuring the signal strengths, the expression of a group of defined genes could be assessed. But it was desirable to be able to analyze the expression of large sets of genes, and thus, at the end of the 1980s, the first array-like techniques appeared (Kulesh *et al.* 1987): cDNA was synthesized from mRNA, then spotted with pins on a membrane, and eventually hybridized with labeled probe cDNA.

Based on this method, spotted microarrays were developed during the 1990s which contained thousands of PCR-amplified cDNAs on one glass slide (Schena 1996). For the construction of these microarrays, data collections of expressed genes were used, which included full-length gene sequences or expressed sequence tags (ESTs). Two problems that remain with this technique are the available space on the slides and the accuracy of the spotting. Because the spotting is performed by pins, at first the maximum density reached only about 1,000 cDNA spots per cm<sup>2</sup>; on one 10 cm<sup>2</sup> slide, only a few thousand transcripts could be analyzed. Since then, the spot resolution has improved, and currently the production of high-density microarrays with up to 10,000 spots per cm<sup>2</sup> are possible, allowing the production of microarrays that contain the genes of a whole genome of one organism. However, two different spotted microarrays always vary slightly in the amount of cDNA in the single spots, because the cDNA is first amplified by PCR and then mechanically spotted on the slides. Therefore, a control sample and a treatment sample may not be analyzed on two separate microarrays and be directly compared afterwards, but have to be analyzed on one single microarray. For this method, two different fluorescent colors have to be used, which brings up new problems, e.g. differences in binding efficiency or degradation rate of the colors.

To evade all these technical problems, more accurate microarrays were developed that make use of *in situ* synthesis of oligonucleotides. The first of these techniques was again devised by the group of E.M. Southern (Southern *et al.* 1992). Later, improved methods were developed: one based on photodegradable cap-structures called photolithography by Affymetrix (Thomas & Burke 1998), and one using procedures similar to that of ink-jet printers by Agilent, a Hewlett-Packard spin-off. These new types of microarrays were not only more accurate, but they had spot densities that allowed the presence of several 100,000 spots on one array. To distinguish this new generation of microarrays from the old spotted type, they are often called ‘chips’, and with these chips the construction of microarrays that contained several probes for every known gene of one species became possible.

## Transcriptomics

In analogy to genomics, the analysis of the genome, the analysis of the entirety of the transcribed genes, the ‘transcriptome’, became known as ‘transcriptomics’. The analysis of the transcriptome will deliver information that bare sequencing of

the genome is not able to rovide, since the information encoded in the genome never changes, but the expression thereof is highly variable. Or in other words: 'The genome is static, but its expression is dynamic' (Knippers 2001).

At the beginning of the era of transcriptomics, microarrays were mainly used in medical research (Chu *et al.* 1996, Mao 1997, McKenzie *et al.* 1998), only to a certain extent was the technique adapted for important model organisms like *Arabidopsis thaliana* (Schena *et al.* 1995). As more genome sequencing projects were completed, microarrays became available for further plant model species such as *Medicago truncatula*, *Nicotiana tabaccum* or *Oryza sativa*. Finally at the beginning of this decade, the genus *Populus* became accepted as a model for woody plants (Strauss & Martin 2004, Taylor 2002), and the first spotted microarrays for *Populus* were produced (Hertzberg *et al.* 2001). Thus, transcription profiling in a tree species could commence.

## Approaching tree specific questions

Research on wood formation continued to employ *Arabidopsis thaliana* as a model plant even after poplar microarrays were available (Li *et al.* 2006). The reasons were many, working techniques were well established, industrially made gene chips available, and the annotation of the genome far advanced. But other workgroups constructed their own cDNA arrays and began the research of expression patterns that where involved in the development of wood cells. Hertzberg *et al.* (2001) separated wood into layers and extracted RNA from the different zones: meristematic cells, early expansion, late expansion, secondary wall formation and late cell maturation. They could thereby show what changes occur in the transcriptome during the formation of wood, and identify important genes for the different zones. Other working groups analyzed the change of the poplar transcriptome throughout whole growing seasons (Sjödin *et al.* 2008), or in tension wood (Andersson-Gunnerås *et al.* 2006). Schrader *et al.* (2004) examined the cambial meristeme during dormancy and discovered that many water, salt and cold stress induced genes were upregulated, and speculated about their relationship to phytohormones like auxin, abscisic acid and even gibberellins.

These examples demonstrate which direction transcriptomic research is taking. It is advancing from simple transcription profiling and single gene studies towards understanding regulatory processes on a tissue specific level. Although characterization of pathways and regulatory networks in trees is still in its initial phase, the huge amount of information generated by a microarray analysis virtually demands interpretation on a grand scale. Until now, the limiting factor in this area of interest is the amount of information available and the quality of annotation of microarrays. Advancement in this field is still lagging behind, but public data availability *via* online databases like the Joint Genome Institutes (JGI) *P. trichocarpa* project site ([http://genome.jgi-psf.org/Poptr1\\_1/Poptr1\\_1.home.html](http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html)) is

progressing. In the near future, we can therefore expect a high increase in data quality as well as quantity from transcriptomic experiments.

## Analyzing Salt Stress

As stated before, advancement of molecular working techniques and genome annotation within the plant kingdom focussed on *Arabidopsis*. However, its relative close relationship to *Populus* allowed transfer of large parts of this knowledge, stress research in poplar could therefore commence at a high level. During the first years after the technique was established in *Arabidopsis*, microarray analyses addressed mainly global transcriptomic changes and characterization of single identified candidate genes (Desprez *et al.* 1998, Ruan *et al.* 1998). In contrast, Herzberg *et al.* (2001) marked the beginning of the era of microarrays for poplars with a fundamental study analysing the pathways of lignin biosynthesis and carbohydrate metabolism. Functional analysis of single candidate genes in trees, especially in the model organism poplar, still has to be conducted, but can be limited to genes which, through mutation, evolved new or better functions (Junghans *et al.* 2006, Ottow *et al.* 2005). Research of stress tolerance in trees should profit from and integrate such elementary studies in *Arabidopsis* as the description of the SOS (salt overly sensitive) pathway (Zhu 2000), or transcriptome comparisons between salt sensitive and salt resistant species (Taji *et al.* 2004). Ma *et al.* (2006) already showed that in *Arabidopsis*, many genes regulated under stress are not stress specific but are universally regulated in response to pathogens, osmotic fluctuations, mechanical interference and cold; less than 25% of all regulated genes were specific for salt stress. Therefore, the underlying mechanisms should be particularly addressed.

Within the genus *Populus*, *P. euphratica* has become a model plant for research of salt tolerance. First microarray studies show promising results, but focus on technical issues and stay on a descriptive level: Gu *et al.* (2004) constructed the first small *P. euphratica* microarray; it contained only 315 selected genes, but they could show upregulation of typical salt stress related genes as well as several promising new candidate genes. An improved approach was conducted by Brosché *et al.* (2005) who constructed a microarray with roughly 6,400 genes. They identified only 22 regulated genes, but even this might be valuable information, since information about plants evolutionally adapted to stress is still scarce. Due to the above-mentioned overlap of different stress responses, we might also gain further insight into the underlying mechanisms of salt tolerance from studies that addressed drought stress. Approaches to this have also been made in *P. euphratica* (Bogeat-Triboulot *et al.* 2007), but no comparison to a stress sensitive species had been conducted. An example for such an experiment is given by Street *et al.* (2006), who analyzed the difference in drought response between two poplar species, *P. trichocarpa* and *P. deltoides*. Future work regarding salt

tolerance mechanisms should take a similar approach and include *P. euphratica* as a stress tolerant species as well as a stress sensitive poplar species.

## Conclusions

Microarrays represent a powerful tool for analyzing gene regulation. The construction of the Affymetrix GeneChip Poplar Genome Array has made this technology available for woody plants and provides a universally applicable platform for comparison of differing experimental conditions. It allows the analysis of tolerance mechanisms in the genus poplar, which encompasses various species adapted to a wide ecological range and which therefore are adjusted to a diversity of stress conditions. *Populus euphratica* is therein predestined to assess salt tolerance, but should be analyzed in respect to salt sensitive poplar species. However, fundamental knowledge on molecular stress responses in non-woody plants is available, and further research in *Populus* should utilize this and focus on mechanisms characteristic for trees which can not be assessed in *Arabidopsis*.

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## **How does fertilization influence trace gas emission?**

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## Abstract

In this field experiment, we studied the seasonal variation of nitrous oxide ( $\text{N}_2\text{O}$ ) and methane ( $\text{CH}_4$ ) emissions after 30 years of different fertilization strategies and how the climate, soil and management factors influence  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions.

Field measurements took place on a long-term experiment near to Darmstadt, Germany, managed by the Institute for Biodynamical Research ([www.ibdf.de](http://www.ibdf.de)). Since 1980 two fertilizer types were compared and applied in different treatments to this sandy cambisol site. On the mineral variant calcium ammonium nitrate and on the organic fertilizer composted cattle manure was applied. Straw remains on the field. Both fertilizer types are applied in three different rates: low ( $50\text{--}60 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ), medium ( $100 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) and high ( $140\text{--}150 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ).

After almost thirty years of different fertilization organic carbon ( $\text{C}_{\text{org}}$ ) and total nitrogen ( $\text{N}_{\text{t}}$ ) contents significantly differed between both treatments whereby the organic fertilized treatments showed both higher  $\text{C}_{\text{org}}$  and  $\text{N}_{\text{t}}$  stocks. Therefore the question arose if there is any effect on  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emission due to these differences.

Results of the first year of measurement, which started in February 2007, are presented here. With the closed chamber method trace gas fluxes are measured weekly in four replicates. Factors like soil moisture content, tillage and mineral nitrogen content and their influence on trace gas emissions were determined. Nitrous oxide emission increases after tillage and with a higher nitrate content. Methane uptake decreases with higher soil moisture content. Long-term fertilizer application did not influence  $\text{N}_2\text{O}$  emission and  $\text{CH}_4$  uptake neither in rate and nor in type.

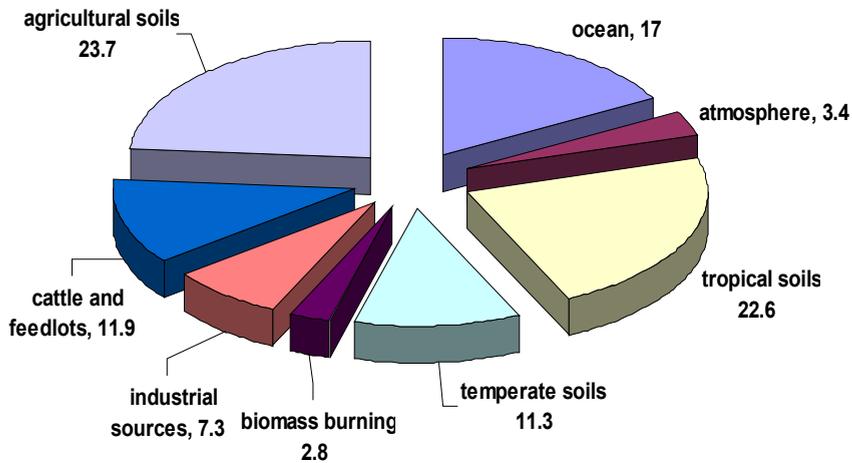
## Introduction

Carbon dioxide, nitrous oxide and methane are the major greenhouse gases responsible for global warming and the depletion of the ozone layer. Nitrous oxide and methane contribute to the global warming with a global warming potential of 310 and 21 (IPCC, 2001). Agriculture occupies about 40–50% of world's terrestrial surface. Globally, agricultural  $\text{CH}_4$  and  $\text{N}_2\text{O}$  emissions have increased by nearly 17% from 1990 to 2005. Current emission rates may escalate in the future due to population growth and changing diets. Greater demand for food could result in higher emissions of  $\text{CH}_4$  and  $\text{N}_2\text{O}$  if there is more livestock and greater use of nitrogen fertilizers.

Therefore it is of major interest to minimize gaseous losses to protect the environment on the one hand and to save resources on the other hand.

## Nitrous oxide

Natural sources for nitrous oxide are the ocean, the atmosphere and natural soils. These emissions are difficult to reduce. However, about 46% of the worldwide  $\text{N}_2\text{O}$  emissions are induced by human beings. Sources like industry, biomass burning and agriculture have to be named. Of global anthropogenic emissions in 2005, agriculture accounts for about 60% of  $\text{N}_2\text{O}$  and about 50% of  $\text{CH}_4$ . Figure 1 shows the different sources of  $\text{N}_2\text{O}$  published by IPCC (2001).



**Fig. 1: Estimates of the global nitrous oxide budget in % from different sources, adopted by IPCC (2001)**

It can be easily seen that the biggest part of the anthropogenic sources comes from agriculture with 24%. The assessment of the emission rates is difficult because they vary greatly in time and space. Increasing soil N availability caused by increased fertilizer use and higher atmospheric N deposition enhances  $\text{N}_2\text{O}$  emission.

Nitrogen is a deficient nutrient in agricultural systems and fertilization of N plays an important role in modern agriculture. The increase of the world population makes a highly productive agriculture more important.

$\text{N}_2\text{O}$  is generated by the microbial transformation of nitrogen in soils and manures, and is often enhanced where available nitrogen (N) exceeds plant requirements, especially under wet conditions (Oenema *et al.*, 2005; Smith and

Conen, 2004).  $N_2O$  is produced via two main processes, nitrification and denitrification. Nitrification is the oxidation of ammonia to nitrate via the intermediate nitrite. The oxidation of ammonia into nitrite is performed by two groups of organisms, ammonia oxidizing bacteria and ammonia oxidizing archaea. Denitrification is the reduction process to nitrogen through a series of intermediate gaseous nitrogen oxide product. Nitrous oxide is an intermediate in the reaction sequence of denitrification and a by-product of nitrification (IPCC, 2001, Duxbury, 1982). Therefore these microbial processes are mainly regulated by temperature, pH, water content, C availability and nitrate content.

Microbial processes depend on water. Denitrification rate increases as soil water content rises. Nitrification also increases with water content up to a level where oxygen is restricted (Granli & Bockman, 1994). Then the diffusivity of soil gas is affected by soil water content (Firestone and Davidson, 1989).

In organic agriculture the application of organic fertilizer like manure or slurry is an important mean to increase the humus content. No synthetic fertilizer is used and existing resources can be applied. Production rests from animal husbandry can close the gap in the agricultural cycle and can be recycled. The use of fertilizer can be regarded as sustainable.

It is supposed that organic fertilized fields emit more  $N_2O$  due to the higher C availability and nitrogen stocks (Groffman & Tiedje, 1991). IPCC (2001) published an emission factor for nitrous oxide from agricultural fields that depends on the nitrogen input which is mainly fertilizer.

$N_2O$  -N emission = 1.25% \* N-input

So far, the calculated emission only depends on the nitrogen input and there is no differentiation between soil texture, fertilizer type etc. A more detailed calculation would permit a better differentiation between sites. Therefore a better basis for extrapolations to countrywide or worldwide emission would be possible.

## Methane

Soil mostly takes methane up, except for the cultivation of rice. Rice fields release methane in large amounts of around 90 Tg  $CH_4$  yr<sup>-1</sup>. Worldwide the uptake of soils is ~ 30 Tg  $CH_4$  yr<sup>-1</sup> (IPCC, 2007).  $CH_4$  is produced when organic materials decompose in oxygen-deprived conditions, notably from fermentative digestion by ruminant livestock, from stored manures, and from rice grown under flooded conditions (Mosier *et al.* 1998). Rice cultivation and animal husbandry are the main sources for methane in agriculture. Soils with a low soil moisture content (not water-logged, no wetlands) are usually sinks for methane. This is a very important fact in the discussion of climate change as soil is able to transform methane. The methane uptake depends on the oxygen availability and therefore on the soil moisture content. The higher the moisture content is the less pores are air-filled.

Therefore methane oxidation does not take place. Soil texture also influences the methane uptake markedly because the sandier a soil is the more aerated it is.

Nitrogen fertilization reduces methane uptake and therefore less methane will be oxidized. Ammonium oxidation and methane oxidation are competing processes because ammonium oxidizers are also able to transform methane and vice versa. Crill et al. (1994) showed that fertilization affected methane uptake negatively but the effect depends on the type of fertilizer.

The objectives of this field study are to determine the influencing factors for methane and nitrous oxide emissions from an agricultural field. A more detailed view on these factors should allow an adaptation of the IPCC-factor for nitrous oxide emission.

Furthermore, the effects of different long-term fertilization strategies on trace gas emissions were studied. Due to organic fertilization carbon accumulates in soils. The effects of a higher  $C_{org}$  content on  $N_2O$  and  $CH_4$  emissions are not determined yet.

Besides, this study should help to find out mitigation strategies for nitrous oxide and methane. So it is of interest if the fertilizer type supports long-term emissions or not. Practical recommendations for lower emissions should follow. Methane uptake is an important task to promote carbon sequestration and can therefore also counteract climate change and enhance soil fertility. Strategies to enhance methane oxidation are of interest for agriculture.

## Material and Methods

### Study site

The field experiment is performed near to Darmstadt (49°50' N, 8°34' E), with an elevation of 100 m above sea level. The mean temperature was 9.5 °C and the mean annual precipitation was 590 mm. The soil is a Haplic Cambisol (WRB 2006) which developed on alluvial fine sand (Bachinger, 1996). The field is a long-term fertilization experiment managed by the IBDF ([www.ibdf.de](http://www.ibdf.de)).

In 1980, six treatments were arranged in a strip design with four replicates, with the factors being type of fertilizer and its application. The fertilizer was applied as composted cattle manure and calcium ammonium nitrate rate and in three different rates (60 kg N ha<sup>-1</sup>, 100 kg N ha<sup>-1</sup> and 140 kg N ha<sup>-1</sup>). Straw remained on the mineral fertilized field after harvest. Besides fertilization, all other management actions were the same.

The crop rotation consists of red clover (*Trifolium pratense* L.), spring wheat, wheat (*Triticum aestivum* L.) potato (*Solanum tuberosum* L.) and winter rye (*Secale cereale* L.). The preceding crop was clover and the intertillage was radish (*Raphanus sativus*

*subsp. oleiferus*). The soil type was a Cambisol with a high percentage of sand. Soil texture consisted of 86% sand, 9% silt und 5% clay in the topsoil.

After almost 30 years of different fertilization history  $C_{org}$  and  $N_t$  contents differed significantly between both treatments. The organic fertilized fields showed significantly higher  $C_{org}$  as well as  $N_t$  stocks.



**Fig. 2: Closed chambers for gas sampling in a wheat field in Darmstadt, Germany in July 2007**

The closed chamber method

Fluxes of  $N_2O$  and  $CH_4$  were measured weekly using closed chambers with a diameter of 30 cm and a height of 30 cm (without plants inside). The soil covers were fixed onto permanently installed PVC collars. During tillage the collars were removed and they were reinstalled afterwards in the same place. Gas samples were taken from the chamber atmosphere using evacuated gas bottles at 0, 20, 40 and 60 minutes after closing the chamber. During the growth period higher chamber were used to save the wheat inside. During this period sampling time was enlarged to 90 minutes due to the higher volume of the chambers. (Flessa et al., 1998)

Analysis of nitrous oxide and methane

Carbon dioxide, methane and nitrous oxide analyses were carried out using a gas chromatographic system with a  $^{63}Ni$  electron capture detector. Further details of the system are shown in Loftfield et al. (1997). The gas analysis was automated by

the use of an autosampler system allowing the fully automated analysis of 64 gas samples and additional calibration gases.

#### Further laboratory analysis

At each time of the gas flux measurement soil samples were taken to a depth of 10 cm. Soil moisture content was determined gravimetrically. Ammonium and nitrate was measured by extraction with  $10^{-2}$  M  $\text{CaCl}_2$  solution. The soil/solution ratio during the extraction was 1:2 due to Wehrmann et al. (1986). The photometric analysis was done using a continuous flow analyser (S/A 20/40 Skalar Analytical, Erkelenz, Germany). Organic C and N contents were determined by an automated C- and N-analyser (Heraeus Elementar Vario EL, Hanau, Germany). Soil temperature was permanently logged in different depths (above ground and 5 cm, 10 cm and 20 cm depth).

## Results and Discussion

### Nitrous oxide emissions

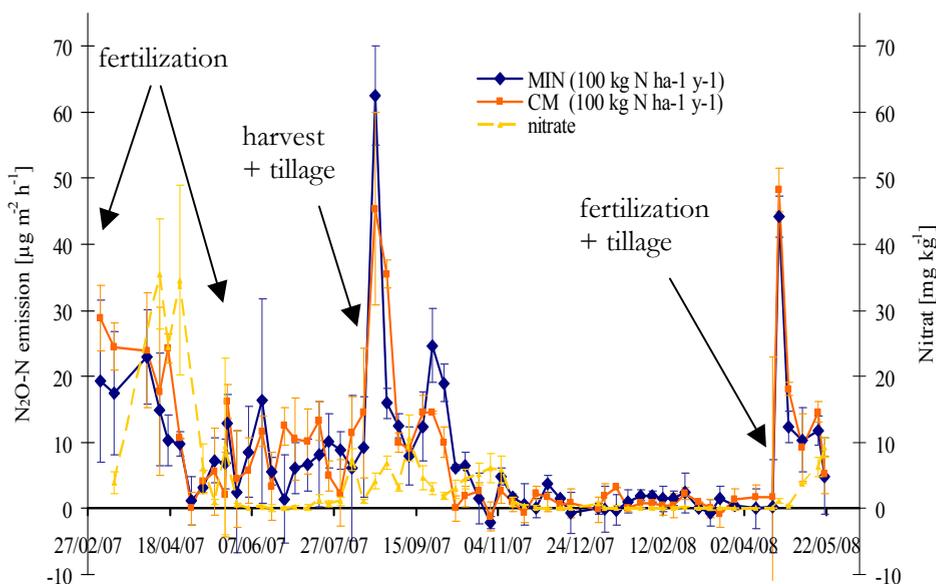
First results from the field experiment are presented in Figure 2. In general,  $\text{N}_2\text{O}$ -N emissions were low, most of the time lower than  $20 \mu\text{g m}^{-2} \text{h}^{-1}$ .

Emission rates increased after fertilization events. The first fertilization took place in March 2007, right before measurements started. Therefore at the beginning of the time series emissions were quite high. The second fertilizer application took place in May 2007 when the emissions rose again but not in the same amount. In August and September 2007 after harvest emissions increased again and reached their highest peak with more than  $60 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ . A second “autumn peak” after harvest was reached when tillage like ploughing was conducted. During winter 2007/2008 emissions were generally low ( $<10 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ ). No typical winter emissions after frost were found. Emissions did not exceed  $5 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ . In 2008, again fertilization induced peak emissions and they stayed on a higher level for a few weeks.

The change in nitrate content during the year was closely related to fertilizer application in spring 2007. Mineral fertilizer was applied in the form of ammonium nitrate and therefore was easily available for nitrification and then for denitrification processes. There was an offset of about two weeks between fertilizer application and the period of higher  $\text{N}_2\text{O}$  emission when nitrate content was very low again. This shows that the nitrate was transformed slowly and for a longer period because emissions were higher during the whole growth period.

In August 2007 the wheat was harvested and tillage operations were done. Tillage and especially ploughing induces very high  $\text{N}_2\text{O}$  emissions. The soil was turned and oxygen availability increased mineralization. This is also the reason for the

high peak in spring 2008. The fertilizer peak occurred more time-delayed which means that ploughing was the main reason for this high emission peak. It can be seen that high nitrate contents were responsible for higher  $N_2O$  emissions. However, mineral nitrogen was not the only factor influencing nitrous oxide emissions. Tillage events and also rain events (not presented here) were major factors influencing emission. Emissions on both treatments, mineral and composted manure, showed the same course. There was no significant difference between the fertilizer strategies which means that a higher  $C_{org}$  content did not influence  $N_2O$  emission.

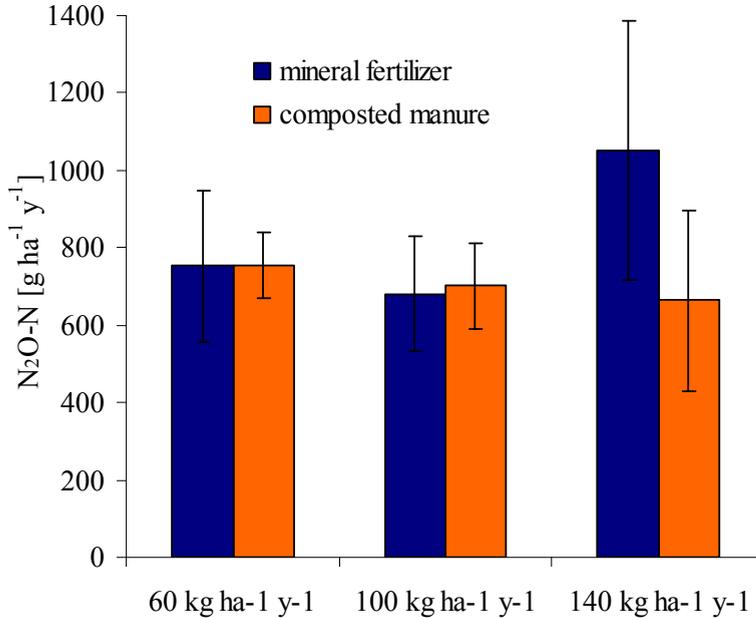


**Fig. 3: Changes in  $N_2O$ -N emission from the treatments organic and mineral fertilizer (means  $\pm$  standard error,  $n=4$ ) within a seasonal cycle from February 2007 to May 2008 (spring wheat). The solid line stands for the mineral fertilized treatments ( $100 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ). The dashed line shows the nitrate contents in the variant. Arrows with the letter show the date of management measure.**

Figure 3 shows the cumulative  $N_2O$ -N emissions for one year (from March 07 to February 08). Cumulative  $N_2O$ -N losses did not exceed  $1 \text{ kg ha}^{-1} \text{ y}^{-1}$  (Fig. 3). The sandy texture might be the most important factor influencing the emissions. As the texture consists of 86% sand, the soil was well aerated. Processes like denitrification, that are anoxic, did not take place.

There was no difference between the fertilizer types. In the lowest variants ( $60$  and  $100 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) emissions were the very same. In the highest fertilized

variant ( $140 \text{ kg N ha}^{-1}\text{y}^{-1}$ ) the mineral fertilized treatments seemed to emit more  $\text{N}_2\text{O}$ . But this difference was not significant since standard deviations were too high. The organic fertilized treatments did not emit more and the differences between the fertilizer rates were also not significant. A higher amount of fertilizer did not lead to higher  $\text{N}_2\text{O}$  losses.



**Fig. 4:** Cumulative  $\text{N}_2\text{O-N}$  emissions (means  $\pm$  standard deviation,  $n=4$ ) for one year from March 2007 to February 2008 on the differently fertilized variants (mineral fertilizer and composted manure) in different rates ( $60, 100, 140 \text{ N ha}^{-1} \text{y}^{-1}$ ).

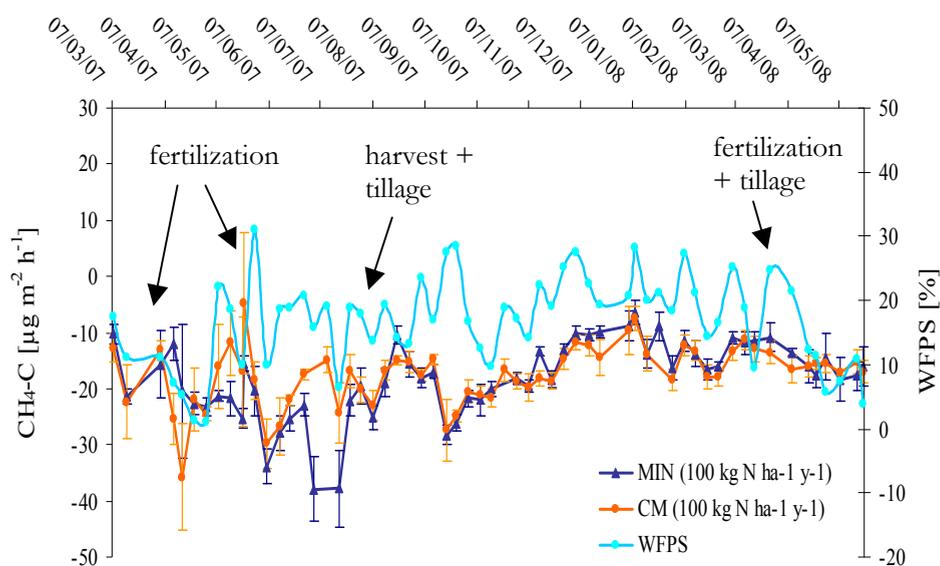
#### Methane uptake

Methane was taken up during the whole year. Figure 4 shows the changes in methane uptake during one year from March 2007 to February 2008.

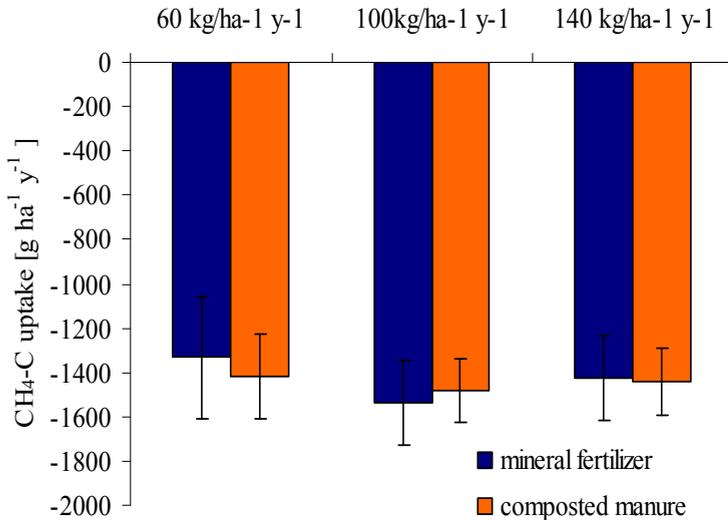
Methane uptake was not different in the variants mineral fertilizer and composted manure. Methane oxidation mostly depended on the water filled pore space and therefore on rain events. Figure 4 shows that most time of the year methane uptake described the same progression as the water filled pore space does. The higher the water filled pore space was the lower the methane uptake got. The more water a soil contained the less oxygen was present. Therefore there was not enough oxygen in the system for methane oxidation.

Except a few dates in June, July and October 2007 and March 2008 methane can mainly be explained by the water content. The measurements that did not fit might be induced by different factors that were not determined yet. Tillage was also a factor that influences soil aeration and therefore methane uptake.

Figure 5 shows the cumulative methane uptake rates for one year from March 2007 to February 2008. For all treatments the uptake was higher than  $1.3 \text{ kg ha}^{-1} \text{ y}^{-1}$ . Neither between the treatments, mineral and organic, nor between the fertilizer rates there was a significant difference. Methane uptake was in all treatments quite high because of the sandy soil texture. This texture led to a well-aerated soil which is a condition for methane uptake.



**Fig. 5:** Changes in  $\text{CH}_4\text{-C}$  uptake (means  $\pm$  standard error,  $n=4$ ) for the different fertilized variants (mineral fertilizer and composted manure) and water filled pore space (WFPS) within a seasonal cycle from February 2007 to May 2008 (spring wheat). Arrows with the letter F show the date of fertilization for the treatments CM and MIN. Arrows with the letter show the date of management measure.



**Fig. 6: Cumulative CH<sub>4</sub>-C uptake (means ± standard deviation, n=4) for one year from March 2007 to February 2008 on the differently fertilized variants (mineral fertilizer and composted manure) in different rates (60, 100, 140 N ha<sup>-1</sup> y<sup>-1</sup>).**

## Conclusion

Nitrous oxide emissions were mainly influenced by tillage and fertilizer. It could be shown that ploughing increased mineralization and that higher nitrate contents led to more N<sub>2</sub>O production. Fertilizer application also heightened the mineral nitrogen content in the soil and high emissions were the consequence. In general, emissions remained on a low level because the sandy soil is always well aerated. Therefore denitrification is not supposed to occur. The different fertilizer strategies did not influence N<sub>2</sub>O emission.

Methane emissions mainly depended on soil moisture content. The higher the water filled pore space was the lower the methane uptake was. The degree of aeration and therefore of oxygen was the main driving factor for methane oxidation. No influence of different fertilizer strategies could be found.

Further work on long-term effects of fertilization will be necessary to develop management strategies, especially to reduce greenhouse gas emissions.

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**“Wood Polymer Composites (WPC) – Processing technologies, chemical modification of wood and natural fiber components and improvement of selected properties”**

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## Introduction

Wood Polymer Composites (WPC) are a relatively new group of hybrid materials which combine the advantages of thermoplastic polymers and wood or natural fibres. In recent years, increasing interest has focused on thermoplastic composites (processing temperature  $<200^{\circ}\text{C}$ ) reinforced with wood fiber or with other lignocellulosic and cellulosic materials (Stark 2003, Mahlberg et al 2001, Balasuriya et al 2002, Borysiak et al, 2006). Lignocellulosics are favored as a new generation of reinforcing materials in thermoplastic since they represent renewable natural resources.

Classical applications for WPC are decking, fencing, industrial flooring, landscape timbers, railings, moldings, roofing, window and door profiles as well as automotive applications such as rearshelf-trim panels, instrument panels, load floors and cabbback panels (Clemons 2000, Rowell 2005). Although WPC decking is more expensive than pressure-treated wood, manufacturers promote its easy maintenance, lack of cracking or splintering, high durability, and environmental preference relative to pressure-treated lumber (Clemons 2002). The actual lifetime of WPC is currently in debate (Morris 1998). Chemically modified wood particles are under investigation for improving the performance of WPC in service.

## Wood Polymer Composites

### Definition

The term “wood-polymer(plastic) composites” refers to any number of composites that contain wood (of any form) and either thermoset or thermoplastic polymers. Thermosets or thermoset polymers are plastics that, once cured, cannot be remelted by heating. These include cured resins, such as epoxides and phenolics, more or less plastics with which the wood products industry is most familiar. Thermoplastics are plastics that can be repeatedly melted, such as polyethylene (PE), polypropylene (PP), and polyvinylchloride (PVC) (Rowell 2005). Thus some plastics need stabilisers (against heat, light, oxygen...), to give them a longer life time. Other additives eliminate problems during production or significantly increase processing efficiency (Richter 2004). The use of lignocellulosic materials with thermosetting polymeric materials, like phenol- or urea-formaldehyde, in the production of composites has a long history. The use of lignocellulosics with thermoplastics, however, is a more recent innovation (Youngquist 1999).

Products typically contain approximately 50% wood, although some composites contain very little wood and others as much as 70%.

## Polymer

Because of the limited thermal stability of wood, it was believed initially that only thermoplastics that melt or can be processed at temperatures below 200°C could be used in WPCs and currently that is state of the art. The plastic is often selected based on its inherent properties, product requirement, availability, costs, and the manufacturer's familiarity with the material. Small amounts of thermoset resins such as phenol-formaldehyde or diphenyl methane di-isocyanate are also sometimes used in composites with a high wood content (Wolcott 2000, Klyosov 2007).

### *Polyethylene*

Discovery 1933, Production 1939 (Elias 1997)

Traditionally, commercial polyethylenes have been classified into three major groups based on both the manufacturing process and the polymer properties. It has a relatively low-melting temperature (typically between 106 – 130°C, depending on density/branching of PE) and can be produced in a very wide range of viscosity of its melts. Polyethylene is a semi-crystalline polymer. It means that at ambient temperatures the polymer consist of two rather distinct fractions, or phases – crystalline and amorphous.

Table 1: Polyethylene, molded or extruded (Vasile 2005)

Polyethylene	Density [g/cm <sup>3</sup> ]	Melt flow index [g/10min]
High- density	0.941 - 0.965	0.2 - 30
Medium-density	0.926 - 0.940	1 – 20
Low-density	0.915 – 0.925	0.3 – 26
Linear low-density	0.915 – 0.925	0.1 – 100
Very low-density	0.870 – 0.914	0.02 - 10

Polyethylene is rather soft, making Pe-based composite deck boards easier to nail, screw, cut, and saw. Polyethylene, as well as polypropylene, shows near-zero moisture adsorption and very high resistance to chemicals, including strong acids. Polyethylene shows a relatively high resistance to oxidation compared to other polyolefins, hence, requires lesser amount of antioxidants for processing and for service indoors. On the contrary, polyethylene is rather flexible and not very strong.

(Elias 1997, Salamone 1999, Vasile 2005, Klyosov 2007, Radovanovic 2007, Wypych 2008).

### *Polypropylene*

Discovery 1954, Production 1957 (Elias 1997)

Polypropylene is a high-volume, commodity polymer possessing characteristics of special materials and has a history of technical innovations. This polymer has numerous grades for specific end uses, and the catalyst is a complex system resulting from major technological achievements. Ironically, the catalyst's sophistication allows the manufacturing process to be simple, economical, flexible, and environmentally friendly. Continuing catalyst developments, such as homogenous catalysts, have great potential for generating new types of polypropylene with expanded applications.

Although technically incorrect, the term polypropylene is widely accepted to mean highly isotactic propylene homo-polymers, propylene-ethylene copolymers, and other co- and terpolymers of propylene that are produced in commercial quantities. Isotactic polypropylene is an important polymer not only commercially, but also scientifically, because of its different morphological behaviour. On the crystal lattice level, iPP exhibits three different morphological forms,  $\alpha$ ,  $\beta$ , and  $\gamma$ , distinguished by the arrangement of the chains. Another form of iPP with a degree of order between crystalline and amorphous phases was first reported by Natta, who named it the "smectic" form. Polypropylenes have a specific gravity (density) of 0.90-0.91 g/cm<sup>3</sup>, which is approximately equal to that of very low-density polyethylene (Awaya 1988, Elias 1997, Salamone 1999, Vasile 2005, Klyosov 2007, Radovanovic 2007, Wypych 2008).

### *Polyvinylchloride*

Discovery 1838, Production 1931 (Elias 1997)

The structural order in PVC, as manifested by a low degree of crystallinity, is of great importance for its mechanical as well as for its rheological properties. PVC exhibits relatively good mechanical properties regarding its molecular weight in the absence of a rigid skeleton or any steric hindrance to prevent molecular motions and flexibility. Therefore, one is forced to admit that special interactions and/or structural order are responsible for its mechanical or rheological behaviour. Commercial PVC exhibits a low level of defects in chemical structure, such as short-chain branching and unsaturation (Salamone 1999). PVC is the heaviest material compared to polyethylenes and polypropylenes. Specific gravity (density) for PVC is 1.32-1.44g/cm<sup>3</sup>. There are two principle forms of PVC, rigid and plasticized, or flexible. Rigid, unmodified PVC is stronger and stiffer than polyethylene and polypropylene. Compared to these two materials, PVC has some inherent disadvantages, among them low thermal stability and high brittleness of PVC at ambient temperatures is caused by a relatively high glass transition temperature of PVC, which is typically in the range of 70-90°C. One of the principal beneficial properties of PVC is that it is inherently flame resistant (Klyosov 2007)

In addition a various number of other matrix materials is used, such as Polystyrene (PS), Acrylonitrile-Butadiene-Styrene (ABS), Polyamid 11, Polyamid 12, Lignin, Nylon, Starch, Polylactic-acid, and other biopolymers (Karus 2005, Gahle 2007, Klyosov 2007, Grüneberg et al 2008)

### Wood or Natural fibers

Wood used in WPCs is most often in particulate form (e.g., wood flour) or very short fibers and bundled fibers rather than long individual wood fibers. The relatively high bulk density and free-flowing nature of wood flour compared with wood fibers or other longer natural fibers, as well as its low cost, familiarity, and availability, is attractive to WPC manufacturers and users (Bodig 1992, Rowell 2005).

Common species used include pine, maple and oak in North America (Rowell 2005) as well as recycled mixed contingents. In Europe mainly spruce and pine is used (Karus 2005). As with most materials, wood particle costs are variable and depend on such factors as quantity, availability, particle size, and shipping/transport distance. Narrow particle size distributions and fine wood flour sizes tend to increase cost (Müller 2008).

Because of the potential of improved mechanical properties with fillers of greater l/d ratios, there has been a continuing interest in the use of individual wood fibers rather than wood flour as reinforcement in WPC. Adding fibers rather than flour increases mechanical properties such as strength, elongation, and unnotched Izod impact energy. However, processing difficulties, such as feeding and metering low-bulk-density fibers, have limited the use of fibers in WPCs. There have been some developments in pelletizing fibers for easy handling and processing (Jacobson 2002).

Along the use of wood fibers a various number of natural fibers are used for WPC. In Table 2 an overview is given. Recently miscellaneous manufacturers of advanced items have been used natural fibers as reinforcement filler; mainly flax, hemp, jute, kenaf, sisal, abaca, grass and stinging nettle (Karus 2006, Gahle 2007).

Table 2: Mechanical properties of some selected natural- and synthetic fibers (Al-Qureshi 1999)

Materials	Density (d) [N/m <sup>3</sup> ]	Cellulose /Lignin [%]	Max. stress (Y) [MPa]	Max. Strain [%]	Mod. Elas. (E) [GPa]	Y / d [Km]	E / d [Mm]
Wood	3.90	54 / 29	34	2.9	13	8.7	3.33
Jute	8.34	63 / 16	95 – 119	1.4	3 – 18	11.4 – 14.3	0.36 - 2.16
Banana	4.02	65 / 15	85 – 150	2.1	3 – 5	21 –37	0.75 – 1.25

Sisal	8.43	67 / 12	300 – 800	2.9	16 – 31	35.6 - 95	1.90 – 3.70
Bamboo	10.80	-	70 – 575	3.2	5 – 29	6.5 – 54.0	0.46 – 2.70
Epoxy	12.26	-	28 – 91	2.1	1.4 – 3.5	2.2 – 7.5	0.11 – 0.29
Asbestos	33.30	-	3100	2.5	170 - 200	93	5 – 6
Polyester	12.26	-	45	3.2	0.35	3.7	0.03
E-Glass	25.00	-	2500	2 – 5	74	100	2.96

### Additives

Plastics are an indispensable part of modern life. A range of additives is necessary, however, to produce them economically and give them their desired properties (Richter 2004). Wood and plastic are not the only components in WPCs. These composites also contain materials that are added in small amounts to affect processing and performance. Although formulations are highly proprietary, additives such as coupling agents, light stabilizers, pigments, lubricants, fungicides, and foaming agents to name several of them are used to some extent (Mapleston 2001).

The following table gives an idea of the large variety of additives used with plastics.

Table 3: Overview of commonly used additives for WPC (Mapleston 2001, Clemons 2002, Richter 2004, Karus 2006, Gahle 2007, Grüneberg et al 2008)

Polymers	Heat stabilizers	Antioxidants	Plasticisers	Coupling agent
Fillers	Bio stabilizers	Antistatics	Blowing agents	Compatibilizers
Lubricants	Light stabilizers UV-absorbers	Flame retardants	Nucleating agents	hydrophobic agents
Pigments	Reinforcements (fibers)			

In order to improve the compatibility between high surface energy lignocellulosic fibers and low-energy polyolefin matrixes, extensive use of coupling agents has been made (Bledzki et al 1998, Hill 2000, Lu et al 2000, Hill 2006). The most commonly employed coupling agents are maleated polymers (e.g. maleic anhydride polypropylene, MAPP) or polymethylene polyphenylene isocyanate (PMPPIC). Coupling agents are added to the wood fibre/polymer ingredients and subjected to energetic mixing involving high shear in extruders or high speed

mixers. Evidence has been represented indicating that the attached anhydride moieties are capable of reacting with the surface of the lignocellulosic fiber (Felix et al 1991, Grüneberg 2006, Hill 2006). Beside maleated polymers, polyisocyanates and silanes are used as coupling agents (Raj et al 1990). Most of the silane/titanate/zirconate coupling agents can be represented as  $R-(CH_2)-X(OR')_n$ , where  $X = Si, Ti, \text{ or } Zr$ ,  $n = 0-3$ , OR is the hydrolyzable alkoxy group, and R and R' are the functional organic groups (Bledzki et al 1996, Nabi Saheb et al 1999).

As soon as the maleated coupling agents were introduced into the WPC, it was noticed that their effect often significantly depends on lubricants employed in the same system. The most striking was a conflicting effect between maleated polyolefin's and metal stearate lubricants. Zinc stearate invariably decreases both strength and stiffness of WPC compared to the non-metal lubricant. However, if without coupling agents this decrease was insignificant. Non-metal lubricants, however, were much less antagonistic, though they also decreased the mechanical properties of the WPC in the presence of the coupling agents by 10-20% for flexural strength and 2-9% for flex modulus (Klyosov 2007). The effect of conflicting of the maleated polyolefin's with the zinc stearate and other metal-containing stearates is commonly known in the industry. Therefore, a number of companies have developed non-metal lubricants (Manolis Sherman 2004, Markarian 2005).

Antioxidants slow down the plastic degradation under high temperature, attrition, and so on, hence, minimize the VOC formation and the respective decrease of density. In order to minimize water adsorption by WPC boards, they should have as high a density that their formulation allows. To achieve this goal, a proper amount of antioxidants should be introduced to the formulation (Klyosov 2007).

## Processing technology

Due to the considerable growth of the WPC market in the last few years, many machine manufactures developed new processing concepts for the production of WPC products. At present the processing procedures are roughly divided into two groups:

1. Two-step processing
  - Process 1: Compounding with a compounder (e.g. co- and/or counter rotating twin screw extruder, planetary-roller extruder) or hot-cool mixer
  - Process 2: Extrusion (single- or twin screw extruder), injection or compression moulding
2. Direct Processing
  - Direct extrusion with a counter-rotating twin-screw extruder (parallel, conical) or using a compounder with a melt pump between the

compounder and the die (currently not with a high volume in the market, but with a large potential)

Direct injection moulding (barely present on the market, require big investments) (Radovanovic et al 2006, Karus et al 2005, Gahle et al 2007)

The manufacture of thermoplastic composites is often a two-step process. The raw materials are first mixed together in a process called compounding, and the compounded material is then formed into a product. Compounding is the feeding and dispersing of fillers and additives in the molten polymer. Many options are available for compounding, using either batch or continuous mixers. The compounded material can be immediately pressed or shaped into an end product or formed into pellets for future processing (Clemons 2002). Combining the compounding and product manufacturing steps is called in-line processing.

The main problem in processing wood-thermoplastic composites is the tendency of untreated wood to form large aggregates, due to a high intramolecular bonding among the fibers. Therefore, the dispersion of the fibers in the polymer matrix is small and the reinforcing ability of the fiber is reduced remarkably. The better the fibers are dispersed in advance in the polymer matrix; the better is its uniformity. Then, the mixing time can be shortened and thus less fibers break during the mechanical mixing process (by shearing)(Raj et al 1989/1991, Bataille 1990, Gatenholm 1993).

The dispersion of wood fibers can be improved by pre-treatment with lubricants or thermoplastic polymers (Raj et al 1989).

Another problem is that the rate of thermal decomposition of lignocellulosics increases exponentially with an increase in temperature. At the normal processing temperature (180-200°C), critical values of decomposition are realized (Bataille 1990). Decomposition of pulp components creates voids inside the composite, causing a decrease in mechanical properties. An optimization of processing temperature and time is necessary. Melt viscosity decreases with increasing shear rate and increases with higher filler content to each temperature (Kokta 1983). The properties of extruded composites are sensitive to a screw configuration and the compounding temperature (Yam 1990).



Figure 1: Various processing technologies for WPC production (a) Twin screw extruder, (b) hot cool mixer unit, (c) injection moulder, (d) compression moulding die

## Wood Modification

Among numerous definitions, Hill (2006) describes wood modification as:

“Wood modification involves the action of a chemical, biological, or physical agent upon the material, resulting in a desired property enhancement during the service life of the modified wood. The modified wood should itself be non-toxic under service conditions and, furthermore, there should be no release of any toxic substances during service or at the end of life following disposal or recycling of the modified wood. If the modification is intended for improved resistance to biological attack, then the mode of action should be nonbiocidal (Hill 2006).”

Wood as hygroscopic, heterogeneous and anisotropic, organic material has some growth properties those act adverse to service life. Over the last decades, wood modification has been investigated as a process to improve the resistance against wood-degrading fungi, to increase dimension stability, and also to improve the weathering stability. The improvement can be achieved by a thermal or chemical alteration of the wood structure (Militz et al 1997 / 2005, Mai et al 2004a / 2004b, Krause 2006, Larnøy 2006).

Subsequently in the project description the focus will be on various silane pre-treatments. Organo-silane impregnations require the presence of an organic group attached to the silicon atom that may be inert, or may be capable of participating in a chemical reaction either with the cell wall polymers, with other silane molecules or with a co-additive (Mai 2004b). It is often assumed that the hydrolysis of methoxy, or ethoxy, groups of the organo-silane produce silanols, which then react with the cell wall (Hill 2006). However, it is important to note that, even if such a reaction takes place, the Si-O-C bond between the silane and the cell wall polymeric groups can be hydrolysed very easily, resulting in the regeneration of silanol. The resistance of the organo-silane to leaching by water then relies upon either bond formation with the cell wall polymers mediated by the organo-functional group, or due to entanglement of the silane polymer within the cell wall matrix (Schneider et al 1985, Hill 2006).

## Project description

Title of the project: “Improvement of dimension stability and other selected material properties of extruded Wood Polymer Composites”

Within this project already established modifications methods from solid wood shall be transferred to the use in Wood Polymer Composites; specially N-methylol-compounds, silanes, silicons and waxes for pre-treatment.

With the modification of the wood particles the following properties shall be enhanced:

- dimension stability
- resistance against basidiomycetes
- resistance against UV-light / weathering
- mechanical properties, substitution of coupling agents

In the first step, screenings on the effectiveness of the modification agent regarding process ability (extrusion) will be done. The modification agent with the best result due to the tested properties will be used for further experiments and tests. Untreated wood particles with and without coupling agent will serve as reference; the coupling agent is powdered MAA. Additionally, biological investigations will be done, in particular on the resistance against fungi and other destroying micro-organisms. As final step, up-scaling from laboratory scale to industrial scales will be done.

Various properties will be tested regarding the following standards:

- Physical-mechanical properties
  - Bending strength, Bending-MOE DIN EN ISO 178
  - Impact bending strength ISO 178
  - Tensile strength, Tensile strength-MOE DIN EN ISO 527

- Water uptake, Dimension stability DIN EN ISO 62
- Biological Durability
  - Resistance against basidiomycetes in Agar-Blocktest
  - Resistance against soil fungi (*Moderfäule*) ENv 807
  - Resistance against mold and stain BS 3900
- Weathering
  - Natural weathering EN 927-3
  - Artificial weathering using fluorescent UV-light and water EN 927-6

At present the project is ongoing and first results and cognitions will be considered in the planning of further experiments. The project duration is until May 2010.

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**Integration concept of Non Timber Forest  
Products (NTFP) into Multiple Use Forests (MUF)  
systems: a strategical workflow based on a case  
study from Hainan Island, P.P. China**

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## Abstract

Main development goals for forestry in China are characterized through 1) enlarging forest land cover, 2) protecting remaining ecosystems and related resources and 3) ensuring productivity and multifunctional uses. However, these fundamental issues are competing with A) the growing demand for biomass and forest related products, B) intensified land use change due to growing urbanisation and C) and the fight against desertification/land degradation. Therefore the establishment of a sustainable multipurpose forest management - and its related resources - is crucial for rural livelihoods to keep socioeconomic demands with ecological needs sustainable and balanced.

This paper discusses a strategical workflow to identify components of a flexible Multiple Use Forest (MUF) management, and shows a framework strategy within changing environment and market situation in China. The focus is set on management options carried out for a case sensitive pine (*pinus caribibaea*) plantation conversion management in Baisha, Hainan, which is aiming on balancing the social- and economic demands with natural resources options and ecosystem services.

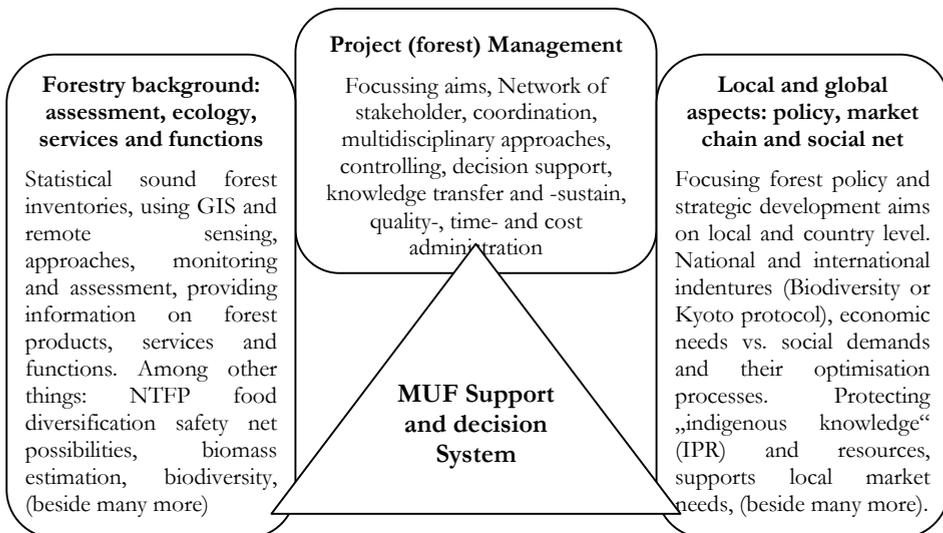
**Key words:** Multiple Use Forestry, Hainan, China, Non Timber Forest Products, NTFP

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## Introduction

This paper gives a first view into an integration concept of Non Timber Forest Products (NTFP) into a Multiple Use Forest (MUF) management approach. Therefore a MUF support system is introduced figuring out the different frameworks it is implemented in and pictures out an optional integration process into a strategic oriented management plan - balancing intensive forest management with surrounding socioeconomic demands. This is based on the idea that on the one hand sustainable decisions need basic and sound data from inventories to carry out informed decisions and systematic management approaches. But on the other hand and not less important any management approach needs a view on the political, socioeconomic and scientific- cultural conditions for a sustainable and long viable decision making process. This combination might bridge the gap between the information basis and decision makers, between policy and international indentures, scientific determinations and the actual needs of local people and global economy. From this point of view the awareness and definition of different needs or aims of involved stakeholders is a useful information basis for an agreed and embedded decision making process. Finally this should result into a continuously monitored strategic planning and implementation process using a permanent adjustable workflow.



**Figure 6: A Multiple Use Forestry (MUF) triangle: implementing Project Management as basic tool for communication, strengthening confidence of stakeholders and assuring sustainable decisions regarding quality, time and cost efficiency.**

A strategical workflow is defined as “predefined activity”. To predefine the Activities and communication interfaces, stakeholders or working environments have to be specified like in figure 1. It shows a Multiple Use Forestry triangle, where local and global aspects, forest information basis and management strategies are combined within a support system, which follows the assignment of supporting all three basic fundamentals of an informed decision making process. In this case described with forest **inventories** and communication processes, focused aims and their continuously assessment (forest **monitoring**) and possible management options for a number of planned scenarios. Socioeconomic observations (**household/stakeholder** or **market surveys**) give additional information about the use, the need and the impact of optional MUF scenarios.

## Methods

The data collection was taken out within a sampling plot survey. In this paper information are presented gathered from the pine forest plantation (*pinus caribaea*) in Baisha country, Hainan province, PR China. The area covers around 16.000mu (approximately 1000ha). The height of this watershed area is about 400 – 800m. Supplemental forest uses and also possible threads where observed and evaluated. Expert interviews and several visits on Baisha markets and pharmacies completed a database of 30 different NTFPs. Forest services and several indigenous tree species had been verified. The overall combination of data is supposed to be discussed for forest management options in more details within the PhD project. The following inventories had been taken out:

### Strip transects

For a basic information pool as source for the pine plantation conversion management, strip transects (2 metres each side) had been laid downhill through the plantation. Starting from the hilltop, information from the indigenous tree species changing along an altitudinal gradient had been collected. More than 20 different confirmed indigenous tree species provide a database which documents, that indigenous tree species were able to “invade” into the pine plantation and may now build a basis for a forest conversion strategy.

### Plot inventory

After defining a random start point the rectangular sampling points where laid in a row downhill into the plantation with a continuous distance of 50 metres to each other and a lateral length of 6\*6 trees. The corner of the plot was defined as starting point and data where collected. This defined due to the plot design the centre of the plot afterwards. In the centre fish eye photos and soil information where carried out, and one up to two trees where regularly sampled with borings for tree ring calculations. Each plot was defined by GPS position. In the pine area

the trees were numbered from the resin collectors. This made the retrieval of data plot information for the second year quite exact. Orientation and ground inclination was recorded and from all trees DBH and height was measured with a two or five meter scale stick and calibrated by laser height measurements. Additional Bitterlich sampling was carried out from the centre of the plot. The data will be analysed regarding the following stratification, e.g.:

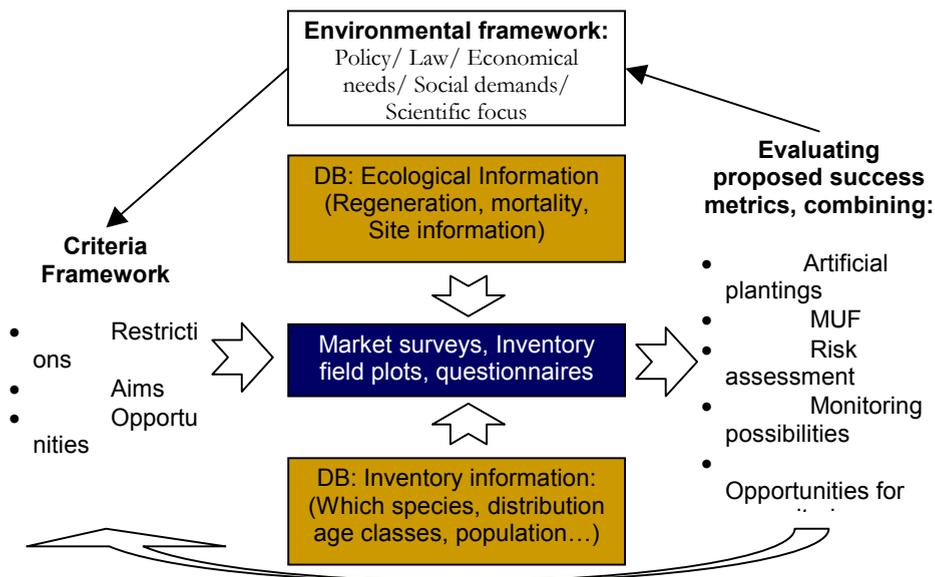
- Orientation north/south (exact orientation measured per sample point and assigned to a north or south direction to get stratified results), averaged tree distances within the sampling plot
- Natural stratification by age classes
- Site information like hilltop, middle hill (steepness measured), downhill
- Soil information (superficial like type and texture, some documented by photograph)
- Forest mountain views (photographs) for biomass prediction
- Aerial photographs (tm 2002, tm 1998) for change detection purposes, in preparation, additional several GIS related data provided by the Hainan Forest Bureau
- Plantation data from Hainan Forest Inventory as an additional information basis
- Weather data from Baisha country for boring correlations

56 square plots with 6\*6 trees had been established in the pine area, repeated by 30 square plots one year after.

## **Study Framework**

### Communication framework

The working and communication process can be seen in figure 2. Around the main focused thesis work of this project, using inventory, assessment and the depiction of optional forest management planning, the surrounding frameworks named “Environment”, “Criteria” (restrictions, aims and opportunities) and the proposed output “success metrics” are defined. It shows a self adjusting process where outputs are used to refine environmental and criteria frameworks for a continuously optimisation process, which will be part of further observations.



**Figure 7: The decision process**

Over all it can be seen, that information are derived out of active surveys and passively provided by existing ecological or forestry databases. They are building the frameworks, where policy or social demands are asking for a special character of the forest resources, may it be strictly (socio-)economical, ecological or something in between. This lead to feedback situations, where environmental, criteria or evaluating frameworks are influencing decision processes and possible management options.

Environmental framework

### China

Several overviews about the difficult environmental situation in China can be found (STERNFELD, 2006; BOHNET 2008). China is in major parts aware about it, like Wen Jibao pointed out again on the 10. Peoples Congress. The environmental protection is seen as major part for sustaining biodiversity in China. But predominantly for this the technical development for nature protection is meant. In fact, many environmental problems could be solved via improving the technical equipment, may it be in the area of air or water pollution control. But regarding to Wen Jibaos postulation, MUF is the consequent forest management approach to be intensified for the upcoming challenges.

Due to the strong economical development in China, the demand for biomass is rapidly growing. However, FAO (2007) reports an increase in forest cover from 157 million ha 1990 to 197 million ha in 2005 (21.2% land cover). Meanwhile China owns the largest plantations worldwide, and the expressed target of a 29% forest land cover in 2050 predicts further strong growing plantation forestry in future. But parallel to this the remaining land suitable for agriculture and forestry decreases due to land degradation, desertification (UNCCD, 2006; KUCHELMEISTER, 2006) and the expansion of cities and industries (LU et al, 2005). The enlargement of forest cover is a common tactic to fight against soil degradation and desertification. For this purpose several afforestation programs had been established. As described by the UNCCD, China is doing a great progress in this field.

One side effect of the strong growing economy, the relative economic importance of agriculture and forestry is more and more declining (2007 12% of the total GDP: NATIONAL BUREAU OF STATISTICS CHINA, 2007). However, 800 million people are still living in the countryside, and about 50% of them do directly depend more or less on agriculture, forestry and their products. Both production fields in China are on reviewed from a great scale still not efficient compared to intensive agriculture or forestry in Europe.

From the forestry point of view, China can still be considered as a country with insufficient stocking volume per capita (0,128Ha per capita, 0,62 Ha per capita on global level. FAO, 2006). This has truly to be seen in an environmental and historical context (1/3 of china consists of mountainous region, approximately more than 11% are deserts) (harvesting impacts of the great leap forward and several intensive felling periods). Political and law criteria for forestry development in China are mainly based on the “Natural Forest Protection Program” (NFPP) (SHEN et al., 2006) and “The Sloping Land Conversion Program” (SLCP) (BENNET et al., date unknown; TRAC et al., 2007). These Programs are told to be the world largest ecological rehabilitation processes, and so many ecological, economical and social impacts can be observed. But despite them several other Programs take effect on environmental development in Great China and Hainan Island:

- NFPP (Natural Forest Protection Program)
- SLCP (Sloping Land Conversion Program)
- CCFP (Conversion of Cropland to Forest Program)
- FGHY (Fast-Growing and High-Yield Plantation Project)
- Sandification Control Program for areas in the vicinity of Beijing and Tianjin
- Key Shelterbelt Development Programs e.g. in the Yangtze River Basin
- Forest Industrial Base Development Program
- The Wildlife Conservation and Nature Reserves Development Program

On a global perspective, the most destructive artificial impacts on Chinese forests are the damage of forests (e.g. in China acid rain, water contamination, see also the “green GDP” discussion (LIU 2007, JING, 2007), which lowers the GDP around the environmental damage rate), land use changes (LI et al., 1997) and still rising biomass demands. Consequently reducing or turning land use change into an opposite direction and additionally fulfilling the future biomass demand are beyond the first targets sustainable forestry aims at. However, due to the logging ban in China its global impact on the forestry situation is still strong, leaving great social-economical and ecological impacts within China (e.g. job losses (SHAO 2006)) and its neighbouring countries (illegal or too intensive large area logging, FAO 2001). Because of this huge supra-regional imbalances of timber use and production a Multiple Use Forestry (MUF) management - simultaneously focussing on the support of environmental services, conservation and additional income for rural people – has to be installed for sustaining the rural fundaments and future biomass needs in China for its economic development in its own homeland. Beside special protective goals this political and socioeconomic reason can be seen as a major incentive for the “use it or loose it” maxim of natural resources (CHOPRA, 1993; NEUMANN et al, 2000). Many Non Timber Forest Products (NTFPs) are commonly used in this manner. Additionally the field of services provided by forests (soil protection, water or CO<sub>2</sub> storage etc.) ought to be typically inserted into MUF systems to generate a permanent supra-regional “pay back”.

### Hainan

The key area focused on in this study is located on Hainan Island, PR China. The geographical position from Hainan is between 18°10' and 20°10' north and 108°37' and 111°03' east at the same latitude as Hawaii. The climate of the island can be described as monsoon tropical. The average temperature range is between 22°C and 26°C with an annual rainfall between 1500mm and 2000mm. The heaviest rainfall can be detected with up to 300mm in September. The topology is mountainous (Wuzhi (1867m) Limu (1412m)), so that mountainous monsoon forest can be found on the 33.940 square kilometres wide island.

The population estimations for Hainan range from 8 million people (7.25 mill. registered) up to 10 million (DAVIES et al, 2007, EVAN et al., 2007). One uncertainty is about the amount of ethnic minorities living in the inner mountainous part of the island. 800.000 people are often guessed in literature, mainly coming from the Li and Miao tribe. The case study was taken out in Baisha country, which is a centre for the Li minority tribe. A pine plantation for Biomass production (paper mill) with about 1000 hectare has been set up 1995, and is nowadays used as well for Non Timber Forest Products (NTFP) production. On this basis the area is ideal to take out exemplary case studies to develop a strategic Forest Management (FM) combining economic demands with social- and ecosystem services.

The logging ban of the NFPP had a great economical impact on rural economy, also on Hainan Island (STONE, 2004). Established in 1994 the missing timber supply of indigenous tree species took effects on rural people in terms of job losses. Especially minorities like the Li or Miao people where former dependant on a steady income based on biological resources by using among other things the timber and non timber resources. The logging ban affected their rural economy badly, which included also obsolete investments in processing equipment and the following market chain.

Supported by subsidy, and assisted by community and government, especially the NFPP lead to plantation afforestations. Former grasslands, but also some high productive agricultural land has been converted to fulfil the aims set by the program. Within a fast process and based on a lack of knowledge and experience, these plantations were often set up inappropriate and achieve nowadays not the optimal return of investment. Unclear property regulations and forest policies, missing sustainable forest management tools, and without an anticipatory risk assessment the need for a change in plantation management to sustain yield and income is obvious. In addition, a sustainable management should comprise the main issues of the Eco-province initiative, which was formulated from the HAINAN PROVINCIAL PEOPLE'S CONGRESS (1999) with basic aims for developing Hainan Island, and additional functions described by foresters during the conducted interviews:

- Prevent forest area from declining on the whole island
- Fight against degradation, biodiversity loss, inshore sand aggradation processes
- Improve plantation output and rotation cycles used mainly for biomass production (e.g. for paper industry, furniture)
- Consider the interests of minorities (mainly Li and Miao)
- Support rural livelihood
- Protect undisturbed forests
- Cultivating NTFPs into established plantations with no optimal output so far. Focussing on multifunctional (e.g. watershed protection or job providing) uses.

#### Scientific focus: NTFP

A focus of this study is set on NTFPs. To define NTFPs we follow the concept of the FAO (UNASYLVA, 1999) as „*Non-timber forest products consist of goods of biological origin other than timber, derived from forests, other wooded land and trees outside forests.*“ Wickens (1991) defines it as “*all the biological material (other than industrial round wood and derived sawn timber, wood chips, wood-based panel and pulp) that may be extracted from natural ecosystems, managed plantations, etc. and be utilized within the household, be marketed, or have social, cultural or religious significance.*”

A common problem while working with NTFPs in science or in the field is that many authors and workers follow several definitions and many expressions. All these used terms cover different aspects based on cultural or scientific background. Species and products may be over all inconsistent defined according to the working focus of the author or organisation (UNASYLVA, 1999). Hardly ever clear definitions of NTFPs and their observed frameworks are explicitly given. More seldom the ecological niche, use and market chain, social impact and management options are coincidental described for each identified species. But MUF approaches are always in the need of up-to-date information about current target species. This might derive changed management options which have to be considered. Only a permanent control of these “use and need” balance on local level can work out for a sustainable management, comprising changing market, cultural and ecological situation.

In fact, focused on timber production this concept is not new and a well known and basic stimulation for forest optimisation management processes. But carried forward on NTFP and MUF concepts the balancing strategy becomes ambitious. In this case it is always indicated to take an overlook over the situation by collecting and stratifying stakeholder interests, resource needs and the associated ecological situation, socioeconomic demands and a number of available management options to optimise the socioeconomic and ecological output in a changing environment. The need is to clarify these variables for each single focused species or stratified foci in MUF management to find the best output options correlating with realistic political or socioeconomic demands. Therefore concepts of MUF systems must be truly flexible, adjustable and have to follow multidisciplinary and highly communicative approaches to implement the overall aim of sustainability. The more aims are defined, the merrier complicated these concepts become. This implies that MUF concepts can become easily ambitious tasks for forest planning and management.

However, especially the market impact of established NTFP production in the forest is often overrated. But it has without doubt under certain conditions a huge impact on local economy. There are numerous examples of special NTFP (Truffles, medicinal plants...) or global marked trends (ecotourism, bio fuels from trees), where on the one hand the demand of the resource exceeds its local biological production or natural regeneration possibility by far. This results into imbalanced markets, where the demand exceeds the sustainable use of the resource. Then prices explode the nearer the resource becomes to extinct, if sufficient cultivation fails. In this way products may disappear from local markets and may only survive as a luxury good if at all.

On the other hand diversified NTFP production can expand the income possibilities beside the timber production and fosters a general improvement in rural areas. They can over all be just one component for lowering rural migration and rising living conditions. This can be benchmarked by accounting all positive local side effects of NTFP use or supported forest services upwards from local to

global level. This means e.g. regarding impacts of biodiversity protection, created pay back systems for environmental services (e.g. the carbon sequestration discussion, ecotourism) or the realistic estimation of the rural outcome possibilities, which are economical hard to estimate and often under represented within an estimated “every days use of (wild) resources” for self subsistence (NEUMANN, 2000).

#### Scientific focus: MUF

MUF is defined as a concept of forest management that explicitly combines two or more overall objectives and tries actively to achieve the best results within the stated management goals (FAO 1960) This concept is not exclusively focusing on economical improvement (EKER, 2007). It makes no differences whether these objectives are applied on a specified biological production or is addressed on boosting environmental services. Over all it includes beside many more aspects e.g. the sustainable management of Non Timber Forest Products (NTFPs), optimized biomass production, support of forest and environmental services as well as biodiversity protection. The oppositional concept focuses only on one aim, e.g. high intensive biomass production in plantation areas.

To balance biomass production with the needs of economy and rural people a constitutional change in plantation management has to be aimed at. The common optimization of plantations regarding rotation cycles, use of (local) adapted tree species or improved biomass production are fundamental and basic approaches, where practical research is needed. But it only will take positive effects if these approaches can be integrated within sustainable long term approaches, considering the protection of ecosystems and ecological service functions on the one hand and aspects of socioeconomic development and policy on the other. To assure positive long term effects on the one hand only coordinated approaches using qualitative sufficient inventory information are needed, based on the principle of good decisions need high qualitative information. Beside technical inventory challenges the information collection should follow the questions of 1) which precise and preciseness of information are needed, 2) what for and 3) how to statistical and analytical interpret and implement results into existing management systems for a long-lasting viability.

Because of this heterogeneous background MUF systems need effective and transparent management planning tools for decision making. The integration of many different stakeholders with their own forest development aims, quality management and social-economic demands need often high communicative skills. Sustainable solutions need approaches from local, technical and specialised forestry knowledge side and are embedded within forest policy and law enforcement, where management decisions have to be implemented. This describes the multidisciplinary influences over MUF systems.

## Criteria Framework

### Aims

The overall aim of a sustainable forest management is a balance between economic needs, social demands and the ecosystem requirements. Based on this several aims and principles can be defined and expressed over a “cross of aims”, table 1. It can be easily adjusted and gives a compacted overview over aims and the framework situation.

**Table 2: The “cross of aims”, showing the relationships between projected aims and involved stakeholders and how the expected results should be measured as success.**

Projected aims	Stakeholder
<ul style="list-style-type: none"> <li>• To optimize biomass output and rotation cycles of Plantation Forests</li> <li>• To detect local species as substitution of plantation species</li> <li>• Identification and further implementation of NTFP in plantation management</li> <li>• Correlated aims: environment protection, support for minorities, better decision support system and planning tools</li> </ul>	<ul style="list-style-type: none"> <li>• Hainan Forest Bureau</li> <li>• Chinese academy of forestry (CAF)</li> <li>• Locals for entrepreneurship</li> <li>• State administration</li> <li>• Minorities</li> <li>• Tourists</li> </ul>
<ul style="list-style-type: none"> <li>• Optimized management plan for the plantation area</li> <li>• Depiction of optional plantation management and strategies</li> <li>• Definition of strategically aims for sustainable forest management</li> <li>• Options for plantation conversion</li> </ul>	<ul style="list-style-type: none"> <li>• Rehabilitation and preservation of close to nature forests and plantations with indigenous trees</li> <li>• Integration of findings into forest management</li> <li>• Reduction of migration into cities</li> </ul>
Expected results	Success metrics

This case study focuses on 1) the development of forest management options for converting pine plantations into close-to-nature forests 2) sustaining NTFP yield and income for rural people and 3) showing management options for a multiple-use forest management. The following points need to be researched into and optimized for the situation on Hainan Island:

- Specification of indigenous tree species suitable for plantation forestry

- Plantation conversion
- Strengthening additional forest services and biodiversity
- A sustainable and simultaneous use of wood, Non Timber Forest Products (NTFP) and social services
- Opening up possibilities for income and viability for the local rural poor.

### Restrictions

Basic restrictions will be given within the project report.

### Opportunities

Opportunities are seen in a sustained multiple usability of Hainan forest resources in combination of enhancing biodiversity and solid ecosystem functions. The output should balance the triangle of ecology, economy and social needs towards forest resources. The opportunity outlook is either part of the project report.

### Findings and inventory databases

The ecological Databases carried out during the inventory will be deeper analysed within the doctoral thesis. Therefore ecological Data will be attached and cross linked with soil and forest stand information, regarding self recorded forest inventory data and remote sensing information. The aim is to identify areas, where the ecological niche of the identified NTFPs and observed forest services may fit into the actual situation to propose management options for a more detailed schema like proposed in Chapter 4.

### Identified NTFP

It is quite clear that beside the here listed NTFPs many more can be found and defined, especially because of 1) the long Chinese cultural and traditional history in using natural resources for many purposes, 2) Hainan owns naturally high divers flora and fauna 3) and scientific discussions and the development of forest services and products pay more and more an important role, as comprehensibly defined within the environmental development goals for the “Hainan Ecoprovince initiative” (DEPARTMENT OF LANDS, ENVIRONMENT & RESOURCES, 1999). Under the time and accessibility limitations this list can be at a first glance on the current situation and function as pilot study. It gives a first view inside the current multifunctional uses by the local people in the considered area of Baisha, where concrete management options for these MUF aims can be designated. On the one hand this means the following NTFPs, which have been artificially introduced with existing market chains:

- Pine resin collecting
- Rattan (*Daemonorops margaritae* and *Calamus tetradactylus*) (LI et al, 2007)
- *Alpina katsumadai* as a medicinal plant, but also as ornamental plant usable

- Silvopastoral uses (grazing etc.)
- Home garden systems
- Agroforestry systems using gum resin, tea plantation and diversified

And on the other hand, table 2 shows the NTFPs found during the field inventory in the Baisha pine plantation. Some more observations were added during market surveys and during visiting several households. Additional services and functions, whose importance was stated out during expert interviews, are additionally added.

**Table 3: List of identified NTFP and services**

No.	Name	No.	Name
1	<i>Lingzhi</i> mushroom	16	<i>Cantella asiatica</i>
2	Bamboo	17	<i>Hedyotis diffusa</i>
3	<i>Alpinia katsumadai</i>	18	<i>Arillus Longan</i>
4	<i>Litsea cubeba</i>	19	<i>Semen Arecae</i>
5	<i>Ganoderma lucidum</i>	20	<i>Eriobotryae Folium</i>
6	<i>Dilleniaceae pentagyna</i> Roxb	21	<i>Fructus Quisqualis</i>
7	Honey	22	<i>Aquilariae Agallocha</i>
8	<i>Daemonorops margaritae</i>	23	A Wood Chip mixture
9	<i>Calamus tetradactylus</i>	24	“Indigenous knowledge”
10	<i>Gossampinus malabarica</i>	25	ecotourism
11	<i>Haemanthus albiflos</i>	26	hunting
12	Several tree fern	27	Firewood
13	<i>Lantana camara</i>	28	resin market trends
14	<i>Schefflera octophylla</i>	29	Low value NTFP
15	<i>Loranthaceae</i>	30	<i>Millettia reticulata</i>

#### Risk Analysis:

During the survey some observations about possible risks have been recorded. They always have to be taken into consideration for optional management plans and their impact on each aspect of the MUF system must be evaluated. E.g. storm damages may certainly have negative impacts on infrastructure (cost factor) and biomass production. On the other hand first observations in the pine plantation indicate, that damaged or uprooted trees get easily and naturally replaced by *Liquidambar formosana*, which is a faster growing light demanding tree species with economical interesting timber growth abilities and additionally assertive against the Grass *Miscanthus floridulus*. Parts of the plantation are therefore facing a segmental slight transformation due to the more or less common storm events. This forest situation can be used as initial seed for MUF.

Further Risks for forestry can be accounted as follows:

- Storm damages (Hainan situated on the “street of typhoons”)
- Pests, fungi, grass (*Miscanthus floridulus*), Asian Longhorn Beetle (ALB)
- Regular fire events
- Mutual disturbing NTFP use and production (e.g. Rattan leaves being cut during daily pine resin collection because of their thorns)
- Knowledge transfer (how to tread and market NTFP if job losses in forestry lead to rural migration?)
- Ownership and policy

### Identified tree species

The tree species identified in the Baisha forests are quite diverse and give only a glimpse on the actual biodiversity of Hainan Island. Because of the special island situation several endemic species are reported. Because of this it makes sense to identify concrete ecological niches of the favoured species and overlap this with the local current situation.

**Table 4: List of identified tree species**

No.	Name	No.	Name
1	<i>Albizia procera</i>	14	<i>Ficus chlorocarpa</i> Benth
2	<i>Antidesma ghaesembilla</i> Gaertn	15	<i>Lannea coromandelica</i>
3	<i>Aporosa dioica</i>	16	<i>Machilus chinensis</i>
4	<i>Bombax malabaricum</i>	17	<i>Melastoma candidum</i>
5	<i>Breynia hyposauropa</i> Croiz.	18	<i>Melastoma Sanguineum</i> Sims.
6	<i>Bridelia insulana</i> Hance	19	<i>Miscanthus floridulus</i>
7	<i>Callicarpa nudiflora</i>	20	<i>Phyllanthus emblica</i>
8	<i>Catunaregam spinosa</i>	21	<i>Radermachera sinica</i>
9	<i>Cratogeomum ligustrinum</i>	22	<i>Syzygium cumini</i> Skeels
10	<i>Engelhardtia colebrookiana</i>	23	<i>Thysanolaena maxima</i> (Roxb.) Kuntze
11	<i>Ervatamia divaricata</i>	24	<i>Zanthoxylum ailanthoides</i>
12	<i>Euodia leptota</i>		
13	<i>Eurya nitida</i>		

### Inventory data

Inventory information will be further discussed as core output of the doctoral thesis.

### Questionnaires and observations: Further information pools

Beside direct household observation and market surveys in Baisha (local market, pharmacies) and 2 bordering villages several expert interviews conducted from forest officers and professors had been carried out. A broader overview was given during excursion to Agroforestry areas near by Haikou (similarities observed to

the “Taungya” system (MENZIES, 1988)) and eco tourism sides at the Wuzhishan Mountains (WEN, 1998; Li, 2001). Further information pools were provided by the Hainan Forestry Bureau and will be an important part of the analysing process.

## **An optional case sensitive multipurpose forest management approach for pine plantation conversion**

The conversion of plantations into MUF management is following three main targets: i) to higher biodiversity for sustainability reasons, ii) to generate and use ecosystem services and new introduced income possibilities and iii) to follow general social and political even higher aims defined within the framework considerations made in Chapter 3.

In this situation, sustainable management of renewable natural resources is a key for the development of rural areas. The combination of indigenous timber tree and NTFP production (in a broader sense also the impact on water, soil, wildlife and others) take direct effects on the social functions of forests. Forest provide living space, supply for daily live and income. On the long run a successful forest management has to combine these issues to guarantee and sustain yield, income and basic living conditions. For the pine plantation in Baisha case sensitive management options are suggested for discussion, covering plantation enrichment (A, B), plantation conversion (C, D) and disaster situation (E) (SPRENGER, 2007).

### A NTFP Enrich Situation:

Basing on already established NTFPs within the pine area, other NTFPs have to substitute in future dropped out ones (like pine resin collecting as a slowly replaced tree species, C,D), or new introduced if a market chance is predictable. Planting NTFP trees along easy to reach and harvestable road sides, e.g. *Gossampinus malabarica* (for pillow filling), *Schefflera octophylla* (medicinal trees) etc. will provide the area with biodiversity and utilizable functions. Due to world wide market possibilities already established Rattan plants can be fostered. If pine resin collection drops out the mutual disturbance may be reduced.

### B Timber production Situation:

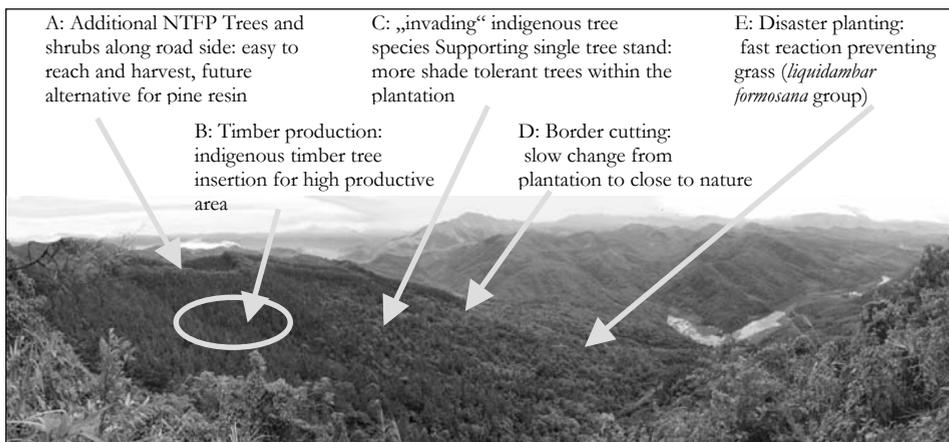
High productive pine areas can be found out during the slight border cuts (D). Areas, where the pines are in a comparatively good condition a substitution with timber trees, e.g. a mixture from *Acacia mangium*, *Liquidambar formosana* etc. can be used for production and reestablish possible small timber entrepreneurship. This should be seen as environmental, but also as economical investment to support rural development.

### C Slow transforming Situation:

To prevent the pine plantation from being clear cut, a single indigenous tree protection and promotion has to be established. In practice- if necessary - an oppressor, mainly nearby pines, has to be cut. The aim in supporting already under the pine trees naturally established trees is to achieve a “seed” tree cover. It protects the area from overgrowth with grass during light changes. Furthermore, the probably more shade tolerant trees provide a first source of biodiversity within the plantation.

### D Slow transforming Situation:

It is suggested that the pine plantation achieves a slight border cut to prevent clear cut. This can also be done within the plantation if small areas get opened by disaster events, and some kind of indigenous tree is supposed to be strong enough to survive this changed situation (or combined with enrichment planting, E). After some time where the edge trees can adapt to the new situation the slight border cut can start over. The aim is to give the left indigenous trees time to adapt to the new light situation and to assure, that the borders will not get destabilized.



**Figure 8: Condensed illustration of the discussed pine plantation conversion options**

### E Disaster Situation:

The disaster situation is owing to the risk management which suspects further destructive impacts on the pine plantation. Opened space from storm/fire/pest events do have often problems with fast growing grass. These high competitive species like *Miscanthus floridulus* often inhibit natural tree growth. In this situation an urgent reaction is appropriate to prevent the grass from growing. Enrichment planting with light demander or pioneer trees (eg: *Liquidambar formosana*, an indigenous tree species with good timber attributes) would be a suitable reaction.

In this situation two starting possibilities are supposable: to plant small trees (ca. 30cm height) is cheaper, but demands further costs and maintenance, e.g. cutting grass. The alternative would be planting bigger trees in acceptance of higher first investments.

## Discussion

The advantages of the suggested optional management system will be mainly discussed within the PhD project. But over all the following points are indicated and merged as follows:

- The ecosystem impact of the plantation conversion will probably carry out enhanced forest services
- It supports NTFP biodiversity within a “use it or loose it” framework
- Generates income after the pine plantation (resin collection) will be removed.
- On a longer run the established timber production areas will provide the rural people with timber resources, so that former working fields of furniture production can be reactivated
- Probably better disaster protection than provided by an even aged homogenous pine forest

One main task is the establishment of a proper knowledge transfer in two meanings. First, because of the logging ban workers if not migrated often come from the former timber industry. They have to be trained how to handle the NTFP (e.g. when to harvest, how to harvest without damaging and with highest yield, etc.) Another uncertainty in the opinion of the workers might be the question, if the NTFP has a market chain over the local market or additionally a market abroad, so that local markets will not get flushed by NTFP products. Second, forest management and policy will become more complex, so that trained foresters have to take more and complex decisions than in a pine plantation situation.

A key sentence regarding the rural development of Hainan was found in STONE et al (2004). In the opinion of the author the sentence has to be extended as follows (added words in bold):

### **To sustain rural development**

*the use of locally made goods and services and the employment of community members whenever possible*

**combined within a diversified market chain and supported by multiple use forestry**

*is absolutely critical for biodiversity and to generate and retain benefits in the community!*

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# **Turbulent fluxes in an Indonesian rainforest. The eddy-covariance method and the role of advection.**

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## Introduction

Energy- and matter-fluxes in tropical landscapes are – in the case of CO<sub>2</sub> and H<sub>2</sub>O – an important part of global biogeochemical cycles and global energy-budgets. At the same time there is till nowadays comparatively little knowledge about these aspects of tropical rainforests and tropical agricultural landscapes. The special research area “STORMA” /”Stability of Rainforest Margins in the Tropics”, a joint German-Indonesian research project under participation of Göttingen University, tries to fill these gaps with comprehensive interdisciplinary research at the margins of Lore-Lindu-National Park on the Indonesian island of Sulawesi.

CO<sub>2</sub>- and H<sub>2</sub>O fluxes are strongly influenced by the kind of landuse and vegetation and clearing of forests will thereby alter the capacity of a landscape to store and circulate carbon as well as water with far reaching influences for the climate of a whole region. The same is true for the energy budget as albedo is changed by altering vegetation and heat fluxes of latent heat depend strongly on the amount of stored water; so fluxes of sensible heat will normally raise after forest clearing (Ibrom et al., 2007, Olchev et al., 2008).

There is different techniques developed for the assessment of energy-fluxes and matter-fluxes of CO<sub>2</sub> and H<sub>2</sub>O, differing between more ground-based techniques to more airborne-tech-niques. The first ones often work on a leaf- and tree-based approach, e.g. measuring fluxes of a single leaf, the latter ones often integrate satellite imaging. A very successful technique situated in a way between these two approaches is the so-called eddy-covariance-technique (EC) with the help of ultrasonic anemometers, as these instruments are normally installed on a mast or on a scaffold above a certain type of vegetation like a wheat field or a forest. (Baldocchi et al., 2001).



**Fig. 1:** The main measurement tower at the site in Bariri rainforest (height: 70m):

In the STORMA-framework the Department of Bioclimatology uses the EC-technique on measurement towers inside and above the highland rainforest of Bariri, in the east of Lore-Lindu National Park. In the period between 2003 to 2007 measurements were done by a single tower (Fig. 1), in the period since 2007 with a triangle of three towers. The set of three towers was especially introduced for clarifying the role of the mean air flow (or advection) for the transport of  $\text{CO}_2$ , as this is a well-known problem to the EC-research generally (see below). As the new advection data from the first year of the three towers have only been processed so far, this paper will present the bioclimatological results from Bariri that could be achieved till end of 2007 and give an outlook to our expectations towards the new data.

## The Boundary Layer and turbulence as the basis of EC-research

With a height of about 1 km in average the Boundary Layer (BL) is the part of the tropo-sphere, that is in direct contact with the earth's surface, including vegetation-atmosphere interactions. It responds to surface forcings like heat- and matter-fluxes and frictional drag with a timescale of an hour or less and shows a diurnal variation of temperature. During the day the *Mixed layer*, usually driven by convection, dominates the whole height of the BL; during the night from the cooling ground a *Stable layer* will grow up, that will only show sporadic or almost none turbulence. Above the *Stable Layer* a *Residual layer* will exist till the morning; it is neutrally stratified and turbulence will therefore go in all directions.

Turbulence as a phenomenon can be described as gustiness that is superimposed on the mean wind; it consists of many irregular swirls called eddies. These eddies occur in many different sizes – from 3000 m to some millimetres - , which are superimposed on each other. Turbulence is produced for example by solar heating of the ground, causing large eddies to rise, or all kinds of obstacles to the mean air flow, causing frictional drag and wind shears. With regard to energy the largest eddies transport most of it, while in the sense of an energy cascade, the smaller ones feed on the larger ones till the most little ones vanish in molecular viscosity.

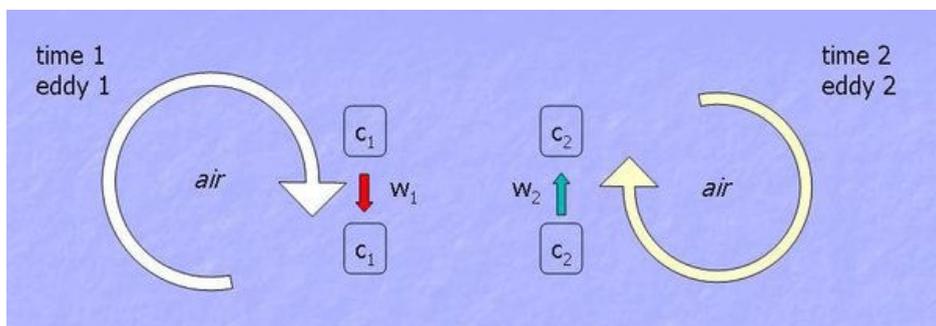
By looking on a windspeed record it is even visually possible to separate between a mean wind speed over a certain time interval and the peaks in the record showing the turbulence of wind. Different sizes of peaks occur in certain frequencies and give a hint on eddies of different extension and speed. Therefore we can as well evaluate this record in form of a wind speed spectrum with the portion of turbulent energy of specially sized eddies as function of their frequency. This spectrum shows an obvious spectral gap at about 1 hour of frequency: motions left of this gap can be associated with the mean flow, motions right of it with turbulent flow. Thus the spectral gap is the usual mathematical instrument for identifying and separating both kinds of flow (Stull, 1988; Kraus, 2004).

## The EC-technique

The EC-technique is based upon the measurement of the movement of eddies with the help of three-dimensional ultrasonic anemometers (USAT, Fig. 2). The USAT measures the speed, size and direction of a single eddy passing through while at the same time an infrared gas analyser (IRGA) mounted nearby will measure the contents of CO<sub>2</sub> and H<sub>2</sub>O of this eddy. By integrating over the time the amount of CO<sub>2</sub> or H<sub>2</sub>O leaving or entering the air volume below the USAT can be calculated (Fig. 3).



**Fig. 2:** Ultrasonic anemometer (Campbell CSAT3, left) and infrared gas analyzer (Li-Cor 7500, right) (Burba, 2007).



**Fig. 3:** At time 1 eddy 1 transports the concentration  $c_1$  downwards with the velocity  $w_1$ ; at time 2 eddy 2 transports concentration  $c_2$  upwards with velocity  $w_2$ . Integration over time gives the net-flux of  $c$  (Burba, 2007).

For correct measurements the EC technique is dependent on more or less homogenous terrain and a turbulent flux that is statistically stationary. Given these preconditions flux can be described by the covariance of the turbulent wind speed in the vertical ( $w$ ) and the concentration of an entity  $C$  (like  $\text{CO}_2$  or  $\text{H}_2\text{O}$ ) with the EC-equation (Falk, 2004):

$$F_C = \overline{w'C'}$$

Results should lead to a closure of the energy balance equation (Falk, 2004):

$$R_n - G - H - \lambda E = 0$$

$R_n$ : Net-Radiation

$G$ : rate of change of storage in the control-volume below the measurements

$H$ : sensible heat flux

$\lambda E$ : latent heat flux

Furtheron EC measurements are dependent on well developed turbulence with a friction velocity  $u^* > 0.30 \text{ m s}^{-1}$ :

$$u_*^2 \equiv \left[ \overline{u'w_s'^2} + \overline{v'w_s'^2} \right]^{1/2}$$

$u, v, w$ : velocities in x, y, z - direction

The measurement of respiratory fluxes at night is a major challenge, as stable atmospheric stratification at night is connected to low wind speed and thereby strongly decreased turbulent exchange. In this connection the friction velocity  $u^*$  is used as a measurement for the degree of atmospheric turbulence (the so-called “ $u^*$ -filter-criteria”) (Stull, 1988; Ross, 2007).

If turbulence is less developed advection, the transport with the mean flow, begins to play a major role and has to be integrated into calculations of mass and energy conservation. This lead from the Conservation equation in a general form (Kraus, 2004):

$$\frac{\partial \overline{\chi}}{\partial t} = -\overline{v} \cdot \nabla \overline{\chi} - \overline{v'} \cdot \nabla' \overline{\chi'} + \overline{Qu}$$

(the term on the left side as the mean local change of entity  $\chi$ , the terms on the right side as advection of  $\chi$  with the mean wind, as divergence of the turbulent fluxdensity of  $\chi$  and as sink or source)

To the Conservation equation developed by Aubinet (2003):

$$\int_0^{z_r} S dz = \int_0^{z_r} \frac{\partial \bar{C}}{\partial t} dz + \overline{(w' C')_r} + \overline{w_r (C_r - \langle \bar{C} \rangle)} + \int_0^{z_r} u \frac{\partial \bar{C}}{\partial x} dz$$

I          II                  III                  IV                  V

I: Source/Sink-term of CO<sub>2</sub> / NEE

II: Storage-flux

III: Turbulent flux

IV: Vertical advection

V: Horizontal advection

## Eddy-covariance results and the role of advection

Eddy-covariance measurements from October 2003 to February 2005 were used for the validation of a SVAT-model (*Soil-Vegetation-Atmosphere-Transport*, MixFor-SVAT), that was introduced to model the manifold landscape around the Bariri rainforest and a cacao-agroforestry system in the Northern part of the rainforest margin. SVAT-models model as well the carbon-exchange of the Soil-Vegetation-Atmosphere-System as the water-exchange, mostly in 1-D-manner for different layers of soil, vegetation and atmosphere (Olchev et al., 2008)(Fig. 4,5).

Comparisons of measured and modelled H<sub>2</sub>O- and CO<sub>2</sub>-fluxes were undertaken for a rainy and cloudy period from 03.02. to 13.02.2004 and for a dry and sunny period from 22.06. to 02.07.2004. Both methods showed good agreement with a coefficient of determination ( $r^2$ ) of 0.62 for CO<sub>2</sub>- and 0.64 for H<sub>2</sub>O-fluxes. Generally, as a daily cycle, H<sub>2</sub>O-flux (= latent heat) will be the highest around midday, when it is warmest and evaporation is high; at night latent heat flux is near 0 W m<sup>-2</sup>. For CO<sub>2</sub> the daily summit will also show at midday, but in this case negative, as CO<sub>2</sub> is flowing downwards in the direction of assimilating leaves (high rate of photosynthesis during the day). A second smaller summit for CO<sub>2</sub> occurs at night, as during night respiring plants and the soil will release CO<sub>2</sub>. All these peaks are stronger for the dry and sunny period, as sun and warmth triggers the described processes.

For the eddy-covariance-data data gaps at night are obvious: at night turbulence is often low and below the threshold of the friction velocity  $u^*$  ( $u^* > 0.30$  m s<sup>-1</sup>), so turbulence can not be measured. In this case even  $u^* > 0.15$  m s<sup>-1</sup> has been used (dark grey line), filling the data gaps to some extent (Fig. 4,5).

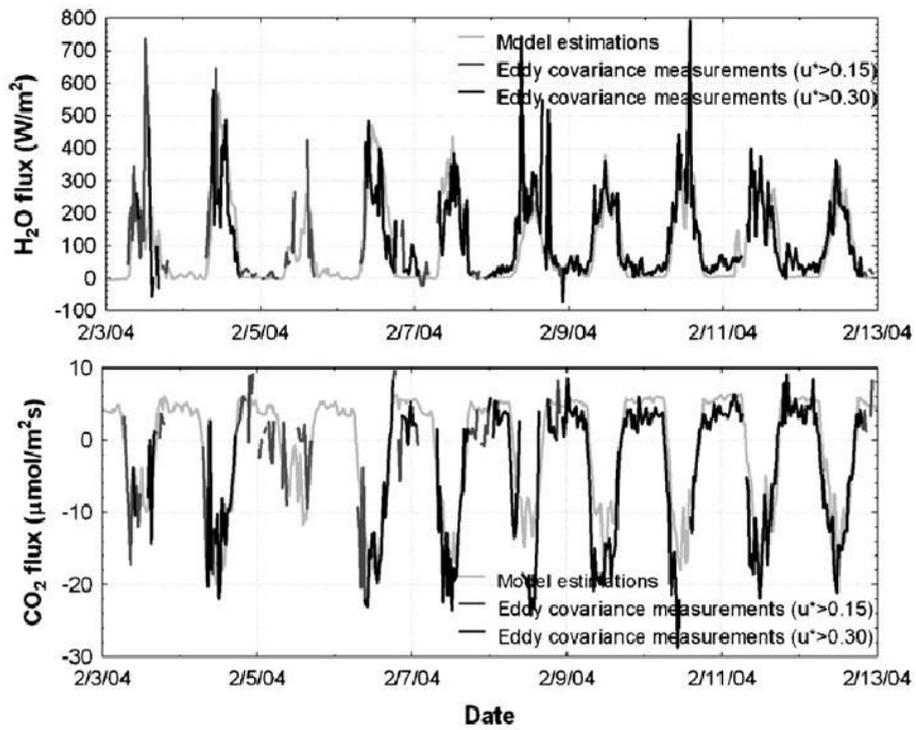
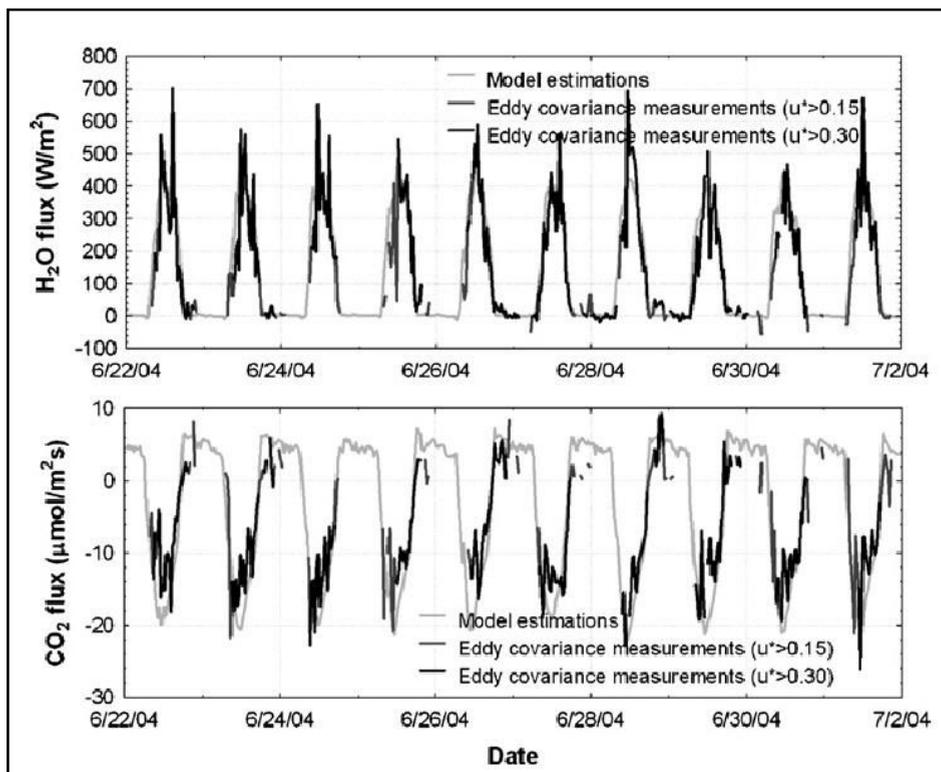
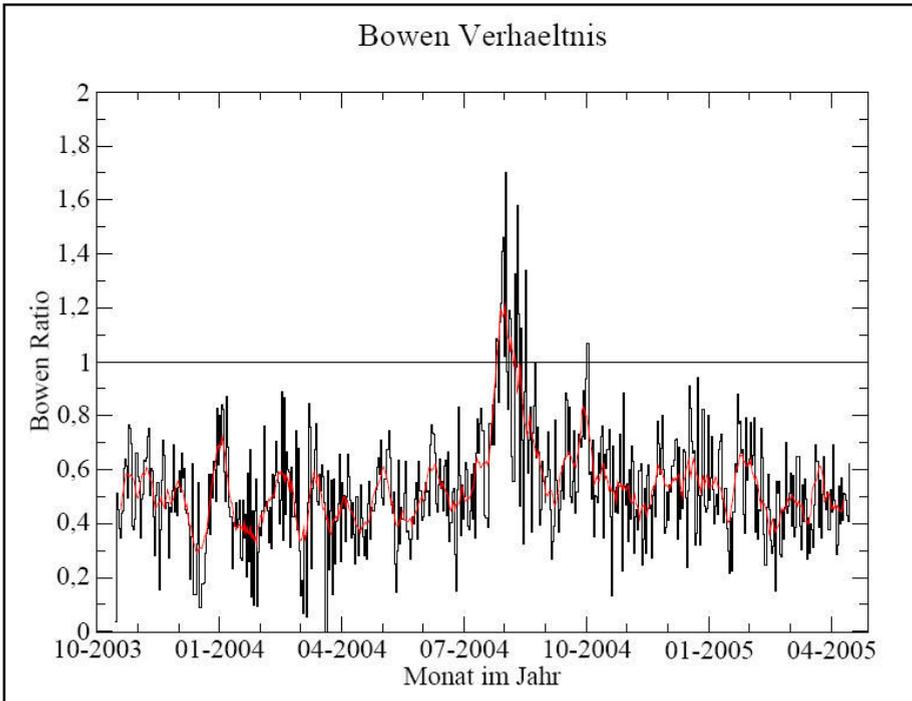


Fig. 4: Eddy-covariance measurements from February 2004 during a rainy and cloudy period (Olchev et al., 2008).



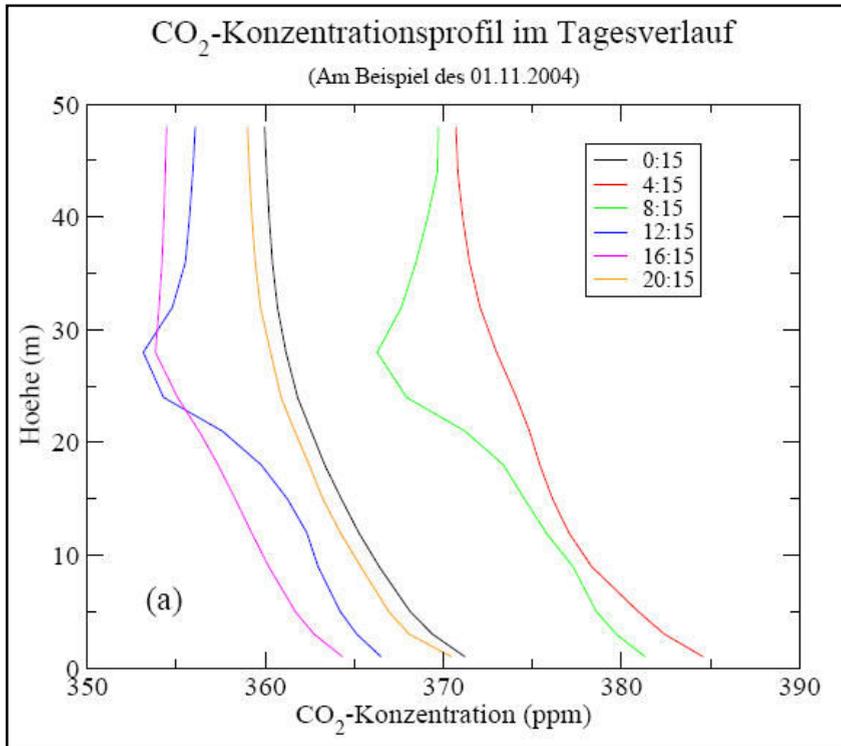
**Fig. 5:** Eddy-covariance measurements from June 2004 during a dry and sunny period (Olchev et al., 2008).

The Bowen ration (ratio between sensible heat and latent heat) (Fig. 6) was calculated by the EC-measurements with a result of 0.47 for the rainy and 0.56 for the less rainy season. In comparison to that the Bowen ratio of a chocolate plantation North of Lore Lindu National park had values from 1.06 to 1.60 (Falk, 2004). As a high Bowen ratio means a superiority of sensible heat flux via latent heat flux the comparison shows clearly that the rainforest has a much higher rate of evaporation and therefore contributes much more to the regional water cycle.



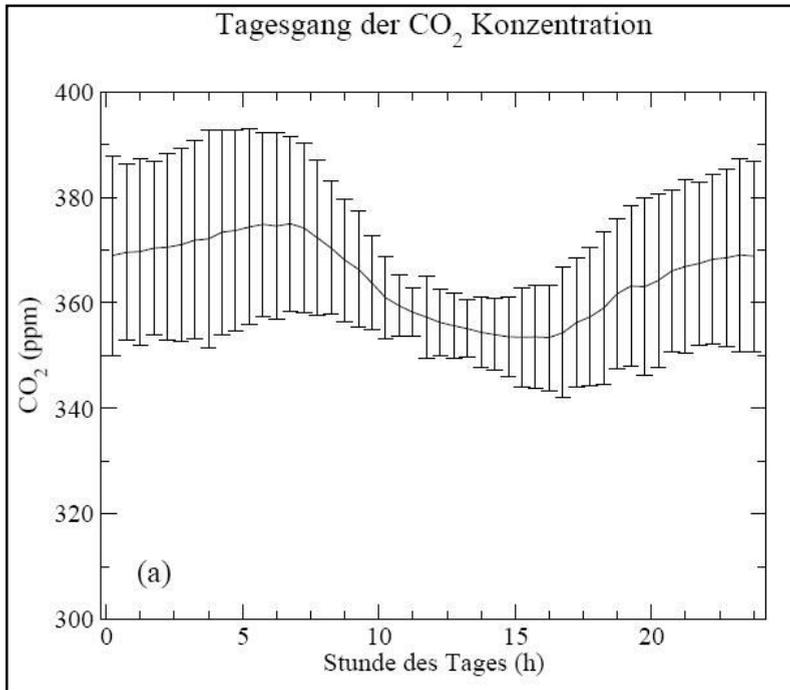
**Fig. 6:** Bowen ratio for the Bariri rainforest (Ross, 2007).

CO<sub>2</sub> profiles during the day could be modelled with MixFor-SVAT as well (Fig. 7). Fig. 7 shows generally lower values at crown-height, where assimilation takes place and higher values near the ground, where especially the soil is releasing CO<sub>2</sub>. The profile with the highest values is found at 4.15 h in the night, when respiration has released CO<sub>2</sub> all through the night; the opposite situation is found at 16.15h in the afternoon, when assimilation during the day has minimized the CO<sub>2</sub> concentration in the air.



**Fig. 7:** CO<sub>2</sub>-concentration profiles in the course of the day (Ross, 2007).

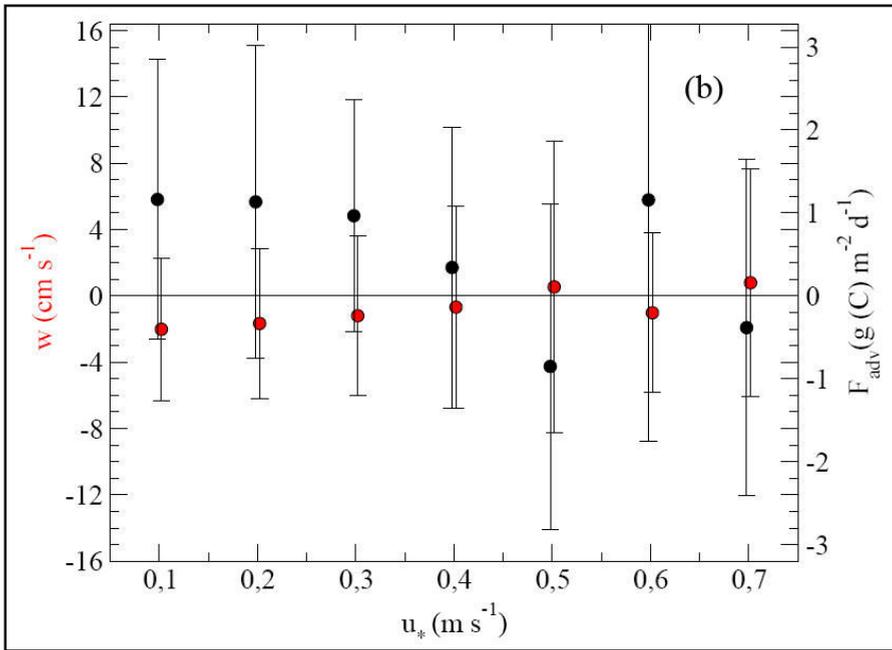
These dependencies are also obvious in Fig. 8. However, the low absolute values at about 370 ppm are remarkable, especially if compared to investigations at other comparable rainforest sites. This can be seen as a strong hint that besides turbulent transport of CO<sub>2</sub> as well advective transport takes place. In rainforest sites without advection night-time values reach between 450 and 550 ppm (Grace et al., 1996).



**Fig. 8:** Daily course of CO<sub>2</sub>-concentration (Ross, 2007).

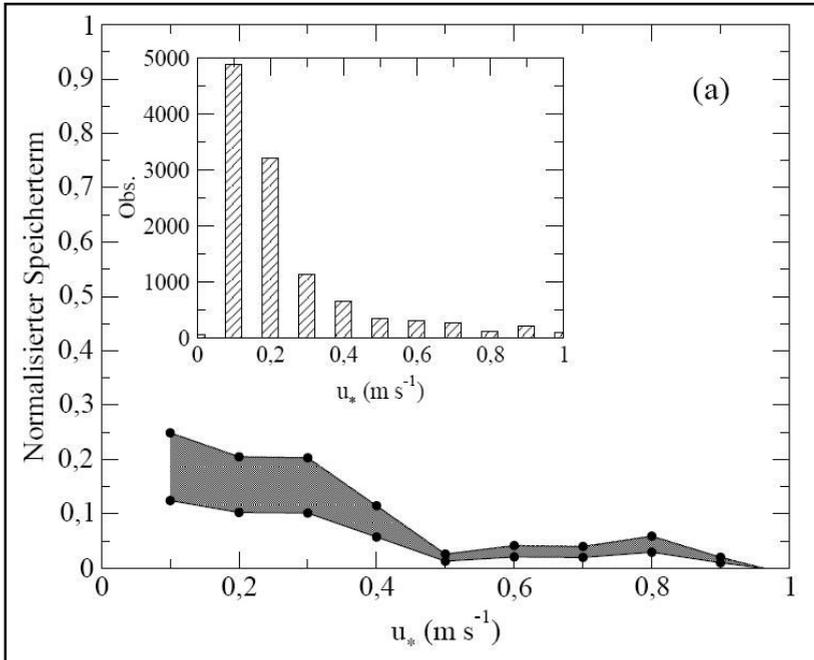
Advection, the transport with the mean flow, has been discovered during the last years as a remarkable part of NEE estimations that cannot be neglected anymore under many site-conditions like in the “traditional” EC-approach (e.g. Feigenwinter et al., 2004). Meanwhile several studies have shown that advection has not only to be taken into account at sites with steep slopes but already at moderately sloping sites (Aubinet et al., 2005; Kutsch et al., 2008). To determine the horizontal and vertical advection in the field a set of measurement towers is essential (Feigenwinter et al., 2008).

Comparable with the above mentioned studies advection in Bariri occurs mainly at night and often due to katabatic flows (cold downhill flows). As the atmosphere gets more stable during the night air is entraining from the upper part of the forest downward into the stem-space. With growing stability friction velocity ( $u^*$ ) is decreasing and therefore vertical velocity becomes increasingly negative (Fig. 9). At the same time the vertical advection ( $F_{adv}$ ) will become positive as highly concentrated air is leaving the control volume (and less concentrated air entraining from the top).



**Fig. 9:** Dependence of Vertical velocity  $w$  and Vertical advection  $F_{\text{adv}}$  from Friction velocity  $u^*$  (Ross, 2007).

A similar hint is given by the normalized storage flux as a function of  $u^*$ , which does not exceed the value 0.2 (Fig. 10). The flux was normalized with the mean flux from soil chamber measurements and can be seen as proportion of respired  $\text{CO}_2$  stored in the control volume below the EC-system. This very low value can neither be explained by cooling of the soil during the night (the temperature is responsible for the rate of respiration) nor by over-saturation of the air near the ground, but only by advection processes that take place within the stem space.



**Fig. 10:** Normalized storage flux as function of friction velocity  $u^*$  (Ross, 2007).

## Conclusion

There are very strong indications for horizontal and vertical advection at the Bariri rainforest site, as well by measurements as by modelling and comparisons with comparable research sites. The data that has meanwhile been collected by three towers with three vertical profiles and four horizontal layers (12 CO<sub>2</sub>-infrared gas analyzers) is offering the opportunity for a detailed description of the governing flow regime and the validation and adaptation of the flux models.

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# **Criteria for the assessment of forest naturalness in nature-conservation-orientated forest management planning in Germany**

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## Introduction

In Central Europe the principles of close-to-nature forest management are widely recognised as being suitable guidelines for sustainable forest management. According to these principles, forest management should be orientated to natural processes, dynamics and structures and is intended to use the natural potentials and imitate the natural development of forest ecosystems (Röhrig et al. 2006). Also in Germany one key goal of forest policy is the further enhancement of close-to-nature forest management in forest management practice (BML 2000, BMVEL 2001). The demand to establish close-to-nature forests, which is a central aim of managing forests in such a nature-orientated manner, was even included in the German Federal Nature Conservation Act in 2002 (§ 5 (5) BNatSchG<sup>1</sup>). The state forest enterprises of the federal government and the *Länder*<sup>2</sup> have a leading role in this process; all of them have committed themselves to manage their properties according to the principles of close-to-nature forest management. Forests with natural and close-to-nature characteristics are assumed to be ecologically more stable than forests that show less of such features due to more intensive human intervention. Furthermore, compared with other forests, natural and close-to-nature forests are particularly valuable habitats of wild fauna and flora in terms of biodiversity conservation and therefore especially worthy of protection.

To support the establishment of sustainable forest management practices at an European level, 37 European countries and the European Community adopted a resolution in which they outlined ‘general guidelines for the sustainable management of forests in Europe’ and committed themselves to the implementation of the guidelines in their national forest policies (Resolution H1) at the Second Ministerial Conference on the Protection of Forests in Europe (MCPFE)<sup>3</sup> in Helsinki in 1993. At the same conference the ministers responsible for forests in these countries stated that the conservation and enhancement of biodiversity in forests is an essential element of sustainable forest management (Resolution H2) (MCPFE 2008b). In the political process following the

<sup>1</sup> Gesetz über Naturschutz und Landschaftspflege (Bundesnaturschutzgesetz – BNatSchG) vom 25. März 2002. BGBl. I S. 1193

<sup>2</sup> The Federal Republic of Germany is formed by 16 federal states that are called *Länder* (sing. *Land*). The state forest enterprises manage 3.7 % of the total German forest area that are under the ownership of the federation and 29.6 % of the total German forest area that are under the ownership of the *Länder* (BMELV 2005).

<sup>3</sup> The Ministerial Conference on the Protection of Forests in Europe (MCPFE) is a high-level political initiative for co-operation in the field of forests and forestry of 46 European countries and the European Community. Since 1990, five Ministerial Conferences on the Protection of Forests in Europe, including a follow-up process after each conference, have taken place (MCPFE 2008a).

conference, the Helsinki process, six pan-European criteria, 35 quantitative indicators and 17 qualitative indicators for sustainable forest management were developed in order to assess progress towards sustainable forest management and conservation of biodiversity in Europe. As one of the 35 quantitative indicators for sustainable forest management 'naturalness' was chosen. Among other indicators, such as tree species composition, regeneration, introduced tree species, deadwood, threatened forest species and protected forests, the indicator naturalness was introduced in order to describe the criterion 'maintenance, conservation and appropriate enhancement of biological diversity in forest ecosystems'. The naturalness of European forests is assessed by classifying the forest area of each country by three classes of naturalness: 'undisturbed by man', 'semi-natural' (including a subclass 'modified natural') and 'plantations' (MCPFE 2007). The three main classes undisturbed by man, semi-natural and plantations originate from the classification scheme used for the assessment of forest naturalness within the Temperate and Boreal Forest Resources Assessment (TBFRA) 2000 (UN-ECE/FAO 2000), the subclass modified natural is taken from the extended five-class scheme of the Global Forest Resources Assessment (FRA) 2005 (FAO 2006) (MCPFE 2007). Within the FRA 2005 the world's forests were classified in terms of their naturalness, according to the following classes:

**Primary forest:** Forest of native species, where there are no clearly visible indications of human activities and the ecological processes are not significantly disturbed.

**Modified natural forest:** Forest of naturally regenerated native species where there are clearly visible indications of human activities.

**Semi-natural forest:** Forest of native species, established through planting, seeding or assisted natural regeneration.

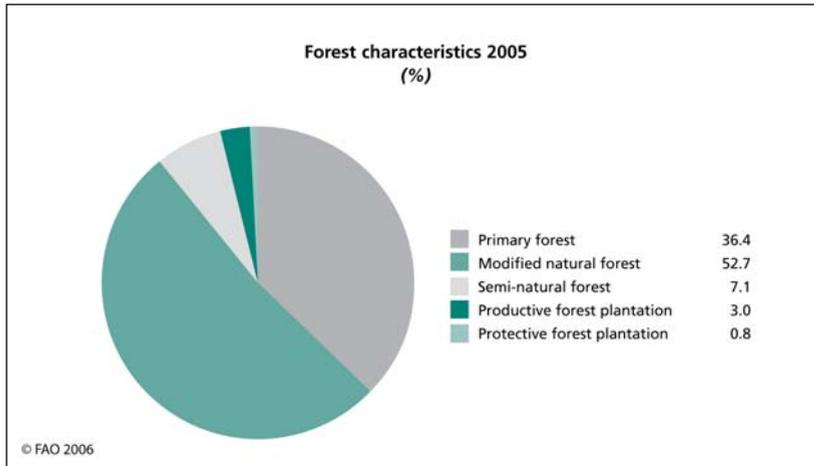
**Productive forest plantation:** Forest of introduced species and in some cases native species, established through planting or seeding, mainly for production of wood or nonwood goods.

**Protective forest plantation:** Forest of native or introduced species, established through planting or seeding mainly for provision of services.

World-wide, primary forests have been reduced to 36.4 % of the total forest area and are further decreasing; today modified natural forests, i. e. forest ecosystems clearly influenced by human activities, dominate (52.7 % of the world's forest area) and semi-natural forests and plantations, that are intensively managed and partly dramatically changed forest ecosystems, comprise already 10.9 % of the world's forest area (Figure 1). Germany reported its complete forest area (11.076 million ha<sup>4</sup>) as semi-natural (FAO 2006).

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<sup>4</sup> corresponds to 31 % of the total German land area (BMELV 2005)



Considering the FAO classification scheme for forest naturalness, some of the most common criteria for defining the degree of naturalness of forests can be identified: the tree species composition (dominance of native or introduced tree species), the type of regeneration (natural or artificial) and, as an overall criterion, the intensity of human influence, especially through silvicultural intervention. After showing that information on forest naturalness is required at different spatial and thematic levels, it shall be explained in the following how data on the degree of naturalness of forests are obtained in Germany.

## Assessment of forest naturalness in Germany

A forest's naturalness is not understood as one single condition. It rather includes a continuum between 'natural' and 'artificial'. To classify this continuum classes which denote varying degrees of naturalness are employed. Several classes together constitute a scale of naturalness. In Germany already in the 1950s considerations about the classification of forests in terms of their naturalness were made (v. Hornstein 1950), but more elaborate concepts and methods for description and assessment of forest naturalness were only devised in the 1980s, when interests of nature conservation started to gain more importance in forest management practice and silviculture began to develop towards close-to-nature forest management.

Until now forest habitat mapping (*Waldbiotopkartierung*) is the most common inventory method for the assessment of forest naturalness in Germany, inspired by a concept developed by Ammer & Utschick (1982) and conducted the first time at a large scale by the forest authorities of Baden-Württemberg (Volk 1987) and Lower Saxony (Hanstein & Sturm 1986) in the middle of the 1980s. Forest habitat mapping was introduced as a new nature-conservation-orientated element of forest management planning. It is intended to provide information on the conservation value of forest habitats, especially in managed forests, shall help to consider the interests of nature conservation in forest management and is assumed to be suitable to monitor the achievements of close-to-nature forest management (Sächsische Landesanstalt für Forsten 1996). Mostly a differentiation between two types of forest habitat mapping, selective and comprehensive forest habitat mapping, is made (Arbeitskreis Forstliche Landespflege 1996). The basic aim of selective habitat mapping is just to identify those habitats within a region that are most worthy of protection. Within the comprehensive forest habitat mapping, however, all habitats of a certain area are investigated more intensively in respect of several habitat features. For the operationalisation of the assessment of those habitat features that are relevant for habitat evaluation normally three criteria, diversity, rarity and naturalness, are used. Between 1986 and 1998 the state forest enterprises of the federal government and of ten *Länder* have integrated comprehensive forest mapping methods into their forest management planning (Figure 2).

In the eleven



Figure 7: The ten German Länder conducting comprehensive forest habitat mapping

comprehensive forest mapping methods of the state forest enterprises three sub-criteria of naturalness are applied: naturalness of the vegetation composition, naturalness of the site development and naturalness of the vegetation development. In all these methods the criterion naturalness of the vegetation composition is used, the naturalness of the site development is only assessed in seven and the naturalness of the vegetation development is only assessed in three of the methods.

#### Naturalness of the vegetation composition

The classification of naturalness of the vegetation composition is based on the comparison of a forest's actual vegetation with its natural vegetation. Under the condition that natural means not affected by human influence, it is very difficult

or even impossible to describe this natural forest vegetation, i. e. the zero point of the scale, for any region in Central Europe. The reason for this is that human influence in Central Europe has been all-pervasive throughout the millenia (Ellenberg 1996) and only very rare, scattered and small relics of virgin forests could remain there due to the continuous use of forests in history and a high population density (Parviainen 2005). Research in the mentioned remnants of virgin forests that have survived in mountainous areas and wetlands, especially in the Balkans, Alps and Carpathians (Parviainen 2005), and in strict forest reserves<sup>5</sup>, however, can help us to obtain a better idea of how natural forests in the temperate zone of Europe look like and which characteristics are typical of them (Bücking 2000). As an approach towards the natural vegetation the ‘potential natural vegetation’ (PNV), which can be derived from the site conditions, is used. According to this concept, the naturalness of the vegetation composition is understood as being the degree of correspondence of the actual vegetation to the potential natural vegetation.

The concept of the potential natural vegetation was created by the German vegetation scientist Reinhold Tüxen (1956). He defined it as:

*“ein gedachter natürlicher Zustand der Vegetation [...], der sich für heute oder für einen bestimmten früheren Zeitabschnitt entwerfen läßt, wenn die menschliche Wirkung auf die Vegetation unter den heute vorhandenen oder zu jenen Zeiten vorhanden gewesenen übrigen Lebensbedingungen beseitigt und die natürliche Vegetation, um denkbare Wirkungen in zwischen sich vollziehender Klima-Änderungen und ihrer Folgen auszuschließen, sozusagen schlagartig in das neue Gleichgewicht eingeschaltet würde.”<sup>6</sup>*

The potential natural vegetation was originally devised as a parameter for expressing a site’s actual biotic potential and should be used especially in forestry and agriculture (Tüxen 1956). Afterwards, however, it additionally became a very important instrument of landscape and nature conservation planning (Kaiser 1996). The potential natural vegetation is an imagined, i.e. constructed and hypothetical vegetation, and is generally outlined for the present time in a sense of ‘present-day potential natural vegetation’ (Tüxen 1956, Kowarik 1987, Kaiser 1996, Schirmer 1999). It must be kept in mind that this present-day potential natural vegetation is not identical with the reconstructed natural vegetation of former times (Kowarik 1987, Härdtle 1995). A site’s potential natural vegetation is understood as being the most developed vegetation possible on this site (Kowarik 1987, Kaiser 1996). The basis of this idea is the climax theory stating that within a

<sup>5</sup> Unmanaged forests reserved for natural processes and left to their self-dynamic development (Bücking 2000).

<sup>6</sup> “imagined natural state of vegetation ... that could be outlined for the present time or for a certain earlier period, if human influence on vegetation was removed – the remaining conditions of life presently existing or having existed during those periods still being valid – and the natural vegetation was imagined as switched into the new balance within a split second to exclude the possible effects of climatic changes and the consequences thereof” (translation by Härdtle 1995)

successional series a site's vegetation inevitably develops towards a stable state corresponding to the climatic and edaphic site conditions (Dierschke 1994). Consequently, a site's potential natural vegetation is characterised by and named after the final plant community (Tüxen 1956). It is assumed that within nearly the whole area of Central Europe the actual vegetation would develop towards final forest communities preceded by pioneer forest communities, if man no longer exerted any influence upon it (Dierschke 1994, Ellenberg 1996). Beech and mixed beech forests of *Fagus sylvatica* would naturally comprise two thirds of the German territory (Bohn et al. 2007).

Defining the potential natural vegetation through final plant communities tends to evoke misunderstandings. It must be stated clearly that the potential natural vegetation is not the anticipated final stage of a succession actually taking place in time and space (Kowarik 1987, Härdtle 1995). The potential natural vegetation does not develop slowly; it rather has to be imagined as coming into existence at once. Site changes that might take place successively must be excluded from the basis of reference for PNV construction. This shows that the traditional concept of the potential natural vegetation excludes succession and other natural dynamics (Kowarik 1987, Härdtle 1995, Zerbe 1997, Schmidt 1998, Reif 2000). With the intention to dispose this weakness an extended PNV concept for forests, that is also widely used within the comprehensive forest habitat mappings of the state forest enterprises, was created. In this extended model the potential natural forest community consists not only of plant species of the site's final forest community, but also includes plant species of forest communities that belong to the successional series leading to this final community (pioneer forest communities, transition forest communities) (Sturm et al. 1999).

The definition of potential natural vegetation as used in nature conservation and forest habitat mapping differs from the traditional definition in a further point. Although the present-day PNV can include not only native but also naturalised non-native tree species (Kowarik 1987, Kaiser 1996, Schmidt 1998), the latter are excluded when using the concept of the potential natural vegetation for describing potential natural forest communities that are used in the context of the assessment of forest naturalness and as development objectives of forest nature conservation. With some exceptions, the scales of naturalness of the vegetation composition as applied in the comprehensive forest habitat mappings of the state forest enterprises comprise normally five classes: natural, close-to-nature, far-from-natural, alien-to-nature and artificial. It is generally accepted that the vegetation of forests is composed of at least three layers: the tree layer, the shrub layer and the ground vegetation layer. In most methods of comprehensive forest habitat mapping, however, only the tree layer is considered when assessing the naturalness of the vegetation composition. Only in Thuringia the ground vegetation is included in the methodology (Landesanstalt für Wald und Forstwirtschaft & Thüringer Landesanstalt für Umwelt 1999). This means that the criterion

naturalness of the vegetation composition is actually reduced to the criterion naturalness of the tree species composition, which is described by the correspondence of the tree species composition of the actual tree layer to the tree species composition of the tree layer of the potential natural forest community. Each potential natural forest community is characterised by a typical set of tree species and a typical tree species composition, which is defined by the proportions of the potential natural forest community's tree species, including principal, secondary, tertiary and pioneer tree species, to each other. The classes in the scales of naturalness of the vegetation composition describe the deviation of a forest's actual tree species composition from the typical tree species composition of the potential natural forest community. This deviation is defined by the proportion of a forest's area that is covered by tree species belonging to the potential natural forest community to its area that is covered by tree species not belonging to the potential natural forest community. In all *Länder* and in the government-owned forests a forest must be composed of tree species belonging to the potential natural forest community to an extent of at least 90 % in order to classify as enjoying the highest degree of naturalness. In the Saarland even an extent of 95 % (Sturm et al. 1999) and in Thuringia of 100 % (Landesanstalt für Wald und Forstwirtschaft & Thüringer Landesanstalt für Umwelt 1999) is demanded. In most methods a PNV tree species share of more than 10 % can prevent assignment of a forest to the naturalness class characterising the lowest degree of naturalness. An additional parameter for evaluating the naturalness of the tree species composition is the proportion of native to non-native, i. e. introduced, tree species within the non-PNV species of a forest. This is of particular importance in Baden-Württemberg (Schirmer 1999), Brandenburg (Steinmeyer 2003), the Saarland (Sturm et al. 1999) and Thüringen (Landesanstalt für Wald und Forstwirtschaft & Thüringer Landesanstalt für Umwelt 1999). In Brandenburg additionally a distinction is made between naturalised and non-naturalised non-native tree species; naturalised non-native tree species are considered to affect a forest's naturalness of the tree species composition less than non-naturalised ones (Steinmeyer 2003). In the methods of the government-owned forests and of Saxony-Anhalt, the participation of site-adequate and non-site-adequate non-PNV tree species in the tree species composition is used to differentiate the lower degrees of naturalness; tree species that does not belong to the PNV but are site-adequate are evaluated more favourably than tree species that does not belong to the PNV and are not site-adequate neither (Bundesministerium der Finanzen 1997, Forstliche Landesanstalt Sachsen-Anhalt 2002).

#### Naturalness of the site development

The aim of the naturalness criterion naturalness of the site development is to describe anthropogenic soil changes of the past, such as deforestation, drainage, ploughing, farming or mining. The most dramatic human intervention in natural

forest ecosystems is the removal of forests and the conversion of forest land to other land use. The consequences are far-reaching and long-lasting, often bringing about irreversible changes in soil structure. The concept of 'ancient woodland' therefore plays a decisive role in determining the degree of naturalness of the site development. First the concept of ancient woodland arose in countries which have been nearly completely deforested for centuries, such as the Netherlands and the United Kingdom, i. e. where sites continuously covered with forests for several hundred years are very seldom. According to Peterken (1994) "ancient woods are woods occupying sites which have been wooded continuously since at least 1600". In Peterken's sense, continuity of woodland is not broken by felling, coppicing, planting nor any other forestry operation, provided that woodland of some kind regenerates immediately, but it is broken by an alternative land use, such as pasturage or cultivation. In Germany ancient forests are understood as "forests which have been in existence for at least several hundred years, according to historical maps, stand descriptions or other circumstantial evidence" (Wulf 1994, translation by the author). Within the comprehensive forest habitat mappings sites on which ancient forests thrive are identified. The minimum time range of continuous forest coverage regarded as sufficient for recognising a forest site as 'ancient' are dependent on the availability of historical documentary records. Lower Saxony, Rhineland-Palatinate, the Saarland and Thuringia, who have integrated the concept of ancient forest sites into their comprehensive forest habitat mapping methods, laid down time ranges of 200 up to 300 years (Landesanstalt für Wald und Forstwirtschaft & Thüringer Landesanstalt für Umwelt 1999, Sturm et al. 1999, Niedersächsisches Forstplanungsamt 2006).

#### Naturalness of the vegetation development

The third naturalness criterion of the German comprehensive forest habitat mappings, naturalness of the vegetation development, is only applied in the government-owned forests (Bundesministerium der Finanzen 1997), in the Saarland (Sturm et al. 1999) and in Schleswig-Holstein (Gemperlein & Heeschen 2002). It is directed towards the question of to what extent a forest has developed or will be able to develop naturally; that is, without being managed. The degree of naturalness of the vegetation development sometimes also includes information on the origin of the forest (natural, by planting or by coppice sprouting).

## Discussion

Knowledge about natural forest structures and processes is restricted. More research, especially in virgin forests and strict forest reserves, will be necessary to increase this knowledge. But even if we knew more about the features of natural forests, we could only imagine what a natural forest on a certain site would be like. We are neither able to reconstruct a former natural state nor to anticipate a future one with precision. Even the potential natural vegetation can only give us a basic idea. This imagined natural state of vegetation is neither identical with the original vegetation, which existed before humans started to change the primeval towards a cultural landscape, nor does it correspond to the vegetation that would actually develop on the site if any direct human influence was removed. All in all, we have no clear notion of the absolute natural state, the zero point of naturalness. Consequently, the assessment of naturalness on the basis of scales of naturalness is always hypothetical. This fact is the crucial weakness of all scales of naturalness. Eleven German state forest enterprises, however, apply scales of naturalness to assess the degree of naturalness of their forest properties. Within an inventory method called comprehensive forest habitat mapping three criteria are used to describe forest naturalness: naturalness of the vegetation composition, naturalness of the site development and naturalness of the vegetation development. It is a subject of criticism that only these three criteria are used and that in some *Länder* even this small set of criteria is further reduced to only one or two of them. Additionally, the criterion naturalness of the vegetation composition usually only takes the tree species composition of the tree layer into consideration. It excludes the ground vegetation. It is obvious that in this way the degree of naturalness of forest habitats cannot be described in a way that is adequate to the complexity of forest ecosystems. Other habitat features, such as age and spatial structure and the amount and distribution of deadwood, should be included in the evaluation of forest naturalness. Despite this criticism, it is appreciated that for a high proportion of the German forest area the comprehensive habitat mappings of the state forest enterprises produce large-scale information on the naturalness of forests that gives valuable orientation to forest nature conservation and close-to-nature forest management.

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# The fall webworm, *Hyphantria cunea* (Lepidoptera: Arctiidae): a new emerging pest insect for forests and agricultural crops of Iran

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## Abstract

The fall webworm, *Hyphantria cunea* Drury is a polyphagous insect native of North America that became an invasive alien species in Italy, Switzerland, Hungary, Romania, Russian Federation, Japan, Korea, China, and New Zealand during last five decades. In August 2002, specimens of *H. cunea* were found in Iran for the first at the Caspian forests nearby the city of Lasht-e-Nesha, Guilan province. In this area, invasive populations of *H. cunea* have become widely established during last few years and the species is now considered one of the most important pests from the western province of Ardebil to the eastern province of Mazandaran. Moreover, new invaded areas are detected annually. The species has a broad host, ranging from various species of forest trees, fruit trees, and ornamentals to annual crops, and weeds. The preferred forest hosts include maple alder, elm, oak, ash, willow, wild plum, and apple trees.

In the northern forests of Iran *H. cunea* has two generations per year. Adults of the first and second generation were found in early May and late June, respectively. Infestations of Iranian forests were recorded on a large number of plant species along forest borders, road sides, forest parks, seed and fruit orchards, annual plants, and home gardens. Furthermore, notably non-host plants were orange trees and most of the coniferous species.

To keep this pest under control, a comprehensive program was recently developed by the Department of Forests and Range Protection, Research Institute of Forests and Rangelands (RIFR) of Iran. A pheromone-based monitoring system was tested to record the population dynamic of *H. cunea*, but the pilot experiments failed to attract any conspecifics since males were not lured by using commercial sex-pheromone traps. Therefore, it is considered that the local populations may belong to a different subspecies or race where a diverse sex pheromone blend has evolved. At present, there is an integrated approach in cooperation between RIFR and Göttingen University, aiming to identify the blend components of the sex pheromone of the *H. cunea* populations in Iran. This study may further confirm the exact taxonomic status of these populations and will be crucial to set up a new pheromone-based monitoring system in order to detect and control *H. cunea* in Iran.

**Keywords:** Arctiidae, Lepidoptera, fall webworm, American white moth, mulberry moth, IPM, biocontrol, invasive insects, quarantine pest, sex pheromone.

## 1. Geographical distribution

The fall webworm, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae), is native to United States, Canada and Mexico, where its distribution was limited before 1940. In these countries the species is not considered to be a pest of economic importance. After the Second World War, invading populations were recorded in central Europe and eastern Asia (Warren & Tadic, 1970; Umeya & Ito 1977) and in Japan (Tokyo) in 1945 (Masaki, 1975). In Europe it was firstly established in Hungary and it is now a serious pest in Bulgaria, Romania, Hungary, former Yugoslavia, Russia and northern Italy. In Switzerland few infestations were found for the first time in 1991, most likely as a progressive migration of the insect from Italy (EPPO n.2, 1996). However, *H. cunea* seems to be unable to establish itself in the northern half of Europe, probably because of climatic constraints (Wittenberg, 2005). In 1979 the species was reported in China (Liaoning province) and it is now established in several eastern provinces of the country (FAO, 2007, Su *et al.*, 2008). In New Zealand few infestations were reported on Mt. Wellington, Auckland, in March 2003 and a second foci was found in early 2005, however at present New Zealand is considered to be free of this pest (Kean & Kumarasinghe, 2007). In 1952, the species was found in Ukraine and at present is a severe pest in several regions of Russia, especially nearby Caspian and Black Sea.

In Iran, the species was firstly recorded in August 2002 nearby Lasht-e-Nesha city, Guilan province. At present is considered an important pest in several northern provinces along the Caspian coast, including Guilan, Mazandaran and Ardebil. In Guilan province, 11 cities and 258 villages are affected by this pest (Rezaei, 2004).

## 2. Taxonomy

*Hyphantria cunea* belongs to the order Lepidoptera, family Arctiidae and subfamily Arctiinae. Two forms, generally designated as "races" by several authors, have been reported. Both races are easily distinguished by larval characters, behaviour and nest structures. The first one, called "black race", is considered as the *H. cunea* holotype, having larvae with a black head capsule. The second is called "orange race" and the larvae have a light orange to dark brown head capsule. The two races usually occur together, although they differ in their frequency on various tree species (Oliver, 1964; Ito & Hattori, 1975). In both races the body colour of the larvae may vary considerably, showing a conspicuous polymorphism. According to Takeda (2005), in USA the black-headed race infests *Morus* spp. and *Salix* spp., while the orange-headed race occurs on *Carya* spp., *Juglans nigra* L., *Prunus serotina* Ehrh., and *Diospyros* spp. Larvae of the black-headed race feed without a clear day-night rhythm, whereas larvae of the red-headed feed mainly at night

(Tadeka, 2005). Moreover, a difference in web formation is observed between the two races. The black-headed race larvae make compact colonial-webs until the 4<sup>th</sup> instar and then they disperse and become solitary during the 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> instar. On contrary, the orange-headed race larvae remain in compact colonial-webs during their whole larval stage (Suzuki *et al.*, 1980). Physiological studies, as critical photoperiod for pupal diapause and pupal development time, together with the previous described differences, suggested that the two races represent a set of ecological adaptations, favouring univoltinism for the black-headed race and bivoltinism for the red-headed larvae (Tadeka, 2005). A recent genetic investigation based on mitochondrial DNA data suggested that the two races are distinct species, being the second a sibling sympatric species of the first one (Gomi *et al.*, 2004). However, the formal taxonomic status remains still unclear. The more invasive black-headed species is the one that has spread through central Europe, eastern Asia, New Zealand, and Japan (Kean & Kumarasinghe, 2007). In the last country bivoltine and trivoltine populations of the black-headed race were described (Gomi, 2007).

### 3. Life cycle

In the native area, the life cycle shows geographic variation, from univoltine in southern Canada (Morris & Fulton 1970), to bivoltine in Virginia and tetravoltine in Louisiana (Oliver 1964). In Japan, a bivoltine life cycle prevailed throughout the distribution area, but trivoltine populations are recorded in the south-western areas (Masaki, 1975; Gomi, 2007). In central Europe there are usually two generations per year as well as in the Iranian Caspian areas (Rezaei *et al.*, 2006). *H. cunea* overwinters as a pupa in a state of diapause (Masaki 1977). The adult moths begin to fly before, or during, the flowering period of apple trees (April-May). At the Caspian coast of Iran (Guilan province) adult moths emerge in early May and mate during the following 2-3 days (Rezaei *et al.*, 2006). Adults are white, bearing black to brownish spots on the forewing and have a full wingspan ranging from 25 to 31 mm. Females lay green, shiny and spherical eggs in batches of 100-600 (maximum 2000) covered by tiny abdominal hairs on the underside of host leaves (Fig.1). Incubation period lasts about a week, during which the eggs turn grey before hatching. The first instar larvae start immediately to make silk webs and feed together on the lower parenchyma of the leaves (Ito & Miyashita, 1968). As the gregarious larvae grow during the six or seven instar stages (but up to 11 instars were also observed), webs enlarge and enclose more foliage. Late instars feed on the whole leaves and build impressive silk webs that sometimes enclose entire branches (Fig.2). In severe cases an infestation leads to a total defoliation of the host trees.

Larval development takes four to eight weeks, varying in function of temperature and food availability. Pupation takes place in grey friable tin cases, and the pupae, brown in colour, are hidden under bark of trees, below ground surface, in the cracks of wooden materials or in debris of the soil. The adults of the first generation emerge in July-August (Wittenberg, 2005), although in Guilan province they already appear in late June (Rezaei *et al.*, 2006). Adults are good flyers, and can spread with vehicles, packing material, host plant material, etc. (CABI Bioscience, 2005). Reproduction and host finding behaviour of the summer adults are similar as for the spring adults.

The second generation of larvae is responsible for the greatest damages on host plants. During August and September extensive webs of *H. cunea* larvae can be seen in several forest locations of Guilan province feeding on the underside of host leaves, (Rezaei *et al.*, 2006). The pupae of the second generation overwinter well under tree barks, fences and soil cracks.

#### 4. Larval development

In Guilan province, experiments were conducted to characterise the larval development of *H. cunea*. Six instars feeding on mulberry leaves (*Morus* sp.) in field cage experiments were recorded (Rezaei *et al.*, 2006). The younger larvae grew faster than the elders. In laboratory rearing condition (25°C, 70% relative humidity), the developing time of each stage was measured. Total developmental time for the 1<sup>st</sup> and the 2<sup>nd</sup> instar larvae was  $6.4 \pm 0.51$  days, whereas the total time for the 5<sup>th</sup> and the 6<sup>th</sup> instars lasted  $9.26 \pm 0.89$  days. In addition, full larval development was reached after  $22.12 \pm 1.27$  days, where prepupal and pupal periods lasted  $1.42 \pm 0.49$  and  $9.5 \pm 1.59$  days, respectively. The mean longevity of male and female adults was recorded as  $4.32 \pm 0.47$  and  $6.31 \pm 0.47$  days, respectively. Therefore, the time of one generation, from first egg hatching to second egg hatching, was estimated equal to  $42.88 \pm 2.02$  days under controlled conditions. Time for one generation under field conditions (Guilan province) was longer than in laboratory, where mean larval and pupal periods were estimated to be  $25.32 \pm 2.34$  and  $13.04 \pm 1.69$  days, respectively.

#### 5. Host species

The fall webworm *H. cunea* is a highly polyphagous Lepidoptera and feed on wide ranges of forest and fruit trees, shrubs and herbaceous plants. Some of 600 host plant species have been reported as potential hosts. In Romania, it was shown that, although *H. cunea* is polyphagous, normal development will occur only on few food plants. The preferred host species are *Morus* spp., *Acer* spp., *Malus* spp., *Prunus* spp. and *Pyrus* spp., rather than *Vitis vinifera*, *Fragaria* spp., *Rosa* spp., *Humulus lupulus* or *Ailanthus altissima* (Iamandei *et al.*, 2004; Smith *et al.*, 1992). Cottonwood (*Populus deltoides*) and *Platanus* spp., are also considered as good host species (Wittenberg, 2005), but larvae are able to develop also on *Salix* spp., *Fraxinus* spp., *Betula* spp., *Alnus* spp., *Carya* spp. (pecan and hickory), *Juglans* spp., *Ulmus* spp., persimmon (*Diospyros* spp.) and sweetgum (Liquidambar) spp. (FAO, 2007). Forests and ornamental host species in Iran include alder, maple, elm, iron tree (*Parrotia* spp.), ash, Persian walnut (*Juglans regia*), Caucasian walnut (*Pterocarya fraxinifolia*), plane, poplar, willow and bald cypress. Experiments showed that the 6<sup>th</sup> instar larvae can consume a daily average of 435 mm<sup>2</sup> of fresh ash foliage while the 7<sup>th</sup> instar larvae brought it to an average of 814 mm<sup>2</sup> (Jarfas & Miklos, 1986).

#### 6. Sex pheromone

Pheromone traps can offer a reliable technique for monitoring and controlling forest pests by intercepting for instance male peak flights. The sex pheromone released from *H. cunea* females has been studied in North America, Europe, New Zealand, and China. The first characterization was achieved by Hill and coworkers (1982) by

investigating pheromone gland extracts of females originating from U.S.S.R. (Kishinev, Bessarabia; black-headed race) and from USA (Geneva, New York, orange-headed race). Three pheromone components were identified: namely (Z,Z)-9,12-octadecadienal (C18:2Ald), (Z,Z,Z)-9,12,15-octadecatrienal (C18:3Ald), and (3Z,6Z)-3,6-9,10-epoxyheneicosadiene (C21-2Epo) with an approximate ratio of 1:1.2:2.6, respectively for the black-headed race and of 1:8:21 for the orange-headed race (Tab. 1). Although these three compounds were precisely identified, no males were caught in the field with any combination of these compounds. Two further trienic epoxide compounds were later identified in the sex pheromone gland of female originating from Hungary, namely (3Z,6Z)-1,3,6-9,10-epoxycosatriene (C20-3Epo) and (3Z,6Z)-1,3,6-9,10-epoxyheneicosatriene (C21-3Epo) (Toth *et al.* 1989). A recent investigation on two *H. cunea* females collected in New Zealand, found a similarity with the USA population; in this case the three compounds already found in the USA population before were reported at a ratio of 1:11:46.50, although a trace quantity of C21-3Epo was also detected (El-Sayed *et al.*, 2005) (Tab. 1). Similar results were achieved very recently in China, where C18:2Ald, C18:3Ald, C21-2Epo and C21-3Epo were found with a ratio of 1:16.8:29.2:3 (Su *et al.*, 2008) (Tab. 1). Field experiments using an equal ternary mixture of C18:3Ald, C21-2Epo and C21-3Epo showed to be attractive to the *H. cunea* male in northern Italy (Trematerra *et al.*, 1993) and a great number of males were captured in China, by a ratio of 8:1:1 of the same three compounds (Zhang *et al.*, 1996). Recently, the quaternary formulation of C18:2Ald, C18:3Ald, C21-2Epo, and C21-3Epo was proved to be very effective for monitoring *H. cunea* populations in China (Su *et al.*, 2008). On the other hand, the commercially available synthetic lure, Nitolure, produced by Nitto Denko (Osaka, Japan) since 1993, showed contradictory reports regarding the efficacy of this lure (Yamanaka *et al.* 2001; Rezaei, 2006). An investigation on the composition of this lure found a ratio 1.2:12.3:1.1 of C18:2Ald, C18:3Ald, C21-2Epo, respectively (El-Sayed *et al.*, 2005) (Tab.1).

## 7. Pest management

The best pest management strategies focus on controlling of the first generation with the ultimate goal of keeping the population of the second generation under the economic threshold level. For this reason removal of infected shoots and branches in early May is an important practice. Monitor of the first flying period of the moth population can be achieved by utilizing light-traps, as a means of sampling moth populations. In case of very high level of populations, pesticides are initially applied as the first control measure because the severe damage may compromise the survival of the host plants. However, the use of pesticides was proved to be less than ideal as a means of controlling this pest, which continue to proliferate also after the treatments (Yarmand *et al.*, 2006). The only written report on the control actions against *H. cunea* in Iran refers to the application of low toxicity pesticides as diflubenzuron (Dimilin) on mulberry leaves and some environmentally safe material including *Bacillus turingiensis* and Neem extract. Application of *B. turingiensis* var. Krustaki against larvae showed the highest mortality rate (Yarmand *et al.*, 2006).

## 8. Natural enemies

The natural enemy complex of *H. cunea* was investigated in Shaanxi province, North-west China, from 1984 to 1986, and over ten natural enemy species were eventually detected from the larvae and pupae of the fall webworm (Yang, 1990). In the period 1996-2000, the insect predators and parasitoids were surveyed throughout the infested areas of China, and 27 natural enemy species were discovered (Yang *et al.*, 2006). Among these, *Chouioia cunea* Yang (Hymenoptera: Eulophidae) (Fig.2), *Coccygomimus disparis* (Viereck), *Coccygomimus parnasae* (Viereck) (Hymenoptera: Ichneumonidae) and *Exorita japonica* Townes (Diptera: Tachnidae) were the most important ones. The endoparasitoid chalcid wasp, *C. cunea*, caused a considerable mortality of *H. cunea* by parasitizing the pupae (Yang, 1990) (Fig.2). Therefore, *C. cunea* was mass-produced on several species of Lepidoptera, and the moth *Antheraea pernyi* Guerin-Meneville (Lepidoptera: Saturniidae) was chosen as the best surrogate host, since a single pupa produced an average of more than 6,000 parasitoid wasps (Yang *et al.*, 2006). Mass-releasing programs were undertaken in several provinces of China and the effect of the biological control program was significant. Damage caused by the fall webworm could usually be controlled within one year after the mass-release with an average parasitization higher than 80% (Yang *et al.*, 2006).

In Iran, natural enemies associated with the *H. cunea* were investigated in the infested areas of Guilan province. Two native parasitoid wasps were detected and identified: *C. cunea* and *Psychobagrus omnivorus* Walker (Hymenoptera: Pteromalidae). The egg parasitoid *Trichogramma dendrolini* Matsumura (Hymenoptera: Trichogrammatidae) and the predator bug *Arma custos* F. (Heteroptera: Pentatomidae) were detected as well (Rezaei, 2006).

## 9. Conclusions

To effectively manage new invasive pest insects and prevent their further expansion, there is a need of study in detail biological and ecological aspects of the local insect populations. In addition, specific studies on the efficacy of the indigenous natural enemies and their ability to control, or reduce, possible outbreaks of the alien species have to be done. In the recent infestations of *H. cunea* in the northern forests of Iran, it will be important to perform complementary investigation of morphological, ecological and biological aspects before implementing control strategies. Follow-up studies should be carried out on distribution pattern and preferred host plants in Iran, different sub-species and forms of the fall webworm are present in other countries.

Sex pheromone traps are an essential tool for monitoring and detecting invasive species, however the commercial luring products for *H. cunea* already showed no efficacy in intercepting male flight peaks, most likely because the ratio of the sex pheromone compounds vary among different populations. Geographical and population differences in sex pheromone components are already documented in several pest species, e.g. *Ostrinia nubilalis* (Klun *et al.*, 1975), *Agrotis segetum* (Löfstedt *et al.*, 1986, Hansson *et al.*, 1990), *Hemileuca eglanterina* (McElfresh *et al.*, 2001) and *Spodoptera frugiperda* (Luciane *et al.*, 2006). In *H. cunea*, it is likely that the two races coexist and several polymorphisms and ecological plasticity of local populations were documented in several countries. The variation in voltinism and number of larval instars are perhaps the clearest examples of the species adaptability. This plasticity may be responsible also for the higher potential of colonisation of diverse biotopes.

There is no published information on the sex pheromone composition of fall webworm in Iran, but the experiments conducted on population from USA, U.S.S.R, New Zealand and China confirmed that different local pheromone "languages" already exist in this species (Hill *et al.*, 1982; El-Sayed *et al.*, 2005; MaoWen *et al.*, 2008). In a field test conducted in Guilan province, attraction of male moths to traps baited with the pheromone lure was investigated. The results were not promising and the efficacy of the blend remained unclear (Rezaei, 2006). Therefore, it is a priority to characterise the sex pheromone components and their exact ratio in the fall webworm populations of Iran.

At present an integrated approach in cooperation between the Research Institute of Forests and Rangelands of Iran and the Department of Forest Zoology of Goettingen University (Germany) is proposed to identify the chemical components of sex pheromone of the Iranian populations of the fall webworm. Pheromone blend extraction and characterisation will be carried out in a joint research activity under the framework of the project "Integrated management of fall webworm in Iran", currently under approval

for funding by Governmental institutions. The new formulation of the sex pheromone blend will be employed to trap and intercept male flight peaks of the *H. cunea* populations in Iran and help to monitor, detect and control this invasive pest insect.

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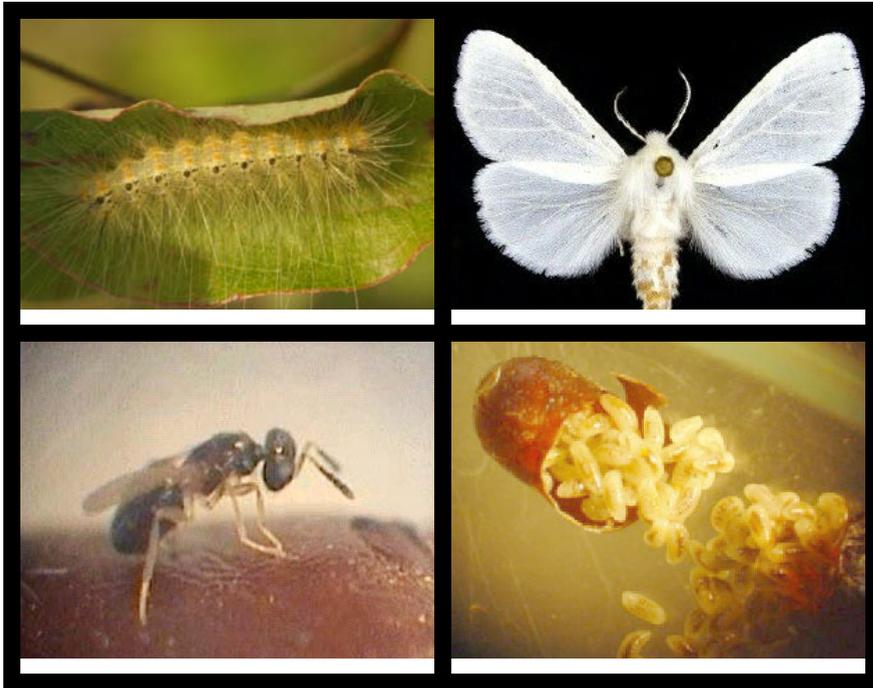
**Table 1** Chemical compositions and sex pheromone blends ratio of the female fall webworm, *Hyphantria cunea*, found in populations from USA, U.S.S.R., New Zealand, China, and in a synthetic attractant (Nitolure).

No.	Compounds	USA orange- headed p. <sup>1</sup>	U.S.S.R. black- headed p. <sup>1</sup>	New Zealand population <sup>2</sup>	China population <sup>3</sup>	Nitolure <sup>2</sup>
1	C18:2Ald	1	1	1	1	1
2	C18:3Ald	6	1.2	11	16.8	10.2
3	C21-2Epo	27	2.6	46.5	29.2	0.9
4	C21-3Epo	n.d.	n.d.	0.2	2.3	n.d.

<sup>1</sup>Hill *et al.* (1982), <sup>2</sup>El-Sayed *et al.* (2005), <sup>3</sup>MaoWen *et al.* (2008). C18:2Ald: (9Z,12Z)-ctadecadienal; C18:3Ald: (9Z,12Z,15Z)-octadecatrienal; C21-2Epo: (3Z,6Z,9S,10R)-9,10-epoxy-3,6-heneicosadiene; C21-3Epo: (3Z,6Z,9S,10R)-9,10-epoxy-1,3,6-heneicosa-triene; n.d.: not detected. Published ratios of blends from U.S.S.R., New Zealand, China and Nitolure were normalised, considering the first compound equal to 1, in order to better compare the different composition. Nitolure stands for the commercial lure produced by Nitto Denko (Osaka, Japan).



**Figure 1** Different development stages of the fall webworm, *Hyphantria cunea*: (above, left) an egg batch of greenish eggs on the underside of host leaf; (above, right) a second egg batch; (below, left) gregarious larvae; (below, right) pupae and the surrounding plant debris.



**Figure 2** Different development stages of the fall webworm, *Hyphantria cunea*: (above, left) late instar larva, (above, right) adult moth. Native parasitoid of *H. cunea* in Iran: (below, left) a female adult wasp of *Chouioia cunea* (Hymenoptera: Eulophidae) ready to lay her eggs into a *H. cunea* pupa; (below, right) parasitoid larvae of *C. cunea* observed after during dissection of a *H. cunea* parasitized pupa.

***In vitro* callus induction and cryopreservation of  
*Secale montanum* embryonic cell follicles**

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## Abstract

*Secale montanum* is one of the important species in Iran rangelands. For breeding programs and genetic conservation through cryopreservation, there was a need for callus production. So, seeds of *S. montanum* collected from rangelands of Iran. The seeds were sterilized by immersing in 2% sodium hypochloride for 15 min after putting under running water for 2 hours and washed three times with distilled sterile water. Then excised embryos cultured on MS medium with  $\frac{1}{2}$  of nitrate supplemented with 0.5, 1, 2, 3  $\text{mg l}^{-1}$  2,4-D. Differences in 2,4-D concentrations had no significant effect on callus induction but the highest growth percentage of embryonic callus was in 3  $\text{mg l}^{-1}$  2,4-D. The cell follicles obtained from calli pieces were placed on liquid MS medium containing 0.5 M sucrose and 75  $\mu\text{mol}$  ABA, at 4 °C and 25 °C for 10 days. Two cryopreservation methods were employed for cold acclimated and non cold acclimated cell follicles 1) Vitrification method: The explants were loaded with 2 M glycerol and 0.6 M sucrose for 20 min at 25 °C and placed in cryotubes having 0.5 ml PVS2 at 0 °C. The tubes immersed in LN. 2) Encapsulation method: Cell follicles were encapsulated in alginate beads. The beads were dried under laminar airflow for 4 h before immersing in LN. Cryopreserved cell follicles in both methods thawed and transferred into 40 °C distilled sterile water for 2 min and post-treated in liquid MS medium with 1.2 M sucrose at 25 °C. The follicles placed on semi-solid MS medium with 0.5 M sucrose as recovery medium for 3 days, subsequently, and then they transferred onto semi-solid MS mediums supplemented with 1  $\text{mg l}^{-1}$  2,4-D and 1  $\text{mg l}^{-1}$  2,4-D + 0.5  $\text{mg l}^{-1}$  GA<sub>3</sub>, for callus development. All cell follicles in both methods were survived and their growth was such as control cell follicles. Calli were transferred on organogenesis medium which encapsulated cell follicles produced root. However, both procedures appear promising for cells cryopreservation of *Secale montanum*.

**Keywords:** Calli, embryonic cell follicles, cryopreservation, vitrification, PVS2, encapsulation-dehydration, alginate beads, liquid nitrogen (LN), *Secale montanum*

**Abbreviation:** 2,4-D: 2,4-dichloropheoxyacetic acid, LN: liquid nitrogen, GA<sub>3</sub>, gibberellic acid, MS: Murashige and Skoog, PVS2: plant vitrification solution 2, 2iP: N<sup>6</sup>(2-isopentenyl)-adenine, BA: 2,6-Benzyl amino purine, TDZ: 1-phenyl-3-(1,2,3-thiadiazol-5YL)-urea

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## Introduction

Of all the plants of the earth the grasses are of the greatest use to the human race. To the grasses belong the cereals, sugarcane, sorghum and the bamboos; and, since they furnish the bulk of the forage for domestic animals, the grasses are also the basis of animal industry (Hitchcock 1935). These words are at least as true today as they were when written about 70 years ago and the primary reason for the high interest in including biotechnological application in their improvement. A fundamental requirement for nearly all application of biotechnology is the regeneration of whole plants from cells and tissue cultured *In vitro*. During the past few decades tissue culture techniques have been developed that could be used for the improvement of crop plants. Comparatively, monocotyledons are regarded as difficult *In vitro* material. The potential value of cell, tissue and anther culture as tool for use in the improvement of crop plants has been described (Vasil, 1987). A key factor in successful callus induction and plant regeneration in the grass family is the use of meristematic tissues as explants. These include mature and immature embryos, unemerged inflorescences, and basal leaf tissue.

The establishment of embryogenic cell suspensions has proven to be an important prerequisite for gene transfer approaches into cereal crops and forage grasses (Vasil, 1987). Embryogenic cell suspensions from monocot species can thus be used as a unique source of totipotent protoplasts and as direct targets for biolistic transformation. Long term maintenance of cultures is not only expensive and time consuming but also incurs risk of loss through microbial contamination, somaclonal variation and human error. Moreover, regeneration capacity decreases severely often after only a few months of culture (Datta *et al.*, 1992). All these risks could be minimized by *in situ* culture cryopreservation, which in recent years, has become an important tool for long-term storage of plant material, without genetic alteration.

During freezing as the internal water is converted into ice, the cell undergoes dehydration, which results in an increase in the concentration of salts and metabolites, as well as in membrane damage (Steponkus and Lynch, 1989). Protection against injury can be achieved by vitrification, i.e. the non-crystalline solidification of water. Two requirements must be met for a solution to vitrify: (i) rapid freezing rates (normally by plunging explants enclosed in a cryotube into liquid nitrogen); and (ii) a concentrated cellular solution. The latter is obtained through air drying, freeze dehydration, application of penetrating or non-penetrating substances or acclimation. Key for successful cryopreservation thus lies not with freezing tolerance but with dehydration tolerance and its induction (Panis *et al.*, 2005). Thus, successful cryopreservation protocols balance cell water

content so that both freezing injury and desiccation damage are minimized (e.g. Suzuki *et al.*, 2006; Shibli *et al.*, 2001; Scocchi *et al.*, 2003).

Methodologies encapsulation-dehydration and vitrification techniques have been employed for a variety of plant tissues like seeds, shoot apices, zygotic embryos, somatic embryos, protoplasts and cell suspensions. The encapsulation-dehydration procedure is based on the technology developed for the production of artificial seeds. Explants are encapsulated in alginate beads, pregrown in liquid medium enriched with sucrose, partially desiccated down to a water content around 20% (fresh weight basis), then frozen rapidly (Dereuddre, 1992; Engelmann, 1997). Although the procedure can be considered rather lengthy and labour-intensive, it is observed that the presence of a nutritive matrix (the bead) surrounding the explants can promote its regrowth after thawing. In vitrification method explants are treated with a concentrated vitrification, followed by a direct plunge into liquid nitrogen. This results in both intra- and extra-cellular vitrification. The vitrification solution consists of a concentrated mixture of penetrating and non-penetrating cryoprotectant substances. The most commonly applied solution is "PVS2" (Plant Vitrification Solution 2) (Panis and Lambardi, 2005). The most common cryoprotectants (used in cryoprotectant solutions) include agents like DMSO, ethylene glycol, 1, 2-propanediol, and glycerol which is a basal component in PVS1, PVS2, and PVS3. While in the preculture stage only a limited number of sugars and polyalcohols such as sucrose, glucose, sorbitol and mannitol have been used on plant tissues (Turner *et al.*, 2001). Vitrification is today by far the most widely used cryopreservation protocol. The success of the procedure can be attributed to its easiness, high reproducibility and to the fact that it can successfully be applied to a wide range of tissues and plant species (Panis and Lambardi, 2005).

*Secale montanum* is one of the important species in Iran rangelands. Whyte *et al.*, (1959) described *S. montanum* as a palatable, leafy, short-lived, tufted perennial which can provide winter grazing in subtropical areas with fair winter rainfall. In Iran, local ecotypes of *S. montanum* were useful for revegetating overgrazed substeppeic rangelands (Peymani-Fard, 1993). Plant genetic resources conservation and preventing erosion and extinction of this species is of utmost importance. So, long-term storage of embryonic cell follicles of this species in liquid nitrogen could provide a secure backup for these collections.

The objective of this study was to cryopreserve *S. montanum* embryonic cell follicles with simple procedures. In this work we present two methods including vitrification and encapsulation-dehydration for cryopreservation of the embryonic cell follicles.

## Materials and methods

**Plant materials:** Seeds of *S. montanum* were collected from Yam, East Azarbayjan, Iran rangelands. Healthy seeds selected and used in the following treatments:

**Callus induction:** For callus induction, sterile leaf discs from *in vitro* propagated plants and half seeds containing embryo were placed on MS medium with  $\frac{1}{2}$  of nitrate supplemented with different concentrations 0.5, 1, 2, 3  $\text{mg l}^{-1}$  2,4-D. The explants were surface sterilized by putting under running water for 2 hours then immersing in 2% sodium hypochloride for 15 min and washed three times with sterilized distilled water. Produced calli were used for cryopreservation experiments.

**Desiccation of cell follicles:** Cell follicles obtained from callus pieces were placed in liquid MS medium containing 0.5M sucrose and 75 $\mu\text{mol}$  ABA, at 4 $^{\circ}\text{C}$  and 25 $^{\circ}\text{C}$  for 10 days.

**Vitrification procedure:** For osmoprotection, cell follicles were first treated with loading solution containing 2 M glycerol and 0.6 M sucrose for 20 min at 25 $^{\circ}\text{C}$ . The treated cell follicles were transferred into 2 ml cryotubes containing 0.5 ml PVS2 solution (15% w/v ethylene glycol, 15% w/v DMSO (dimethyl sulfoxide), 30% w/v glycerol, 13.7% w/v sucrose supplemented with  $\frac{1}{2}$  strength MS salts) at 0 $^{\circ}\text{C}$  and immediately immersed in LN for a week.

**Encapsulation-dehydration procedure:** Initially, 3 gr alginate powders was dissolved in 100 ml modified MS medium (all salts in full strength except macro elements were at  $\frac{1}{2}$  strength, 0.75 M sucrose and 50  $\text{mg l}^{-1}$  ascorbic acid). The cell follicles transferred into the alginate solution for 15 min subsequently, the cell follicles quickly transferred into 100 mM  $\text{CaCl}_2$  solution for the same duration. The coated cell follicles were dried at room temperature for 4 h and transferred into 2 ml cryotubes and plunged into LN (liquid nitrogen) for one week.

**Thawing and recovery:** The cell follicles thawed in sterile distilled water bath at 40 $^{\circ}\text{C}$  for 2 min. The cell follicles were rehydrated and detoxified using liquid MS medium containing 1.2 M sucrose and, subsequently, the follicles placed on semi-solid MS medium with 0.5 M sucrose as recovery medium for 3 days, then transferred onto semi-solid MS medium supplemented with two hormone treatments: A) 1  $\text{mg l}^{-1}$  2,4-D and B) 0.5  $\text{mg l}^{-1}$   $\text{GA}_3$  + 1  $\text{mg l}^{-1}$  2,4-D, for callus development. Developed calli were transferred on organogenesis medium.

**Statistical analysis:** In callus production experiments, the effectiveness of 2,4-D as auxin for callus induction and growth (in length and width) has been demonstrated. There were six replications for each treatment and 4 embryos per each replication.

In cryopreservation experiments, there were three replications for each treatment. No liquid nitrogen was used in the control treatments. Means of growth of cells was expressed as quantitative index and was measured after one month (Table 1).

In all of the experiments, the results were analyzed by analysis of variance (ANOVA) and means were compared with Duncan's multiple range test.

**Table 1:** Quantitative growth of cell follicles

Quantitative value	1	2	3	4	5	6
Qualitative growth value	Very low	Low	Intermediate	High	Very high	Extremely high

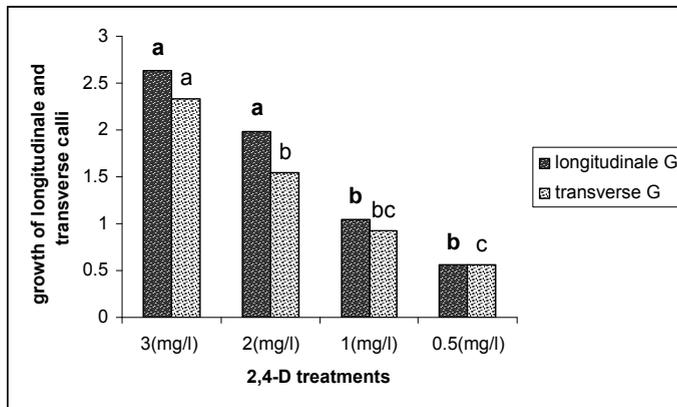
## Results

**Callus induction:** The callus induction only produced from mature embryos of *S. montanum* on MS medium with  $\frac{1}{2}$  of nitrate supplemented with 0.5, 1, 2, 3 mg $l^{-1}$  2,4-D. There was no significant difference on callogenesis but the highest callus growth was observed on medium containing 3 mg $l^{-1}$  2,4-D (Figure 1 and Table 2). But, the media treatment containing 1 mg $l^{-1}$  2,4-D was used as the best treatment, because of less toxicity.

**Table 2:** Analysis of variance of callus induction and growth of *Secale montanum* cultured on MS medium supplemented with different concentration of 2,4-D.

Source of variation	C.V.	M.S.	Pr>F
Effect of hormone on callus induction	24.96	0.041	0.975ns
Effect of hormone on callus length (mm)	33.50	5.21	0.0001**
Effect of hormone on callus width (mm)	31.79	3.6	0.0001**

NS: Not significant \*\*Significant at  $\alpha \geq 0.01$



**Figure 1:** The effect of 2,4-D concentrations on callus growth

All cell follicles in both cryopreservation methods were survived completely (Figure 2). There was not significant difference on callus growth between cryopreservation methods but calli on control treatments had better growth than calli on cryopreservation treatments (Table 3 and Figure 3). So, LN treatment decreased callus growth in both encapsulated and vitrified cell follicles. However, cells growth and rooting was better in encapsulated calli than in vitrified calli. The cold-dark (4°C) pre-treatment had heist growth and calli incubated in 16 hours light pre-treatment at 25°C had the most roots in encapsulation method (Figure 4).

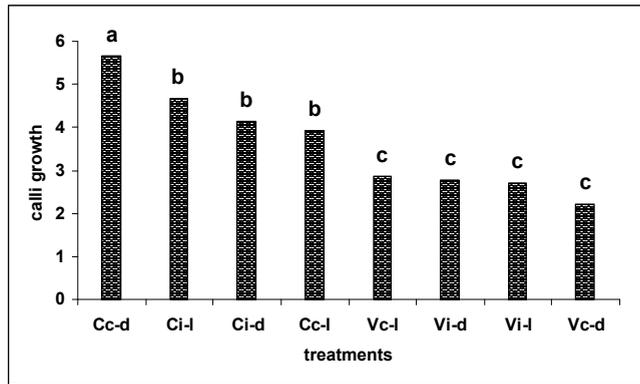


**Figure 2.** Survival of cell follicles in both cryopreservation methods: (a) encapsulated cell follicles, (b) control cell follicles and (c) vitrified cell follicles

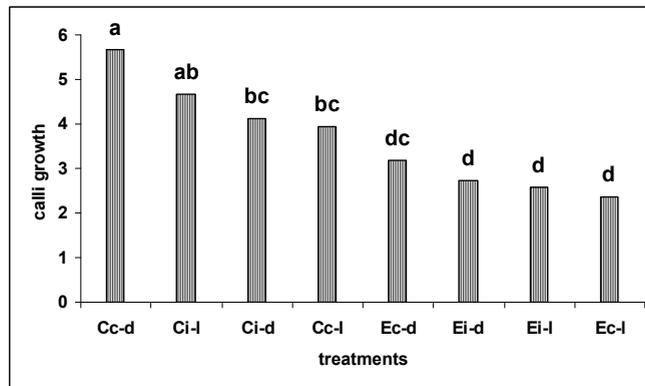
**Table 3:** Analysis of variance effect of pre- and post-treatments on growth cell follicles in vitrification and encapsulation methods

Source of variations	C.V.	M.S.	Pr>F
Effect of vitrified pre-treatments on growth cell follicles	14.89	4.84	0.0001**
Effect of post-treatments on growth cell follicles in vitrification method	14.89	0.54	0.146 <sup>ns</sup>
Interaction between pre- and post-treatment in vitrification method	14.89	0.117	0.692 <sup>ns</sup>
Effect of encapsulated pre-treatments on growth cell follicles	16.45	3.94	0.0001**
Effect of post-treatments on growth cell follicles in encapsulation-dehydration method	16.45	0.011	0.84 <sup>ns</sup>
Interaction between pre- and post-treatment in encapsulation-dehydration method	16.45	0.03	0.943 <sup>ns</sup>

NS: Not significant and \*\*Significant at 0.01 level

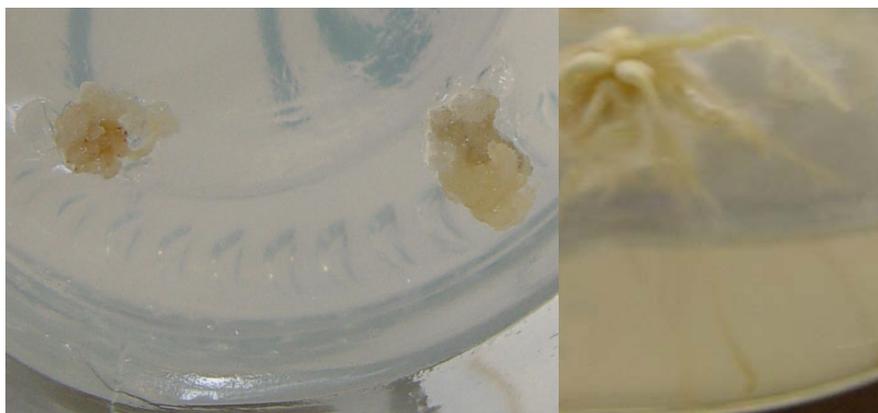


(A)



(B)

**Figure 3:** The effect vitrification and encapsulation-dehydration methods on cell follicles growth. A: The effect pre-treatments in vitrification method on cell follicles growth. [Cold-dark (Vc-d), Cold-light (Vc-l), Incubator-dark (Vi-d), Incubator-light (Vi-l)]; B: The effect pre-treatments in encapsulation-dehydration method on cell follicles growth. [Cold-dark (Ec-d), Cold-light (Ec-l), Incubator-dark (Ei-d), Incubator-light (Ei-l)]; and Cold-dark (Cc-d), Cold-light (Cc-l), Incubator-light (Ci-l), Incubator-dark (Ci-d) used as control treatments for cell follicles.



(a) (b)  
**Figure 4:** a) The cold-dark (4°C) pre-treatment had heist growth and b) calli incubated in 16 hours light pre-treatment at 25°C had the most roots in encapsulation-dehydration method.

## Discussion

The callogenesis from mature embryos of *Secale montanum* produced on MS medium with  $\frac{1}{2}$  of nitrate supplemented with 0.5, 1, 2, 3 mg $l^{-1}$  2,4-D. Mohmand (1993) found good callus from mature seed embryos of spring and winter wheat genotypes. Also, Barabanova *et al.*, (1988) and Bartok and Sagi (1990) induced callus on 1.5 and 6.0-8.0 mg/l 2,4-D, respectively on wheat. Growth regulator concentrations in culture medium are critical for the control of growth and morphogenesis. Generally, high concentration of auxins and low cytokinins or auxins alone in the medium promotes abundant cell proliferation with the formation of callus (Ihsan Shah *et al.*, 2003).

In cryopreservation experiments, cold acclimation is often used to increase the survival of cryopreserved cells and tissues (Martines and Reville 1998). Keith and McKersie (1986) suggested that the freezing tolerance of cultured cells may reflect whole-plant freezing tolerance and resemble the genetic potential of plant cells to acclimate to cold (Keith and McKersie, 1986).

A number of authors have addressed the influence of ABA and sugars on cell follicles desiccation and cryopreservation tolerances (Nieves *et al.*, 2001). Exogenous application of ABA, or the increase in endogenous ABA level under stress conditions (osmotic or water loss) has been associated with synthesis of proteins (Pelah *et al.*, 1997) and compatible solutes (Nieves *et al.*, 2001),

components which play important roles in plant stress tolerance. The accumulation of sucrose inside tissue helps in maintaining cell viability during dehydration and cryopreservation by stabilization of membranes (Oliver *et al.*, 1998) and enhancement of vitrification (Gonzalez-Arno *et al.*, 1996). The superiority of sucrose over other sugars was also reported in cryo-tolerance of oil palm somatic embryo clumps (Dumet *et al.*, 1994). ABA and sucrose in the culture medium decreased water content and osmotic potentials, but only slightly increased cold hardiness and tolerance to liquid nitrogen. ABA and sucrose effects were greatly enhanced when combined with low temperature treatment (Chang and Reed., 2000). The present work demonstrated the importance of low temperature (cold acclimation), sucrose and ABA pretreatments, in desiccation and cryopreservation tolerance of cryopreserved cell follicles.

Vitrification provides the benefits of cryopreservation without the damage due to ice formation. In this method, cell follicles are sufficiently dehydrated osmotically by exposure to a highly concentrated vitrification solution 2 (PVS2) and can let the cell solidity into a non-crystalline, glassy state (Lambardi *et al.*, 2000, Touchell *et al.*, 2002). Evidences show that PVS2 acts by three mechanisms. 1) Replaces cellular water, 2) changes freezing behavior in cells and 3) impedes water loss during air drying. Since some components of PVS2 such as glycerol and DMSO penetrate into cells as well as lower lethal temperature (Volk and Walters, 2006). However, the direct exposure of cells to PVS2 solution causes harmful effects due to osmotic stress. Thus, the key to successful cyopreservation by vitrification is to induce the osmotolerance to PVS2 solution by pre-treatment of explants.

Pre-treatment reduces the cell size and the cytoplasm to vacuole ratio, enhances the ability of cells to take up cryoprotectants during prolonged exposure and/or modifies cell walls and membranes to resist dehydration injury and deformation during freezing. So, we used loading solution to reduce negative effect of PVS2. This solution with glycerol and sucrose lower the melting temperature and water content of cells then exposure to PVS2 lower them more than loading solution (Volk and Walters, 2006).

In encapsulation/dehydration method, cells surrounded in calcium alginate capsule. A possible explanation for the positive effect of encapsulation on survival and growth could be a greater availability of mineral and hormonal nutrients (Flachsland *et al.*, 2006). It is, also, possible that endogenous substances might be more slowly released to the medium because of the presence of the beads thus remaining around the explants for the longer time and affecting favorably the response (Flachsland *et al.*, 2006).

In our study, the observation showed that both methods had complete survival and cells growth almost resembled. So, they are easy to perform and often has a high recovery percentage, which makes them widely applicable, particularly to the conservation of plant species sensitive to low temperature (Chen *et al.*, 1985).

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# **Climatic variation causes genetic variation in Iranian rangeland plant species**

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## Abstract

*Bromus* L. is one of the most important rangeland plant genus in Iran which contains several wide spread species all around the country. Several perennial species of the genus are very nutritive and palatable and several others are very effective in soil conservation. A national research project was carried out at Forests and Rangelands Research Institute by which we have been evaluating and estimating genetic and cytogenetic variation between several *Bromus* species. *B. tomentellus*, *B. hankegnus*, *B. sterilis*, *B. inermis*, *B. cappadocicus*, *B. persicus* and *B. biebersteinii*, were studied for their karyotypic characteristics. Then six populations of *Bromus tomentellus* were selected based on their morphological characteristics to investigate their karyotypes. Morphological and seed storage protein profile were also used to investigate genetic variation of the species.

Within the studied populations, they varied from diploid (*B. sterilis*,  $2n=14$ ) to dodecaploid (*B. tomentellus*,  $2n=84$ ). This study confirmed the existence of high levels of ploidy in this genus. The size of the chromosomes among the recorded species varied from 2.6  $\mu\text{m}$  in *B. inermis* to 7.7  $\mu\text{m}$  in *B. biebersteinii*. Chromosome length grand mean of the populations varied from 3.8  $\mu\text{m}$  in *B. inermis* to 5.7  $\mu\text{m}$  in *B. cappadocicus*, and *B. biebersteinii*. In all of the populations the chromosomes were mainly of m type (centromers at median region). However, several populations possessed sm type (sub-meta centric) chromosomes. Among the studied populations the highest TF% was estimated on *B. sterilis* species (44.84). The lowest TF% value was estimated on *B. hankegnus* (41.44). The populations of *Bromus tomentellus* possessed three ploidy levels ( $2n=42$ , 70 and 84). Two populations showed the highest ploidy level so far recorded on the species (dodecaploid,  $2n=84$ ). Providing protein profile, the population varied a lot based on the profile. The most number of bands was found in one of *Bromus tomentellus* populations and the least number in a population of *B. benekenii*. Several species of the genus are completely recognizable from other species based on the profile but more criteria are required for other species. This would indicate an active evolution process within and between populations of the species. Analysis of variance of the recorded data on the progeny families of *Bromus inermis* indicated that the families were highly uniform and not enough genetic variation was estimated on the population. The population may have been under breeding progress so that the genetic variation has vanished. Insufficient sampling during germplasm collection may be another reason for the problem.

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## Introduction

### *I.R. Iran's overall climate*

Islamic Republic of Iran covers about 1.65 million km<sup>2</sup>. Mountains and deserts cover more than half part of the country's area. There are two major mountain ranges of Alborz in the north and Zagros in the west and southwest, together with lower mountain ranges in centre and east of the country close to the central plateau. The variation in precipitation and evaporation indicates a wide variety of climates within the country. Iran is categorized as generally having arid and semiarid climates. This implies that the annual precipitation is less than the potential annual loss of water through evapotranspiration. The occurrence of rainfall is unreliable and deviations from the mean are generally more than 40%. The average annual precipitation over the country is estimated to be about 250 mm, about one-third of global annual precipitation. Annual precipitation within the country varies between 26mm in Lut Salt Desert in the southeast to 2000 mm in the Caspian Sea region. Winter is normally the rainy season for the country. More than 50% of the annual precipitation occurs in that three months period. Except for the northern mountains, summer is dry season. The pan-evaporation varies between 700 and 4200 mm per year. It is considered that of the total precipitation some 70% evaporates, about 20 percent constitutes surface runoff, and the rest infiltrates to recharge the under ground water.

The incidence of drought in Iran is common with substantial consequences on food security, livestock production, environment and natural resources. During past ten years the country suffered from the worst droughts in the past 40 years. The latest drought is said that affected some 37 million people, and hit at least 25 out of 30 provinces of the country. This would imply the necessity of optimised utilization of the resources.

### *Iran is a centre of diversity*

Iran is one of the worlds major centres of origin and diversity for many important field crops such as wheat, barley, oat, rye, chickpea, lentil, sunflower, many vegetable species, forages, fruit trees and nuts. In fact due to diversity in geomorphology, topography and climate, Iran has a unique situation with its richness in genetic diversity in forests and range plant species. These plant species include many wild and domesticated forms of annual and perennial, herbaceous and woody plants. This richness is very useful for a sustainable agriculture and crop improvement. Efficient investigation of genetic variability and conservation of genetic diversity of forage and rangelands plant species including wild relatives of the agronomic plants and fruit tree species are required. There are so many other endemic plant species in tree and shrub forms, which are very unique for their characteristics. Many of these genetic resources, because of their adaptation to an often harsh environment, may contain desirable characteristics such as tolerance to drought, salinity, heat or other biotic and abiotic stresses. They may

therefore, be very important resources for plant breeding, which may be conducted in some form of association with international interests.

### ***Erosion of genetic diversity***

These valuable germplasms are sometimes under intensive exploitation. In some cases the exploitation is so intensive that the species is under danger situation. We are committed to prevent the extensive exploitation of these genetic resources even in collaboration by other international organizations that may benefit from the collection and use of these resources. There are several other factors contributing to the process of erosion include long periods of drought, high pressure of grazing, urbanisation, land degradation, and replacement of land races. Due to a long term improper utilization of natural resources, the original structure of plant genetic diversity have been continuously changing during last century. Not for a long time ago, there was no efficient strategy for conserving, assessing and utilizing the plant genetic resources. Whereas, due to various reasons such as industrialization, construction of highways and dams and urbanization, there is an increasing pressure on forests and rangelands. With the current population growth rate, the demands on the utilization of the natural resources would be increased in the near future.

It's worth mentioning that Iranian Forests and Rangelands Research Institute is in a position to be in a close collaboration with other countries and serve as regional centre for technical supports, required collection, evaluation, preservation, reclamation and exploitation of the valuable germplasm.

Several national program and research projects were carried out at the research institute by which we have been evaluating and estimating genetic variation between several important forests and rangeland plant species and then characterizing genetic architecture of the best population of the species. *Bromus* species are among the most important rangeland plant species that can be found in vast areas of the country. In this article genetic and karyotypic variation of the species are to some extent described.

### ***Bromus species***

*Bromus* L. is one of the most important rangeland plant genus in Iran which contains several wide spread species all around the country. The genus comprises about 160 species distributed all over the world (Acedo and Lamas, 2001). Iranian Natural Resources National Gene Bank has collected a great number of accessions of several locally adopted species from the nature. Several perennial species of the genus are very nutritive and palatable and several others are very effective in soil conservation. The palatable species are usually used for grazing domestic animals in the region. The species vary to some extent in ecological requirements, so that they form different ecotypes with different characteristics.

Within and between population genetic variation were recorded on morphological characteristics of elsewhere (Mirzaie-Nodoushan *et al.* 2000)

*Bromus tomentellus* Boiss is one of the most important species of the genus, which is scattered all around the country. The species is very nutritive and palatable as well as being important in soil conservation. The species is usually used for grazing domestic animals in the region. Different populations of the species vary to some extent in ecological requirements, so that they form different ecotypes with different characteristics. Primary information such as karyotypic characteristics and ploidy level of the ecotypes may end to some unknown information interested to breeders. *Bromus* species are known as the species with various intra-specific ploidy levels. Long time ago, Hill (1965) recorded up to 112 chromosomes for *B. erectus* Hudson. Yang and Dunn (1997) also recorded various levels of polyploidy in *B. inermis* Leyss.

However, there are complex relationships between the species of *Bromus*. In some cases, autopoloidy, allopoloidy, interspecific hybridization and genetic introgression between related populations have all contributed to the evolution of species or cultivars (Armstrong 1985). As well as the relationships, possible presence of genetic systems that suppress homologous chromosome pairing would increase the complexity of the relationship (Armstrong 1991). Thus, all these would make the investigation of the relationships between the species of *Bromus* and its interspecific hybridization more problematic. Researchers have made many attempts for identifying even individual chromosomes of several *Bromus* species, for instance, by using C-banding procedures and chromosome dimensions (Tuna *et al.* 2001).

Primitive studies on *Bromus* species in Iran revealed high genetic and cytogenetic variation within and between the species. Since species such as *B. tomentellus* is scattered in a vast area, which form various ecotypes, it was assumed different karyotypic characteristics might be one of the main reasons for the variation. Therefore, highly variable populations were selected to investigate their karyotypic and morphologic characteristics.

Differences in chromosome number, shape and size at metaphase of mitosis division can express genetic variation between the individuals. These differences are much greater at interspecific level. We may be able to produce more details for the species under investigation with the aid of chromosome analysis procedures, on which the possible compatible karyotypes are more easily recognized. On the other hand, karyotypic data are of importance in showing the species inter-relationships.

Researches overcome sexual breeding barriers of plant species by somatic hybridization through protoplast fusion (Yamaguchi and Shiga, 1993, Kunitake *et al.* 1996 and Tsukatani *et al.* 2002), but different genomic size and characteristics are the main obstacles for next generations. Although such complexity may allow the maintenance of large reserves of genetic variability within and among populations, favouring potential long-term breeding progress, but limiting genetic

gains in short- and long-term breeding programs (Casler *et al.* 2000). However, the objective of this study was to determine and compare karyotypic characteristics of several populations of locally adopted *Bromus* species.

### ***Within and between species karyotypic variation in Bromus***

Seven *Bromus* species, *B. tomentellus*, *B. bankegnus*, *B. sterilis*, *B. inermis*, *B. cappadocicus*, *B. persicus* and *B. biebersteinii*, were either collected from the nature or obtained from National Natural Resources Gene Bank, Iran. Based on an ongoing breeding project, Cytogenetic studies on the populations were performed in order to specify their karyotypic characteristics. Preparations were made using fresh grown root tips for the karyotypic studies. Different pre-treatments were used and obtained the best results for treating the root tips with saturated  $\alpha$ -Bromonaphtaline for three hours followed by fixation in a glacial acetic acid and ethanol (1:3 volume ratio) for 17 to 24 hours (Mirzaie-Nodoushan and Asadi-Corom, 2002). The root tips were then hydrolyzed in 1N HCl at 60 degree centigrade for 4-5 minutes and used Hematoxiline as the preferred agent for chromosome staining. Karyological data such as long arm (L) and short arm length (S) on all chromosomes of five well-prepared cells were recorded at metaphase stage containing a complete set chromosome, for all populations using photomicroscope equipped with camer lucida and micrometer. Then total length of the chromosomes, L/S and S/L ratios were driven on the recorded data. High number of chromosomes and close morphological similarities of the chromosomes of the species prohibit the exact identification of homologous pairs. However, chromosome pairs were identified and arranged based on their total length and arm ratios. The studied populations of the species differed in their ploidy levels. A haploid set of chromosomes from each population were used for analyzing the data. The chromosomes were described according to Levan terminology (Levan *et al.* 1964), then we used factorial analysis of variance on the data groups based on completely randomized design model for each ploidy level, regarding the populations and their chromosomes as two different factors (Mehrpur *et al.* 2002). Several statistical parameters such as total form percentage, TF% (Huziwara 1962), the mean chromosome length (Forni-Martins *et al.* 1994) S% and S/L (ratio of the shortest to the longest chromosomes) were estimated to compare the karyotypes of the populations..

### ***Within species karyotypic variation***

Six populations of *Bromus tomentellus* were selected based on their morphological characteristics to investigate their karyotypes. Preparations were made using fresh grown root tips for the karyotypic studies. Pre-treatments, fixation, hydrolyzation, and staining were done as mentioned above. The same Karyological data such as mentioned above were recorded on all chromosomes of three to five well-prepared cells of the populations at metaphase stage containing a complete set of

chromosomes. Chromosome pairs were identified and arranged based on their total length and arm ratios. The studied populations differed in their ploidy levels and karyotypic characteristics. The same analysis were performed on the data as performed on the between karyotypes data. Finally, Karl Pearson correlation coefficients were estimated for all paired combinations of populations at each ploidy level based on their chromosome dimensions

## Results and discussion

### *Ploidy level and chromosome number of different species*

The somatic chromosome number and details of the karyotypes are presented in Table 1. Within the studied populations, *B. sterilis* was the only diploid species ( $2n=14$ ). This is in agreement with the results of an investigation recorded by Sheidai and Fadaei (2005). *B. tomentellus* showed the highest ploidy level (dodecaploid,  $2n=84$ ). To our knowledge this ploidy level has not been reported on this species. Oja and Laarmann (2002) recorded different ploidy levels within one species of *Bromus*. In this study *B. hankegnus* and *B. cappadocicus* were hexaploid ( $2n=42$ ). *B. inermis* was octaploid ( $2n=56$ ), and *B. persicus* was tetraploid ( $2n=28$ ). *B. biebersteinii* was decaploid ( $2n=70$ ). Ploidy level in *Bromus* species were reported to be varied from diploid to decaploid (Armstrong, 1991, Tuna *et al.* 2001). This study confirmed the existence of high levels of ploidy in this genus. Parts of this study were published elsewhere in details (Mirzaie-Nodoushan and Shariat, 2002, Mirzaie-Nodoushan *et al.* 2005b)

### *Size of the chromosomes*

For a long time difficulties are reported in studying *Bromus* karyotypic characteristics that is due to the large number of chromosomes, small morphological differences between the chromosomes and variability from cell to cell for chromosome length and arm ratio (Tuna *et al.* 2001). The size of the chromosomes among the recorded species varied from 2.6  $\mu\text{m}$  in *B.inermis* to 7.7  $\mu\text{m}$  in *B. biebersteinii*. Chromosome length grand mean of the populations varied from 3.8  $\mu\text{m}$  in *B. inermis* to 5.7  $\mu\text{m}$  in *B. cappadocicus*, and *B. biebersteinii* (Table 1).

### *Karyotypic characteristics*

Karyotypic formula and symmetry parameters are presented in Table 1. In all of the populations the chromosomes were mainly of m type (centromers at median region). However, several populations possessed sm type (sub-meta centric) chromosomes. *B. hankegnus* possessed the most sm type chromosomes ( $2(6m+16sm)$ ). Among the studied populations the highest TF% was estimated on

*B. sterilis* species (44.84). This species possessed only m type chromosome that is the main reason for the population to have the most symmetric karyotype based on TF%. The lowest TF% value was estimated on *B. hankegnus* (41.44). This species contains 16 sm type chromosomes that make its karyotype asymmetric. DRL presents different scheme of asymmetry for the populations. That is due to different basis of the estimation of the two parameters, TF% and DRL. TF% estimates the symmetry based on the ratio of total short arm lengths of the chromosomes of a genotype to its total chromosome length, whereas, DRL estimates the symmetry based on the difference between relative length of the shortest and the longest chromosomes of a genotype. However, based on DRL, *B. sterilis* showed the most asymmetric karyotype, (DRL= 2.93) while *B. tomentellus* showed the most symmetric karyotype, (DRL= 0.64). The highest ratio between the shortest to the longest chromosomes (S/L) was observed on *B. sterilis*, the diploid population. In contrast the lowest S/L was observed on *B. biebersteinii*, the decaploid species (2n=70). The studied populations and species are cytogenetically varying complex of mainly polyploidy genotypes. To get further insight into this issue, more research is necessary on the species to find out the general trend of evolution in the species as well as inter-relationship between the species.

In this genus, the basic chromosome number is  $x=7$ , as in related genera of Poaceae, such as *Lolium* and *Triticum*. This study confirmed that the *Bromus* species highly vary karyologically in number of chromosomes. This kind of genetic and cytogenetic variability can confer an adaptive advantage against variable climate and other ecological elements in the region. Therefore, cytologically there is not a high degree of uniformity in chromosome number between the species of *Bromus*.

Karl Pearson correlation coefficients, estimated for karyotypic dimensions showed a high r-value ( $>.95$ ) for total length of the chromosomes (data are not shown). However, lower r-values were estimated for other karyotypic characters, particularly arm ratios. These indicate occurrence of structural changes within the chromosomes of the studied populations.

In general the studied populations of the species were not only highly differentiated from each other based on ploidy level and chromosome numbers but also there were highly differentiation between the populations with similar ploidy levels. These karyotypic data are highly useful in taxonomic studies on the species. Moreover, these results revealed that interspecific hybridisation requires a precise selection of the parents for the crosses.

### ***Detecting genetic variation in Bromus species by electrophoresis***

Several populations of *Bromus* species were studied for their seed storage protein variation utilizing SDS-PAGE method in order to investigate genetic variation between the species as well as finding the ability of the method in identifying the different species. Providing the mentioned protein profile, the number of bands

was determined and the populations were evaluated based on the profile. Twenty seven different protein bands were recognized in the profile. The population varied a lot based on the profile. The most number of bands was found in one of *Bromus tomentellus* populations and the least number in a population of *Bromus benekenii*. Several species of the genus are completely recognizable from other species based on the profile but more criteria are required for other species. This study was published in detail somewhere else (Mirzaie-Nodoushan *et al.* 2005)

### ***Karyotypic variation within Bromus tomentellus***

The populations possessed three ploidy levels ( $2n=42$ , 70 and 84). Two populations showed the highest ploidy level so far recorded on the species (dodecaploid,  $2n=84$ ). There were several cells with higher numbers of chromosome ( $2n>160$ ). Since they were not consistent within a population they were not regarded as a ploidy level for the species. This would indicate an active evolution process within and between populations of the species. Ploidy level in *Bromus* species were also reported to be varied from diploid to decaploid, Armstrong, (1991), and Tuna *et al.* (2001). This study confirmed the existence of high levels of ploidy as well as existence of dodecaploid karyotypes in the species (Mirzaie-Nodoushan *et al.* 2006).

Due to the large number of chromosomes, small morphological differences between the chromosomes and variability from cell to cell for chromosome length and arm ratio difficulties are reported in studying *Bromus* karyotypic characteristics (Tuna *et al.* 2001). The size of the chromosomes among the studied populations varied from 3.1  $\mu\text{m}$  in Ardabil population to 7  $\mu\text{m}$  in Tabriz population. In general, the studied populations are not only highly differentiated from each other based on ploidy level and chromosome numbers but also there is highly differentiation between the populations with similar ploidy levels.

### ***Karyotypic characteristics***

All of the populations had mainly m type chromosomes (centromers at median region). However, two populations possessed several sm type (sub-meta centric) chromosomes. Hoomand population, one of the hexaploids, and Lar Lake population, one of the dodecaploid populations, each possessed four sm type chromosomes. Among the studied populations the highest TF% was estimated on Kermanshah population (45.05). The lowest TF% value was estimated on Lar Lake population (43.45). This population contains 4 sm type chromosomes that is one of the main reasons to make its karyotype asymmetric. Based on DRL, Ardabil population showed the most asymmetric karyotype, (DRL= 1.28) while Tabriz population showed the most symmetric karyotype, (DRL= 0.62). The highest ratio between the shortest to the longest chromosomes (S/L) was observed on Sirachal population. In contrast the lowest S/L was observed on Lar Lake and Tabriz, the two dodecaploid populations. On the whole the higher

ploidy levels showed lower DRL, or more symmetric karyotypes based on their longest and shortest chromosomes.

### ***Morphologic genetic variation of Bromus tomentellus***

In order to investigate morphological genetic variation in *Bromus tomentellus* species twelve genotypes of the species available in forage gene bank which were collected from different parts of the country were surveyed for eleven morphological traits. The data were analysed using RCBD analysis of variance. After confident result of differences between populations for the morphological traits, the genotypes were classified based on the mentioned traits. Correlations between all combinations of genotypes were also estimated.

Principal components analysis (P.C.A) presented two components, containing about 98 percentage of the total existing variation of the data. The two main components were used to produce scatter plot of the populations. Path analysis showed that leaf length, flowering date, and plant vigour were the most important factors affecting plant yield, and had direct effects of 1.04, 1.11 and 0.56 respectively. Applying UPGMA method of cluster analysis on the studied attributes, four groups or clusters were obtained. Regarding the morphological characteristics the least and the most similar populations were identified to be used in the future breeding projects. The details of the results of this part of the experiment were published elsewhere (Mirzaie-Nodoushan *et al.* 2001).

### ***Genetic architecture of a population of Bromus inermis.***

Genetic architecture was studied on one accession of *Bromus inermis* to investigate the suitable breeding method and then to estimate the genetic gain based on the genetic variation of the plant population. By selecting the best population of the species in a primitive study, single plants were used to harvest half-sib families' seed. The seed were planted in a randomized complete block design field experiment to investigate genetic architecture and suggest proper breeding method for the population. Fourteen half-sib families were compared in the three times replicated field experiment and a number of characteristics including fresh and dry yield were recorded on the single plants. Analysis of variance of the recorded data on the progeny families indicated that the families were highly uniform and not enough genetic variation was estimated on the population. Various reasons may be regarded to justify this result. The population may have been under breeding progress so that the genetic variation has vanished. Insufficient sampling during germplasm collection may be another reason for the problem. In this case the existing genetic variation depends on the number of plants on which the seed was collected. Both the factors may also be involved in decreasing the genetic variation.

## Conclusion

The studied populations are morphologically and cytogenetically varying complex of genotypes. To get further insight into this issue, more research is necessary on the species to find out the general trend of evolution in the species. This study confirmed that the species particularly *Bromus tomentellus* highly vary in ploidy level and number of chromosomes. This kind of genetic and cytogenetic variability can confer an adaptive advantage against variable climate and other ecological elements in the region. This might be one of the main reasons for the species for being such an adaptive species, which is highly scattered all around the country. Being so variable in karyotypic characteristics may end to new species with completely different morphological characteristics.

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Table 1: Mitotic characteristics of the studied populations of *Bromus* species.

<b>Species</b>	2n	M ( $\mu\text{m}$ )	S ( $\mu\text{m}$ )	L ( $\mu\text{m}$ )	S/L	S%	TF%	DRL	KF
<i>B. tomentellus</i>	84	5.4	3.8	6.7	0.57	0.84	43.45	0.64	80m+4sm
<i>B. hankegnus</i>	42	4.6	3.3	6.0	0.55	1.71	41.44	1.40	26m+16sm
<i>B. sterilis</i>	14	4.4	3.6	5.4	0.67	5.84	44.86	2.93	14m
<i>B. inermis</i>	56	3.8	2.6	4.0	0.65	1.22	42.63	1.88	56m
<i>B. cappadocicus</i>	42	5.7	4.4	7.2	0.61	1.84	44.54	1.17	42m
<i>B. persicus</i>	28	4.9	3.4	6.4	0.53	2.48	43.47	2.18	28m
<i>B. biebersteinii</i>	70	5.7	3.8	7.7	0.49	0.95	43.06	0.98	64m+6sm
<b>Location of collection</b>	<b>Populations of <i>Bromus tomentellus</i></b>								
<i>Hoomand</i>	42	4.7	3.8	6.0	0.63	1.91	44.31	1.10	38m+4sm
<i>Sirachal</i>	42	5.5	4.3	6.7	0.64	1.86	44.32	1.04	42m
<i>Ardabil</i>	42	4.1	3.1	5.3	0.58	1.80	43.74	1.28	42m
<i>Kermanshah</i>	70	5.5	4.1	6.8	0.60	1.06	45.05	0.71	70m
<i>Lar Lake</i>	84	5.4	3.8	6.7	0.57	0.84	43.45	0.64	80m+4sm
<i>Tabriz</i>	84	5.7	4.0	7.0	0.57	0.84	44.44	0.62	84m

TF% = Total form percentage, M= grand mean of chromosome length, L. = length of the longest chromosome, S = length of the shortest chromosome, DRL = Differences between the maximum and minimum relative length of the chromosomes, S% = relative length of the shortest chromosome, S/L = ratio of the shortest to the longest chromosomes, KF = Karyotypic formulae

# **The effects of Poplar clones age variations and production conditions on medium density fiberboard (MDF) properties**

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## Abstract

In this study, MDF was produced from three clones of poplar (*P.e. vernirubensis*, *P.e. costanzo*, *P.e.561.41*). Age clones of poplar were 4 and 12 years. In addition to age variation, the influence of three production variables (steaming time, press time and resin content on MDF properties were investigated. Variations of each variable were as follow:

- Steaming time (5, 10, 15 minutes)
- Press time (3, 4, 5 minutes)
- Resin content (9%, 11%)

Fiber length and fiber diameter of 4 and 12 years old poplar clones (*P.e. vernirubensis*, *P.e. costanzo*, *P.e.564.41*) were measured 746, 25.23, 805, 25.76, 751, 25.44 and 902, 31.62, 995, 29.23, 899, 26.83 micron respectively.

MOR, MOE, IB, TS2, TS24 were determined according to EN standard.

Increasing steaming time decreased board's internal bond and bending properties. Also increasing press time increased board's internal bond. The effect of press time on bending properties was not significant. As expected, boards demonstrated better properties at higher resin consumption. Minimum of boards thickness swelling were obtained at 15 minutes steaming time because of fiber hydrophilic properties were decreased. There was no significant difference between boards' strength properties made up of 4 and 12 year old Poplar clones except thickness swelling. The effect of clones' age on thickness swelling after 2 and 24 hours was significant and thickness swelling of boards made by Poplar clones 4 year old was less than boards made by Poplar clones 12 year old.

**Keywords:** Poplar, Fiberboard, Fiber, Resin, Clone, Steaming

## 1- Introduction

With increasing of consumption of wood and paper productions in Iran due to population growth, changes in consumption pattern and developing demands therefore a modern management together comprehensive view should be searched and applied in order to provide for suitable materials to produce qualified goods. It present new occupations and response to internal needs too, meanwhile Iranian limited forests with important ecological and environmental aspects will be protected from over utilization.

MDF is one of the wood productions which can be produced by lignocelluloses material ever from some annual plants as raw material. Of course qualified boards may be produced by suitable fiber material combination which prepares

homogenous material. In this case a cheap material may become to a valuable production. MDF is very applicable because of its characteristics such as technological and physical properties. MDF with low thickness can be replaced with plywood, particleboard and HDF for interior uses in structures. Since MDF has no natural defects which are in massive wood and are suitable for production in big dimensions. Therefore is most considerable for furniture and carpentry industries, so that is more profitable than massive wood.

According to MDF properties, its worldly productions have been recently developed rapidly. In Iran, MDF consumption was increased from 135 cubic meters in 1996 to 300000 cubic meters in 2005. Therefore new MDF factories will be established in future in order to increase production together decreasing of imports.

According to policies of forests organization for preservation of Iranian forests, harvesting of wood has been recently decreased and decreasingly yearly. To rely on statics in 10 years ago harvesting have been decreased about 50% so that annual harvesting was 985000 cubic meters in 2003 and decreased again about 25% in 2004 [3]. Therefore wood industries can be depended on forests of north of Iran so that Iranian wood and paper factories will be faced with deficit for supply of raw materials. Therefore alternative resources should be followed. One of these resources is Poplar wood which is one of fast growth trees in Iran. As Poplar is one of the flexible and adaptable species, from ecological aspects, it can be cultivated in different areas. This is a communitical tree so that it is possible to cultivate behind together. Therefore dense nursery can be established by some of species and varieties of Poplar. Poplar wood is white and debarked easily.

According to above poplar researches division in research institute of forests and rangelands of Iran achieved and performed many research projects in order to offer successful clones related to short rotation for supply of wood. Such as *P.e.vernirubensis*, *P.e. costanzo*, *P.e.561.41* clones [15]. Wood science research division of above institute focuses their researches on successful clones of Poplar so that with studying on their properties find appropriated applications.

As MDF properties are affected by fiber treatment and production conditions of board, therefore boards of MDF were made under different fiber treatment and production conditions. Also in order to survey of effects of Poplar clones age on MDF properties, 4 and 12 year old Poplar clones were used.

Some related researches are as follow:

Ramezani (2001) in his master science thesis studied fiber dimensions, chemical compound and pulping (APMP) of 3 species (*P. deltoids*, *P. euroamericana*, *P. nigra*) in Kermanshah province. He reported fiber length, fiber diameter, lumen cell diameter, cell wall thickness for

*P. euroamericana* 1190, 25.68, 17.28, 4.21 micron respectively.

Nazarnejad (1997) studied on anatomical, physical and chemical aspects of *P. deltoiedes* (clones of 77.51) and *P. euroamericana* (clones of 45.51) with 12 year age in

Safrabaste station. He appointed fiber length, cell wall thickness of *P. euroamericana* 1290, 4.49 micron respectively.

Dean S. Deabell et.al (2002) studied effects of clones, age, growth rate and pruning of juvenile Poplar trees on wood density and fiber length. Age of trees was at 9 years and discs were cut from 1.5, 3 meters height of trees. Fiber length was increased from 0.57 mm at 1 year old to 1 mm at 9 years old. Fiber length of different clones was also varied.

Mahdavi (2002) studied using of Poplar and Eucalyptus wood combination in order to produce mechanical pulps. In this research he determined fiber biometry of with 8 and 18 years. He reported fiber length of *P. deltoides* 0.897, 0.995 mm for these respectively. He resulted with increasing in age fiber length was increased.

Zobel (1998) pointed out wooden tissue formed near to core in first years of growth is juvenile wood with special properties. In other hand wooden tissue formed near to bark in several years after beginning of growth is mature wood whose properties is difference in compared with juvenile wood. Juvenile wood has lower density, fiber length shorter and less cell wall thickness than of mature wood. Angle deviation of microfibril in S2 layer of juvenile wood is more than of mature wood. In addition cellulose and hemicelluloses content are less than of mature wood and lignin content of juvenile is more than of mature wood.

Todd and collaborators (1999) studied effects of forestry manner and kind of wood (heart wood, sap wood) on particleboard and MDF properties made by wood of *Pinus.teda*. Researchers found thickness swelling and water absorption of these productions made by heart wood (juvenile wood) were less than by sap wood. Bending properties and internal bond of composite wood made by heart wood and sap wood were also comparable with together and there is insignificant difference between them. Although MOR of MDF made by heart wood was partly more than of sap wood.

Les Groom et.al (1999) studied relationship between fiber properties and fiber board properties. They used juvenile and mature woods of *Pinus.teda* for MDF production. Mixture ratio of juvenile and mature wood included (100- 0, 75- 25, 50-50, 25- 75, 0- 100) respectively. Results of this research indicated that with increasing in juvenile wood bending properties of MDF were improved.

Habibi et.al (2002) in a research titled as the effect of Bagasse fiber properties on the medium density fiberboard (MDF) quality noted that the fibers strengths properties decrease and their water absorption improve by increasing steaming time and steaming temperature. They ascribed this problem to the destruction of cellulose and hemi-cellulose chains caused by hydrolyze reactions.

Habibi et.al (2003) in another research titled as study on effects of resin content and press time on MDF properties found that effects of increasing in resin content and press time on mechanical and physical properties were significant so that maximum of strength properties of boards were obtained in 11% resin

content and press time of 6 minutes. In these conditions, water absorption properties of boards were least.

Short et.al (1978) determined the physical and mechanical properties of fiberboards made out of (a) wet chips and (b) chips of up to 50% moisture. MDF was made out of the mixture of *Pinus teda* and hard woods. In the case (b) the boards of hard woods indicated better internal bond and the values of MOR, internal bond, water absorption, and thickness swelling in the boards of case (b) showed an increase with compared to boards of case (a), but the linear expansion and MOE decreased. Refining of under pressure chips of case (b) showed better fiber for hard wood and soft wood than case (a) and consequently the refining dried *Pinus teda* chips indicated an improved MDF board.

Roffael and Dix (1992) studied the properties of MDF made out of young poplar woods. These researchers noted that fiberboards made out of 16-year poplar fibers had more mechanical strength and less thickness swelling than fiberboards made out of 5-years poplar woods with the same colon. Results show that it is possible to produce the medium density fiberboard with acceptable strength properties, out of poplar wood juvenile in the proper construction process condition.

Laboskey et.al (1993) studied the influence of various levels of steam pressure inside the double disk refiner (50, 60, 70, 80, 90, 100 psi) and the resin content of formaldehyde urea (6%, 8%, 10%, 12%) on the MOR, MOE, internal bond, water absorption, and thickness swelling in medium density fiberboard resulted from *Acer rubrum* and concluded that increasing the steam pressure of refining didn't have a considerable influence on the MDF strength properties and dimensional stabilities of the board, while the resin content had a considerable influence on the all of the board properties, so that increasing resin content from 6 to 12% lead to 174 % increase in internal bond, 68 % increase in MOR and 40 % increase in MOE. They also concluded that there is another important factor, other than resin, that affects MDF properties and that is the moisture content of chips during the refining of fibers.

Okamoto et.al (1994) investigated the influence of high steam pressure on the mechanical and physical properties of MDF boards as well as steaming pressure treatment effects on the chemical composition of MDF. They concluded that the dimensional stabilities of MDF improve by increasing the steaming time as well as increasing steaming pressure and mechanical properties of MDF decrease. It has also been observed that the longer the steaming time and the greater the steaming pressure, the less amounts of hemi-cellulose and alpha cellulose will be, while lignin composition doesn't so vary. The best condition for under pressure steam injection in range of 60 – 90 sec is identified to be in steam pressure of 11 kgf / cm<sup>2</sup> or 90 -180 sec in steam pressure of 6 kgf / cm<sup>2</sup>.

Maloney (1989) from his researches concluded that increasing of resin content causes improving of mechanical properties and dimensions stability of wood composite production. He also pointed out that when temperature of mat core

reach to optimum point for UF resin polymerization, strength properties of these productions will be improved.

Suzuki et.al (1989) studied effect of independent variables on MDF properties. Thickness of boards was 4-5 mm and used resins were UF, UMF and lignosulfonate. Bending and internal bond properties were improved by increasing of resin content especially when UF resin and lignosulfonate were used. Meanwhile thickness swelling was also decreased by increasing of resin content.

## 2- Material and Methods

In this study samples were selected after consultation with Poplar research institute of forests and rangelands. Therefore 3 clones include *P.e 561.41*, *P.e.costanzo*, *P.e.vernirubensis* with 4 and 12 years old were selected in Alborz station. For measuring of fiber length and fiber diameter a disc was cut from breast height of trees. Then slices were provided by them in based on Franklin method and finally fiber length and fiber diameter were measured.

**Variable parameters:** In this survey three press times 3, 4, 5 minutes and three steaming time 5, 10, 15 and two resin content 9%, 11% were used.

**Constant parameters:** In this survey the press temperature of 165 °C, board density of 0.7g/cm<sup>3</sup>, were used constantly for all treatments.

### 2-1- Stages of Experimental Boards Construction

In order to prepare fiber, samples were chipped into proper chips by a drum chipper of the type Pallman and treated with variables steaming times. Then chips were refined by an experimental refiner and their fibers were separated. After that drying process was done by a rotary dryer with an angular velocity of 3rpm. The final fibers moisture content before spraying resin was about 1%.

Spraying resin was performed horizontally and with angular velocity of 20rpm. To form the fibers mat, the wooden cast of the dimensions 30cm\*32cm\*25cm were applied. Fiber were weighted using the scale with the accuracy of 1g and were sprinkled within the cast uniformly. The height of fibers mat was smoothed and balanced in all directions. After spraying resin stage, two samples from fibers mat were prepared, to control the mat moisture content. After forming the fibers mat, the experimental press of the type Burkle-L100 was used for compressing and constructing boards.

The boards were put in the experimental condition for 2 weeks and then were cut based on EN standard from test samples. Then the physical and mechanical properties of boards including MOE, MOR, internal bond and thickness swelling after 2 and 24 water immersions were determined. The results of this survey were analyzed using Factorial experiment in the form of a completely randomize design and comparing the average values using Duncan test.

### 3- Results

**Anatomical characteristics:** mean of fiber length and fiber diameter for 3 clones with 4 and 12 year old are as follows:

Table 1- fiber length and fiber diameter of 4 year old Poplar clones

clone	<i>costanzo</i>	<i>vernirubensis</i>	561.41
Fiber length(micron)	805	746	751
Fiber diameter(micron)	25.76	25.23	25.44

Table 2 - fiber length and fiber diameter of 12 year old Poplar clones

clone	<i>costanzo</i>	<i>vernirubensis</i>	561.41
Fiber length(micron)	995	902	899
Fiber diameter(micron)	29.23	31.62	26.83

The tables shows fiber length and fiber diameter were increased with trees age. In other words, amount of juvenile wood of 4 year old Poplar was more than 12 year old. This finding has been already approved by other researchers (11, 13, 23).

**3-1- Physical and Mechanical Properties of fiberboards:** All of strength properties and stabilities dimension of boards in different treatments are summarized in tables 3 and 4.

Table 3 - Mechanical and physical properties of MDF made up of 4 year old Poplar clones in various treatments

Steaming time (min)	Press time (min)	Resin content (%)	MOR (MPa)	MOE (MPa)	IB (MPa)	T.S.2 (%)	T.S.24 (%)
5	3	9	19.44	1828	0.279	38.87	45.37
		11	20.51	2171	0.347	32.07	38.37
	4	9	19.01	1608	0.367	38.45	44.41
		11	25.23	1973	0.528	30.36	36.13
	5	9	19.82	1817	0.481	40.75	50.56
		11	21.19	1911	0.55	31.33	38.29
10	3	9	16.18	1607	0.243	35.32	42.45
		11	19.11	1786	0.324	29.17	36.37
	4	9	17.12	15.48	0.167	44.21	51.75
		11	20.1	1723	0.395	30.67	37.28
	5	9	15.4	1518	0.32	39.01	46.24
		11	21.67	1770	0.48	32.46	39.32
15	3	9	15.71	1482	0.179	30.86	35.98
		11	17.51	1544	0.277	25.38	29.36
	4	9	13.61	1319	0.267	28.2	33.61
		11	16.61	1595	0.325	24.14	28.6
	5	9	15.75	1460	0.323	30.16	36.36
		11	16.17	1588	0.413	23.67	29.1

Table 4 - Mechanical and physical properties of MDF made up of 12 year old Poplar clones in various treatments

Steaming time (min)	Press time (min)	Resin content (%)	MOR (MPa)	MOE (MPa)	IB (MPa)	T.S.2 (%)	T.S.24 (%)
5	3	9	18.5	1748	0.271	40.18	45.37
		11	21.01	1810	0.353	35.24	40.43
	4	9	21.76	2015	0.338	41.91	45.4
		11	19.36	1860	0.54	31.06	37.33
	5	9	18.94	1799	0.458	40.08	45.77
		11	23.78	2020	0.571	31.38	34.94
10	3	9	15.06	1442	0.237	44.7	50.56
		11	17.95	1682	0.315	31.47	36.15
	4	9	16.86	1705	0.265	43.36	48.94
		11	19.67	1825	0.386	36.63	41.53
	5	9	16.91	1590	0.34	34.07	38.67
		11	19.2	1834	0.435	30.65	34.34
15	3	9	15.15	1496	0.192	35.06	39.07

		11	15.9	1474	0.27	29.18	32.66
	4	9	14.16	1360	0.255	33.79	38.46
		11	17.41	1707	0.335	26.51	32.8
	5	9	16.03	1540	0.3	33.99	35.35
		11	17.14	1660	0.41	25.36	30.96

**MOR:** The results derived from variance analyses showed that the independent effects of steaming time and resin content on the MOR is statistically significant at the level of 1%(CV=9.07%). The figure 1 shows effect of resin content on MOR. The results derived from the comparison of averages by Duncan test are presented in table 5 for different steaming times. Also the effects clones age and press time on MOR are not statistically significant. Maximum MOR was observed in resin content of 11% (fig 1). MOR of Boards made up of 4 and 12 year old Poplar clones were 18.64 and 18.01 MPa respectively (fig 2). Although effect of press time was not significant on this property but maximum of MOR was obtained at 5 minutes press time.

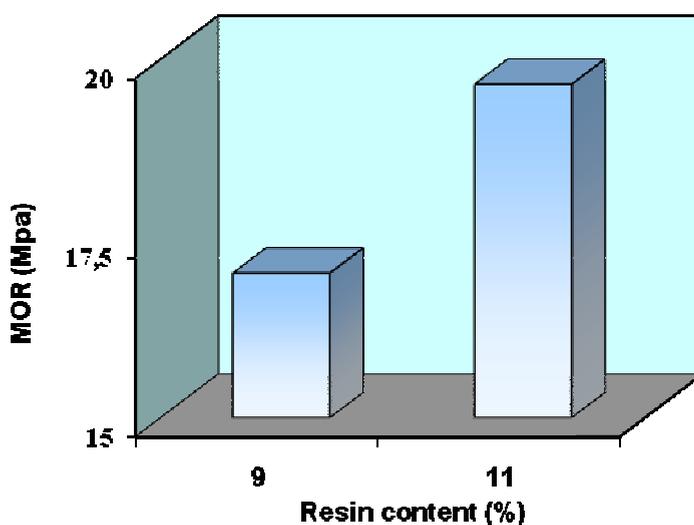


Fig 1: The effect of resin content on MOR

Table 5 - Duncan's grouping test of MOR based on different steaming time

Steaming time (min)	MOR (MPa)	Duncan's grouping
5	20.96	A
10	18.16	B
15	15.93	C

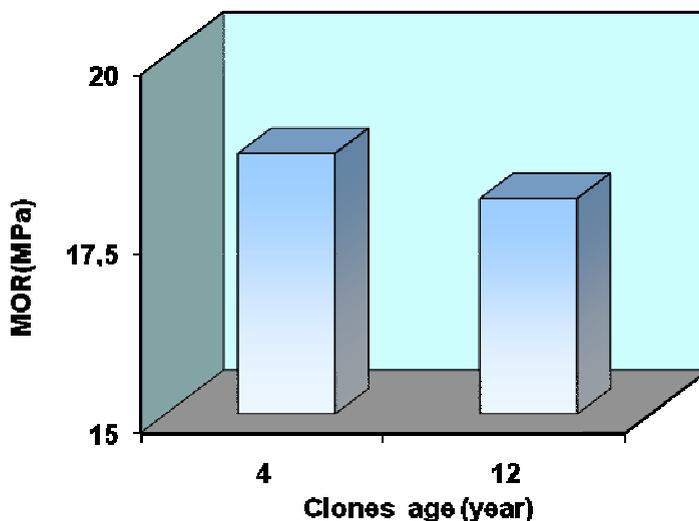


Fig 2: The effect of clones' age on MOR

**MOE:** The results derived from variance analyses showed that the independent effects of steaming time and resin content on the MOE is statistically significant at the level of 1% (CV=7.49%). The effect of clones' age and press time on MOE is not statistically significant. Maximum MOE was observed at 5 minutes press time. Figures 3 and 4 show effect of resin content and clones age on MOE respectively. The results derived from the comparison of averages by Duncan test are presented in table 6 for different steaming times. MOE of Boards made up of 4 and 12 year old Poplar clones were 1693 and 1700 MPa respectively.

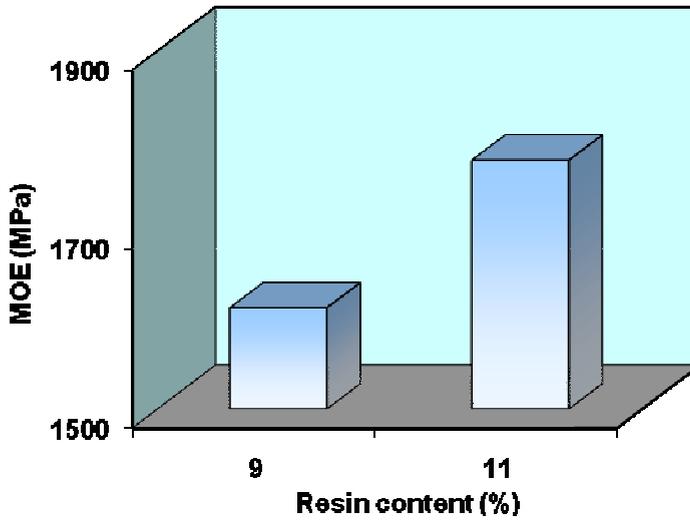


Fig 3: The effect of resin content on MOE

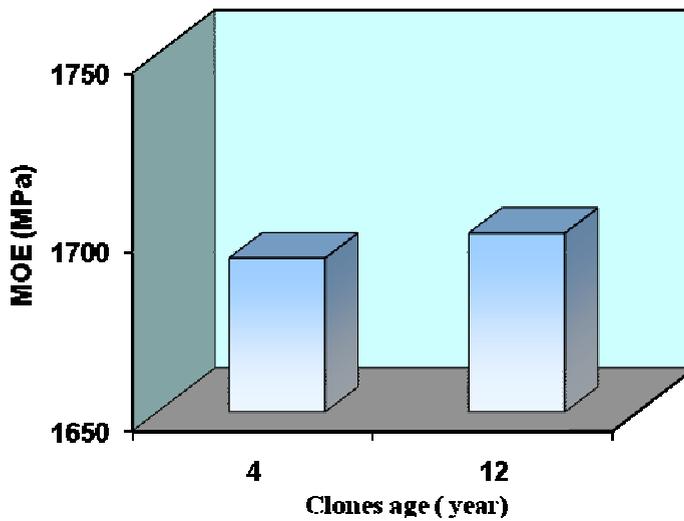


Fig 4: The effect of clones' age on MOE

Table 6 - Duncan's grouping test of MOE based on different steaming time

Steaming time (min)	MOE (MPa)	Duncan's grouping
5	1880	A
10	1698	B
15	1518	C

Internal bond: The results derived from variance analyses of the variable's effect on the internal bond showed that the independent effects of steaming time, press time and resin content on the internal bond are statistically significant at the level of 5% (CV = 21.97%). The effect clones age on IB is not statistically significant. The figures 5 and 6 show effect of resin content and clones age on IB. The results derived from the comparison of averages by Duncan test are presented in tables 7 and 8 for different steaming times and press times respectively.

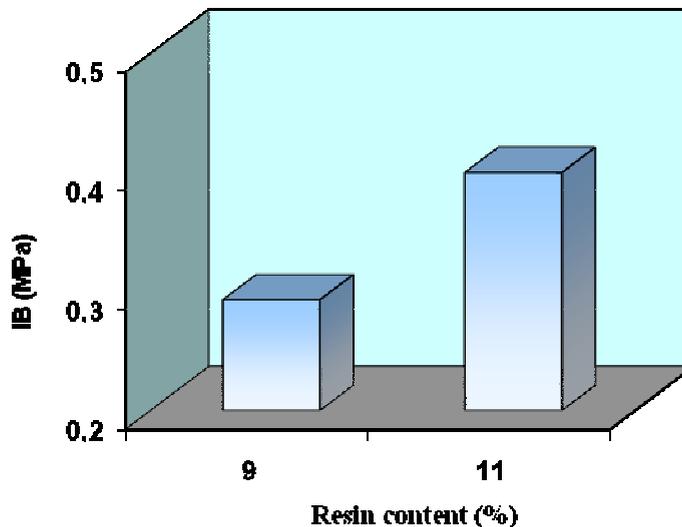


Fig 5: The effect of resin content on IB

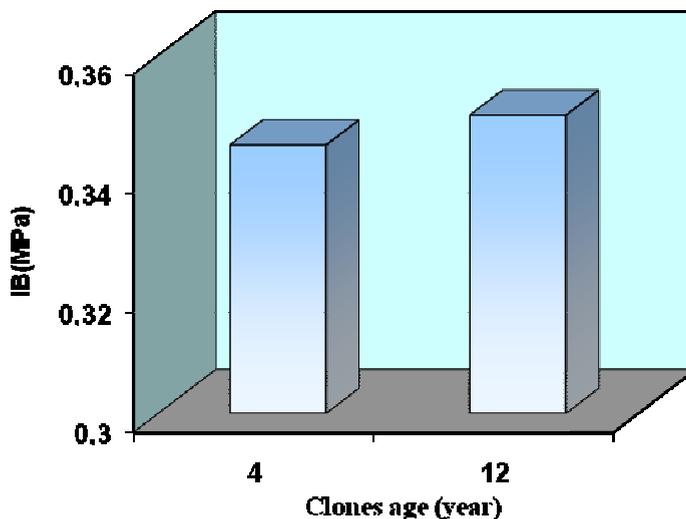


Fig 6: The effect of clones' age on MOE

Table 7 - Duncan's grouping test of IB based on different steaming time

Steaming time (min)	IB (MPa)	Duncan's grouping
5	0.423	A
10	0.321	B
15	0.295	B

Table 8 - Duncan's grouping test of IB based on different press time

Press time (min)	IB (MPa)	Duncan's grouping
3	0.273	C
4	0.347	B
5	0.419	A

Thickness swelling after 2 and 24 hours: **The results derived from variance analyses of the variables' effect on the Thickness swelling after 2 and 24 hours showed that the independent effect of steaming time, clones age and resin content on the above properties is statistically significant. The coefficient of variance for thickness swelling after 2 and 24 hours are 8.28% and 8.55% respectively. Although the effect of press time on these properties is not statistically significant but minimum these properties were observed at 5 minutes press time.**

Figures 7, 8, 9, 10, 11 and 12 show the effects of clones age, steaming time and resin content on thickness swelling after 2 and 24 hours respectively.

The interaction effect of the said variables on the above properties is not statistically significant. Figures 4 and 5 show the effect of steaming temperature on the thickness swelling after 2 and 24 hours, respectively. The results derived from the comparison of averages by the Duncan test on the said properties are presented in tables 9 and 10 for interaction effects of steaming time and press time on these properties.

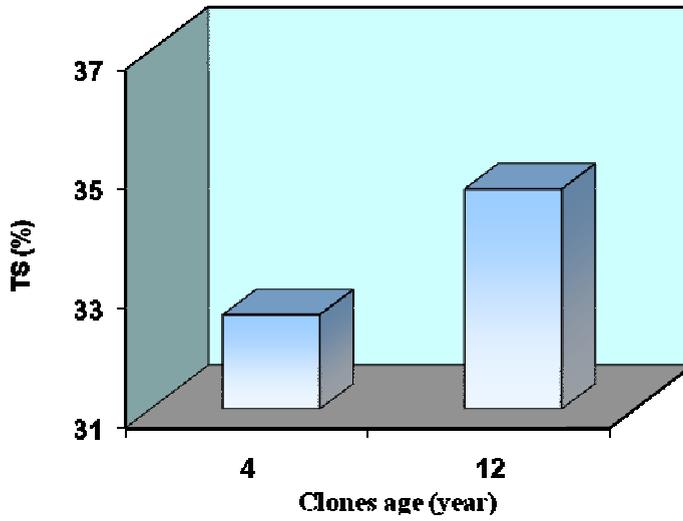


Fig 7: The effect of clones' age on T.S. after 2 hours

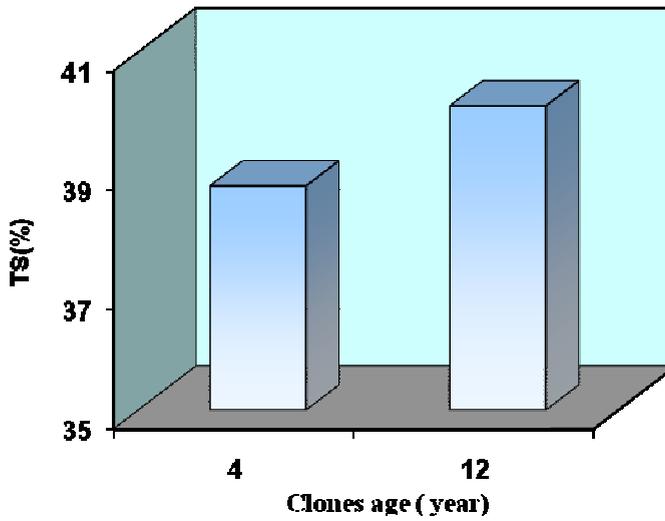


Fig 8: The effect of clones' age on T.S. after 24 hours

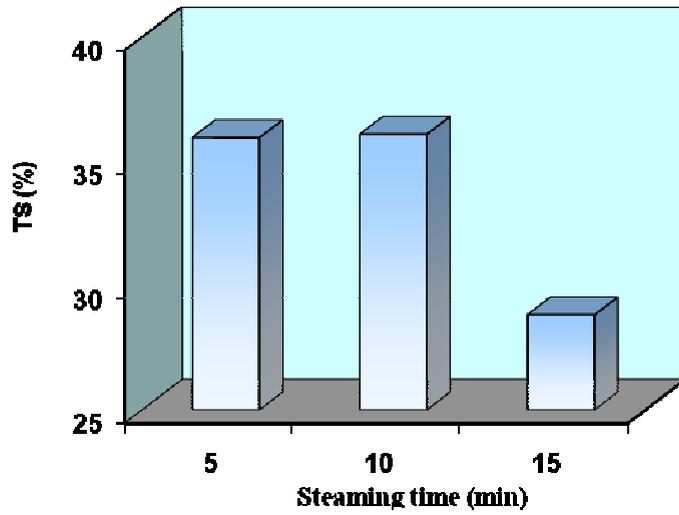


Fig 9: The effect of steaming time on T.S. after 2 hours

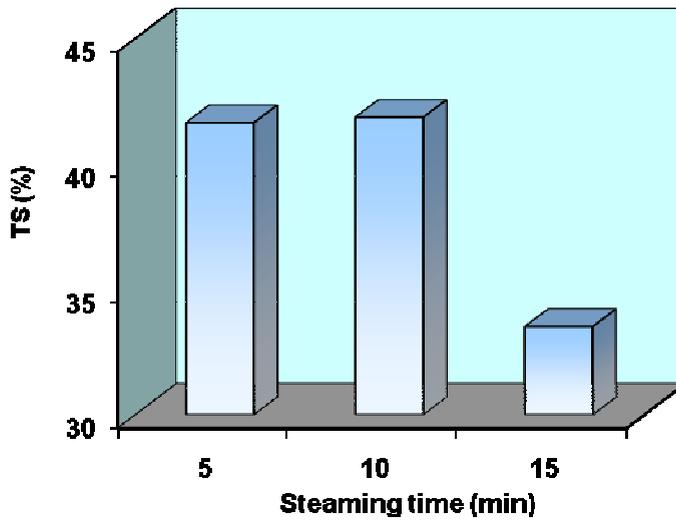


Fig 10: The effect of steaming time on T.S. after 24 hours

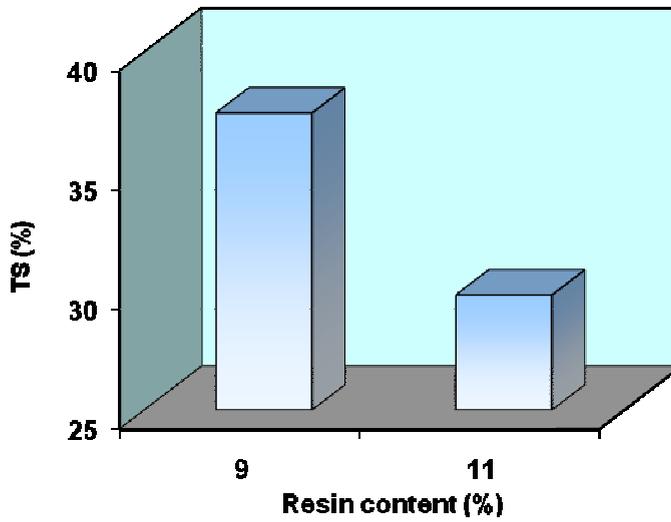


Fig 11: The effect of resin content on T.S. after 2 hours

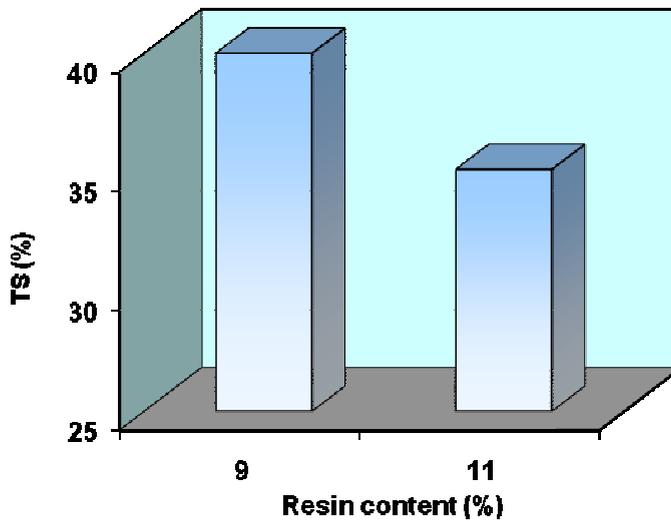


Fig 12: The effect of resin content on T.S. after 24 hours

Table 9 - Duncan's grouping test of T.S.2 based on interaction effect of steaming time and press time

<b>Steaming time (min)</b>	<b>Press time (min)</b>	<b>T.S.2 (%)</b>	<b>Duncan's grouping</b>
5	3	36.58	AB
5	4	35.44	B
5	5	35.88	B
10	3	35.29	B
10	4	38.8	A
10	5	34.23	B
15	3	30.09	C
15	4	28.16	C
15	5	28.3	C

Table 10 - Duncan's grouping test of T.S.24 based on interaction effect of steaming time and press time

<b>Steaming time (min)</b>	<b>Press time (min)</b>	<b>T.S.24 (%)</b>	<b>Duncan's grouping</b>
5	3	41.72	AB
5	4	40.82	B
5	5	42.39	AB
10	3	41.51	B
10	4	44.87	A
10	5	39.2	B
15	3	34.21	C
15	4	33.37	C
15	5	32.94	C

## 4- Discussion

**Anatomical characteristics:** Fiber length and fiber diameter for 4 and 12 year old Poplar clones are as follows:

Table 11- fiber length and fiber diameter of 4 year old Poplar clones

clone	<i>costanzo</i>	<i>vernirubensis</i>	561.41
Fiber length(micron)	805	746	751
Fiber diameter(micron)	25.76	25.23	25.44

Table 12 - fiber length and fiber diameter of 12 year old Poplar clones

clone	<i>costanzo</i>	<i>vernirubensis</i>	561.41
Fiber length(micron)	995	902	899
Fiber diameter(micron)	29.23	31.62	26.83

The tables shows fiber length and fiber diameter were increased with trees age. In other words, amount of juvenile wood of 4 year old Poplar was more than 12 year old. This finding has been already approved by other researchers [4, 14, 25].

**Mechanical and physical properties of boards:** Mechanical properties of wood composite materials are affected factors such as fibers strength properties, transmission form of stress from one fiber to another and fiber orientation in mat. With increasing of steaming time, bending properties (MOE & MOR) of boards were decreased so that maximum and minimum of these properties were observed at 5 and 15 minutes steaming time respectively. Increasing the steaming time, the wood chips were under pressure for longer time and this probably caused more hydrolyze reactions. Therefore hydrogenised bonds of cellulose and hemicelluloses chains were more broken finally fibers strength properties and boards bending properties were decreased [9, 18]. MOR and MOE of boards made by 4 and 12 year old Poplar clones were 18.64, 18.01 and 1693, 1700 MPa respectively. Effect of clones' age was not significant on these properties. Results of other researches indicated strength properties of fiberboards made by juvenile and mature woods were comparable with together so that difference between them was insignificant [24].

Increasing resin content increased bending properties (MOE, MOR) and internal bond of boards so that maximum of these properties were observed in 11% resin content. It can be noted in this case that by increasing resin content, number of joint point was increased. Therefore strength properties of boards were improved [10, 11, 13, 24]. Although effect of press time is not significant on bending properties but maximum of these properties were obtained at 5 minutes.

The internal bond is an indicator of connection within fibers. The results derived from the investigating of the influence of steaming time on the internal bond, showed that increasing this factor, decreased the internal bond so that maximum of this property was observed at 5 minutes steaming time. Increasing the steaming time, decreased the strength properties of fiber by destructive reactions, and this reduction caused dropping of internal bond [9, 18]. The internal bond was improved by increasing of press time so that maximum this property was observed at 5 minutes press time. Generally, increasing of press time cause heat transmission to throughout of board thickness concluding complete polymerization of resin in inner layers of mat. Therefore internal bond is improved [10, 13]. Internal bond of fiberboards made by 4 and 12 year old Poplar clones were 0.345 and 0.35 MPa respectively that there was no significant difference between them [24].

Changing the dimensions of lingo-cellulosic productions caused by the absorption and desorption of water by the cell wall, particularly the productions with high density is considered as unfavorable properties. Productions such as fiberboard are swelled in the direction of thickness. The results derived from investigating the steaming time on the thickness swelling after 2 and 24 hours showed that increasing this factors, have improved these two properties, so that the minimum value of each noted properties was observed in steaming time of 15 minutes. Increasing the steaming time apparently causes the destruction of hemicelluloses chains, and therefore by destructing OH agents, the water absorption of fiber and consequently the boards, decreases [9, 18]. Thickness swelling after 2 and 24 hours was decreased by increasing of resin content. This is probably due to increasing joint points between fibers and increasing of connection strength [10, 11, 13, 23]. Minimum of thickness swelling was observed in 11% resin content. The effect of clones' age on thickness swelling after 2 and 24 hours was significant and thickness swelling of boards made by Poplar clones 4 year old was less than boards made by Poplar clones 12 year old. This is due to more juvenile wood percent in 4 year old Poplar clones in compared with 12 year old Poplar clones. Hemicelluloses and celluloses content are more in mature wood than juvenile wood. Therefore this cause less thickness swelling in boards made by 4 year old Poplar clones [25].

Although effect of press time was not significant on thickness swelling but minimum these properties were observed in press time of 5 minutes.

**Maximum of strength properties of boards, obtained in the steaming time and press time of 5 minutes and resin content of 11%. Also minimum of**

thickness swelling of boards, obtained in the steaming time of 15 minutes and press time of 5 minutes and resin content of 11%. There is no significant difference between boards' strength properties made up of 4 and 12 year old Poplar clones except thickness swelling. Therefore using the said condition and 4 year old Poplar clones is advised to produce the medium density fiberboard.

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## **Spatial structure of trees in old - growth oriental beech stands of Hyrcanian forests**

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## 5VgfUW

In this investigation, coordination of 454 oriental beech (*Fagus orientalis* Lipsky) trees in 7 different types of old-growth stands was studied. The study area is located in Safarood – Ramsar in Hyrcanian forest of northern Iran. Using data of crown dimension and height of trees, the horizontal and vertical distribution of trees were prepared.

The results showed that trees are usually oriented in groups and seldom in random pattern. In other word, the horizontal distribution of trees usually appears in cluster form but in randomized group and seldom it was poison or random. Analyzing of spatial structure of stands can be very useful for demonstrating and modeling the actual state of the stands with hypothetical mathematical models. Moreover, monitoring of the studied stands can deliver sufficient data for understanding of further development of the stands. We determined 8 structure types on the structure triangle with the aid of our identification key.

**Key word:** oriental beech, Iran, coordination, spatial structure, horizontal and vertical distribution

## Introduction

The analysis of spatial structure is now commonly used in plant ecology (Tomppo 1986, Haase 1995, Pelissier & Goreaud 2001, Wiegand & Moloney 2004), which allows characterizing the structure of different populations, as well as the spatial interactions between populations. It can also be used to infer some information on the biological processes that are highly linked to the spatial structure of an ecosystem (Moeur 1993, Barot et al. 1999, Goreaud 2000). Indeed, the observed spatial structure results from past biological processes (especially birth and mortality of plants), and in return it defines the variety of local neighborhoods of each plant, which will influence future processes such as competition and mortality.

The spatial structure of a forest stand (in other words the organization of the trees in space) plays a key role in the dynamics of forest stands. The classic models are not sufficient for recognition and prediction of stand dynamics, therefore new models are required to study the dynamic of stands and interactions between trees. Spatial structure of real stands is well known to be non poisson. Many natural or human processes (competition, growth, mortality, thinning, regeneration, etc.) result in a very complex and highly variable structure (Goreaud et al. 1997). The relative location of young and old trees of the same species can help us to understand the dynamics of regeneration (Pelissier 1995, Collinet 1997). The point process formulation can be used to simulate virtual stands of various structures. As far as trees are concerned, where slow growth hinders experimentation,

simulation on virtual stands seems particularly interesting, for instance, in order to test an experimental design before creating it, to compare various silviculture scenarios (Pukkala 1989), or to predict the effect of environmental changes (Pretzsch and Kahn 1995).

The patches forming the mosaic are distinguishable on the basis of stand volume accumulation, age and size structure, canopy openness, occurrence of regeneration, and /or eventually, species composition (Leibundgut 1979, Korpel 1993, Podlaski 2004). However, to date, little attention has been paid to a fundamental difference between "patchiness" as a general feature of forest pattern resulting from the spatial and temporal correlation of ecological processes and 'accidental occurrence of less or more homogenous patches', which may be an effect of quite random natural phenomena (Jaroslaw & Paluch 2007).

The spatial relation between canopy openness and the occurrence of regeneration did induce researchers to frame the concept of gap dynamics (Watt 1947, Shugart 1984, Remmert 1991), which assumes that the death of a canopy tree creates a gap of up to a few hundred square meters that becomes a localized site of regeneration and subsequent growth. As a rule, the cohort of trees filling the gaps exhibits limited age and size variety, and a complex stand structure results from the asynchronous formation and filling of canopy openings rather than structural diversification of single patches. The basic spatial unit of the mosaic, characterized by a certain structural homogeneity, is an area corresponding to the crown width of large canopy trees. However, some authors have argued that structural similarity may be attributable to much larger areas, ranging from one to a few thousand squares meters (Leibundgut 1979, Korpel 1993, Emborg et al. 2000). In this case, the patchiness results from some general spatial and temporal correlation of tree die-off processes, which then sets in motion regeneration and recruitment (Jaroslaw & Paluch 2007).

This paper is aimed at reviewing information on natural stand dynamics in beechwoods of Safarood – Ramsar in Hyrcanian forest of northern Iran. This information can contribute to defining the reference point for natural-based management of beech forest.

## Materials and Methods

This study is carried out in a pure oriental beech (*Fagus orientalis* Lipsky) stands which is located in an altitude of 1200 to 1300 m.a.s.l. in "Eshkete – chal" of Ramsar region in the Caspian forests of northern Iran.

The experimental design was completely randomized design with 20 sample plots, each covering one ha (Fig. 1). Each sample plot was divided into 0.25 ha (= sum of 80 sub-plots). All trees were assessed within the sub-plots, which were then divided into four diameter classes of small size timber (ST = dbh<30cm), medium size (MT= 30<dbh<50 cm), large (LT= 50<dbh<65 cm) and extra large (XLT=

dbh>65cm). The spatial coordination of all trees were determined as well and were transferred over the maps later.

A total of 454 trees (8 selective sub-plots) were studied and 7 different forest types were recognized considering different combinations of proportion of timber size classes.

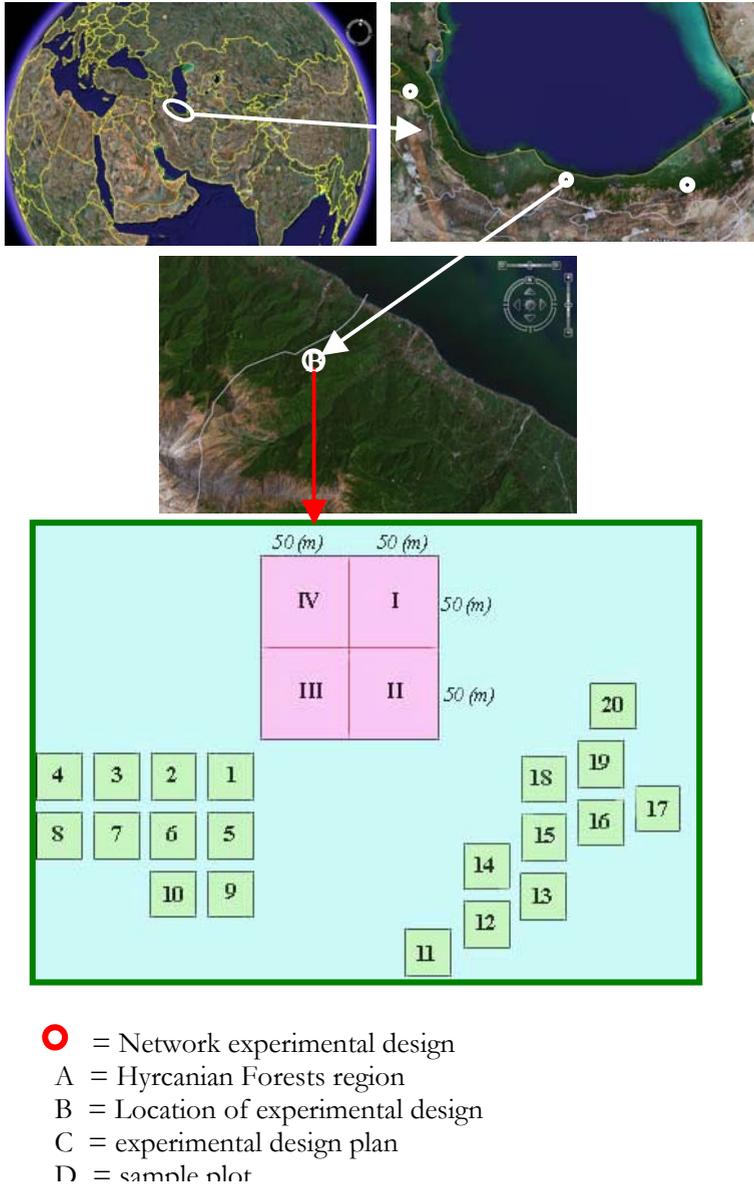


Fig. 1. The Hyrcanian Forests region and the experimental design.

## Results and Discussion

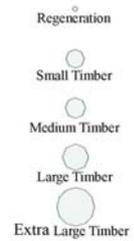
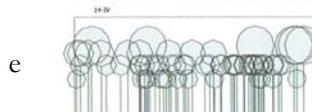
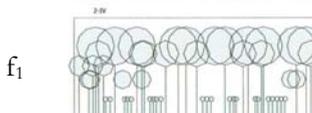
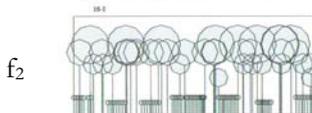
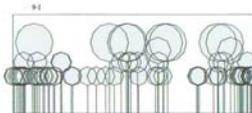
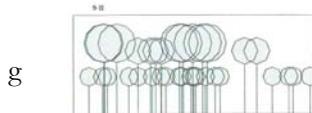
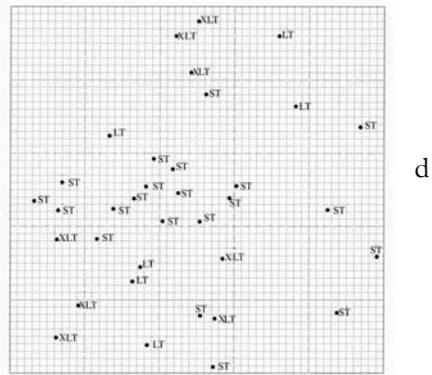
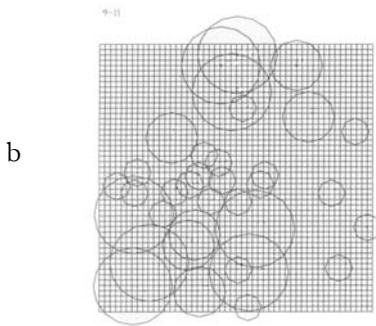
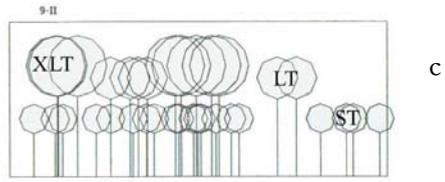
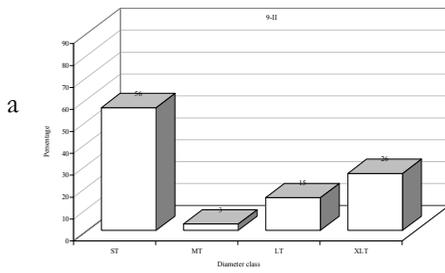
The horizontal distribution of trees usually occurs in cluster form but in randomized group and seldom it was poison or random. The randomized groups covered an area of 400 to 800 m<sup>2</sup> which were made of one or two neighbouring diameter classes. In some cases, individual trees of other diameter classes were observed within the randomized groups.

Table 1: Identification key for different types.

Development	Structural type	Identification key
Initial	Initial uneven – aged or young stands in regeneration stage	ST>70%
	Typical uneven – aged stand	%50 <ST<%70 and MT<30% and LT & XLT<30%
	Uneven – aged stands tending to even-aged from and homogeneity	%30<ST<%50 and MT<30%
Optimal	Even – aged like (= regular) stand in ST/MT classes	ST&MT>60% ST>30% and MT>30% and LT & XLT<50%
	Even – aged stand in MT classes	ST<30%
	Even – aged in MT/LT classes	ST< 20% and MT&LT>60%
	Even – aged in LT/XLT classes	ST<20% and MT<30% and LT & XLT< 50%
Decay	Temporary decay stage	%20<ST<%60 and MT<%20 and %20 <LT&XLT<%60

We determined identification key for different possible types (table 1). As an example, figure 2 shows the proportion of timber size classes, horizontal and vertical profile as well as point pattern map of the type 7. Figure 3 shows some examples of vertical profile in all different seven recognized types.

This study within the 80 sub-plots (50\*50 m) results in classification of only 7 different structural types within different development stages as following:



Initial stage:

- 1) Initial uneven – aged or young stands in regeneration stage,
- 2) Typical uneven – aged stand,

Optimal stage:

- 3) uneven – aged stands tending to even - aged from and homogeneity,
- 4) even – aged like (= regular) stand in ST/MT classes,
- 5) even – aged stand in MT classes,
- 6) even – aged in LT/XLT classes,

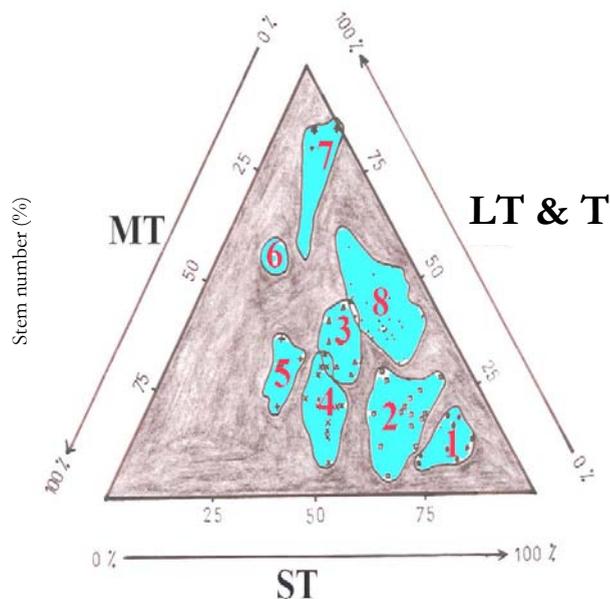
Decay stage

- 7) temporary decay stage.

Fig. 2, a) Mathematical structure (histogram), b) horizontal profile, c) Vertical profile and d. point pattern map of the type 7. Fig. 3. vertical profile a) Initial uneven – aged or young stands in regeneration stage, b) Typical uneven – aged stand, c) uneven – aged stands tending to even - aged from and homogeneity, d) even – aged like (= regular) stand in ST/MT classes, e) even – aged stand in MT classes, f<sub>1</sub>) even – aged in LT/XLT classes with extreme XLT class, f<sub>2</sub>) even – aged in LT/XLT classes with extreme LT class, g) temporary decay stage.

We calculated identification key for each types and determined 8 structure types on the structure triangle (Fig. 4). Types 6 was not observed in this study. The structure types of temporary decay stage (type 8) and typical uneven – aged stage (type 2) occupied larger areas in the structure triangle, whereas the even – aged in MT/LT class (types) occupied smaller area. It should be mentioned that the type 6 was not observed in our study.

Study of relation between over – storey and under – storey (regeneration) showed different patterns and structures. For example, no regeneration were observed in type 4 (even – aged in ST/MT classes) as a young stand, while groups of thicket were observed in type 6 (even – aged in LT/XLT classes) as an old stand. This depends on the age of the stand and receiving of light in the understorey.



Distance between trees were measured by triangular method and the results are given in table 2.

Table 2: Distance between trees in the diameter classes

Diameter class	Structural type	Distance between trees(m)		
		Max.	Min	Mean
Small timber (ST)	Initial uneven – aged or young stands in regeneration stage	5.50	0.75	2.70
Medium timber (MT)	even – aged stand in MT classes	7.50	1.00	4.20
Large timber (LT)	even – aged in LT/XLT classes with extreme LT class	12.75	4.00	8.35
Extra large timber (XLT)	even – aged in LT/XLT classes with extreme XLT class	16.25	5.00	11.20

By using of frequency distribution of trees in diameter classes and using of French and Belgium references (Collet et al. 1998, Bary-Lenger et al. 1993) we could define the typology better. Spatial structure considering European (Goreaud 2000) and Canadian studies (Boucher et al. 2002) illustrates the horizontal and vertical structure of the stand and demonstrates the situation of a tree beside its neighbours. We could also study and understand the competition, growth and development of the individual trees, as well as development of the stands and the succession of different types (Wijdeven 2003, Cemagref 2001).

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# **Investigation on Durability of Eucalypt Woods by Field test Stakes**

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## Abstract

Termites damage and destroy wood and wood products of all kinds. Wood samples of this research were four eucalypt woods, *Eucalyptus camaldulensis*, *E. microtheca*, *E. striatycalyx* and *E. viminalis* against termites in cases of untreated and treated with Creosote and a water-borne preservative (ACC). The dimensions of the samples were 2 by 2 by 47 cm. treated by Creosote and Celcure using full cell process method. Retentions of Creosote were 16.96, 14.16, 35.05 and 156.45 kg/m<sup>3</sup> and Celcure 3.24, 5.65, 5.71 and 18.56 kg/m<sup>3</sup> respectively. Both control samples (untreated) and preservative treated samples were placed in two stations, in the Albaji through field test and Mishmast. After 1, 2, 4, 5, 6, 7, 8 and 9 years exposure to termites, the samples were evaluated according to ASTM Standard D-1758 - 01.

As a result of this research, all of the Creosote impregnated samples (*E. camaldulensis*, *E. microtheca*, *E. Striatycalyx* and *E. viminalis*) were sound after about 95 months, installation in Albaji and in Mishmast station. The neutral samples; *E. striatycalyx* and *E. microtheca* species are not resistant. *E. viminalis* is perishable but *E. camaldulensis* is resistant against termites. Impregnation with Celcure doesn't increase the durability against termites. Although, the damage by termites were similar in Khuzestan and Qom, but the activity rate of termites in Qom, is more than that of in Khuzestan.

**Keyword:** durability, Creosote, Celcure, Bethel, termite-attack wood resistance, Eucalyptus.

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## Introduction

The resistance of four eucalypt wood Species including; *E.camaldulensis*, *E. microtheca*, *E. striatycalyx* and *E. viminalis* was measured against termites in cases of untreated and treated with Creosote and a water-borne preservative (Celcure 5%). Two stations were considered for installing samples, in the Albaji (Khuzestan province) and Mishmast (Qom province) through field test. This project has been done with the aim of investigation on treated and untreated resistance of eucalypt wood species against termites.

## Materials and methods

Wood samples of the research; *E. camaldulensis*, *E. striatycalyx*, *E. microtheca*, and *E. viminalis* logs with no visible evidence of infection by stain, insect, mold, holes and decayed fungi (Fig 1), were prepared from Mamassany in Fars province (Fig 4) of Iran and carried to wood division sawmill. This study was done according to recommendation of ASTM Standard D-1758 – 01(1).



**Fig 1- Logs and cut of eucalypt woods**

Test specimens were sawed from heartwood of lumber in 100 cm length. Freshly cut lumbers were stored in laboratory until the MC reached to about 20% or less (Fig. 2).



Fig 2- Cut lumber stored in laboratory for drying

### **Test stake dimensions**

Two test procedures are outlined, employing two specimen types, 0.75 - in. square and nominal 2 by 4 -in. stakes, respectively Method A and Method B. Method A using smaller and more numerous specimens, is preferred for possibly rapid results. Method B for longer term test of a quasi service nature. Because of limited time, Method A (2 by 2 by 47 cm) was used in this research (1).

### **Preservatives (Celcure and Creosote)**

The combination of water-borne wood preservative of Celcure (ACC) was: Chromic acid<sup>1</sup>, Copper sulfate<sup>2</sup>, and Sodium dichromate<sup>3</sup>. Stakes were stored in laboratory until their moisture acceded to about 18% by air-dried. Then stakes were numbered and weighted before impregnation (7).

### Treatment styles

Impregnation was done by the Bethel method (full-cell process) with the initial vacuum 635 mm Hg (-0.84 bar) for 30 minutes, with pressure 12 bar, for 3 hours. Then some of stakes were impregnated by Celcure of 5 percent consistency and some were treated with creosote. It is worthy of mention that the Celcure temperature was 25°C and the creosote temperature was 90°C and it was done with unit impregnation laboratory scale (fig 3). All these treated samples were stored in laboratory to be stabilized (7).

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1- CrO <sub>3</sub>	1.7%
2- CuSO <sub>4</sub> .5H <sub>2</sub> O	50%
3- Na <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> .2H <sub>2</sub> O	48.3%



Fig 3: Equipment and Impregnation laboratory scale

The average retention of pressure impregnated with Celcure and Creosote in *E. camaldulensis*, *E. microtheca*, *E. striatycalyx* and *E. viminalis* samples were, as follows (Table 1).

Table 1-Average charge retentions of Creosote and Celcure

Species	Average retention (kg/m <sup>3</sup> )	
	Creosote	Celcure
<i>E. camaldulensis</i>	16.96	3.24
<i>E. microtheca</i>	14.16	5.65
<i>E. striatycalyx</i>	35.05	5.71
<i>E. viminalis</i>	156.45	18.56

### Installation of stakes

Two sites (Fig4) were considered for installing samples (field test) for termite attack testing: Albaji in Khuzestan province and Mishmast in Qom province.

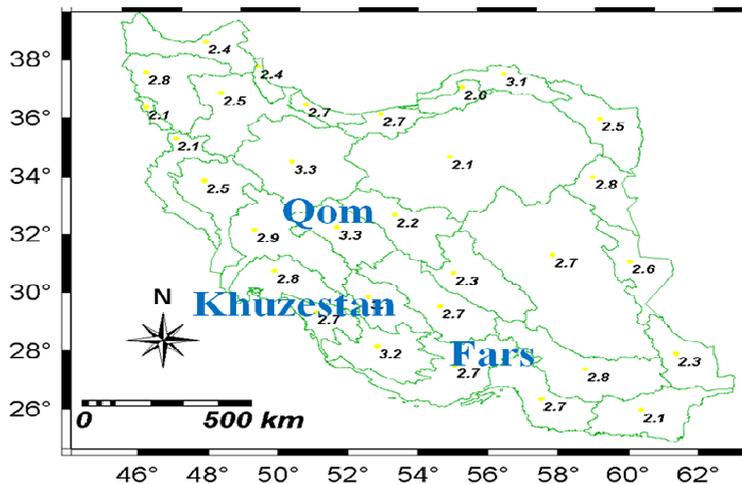


Fig 4-Map of Iran, the place of Logs preparation (Fars) and sites of Installing samples (Khuzestan and Qom)

### Installation place of stakes

The treated stakes were exposed in the ground where wood-destroying termites existed in field test plots. Station of installing samples for termites attack testing was in Albaji and Mishmast (Fig 5&6).



Fig 5–Installation site of samples in Albaji station



Fig 6–Installation site of samples in Mishmast

### Samples evaluation standard

Evaluation of samples was done based on ASTM D –1758 – 01(1). In any inspection of sample, some of stakes were removed from the ground carefully by a straight upward pull and Graded to above standard recommendation (Table 2).

Table 2-Grading system for Below Ground Condition Termite Grades

Grades No.	Description of condition
10	No attack, 1 to 2 small nibbles permitted
9	Nibbles 3% of cross-section
8	Penetration 3 to10 % of cross-section
7	Penetration 10 to 30 % of cross-section
6	Penetration 30 to 50 % of cross-section
4	Penetration 50 t0 75 % of cross-section
0	Failure

**Demolition average grades No. of samples (formula 1):**

Formula 1

$$I = \frac{\sum nY}{\sum n}$$

Where:

I = Average grades No.

n = Number of sample

Y= Grades No. Results

**Results**

Retentions of Creosote and Celcure were 16.96, 14.16, 35.05, 156.45 kg/m<sup>3</sup> and 3.24, 5.65, 5.71, 18.56 kg/m<sup>3</sup> for *E. camaldulensis*, *E. microtheca*, *E. Striatycalyx* and *E. viminalis* respectively. Thus, the above mentioned species (*E. cam.*, *E. mic.* and *E. Str.*) are resistance impregnating and with 3- treatability code, but *E vim.* species is moderately resistance impregnating and with 2- treatability code.

All of the Creosote impregnated samples were sound after about 8 years (95 months) installation in Albaji (Khuzestan province) and in Mishmast stations (Qom province) (Fig 8).



Fig 7- Samples impregnated with Creosote were sound after about 95 months  
Situation of *E. camaldulensis* samples (Table 3, Fig 8 & 9).

Table 3- Average index of conditions for *E. camaldulensis* samples in Albaji

Installation time (month)		36	48	60	72	84	96	118
Grades No. Samples	Control	10	9	9.2	8	7.3	7	6.7
	Celcure	10	10	10	10	9.7	9.4	*-
	Creosote	10	10	10	10	10	10	-

\* - No evaluation



Fig 8-Control samples of *E. camaldulensis* in Albaji and Mishmast station, the attack by termites started after 36 month and then the grade No. reached to 6.7 after 118 month



Fig 9-In control samples of *E. camaldulensis*, the attack by termites started after 36 month, then the grade No. reached to 6.8 after 118 month installation in Mishmast station

Situation of *E. microtheca* samples (Table 4, Fig 10 & 11).

Table 4 - Average index of condition for *E. microtheca* samples in Albaji

Installation time (month)		36	48	60	72	84	96	118
Grades No. Samples	Control	10	9	8.2	6	5.4	2	-
	Celcure	10	10	9.3	8.5	5.7	3	-
	Creosote	10	10	10	10	10	10	-

- No evaluation



Fig 10-Control samples of *E. microtheca* in Albaji and Mishmast station, the attack by termites started after 36 month, then the grade No. reached to 0 and 2 after 96 month



Fig 11- Treated with Celcure samples of *E. microtheca*, the attack by termites started after 60 month, then the grade No. reached to 3 after 96 month installation in Albaji

Situation of *E. striatycalyx* samples (Table 5, Fig 12 & 13).

Table 6 - Average index of condition for *E. striatycalyx* samples in Albaji

Installation time (month)		24	36	48	60	72	84	96	118
Grades No. Samples	Control	9.7	9	9	8.2	6	4	3.2	2
	Celcure	10	10	10	9.4	9	7.7	5.4	-
	Creosote	10	10	10	10	10	10	10	-

- No evaluation



Fig 12- Control samples of *E. striatycalyx*, the attack by termites started after 24 month, then the grade No. reached to 2.4 after 118 month installation in Albaji and Mishmast station



Fig 13- Treated with Celcure samples of *E. striatycalyx*, the attack by termites started after 36 month, then the grade No. Reached to 3 after 96 month installation in Albaji

Situation of *E. viminalis* samples (Table 5, Fig 14, 15& 16).

Table 6 - Average index of condition for *E. viminalis* samples in Albaji

Installation time (month)		8	14	18	24	36	48	60	72
Grades No. Samples	Control	8.3	7	5.1	1.7	*-	-	-	-
	Celcure	10	10	10	9.1	9	7.3	4.6	-
	Creosote	10	10	10	10	10	10	-	-

- No evaluation



Fig 14- Control samples of *E. viminalis* in Albaji, the attack by termites started after 8 month, and then the grade No. reached to 5.1 after 18 month



Fig 15- Control samples of *E. viminalis* in Albaji, the attack by termites, and grade No. reached to 0 after 24 month



Fig 16- Treated with Celcure samples of *E. viminalis*, the attack by termites started after 24 month, then the grade No. Reached to 4.6 after 48 month installation in Albaji



Fig 17- Inside the ground part of control sample is attacked by termites



Fig 18-Samples are covered with mud by termites for protecting them against sunlight



Fig. 19- Treated with Creosote samples are sound and Control samples are attacked

#### The following 5 termite species exist in Khuzestan:

- 1- *Microcerotermes gabrielis* (Weidner)
- 2- *Microcerotermes buettikeri* (Chhotani & Bose)
- 3- *Microcerotermes diversus* (Silvestri)
- 4- *Amitermes vilis* (Hagen)
- 5- *Anacanthotermes vagans* (Hagen)

The species of lines 3, 4 and 5 are spread throughout Khuzestan province. *Microcerotermes diversus* is a more aggressive species which attacks to all of the wood and non wood plant and also to wooden furniture in houses (5&8).

The following three termite species are spread throughout Qom (2):

- 1- *Microcerotermes gabrielis* (Weidner)
- 2- *Amitermes vilis* (Hagen)
- 3- *Anacanthotermes vagans* (Hagens)

## Conclusion

According to the results of periodical evaluation of the samples: Neutral samples of *E. striatycalyx* and *E. microtheca* species are, non-durable and also not resistant against termites, but *E. camaldulensis* species is durable and B- natural durability code. *E. viminalis* is perishable and E- natural durability code. The grades No. of all the samples (*E. microtheca* and *E. striatycalyx*) impregnated with Celcure reached to 3 and 5.4 after 96 month installation in Albaji and Mishmast. The grades No. of all the samples *E. viminalis* impregnated with Celcure, reached to 6.4 after 60 month installation in Albaji and Mishmast. The grades No. of all the *E.*

*camaldulensis* samples, impregnated with Celcure, were 9.4 after 96 month installation in Albaji and Mishmast. All of the samples impregnated with Creosote were sound after 95 months installation in Albaji and Mishmast.

Treatment with Celcure did not increase durability, but impregnation with Creosote increased the durability of timbers up to 96 month. Based on the results of this study, natural durability of *E. camaldulensis* against termites is more than 72 month. Although, the damage by termites was similar in Khuzestan and Qom, but the activity rate of termites in Qom, is more than that of in Khuzestan (9).

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# The Forests of the Southern Iran

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## **Abstract**

For identification of the forest Association in southern Iran the general information of this region was collected using the existing scientific resources and the geological, pedagogical and topographical maps of the region. At the next stage the information of the sites was surveyed through field operation. After date analyze, the most important forest associations was identified. Southern Iran is part of saharo-sindian and sudanian-Dakani regions. In view of the scope of the changes of ecological factors of southern Iran, there are two main forest regions including Persian-Gulf and Omani region.

The main elements of percian-Gulf region including *Ziziphus spina-christi*, *populus euphratica*, *prosopis cineraria* and *P. koelsiana*. The main elements of omani region in cluds *Acacia spp.*, *Avecina marina*, *Rbizophora macronata*, *Capparis deciduas* and *Nanorbops pitschi*ena. Intwo regions 27 forest association Identified.

## **Introduction**

The area of Iranian forests amounts to 12.4 million hectares and have covered 7.4% of the total area of Iran. As a result Iran is considered to be amongst the low forest cover countries(LFCC). if we compare and contrast it with other parts of the world but it is unique in terms of plant diversity and the reserves of the plant genetics. Approximately 8000 plant species have been identified in Iran.(Sagheb Talebi-2005).

Iran is located in the junction of three important plant regions due to natural conditions and geographical position. The Irano-Touranian region which includes two foreste areas of Zagros and Irano-Turanian, Hyrcanian region which is part of the plant zone named Euro- Siberian and is composed of two regions of Hyrcanian forest and Arsbarani forest (Sagheb Talebi,2005) and the Saharo-Sindian region which covers the southern parts of Iran and includes two forest regions of Persian Gulf and Omani (Javanshir, Karim, 1976).

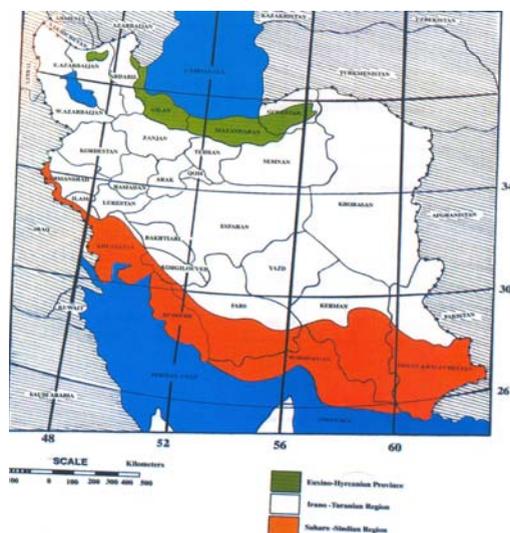
### **The Saharo-Sindian region:**

This region which has the Sub-tropical vegetation encompasses the southern part of Iran. In this region the famous Saharo-Arabian.Sudanian and also Irano-Tuoranian species. Precipitation occurs in Autumn and Winter and does not exceeds 100 mm. In some parts precipitation amounts to 250 mm annually. The amount of annual evaporation is around 3200-4200 mm and even more than that. The rainfalls are flood-like and irregular. Sometimes more than 100 mm of rain falls within 24 hours and causes lots of floods. Dry season is very long and warm and allocates around 7 through 8 months of the year to itself. The vegetation is very poor in terms of species. In the eastern parts the rainfall is less than usual but more regular and has a richer flora. The ecological reasons of the concentration of

these Sub-tropical plant elements in this part of Iran include the height from the sea level, type of precipitation and temperate winters.

This region which is regarded as the Persian-Gulf and Omani forest region has an area of 2130000 hectares beginning in Ghasre-Shirin and stretches into the south in form of a narrow band and continues up to the Iranian-Pakistani borders in the east (map 1). The altitude ranges from sea-level up to 800 meters from it. The main vegetation elements are among Sub-tropical elements and have a Saharo-Sindian origin. Due to ecologic differences this region is divided into two regions, Persian-Gulf and Omani regions.

Persian-Gulf stretches from the west to the boundaries of Bushehr and Hormozgan and includes the Omani regions of Hormozgan and Sistan-Baluchestan provinces.



Map: Three phytogeographical regions of Iran (Sagheb-Talebi, 2004)

**Geology:** in accordance with Stocklin division

There are three geological zones in this region. Khuzestan plain is a limited region in the west of this region and the continuation of the Arabian platform and has been covered by alluvial sediments.

**Zagros folded zone:** this region is 150 to 250 kilometer wide and has been formed by the sediments of the covers of the continental border of the east of the Arabian Platform.

**The eastern Iranian zone, Makran mountain chain:** The most important geological limitation of this region is the erosion of its geological structures. In this zone there are the marine sediments 10000 meters high related to Cenozoic age.

### **Pedology**

There are 11 types of soil in this region. Most of soils are deep, semi-deep and low to average depths alluvial calcareous with PH 7-8.5 and EC 0.18-126ds/m and it is impossible for any plant to grow in some regions due to excessive saltiness. In some regions the halophytes grow. The low depth of soil, the presence of a great deal of gravels, slope, physiographic and erosion are among the factors which limit the soil of this region. The soils of this region are poor in organic materials.

The soils of the high and mountainous regions are shallow and underdeveloped along with extreme erosion. In some regions where the soil enjoys a sufficient depth the forest stands have been formed. The soils of the eastern parts which belong to Makran-zone are mostly salinity with 7 to 8.5 pH. Most of these soils are composed of the riverbeds on which water runs at the time of rainfall or of the sediments caused by the flooding of the permanent rivers. The texture of the soils of these lands is light and medium and the heavy soils can be seen in some regions especially in the low lands (Sagheb Talebi, 2004).

### **The Climate of the region**

The quantity of precipitation increases from east to west. In Chahbahar the rainfall amounts to around 100 millimeters and in Abadan it amounts to around 155 millimeters and the greatest amount of precipitation is in Bushehr City with the average 250 millimeters. The relative humidity of air increases from east to west. The difference between maximum and minimum relative humidity in Chahbahar and Abadan is 44.5% and 72.6 % respectively.

**Evaporation:** the annual average evaporation is 3200 to 4200 millimeters in this region.

**Temperature:** in this vegetative region the maximum average temperature decreases from east to west and the minimum average temperature decreases in such a way the difference between the maximum and minimum temperature in Abadan is 40.3 degrees centigrade and equal to 17.1 degrees centigrade. The minimum absolute temperature occurs in Abadan which is 0 degrees centigrade (in January and February) and in Chahbahar which is 10.6 degrees centigrade (in December).

In this region the direction of the dominant winds is northwesterly to southeasterly and the amount of evaporation and perspiration is also increased due to the presence of winds.

Climatically this region has hot and scorching summers and mild winters. In accordance with the Ambergree method the climate of this desert region is extremely warm, average and slight and in some high parts it has a temperate desert climate.

## Vegetation

The vegetation of southern Iran displays tangible and significant changes from east to west and from south to north. The eastern part of Iran (Baluchestan) is part of Sudanian-Dakani region and its western part ( Hormozgan, Boushehr, and Khuzestan) is part of Saharo-Sindian region (Sabeti, 1994) and as we go from south to north we will view the permeation of the plant species of Irano-Touranian region.

Due to changes temperature and humidity from east to west we view the changes of vegetation. Due to distance from sea and the increase of height above-sea level we also view the changes of vegetation from south to north. In the seaside of Persian Gulf and Oman Sea the halophytes communities grow. Due to increase of distance from the sea and decrease of salinity of soil we view non-halophyte species. In high regions such as Geno mountain in Hormozgan Province, Siah Mountain in Boushehr province we can observe the Irano-Touranian species such as *Amygdalus scoparia*, *Pistia atlantica* and *Juniperus polycarpus*. In Khuzestan province in high regions and Boushehr Province in the Posht Par plain (1250m above sea-level) we can view *Quercus persica* besides the mentioned species. In view of the scope of the changes of ecological factors of Southern Iran there are two main forest regions including Persian-Gulf and Omani region. Now we embark upon description of the main forest communities which are in these regions.

The main vegetative elements in Persian-Gulf including *Ziziphus spina-christi*, *Populus euphratica*, *Prosopis cineraria* and *P. koelsiana*. The main vegetative elements of Omani forest region includes *Acacia* species, *Avecina marina*, *Rehizophora macronata*, *Capparis decidua*, and *Nannorbops pitschieana*.

## Materials and methods

For identification of the forest Associations in Southern Iran the general information of this region was collected using the existing scientific resources and the geological, pedological, and topographical maps of the region. At the next stage the information of the sites was surveyed through field operations from Dehloran (south-west), to Chahbahar (south – east) and from south direction to north direction in 6 information bands. Moreover quantitative and qualitative statistical-survey was carried out in some Associations including:

- 1- Seashores up to 1500 meters heights of Geno mountain in Bandar Abbas.
- 2- Seashores up to heights 1600 meters above sea level, Haft-Chah mountain located in Taheri port.
- 3- Seashores up to the height 800 meters above sea level from Dorahak-Riz path(kangan port).
- 4- Seashores(Ziarat-Dashti village) up to 1950 meters height of Siah-mountain in Khormoj(Bushehr).

- 5- Seashores up to 1250 meters height of the Kheirak mountains in Dashtestan.
- 6- Seashores up to 1250-meter height of the path to Masjed-Soleiman up to Naser- Khosrow village located in Khuzestan.

After analysis of the information the forest Association of southern Iran is introduced as follows:

### **Mangrove forests:**

In Iran mangrove forests grow in form of big and small stands in the shores of Persian Gulf and Oman Sea. They are pure forests which are composed of black mangrove (*Avecina marina*) species. In part of the shores of the ports of Sirik and Jask the species of white mangrove (*Rhizophora macronata*) grows along with it. These forests are seen along the ebb and tide shore of the sea and the estuary of the seasonal and permanent rivers (Safiari, 1994). These forests begin in the easternmost part of the Oman Sea located in Gowater gulf and finish in the west of Persian-Gulf beside the Mal-Gonzeh village located in around 30 kilometer of the northwestern part of Dayyer port in Boushehr province. From this part toward the southwestern part of Iran these forests are not seen due to decrease of temperature of the environment. The 0-degree centigrade temperature is dangerous for mangroves and destroys them. The optimum growth and establishment occurs in the 25-27 degrees centigrade for 250 days per year.

The mangrove and white mangrove have penomatopher and viviparous property. The soil of the floor of the mangrove forests are fine-aggregate and inundated with clay-loamy texture and Ec is between 36 to 42 ppt, 6.7 to 7.6 pH.

From the center of forest toward the shore and land the vegetation has more diversity along with the mangrove forests as follows:

The vegetation of the central core of the forest: *Avecina marina* and *Rhizophora macronata*.

The adjacent cover or peripheral cover including the halophyte species such as:

- 1- *Arthrobenum macrostachym*
- 2- *Bientrica cycloptra*
- 3- *Holocnemum strobilaceum*
- 4- *Sueda fruticosa*
- 5- *S. Vermiculata*
- 6- *Salicornia europea*

Tree cover and the peripheral shrubs near the forests including:

- 1- *Acacia tortilis*
- 2- *Prosopis cineraria*
- 3- *Tamarix sp.*
- 4- *Ficus bengalensis*

The average number per hectare of mangrove forests is 431 root-stocks; the average diameter of the crown of shrubs is 2.5 meters, the average height is

around 3 meters, the average of the crown-cover is around 4.9 square meters, and the percentage of its crown-cover is around 21% (Rashvand , 2000).



Figure 1: Mangro forest(*Avesina marina*, Asalooyeh)



Figure 2: Mangro forest (*Avesina marina*) Asalooyeh

Iranian Prosopis Forests(*prosopis cineraria*)associatons:

These forests grow on light alluvial rough-grained soils along with special gravel in regions where there is fresh underground water. They are seen on the outskirts of the seasonal rivers for the easternmost border of Iran and Pakistan toward west around Borazjan (Nanizak village).

These forests are viewed around Iranshahr, Karvandar and Bampour, south of Bam, Narmashir, Jazmourian, Jiroft, Nikshahr, Ghasre-ghand,Asalooyeh,Kangan port and Bandar-Abbas.

The climate of the site of these forests is intensive-warm-desert (terrestrial). The average annual temperature is 21 degrees centigrade and the average maximums and minimums are 32.2 and 17.8 degrees centigrade respectively. The number of trees per hectare is 3-10 root-stocks and the percentage of the canopy cover is 2.9 to 3.3 and the average of the trees height is 7 to 9.4 meters and regeneration in the forest is very rare.

The soil of the Iranian *Prosopis* forests has light texture (sandy-loam), is loamy and nearly heavy (silty-loamy), heavy (silty-clay-loam) up to very heavy (silty-clay), having 6.3-7.7 pH and its Ec is 0.4 to 55 ds/m (Sadeghi, 2000).

- 1- *Capparis spinosa*
- 2- *Zizyphus spina-chrisi*
- 3- *Prosopis koelsiana*
- 4- *Taveriniera spartea*
- 5- *Suaeda egyptica*
- 6- *Stipa cappensis*
- 7- *Erodium sp.*
- 7- *Plantago sp.*
- 8- 9- *Pteropyrum Oliveri*
- 10- *Hamada salicornia*
- 11- *Pennisetum divisum*
- 12- *Cenchrus ciliaris*
- 13- *Medicago spp.*
- 14- *Calotropis procera*



Figure 3: *Prosopietum cineraria*, Gavbandy port.



Figure 4: *Prosopis cineraria*, Kangan.



Figure 5: *Ficus Bengalensis*, Asalooeyeh

*Prosopis koelsiana* associations:

This association extends from the easternmost border of Iran (Goater Port) up to Boushehr province, around Borazjan Town such as Abolfiroz village. It grows in such areas as Iranshahr, Bampour, Bam, Jazmourian, Kahnoj, Jiroft, Nikshahr, Konarak, Banadar Abbas, Kangan, Bordkhood, Khormoj, and Borazjan. This shrub has a powerful outshoot power and is reproduced by help of root shoot.

Accompanying plants:

- 1- *Stipa cappensis*
- 2- *Capparis spinosa*
- 3- *Prosopis stephaniana*
- 4- *Tamarix sp.*
- 5- *Zizyphus spina-christi*
- 6- *Calotropis procera*
- 7- *Ephedra foliata*
- 8- *Geranium mascatensis*
- 9- *Chenopodium mural*

10- *Erodium sp.*

11- *Medicago spp.*

*Zizypus spina-Christi* (Konar associations):

This tree grows in such provinces as Fars, Hormozgan, Baluchestan, Boushehr, Khuzestan, Keramn, Ilam, Lorestan, from the 4 to 1200 meters height above sea level.

Konar(Christ-thorn) grows on the gravelly alluvial, fine grained alluvial, sandy-wind-laid deposits (Aeolian deposits) in form of dispersed-stands (canopy cover 1-5%) and grows in other regions in form of rare-stands in southern Iran. It does not grow on low lands.

The sites of Konar (*Zizypus spina-Christi*), in accordance with the Ambergee method, is mildly desert warm, extremely desert warm, average and semi-average warm desert, and semidry temperate and dry temperate.

The soil of the sites of Konar has a loamy, loamy-sandy, loamy-silty, clay-silty texture, and the saltiness of 0.18 to 3.9 ds/m and PH of 7 to 8.2.

The average height of the trees is three meters, the average number per hectare is 23 single roots; the average of the canopy cover is 2.5 to 4.55, the percentage of the number of the sapling per hectare is 11 single roots and the measured tallest tree is 13 meters in Dashtestan.

The species accompanying Konar(*zizyphus spina-christi*):

78 plant species related to 27 families have been collected and identified from the sites of Konar in Boushehr Province and some of them are as follows:

1- *Stipa cappensis*

2- *Lycium deressum*

3- *L.shavil*

4-*Calotropis procera*

5- *Prosopis cineraria*

7- *P.koelsiana*

8- *Ephedra foliata*

9- *Prosopis stephaniana*

10- *Crepis kotschiana*

11- *Echinops cephalotes*

12- *Erodium cicutarium*

13- *Gallium sectaceum*

14- *Hippocrepis constrictae*

15-*Malva silvester*

16- *Medicago minima*

17- *Periploca aphylla*

18- *Plantago ovata*

19- *Rumex congolomeratus*

20- *Veronica sp.*



Figure 6: *Ziziphus spina-christi*, Dashtestan, 2000



Figure 7: fire Christ-thorn(*Ziziphus spina-christi*) forests, Dashtestan, 1995.



Figure 8: Budding Garden Jujube(*Ziziphus spina-christi*\**Z. mauritanica*), Khormuj, 2006.

Semeng associations(*Tecomella undulate*):

This tree grows in Baluchestan and between Sirjan and Bandar-Abbas, Lar, Saeid-Abad, Haji Abad, and Andimeshk and the valleys of Khuzestan (Sabeti , 1994). It is widespread in the following regions(Bushehr province):

Safieh, Soheil, Shahniah, Mokhdan, Aliabad, Keshtoo, Lavar, Goorak-Sadat, Tange-Eram, Boushkan, Fariab, and the Shirino village.

This tree has turned out to be the dominant tree in some regions and constitutes Semeng association. The sites of Semeng are geologically located in the seashore deserts, rough-grained conglomerates, marine faults and terraces up to the sediments of the 4<sup>th</sup> geological age.

The sites of Semeng is , in accordance with the Ambergee method, the extremely warm desert and average warm.

The soil of the sites of Semeng has sandy texture or loamy-sandy, and loamy. pH is 7.1 to 7.9 and Ec is equal to 0.8 to 8.8 ds/m.

This tree is sensitive to cold and if the temperature drops the plant will be damaged. This tree grows on the slopes 20 to 850 meters above level. The average of the number per hectare for this tree is 90 to 400 . The height of trees has been reported up to 13 meters in Boushehr. (Hosseini and the colleagues, 2001)

Accompanying species:

- 1- *Ziziphus spina-Christi*
- 2- *Tamarix sp.*
- 3- *Prosopis juliflora*
- 4- *Amygdalus scoparia*
- 5- *Lycium shawii*
- 6- *Artemisia scoparia*
- 7- *Heliantemum lippii*
- 8- *Centaurea depressa*
- 9- *Albagi camelorum*
- 10- *Capparis spinosa*
- 11- *Convolvulus spinosa*
- 12- *Ephedra foliata*
- 13- *Astragalus sp.*
- 14- *Medicago minima*
- 15- *Sinapis alba*
- 16- *Stipa cappensis*
- 17- *Hordem violaceum*
- 18- *Cenchrus ciliaris*
- 19- *Panicum sp.*



Figure 9: *Tecomella undulate*, Tangestan, 2005

Estabraq associations (*Calotropis procera*):

This plant is seen in southern Iran from Khuzestan to Makran, Baluchestan from the seashores up the regions 1100 meters above sea level. The soil of the sites of this plant has a very light sandy texture with suitable draining and Ec is 0.43 to 1.92 ds/m and its pH is 8.1 to 7.4.

in accordance with Ambergée method the climate of these sites is extremely warm desert. The sites of *Calotropis procera* have 7.5 to 35.75 stands per hectare approximately, the average height of the shrubs is 1.5 to 2 meters, the average of the area of the canopy cover per hectare is equal to 32.7-106.9 square meters.

Accompanying species (Hosseini & Colleagues, 2000):

- 1- *Hamada sp.*
- 2- *Convolvulus spp.*
- 3- *Asphodelus sp.*
- 4- *Lycium depressum*
- 5- *Prosopis stephaniana*
- 6- *Albagi sp.*
- 7- *Prosopis koelsiana*
- 8- *Atriplex leucoclada*
- 9- *Ziziphus spina-Christi*
- 10- *Astragalus sp.*
- 11- *Capparis spinosa*



Figure 10: *Calotropis procera*, Khaki

Ligaji associations (*Capparis spinosa*):

This plant is one of the elements constituting the vegetation of the Mediterranean ecosystem whose growth period occurs in spring, summer, and early autumn (in the end days of September). It is seen in a wide area of the country including the Northern, northwestern, western, central, eastern and southern regions. In southern Iran in some regions it has turned out to be the dominant species and constitutes a association.

The most effective factors in dispersion and abundance of this plant are soil texture, draining, soil humidity, and precipitation and soil saltines respectively.

The soil texture of the sites of this plant is very light (sandy), and its  $E_c$  is 0.98 to 6.2 ds/m and pH is 7-7.4. The average of the number per hectare is equal to 7-64 stands and the percentage of canopy cover is 0.23 to 4.6 % and the average height of the shrubs is equal to 1.03 to 3.02 meters. The climate of the sites of *Capparis spinosa* is, in accordance with Ambergree method is extremely warm desert.

Accompanying plants (Fakhri, 2006):

- 1- *Calotropis procera*
- 2- *Stipa capensis*
- 3- *Carthamus tinctorius*
- 4- *Lycium depressum*
- 5- *Ziziphus spina-Christi*
- 6- *Artemisia scoparia*
- 7- *Prosopis juliflora*
- 8- *Albagi camelurum*
- 9- *Citrulus colocynthis*
- 10- *Peganum harmala*
- 11- *Acacia nilotica*
- 12- *Colchicum speciosum*
- 13- *Prosopis stephaniana*
- 14- *P. koelsiana*

15- *P. cineraria*Figure 11: *Capparis spinosa*, Dashtestan, 2005.*Acacia associations:*

One of the plants which are special to this region is *Acacia* which has 5 endemic species and several exotic species. The species of this genus have been naturally disseminated from the easternmost borders of Iran to Boushehr Province, around Boushehr Town and are not naturally seen in Khuzestan.

Karat associations (*Acacia nilotica*):

This plant is seen from the eastern borders of Iran to outskirts of Boushehr Town (Kangan, Bandar Lengeh, Bandar Abbas, Haji-Abad, Cha-Bahar, and Bampur) on the deep alluvial soils and on the sidelines of the seasonal rivers and near the ponds. The height of the trees is up to 14 meters and the diameter of the crest has been measured to be equal to 1 meter. The climate of sites of the tree is extremely warm desert and the average annual precipitation is 140 to 250 millimeters. This plant grows well in wet seasons and its growth is decreased in dry seasons. Each year it produces abundant seeds. The soil of the sites of this plant is alkaline.

## Accompanying plants:

- 1- *ziziphus spina-christi*
- 2- *stipa cappensis*
- 3- *prosopis cineraria*
- 4- *pennisetum divisum*
- 5- *calotropis procera*
- 6- *Hamada salicornica*
- 7- *Rhazya stricta*
- 8- *lycium shanvi*
- 9- *prosopis stephaniana*
- 10- *Medicago spp.*

*Acacia tortilis* associations:

This plant is seen between Jask to Bandar Gavbandi (Gavbandi port) in Hormozgan Province on sandy, loamy and shallow soils and some southern slopes. The height of this plant is 4-10 meters. It has an umbel crown and is seen in the regions with the 100-millimeter precipitation. it bears 50 degrees centigrade as the maximum temperature.

Accompanying plants:

- 1- *Lycium shawii*
- 2- *Ochradenus baccatus*
- 3- *Ephedra foliata*
- 4- *Hamada salicornica*
- 5- *Stipa cappensis*
- 6- *Calotropis procera*
- 7- *Prosopis cineraria*
- 8- *Ziziphus spina- christi*
- 9- *Acacia nilotica*
- 10- *Medicago spp.*



Figure 12: *Acacia tortilis*, Bandar – Lengeh, 2006.

*Acacia nubica* associations:

This plant is seen in Hormozgan province around Bandar Lengeh to Bandar-Abbas on the alluvial rough-grained plains. This shrub loses its leaves in dry season and its leaves appear after first rainfall in autumn. It is a resistant plant in the dry and arid conditions.

## Accompanying plants:

- 1- *Acacia ebrenbergiana*
- 2- *Prosopis cineraria*
- 3- *Stipa capensis*
- 4- *Medicago spp.*
- 5- *Acacia nilotica*
- 6- *Capparis spinosa*
- 7- *Heliantemum lippi*
- 8- *Acacia tortilis*
- 9- *Ziziphus spina-Christi*



Figure 13: *A. nilotica*, Bushehr, 1995.

*Acacia ebrenbergiana* associations:

This shrub is seen in southeastern Iran in Sistano-Baluchestan , and Hormozgan provinces on the alluvial soils and slopes and becomes dominant in some regions and makes up a association. It is seen toward south to north to Sarheh heights and the hilly areas around Sarbaz river, Espakeh and Maskootan hilly areas, the mountains around Bent and fanooj, Nespooran and Dahan in southwestern regions of Iranshahr. It is resistant to cold.

## Accompanying plants:

- 1- *Periploca aphylla*
- 2- *Moringa peregerina*
- 3- *Ziziphus spina-Christi*
- 4- *Prosopis cineraria*
- 5- *Acacia nubica*
- 6- *Acacia tortilis*

7- *Calotropis procera**Acacia albida* associations:

This association is seen only in Boushehr Province near Razm-Abad village in Dashti Town. The soil of this site is fine-grained alluvial with a slope less than 3% and grows in heights around 40 meters above sea level. The annual precipitation is around 250 millimeters and the annual evaporation is around 3500 millimeters. There is rainfall in autumn and winter. The main species of this site is *Acacia albida* which is also reproduced through root shoot.

## Accompanying plants:

- 1- *Zizyphus Spina – Christi*
- 2- *Lycium shawii*
- 3- *Calotropis procera*
- 4- *Stipa Capensis*
- 5- *Medicago Spp.*

*Nannorhops Ritchieana* associations:

This shrub grows in Makran of Sistano-Baluchestan province. It is seen the sidelines of the temporary and permanent watercourses which have a cobble bed or subterranean current of fresh water. Moreover it is also seen in the estuaries of such rivers as Sarbaz, Nikshahr, and Zarabad and in all the water courses leading to the mountains in south Espakeh, Medonch, Esfand, and Bent (keneshloo,2005).

## Accompanying plants:

- 1- *Nerium indicum*
- 2- *Saccharum ravennae*
- 3- *Acacia ebrenbergiana*
- 4- *Razia stricta*
- 5- *Tamarix sp.*
- 6- *Prosopis cineraria*
- 7- *Prosopis koelsiana*
- 8- *Colotropis procera*

*Tamarix mascatensis* associations:

This shrub is seen in the sidelines of the seasonal and permanent rivers and in regions where the texture of the soil is light and without gravels and near the ponds where the flood caused by rainfall flow or gather.

## Accompanying plants:

- 1- *Lycium shawii*
- 2- *Tamarix dioica*
- 3- *Populus euphratica*

- 4- *Suaeda vermiculata*
- 5- *S. egyptica*
- 6- *Chenopodium murale*
- 7- *Aeluropus lagopoides*
- 8- *Tamarix aphylla*
- 9- *T. ramosissima*
- 10- *T. paseroiadae*
- 11- *T. tetraunder*

This association is seen around such rivers as Heleh, Mond, Dalaki, and Bahoush in Boushehr Province.



Figure 14: *Tamarix sp.*, Khuzestan, 2007

*Populus euphratica* associations:

This association is located around such rivers as permanent rivers or regions which have permanent water resources. It makes up its association in the sidelines of such rivers as Karoun, Shapour, Dalki, and Mond in areas which have fine-grained and cobble-free sediments. The presence of sufficient underground water or permanent surface water plays an effective role in the establishment of this species.

Accompanying plants:

- 1- *Salix sp.*
- 2- *Nerium oleunder*
- 3- *Vitex negundo*
- 4- *Tamarix spp.*
- 5 *Stipa cappensis*

And other grass-like species.



Figure 15: *Populus euphratica*, Khuzestan, 2007.

*Capparicetum-calligonetosum* associations:

This association is seen around Bmpour plain on sandy hills and *capparis decidoa* is seen as the dominant species. These two species (*Capparis decidoa* & *Calligonum bungi*) play a vital role in stabilization of shifting sands.

Accompanying plants:

- 1- *Prosopis koelsiana*
- 2- *Prosopis cineraria*
- 3- *Calligonum bungi*
- 4- *Tamarix aphylla*
- 5- *Hamada* sp.
- 6- *Haloxylon persicum*

And other annual Geramines grow under the crown of the above-mentioned plants (Assareh and Keneshloo, 2005).

Reed bed associations (*Phragmites australis*):

This association is seen on the sidelines of the permanent rivers and the regions which have fresh water (soft water) most of the year and have a fine-grained soil texture. This association is seen in Minou Island, in the outskirts of Sarbandar-Mahshahr road, Mahshahr-Hendijan road, Shadegan and Hoorol-Azim (Alazim ponds) and Hoor-e-Shadegan and around Boushehr-Alishahr road.

Accompanying plants:

- 1- *Aeuroopus* sp.
- 2- *Cyperus* sp.
- 3- *Suaed* sp.
- 4- *Juncus* sp.
- 5- *Typha latifolia*
- 6- *Tamarix* sp.

7- *Salsola baryosna*  
(Assareh & Koneshkoo, 2005)

*Dodonea viscosa* associations:

This perennial shrub is seen in the mountainous heights between Kangan and Bandar Abbas 90 to 800 meters above sea level. This association can be seen on the sidelines of Shirino-Haftchah road, and Dorahak-Riz and Genoo-mountain on the limestone parent.

Accompanying plants:

- 1- *zizhus spina – Christi*
- 2- *Amygdalus scoparia*
- 3- *Ephedra foliata*
- 4- *Amygdalus eburnea*
- 5- *Astragalus sp.*
- 6- *Capparis sp.*
- 7- *Stipa cappensis*
- 8- *medicago sp.*



Figure 16: *Dodonea viscosa*, Kuhe – Geno, Bandar-Abbas, 2007.

*Periploca aphylla* associatins:

This shrub is seen on the rough-grained alluvial soils and the steep slopes with limestone parent on the heights 100 to 800 meters above sea level in southern regions of Iran. This shrub is seen in form of rare stands (canopy cover less than 1%).

Accompanying plants:

- 1- *zizhus spina – Christi*
- 2- *capparis spinosa*
- 3- *Convolvulus sp.*
- 4- *Stipa cappensis*
- 5- *Dodonea viscosa*
- 6- *Zataria multiflora*
- 7- *Ferula sp.*

- 8- *Lycium shawii*
- 9- *Medicago sp.*



Figure 17: *Periploca aphylla*, Kuhe – Geno, Bandar-Abbas



Figure 18: *Periploca aphylla*, 1995.

Almond (*Amygdalus scoparia*) associations:

This association is seen in the type of the lands which are high plateaux and upper terraces on the heights 450 to 1950 meters above sea level. The soil of the sites is shallow and sometimes without soil in such a way that the shrubs are located on the limestone parent with PH of 6.8 to 7.4 and EC of 0.8 to 1.7 decisiemens per meter(ds/m). in accordance with Ambergee method the sites of almond have a dry and temperate climate, have 200-600 mm precipitation per year and average temperature of 22.5 degrees centigrade and the annual relative humidity of around 60%.

Accompanying plants:

- 1- *Daphne mucrunata*
- 2- *Ficus carica*

- 3- *Ziziphus nummularia*
  - 4- *Allium* sp.
  - 5- *Calendula* sp.
  - 6- *Convolvulus* spp.
  - 7- *Ebenus stellata*
  - 8- *Ruta graveolens*
  - 9- *Aegilops columnaris*
  - 10- *Poa bulbosa*
  - 11- *Medicago* spp.
- (Rashvand , 2000)



Figure 19: *Amygdalus scoparia*, Haft-Chah, Bushehr, 1995.



Figure 20: *Amygdalus scopari* & *Salix alba*, Posht-Par, Bushehr, 1995.

#### *Amygdalus eburnean* associations:

The dominant species of this association is *Amygdalus eburnean*. This association is seen in the heights more than 600 meters above sea levels in southern Iran.

The average number per hectare is 462 single roots, the average height of shrubs is 1.2 meters, and average of percentage of canopy cover is 18.1%.

Accompanying plants:

- 1- *Amygdalus iranshabrii*
- 2- *Convolvulus spinosa*
- 3- *Acantholimon sp.*
- 4- *Astragalus fasciculifolius*
- 5- *Carthamus sp.*
- 6- *Rumex sp.*
- 7- *Aristida adscensionis*
- 8- *Stipa cappensis*
- 9- *Ziziphus spina – Christi*

(Rashvand , 2000)

Wild pistachio associations (*Pistacia atlantica*):

This association is seen in the northwest regions, central regions, northeastern regions and southern regions of Iran (in such provinces as Khuzestan, Boushehr, Hormozgan, and Sistano-Baluchestan). The dominant species of this community is *Pistacia atlantica*. The soil of the *Pistacia atlantica* sites have a light texture without structure, PH is 6.8 to 7.4. Its climate is mild warm desert and temperate dry. The *Pistacia atlantica* forests grow on Mishan geological formations (Gouri-Mambar district), Gachsaran, Asmari, Jahrom and Bangestan.

This association grows in the heights 700 to 1950 meters above sea level. The average number of trees per hectare is two to five stands. The diameter of the crest is 38cm (Rashidi, 2006).

Accompanying plants:

- 1- *Amygdalus scoparia*
- 2- *Amygdalus eburnea*
- 3- *Astragalus spp.*
- 4- *Ebenus stellata*
- 5- *Ziziphus nummulana*
- 6- *Ziziphus spina – Christi*
- 7- *Convolvulus spp.*
- 8- *Bromus tectorum*
- 9- *Medicago rigidula*
- 10- *Stipa cappensis*



Figure 21: *Pistacia atlantica*, Posht-Par, Bushehr, 2005, Rashidi



Figure 22: *Pistacia Atlantica*, Ilam, 1996.

*Ephedra pachyclada* associations:

The main elements of this association are *Ephedra pachyclada* and *Ephedra major*. This community grows in the upper heights between 800 to 1500 meters above sea level. For example it is seen in the heights (1500 meters above sea level) of Haft-Chah around Taheri port in Boushehr province.

This association grows on the limestone parent.

Accompanying plants:

- 1- *Amygdalus scoparia*
- 2- *Stipa cappensis*
- 3- *Amygdalus eburnea*
- 4- *Medicago spp.*

Olive associations (*Olea ferrugina*):

This association grows in the heights of Geno-mountain in Hormozgan province.

*Juniperus polycarpus* associations:

This association grows in the heights of Geno Mountain in Bandar Abbas.



Figure 23: *Olea ferrugina*, Kuhe – Geno, Bandar-Abbas, 2007.

*Dalbergia sisoo* association:

The main constituent plant of this association is *Dalbergia sisoo*. This plant is seen inside the wet valleys of the estuaries of the Chegin river around Bashagard and on a limited manner inside the valleys around Dezful. Its wood is applied in industry. (Assareh Koneshloo, 2005)

*Salicetum* – *Myrtosum* associations:

The dominant species of this association is *Salix alba* and *Myrtus comminus*. This association can be seen on the sidelines of permanent rivers and springs in southern Iran. Khirak River (1100 meters above sea level) located in Boushehr province.

Accompanying plants:

- 1- *Nerium oleander*
- 2- *Tamarix sp.*
- 3- *Vitex negondu*

*Ziziphus nummularia* associations:

This association grows in the heights 300 to 1300 meters above sea level in southern Iran. The dominant plant of this association is *Ziziphus nummularia* which grows in such provinces as Ilam, Lorestan, Khuzestan, Boushehr, Fars, and

Hormozgan. Geologically the best associations of *Ziziphus nummularia* grow on the quaternary cobbled alluvial formations belonging to the fourth geological age. The natural sites of *Ziziphus nummularia* have mild warm desert climate and semidry temperate climate.

These associations are seen on the gravelly alluvial fans and piedmont plains. The soil of these sites has EC less than 2 ds/m, pH of 7.6 to 8.1.

The average number per hectare of *Ziziphus nummularia* association is 38-47 stands, the average high of shrubs is 1.9 meters and the percentage of canopy cover is 3.5%.



Figure 24: *Ziziphus Nummularia*, Posht-Par, Dashtestan, 1995.

### Problems forest of the Iran

- 1- Rainfall shortage (50-250 mm/year).
- 2- Evaporation is very upwards (about 3200-4200 mm).
- 3- In this region exist some diapers that they make salinity soils.
- 4- Sea water acts as a main factor in soil Stalinization in sea-bank lands.
- 5- Soil erosion is one of the most important limitations in this region.
- 6- Domestic animals are in forests permanently.
- 7- In this region Bahman (*stipa cappensis*) is very large in amount.
- 8- Non – existence of forests – regeneration.
- 9- In this region harvesting of mines (Petroleum, Gas and others) to demolish forests.
- 10- To set on fire intentional and un-intentional in forest.

### Results and conclusion

Existence forests species (Trees and shrubs) which they are resisting on salinity and Dryness in southern – Iran is a natural potential in this region. Rainfall shortage, excess of evaporation, salinity and soil erosion, existence of domestic

animals in forest constantly and Non-existence of forests – regeneration are the most important limitation of these forests.

Based on natural potentials and natural and artificial limitations in this region, there is some suggestions for programmed and doing.

- 1- Applied research to be accomplished.
- 2- Establishment of station for seed product of forest and rangeland plants in this region.
- 3- Programmed on harvesting of Rainfalls, specially doing programs for flood and erosion control and to reserve Rainfall.
- 4- Increasing and production wild Alfa Alfa (*medicago spp.*) seed in this region.

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## Forest Association of southern – Iran

No.	Forest association	Name of sites	Pedology	Geology	Climat based on Amberyee method	Dominant species	Accompanying species	Silviculture characteristics
1	<i>Mangrove forests</i>	Seashores of Srik, Jask, Qeshm – Irland, Bandar – Khamir Asalooyeh (Nayband – Gulf) Dayyer port, Male-Gonzeh village.	clay-loamy texture, Ec= 36-42 ppt, Ph = 6.7-7.6 and fine aggregate	Quaternary Alluvial formation	Extremely warm – desertical	Avecina marina Rhizo phora macronata	1- <i>Arthrochennum macrostachym</i> 2- <i>Bienertia cycloptira</i> 3- <i>Holocuennum striluceum</i> 4- <i>Sueda fruticosa</i> 5- <i>S.V.ermiculata</i> 6- <i>Salicornia europea</i>	Number perha= 431 Percentag of crown canopy = Height (m) = 3 Height of crown (rm) = 2 Regeneration N/ha = 21% Variety of stands: pure and mixed
2	<i>Iranian – Prosopis forest</i>	They are seen on eastern most border of Iran and Pakistan toward west around Borazjan (Bushehr p.) examples: Iranshahr, Bampur, Jazmourian, Bandar-Abbas, Kangan, Asalooyeh	light alluvial rough –grained soils, light texture (sandy-loam) and heavy (silty-loamy) Ec = 0.4-55ds/m pH = 6.3-7.7	Quaternary Alluvial formation	Intensive – warm – desertical	Prosopis cineraria	1- <i>Capparis spinosa</i> 2- <i>Ziziphus spina-christi</i> 3- <i>Prosopis koelsiana</i> 4- <i>Taverniera spartea</i> 5- <i>Suaeda oxyptira</i> 6- <i>Stipa capensis</i> 7- <i>Erodium sp.</i> 8- <i>Plantago sp.</i> 9- <i>Prenopyrum oilberi</i> 10- <i>Hamada salicornia</i> 11- <i>Pennisetum divisum</i> 12- <i>Cenchrus ciliaris</i> 13- <i>Medicago spp.</i> 14- <i>Calotropis procera</i>	N/ha = 3-10 Pc = 2.9-3.3% HT = 7-9.4 m HCT= 5-7.3 m Regeneration = rare VS: Mixed
3	<i>Prosopis koelsiana</i>	They are seen on easternmost border of Iran (Goater Port) up to Boushehr province. Examples: Iranshahr, Bampur, Bam, Jazmourian, Kahnoj, Jiroft, Nikshahr, Konarak, Banadar Abbas, Kangan, Birdkho on, Khormoj, an Borazjan.	Light texture (Sandy lomy)	Quaternary Alluvial formation	Intensive – warm – desertical	Prosopis koelsiana	1- <i>Stipa capensis</i> 2- <i>Capparis spinosa</i> 3- <i>Prosopis stephaniana</i> 4- <i>Tamarix sp.</i> 5- <i>Ziziphus spina-christi</i> 6- <i>Calotropis procera</i> 7- <i>Ephedra foliate</i> 8- <i>Geranium mascatense</i> 9- <i>Chenopodium mural</i> 10- <i>Erodium sp.</i> 11- <i>Medicago spp.</i>	It has a powerful outshoot power and reproduced by help of root shoot
4	<i>Ziziphus spina-christi</i>	They are seen in suchprovince: Fars, Homozgan, Baluchestan, Boushehr, Khuzestan, Keram, Ilam, Lorestan	Gravelly alluvials, fine gained alluvials and sanywind – Hills soil texture is loamy, loamy-silty, loamy – sandy and clay-sily. Ec=0.18-3.9ds/m pH= 7-8.2	Quaternary Alluvial, siltsone and conglomerates formation	Mildly warm, deser, extremely – warm desertical average and semi – average warm-desertical and dry-temperate.	Ziziphus spina-christi	1- <i>Stipa capensis</i> 2- <i>Lycium dereessum</i> 3- <i>L.shavil</i> 4- <i>Calotropis procera</i> 5- <i>Prosopis cineraria</i> 7- <i>P.koelsiana</i> 8- <i>Ephedra foliate</i> 9- <i>Prosopis stephaniana</i> 10- <i>Crepis kotschiana</i> 11- <i>Echinops aphyalotes</i> 12- <i>Erodium aictarium</i> 13- <i>Gallium saxaceum</i> 14- <i>Hippocrepis constricta</i> 15- <i>Malva sibirer</i> 16- <i>Medicago minima</i> 17- <i>Periploca aphylla</i> 18- <i>Plantago ovata</i> 19- <i>Rumex conglomeratus</i> 20- <i>Vernicia sp.</i>	N/ha = 23 PCC = 2.5-4.55% HT (m) = 3 m HCT = 2m NR/ha = 11 The highest tree = 13m

5	<i>Semang (Tecomella undulata)</i>	Baluchestan and between Sirjan and Bandar Abbas, Lar, Saicid-Abad, Haji Abad, and Andimeshk Dashtestan, Kangan, Dashedi and Tangestan	Alluvial soils with sandy, loamy – sandy and loamy texture. pH = 7.2-7.9, Ec = 0.8-8.8ds/m	Quaternary alluvial and conglomerates formations	The extremely warm - desertical	<i>Tecomella undulata</i>	21- <i>Ziziphus spina Christi</i> 20- <i>Tamarix sp.</i> 21- <i>Prosopis juliflora</i> 22- <i>Amygdalus scoparia</i> 23- <i>Lycium shawii</i> 24- <i>Artemisia scoparia</i> 25- <i>Helianthemum lippii</i> 26- <i>Centaurea depressa</i> 27- <i>Alhagi camelorum</i> 28- <i>Capparis spinosa</i> 29- <i>Convolvulus spinosa</i> 30- <i>Ephedra foliata</i> 31- <i>Astragalus sp.</i> 32- <i>Medicago minima</i> 33- <i>Sinapis alba</i> 34- <i>Stipa cappensis</i> 35- <i>Hordeum violaceum</i> 36- <i>Cenchrus ciliaris</i> 37- <i>Panicum sp.</i>	Number per perha = 90-400 Percentag of crown canopy = 26% Height of trees shrubs (HT/S)(m) = 8-9 Height of crown (HC)(m) = 5 Number of regeneration per hectar (NR/h) = - Variety of stands (VS): pure
6	<i>Calotropis procera</i>	Khuzestan, Bushehr, Bandar-Abbas, Baluchestan and Fars province	Alluvial soils with sandy texture. pH = 7.4-8.1, Ec = 0.43-1.92ds/m	Quaternary alluvial formations	The extremely warm - desertical	<i>Calotropis procera</i>	1- <i>Hamada sp.</i> 2- <i>Convolvulus spp.</i> 3- <i>Asphodelus sp.</i> 4- <i>Lycium depressum</i> 5- <i>Prosopis stephaniana</i> 6- <i>Alhagi sp.</i> 7- <i>Prosopis koelsiana</i> 8- <i>Atriplex leucoclada</i> 9- <i>Ziziphus spina-Christi</i> 10- <i>Astragalus sp.</i> 11- <i>Capparis spinosa</i>	N/ha = 7.5-35.75 PCC = 1% HS (m) = 1.5-2 m NR/ha = rare VS: mixed
7	<i>Capparis spinosa</i>	In all parts of Iran	Soil texture is very light (Sandy) Ec = 0.98-6.2 ds/m pH = 7-7.4	Quaternary alluvial formations	The extremely warm - desertical	<i>Capparis spinosa</i>	16- <i>Calotropis procera</i> 17- <i>Stipa capensis</i> 18- <i>Carthamus tinctorius</i> 19- <i>Lycium depressum</i> 20- <i>Ziziphus spina Christi</i> 21- <i>Artemisia scoparia</i> 22- <i>Prosopis juliflora</i> 23- <i>Alhagi camelorum</i> 24- <i>Citrullus colocynthis</i> 25- <i>Pegannum harmala</i> 26- <i>Acacia nilotica</i> 27- <i>Colobium speciosum</i> 28- <i>Prosopis stephaniana</i> 29- <i>P. koelsiana</i> 30- <i>P. cineraria</i>	N/ha = 7-64 PCC = 0.23-4.6% HS (m) = 1.03-3.02 m NR/ha = rare VS: mixed
8	<i>Acacia nilotica</i>	This tree is seen from the eastern borders of Iran to outskirts of Bushehr town. (Kangan, Bandar-Lenge, Bandar-Abbas, Haji-Abad, chabandar and Bampoor	Deepalluvial – alkaline soils	Quaternary alluvial formations	The extremely warm - desertical	<i>Acacia nilotica</i> <i>Ziziphus spina-christi</i>	1- <i>Ziziphus spina-christi</i> 2- <i>stipa cappensis</i> 3- <i>prosopis cineraria</i> 4- <i>pennisetum divisum</i> 5- <i>calotropis procera</i> 6- <i>Hamada salicornica</i> 7- <i>Rhazya stricta</i> 8- <i>lycium shawii</i> 9- <i>prosopis stephaniana</i> 10- <i>Medicago spp.</i>	HT = 14m NR/ha = 7.5-35.75 NR/ha = rare VS: mixed
9	<i>A. tortilis</i>	This tree is seen between task to Bandar-Gavbandy in Hormozgan province	Soil texture is sandy, loamy and shallow soils	QAF	The extremely warm – desertical, annual Rainfall = 100mm	<i>Acacia tortilis</i>	11- <i>Lycium shawii</i> 12- <i>Ochradenus baccatus</i> 13- <i>Ephedra foliata</i> 14- <i>Hamada salicornica</i> 15- <i>Stipa cappensis</i> 16- <i>Calotropis rovera</i> 17- <i>Prosopis cineraria</i> 18- <i>Ziziphus spina-christi</i> 19- <i>Acacia nilotica</i> 20- <i>Medicago spp.</i>	HT = 4-10m NR/ha = rare VS: mixed

10	<i>Acacia nubica</i>	This plant is seen in Hormozgan province around Bandar – Lengeh to Bandar-Abbas	Gravelly – Alluvial plans	Quaternary alluvial formation	The extremely warm – desertical	Acacia nubica	10- <i>Acacia ehrenbergiana</i> 11- <i>Prosopis cineraria</i> 12- <i>Stipa capensis</i> 13- <i>Medicago spp.</i> 14- <i>Acacia nilotica</i> 15- <i>Capparis spinosa</i> 16- <i>Heliantemum lippi</i> 17- <i>Acacia tortilis</i> 18- <i>Ziziphus spina-Christi</i>	Number per perha= Percentag of crown canopy = Height of trees shrubs (HT/S)(m) = Height of crown (HC) (m) = Number of regeneration per hectar (NR/h) = - Variety of stands (VS): mixed This shrub loses its leaves in dry season and its leaves appear after first rainfall in autumn
11	<i>A. ehrenbergiana</i>	Sistano Balochestan and Hormozgan province	Alluvial soils	Quaternary alluvial formation	The extremely warm – desertical	A. ehrenbergiana	1- <i>Periploca aphylla</i> 2- <i>Moringa peregriana</i> 3- <i>Ziziphus spina Christi</i> 4- <i>Prosopis cineraria</i> 5- <i>Acacia nubica</i> 6- <i>Acacia tortilis</i> 7- <i>Calotropis procera</i>	-
12	<i>A. albida</i>	This tree is seen only in Bushehr province near Razm-Abad village in Dashti town	Alluvial fine gravelly	Quaternary alluvial formation	The extremely warm – desertical	A. albida	6- <i>Ziziphus Spina-Christi</i> 7- <i>Lycium shawii</i> 8- <i>Calotropis procera</i> 9- <i>Stipa Capensis</i> 10 <i>MediCago Spp.</i>	Acacia albida also regeneration with root – shoot
13	<i>Nannorrhops Ritchiana</i>	This shrub grows in makra of sistano Balochestan province	-	-	The extremely warm – desertical	Nannorrhops Ritchiana	1- <i>rium indicum</i> 2- <i>Saccharum ravennae</i> 3- <i>Acacia ehrenbergiana</i> 4- <i>Razia stricta</i> 5- <i>Tamarix sp.</i> 10- <i>Prosopis cineraria</i> 11- <i>Prosopis koelsiana</i> 12- <i>Calotropis procera</i>	-
14	<i>Tamarix mascatensis</i>	This shrub is seen in the sidelines of the seasonal and permanent rivers and near the ponds where the flood caused by rainfall flow or gather. Example : around such rivers as Heleh, mond, Dalaki and Bahoush.	Texture of soil is high and without gravels	Quaternary alluvial formation and etc.	The extremely warm – desertical and mildly warm-desertical	Tamarix mascatensis	12- <i>icum shawii</i> 13- <i>Tamarix dioica</i> 14- <i>Populus euphratica</i> 15- <i>Suaeda vermiculata</i> 16- <i>S. egyptica</i> 17- <i>Chenopodium murale</i> 18- <i>Aeluropus lagopoides</i> 19- <i>Tamarix aphylla</i> 20- <i>T. ramosissima</i> 21- <i>T. paseroides</i> 22- <i>T. tetrandr</i>	NR/h : Rare VS: pure and mixed
15	<i>Populus euphratica</i>	<i>Khuzestan, Bushehr and Fars province around such rivers: Karoun, shapour, Dalak, and Mond and other areas wich have underground water or permanent surface water</i>	<i>Soil texture is light (sandy) , sandy loomy) without gravell.</i>	<i>Quaternary Alluvial formation</i>	<i>The extremely warm – desertical and Mildly warm-desertical.</i>	<i>Populus euphratica</i>	6- <i>Slix sp.</i> 7- <i>Nerium oleunder</i> 8- <i>Vitex negundo</i> 9- <i>Tamarix spp.</i> 10- <i>Stipa appensis</i>	<i>Number per hectar (N/ha)= Percentage of crown canopy (PCC)= Height of trees/shrubs (HT/S)<sub>av</sub>= Height of Crown (HC)<sub>av</sub> = Number of regeneration per hectar (NR/h)= Variety of stands (VS): pure and Mixed</i>
16	<i>Cappariet unculligonetus om</i>	<i>This is seen around Banpoor plain</i>	<i>Soil texture is light (sandy) , on sandy Hills.</i>	<i>Quaternary formation</i>	<i>Theextremely warm-desertical.</i>	<i>Capparis deciduas and calligonam bungi.</i>	7- <i>osopis koelsiana</i> 8- <i>Prosopis cineraria</i> 9- <i>Calligonum bungi</i> 10- <i>Tamarix aphylla</i> 11- <i>Hamada sp.</i> 12- <i>Haloxylon persicum</i>	<i>V/S: Mixed These two species (Cpparis deciduas and calligonum bungi) play a vital role in Stabilization of shifting sands.</i>

17	Almond associations ( <i>Amygdalus Scoparia</i> )	Bushehr, Bandar- Abbas, Fars and Khuzestan province	This association is seen in plateaux and upper terraces types of soil the soil of the sites is shallow or with out soil. PH=6.8-7.4 EC=0.8 to 1.7 ds/m	Asmary and Aghajari formation and other formations with limestone	Dry and temperate, annual rainfall is 200-600 mm	<i>Amygdalus Scoparia</i>	8- 9- 10- 11- 12- 13- 14- 15- 16- 17- <i>Daphne micrumata</i> <i>Ficus carica</i> <i>Ziziphus nummularia</i> <i>Allium</i> sp. <i>Calendula</i> sp. <i>Convolvulus</i> spp. <i>Ebenus stellata</i> <i>Ruta graveolens</i> <i>Aegilops columnaris</i> <i>Poa bulbosa Medicago</i> spp.	N/ha=420 PCC= 37% HS = 1.8-2.6 m VS=Pure and Mixed
18	<i>Amygdalus eburnea</i>	In Khuzestan, Bushehr, Bandar -Abbas provinces is seen.	This is seen in plateaux and upper terraces types of soil. The soil is shallow or with out soil.	Asmary and Aghajari formation and OFWL	Dry and temperate,	<i>Amygdalus eburnea</i>	10- 11- 12- 13- 14- 15- 16- 17- 18- <i>Amygdalus iranbahrii</i> <i>Convolvulus spinosa</i> <i>Acantholimon</i> sp. <i>Astragalus fasciculifolius</i> <i>Carthamus</i> sp. <i>Rumex</i> sp. <i>Aristida adscensionis</i> <i>Stipa capensis</i> <i>Ziziphus spina - Christi</i>	N/ha=462 PCC= 18.1 % HS= 1.2 m VS = Pure and Mixed NR/h= rare
19	Wild <i>pistachio</i>	This is seen in the Northeastern and southern regions of Iran (Khuzestan, Bushehr, Hormozgan and sistano-Baluchestan provinces) Altitude = 700- 1950m	The soil texture is light and without structure PH= 6.8 – 7.4	Mishan (Gour- Member) Gachsaran, Asmari, Jahrom and Bangestan formations.	Mild warm- desertical and temperate dry.	<i>Pistacia atlantica</i>	11- 12- 13- 14- 15- 16- 17- 18- 19- 20- <i>Amygdalus scoparia</i> <i>Amygdalus eburnea</i> <i>Astragalus</i> spp. <i>Ebenus stellata</i> <i>Ziziphus nummulana</i> <i>Ziziphus spina - Christi</i> <i>Convolvulus</i> spp. <i>Bryonia tectorum</i> <i>Medicago rigidula</i> <i>Stipa capensis</i>	Number per hecтар (N/ha)=2.5 Percentage of crown canopy (PCC)= Height of trees/shrubs (HT/ s) <sub>m</sub> =4.4 Diameter on chest = 38 cm Height of crown (HC <sub>m</sub> )= Number of regeneration per hecтар (NR/h)= Variety of stands (VS): Mixed
20	<i>Ziziphus nummulari a</i>	Ilam, Lorestan, Khuzestan, Bushehr, Fars and Hormozgan provinces (altitude= 300-Boom)	The soil type is Alluvial gravelly fans and piedmont plains with light texture. PH=7.6-8.1 EC=about 2ds/m	Guaternery Alluvial formations	Mild warm- desertical and semidry temperates.	<i>Ziziphus nummularia</i>		N/h=38-47 PCC=3.5% HS=109m NR/ha=Rare V.S: Pure and Mixed

**Effects of drought stress on *Eucalyptus camaldulensis* at germination and seedling stage**

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## Abstract

*Eucalyptus* is an important exotic species in Iran in respect to, industrial, medicinal and ornamental points of view. Different species of *Eucalyptus* demonstrate different responses to drought. This research was carried out to estimate drought resistance of *Eucalyptus camaldulensis* at germination and seedling stages. Five potential osmotic level (0, -0.1, -0.3, -0.6 and -1.2 MPa) treatments were used in five replications. Polyethylene glycol 6000 was used as diminutional osmosis in hydroponic culture in greenhouse. Radicule length, plumule length, vigour index, germination percentage, germination speed and germination index were estimated at germination stage. Osmoprotectants, pigments, abaxial and adaxial number, length and width of abaxial and adaxial stomata, and growth parameters, withering, falling, drying leaves were measured in growing stage on six month plant. Increased of drought stress leads to increased osmoprotectants, weathering, falling and drying leaves and decreased of radicle length, plumule length, vigour index, germination percentage, germination speed and germination index, pigments and growth parameters. Osmoprotectants have important plane in osmosis regulation and osmosis protection so increase in content of these substance led to tolerance of plant against osmosis stress. Decrease in pigments is because of decomposition enzymes activity.

**Key words:** drought stress, *Eucalyptus*, vigour index, pigments, osmoprotectants.

## Introduction

Drylands cover about 41% of Earth's land surface and are inhabited by more than 2 billion people (about one third of world population). Drylands are limited by soil moisture, the result of low rainfall and high evaporation, and show a gradient of increasing primary productivity, ranging from hyper-arid, arid, and semiarid to dry subhumid areas. Deserts, grasslands, and woodlands are the natural expression of this gradient. The annual rainfall in Iran is about 50 mm in some low lying plains of the central plateau while the annual evaporation may exceed 4000 mm. The country-wide average precipitation is about 250 mm. It is estimated that Iran has about 23 million ha of salt affected lands (Dewan, et al 1964, Roozitalab, 1987, Siadat et al 1997). About 21% of the lands in Iran are classified as desert and degraded lands. Close to 90% of the country is semi-arid, arid and hyper-arid. Saline water resources in Iran are also available in large quantities either as surface water bodies, or saline groundwater.

There are over 500 species of *Eucalyptus* described in the Flora of Australia. The establishment of trees that tolerate drought and provide biomass for fuel and environmental services, including watershed management, soil nutrient and water retention. Fodder for livestock, and construction material, is basic for sustained

livelihood of farmers. The success of *E. camaldulensis* as exotic evergreen species has been attributed to its high biomass production on poor and dry lands, tolerance to drought and rapid growth when water is available (Florence, 1996; Gindaba et al., 2005).

Plants possess three mechanisms that may counteract water deficit stress: (1) reduction of water loss through stomatal control and morphological changes; (2) increase extraction of water from the soil and; (3) increased ability to tolerate low tissue water potential (Nilsen and Orcutt, 1996). During the course of drought stress, active solute accumulation of compatible solutes such as amino acids, polyamines, and carbohydrates is claimed to be an effective stress tolerance mechanism (Rosa-Ibarra and Maiti, 1995; Kerepesi and Galiba, 2000). Soluble sugar changes are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation and respiration. Among soluble carbohydrates, sucrose and fructans have a potential role in adaptation to these stresses (McKersie and Leshem, 1994). Proline is another widely studied compatible solute for plants grown under stress conditions (Delauney and Verma, 1993), which is important in osmotic adjustment in plant tissues (Hare and Cress, 1997) and is also a nitrogen source during recovery from stress (Trotel et al., 1996). However, proline accumulation appeared to be merely a result of salt stress damage rather than an indication of salt tolerance (De Lacerda, et al., 2003; Claussen, 2005; Zhou et al., 2007).

We examined the growth of *Eucalyptus camaldulensis* in response to drought in a controlled environment to elucidate mechanisms and relations between both physiological (proline, soluble sugar, Glycine betaine,...) and morphological (biomass,...) response to biophysical environmental stress.

## Material and methods

*Eucalyptus camaldulensis* grew in well-controlled experiments to measure some physiological characters. Seeds of *Eucalyptus camaldulensis* were purchased from Kim seed Co. south west of Australia. Seeds of all species were divided into two groups for germination test and seedling test.

a) Germination test: Germination tests were carried out in a plant growth chamber (LabTech, Korea). Batches of 30 seeds were placed in 9-cm Petri dishes on two folds of Whatman filter paper #1 and wetted with 5 ml of different treatments of polyethylene glycol 6000 separately. There were five replicates per treatment. The seeds germinated within 5-15 days without pretreatment. Petri dishes were inspected daily until the first seeds had germinated (Baskin and Baskin, 1998) to identify the onset of germination, after that every four days were counted and plumule and radicle length were estimated.

b) seedling test: the seeds were germinated in a sterilized silt pot in a controlled temperature glasshouse (20° C day/15°C night), in the Institute of Forest and

Rangeland of Iran, from January 2007 to February 2008. When seedlings reached the two-leaf stage the half-strength Hogland solution was used for irrigation (Moor, 1960). After 10 weeks, seedlings were transplanted to small pail of nutrient solution and polyethylene glycol with 0, -0.1, -0.3, -0.6 and -1.2 MPa osmotic potential. Each pail was considered as an experimental unit, and each treatment was replicated 30 times (three pails, 30 seedlings). Polyethylene glycol increments at 3 d intervals to reach the maximum osmotic potential of -1.2 MPa after 15 d.

Sampling were carried out from stamen leaves of different treatments of first and second experiment after one month intervals after the highest treatment concentration was reached. Pigments (total chlorophyll, chlorophyll a, b, carotene), osmoprotectants (soluble sugar, prolin and glycin betain) and growth parameters (included biomass, leaf area, relative water content, water saturation different, specific leaf area) and other characters included withering, falling, drying leaves and stomata number and size were calculated

RWC was measured on leaf discs collected by driving a circular leaf punch of 1cm<sup>2</sup> - 2cm<sup>2</sup> internal diameter into randomly selected leaf blades. Part of the blade close to the apex was punched to avoid the large mid-veins close to the base of the leaves. Each leaf disc or leaflet was placed in an airtight vial and immediately taken to the laboratory where it was weighed. The discs or leaflets were then hydrated to full turgidity by floating on de-ionized water in a closed Petri dish in the dark for 6 h. The samples were removed from the water, surface moisture blotted off using filter paper, and weighed to get turgid weight. Samples were then oven dried at 85 C for 24 h and dry weights determined. RWC was then calculated using the equation:

$RWC(\%) = (W_f - W_d) / (W_f - W_d) * 100$  where  $W_f$  is the fresh weight,  $W_d$  is the dry weight and  $W_t$  is the turgid weight (Beadle et al., 1993).

Chlorophyll a, b and carotenoid contents were estimated by Wintermans & Motes (1965) and Jason (1978) in leaf samples (0.25 g) homogenized in 4.5 cm<sup>3</sup> of acetone (80%). Absorbance was recorded at 645, 663 and 470 nm (Spectrophotometer CECIL Model 3000, Cambridge, UK). Free prolin content in the leaves was determined following the method of Bates et al. (1973). Total soluble sugar was estimated by anthron reagent (Irigoyen et al., 1992). Glycinbetaine estimation was done according to Grattan and Greive (1983). The absorbance was measured at 365 nm with UV- visible spectrophotometer. Reference standards of GB(50- 200 µg cm<sup>-3</sup>) were prepared in 2 M sulfuric acid. The experimental design was completely randomized design with 5 replications of 5 treatments. Stomata size and stomata number per unit leaf area were measured by microscope.

**Biomass analysis:** The leaves, branches and stems of each harvested ramet were separated and dried at 70°C for 48 h before weighing. At each harvest, 10 fully-expanded leaves per plant were collected from up to four crown positions for analysis of leaf area: weight ratios (specific leaf area, SLA). The

sample were representative of the range of leaf sizes found on plants. The single side area of fresh leaves was measured using an area meter, and weight was determined after drying at 70°C for 48 h.

**Data analysis:** Duncan test at confidence level of 95% was used to separate means using SPSS 13. Standard error of mean (SE) was used to indicate variability. variables were tested for normality and homogeneity of variances and transformations were made when necessary to meet the underlying statistical assumptions of ANOVA.

## Result

*Eucalyptus camaldulensis* showed significant impacts of osmotic potential on relative water content, specific leaf area, biomass, total chlorophyll, carotene, glycine betain, prolin, soluble sugar, weathering leaves, falling and drying leaves, height of ridicule and plumule, germination percent, vigour index, germination speed and germination content. Glycine betain, prolin, soluble sugar, weathering, falling and drying leaves which increased by increasing drought. The relative water content decreased as osmotic potential concentration increased. No significant difference between control and -0.1 MPa was observed for RWC, but it was significantly decreased at -1.2 MPa (Table 1). Specific leaf area decreased by decreasing osmotic potential.

Species did not show variations in biomass production in the mild water deficit and well-watered treatments. Biomass decreased as osmotic potential decreased, it wasn't significantly differences between control, -0.1 MPa and -0.3 MPa but it was significantly decreased at -0.6 and -1.2 MPa (Table 1). The maximum contents of chlorophyll a, b and carotenoids were in control treatment and the greatest reduction occurred at -1.2 MPa.

To preserve sufficient water uptake osmotic adjustment occurred by production of prolin, glycinebetain and soluble sugars. The content of prolin and soluble sugars were raised by decreasing osmotic potential. The content of soluble sugars was significantly enhanced at -1.2 MPa. The tree and half fold increase in proline content was observed at -1.2 MPa compared to control. The concentration of glycinebetaine was significantly increased by decreasing osmotic potential, it increased 3 fold in comparison with control treatment. prolin, glycinebetain and soluble sugars are accumulated in cytoplasm and its organelles in order to maintain photosynthesis (Schroppel-Meier and Kaiser, 1988)

The test species differed greatly in their response to drought, as quantified by the percentage germination. Best germination was obtained in distilled water controls. Decrease in osmotic potential progressively inhibited germination. Almost all viable seed germinated at -0.6 Mpa while at -1.2 MPa didn't germinate. An inspection of the ungerminated seed revealed that most of them were non-viable in -1.2 MPa treatment, perhaps due to detrimental affect of the

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treatment. The number of stomata per unit of leaf area in adaxial and abaxial epidermis was significantly decreased by decreasing osmotic potential. Stomata size (length and width) was decreased as osmotic potential decreased.

Drought adaption depends on the severity of water deficit under natural habitats, and different water- use strategies maybe employed (Passioura, 1982). In general, *E.camaldulensis* have evolved two contrasting water-use strategies for survival and growth under limited water availability. *E. camaldulensis* have conservative water-use strategies, are adapted to conditions where drought period are prolonged, and are associated with high capacity for drought resistance and slow intrinsic growth rates. *E. camaldulensis* with a prodigal water use strategy, is adapted to mild drought of short duration where plants consume available water rapidly until almost all water from the soil is exhausted.

Table 1: The effects of osmotic potential with polyethylene glycol 6000 on the growth and physiological parameters of *Eucalyptus camaldulensis*. Means  $\pm$  SE, n=5, means in a column followed by a different letter are significantly different ( $P < 0.05$ ) according to Duncan test.

RWC	SLA	Biomass	Total chlorophyll	Chlorophyll a	Chlorophyll b	Carotene	glycinbetain	Soluble sugar	Prolin
control	33.3 $\pm$ 3a	80 $\pm$ 7a	2.4 $\pm$ 0.4a	1.2 $\pm$ 0.1a	1.2 $\pm$ 0.1a	24.8 $\pm$ 2.2a	202 $\pm$ 15d	0.4 $\pm$ 0.04b	0.4 $\pm$ 0.04b
-0.1 MPa	31.6 $\pm$ 2.8ab	76 $\pm$ 8a	2.2 $\pm$ 0.06a	1.1 $\pm$ 0.13a	1.1 $\pm$ 0.06a	10.7 $\pm$ 0.9cd	209 $\pm$ 18d	0.6 $\pm$ 0.04b	0.6 $\pm$ 0.04b
-0.3 MPa	31 $\pm$ 3ab	72 $\pm$ 5a	2.2 $\pm$ 0.2a	1.06 $\pm$ 0.1a	1.1 $\pm$ 0.05a	9.1 $\pm$ 0.7d	224 $\pm$ 12 c	0.9 $\pm$ 0.05b	0.9 $\pm$ 0.05
-0.6 MPa	29 $\pm$ 2b	59 $\pm$ 6b	1.9 $\pm$ 0.06b	0.9 $\pm$ 0.08b	0.9 $\pm$ 0.03b	16.7 $\pm$ 1.57b	255.2 $\pm$ 9 b	0.77 $\pm$ 0.13b	0.77 $\pm$ 0.33b
-1.2 MPa	25 $\pm$ 1.9c	58 $\pm$ 4b	1.4 $\pm$ 0.06c	0.7 $\pm$ 0.03c	0.71 $\pm$ 0.03c	11.6 $\pm$ 1.8c	686.3 $\pm$ 29 a	1.4 $\pm$ 0.1a	1.4 $\pm$ 0.04a

Weathering	Falling	Drying	Height of ridicule	Height of plumule	Germination percent	Vigour index	Germination index	Germination speed
control	0c	0d	15.6 $\pm$ 3.2a	11 $\pm$ 0.6a	40.1 $\pm$ 4.5a	10.9 $\pm$ 2.6a	4.16 $\pm$ 0.4c	5.2 $\pm$ 0.36a
-0.1 MPa	0.04 $\pm$ 0.03c	0.05 $\pm$ 0.03bc	10.6 $\pm$ 2.6a	5 $\pm$ 0.58b	36.5 $\pm$ 3.4a	5.6 $\pm$ 0.75b	4.7 $\pm$ 0.67bc	3.04 $\pm$ 0.17b
-0.3 MPa	0.15 $\pm$ 0.03b	0.12 $\pm$ 0.04ab	12 $\pm$ 1.53ab	4.67 $\pm$ 0.3b	41.2 $\pm$ 1.9a	6.9 $\pm$ 0.8ab	5.7 $\pm$ 0.2ab	3 $\pm$ 0.16b
-0.6 MPa	0.2 $\pm$ 0.03b	0.15 $\pm$ 0.03ab	5 $\pm$ 1.15bc	3.7 $\pm$ 1.2b	39 $\pm$ 1.27a	3.4 $\pm$ 0.8cd	6.4 $\pm$ 0.26a	2.7 $\pm$ 0.12b
-1.2 MPa	0.3 $\pm$ 0.03a	0.2 $\pm$ 0.04a	0.5 $\pm$ 0c	1.17 $\pm$ 0.2c	3 $\pm$ 0.58b	0.05 $\pm$ 0.01d	0.56 $\pm$ 0.06d	0.2 $\pm$ 0.02c

	Adaxial number	Adaxial length	Adaxial width	Abaxial number	Abaxial length	Abaxial width
control	1000 $\pm$ 140 c	2.9 $\pm$ 0.3 a	1.9 $\pm$ 0.2 ab	2000 $\pm$ 150 c	2.8 $\pm$ 0.4 a	A. 2.05 $\pm$ 0.19 a
-0.1 MPa	1100 $\pm$ 100 c	2.7 $\pm$ 0.55 ab	1.8 $\pm$ 0.24 ab	2350 $\pm$ 200 bc	2.7 $\pm$ 0.25 b	1.95 $\pm$ 0.22 a
-0.3 MPa	1330 $\pm$ 150 b	2.66 $\pm$ 0.65 ab	1.8 $\pm$ 0.31 a	2450 $\pm$ 260 bc	2.7 $\pm$ 0.32 b	1.9 $\pm$ 0.2 a
-0.6 MPa	1450 $\pm$ 100 b	2.6 $\pm$ 0.5 ab	1.7 $\pm$ 0.15 b	2600 $\pm$ 300 b	2.6 $\pm$ 0.3 b	1.73 $\pm$ 0.25 b
-1.2 MPa	1700 $\pm$ 150 a	2.4 $\pm$ 0.4 b	1.7 $\pm$ 0.1 ab	3050 $\pm$ 200 a	2.6 $\pm$ 0.2 b	1.68 $\pm$ 0.14 b

## Discussion

Our results demonstrate a distinct difference in response to drought stress between different treatments. Under field conditions such factors as age of plant, symbiotic associations with microorganisms, competition with other species, nutrient availability, soil structure and the absence of restriction to root growth could also play a substantial role in determining the degree to which species may succeed in a water stressed environments (Gindaba et al., 2005). Although these biotic and abiotic interactions may have considerable effects on the growth and biomass allocation of trees under natural conditions, plants with extensive root systems are favored to survive in resource poor environments, because they are more likely to tolerate occasional severe droughts and encounter the nutrients that are distributed irregularly in many soils. Shallow rooted trees with extensive lateral root system are, however, more competitive and may not be good candidates for instance in agroforestry systems (Gindaba et al., 2005). Similar to the current observations at the seedling stage, *Eucalyptus camaldulensis* also produce extensive root system under natural conditions. These morphological and physiological responses to water availability showed that different survival strategies under drought stress at the initial phase of seedling growth and establishment may employ. Several adaptations may increase the seedling's ability to cope with the consequences of drought: the ability to maintain viability at water potentials below the turgor loss point (Richards and Lamont, 1996), Rapid root growth to exploit the temporally- and spatially- restricted soil water resources (Schutz, 1999), and a high root: shoot ratio (Osunkoya et al., 1994).

The reduction of productivity in many plants subjected to excessive salinity or drought is often associated with reduced photosynthetic capacity as determined by lower chlorophyll contents (Netondo et al., 2004, Zhou et al. 2007). Under drought stress, the reduction of chlorophyll contents in our treatments was more obvious than that control plants, showing a more severe drought damages evidenced more pale green leaves and visible loss of turgor. The increased activity of the chlorophyll degrading enzyme or the suppression of the specific enzyme responsible for pigment synthesis may attribute to the decreased chlorophyll contents in drought stressed plants (Reddy and Vora, 1986). Osmotic adjustment by accumulation of compatible solutes, which include sugars, glycerol, amino acids, sugar alcohols and other low molecular weight metabolites, is one of the mechanisms evolved by plants to overcome salin and drought stress (Verslues et al., 2006). Sucrose can act in water replacement to maintain membrane phospholipids in the liquid crystalline phase and to prevent structural changes in soluble proteins. The role of reducing sugars (glucose and fructose) in the adaptive mechanism is more controversial, and even their accumulation can be detrimental from several points of view (Kerepesi and Galiba, 2000).

Our data, while providing further evidence on relationship between soluble sugar accumulation and degree of drought tolerance, indicated that total soluble sugar content might be useful trait to select drought tolerant eucalyptus genotypes. Tolerance mechanisms can be categorized as those that function to minimize osmotic stress or ion disequilibrium or alleviate the consequent secondary effects caused by these stresses (Bohnert et al., 1995).

A holistic approach to determine the effects of other environmental factors and their interactions on *Eucalyptus occidentalis* and *Eucalyptus delegatensis* germination was described by Zohar et al. (1975) and Battaglia (1993). At the cellular level, reduced water potential and RWC affect the physiology of the cell in several ways, including changes in intercellular organelle positions, transport channels, enzyme biochemistry; as well as cell wall shrinkage (Hall, 2001; Lawlor and cornic, 2002). This result is important in the planning ecologically and economically advantageous *Eucalyptus* agriculture. Further research should be conducted on the tolerance of other species. This study confirms several aspects of the physiology of *Eucalyptus camaldulensis* with respect to water deficits. It demonstrates that some degree of drought tolerance may occur in *E.camaldulensis*.

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# **Micropropagation of the Iranian woody lants**

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## Abstract

Iran is a unique country regarding plant diversity and genetic reserves but the forest cover is considered poor as compared with other countries, because of rapid urbanization, intensive grazing, firewood production and farming in wooded areas, thus we need for creating of hand cultured forests. Asexual propagation with conventional methods wasn't high success, therefore micropropagation methods are employed for product of clonal plants from superior genotypes of interesting forest trees. *Eucalyptus grandis* and *Eucalyptus globulus* are important fast growing trees in the world. These are cultivated for their wood fibers which is widely used in the paper industry, domestic fuel and charcoal production. Apical and axillary buds from adult elite trees in the forests of northern Iran were collected in different seasons. After surface sterilization of explants, these were cultured on a modified MS medium containing different growth regulators. The rate of shoot multiplication, shoot length growth and other responses were studied. *Eucalyptus* showed rapid multiplication, when cultured on MS medium (half strength Nitrate) containing 1 mg<sup>l</sup><sup>-1</sup> Zeatin, 0.1 mg<sup>l</sup><sup>-1</sup> IAA for shoot proliferation. Elongated shoots excised at 1.5 cm in length were rooted in half MS medium with 0.5 mg<sup>l</sup><sup>-1</sup> NAA and 0.5 mg<sup>l</sup><sup>-1</sup> IBA. These plantlets were established successfully in the greenhouse conditions and finally these plants transferred in the field.

**Key words:** Micropropagation, *Eucalyptus grandis*, *Eucalyptus globulus*.

## Introduction

Eucalyptus trees are native of Australia which are of fast growing and corpulent trees. Nowadays great forestry from kinds of these trees species has been created in the most region of the world. Eucalyptus trees entered to Iran from about half century ago and plantation many of those species in the north and south had very satisfactory results and yet great lands in Iran are susceptible of this species expansion. The large number of seedlings from those species is needed for creating of hand-cultured forests. *Eucalyptus grandis*, *Eucalyptus globulus* are tall, straight and rapidly growing trees, up to 150 feet high under good conditions. these have been used for a large number of purposes: as a source of pulp in paper production, for charcoal production, for building and transmission, telephone poles and good honey (Lakishma Sita, Shobharani, 1985).

Asexual propagation with conventional methods such as cutting and graft wasn't possible or obtained success was very low because of hard proliferation of adventitious roots (Assareh, 1998). This method is employed for product of virus

free plants, gene bank and genetically uniform plants, that its better than other vegetative methods.

Although successful results for *in vitro* cloning have been reported for some species (Mccomb, Bennet 1986). The rooting phase is a critical and limiting step (Hartney, 1980).

Micropropagation of *Eucalyptus* may be an important tool for tree improvements and reforestration programs. Also large number of clonal plantlets may be micropropagated for physiological and silvicultural studies when genetic uniformity is desired. Foresters have in recent years appreciated the value of vegetative propagation as a source of uniform trees of known genotype and as a means of obtaining genetic and physiological information. In most of these micro methods, organogenesis was obtained from embryonic or juvenile seedling tissues. A few reports about shoot regeneration of explants taken from mature *Eucalypt* trees. In view of their highly heterozygous nature and long duration with respect to flowering, it is all the more important to develop methods for clonal propagation of *Eucalyptus grandis*. Conventional vegetative propagation is not successful for *Eucalyptus grandis* and use of seed propagation is yielding highly variable progeny for continuous pulpwood supplies. It is important to have plantations of uniform bole size and quality. Hence it is desirable to develop reliable methods for clonal propagation.

In this paper, we report the production of plantlets from nodal stem segments of mature *Eucalyptus grandis* and *Eucalyptus globulus*.

## Materials and Methods

Nodal segments collected from 30 years old trees growing in chamestan forests. Shoots measuring 10-20 cm from two genotypes were cut and brought to the Lab and were washed thoroughly with detergent and distilled water before sterilizing in 0.1% mercuric chloride solution for 7-10 min. After sterilization, the shoots were washed repeatedly were cut and cultured under aseptic conditions. Mourashige & skoog (1962) mineral salts and vitamins with 3% sucrose and 100 mg<sup>l</sup><sup>-1</sup> ascorbic acid were used as basal medium. Nodal explants were cultured on nutrient media supplemented with a wide range of combinations of cytokinin such as Kin, BA, 2ip, Zeatin and auxins including IAA, IBA and NAA. Initial experiments showed that shoot formation was most consistently induced by BA (0-1) mg<sup>l</sup><sup>-1</sup> with Kin (0-0.2), GA (0-0.25), IBA (0-0.1) mg<sup>l</sup><sup>-1</sup>.

Media were solidified with 0.68 % agar. Culture media were autoclaved under 1.06 kg/cm<sup>2</sup> pressure for 20 min. Cultures were incubated in 16 h light (3000-5000) lux at 25 ± 2°C. In all experiments 30 replicants were used and each experiment was repeated at least three times. The subculturing intervals extended from 4 weeks. All chemicals used were of analytical grade (BDH & MERCK). Explants from mature sources were identical in their response to shoot induction. ANOVA was

performed using shoot number, shoot length and percentage rooting. Tukey multiple range test was used to compare treatments with in clones when a significant difference was found between treatments. The results of statistical analysis were considered significant when they were outside 95% confidence intervals. A final experiment examined the effect of the combination of Kin, BAP, 2ip and zeatin in other ratios.

For rooting initiation, shoots were separated and individually subcultured to half strength MS medium supplemented with IBA, NAA, IAA separately (0.1-1) mg<sup>l</sup><sup>-1</sup>. The plantlets from each of genotypes transferred in steril soil pots in the green house and finally planted in the field.

## Results

The response of nodal segments to different cytokinin - auxin combinations was determined using MS medium. Shoots measuring 10-15 mm were formed in 5-6 weeks time. Shoot proliferation was not as good on Kin and 2ip media, shortening internode and growth inhibition also was observed. When shoots were transferred to BAP and Kin, good multiplication and growth obtained than the continuous BAP medium. For multiple shoot production, MS medium (half strength nitrate) supplemented with 0.1 BA, 0.1 Kin, 0.1 GA and 0.01 mg<sup>l</sup><sup>-1</sup> IBA was used. Up to 20- 40 shoots could be obtained from one nodal culture. Shoots were sub cultured to MS supplemented with Zeatin 1, IAA 0.2 mg<sup>l</sup><sup>-1</sup> for elongation prior of shooting (warrag, 1990). Treatment of cultures with GA 0.1 mg<sup>l</sup><sup>-1</sup> resulted in rapid elongation of shoots (Mccomb, Bennet 1986).

Medium contained 0.5 mg<sup>l</sup><sup>-1</sup> of IBA and NAA was also found to be very good in rooting. Roots could be induced on shoots taken from multiplication media containing Kin or Zeatin but not BAP.

The plantlets were successfully transferred in the pots contained steril soil in green house. After acclimation of plants, these were planted successfully in the field.

## Discussion

The use of two different multiplication media improves shoot condition and subsequent rooting of *E.grandis* and *E.globulus* even if these do not always increase shoot multiplication (Bunn,2005). The rooting response were correlated with the production of particular flavonoids under the influence of the cytokinin in the shoot multiplication medium (Curir, 1990). Endogenous cytokinins appear to have a role in adventitious root production.(Bullmark,Kubat,1988). It is possible that BAP is more effective than Kin in switching on endogenous cytokinin production (Vankova 1991).

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With *E.grandis*, the nodal explants from juvenile as well as adult trees responded alike to shoot induction, however shoots induced from juvenile material were more amenable to rooting than those from mature trees. Mascarehans, Gupta in 1987 the same results reported.

Shoots were subcultured to MS medium contained active charcoal (2.5 g<sup>l</sup><sup>-1</sup>), GA (1 mg<sup>l</sup><sup>-1</sup>) and without auxines for elongation prior to rooting (Jones, 1993).

In woody species, the frequency of regeneration *invitro* generally declines with the age of the explant source (Lakishmasita, 1985). Micropropagation by induction of shoots from pre- exciting meristems is a useful technique in vegetative propagation since it guarantees that the characteristics of the source plant are conserved. The present study provides a method that ensures a high frequency of multiple shoot induction from mature tree nodal meristems of *E.grandis* and *E.globulus*. the subsequent rooting of shoots induced in culture. Furthermore the present study shows that with at low auxin levels a higher cytokinin concentration was needed.

Roots could be induced on shoots taken from multiplication media containing Kin or Zeatin but not BAP. Curir et al (1990) reported for *E.gunnii* Hook.

Season	Time of Treatment (M) per minute	Viaability (%)	Contaminate (%)	Necrosis (%)
<b>Spring</b>	1	76	24	0
Summer	6	73	18	9
Automn	5	26	60	14
Winter	15	12	62	26

M= HgCl<sub>2</sub> 0.1 %

Table 1 : Strelization of treatments in *Eucalyptus globules*

season	Time of Treatment (M) per minute	Viaability (%)	Contaminate (%)	Necrosis (%)
Spring	3	78	13	9
<b>Summer</b>	7	82	11	5
Automn	9	27	71	20
Winter	30	22	39	39

M= HgCl<sub>2</sub> 0.1 %

Table 2: Strelization of treatments in *Eucalyptus grandis*

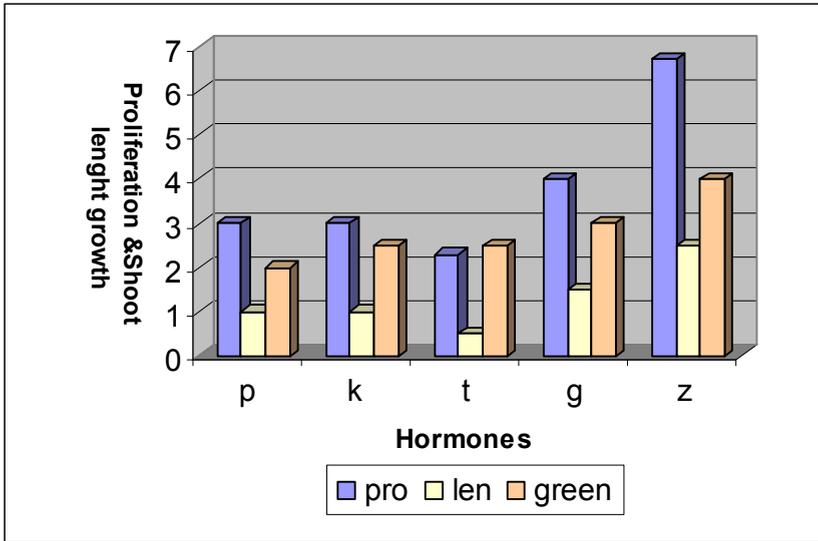


Fig1 : Effect of Hormones on Prpliferation & Shoot length growth of *E.grandis*

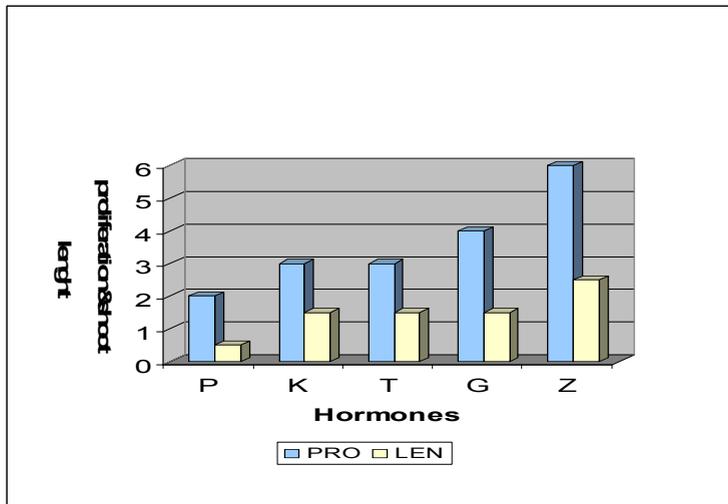


Fig2 : Effect of Hormones on Prpliferation & Shoot length growth of *E.globulus*

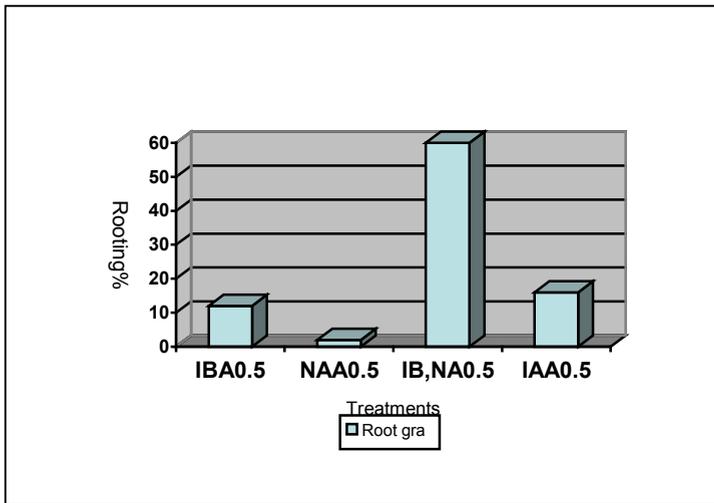


Fig 3: Rooting of *E.grandis* in different treatments

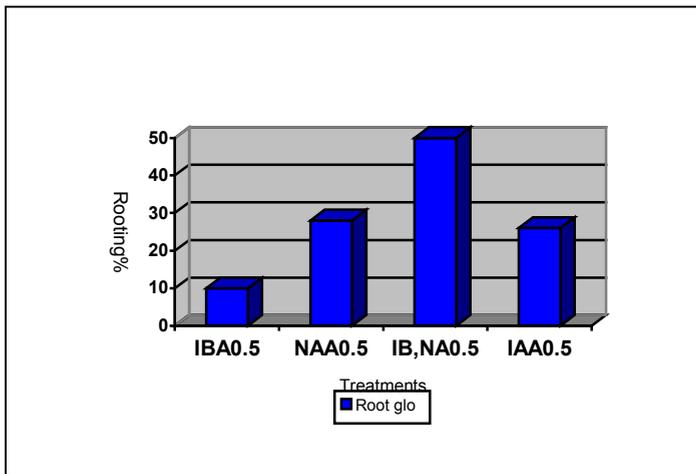


Fig 4: Rooting of *E.globulus* in different treatments



**Culture of buds**



**Rooting stage**



**Shooting Phase**



**Plants in field**



**Proflerated stage**

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# **Insects associated with forest communities and poplar plantations in Iran**

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## Abstract

Based on outcome of several research projects conducted in the forests communities in five ecological zones of Iran, since 1993, vast number of important pests, a number of potentially dangerous insects (at the moment are responsible for few damage) and the others which expect to cause problems in the future as well as beneficial insects such as parasitoids, predators, pollinators and sarcophagus insects were collected and maintained in Museum of the Research institute of forests and rangelands of the country (RIFR). Conventional collecting methods applied e. g. sweep net, hand picking, aspirator, pitfall trap and collections were made weekly during the spring and summer. Specimens were collected and sealed in plastic bags and returned to the lab, pinned or preserved in alcohol. Immature stages were retained until adult emerge. So far twenty five thousand specimens representing 4031 species and 18 orders have been recorded of which, 600 species are identified. Three categories of insects were distinguished: Key pest, predator or parasite of the key pest and occasional visitor.

Important forest pests in northern Iran include: *Lymantria dispar*, *Galerucella lineola*, *Operophtora brumeata*, *Ennemus* sp. *Cryptococcus fagi*, *Tortrix viridana* and *Hyphantria cunea*. Key pests in western oak forest include *Leucoma wiltshieri*, *Porthesia melania*, *Laspyrecia fagiglandana* and *Balaninus glandium*. The main pests in Arasbaran (East Azarbaijan province), Fandoghloo and Andabil (Ardabil province), Tarom Olya (Gazvine) and Tarom Sofla oak forests (Zanjan), are *Euproctis chrysorrhoea*, *Laspyrecia fagiglandana* and *Balaninus glandium*. The study related to insects association with forests of Iran can be categorized in two groups: First, Insects collected based on Taxonomic classification, second, we have been studied insect fauna based on specific plant cover tree species." Oak gall wasps of Iran and their associated parasitoids and inquilines project is an example for the first and " Poplar Integrated pest Management (PIPM) project for second group.

In the period 2000 to 2008 a joint research project titled "Studying oak gall wasps of Iran" were carried out between RIFR of Iran and Edinburgh university of Scotland and Systematic parasitoid laboratory of Hungary. In this project, about 83 wasps species of Cynipidae family were collected and scientifically determined as gall makers on different oak species (*Quercus infectoria*, *Q. brantii*, *Q. libanii*, *Q. castaneifolia*, *Q. macranthera* and *Q. petraea*). Of collected and identified gall wasps, 20 species are new for world science, 10 species are under description and 20 species are reported for the first time from the country. The names of identified gall wasps are mentioned in this paper. Meanwhile other wasps associated with oak galls include parasitoid wasps of gall makers and symbiotic wasps with gall maker (Inquilines) are presented here.

About 200 species belonging to Arthropod's phylum (insect and Acari) were collected on different poplar species and clones in different ecological zones

of the country. Key pests of poplar including; phytophagous pests, sucking and xylophagous pests are discussed in this paper.

**Key words:** Key pest, predators, parasitoids poplar, gall, Gall makers.

## Introduction

The total forest area of Iran was estimated approximately 18 million hectares about three decades ago. Unfortunately, a tremendous proportion of those forests have been destroyed. The main factors in this destructive trend have been shifting cultivation and heavy overgrazing. Along the southern coastline of the Caspian Sea temperate to humid deciduous forests thrive. Drought-adapted woodlands cover the Zagros Mountains in the west and ranges of northern Khorassan. Thorn-cushion formations are dominant in the sub alpine zones of these mountains. Drier parts of the country are home to dwarf-scrub formation with many halophytic communities. On the coastline-of the Persian Gulf, semi-desert shrub lands belonging to the Paleotropic kingdom thrive. With respect to the vegetations association, five different regional types of forest may be distinguished in I. R. of Iran which is sited in table 1.

**Table 1: Different forest types in I. R. Iran.**

Type of Forests	location	Annual	Area	
		Rainfall (mm)	Million ha.	%
Caspian Forests	N.	600-2000	1.9	1.15
Arasbaran Forests	N.W.	400-700	0.2	0.12
Zagros Forests	W.	300-600	3.5	2.12
Central Forests	C.			
Almond and Pistachio Forests	S.W.	100-150	5.5	3.33
Juniper Forests	N.E.			
Subtropical Forests:				
-Mangrove	S.	125	0.5	0.30
-Others	S.	-	0.8	0.48
<b>Total</b>	-	-	<b>12.4</b>	<b>7.5</b>

The total forest and woodland cover of Iran is estimated at 12.4 million ha. Out of this amount 3.8 million ha is forest and the rest is

categorized as woodlands. Most of the commercial forests of the country are located in the north. Recent studies indicated that out of 1.9 million ha of northern forests, only 1.2 million ha can rank as commercial forests.

Reduction of the forest areas of Iran from 18 million hectares in the year 1948 (Saei, 1948) to the present 12.4 million hectares and accelerating demand of the country to wood and wood based products, call for alternative approaches like planting of fast growing trees, as great importance. Forest plantation by fast growing species like different kinds of pine and poplar in suitable climates and areas for their plantation and growth are successful methods in preparing a part of increasing needs of the human to wood.

Poplar planting in Iran is among the oldest traditional agronomy that from the very early years has been developed by the aims of wood production, wind breaker and even ornamental usage. With attention to great usage of poplar products in rural and urban buildings and match production industry of the country and its suitable characteristics such as species variation and adaptability of different poplar species to different climates of Iran, poplar plantation has been gratefully accepted by Iranian farmers and also wood and paper companies and factories.

The key to any IPM program in forestry is the study of forest insect fauna of the country. Thus, principal objectives of the present study were to identify insects associated with Iran's forests and poplar plantation and to assess their relative importance, host preference and distribution.

Each of the different ecological zones and special forest type of Iran present special challenges in terms of forest entomology. Entomological inventories are a fundamental aspect and a basis for better understanding of biological diversity. As conservation of natural ecosystem and bio-diversity is an important mandate for Research Institute of Forests and Rangelands, several national and local research projects were carried out since 1993 to study forest insect fauna of Iran for developing IPM programmes for every forest community.

## **Methods and process**

The survey is based on a number of fixed locations distributed through the country to represent major vegetation zones of Iran, e.g., insects associated with oak forests. Each location (collecting stations) could be visited at least four times during the year to sample the changing fauna in different seasons; obviously this is not always possible. The chosen location, plantation or natural forest, all include good representative of the most important natural forest ecosystems, unmodified or hardly modified by human activity.

To fulfill the study programmes various collection methods are applied over different regions. On each visit collections are made of all insects pests non pests

and beneficial arthropods, associated with specific tree stands, whether they are responsible for obvious damage or not. Further to specimens collected by light trap and pitfall traps, insects resting on the plants were captured directly by hand or with an aspirator or by a sweep net. Where immature stages are gathered, these are taken back to laboratory for rearing. At the same time assessment of damage is made and if possible examples of damaged plant material are removed for inclusion in the collections at research centers in provinces. At centers material is pinned or preserved in alcohol, immature stages are retained until adult emerge. Subsequently species are sorted and preliminary identification of selected arthropods group is performed followed by transferring to head office for further identification.

All material collected after coding and naming are included in the Forest Arthropod Museum housed at Alborz Research Complex in Karaj. The information's is supplemented by observations made in plantations and nurseries in different parts of the country. Special survey made of many plantations distributed through the mountains, foothills and plains allows comparison of the influence of climate and altitude on insect fauna.

Various mechanisms will be employed to identify specific insects including use of taxonomic keys, collaboration of the entomologist colleagues and contribution of national museums inside and outside of the country.

So far a vast number of insect specimens have been collected and are currently maintained at Forest Arthropod Museum housed in Research Institute of Forests and rangelands of Iran.

The museum established in the year 2000 is the principal repository for forest insects in Iran. Significant strengths of the collection include Coleoptera (28%), Lepidoptera (31%). Each collection is presented with its code, Our current listing follow the 4- letter formula for codes of collection depositories. We use the letter abbreviations/ combinations to indicate a particular depository. A comprehensive data bank has established and a web link is under construction.

The program comprises more than 40,000 specimens, with references, geographic distribution and important ecological information. Several hundred original digital images are also available. A searchable database of user-oriented, call up any number of records from among over 50000 specimen's records. Records are chosen based on user- specified criteria such as province or collector name, elevation range, order and family of the species.

To some extent the content of our museum reflects the interests and expertise of the collectors and taxonomists who have been cooperating with us. Heteroptera have benefited from the presence of Dr. Armand Matoque of Paris Museum of Natural History in the year 2003. So far 200 species of 11 families of Heteroptera have been identified. Some are new record for Iranian fauna.

Based on our collaboration the result of our joint research project with Dr.Graham Stone in Edinburgh university of UK and Dr. George Melika of Systematic Parasitoid Laboratory of Hungary, the fauna of oak gall wasps of the

country were determined and result of this joint project has been published in the scientific periodicals (Askew *et al.*, 2006; Azizkhani *et al.*, 2006a, 2006b; Melika *et al.*, 2004; Nazemi-Rafie *et al.*, 2007; Richard *et al.*, 2007; Sadeghi, 2008a; Sadeghi *et al.*, 2006a, 2008, 2009; Stone *et al.*, 2007; Tavakoli *et al.*, 2008; Zargaran *et al.*, 2006a, 2006b)

## **Outcomes and Results**

Each of the different ecological zones and special forest types of Iran present special challenges in terms of forest entomology which account as a fundamental but often under-considered aspect. So far a vast number of insect specimens have been collected and are currently maintained at Forest Arthropod Museum.

Currently our museum contains over 620 drawers of insects. In all, The museum holds 50,000 pinned specimens. We are still in the process of sorting and organizing the collection. Some of obtained results of forests fauna project have been published (Farashiani *et al.*, 2007; Sadeghi *et al.*, 2004; Yarmand 1991, 1997; Yarmand *et al.*, 2006a, 2006b, 2008). Figure 1, shows insect abundance in any order. Table 2 shows number of insect specimens and species in any order present in our museum. Name, major hosts and geographic distributions of some of the most important of forests pests in Iran are present in table 3.

**Table 2: Survey of forest insect fauna of Iran (1994-2003).**  
No. of insects specimens and species for any order (June 2004)

	<b>Order</b>	<b>Species (estimated)</b>	<b>Specimen</b>
1	Lepidoptera	1000	6901
2	Coleoptera	1500	6848
3	Heteroptera	140	2930
4	Hymenoptera	500	2449
5	Diptera	350	1821
6	Homoptera	200	1400
7	Orthoptera	200	2043
8	Neuroptera	40	359
9	Odonata	28	326
10	Isoptera	6	106
11	Mantodea	12	94
12	Dermaptera	7	91
14	Tricoptera	12	41
15	Plecoptera	10	22
16	Mecoptera	5	7
17	Thysanura	4	11
18	Phasmida	2	4
	<b>Sum</b>	<b>4031</b>	<b>25513</b>

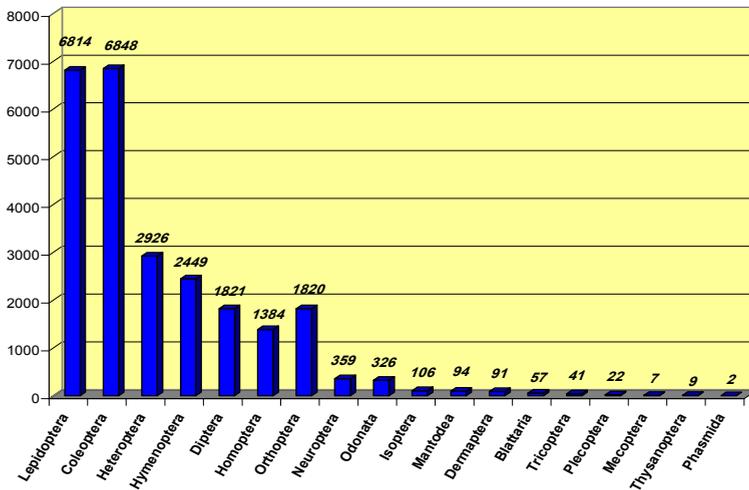


Fig 1: Insect abundance in any order (Year 2004)

Table 3: Primary Insect pest's problems in Iranian forests.

Species	Common name	Major hosts	Current geographic distribution
1- <i>Lymantria dispar</i> L.	Gypsy moth	Many deciduous	Northern, Central and Tehran
2- <i>Cryptococcus fagi</i>	beech scale	and conifers	provinces
3- <i>Phyllaphis fagi</i>	beech aphid	<i>Fagus orientalis</i>	Northern provinces
4- <i>Hyphantria cunea</i> Du.	Fall web-worm	<i>Fagus orientalis</i>	Northern and Central provinces
5- <i>Tortrix viridana</i> L.	Oak bud moth	Many deciduous	Guilan provinces
6- <i>Euproctis chrysorhoea</i>	Brown tail moth	trees	North, South-West and Western
7- <i>Portesia melania</i> Stgr.	Leaf eater moth	<i>Quercus</i>	provinces
8- <i>Leucoma wiltshire</i>	Oak defoliator	<i>Quercus</i>	North- West, Tehran and Central
9- <i>Laspeyresia fagiglandana</i>	Codling moth	<i>Quercus</i>	provinces
10- <i>Balaninus glandium</i>	Oak weevils	<i>Quercus</i>	Western and South- Western
11- <i>Thaumetopoea solitaria</i>	Tent caterpillar	<i>Quercus</i>	Fars, Khozestan, Kohgiluyeh and

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12- <i>Ocneria terebinthina</i>	Pistachio defoliator	<i>Quercus</i>	Lorestan provinces
13- <i>Eurytoma amygdali</i>	species	<i>Pistachia mutica</i>	Western and South- western provinces
14- <i>Megastigmus pistaciae</i> Walker.	Almond seed Pistachio seed	<i>Pistachia mutica</i> <i>Amygdalus sp.</i>	Zagros area
15- <i>Eurytoma plotnikovi</i>	chalcid		Western, Fars and central provinces
		<b>Pistachia vera</b>	Western, South- western and Central provinces
		Wild and edible pistachio	Generally distributed Central Fars, Khorasan and Central provinces

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## 1-Oak

### 1-1- Oak gall wasps Fauna:

Several arthropod species especially in insect (Insecta) and acaroids classes and microorganisms such as plant fungi, bacteria and viruses induce hypertrophy on different plant organs that so-called Galls. In Arthropoda phyla, the plant gall makers mainly belong to Cynipidae, Aphididae, Psyllidae, Cecidomiidae families, Coleoptera order and Eriophidae (Acari) family. Among insect gall makers, the wasps of Cynipini tribe (Hym. Cynipidae), induce gall of diverse shapes on different plant species mainly on oak trees (Fig:2).

This study was carried out during years 2001-2007. Gall wasps fauna were studied on six oak species included; *Q. infectoria*, *Q. brantii*, *Q. libani*, *Q. macranthera*, *Q. petraea*, *Q. castaneifoliae* distributed in Zagross chain mountain, Arasbaran protected forest, Fandoghloo, Andabil, Tarom Olya and Sofla and Caspian forests of Iran. Among 83 gall wasps species collected and identified in this study, 20 species are the new for world science, 10 species are under description and 20 species are reported for the first time from the country. The names of collected and identified gall wasps are as below: *Andricus anthracinus*, *A. quercusradisis*, *A. caliciformis*, *A. conglomeratus*, *A. dentimitratus*, *A. fecundator*, *A. gemmus*, *A. kollari*, *A. lucidus*, *A. quercustozae*, *A. crispator*, *A. tomentosus*, *A. caputmedusae*, *A. sadeghii* sp. Nova, *A. infectorius*, *A. istvan* sp. Nova, *A. coriariformis* sp. Nova, *A. libani* sp. Nova, *A. stellatus* sp. Nova, *A. atkinsonae* sp. Nova, *A. pujadevillari* sp. Nova, *A. schoenroggei*, *A. mayri*, *A. cerrocola*, *A. grossulariae*, *A. megalucidus*, *A. moreae*, *A. ceconii*, *A. curtisii*, *A. megatruncolus* sp. Nova, *A. polycerus*, *A. coriariformis* sp. Nova, *A. sternlichti*, *A. multiplicatus*, *A. stonei* sp. Nova, *A. askewi*, *A. galeatus*, *A. curator*, *A. istavani* sp. Nova, *A. coriarius*, *A. hystrix*, *A. csokai* sp. Nova, *A. schoenroggei* sp. Nova, *A. assarehi* sp. Nova, *A. pseudoaries* sp. Nova, *A. paracoriarius* sp. Nova, *A. chodjaji* sp. Nova, *A. solitarius*, *A. aries*, *A. giraudianus*, *A. theophrastea*, *Biorhiza pallida*, *Cynips quercusfolii*, *C. korsakovi*, *C. loricatus*, *C. quercus*, *C. divisa*, *C. cornifex*, *Dryocosmus tavakolii* sp. Nova, *D. mikoi* sp. Nova, *D. caspiensis* sp. Nova, *D. israelii*, *Neuroterus lanuginosus*, *N. saliens*, *N. glandiformis*, *N. quercusbaccarum*, *N. lenticularis*, *N. numismalis*, *N. albipes*, *Pseudoneuroterus macropterus*, *Synophrus politus*, *Trigonaspis synaspis*.

### 1-2- Parasitoides fauna of gall wasps:

During study period, following parasitoid wasp species were emerged from different gall wasp species in the laboratory. Distributions and associated gall wasp of these parasitoids have been studied (Sadeghi, 2008b). The species reported for the first time from the country are asterisked.

**EURYTOMIDAE:** *Sycophila biguttata* Swederus, 1795, *Sycophila flavicollis* Walker, 1834, *Sycophila iracemae* Nieves, 1983, *Sycophila variegata* Curtis, 1831, *Eurytoma brunniventris* Ratzeburg, 1852, *Eurytoma pistacina* Rondani, 1877.

**TORYMIDAE:** *Megastigmus dorsalis* Fabricius, 1798, *Megastigmus stigmatizans* Fabricius, 1798, *Torymus affinis* Fonscolombe, 1832, *Torymus auratus* Müller, 1764,

*Torymus cyaneus* Walker, 1847, *Torymus erucarum* Schrank, 1781, *Torymus flavipes* Walker, 1833, *Torymus geranii* Walker, 1833.

**ORMYRIDAE:** *Ormyrus nitidulus* Fabricius, 1804, *Ormyrus pomaceus* Geoffroy in Fourcroy, 1785

**PTEROMALIDAE:** *Pteromalus* sp., *Cyrtopyx robustus* Masi, 1907, *Caenacis lauta* Walker, 1835, *Cecidostiba fungosa* Geoffroy in Fourcroy, 1785, *Cecidostiba semifascia* Walker, 1835, *Hobbya stenonota* Ratzeburg, 1848, *Mesopolobus albitarsus* Walker, 1834, *Mesopolobus amaenus* Walker, 1834, *Mesopolobus fasciventris* Westwood, 1833, *Mesopolobus sericeus* Förster, 1770, *Mesopolobus tibialis* Westwood, 1833.

**EUELMIDAE:** *Eupelmus annulatus* Nees, 1834, *Eupelmus cerris* Förster, 1860, *Eupelmus rostratus* Ruschka, 1921, *Eupelmus matranus* Erdős, 1947, *Eupelmus urozonus* Dalman, 1820.

**EULOPHIDAE:** *Aulogymnus arsames* Walker, 1838, *Aulogymnus gallarum* Linnaeus, 1761, *Aulogymnus testaceoviridis* Erdős, 1961, *Aulogymnus trilineatus* Mayr, 1877, *Aulogymnus* sp., *Cirrospilus viticola* Rondani, 1876, *Aprostocetus* sp., *Baryscapus anasillus* Graham, 1991, *Baryscapus berhidanus* Erdős, 1954, *Baryscapus pallidae* Graham, 1991, *Pediobius lysis* Walker, 1839, *Pediobius rotundatus* Fonscolombe, 1832, *Pediobius* sp., *Closterocerus trifasciatus* Westwood, 1833.

Sixteen inquiline wasp species emerged from collected galls belonging to different gall makers species in the laboratory. Among collected inquilines species five species are described for world science and the resting species are reported for the first time from the country. The species that are reported for the first time from the country are asterisked.

### 1-3- Inquilines symbiotic fauna associated with oak gall wasps:

#### Synergini:

*Ceroptres cerris*\* Mayer, *C. clavicornis*\* Hartig, *Saphonecrus haimi*\* Mayer, *S. irani* sp. Nova Melika and Pujade-Villar, *Synergus acsi* sp. Nova Melika and Pujade-Villar, *S. bechtoldae* sp. Nova Melika and Pujade-Villar, *S. gallaepomiformis*\* de Fonsc, *S. mikoï* sp. Nova Melika and Pujade-Villar, *S. palmirae* sp. Nova Melika and Pujade-Villar, *S. pallidipennis*\* Mayrer, *S. pallipes*\* Hartig, *S. thaumacerus*\* Dalman, *S. umbraculus*\* Olivier, *S. variabilis*\* Mayre, *S. reinhardi*\* Mayr, *Synophrus olivieri*\* Kieffer, *S. syriacus*.

## 2-Poplar

### 2-1- Arthropods Fauna of the poplar:

Several research projects have been done about introduction poplar species and clones in different provinces of Iran and their results has been published. Arthropoda fauna of poplars have been collected and reported by different researchers. Afshar was the first person who studied poplar pests of Iran and published the results under the title "Important Pests of Poplar and Salix in Iran" (Shojaee, 1984). After that, Farahbakhsh (1961) has named 104 animals for the poplar fauna which the majority of them are arthropods specially insects. Abaï

and Adeli in 1983 collected and reported 119 animal species mostly insects on poplar trees in different regions of Iran. Rezvani and Termeh (1983) have also reported different species of aphids on poplar trees. In a preliminary research, Babmorad (1993) has introduced 51 species of poplar pests and useful insects in Karaj. In the year 2000 Babmorad *et al.* (2000c) have collected 22 spider species and 2 species of parasitoid mites as predators and parasitoids of poplar pests. In another report (2000b) they mention 9 insects as predators of poplar lace bug. Biology, natural enemies and control methods of some poplar pest species such as *Melasoma populi*, *Gypsonoma aceriana*, *Nycteola asiatica*, *Cerura vinula*, *Steraunematus compressicornis*, *Aeolesthes sarta*, *Monosteira unicastata* and *Melanophila picta* have been studied by a number of researchers in different parts of Iran (Adeli, 1967; Babmorad *et al.*, 2000a; Babmorad and Sadeghi, 2004; Behdad, 1987; Davachi, 1947; Dordaei *et al.*, 2000; Farashiani *et al.*, 2000a, 2000b; Hojjat, 1983; Khial and Sadrayi, 1984; Kiadaliri *et al.*, 2000; Sadeghi *et al.*, 2000, 2006b; Sadeghi and Askary, 2002; Sadeghi and Ebrahimi, 2001; Sadeghi and Tarasi, 2002; Sadrayi, 1994a 1994b; Sadrayi and Khial, 1994; Salehi, 2000; Salehi and Babmorad, 1998).

## 2-2- Key pests of poplar, their distribution and feeding behavior in Iran:

Considering the distribution, some pests are present only in particular areas. For example *Platymycterus marmoratus* is only in Guilan province a serious pest and is not yet reported from other regions of Iran, or root worms of *Melolontha* spp. are in northern provinces of Iran considered as poplar pests, while xylophages like *Melanophila picta* and *Capnodis miliaris* are serious and important pests in dry regions like Tehran, Hamedan, West-azarbajjan, Zanzan and other provinces where poplar is widely planted (other than northern provinces).

A group of poplar related arthropods are generally distributed across Iran but they are just in particular regions considered as poplar pests. For example *Melasoma populi* or *Monosteira unicastata* are collected from most parts of Iran, but are considered as serious in particular provinces like Tehran, Isfahan, Hamedan and Zanzan pests (Sadeghi, 2008a) Important poplar pests and their distribution are provided in the following three groups:

**I- Phytophagous pests:** A number of insect species of the orders Lepidoptera and Coleoptera feed at least in one of their growing stages (larvae or adult insect), on the leaves and leaf buds of different species of poplar. Around 30 species of arthropods with philophage activity has been collected on poplar which some of them are monophage and feed on just one poplar species and some are oligophage and feed on different poplar species and some are polyphag which feed not only on poplar, but also on several other plant species. The most important phytophagous pests of poplar are mentioned in table 4

**II- Sucking pests:** Some species of insects in the orders Homoptera, Hemiptera and Thysanoptera suck the plant sap inside their digestive system by penetrating

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their stylets in leaf tissues or in trunks of the poplar trees and obtain their essential nutrient materials. Some species from *Pemphigus* spp. genus induce galls on the leaves and petioles of some poplar species. are example of this group. The most important sucking pests of poplar are mentioned in table 4.

**III- Xylophagous pests:** Important xylophagous pests of poplar belong to Coleoptera and Lepidoptera orders. The larvae of some of these species like *Polyphilla olivieri* feed on poplar roots and also cause weakness and drain of the host tree. Nymphs of *Cicadatra ochreatea* have been also collected on roots of some poplar species. Xylophagous pests are of the most important pests of poplar in most provinces of Iran. Some of these pests cause severe damage in poplar trunks, so that in some regions of Iran this severe damage has caused unwillingness of the farmers in poplar planting. The best example of these pests is *Melanophila picta*. The most important xylophagous pests of poplar are mentioned in table 4.

**Table 4- Important poplar pests and their distribution in Iran.**

Scientific name	Order/family	distribution	Feeding behavior
<i>Melasoma populi</i> L.	Col.: Chrysomelidae	General distribution in Iran	Philophage
<i>Zeogophora scutellaris</i> Suff.	Col.: Chrysomelidae	Northern provinces and Tehran	Philophage
<i>Gypsonoma aceriana</i> Dap.	Lep.: Tortricidae	Kermanshah, Markazi, Azarbaijan and Northern provinces	Philophage
<i>Lithocoletis populifoliella</i> Tr.	Lep.: Gracilariidae	Northern provinces	Philophage
<i>Nycteola asiatica</i> Krul.	Lep.: Noctuidae	Markazi and Azarbaijan provinces	Philophage
<i>Stilpontia salicis</i> L.	Lep.: Lymantriidae	Markazi and northern provinces	Philophage
<i>Cerura vinula</i> L.	Lep.: Notodontidae	Azarbaijan, Tehran and northern provinces	Philophage
<i>Lymantria dispar</i> L.	Lep.: Lymantriidae	West-Azarbaijan and northern provinces	Philophage
<i>Euproctis chrysorrhoea</i> L.	Lep.: Lymantriidae	Zanjan and Azarbaijan provinces	Philophage
<i>Platymicterus marmoratus</i>	Col.: Curculionidae	northern provinces	Philophage
<i>Adoretus persicus</i> Reiller.	Col.: Scarabaeidae	Azarbaijan, Markazi and northern provinces	Philophage
<i>Stauronematus compressicornis</i> (F.)	Hym.: Tentredinidae	Northern provinces and Tehran	Philophage
<i>Monosteira unicastata</i>	Hem.: Tingidae	General distribution in Iran	Sucking
<i>Pterocomma populeum</i> (Kalt.)	Hom.: Aphididae	Northern provinces	Sucking
<i>Chaitophorus populi</i> L.	Hom.: Pemphigidae	Markazi, Azarbaijan and Guilan provinces	Sucking
<i>Empoasca decedens</i>	Hom.: Cicadellidae	Markazi, western and northern provinces	Sucking
<i>Chionaspis salicis</i> L.	Hom.: Coccidae	General distribution in Iran	Sucking
<i>Phloemyzus passerinii</i>	Hom.: Phloemyzidae	Markazi, Azarbaijan, Hamedan and Zanjan provinces	Sucking
<i>Lepidosaphes ulmi</i> L.	Hom.: Diaspididae	Markazi, Azarbaijan and northern provinces	Sucking
<i>Paranthren tabaniformis</i> Rott.	Lep.: Aegeriidae	Northern and Markazi provinces	Xylophages
<i>Polyphylla olivieri</i>	Col.: Scarabaeidae	Northern provinces	Xylophages
<i>Melalontha</i> spp.	Col.: Scarabaeidae	Northern and Zanjan provinces	Xylophages
<i>Cossus cossus</i> L.	Lep.: Cossidae	Markazi, Azarbaijan, Hamedan, Zanjan, Tehran and Isfahan provinces	Xylophages
<i>Melanophila picta</i>	Col.: Buprestidae	Markazi, Azarbaijan, Hamedan, Zanjan, Tehran and Isfahan provinces	Xylophages
<i>Capnodis miliaris</i>	Col.: Buprestidae	Khorasan, Isfahan, Markazi and Northern provinces	Xylophages
<i>Zeuzera pyrina</i> L.	Lep.: Cossidae	Northern and Markazi provinces	Xylophages

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Fig 2: Some of the new reported species of oak gall wasps from Iran



*Andricus aries*



*Andricus crispator*



*Andricus curator*



*Andricus dentimitratus*



*Cynips cornifex*



*Andricus infectarius*



*Cynips divisa*



*Cynips korsakovi*



*Cynips loricatus*

# **National Park Hainich – Primeval Beech Forests in the Centre of Germany**

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## Abstract

In the western part of the Federal State Thuringia, between the cities Eisenach, Bad Langensalza and Mühlhausen Hainich National Park is located. With about 16.000 ha it is one of the biggest areas of deciduous forests in Germany. About half of that area, situated in the southern part, was declared a National Park in 1998.

Hainich National Park offers unique forest areas with a high biodiversity and old growth forest in combination with a highly attractive cultural surrounding. It was the first National Park to fulfil the special German responsibility in concern of the conservation of natural European Beech Forests.

Since its foundation 10 years ago the National Park proved to offer both, ecological and economical success for the region and the Federal State of Thuringia. Research projects indicate highly interesting effects of unused forests and use innovative methods and concepts. Environment education and touristic development are well accepted by people of every age and altogether Hainich National Park achieves very good reputations.

## Introduction

This first German National Park of Beech Forests covering 7.500 ha already has had a changeful history with a long time of being a Military Training Area. Different States used Hainich Forest in different ways, but mostly military.

In the Second World War tanks, built in Eisenach, were tested in Hainich. After the war Thuringia was made a Soviet Occupation Zone and due to the fact that the Soviets were eagerly looking for a Training Area close to the inter-Germany border, Hainich, which was only some kilometres distant from Western Germany, became a Military Training Area. Until 1990 the Soviet army intensively used the southern part of Hainich, which is called "Kindel". In 1980 about 600 ha of highly productive beech forests were cleared to construct a firing range. Because there are extended unused areas of natural succession, today "Kindel" is one of the most exciting areas in Hainich National Park. There is a big amount of ephemeral small ponds which are of high ecological interest and give room for many special and rare animals and plants. Still one of the big problems in that area is the contamination with Soviet munitions.

In contrary the northern part of today's National Park was used by the National People's Army of the German Democratic Republic also as a Military Training Area. The village "Ihlefeld", which was situated in midst of Hainich, was evacuated and in the North-Eastern part many firing ranges were established.

Before takeover by the Militaries, the forests were mainly used extensively. Single tree logging, coppice-with-standards and regular "Plenter" forest management

dominated the utilisation and already provided a high horizontal and vertical structure in the forests. Number of non-native conifers was very low, whereas number of species of deciduous trees even reached 11 species/ha in some places, which is an enormous amount for typical Middle-European deciduous forests.



**Figure 1: Hainich forest stand close to primeval forests**

Due to the general lack of usage on large parts of the forest during the military activities, for about 40 years near-natural forest could develop, partly with primeval forest character, which is in that extension unique in Germany.

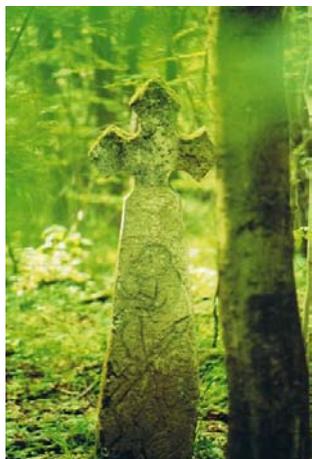
After the Reunion of the two German States the Soviets left the area and the German Armed Forces took over the management of the forests. This allowed experts of forest and conservation to investigate the former inaccessible area and they quickly realised that in the background of the Cold War a beech forest stand unique for Germany could have been growing. Naturally beech forests would cover about 80 percent of Germany's area, but actually only 4, 8 percent of the area represent that plant community. Consequently Germany has a special responsibility in the conservation of beech forests.

In the early 90's a discussion about the protection of Hainich forest began, initiated by the realisation of the high structure, the amount of dead-wood, the biodiversity and the continuity of the forest. This resulted in the 1998s integrated protection concept, which allowed traditional extensive usage in the northern part, while declaring the southern parts a National Park.

Since foundation of the National Park many things happened: former firing ranges are captured by succession, a variety of research projects investigate the forest, more than 120 km of hiking trails were developed and by building the "Treetop Pathway" one of the major touristic attractions in Thuringia was created. The high number of yearly visitors, which increased tenfold since foundation, reflects the success of sustainable near-nature tourism.

## History

As far back as in the early Neolithic Age people left their traces in Hainich. Barrows, which were again used during the Bronze Age, bear witness to the existence of settlements 4.000 years ago. There are a lot of medieval Penitence Crosses to be found. Those are reminders of long gone events. The “Rennstieg”, one of today’s hiking trails, is documentary mentioned already in 1448 as a major trading route. High amounts of landmarks picture the changeful history of owners in Hainich forest.



**Figure 2: Medieval Penitence Cross in Hainich National Park**

To prevent the forests from unregulated logging, the first Forest Order regulated harvesting in 1569. In 1786 and 1872 the second and third Forest Order followed and clearly determined harvesting practices in the Hainich. Coppice and coppice-with-standards were common treatments until the 1850s where the first “Plenter”-usage (single tree extraction) was introduced. Regulated foresting ended by 1946 when Soviets entered the area.

## Location, climate and geology

Hainich forest is situated close to the geographical centre of Germany and covers a total area of 16.000 ha, of which 7.500 ha are protected as National Park. About 2.500 ha of Hainich National Park are open grasslands of former firing ranges, about 5.000 ha are covered by forest. About 94 percent of the area belong to the Federal State of Thuringia, about 4 percent belong to local authorities and about 2 percent belong to the German State and private owners.

Altitude is between 225 – 494 m a.s.l. Yearly rainfall differs between 600 – 800 mm, while average temperature is about 7 – 8 °C. Climate is specified as sub-continental to sub-atlantic with as much as 30 – 40 days of fog. Main



**Figure 3: Ammonite fossil**

wind direction is south-west with an average speed of 3,4 m/s.

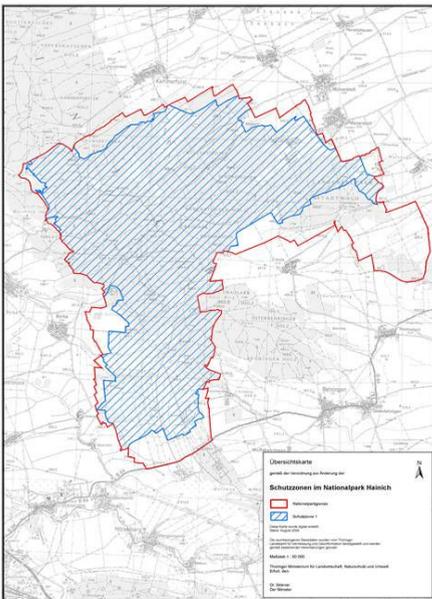
Hainich forest's geologic basis is a Shell-limestone mountain range bordering the Thuringian basin and originating in the Triassic Age. Lower, middle and upper limestone are to be found, while upper limestone dominates the region. Many ammonite fossils demonstrate Triassic live-forms and dolines represent the geologic underground. Landforms were additionally formed during the Pleistocene, where big amounts of loess were accumulated.

Soils differ between several limestone-rendzina-types at higher altitudes and brown earth with accumulations of alluvial clay at lower altitudes and in basins.

## Management and Research

Hainich National Park subdivides in two zones.

Zone 1 is free of usage, according to the IUCN-guidelines. Unused forest area occupied about 1.255 ha in 1997 and was extended to 4.995 ha in 2008. According to this forest areas with utilisation were minimized from 2.982 ha in 1997 to 20 ha in 2008. Additionally unused open grasslands were extended from 1.418 ha in 1997 to 1.828 ha in 2008. In total zone 1 covers 91 percent and thus easily meets the criteria of IUCN for protected areas of category 2. In comparison to other German National Parks Hainich National Park is executing the primary goal of conservation of natural processes quite strictly and consists with about 5.000 ha of Germany's largest forest area free of usage.



**Figure 4: Map of Hainich National Park showing the boundaries (red line) and zone 1 (blue section lining)**

Zone 2 is managed area where the protection state is to be established during the next years. There were several land use forms according to table 1. Some acres,

which remained from times before foundation of the National Park, were reduced to 0 ha, while areas of grassland and pastures are slowly reducing from 1.754 ha in 1997 to 634 ha in 2008 due to maintaining of local extensive sheep-pasturing, but will be reduced to 0 ha in 2015. In a long-term, extensive pasturing should build up a belt around the National Park area and no further pasturing will be found inside the National Park. This will on the long run surely result in a loss of habitats and species, which is diversely discussed in conservationist lobbies, but which is due to the conservation of natural processes in National Parks.

**Table 1: Land use forms in Hainich National Park comparing 1997 and 2008.**  
(\* streets, cart roads, built-over areas)

Usage	Point of time 31.12.1997	Area in %	Point of time 01.07.2008	Area in %
Forest – used	2.982 ha	39,7	20 ha	0,3
Forest – unused	1.255 ha	16,7	4.995 ha	66,2
Open space- unused	1.418 ha	18,9	1.828 ha	24,4
Pasture	1.700 ha	22,6	622 ha	8,2
Grassland	54 ha	0,7	12 ha	0,2
Acre	65 ha	0,9	0 ha	0
Infrastructure*	38 ha	0,5	35 ha	0,4

Research in Hainich National Park is on one hand realised by the National Park Service and additional freelance contractors and on the other hand completed by external research projects of German universities and institutes.

**Table 2 outlines research projects of Hainich National Park Service and additional contractors in 2007.**

Year	Topic
2007	Bird monitoring (resumptions: territories, counting, acquisition of raptors and owls)
	Wood-pecker counting (resumption monitoring)
	Acquisition of fishes at three ponds
	Acquisition of insects at the Treetop-Pathway (resumption)
	Acquisition of wild-bees and other Aculeata (resumption)
	Acquisition of beetles (resumption)
	Acquisition of Diptera (resumption)
	Acquisition of fungi (resumption)
	Photo-documentary (resumption)
	Study: “Economic influence of the National Park”
	Counting of visitors (resumption)

The acquisitions of animals and plants highly increased the knowledge base about the different groups and indicate topics for monitoring in the next years.

Numbers of species steadily increase every year of monitoring.

There are:

- 46 mammal species
- 185 bird species
- 12 Amphibian species
- 11 fish species
- 4.742 Insect species, with:
  - 2.028 beetle species (nearly one thirds of Germany's beetles)
  - 797 butterfly species
  - 1.047 Diptera species
- 1.164 plant species, with:
- 1.620 fungi species

In total there are 7.893 species recognised in Hainich National Park. Some groups are fairly well researched, while others remain at a low level of investigation. Assumptions of numbers of species are predicting about 10.000 animal species, 1.200 plant species and more than 2.000 fungi species. Thus the numbers investigated till now are about two thirds of the assumptions. Acquisition of species will be continued and will probably further enhance the number of investigated species in Hainich National Park.

In 2001 there was a complete acquisition of forest structures based on a 200 x 200 m grid, which will be repeated every ten years. This offers the great opportunity to observe the ongoing dynamic processes in large parts of the forest.

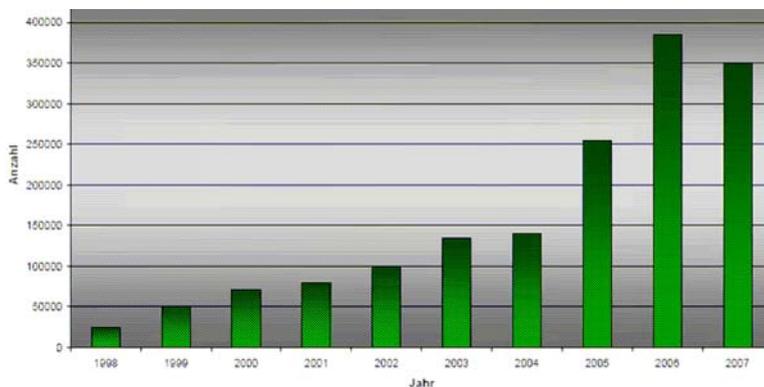
Research of external institutes is outlined in Table 3. These projects resulted in 16 releases in research journals and 9 diploma thesis in 2007. Research reaches from carbonic balances in unused forests up to comparisons of Anthropoid assemblages in concern of age-classes in unused forest stands. To sum up, Hainich National Park offers interesting possibilities especially for research concerning the effects in the development of secondary primeval forest stands and the dynamics in unused forests for Middle Europe. Also there are highly interesting efforts to investigate in animal and plant species-interactions and dynamics.

**Table 3: Research projects of external partners in Hainich National Park in 2008.**

Partners	Topics	Time scale
Albert-Ludwig-University Freiburg Prof. Dr. Huss, Prof. Dr. Bauhus	Long term dynamics of unused beech forests	1999 – ongoing
Georg-August-University Göttingen Prof. Dr. Leuschner	Predictions on dynamics of forests in Hainich National Park, basic ecological research of forests, interspecific concurrence	2000 - ongoing
Max-Planck-Institute of Biogeochemistry Jena Prof. Dr. Schulze	Carbon-balance in unused forest stands	1999 – ongoing
University of Potsdam, Institute of Biochemistry and biology German Research Union Bonn	Biodiversity-Exploratories: assemblage of innovative biodiversity and ecosystem research project in different protected areas	2006 – 2009

## Public Relations and Environment Education

Public relations of the National Park Service result in an ongoing increase of numbers of visitors. This proves for a reliable public concept in Hainich National Park.



**Figure 5: Number of visitors from 1998 to 2007**

The “Treetop-Pathway” (length: 400 m) in the National Park is on one hand the main attraction for most tourists, which indeed is for many nature lovers a pity, but on the other hand offers a great opportunity to realise an effective visitor-guiding and -concentration to relax the touristic pressure in more valuable areas.



**Figure 6: Picture of the “Treetop Pathway”**

Environment education is based on a concept, which should enable the visitor to experience nature with all senses. Hiking trails are partly built barrier-free to offer the possibility to have access to the trails on wheelchairs. There are special programs on several trails, for example school-class nature-guiding and a fairy-tale-tour. All these programs of the National Park Service are offered free of any charge to the visitors.



**Figure 7: Picture of barrier-free nature-experience trail “Brunstal”**

Another main attraction especially for children is the “Environment Education Station”, which arose from a former military garage, and the attached “Wildcat Playground”. The station offers the opportunity to learn about the ecosystem forest and the animals even by rainy weather and the playground is built in an adventurous fashion with creative wood art.



**Figure 8: Pictures of the “Environment Education Station”, left: actual state, right: military garage before restoration**

***In silico* expression analysis of the Arabidopsis  
*KNAT1* gene and its homologs in poplar**

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## Introduction

Plant development requires a tightly controlled balance between undifferentiated dividing cells and cells, which are subjected to undergo differentiation. In the shoot apical meristem, this process is governed by a complex signaling network involving several classes of transcription factors, which are often expressed in highly distinct patterns (Shani et al., 2006). Key-players of differentiation control are the *KNOX* genes (*Knotted-1* like genes; e.g. *KNAT1*, *KNAT2*, *STM*), which comprise a small gene family with eight members in *Arabidopsis thaliana*. *KNOX* genes can be divided into two subclasses, class I and II *KNOX* genes, based on phylogenetic analyses (Scofield and Murray, 2006). A well-characterized member of the class I *KNOX* genes is *SHOOT MERISTEMLESS* (*STM*), which is expressed in the centre of the shoot apical meristem (SAM) but not in the newly formed leaf primordia and in the incipient leaf (Long et al., 1996). Loss-of-function mutations in *STM* lead to premature differentiation of meristematic cells and eventually to cessation of the SAM (Long et al., 1996); but its simultaneous over-expression together with the homeodomain transcription factor *WUSCHEL* induces meristem formation at ectopic places (Lenhard et al., 2002). Taken together these findings indicate that *STM* is a critical regulator of differentiation, whose expression is required to keep cells in an undifferentiated state. The other characterized members of the class I *KNOX* genes fulfill partly redundant functions to *STM* and are generally suggested to be involved in preventing differentiation of the tissue where they are expressed (Scofield and Murray, 2006). In contrast to the class I *KNOX* genes, the members of class II *KNOX* genes are only scarcely described and functional data is mostly lacking.

In the vascular cambium similar decisions as in the SAM have to be taken; an equally tight balance between meristematic cells and cells, which undergo differentiation, is required. Some daughter cells of the cambial meristem differentiate into xylem or phloem, whereas others stay undifferentiated and maintain the pool of meristematic cells. In the model tree poplar, functional evidence for an involvement of *KNOX* genes in controlling differentiation of cambial daughter cells is still lacking. However, high resolution transcript analyses of the poplar cambium showed several *KNOX* genes with strong cambial expression (Hertzberg et al., 2001; Schrader et al., 2004). Furthermore, the poplar *KNOX* gene *ARBORKNOX1* (*ARK1*), which is a close homolog of the *Arabidopsis* *STM*, was shown to be expressed in the cambium (Groover et al., 2006). Over-expression of *ARK1* leads to an inhibition of differentiation of vascular cells. This is in line with the proposed role for *KNOX* genes of keeping cells undifferentiated. However, it has not been shown that the endogenous *ARK1* function is required for the indeterminate state of cambial cells.

The current understanding of the regulation of differentiation in vascular development was greatly enhanced by the study of *Arabidopsis* mutants in the *KNAT1/BP* (*BREVIPEDICELLUS*) gene (Mele et al., 2005). The *bp* mutants show among various developmental defects an increase in lignification of the cambial daughter cells; whereas over-expression of *KNAT1* leads to a decrease in lignin deposition, which is a hallmark of terminal differentiation. These results show that *KNAT1* is regulating the lignification of pro-cambial derivatives and that it is playing a similar role as a repressor of differentiation processes in the procambium as *STM* in the shoot apical meristem.

Cell walls of woody plants constitute an important resource of fixed carbon, and are the base for a manifold of products as paper and panels. Secondary cell wall formation contributes to a large extent to the biomass of wooden tissues. The major compounds of secondary cell walls are cellulose, hemicelluloses and lignin of which the latter is unwanted for many industrial downstream processes. The wood of poplar trees typically consists of 45 % of cellulose, 25 % hemicelluloses and 20 % of lignin (Timell et al., 1969; McDougall et al., 1993). Upon gravistimulation however, the lignin content is drastically lowered and the S2 and S3 layer are replaced by the so called G-layer (gelatinous-layer), which is characterized by highly crystalline cellulose and greatly reduced lignin content. The resulting wood is called tension wood and can contain up to 20 % more cellulose and correspondingly lower levels of lignin and hemicelluloses (Timell et al., 1969). Especially the lignin content is remarkably reduced in gravistimulated wood; albeit if lignin deposition in secondary cell walls of tension wood is completely absent is still a matter of debate (Joseleau et al., 2004). A very similar syndrome of hypolignification, as observed during tension wood formation, occurs in a *35S::KNAT1* over-expressor (Mele et al., 2005). However, whether *KNAT1* up-regulation is required during tension wood formation is not known. Here, we identified the putative homologs of *KNAT1/BP* in poplar and reanalyzed publicly available microarray data in order to test if differential regulation of *KNAT1* genes can explain the repression of lignin deposition during tension wood formation in poplar.

## Materials and Methods

Amino acid sequence alignments were performed with the help of ClustalW (<http://ch.embnet.org>; Thompson et al., 1994) and phylogenetic trees were drawn with Treeview (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>) and afterwards graphically modified with Adobe Illustrator. Sequences were retrieved from the TAIR ([www.arabidopsis.org](http://www.arabidopsis.org)) or from the JGI ([http://genome.jgi-psf.org/Popotr1\\_1/Popotr1\\_1.home.html](http://genome.jgi-psf.org/Popotr1_1/Popotr1_1.home.html)) websites. For alignments the amino acid sequences were used over their entire length. Opening and ending gap penalties

were set to the value of 10, extending and separation gap penalties to 0.05 and "blocks substitution matrix" (BLOSUM) was used as a scoring matrix. BLAST searches were done on the JGI server ([http://genome.jgi-psf.org/Poptr1\\_1/Poptr1\\_1.home.html](http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html)), with a word size of 3 and BLOSUM62 matrix.

For clustering of the Arabidopsis expression data GENEVESTIGATOR (Zimmermann et al., 2004) was used. The Pearson Correlation was applied to estimate the distance between nodes. Expression data for the poplar *KNOX* genes was obtained from the UPSC BASE (Sjödín et al., 2006) or directly from the respective publications.

## Results and Discussion

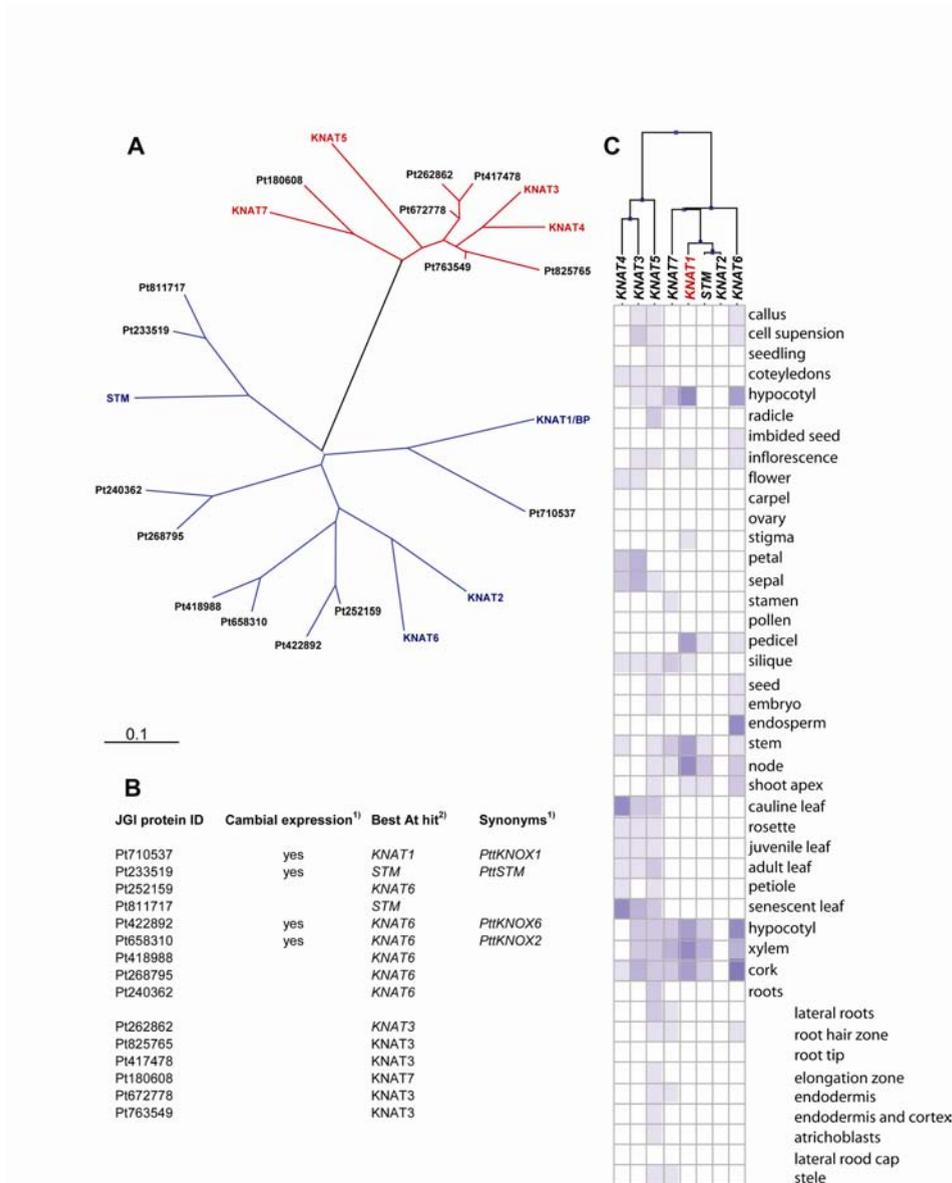
In order to identify sequences homologues to the Arabidopsis *KNAT1* gene we performed a BLAST search with the *KNAT1* amino acid sequence on the entire annotated *Populus trichocarpa* genome (Tuskan et al., 2006). The BLAST search resulted in 15 poplar gene models with an e-value < 10<sup>-15</sup> (data not shown). These sequences and the Arabidopsis *KNOX* sequences were aligned and a phylogenetic tree calculated (Figure 1A.) The topology of the tree showed two different clades separating the class I from the class II *KNOX* genes, with nine poplar gene models classified as class I *KNOX* genes. Interestingly, the clade of *KNAT2/KNAT6* contains six poplar homologs, indicating a recent gene amplification in poplar. In contrast, *KNAT1* only pairs with one poplar gene model; favoring the idea that one of the paralogs got deleted after the recent whole genome duplication (salicolid duplication, Tuskan et al., 2006) in poplar.

Publicly available microarray of Arabidopsis gene expression studies was used to determine which *KNOX* genes are expressed in the developing xylem, a place where lignification takes place (Figure 1B). Strongest expression was found for *KNAT1* and *STM*, whereas *KNAT7* reached half of their expression levels (data not shown). The other *KNOX* genes were hardly expressed in the Arabidopsis xylem. Surprisingly, clustering of the expression data including 3110 arrays grouped *KNAT7* together with the class I *KNOX* genes (Figure 1B), suggesting that its regulation is more similar to class I than class II *KNOX* genes. Interestingly, the *KNAT7* mutant *irregular xylem11 (irx11)* shows collapsed xylem elements in the inflorescence (Brown et al., 2005) indicating that *KNAT7* together with *KNAT1* are required for proper xylem differentiation. If the collapsed xylem elements are due to impaired lignification is however not yet clear.

In order to study the gene expression of *KNOX* genes in poplar we identified nine ESTs (expressed sequence tags) within 17 345 poplar gene models in the UPSC

BASE (Sjödin et al., 2006). The nine ESTs correspond to six different gene models (Figure 2D). We made use of a recent high resolution microarray analysis of the poplar vascular cambium (Schrader et al., 2005) in order to test the expression pattern of these *KNOX* genes. Two different expression clusters could be identified (Figure 2A), genes with high expression in the cambium but low in zone of secondary wall formation (group I) and an inverse complementary expression pattern, representing genes with low cambial expression but high expression in the zone of secondary wall formation (group II). Group I genes constitute of class I *KNOX* genes only, whereas the group II expression cluster contains both class I and II *KNOX* genes. Interestingly, the two groups are also reciprocally expressed in the zone of final cell maturation, where programmed cell death is induced. The finding that group II genes are up-regulated at sites of terminal differentiation is in sharp contrast to what is known of class I *KNOX* genes in several other plant species. Normally, class I *KNOX* genes are highly expressed in undifferentiated dividing cells and have partly redundant function (Scofield and Murray, 2006; Byrne et al., 2002). It will be interesting to see if the group II genes work additively to the group I genes or antagonistically by e.g. competing for the same binding sites on target genes and therefore enhance the effect of down-regulation of group I genes.

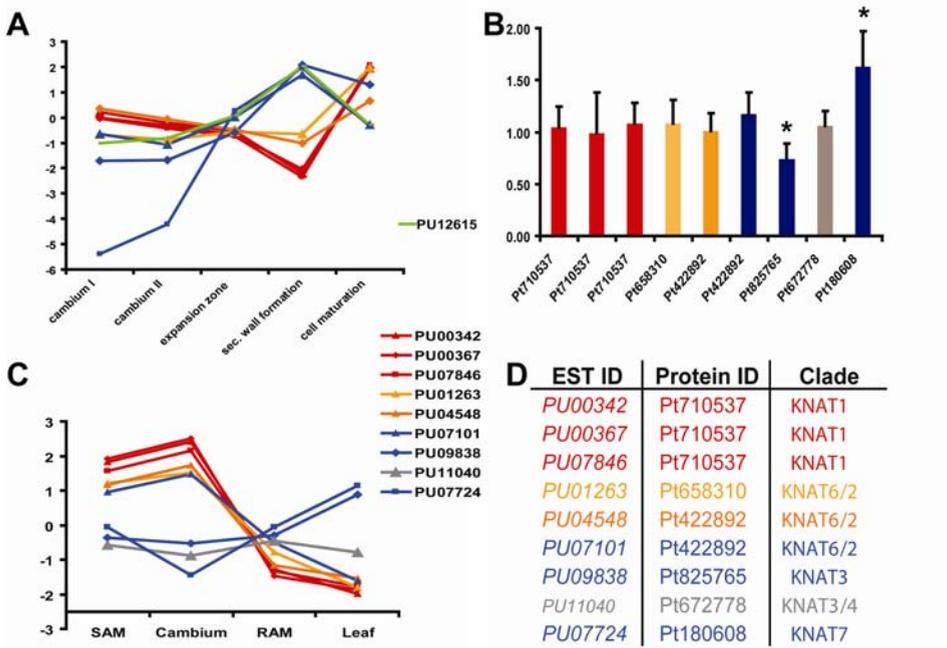
At the site of lateral organ formation, *KNAT1* and *KNAT2* expression is repressed by the MYB domain transcription factor *ASYMMETRIC LEAF1* (*AS1*; Byrne et al., 2002). This mechanism allows cells in the flank of the meristem to differentiate and to give rise to lateral organs. The group I genes behaved similarly than *KNAT1* and *KNAT2* in respect of their down-regulation in the zone of terminal differentiation (Figure 2A). We could identify four different *AS1/2* ESTs in the UPSC BASE of which only *PU12615* gave readable results on the microarrays performed by Schrader et al. (2005). Intriguingly, *PU12615* is reciprocally regulated to the group I genes and therefore a candidate for their repression, suggesting a similar mechanism of transcriptional control as *AS1* performs over *KNAT1* and *KNAT2*.



**Figure 1.** A) Phylogenetic tree of all *KNOX* genes of Arabidopsis and poplar. Class I *KNOX* genes are in blue. Class II *KNOX* genes in red. B) Protein models of the identified poplar *KNOX* homologs. Cambial expression and synonyms (1) according to Scharder et al. (2005). (2) refers to best hit in a BLAST search. C) Clustering of microarray data including more than 3000 microarray slides.

The closest poplar homolog of *KNAT1* is strongly down regulated in the zone of secondary cell wall formation (group I expression pattern); the place where the major lignification takes place. Given the role of *KNAT1* in the Arabidopsis vascular tissue as a repressor of lignification, a similar function can be proposed for the poplar *KNAT1* homolog. In order to test if the expression *KNOX* genes within the group I expression cluster correlates with the repression of lignification in tension wood; we re-analyzed the microarray data published by Andersson-Gunnerås et al. (2006). The authors of this study induced tension wood formation by leaning poplar trees to the side under greenhouse conditions. RNA was extracted from tension wood and compared to un-induced wood on the opposite side of the place of tension wood formation. We analyzed the above identified *KNOX* ESTs on their expression in tension wood. None of the group I genes, which are down-regulated during lignification in cambial derivatives, was differentially expressed in tension wood (Figure 2B). However, two of the group II genes showed regulation. *PU09838* was down-regulated and, more interestingly, *PU07724* showed a significant up-regulation in tension wood (Figure 2B). A role of *PU07724* in repression of lignification is nevertheless unlikely, since under un-induced conditions it shows low cambial expression and strong expression in the zone of secondary cell wall formation, where lignin is deposited (Figure 2B, C).

The initially formulated hypothesis that repression of lignification during tension wood formation could be due to up-regulation of *KNAT1* homologs can be rejected on the basis of the analyzed data. Nonetheless, it should be kept in mind that only about half of the poplar *KNOX* genes could be analyzed in this work. Additional experiments will be required to finally show if default lignification in cambial derivatives and repression of lignin deposition during tension wood formation is co-regulated by the activity of *KNOX* genes. In order to gain a conclusive picture of *KNOX* action on lignification, it will be essential to extend the comparative study of gene expression to functional analyses. Gene knock-down strategies but also over-expression of the *AS1/2* homolog *PU12615*, which we identified as a putative repressor of group I *KNOX* genes, will provide a handle to achieve better understanding of regulatory networks governing lignification.



**Figure 2.** Scales  $\log(2)$ . A) Expression of *KNOX* genes in the vascular cambium and its derivatives, data re-analyzed (Schrader et al., 2005). Genes corresponding to the group I expression cluster are in red. Group II genes in blue. The *AS1/2* homolog *PU12615* behaved like a group II gene and is shown in green. B) *KNOX* gene expression during tension wood formation, data re-analyzed (Andersson-Gunnerås, et al., 2006). Red bars correspond to group I genes, blue bars to group II. C) *KNOX* gene expression in different tissue, data from Schrader et al. (2005). D) Poplar protein models, corresponding EST numbers and phylogenetic clade.

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# **Research in biotechnology of basidiomycetes: mushrooms and enzymes**

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## 1 Abstract

Mushroom-forming Agaricomycetes have important ecological roles i. as saprotrophs in the degradation and recycling of recalcitrant lignocellulosic materials, ii. as symbionts in promoting growth of woody plants through formation of ectomycorrhiza and iii. as pathogens in promoting rejuvenation and biodiversity of ecosystems. In biotechnology, main fields of applications are in mushroom and in enzyme production. Here, we summarize research in these fields as performed by our group. Work for biotechnological applications is supported by basic research on understanding physiology, growth, development and genetics of specific species.

## 2 Introduction

Within the class of basidiomycetes, the mushroom-forming Agaricomycetes with so far about 16.000 described species represent the highest developed organisms in the fungal kingdom (Hibbett and Thorn 2001, Hibbett et al. 2006). In nature, the Agaricomycetes have important ecological roles:

Saprotrophic species degrade dead organic material for their nutrition (Fig. 1A). Often, the organic material is litter that comes from plants such as straw from herbaceous plants and leaves and needles from trees and shrubs. For this, litter-decomposing fungi secrete various types of enzymes that attack the recalcitrant lignocellulosic cell walls of the plant material (see below). Other saprotrophic species are specialized on wood. Thereby, the white-rot fungi attack preferentially lignin and the brown-rot fungi cellulose and hemicellulose (Hoegger et al. 2007). Without these fungal saprotrophs, wood would resist the natural recycling of the



Figure 1: Fruiting bodies of A. the saprotrophic species *Coprinopsis picacea*, B. of a pathogenic *Armillaria* species on the base of a broad-leaf tree and C. of the mycorrhizal species *Amanita muscaria*

organic material, humus would not be generated and there would be no new plant growth. The logical consequence of a break in nutritional recycling through the fungi would ultimately be the complete break-down of the ecosystem (Lonsdale et al. 2008). Some species of the Agaricomycetes are pathogenic to plants (Fig. 1B). In forests of the Northern hemisphere, root rot species of the genera *Heterobasidion* and *Armillaria* are amongst the most feared pathogens (Kharazipour et al. 2007, Vasaitis et al. 2008). Losses through this species can be enormous – worldwide, alone the losses of wood by *Heterobasidion* infections and decay are estimated to be € 7.9 x 10<sup>8</sup> (Asiegbu et al. 2005). However, from the ecological point of view, the pathogenic species have also their importance and, against cursory human feeling, their positive functions: they are beneficial for forest rejuvenation and biodiversity (Winder and Shamoun 2006, Kharazipour et al. 2007).

The third important group of Agaricomycetes exhibit symbiotic functions by forming ectomycorrhiza with woody plants (Martin et al. 2008). The fungi supply required nutrients to their hosts (Müller et al. 2007) whereas the plants support the fungi in yet unknown physiological way in fruiting body formation (Guidot et al. 2001; Fig. 1C).

The fruiting bodies of many species, commonly called mushrooms, are edible (Kües and Liu 2000). Mushrooms have a good nutritional value (Chye et al. 2008) and many mushrooms are said to have medicinal properties (Wasser and Weis 1999, Wasser 2002). Interestingly, amongst the mushrooms used in traditional folk medicine for curing specific illnesses are also toadstool species that can be toxic when consumed by humans (Guzman 2008).

The most expensive mushrooms are from mycorrhizal species, for examples matsutake (*Tricholoma matsutake*), king bolete (*Boletus edulis*) and chantarelles (*Cantharellus cibarius*) (Yun et al. 1997, Giovannetti et al. 1998, Alexander et al. 2002). The high prices base in the unpredictable yearly yields which are influenced by the prevailing climatic conditions and by the dependence of the fungi in fruiting on trees (Pilz and Molina 2002).

In contrast, for a number of saprotrophic species it is possible to produce fruiting bodies in culture. Since the 1980s, much progress has been made in culturing techniques allowing on the one hand high reliable production rates of culinary mushrooms throughout the year and on the other hand to continually broaden the range of the species offered to the consumers. Worldwide, from year to year the mushroom production rates are still rising. Cheap agricultural and forestal organic wastes are used as substrates for mushroom cultivation which therefore can provide a good income to mushroom farmers (Rühl and Kües 2007). After production, spent mushroom substrate (SMS) has still further value – it might for example be used as a peat substitute in gardening (Kharazipour et al. 2007) or in the production of ligninolytic enzymes as recently demonstrated for SMS left from *Pleurotus ostreatus* cultivation (Rühl et al. 2008).

One species cultured and eaten in tropical countries (Fig. 2) is *Coprinopsis cinerea* (Kües et al. 2007b). The saprotrophic *C. cinerea* naturally grows on horsedung. The species has been cultured already in the 19<sup>th</sup> century in the laboratory (Fig. 3) and serves for more than hundred years as the model species for studies in physiology, growth, development and genetics of the basidiomycetes (Kües 2000). With understanding the basics of the life-form of this species, the accumulating knowledge is transferred to other species that are less approachable by experimental research, e.g. mycorrhizal species (Kües et al. 2007b). This strategy is supported by recent sequencing programmes of fungal genomes which allow comparisons between gene functions of closely related fungi (Martin et al. 2008).



**Figure 2:** A jar with pickled young mushroom of *Coprinopsis cinerea* as sold as a speciality in Thai supermarkets after growing the fungus on agricultural wastes, harvesting and preserving them by local farmers (pictures taken from Kües et al. 2007b).



**Figure 3:** A fruiting body of *Coprinopsis cinerea* grown in the laboratory on an artificial agar medium.

### **3 The reproductive system of heterothallic Agaricomycetes: multiple mating types control formation of the fertile dikaryotic mycelium and subsequent fruiting body development**

Reproduction in higher eukaryotes is usually connected to two sexes, i.e. to the occurrence of male and female (Hurst and Hamilton 1992). Male and female distinguish morphologically by the development of specific reproductive organs as well as often by secondary features (for examples see Fig. 4). Male and female are common in the animal kingdom. In the plant kingdom, the female ovary and the male stamina producing the pollen are found together in the same flower or separated in two different flowers on the same or on different plants (Hurst and

Hamilton 1992, Hiscock and Kües 1999). In the fungal kingdom, separation into male and female in contrast is not standard. Nevertheless of lack of sexual morphological differentiation, fungal individuals distinguish from each other separating them into groups of sexually incompatible and of sexually compatible strains (Hiscock and Kües 1999).

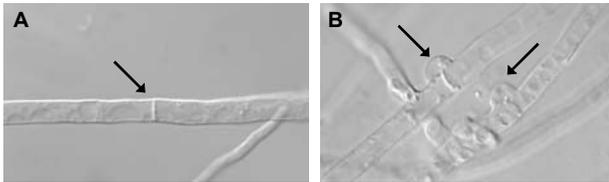


**Figure 4: Already at a young age, boys and girls are easy to distinguish: Bernd (4 years) and Uli (3 years) sitting at the flanks of the group of German children are clearly boys. Mia (3 years) and Ursel (1 year, 8 months) are girls and this may not only be clear to the observer by the dresses they wear. More difficult to guess is the baby (9 months) in the middle of the photos: it is a boy named Heinz. Bernd, Mia, Ursel and Heinz are siblings and Uli is their cousin.**

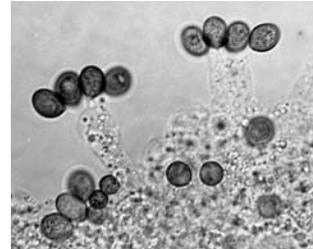
The fungal colonies germinating from spores of Agaricomycetes (primary mycelia called monokaryons) grow in form of undifferentiated cellular filaments (hyphae, Fig. 5A). However, hyphae of different colonies can fuse and, in certain cases when they are sexually compatible, a reaction follows that changes the hyphal morphology (Fig. 5 B). After such compatible fusion, i.e. mating, hyphal cells of the arising secondary mycelium (dikaryon) have two haploid nuclei (one from each parent colony) and specialized clamp cells are formed at the hyphal septa. Most importantly, fruiting bodies can develop at the fertile dikaryon but not at the sterile monokaryons. Within the fruiting bodies, the basidiospores are formed by karyogamy and meiosis as a mean of sexual reproduction by the fungi (Kües et al. 2007b, Rühl and Kües 2007; Fig. 6).

Species that for sexual reproduction do form dikaryons by fusion of compatible monokaryons are called heterothallic. Compatibility between fungal strains, respectively incompatibility between strains is physiologically determined. The given physiological state of a monokaryon is defined a mating type. Only if two monokaryons are different in their mating types, they can successfully mate to form a dikaryon. Most of the Agaricomycetes are heterothallic. Heterothallic species do however not have only two mating types which would be analogous to the situation male and female in other groups of eukaryotes. Instead, Agaricomycetes developed in their populations multiple mating types allowing

manifold different compatible mating reactions. For example, for *C. cinerea* it is estimated that more than 12.000 different mating types exist in nature (Casselton and Kües 2007).



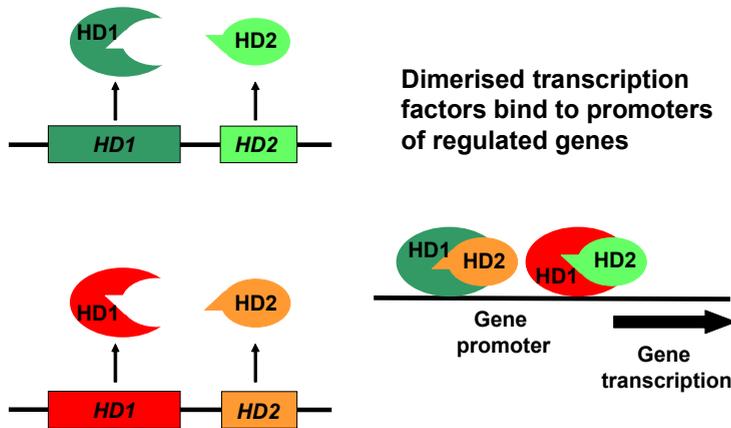
**Figure 5:** A. A hypha from a monokaryon of *Coprinopsis cinerea* with a simple septum (perforated cross cell wall between two hyphal segments; see arrow) and B. hyphae from a dikaryon having clamp cells at the hyphal septa (see arrows).



**Figure 6:** Basidia with four meiotic basidiospores formed in a fruiting body of *Coprinopsis cinerea* (photo from Kües 2000).

How are these multiple mating types achieved? Gene cloning and DNA analysis unravelled that in the Agaricomycetes there are two principle mechanisms that control sexual compatibility:

One set of genes known as the *A* mating type genes encode two types of transcription factors (called HD1 and HD2), i.e. proteins that regulate expression of dikaryon-specific sets of genes within the fungal nuclei by binding to the promoters of the genes. In order to do so, transcription factors of the two different types have to aggregate with each other to form functional transcription factor dimers. Every strain has genes for the two types of transcription factors but these cannot interact with each other to dimerise unlike proteins that come from nuclei of different mating type. Transcription factors from own and foreign origin are distinguished from each other by differences in the amino acid sequence. This makes possible that they function in form of a lock-and-key principle: compatible combinations of transcription factors from different origin (foreign, non-self) match with each other like a key in its fitting lock whilst incompatible combinations (innate, self) do misfit like a key does not function in a lock when having the wrong teeth (Casselton and Kües 2007; Fig. 7). It has to be noted in the *A* mating type system that the rejection between the two types of transcription factors from incompatible, non-fitting self-combinations (same *A* mating type specificity) is the most specific reaction. All non-self combinations (different *A* mating type specificities) are functional and lead to a positive reaction. Transferred to the lock-and-key principle, this would mean that any given key will not function in one specific lock but in all others. In total, about 160 different *A* mating type specificities are believed to exist in *C. cinerea* in nature (Casselton and Kües 2007).



**Figure 7:** Each *A* mating type locus has genes (shown as boxes in green, respectively in red colours) that encode either HD1 or HD2 transcription factors (marked as oval symbols). HD1 and HD2 proteins from different *A* loci (indicated by green and red colours) distinguish in amino acid sequence and thus in their folded structure. HD1 and HD2 proteins from different *A* mating type loci match with each other and form protein dimers that bind to promoters of regulated genes. Binding of the dimers to the promoters controls gene transcription.

Another set of genes called the *B* mating type genes encode pheromones and pheromone receptors. Pheromones need to bind to pheromone receptors in order to start within the hyphal cells a signalling cascade that controls expression of dikaryon-specific functions. As the *A* mating type system, also the *B* mating type system functions according to the lock-and-key principle: Pheromones and pheromone receptors encoded in the same *B* locus do not interact with each other but pheromones and pheromone receptors encoded in different *B* loci. Again, this bases on differences in amino acid sequences: Pheromones from different *B* mating type loci distinguish in amino acid sequence as well as pheromone receptors from different *B* mating type loci (Casselton and Kües 2007; Fig. 8).

In *C. cinerea*, there are an estimated total of 80 different *B* mating type specificities in nature. Multiplying the estimated 160 *A* and the estimated 80 *B* mating type specificities with each gives the high number of 12.800 mating types believed to exist in nature. Only when two monokaryons are different at both the *A* and the *B* mating type locus, they are sexually compatible and can thus form a dikaryon (Casselton and Kües 2007). Dikaryon formation can be crucial for the life style of the fungi. Mycelia of the Agaricomycetes isolated from nature are normally dikaryons and detection of monokaryons is rather seldom. This is, because in the dikaryon with two sets of genes (one per parental monokaryon) there is a more stable genetic situation that may help to better react on changing environmental

conditions. In ectomycorrhizal species such as *Laccaria bicolor*, the dikaryon plays an important role in the formation of mycorrhiza. Only the dikaryon is well able to establish mycorrhiza with roots of trees (Niculita-Hirzel et al. 2008).

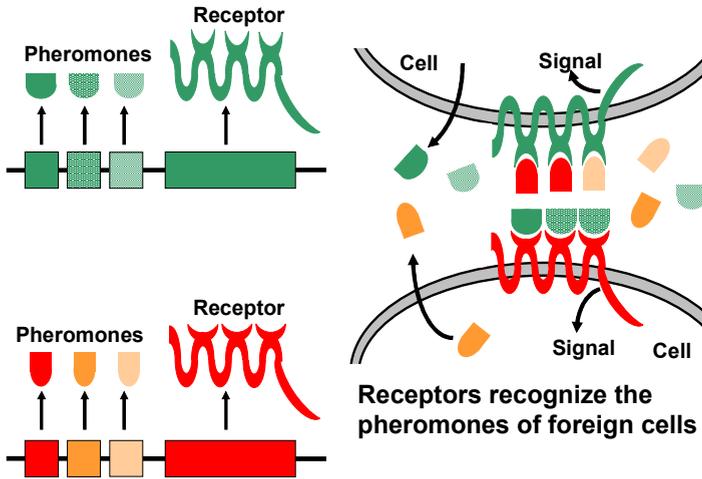
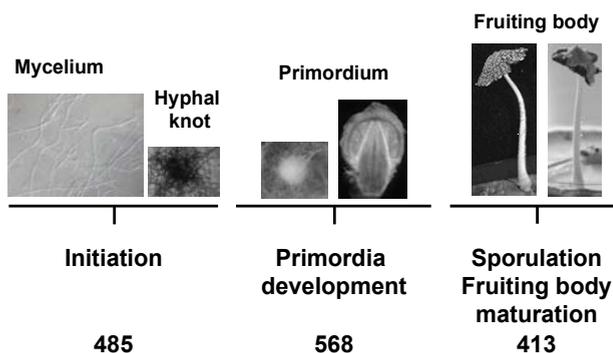


Figure 8: Each *B* mating type locus has genes (shown as boxes in green, respectively in red colours) that encode either pheromones (marked as semi-oval symbols) or pheromone receptors (marked by the sinuous lines). Pheromone receptors are believed to integrate into the cell membrane in order to interact with pheromones from another *B* mating type locus (indicated by green and red colours). Pheromone binding to a pheromone receptor initiates an intracellular signalling cascade. Specificity of the system is verified via different amino acid sequences (indicated by green and red colours).

As shown in *C. cinerea*, the *A* and *B* mating type genes are however not only responsible for dikaryon formation and maintenance but they are also the master regulators of fruiting body development. Compatible *A* mating type products are required to initiate fruiting body development and compatible *B* mating type products are required for fruiting body maturation at the stage of karyogamy, meiosis and spore formation. Light is an important external signal for fruiting body development and the *B* mating type products help to synchronize external and internal signals for proper fruiting body formation (Kües et al. 1998, 2002).

## 4 Production of mutants in fruiting body development of *Coprinopsis cinerea*

Since the *A* and the *B* mating type loci control in the dikaryon the fruiting body development, it is not surprising to find that specific mutations in the *A* and the *B* mating type loci can render a monokaryon into a self-compatible strain that develops fruiting bodies without prior mating to another monokaryon (Boulianne et al. 2000; Fig. 9). Since unlike the dikaryons such self-compatible strains have only one type of nuclei such mating type mutants are ideal strains for induction of mutations in the fruiting body pathway. Mutants in fruiting can easily be found by screening after mutagenesis. In such way, we generated a large collection of fruiting body mutants with defects in initiation of fruiting body development, in formation of fruiting body primordia and in fruiting body maturation including sporulation (Kües et al. unpublished; Fig. 9) and these can be used to clone and characterise genes acting in fruiting. Using one of the initiation mutants, recently a gene for an unusual fatty acid synthase (a cyclopropane fatty acid synthase) has been found to be required for initiation of fruiting body development. In bacteria this enzyme is known to alter properties of cell membranes (Liu et al. 2006). These results coincide well with the fact that addition of certain surfactants rendering membrane properties can positively trigger fruiting body initiation in various Agaricomycetes including *C. cinerea* and *P. ostreatus* (Kües and Liu 2000, Berne et al. 2008). From this, we may learn how in commercial cultivation more mushrooms in faster time can be produced, for example by applying non-toxic surfactants. Analysing the various mutants with defects in fruiting body development (Fig. 9) over the time will give us much insight as how fruiting body development functions and this should help us to further improve culture



**Figure 9: Mutants in fruiting body development of a self-compatible *Coprinopsis cinerea* strain.** About 10.000 clones were screened and the numbers of mutants obtained are indicated at the bottom of the figure. The high numbers indicate that fruiting body development is a very complex process in which many different genes will play a role (Kües et al. unpublished results).

conditions and to introduce in the future more species, possibly even mycorrhizal species, into commercial mushroom cultivation. As stated already above, comparisons between the sequenced genomes e.g. of *C. cinerea* and *L. bicolor* (Martin et al. 2008) and their expressed genes during the fruiting periods (Deveau et al. 2008) will help in optimizing established processes and possibly also in initiating new commercial mushroom productions.

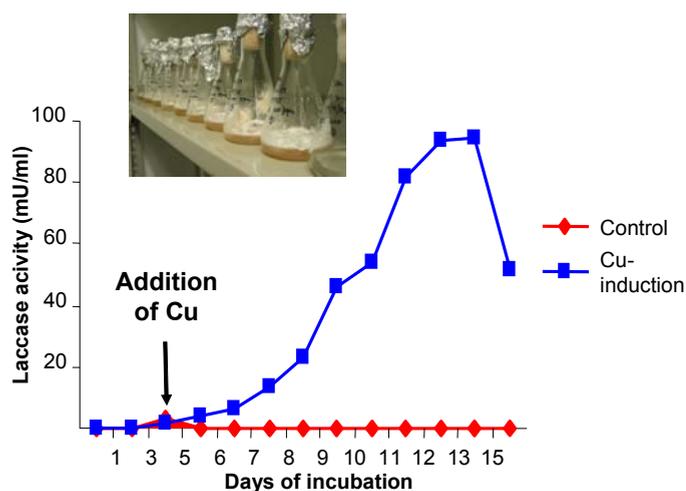
## 5 Enzyme production by *Coprinopsis cinerea* and other Agaricomycetes

Wood-rotting and saprotrophic Agaricomycetes are of high interest as sources of different types of high quality enzymes to be used in various kinds of industrial processes. One group of biotechnologically interesting enzymes are represented by redox-enzymes that attack phenolic compounds including lignin. Particularly white-rot fungi produce such enzymes for degrading lignified plant cell walls. Such lignin-degrading enzymes may for example be applied in the production of wood composites (Kües et al. 2007a; Fig. 10). Two major groups of phenoloxidases are known from fungi: laccases and peroxidases (Hoegger et al. 2007). For application in wood composite production, laccases are more suitable by the fact that they do not need for function the addition of dangerous peroxide unlike the peroxidases (Kües et al. 2007a).



Figure 10: Wood of low quality might be used to produce particles or fibres that subsequently might be pressed into particle boards (shown at the left) or MDF (medium dense fibreboards shown at the right). Kharazipour and colleagues developed processes in which fungal laccases from cultural supernatants (the brown liquid seen in the flask) are applied in glueing particles or fibres in order to replace chemical binders made from non-sustainable crude oil (further information in Kües et al. 2007a).

*P. ostreatus* produces in solid state fermentation as well as in submerged fermentation both types of enzymes, laccases and peroxidases, at reasonable yields (Rühl et al. 2007, 2008). In contrast, *C. cinerea* can naturally produce laccase but this is usually at quite low levels even if an inducer such as copper is added (Fig. 11). In industry, strains of the white-rot *Trametes versicolor* are used for laccase production but there is also an interest in recombinant production through gene cloning and protein expression in heterologous hosts. Gene cloning and heterologous protein expression gain special attention when the protein of interest comes from a fungus of which no fermentation system has been established (Kilaru 2006, Rühl et al. 2007) and there are many different fungi possessing very interesting laccases (Hoegger et al. 2006). Attempts in the past of recombinant laccase production made use of ascomycetes fungi such as the baker's yeast *Saccharomyces cerevisiae* or the filamentous fungus *Aspergillus nidulans*. However, laccases from Agaricomycetes tend to become modified in the heterologous hosts by attaching in an inappropriate way sugar-residues to the proteins. Such wrong glycosylation unfortunately affects negatively the yields, stability and enzymatic characteristics of the laccases (Kilaru 2006, Rühl et al. 2007).



**Figure 11: Laccase production by a strain of *Coprinopsis cinerea* in standing submerged culture with (red line) and without (blue line) addition of copper to the cultures (data are from Rühl et al. 2007).**

To overcome the problem given by recombinant expression in ascomycetes, it was apparent to test heterologous expression within species of the Agaricomycetes (Kilaru 2006, Rühl et al. 2007). We chose the easy to transform *C. cinerea* and developed a system of efficient expression of laccase genes under control of a constitutive highly active promoter from the edible species *Agaricus bisporus* (*gpdII*

promoter). Laccase production by positive transformants can easily be detected by converting the colourless substance ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] into a stained compound when it is added to the growth medium of the fungi (Kilaru et al. 2006b,c; Fig. 12). First expression attempts of laccase genes in *C. cinerea* were done with laccase sequences originating from the fungus – *C. cinerea* has in total 17 different laccase genes (Hoegger et al. 2004, Kilaru 2006, Kilaru et al. 2006a). Current work in the laboratory deals with expression of laccase genes coming from other species and with identification of new enzymes by modern genomics and proteomics techniques (Hoegger et al. 2007). Other lines of research target at optimizing fermentation processes of *C. cinerea* in submerged culture and increasing the yields of enzymes in recombinant production.

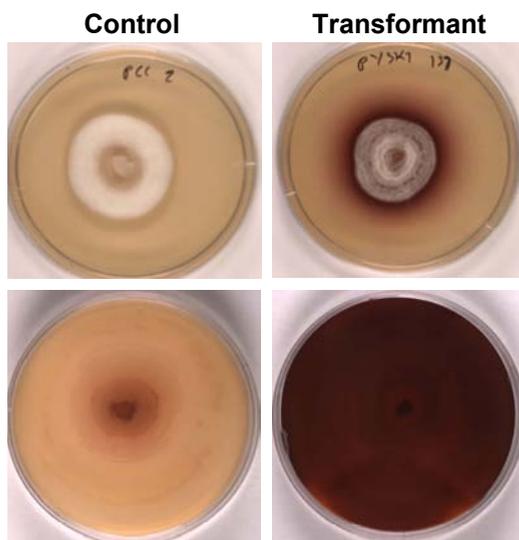


Figure 12: Clones of a *Coprinopsis cinerea* monokaryon after transformation with a control DNA without extra laccase gene (left panel) and with a laccase gene under control of the *Agaricus bisporus gpdII* promoter (right panel). Already the young colony of the transformant with the introduced laccase gene (photographed from top, upper right) secretes high amounts of enzyme which stains by conversion of ABTS the agar brown unlike the control transformant having no extra laccase gene (photographed from top, upper left). The cultures at older age (photographed from the reverse) are shown below. The agar is fully stained dark-brown only in case of the clone transformed with the laccase gene (further details in Kilaru et al. 2006b,c).

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In the year 2008 the research activities in the project “Deutsch–Arabisch/Iranischer Hochschuldialog” between the Research Institute for Forests and Rangelands, Teheran and the University of Göttingen were successfully continued. The project was financed by the Department of Foreign Affairs in the context of the „Europäisch/Islamischen Kulturdialog“ (EIK) supervised by the German Academic Exchange Service (DAAD). Now, like in the two years before, the present book gives again an overview of the common research themes in which the Iranian and German young scientists and senior scientists of both countries works are summarised. The themes cover a wide variety in the research fields of forestry, mircobiology, forest zoology, wood science and wood biotechnology.



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