Studies on physiological parameters for drought tolerance in beech (*Fagus sylvatica L.*) and Poplar (*Populus canescens*)

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Affectionately Dedicated
to
My Revered Father
Late Sh. Didar Singh

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Hisar ***(RAJENDER SINGH BENIWAL)***

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CERTIFICATE-I

This is to certify that this thesis entitled, **"Studies on physiological parameters for drought tolerance in beech (*Fagus sylvatica L.*) and Poplar (*Populus canescens*)",** submitted for the degree of **Doctor of Philosophy** in the subject of **Agroforestry** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Rajender Singh Beniwal** under my supervision and that no part of this thesis has been submitted for any other degree.

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CERTIFICATE-II

This is to certify that this dissertation entitled, **"Studies on physiological parameters for drought tolerance in beech (*Fagus sylvatica L.*) and Poplar (*Populus canescens*)"**, submitted by **Rajender Singh Beniwal** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar in partial fulfillment for the degree of **Doctor of Philosophy** in the subject of **Agroforestry**, has been approved by the student's Advisory Committee after an oral examination with the External Examiner on the same.

MAJOR ADVISOR **EXTERNAL EXAMINER**

HEAD OF THE DEPARTMENT

DEAN, POST-GRADUATE STUDIES

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INTRODUCTION

European beech (*Fagus sylvatica* L.), is a late successional, and the most abundant and dominating tree species of the potential natural vegetation in the central Europe (Ellenberg, 1992). However, in the past, reforestation in Central Europe was mainly performed with conifers. One of the most important aims of the current forest policy is to reverse the mono-cultures of conifers by supporting natural and artificial regeneration of beech and by applying forest management practices that promote growth and wood quality of adult beech trees (Tarp *et al.*, 2000). But there is a problem of losses of beech seedlings establishment, up to 60 % in the field in the first year of plantation. One possible reason for these high losses is probably inadequate treatment of the planting stock during transfer from the nursery to the field site exposing the roots to the air.

Populus canescens (*Ait*.) *Sm.* is a large deciduous tree of height 50-90 ft., grows annually more than 18 inches and has spread of only 10 feet. This species is a spontaneous natural hybrid of *Populus alba x Populus tremula* and prefers light (sandy), medium (loamy), heavy (clay) and well drained soils. It also requires acidic, neutral and basic (alkaline) soils but cannot grow under shade. Members of the genus *Populus* are among the fastest growing trees. Biomass yields of 45 Mg ha^{-1} and heights of more than 3.5 m have been reported for *Populus* hybrids

following four years of growth in intensive plantation culture (Dawson, 1976). They can produce annual yields between 8 and 12 t of biomass dry matter ha^{-1} (Makeschin, 1999). Poplar clones are extensively grown in agro-forestry in Haryana to produce biomass for wood and energy. There is further need to develop parameters to identify most tolerant reproductive material of this species, which thrives best on calcareous soils and degraded sites, which are deficient in water, affecting the nutrient requirement of a tree.

Tree plantation establishment in India must confront a series of biological, climatic and social difficulties. As is often the case in many countries, forest plantation sites are usually located on poor soils in terms of water holding capacity, fertility and/or damage; and also many locations of our tropical region experience frequent drought of varying intensity and duration due to erratic and/or scarce amount of rainfall. Water is one of the major environmental factors limiting growth and productivity. Water stress in plant, affects many important physiological processes, which counts much to the growth and total production of almost all the tree species. For this reason, drought adaptation attributes could become critical for successful poplar establishment and acceptable growth. These problems are not easily resolved. Certainly climatic and soil factors cannot be modified. These factors may, however, be partially compensated by improved seedling quality. Maximizing the physiological quality of the planting stock is the best method, one can think of, to compensate for adverse climate, site or management considerations.

Also the current climatic projections for the Central Europe predict not only increasing air temperatures but side by side a rise in frequency and duration of intensive summer drought (IPCC, 2001). However, the effects of these changing environmental conditions may have adverse affect on the physiological performances, growth and competitive ability of drought sensitive species particularly European beech (Peuke *et al.*, 2002) and poplar; and thus drought is considered to be one of the most important environmental constraints to plant survival and productivity (Gifford and Evans, 1981; Boyer, 1982). Various measures have been suggested for drought management, which are either costly or not feasible to adopt on a large-scale plantation. So in order to harvest the maximum benefits from the limiting resources of water, it is important to know how these stresses affect the plant physiological system, and to find out where ameliorative approaches should be adopted to minimize their deleterious effect.

Planting of bare-root seedlings is a widely used practice of artificially regenerating the forest crops in the Temperate Zone. For successful field establishment, seedlings have to overcome a transplanting shock, which is primarily caused by water stress (Burdett, 1990; Margolis and Brand, 1990; McKay, 1992). This stress is primarily caused by exposure of seedlings to drying ambient conditions, as may occur during the period between lifting in the nursery and planting in the field, exacerbates the effects of transplanting stress. Many studies have shown the harmful effects of root exposure to the air. Air drying conditions may affect plant water status and subsequent survival (Tabbush, 1987) and thus only a few hours of exposure may cause a significant decrease in growth

parameters and survival of the seedlings (Hermann, 1967; Coutts, 1981). Girard *et al.* (1997a) found that 12 day exposure at 8^0C and 60% relative humidity prevented root regeneration and resulted in mortality in 50% of red oak (*Quercus rubra* L.) seedlings. To overcome the planting shock and to regain a physiological status, they must be able to produce new roots (Grossnickle, 1988; Haase and Rose, 1993).

Keeping in mind, the problems of transplanting shock and afforestation on problematic site which are also drought prone; the incorporation of factors which enables plants to withstand drought stress and planting shock, would be helpful in improving the productivity under drought conditions. Inoculation of plant roots with mycorrhizal fungi in combination with hydrogel may be effective in improving growth under drying conditions.

Super absorbent hydrogels, a water retaining polymer (Stockosorb K 400, a highly cross-linked polyacrylamide with about 40% of amide group hydrolysed to carboxylic groups), can absorb a large amount of water (100 to 150 times of its own weight) which is then released slowly to adjacent plant roots and 95% of this absorbed water is available to plants. Its use for agricultural applications (Mohana Raju *et al.*, 2002) and forestry applications (Viero *et al.*, 2002 for *Eucalyptus grandis*, Huettermann *et al.*, 1999 for *Pinus halepensis*, Davies and Castro-Jimenez, 1989 for *Lagerstroemia indica* L. cultivar Natches) has shown encouraging results as it has been observed to increase the water retention of media, reduce irrigation frequency, lower the death rate of plants, improve fertilizer retention in the soil and increase plant growth rate. This property of

hydrogel would be of great use in the production of more biomass of a species in question under the conditions of drought.

In addition, colonization of roots by ecto-mycorrhizal fungi has been shown to improve productivity of various plants in soils under drought stress. Ecto-mycorrhizae are symbiotic association between the roots of higher plants and fungi that frequently provide the host plant with the benefits of improved nutrition, water relations and physiological processes. Seedlings planted on dry sites must possess characteristics that confer tolerance to water stress if they are to survive. Nursery management practices, including inoculation with ecto-mycorrhizal fungi may improve seedling establishment after outplanting. Mycorrhizal colonization is known to improve the absorption of nutrients and water, and can lead to improved growth rates (Bjorkman, 1970; Amaranthus and Perry, 1987). Growths of oak seedlings on two sites in northeastern France are significantly improved following inoculation with ecto-mycorrhiza forming *Paxillus involutus* (Jean and Jean-Louis, 1997). The high nutrient uptake following mycorrhizal colonization can be explained by the increased absorptive surface per unit root weight (Unestam, 1991). So this improved production of mycorrhizal plants was attributed mainly to enhanced uptake of immobile nutrients like phosphorous, zinc and copper and also through improved leaf water potential and maintenance of stomatal opening.

The systematic studies on physiological parameters under drought conditions are scanty in case of trees. By considering all these factors, the two experiments namely '*Drought stress of planting stock of beech and their survival in the field plantation*' and '*Drought and re-watering cycle under controlled*

conditions in Poplar' were carried out to sort out the problems of afforestation in drought prone conditions keeping in view the following objectives:

1. To investigate the effect of drought stress on various physiological parameters.
2. To find out physiological parameters responding to drought stress.
3. Evaluation of field establishment of screened planting stock.

REVIEW OF LITERATURE

The study of plant responses to stress has been a central feature of plant biologist attempts to understand how plants function in their natural and managed environments. In recent years, much progress has been made in understanding how stresses affect plant performance and consequently we consider it timely to examine the eco-physiological and bio-chemical responses to plants to a variety of stresses with a view to identify common principles. In particular, we are interested to assess the drought stress tolerance of a particular species like *Fagus sylvatica* L. and *Populus canescens* with the help of many physiological parameters and whether their use could help in drought avoidance which will ultimately help the seedling to survive in the field.

Effect of drought/air exposure and mycorrhizal and/or hydrogel inoculation on the mycorrhization, survival, growth and development

Almost all aspects of plant growth are affected due to drought (Hsiao,1973), among them the dry matter production is the most important which count much to the total production of any crop. Black spruce families varied in growth rate on drier but not on moisture sites as a result of water stress (Weixing *et al.,* 1995), suggesting early growth traits can be used for early selection. Environmental stress such as drought, however, can cause temporary change in root:shoot ratio that may have important effects on seedling quality and survival (Ritchie, 1984) and overall

productivity (Cregg, 1994). It would be more logical to see if some technique could be devised and adopted for ensuring most effective application and efficient utilization of limited water (Mathur and Mahnot, 2000). Rood and Heinze-Milne (1989) and Rood and Mahoney (1990) reported that a rapid lowering of the riparian water table caused by an abrupt reduction of river flow, lead to drought-induced mortality; particularly for young and very old poplars. Trees respond to limited water supply by altering number and size of leaves, and specific leaf area (Van Splunder *et al.*, 1996; Khalil and Grace, 1992). Moreover, a reduction in leaf area, limits water loss by transpiration and the risk of xylem embolism (Braatne *et al.*, 1992). Hees (1997) reported that drought reduced growth, decreased partitioning of carbon to leaves and increased partitioning to fine root biomass (Tschaplinski *et al.*, 1994), has been described as an adaptation to drought stress in beech and is probably related to the slower development of water stress in xeric populations, when compared with the mesic ones (Tognetti *et al.*, 1995). In beech, drought increased the diameter of fine roots, however the increase partitioning to these roots did not increase the fine root length (Hees, 1997). Garcia and Becerril (2000) reported lower leaf area/fine root ratio in the xeric populations of European beech, and similarly the ratio of leaf to fine root biomass was significantly diminished in the drought stressed seedlings of *Quercus petraea* and *Q. robur* (Frank and Thomas, 2000).

When competition for water is not severe, increases in water use efficiency (WUE) may lead to a higher production, such positive correlations between WUE and productivity have been reported by Zhang *et al.* (1993, 1996). In case of

severe competition for water, optimal strategies of water use are less clear because saved water may be used by other competing plants. Larcher (1995) showed that greater allocation to roots might increase the amount of soil water accessible for a plant.

Fast-growing trees, such as poplar (*Populus* L.), raised in short-rotation intensive cultures, represent an alternative use for agricultural land (Bisoffi and Gullberg, 1996). However, drought episodes may severely reduce poplar productivity and increase sensitivity to various pathogens (Souleres, 1992; Pinon and Valadon, 1997). Significant differences in *Populus davidiana* seedlings in response to progressive drought stress were observed by Zhang *et al.* (2004) in height growth, total biomass, total leaf area, root:shoot ratio and specific leaf area. Zhang *et al.* (2005) also reported that water stress affected dry matter accumulation and allocation more in the dry climate *Populus davidiana* ecotypes than in the wet climate ecotypes.

Whole plant responses to water stress range from stomatal closure to increased root/shoot ratio, leaf area reduction and osmotic adjustment. Several physiological and morphological traits of poplar clones, including stomatal sensitivity to water stress (Liu and Dickmann, 1992; Blake *et al.*, 1996; Harvey and van den Driessche, 1997), sensitivity of leaf expansion, extent of leaf abscission and root/shoot ratio increase (Liu and Dickmann, 1992; Chen *et al.*, 1997; Ibrahim *et al.*, 1997; Tschaplinski *et al.*, 1998) vary widely among clones and this variation is receiving increased attention in breeding programs.

Exposure of seedlings to drying ambient conditions, as may occur during the period between lifting in the nursery and planting in the field, exacerbates the effect of transplanting stress. Symeonidou and Buckley (1999) reported that increased drying period of 6 h, resulted in lowering the vitality of fine roots of *Prunus avium* and *P. cerasifera*, but did not affect their immediate survival, however caused significant reductions in shoot and root growth. Kaushal and Gilbert (2003) also reported the reduced bud break below pre-dawn xylem water potential of –1.5 MPa in cedar and -1.1 MPa in pine.

Critical levels of exposure to drying conditions vary. Based on a review of early literature, Hermann (1967) concluded that dormant hardwood plants could generally withstand prolonged root exposure without appreciable damage but shorter i.e. 5 h or less, periods of root exposure were critical for dormant evergreen coniferous plants. Exposure can lower internal water status and, below –0.5 MPa, can cause death of fine *Pinus radiata* roots (Balneaves and Menzies, 1988). Only a few hours of exposure cause a significant decrease in the survival. Exposure induced bud abortion and formation of epicormic shoots (Girard *et al.*, 1997a). Days to bud break increased in bare root Washington hawthorn and Norway maple when stored with exposed roots (Bates and Niemiera, 1997). Sabine *et al.* (1997) in bare root *Pinus nigra*, Didier *et al.* (2000) in pine and oak, SongZhu *et al.* (2004) in *Toona ciliata*; and Kalliopi and Yannis (2002), Hermann (1967), Coutts (1981) and Tabbush (1987) in many woody plant species reported that survival, growth and physiological parameters decreased with desiccation duration after outplanting. McKay *et al.* (1999), also reported the adverse effect of drying on the

survival of beech and birch, however, ash and oak survival was high irrespective of desiccation. Hermann (1967) and Coutts (1981) reported the sensitivity of seedlings to desiccation depends upon the dormancy status and on the part of the plant that is exposed (Sucoff *et al.*, 1985). Ritchie (1986) reported that Douglas-fir root exposure reduced seedling vigour and terminal growth even though survival was not affected. Re-wetting mitigated the effects of desiccation to *Betula pubescens* (Insley and Buckley, 1985) but in most cases seems to be ineffective. For example, growth of *Pinus radiata* Don. Seedlings did not recover after re-wetting even though shoot water potential did (Balneaves and Menzies, 1988).

Fungal mycelia are capable of rapid and comprehensive exploration, in improving the absorption of nutrients and water, and can lead to improved growth rates (Boddy, 1999; Bjorkman, 1970; Amaranthus and Perry, 1987; Smith and Read, 1997). They are particularly important for slowly diffusing ions such as PO_4^{3-} (Jacobsen *et al.*, 1992), although the uptake of highly mobile nutrients such as NO^{3-} can also be enhanced by mycorrhizal association under drought conditions (Azcon *et al.*, 1996; Subramanian and Charest, 1999). Colonization of roots by ecto-mycorrhizal fungi also has been shown to improve productivity of various plants in soils under drought stress. Ortega *et al.* (2004) reported that mycorrhizal inoculation significantly improved above-ground growth in the 2 years *Pinus radiata* after planting in areas that differed in water availability, especially at the drier site. Growths of oak seedlings on two sites in northeastern France are significantly improved following inoculation with ecto-mycorrhiza forming *Paxillus involutus* (Jean and Jean-Louis, 1997).

Fagbola *et al.* (2001) reported that in the subsoil, inoculation of *Gliricidia sepium* with the mycorrhizal fungus increased root colonization by 89% and 73% under adequate watering and drought, respectively, whereas *Leucaena leucocephala* had only a 38% and 42% increase in root colonization under comparative conditions in the subsoil. However, Shi *et al.* (2002) reported that decreased soil water availability did not significantly change the degree of fungal colonisation of beech roots measured by the amount of ergosterol content.

Ecto-mycorrhizae are symbiotic association between the roots of higher plants and fungi that frequently provide the host plant with the benefits of improved nutrition, water relations and physiological processes (Jesus *et al.*, 2004; Ruiz-Lozano *et al.*, 1995; Burgess *et al.*, 1993; Davies *et al.*, 1993, Dixon *et al.*, 1983; Dixon *et al.*, 1980) by increasing root hydraulic conductivity (Safir *et al.*, 1971) or by modifying root architecture (Kothari *et al.*, 1990). Ecto-mycorrhizal fungi can aid seedlings in overcoming moisture and nutrient stress and can decrease transplant shock (Marx, 1991), especially on degraded sites such as landings (Perry *et al.*, 1987). Positive growth results in the Pacific Northwest using Douglas-fir have been observed in seedlings inoculated with *Rhizopogon parksii* Smith and *Rhizopogon vinicolor* (Castellano, 1996). However, Francois *et al.* (2004) reported that Inoculation treatments did not significantly increase survival and growth of Douglas-fir seedlings 2 years after outplanting, it might be because the average per cent EM colonization of inoculated seedlings at time of outplanting was low (36%), and the beneficial effects of these inoculants may not have been attained. Nursery management practices, including inoculation with ecto-

mycorrhizal fungi, can improve seedling vigor after outplanting (Duryea, 1985; Duryea and Landis, 1984). Increased mycorrhizal colonization can cause increased biomass production of Poplar (Helm and Carling, 1993a, b) and *Pinus halepensis* (Morte *et al.*, 2001).

Viero *et al.* (2002) reported a highly significant interaction between hydrogel and water which had a positive impact on *Eucalyptus grandis* survival and growth. Huettermann *et al.* (1999) reported the exponentially increased water retention capacity of the soil with increasing additions of hydrogel, and greatly enhanced the drought tolerance of *Pinus halepensis* growing on this substrate. Shoot dry weight of stressed and non-stressed *Lagerstroemia indica* plants increased in starch hydrogel amended media under drought stress (Davies and Castro-Jimenez, 1989). Jobin *et al.* (2004) also have shown the potentialities of hydrogels in increasing water retention of the media and in the reduction of irrigation frequency. They also reported that shoot dry weight in *Petunia hybrida* was affected by substrate and hydrogel and was positively correlated to water content between container capacity and –10 KPa of water potential. Mohana Raju *et al.* (2002) reported that superabsorbent could be considered for water managing materials for agriculture and horticulture purposes in desert and drought prone areas.

Effect of drought/air exposure and mycorrhizal and/or hydrogel inoculation on the photosynthetic parameters

At the whole plant level, the effect of drought is usually perceived as a decrease in photosynthesis and growth, and is associated with alterations in carbon and nitrogen metabolism (Mwanamwenge *et al.*, 1999). Drought stress is one of

the most important limiting factors of the photosynthesis (Hsiao, 1973), as it induces stomata to close, reducing gas diffusion between the mesophyll and the surrounding environment. Stomata closure is the first line of defence against desiccation, since it is much quicker than changes in root growth, leaf area and pigment proteins. The decline in net photosynthesis in desiccated leaves is largely caused by stomatal closure. The complete cessation of CO_2 assimilation, however, is due to the breakdown of chlorophylls and thylakoids (Tuba *et al.*, 1996). Genetic variation in traits associated with adaptation to local conditions, such as optimal temperature for photosynthesis and drought tolerance, exists not only at the species level but also at the provenance, family and individual level (Thompson, 1999). Strong and Hansen (1991) suggested the clonal variation in drought resistance within *Populus* species.

When drought limit CO_2 assimilation, chloroplasts may be subjected to an excess of energy, resulting in photo-inhibition or photo-oxidation (Demmig-Adams and Adams, 1992). There are evidences that drought stress leads to an increase in the production of free radicals in leaves (Quartacci and Navari, 1992; Biehler and Fock, 1996), which may induce oxidative stress and contribution to leaf injury (Smirnoff, 1993). Protection of the photosynthetic systems against excess excitation energy may be achieved by direct dissipation of energy in the photosynthetic antenna complexes of photosystem II (Demmig-Adams and Adams, 1992; Foyer, 1997). Chlorophyll fluorescence has proven to be a useful screening test for drought tolerance in wheat (Flagella *et al.*, 1998), Cocoa (Balasimha and Namboothiri, 1996) and *Eucalyptus* (Mescht *et al.*, 1997). Flagella

et al. (1998) showed that the quantum yield of PS II, as related to Calvin cycle metabolism, is reduced only under drastic water deficit. Michael *et al.* (2001) in Douglas-fir reported that the post storage fluorescence assessments indicated down regulation and/or damage of the plant's photosynthetic light harvesting complex, which depend upon storage temperature and duration. Drought tolerance was positively correlated with chlorophyll fluorescence according to Mescht *et al.* (1999). Moderate and strongly conditioned *Pinus halepensis* showed a significantly lower minimum transpiration rate than the control and mildly conditioned seedlings (Pedro *et al.*, 1999). Jan *et al.* (1996) reported the higher net assimilation rate in *Pinus sylvestris*, Davies *et al.* (1996) higher transpiration rate in *Pinus taeda* and Jesus *et al.* (2004) in *Rosmarinus officinalis* by the mycorrhizal plants compared to the non mycorrhizal, as a result of greater root formation and lower shoot mass. The photochemical efficiency of PS II in *Rosmarinus officinalis* was lower in non mycorrhizal plants than mycorrhizal plants under water stress conditions (Jesus *et al.*, 2004). Deltoro *et al.* (1998) found a fast recovery of chlorophyll fluorescence parameters when *Frullania dilatata* were re-hydrated.

Zhang *et al.* (2005) found the higher net photosynthesis and transpiration rate in the wet climate *Populus davidiana* ecotypes compared to the dry climate ecotypes. Tognetti *et al.* (1995) also reported that photosynthesis of water stressed beech (*Fagus sylvatica* L.) plants from both the populations appeared to be reduced primarily by carbon dioxide diffusion through stomata and perhaps secondarily by changes in the chlorophyll concentration rather than by efficiency of photosystem. Lindqvist and Bornman (2002) and Yong Jiang *et al.* (1995)

showed that the plants of silver birch, common oak and *Picea glauca*, respectively independently of lifting date and time in storage, were stressed and had a low photosynthetic efficiency.

Effect of drought/air exposure and mycorrhizal and/or hydrogel inoculation on the plant water relations and ion concentration

Exposure of the plant in a drying atmosphere can affect internal water status (Tabbush, 1987; SongZhu *et al.*, 2004). Coutts (1981) showed that 4.5 h root exposure of bare root Sitka spruce seedlings at 11.5^0C and a wind speed of 0.3 ms^{-1} caused a greater change in the moisture content of fine roots than of woody roots or shoots. Plants inoculation with ecto-mycorrhizal fungi have shown increased resistance to water stress from drought. Stomatal conductance, transpiration rate and leaf water potential is often higher in mycorrhizal plants under drought because of a higher water uptake (Auge *et al.*, 1987; Subramanian *et al.*, 1995; Duan *et al.*, 1996; Rosa and Ruiz-Lozano, 2004), however, Pallardy *et al.* (1995) suggested that ectomycorrhiza may cause an increase in water stress, since stimulation of transpiration may cause lower leaf water potentials. Walker *et al.* (1989) showed that *Pinus taeda* seedlings colonized with eccto-mycorrhizal fungi showed less negative water potentials. In the same way, mycorrhizal plants had postponed declines in leaf water potential during drought stress (Davies *et al.*, 1992; Subramanian *et al.*, 1997 and El-Tohamy *et al.*, 1999) and leaf water potential returned to control level more quickly in mycorrhizal plants than non mycorrhizal maize plants after the relief of drought (Subramanian *et al.*, 1997). In contrast, leaf water potential was similar in mycorrhizal and non mycorrhizal

plants under the conditions of normal watering (Ebel *et al.*, 1996; Bryla and Duniway, 1997).

Morte *et al.* (2001) and Jesus *et al.* (2004) also showed that the decreases in leaf water potential due to drought were higher for non-mycorrhizal than the mycorrhizal *Pinus halepensis* and *Rosmarinus officinalis* plants. Schraml and Rennenberg (2000) reported the leaf water potential of -1.18 MPa in controls and –1.96 MPa under the drought conditions in beech ecotypes. Similarly sharp decrease in plant stomatal conductance and leaf water potential were observed during drought in oak (Fort *et al.*, 1997). Sabine *et al.* (1997) reported that pre-planting exposure of bare root Corsican pine seedlings, decreased seedling water potential and CO_2 assimilation rate after planting. Hsiao (1973) in plant tissues and Pedro *et al.* (1999) in *Pinus halepensis* found the high pre-dawn leaf water potential in the control and mildly conditioned seedlings compared to moderate and strong water conditioned treatments.

Similarly, a strong reduction of pre-dawn water potential in roots and shoots, as well as on transpiration rate in beech ecotypes were found under the influence of drought. It also reduced the water content to 97% of controls in leaves and axes and to 92% in roots (Peuke *et al.*, 2002). Franck *et al.* (2000) and Nicolas *et al.* (2002) reported the differences in reduction of leaf water potential and relative water content in the *Populus euramericana* clone owing to drought acclimation. Frank and Thomas (2000) reported that in the drought stressed seedlings of *Quercus petraea* and *Q. robur*, the relative foliar water deficits were significantly higher, and the pre-dawn leaf water potentials were significantly

lower than in the controls. Root moisture content and root water potential were related to survival only in case of desiccation treatment in broad leaved tree species (Kalliopi and Yannis, 2001).

Nutrient availability declines with soil water content as mineralization rate and mass flow in the soil decline (Nye and Tinker, 1977), both of which may result in decreasing element uptake and release into xylem conduits. Osonubi *et al.* (1988) reported a decline in element concentrations with increasing over-pressure i.e. a strong positive relationships were reported by them between xylem element concentrations and plant water potential. Positive and negative relationships, as well as no relationship between soil water availability and element concentrations in xylem sap have been observed (Osonubi *et al.*, 1988; Gollan *et al.*, 1992; Stark 1992).

Effect of drought/air exposure and mycorrhizal and/or hydrogel inoculation on carbohydrate levels and C:N ratio

Osmotic adjustment using soluble sugars has been observed among poplar clones subjected to drought, and contribute to both plant survival, growth and maintenance (Meyer and Boyer, 1981; Tschaplinski and Blake, 1989; Tan *et al.*, 1992; Gebre *et al.*, 1994). In several broad leaved tree species, osmotic adjustment has been associated with high dehydration tolerance (Auge *et al.*, 1998). Insufficient supply of carbohydrates for metabolic and growth processes (Guehl *et al.*, 1993; Puttonen, 1986) under the conditions of limited water supply are the main causes of transplanting stress. Mildly and moderately water conditioned *Pinus halepensis* seedlings accumulate more nitrogen in shoots and roots, respectively; however shoot starch was concentrated more in the moderate and

strong conditioned treatments while no differences were observed in roots. Soluble sugar and other osmolytes showed the reverse trend, the moderately and strongly water stressed plants exhibited a higher concentration than control plants (Pedro *et al.*, 1999; Bray, 1991; Quick *et al.*, 1989). Peuke *et al.* (2002) in drought sensitive beech ecotypes and Gebre *et al.* (1997) in *Populus deltoides* also reported the high accumulation of glucose and fructose concentrations under the influence of drought, however, Gebre *et al.* (1997) reported that the re-watering of conditioned plants resulted in the decreased accumulation of concentration of solute, whereas Johnson *et al.* (1984) reported increased sugar accumulation in re-hydrated wheat cultivars. But, Girard *et al.* (1997a) reported that non-structural carbohydrate concentrations were not affected during the exposure phase in oak seedlings.

Water stress may impair the nitrogen uptake by the seedlings and adult trees, and these changes in nitrogen uptake may influence the photosynthetic and growth pattern of plants. Alguacil *et al.* (2003) reported the increased contents of nitrogen, phosphorus and potassium in shoot of mycorrhizal plants, and Davies *et al.* (1996) reported that mycorrhizae tended to alter root morphology and carbon allocation pattern of shoots and roots, however, Jan *et al.* (1996) reported the significantly reduced nitrogen assimilation by the host plant growing in association with mycorrhiza.

Effect of drought on wood anatomical properties

The surrounding environment such as drought affects certain wood anatomical properties. Plant tissue responses to water stress depend on the physiological properties of the cell components and the anatomic characteristics

that regulate the transmission of the water stress effect to the cells. The anatomy of water-transporting tissues varies widely among tropical hardwood trees. Parenchyma cells are largely confined to the rays and constitute a relatively small fraction of the wood (Braun, 1970). Difference in response to water stress among mature regions and regions of tissue growth seem to be due to anatomical differences (Matsuda and Rayan, 1990). Leyre *et al.* (2004) reported that mean and maximum vessel diameter in *Quercus ilex* declined due to the severe summer drought recorded in 1994. Matthew *et al.* (2004) and Sofia *et al.* (2004) found that water-limitation significantly reduced mean vessel lumen area, smaller vessels and increased vessel frequency in water stressed eucalyptus plants. Matthew *et al.* (2004) also showed that wood density was negatively correlated with vessel lumen fraction in well-watered plants, but this relationship broke down in the water-limited plants. Similarly, Carlquist (1975) reported that hardwood responded with smaller vessels and larger vessel abundance, which reduces vulnerability to water stress. Harvey and Van Den Driessche (1997) reported that the vessel diameter and specific conductivity were greater in the drought-resistant clones of hybrid poplar than in the drought-susceptible clones. Baas *et al.* (1983), Baas and Schweingruber (1987), Zhang *et al.* (1992), Woodcock and Ignas (1994) and Sass and Eckstein (1995) also reported changes in mean vessel diameter, especially in species with diffuse-porous wood along climatic gradients of water.

Effect of drought on volatile release

Emission of a particular volatile compound into the atmosphere depends upon the rate of its biosynthesis and the rate of its release. Trees emit numerous

volatile organic compounds including a large number of oxygenated compounds, into the atmosphere (Fehsenfeld *et al.*, 1992; Kesselmeier and Staudt, 1999). As volatile organic compounds alter the concentrations of hydroxyl radicals, they are supposed to increase the lifetime of climate sensitive trace gases such as methane (Brasseur and Chatfield, 1991). Environmental factors such as light, temperature, and moisture status can greatly influence the emission of volatiles and the yield (Staudt and Bertin, 1998; Gershenzon *et al.*, 2000). Jin-YouJu *et al.* (2004) reported the release of Hexenl volatile compound under the influence of drought from ash-leaf maple (*Acer negundo*). 3-cis-hexenal is an important component of green leaf volatiles, the mixture of compounds that are emitted when the leaf is damaged (Pichersky and Gershenzon, 2002). Li-JiQuan *et al.* (2000) reported the release of ten volatiles including butyl alcohol, pentyl alcohol, E-2-hexen-1-al, Z-3-hexen-1-ol, pentanal, pentanoic acid, hexanal, hexanoic acid and acetophenone were significantly increased by water stress. However, cyclohexyl isothiocyanate, cinnanittrile, heptanal, phenol, napthalene and benzothiazole decreased during drought in ash-leaf maple.

MATERIALS AND METHODS

Experiment I: Drought stress of planting stock of beech and their survival in the field plantation

To conduct this experiment, one-year-old bare rooted beech seedlings were lifted from Forstbaumschule, Boesinghausen on 11.11.2003 and transported to the Institute of Forest Botany, Georg August University of Goettingen (transportation time: 20 minutes) and planted outside in the field in groups over winter, for using them later on in the next growing season. Then in the spring season (10.03.2004) the seedlings were selected on the basis of similar collar diameter range after careful excavation from field. The soil from the seedling's roots was gently washed off and drip water was removed.

Then, these bare rooted seedlings were exposed to air in the climatic chamber with 50% humidity and 20^0C temperature for 0, 2 and 6 h under full light illumination. Whole plants were laid horizontally on wire mesh in the climate chamber to simulate air exposure before planting. Control plants were not subjected to any exposure and planted immediately after measuring morphological parameters.

For each treatment, one batch of seedlings was harvested for determination of vitality (Table 1) and carbohydrate contents in the roots and buds in the laboratory (Table 2), and the other batch was transplanted randomly in multiple

plots in an experimental area after determination of morphological parameters (Table 3). The seedlings were shovel transplanted in rows at a distance of 40 cm in multiple plots with the presence or absence of *Paxillus involutus* Maj. Strain and hydrogel. The weeds were controlled manually. No additional fertilization was applied to the seedlings and the irrigation was applied to the plants to field capacity, as and when required on the basis of soil conditions.

Mycorrhizal preparation

Paxillus involutus Maj. strain was grown on ½ MMN agar medium (Table 4) over cellophane for one month. Then grown in different flasks for 2 week over shaker, having 100 ml ½ MMN medium with 2 g glucose (without Malt extract and Agar) for liquid preparation of ecto-mycorrhizae. Then prepared homogenized material of the ecto-mycorrhizae for soil mixing.

Preparation of microscopic slides for ecto-mycorrhizal section

First the slides were cleaned in acetone. Then cleaned with brush and detergent, and rinsed with tap water first and with the distilled water later. Then the slides adjusted on glass stand were placed in 2% Mucasol solution for 3 days. On the third day, rinsed for 2 h first under tap water and then for 5 minutes under distilled water. Then a solution was prepared from 0.125 g Chromium (III) Potassium sulphate + 1.25 g gelatine sheets in 250 ml double distilled water. For homogenous mixing, the solution was put in oven at 50^0C for 1 h. This solution was then taken into a long beaker after passing through a filter. Then the slides were leaned against a steel stand for drying, after dipping in the above prepared Chromium Potassium sulphate and gelatin solution.

Styrol-Methacrylate embedding

After the final harvest of the beech seedlings, the ecto-mycorrhizal and non-mycorrhizal root tips (Plate 1 and 2) were collected in 1.5 ml e-cups having formaldehyde acetic acid (FAE) solution (90 Part 70% Ethanol + 5 part Acetic acid + 5 part 37% Formaldehyde). These root tips were then embedded in Styrol-Methacrylate after adopting the following procedure:

Day 1st

First, all the roots were transferred from the FAE solution to 70% Ethanol and were kept overnight. Then transferred again in the following solutions as below

Day 2nd

70% Ethanol for 15 minutes
80% Ethanol for 15 minutes
90% Ethanol for 15 minutes
96% Ethanol for 15 minutes
100% Ethanol for 30 minutes
100% Ethanol for 30 minutes
100% Ethanol/ 100% Acetone for 30 minutes
100% Acetone for 30 minutes
100% Acetone for 30 minutes
Acetone/ Plastic (1:1) during lunch time
Acetone/Plastic (1:3) over night

Day 3rd

100% Plastic in the morning
100% Plastic over night

Day 4th

Individual ecto-mycorrhizal/non-mycorrhizal root tip was transferred into gelatine capsules having 100% Plastic and then polymerized at 60^0C for 3-4 days. Plastic is a solution of the combination of 50 ml Styrol (C_8H_8), 50 ml Butyl methacrylate 99% ($C_8H_{14}O_2$) and 2 g Dibenzoylperoxid with 50% Phthalat.

Then after polymerization, fixed the root tips on the plastic sticks for making cross sections. Then the ecto-mycorrhizal and non-mycorrhizal root tips 1 µm thick cross sections were made on the rotary microtome. The ecto-mycorrhizal sections were put on the drop of distilled water, and expanded on the slides by exposing them to the chloroform for 20 seconds and then were put at 45^0C on the hot plate for fixing on the slide, and also for the evaporation of water. Then stained with 0.1% Toluidine blue in 0.1% Borax, again at the same temperature for 5 minutes. Then washed the stain with distilled water and the glass slide was dried with gentle air pressure. Then embedded in the Euparal and covered with glass cover slips. The glass slides were then put again on the same temperature for overnight, with lead blocks on the cover slips. Then the sections were photographed under a light microscope (Axioskop, Zeiss, Oberkochen, Germany) with a digital camera (Nikon Coolpix 4500, Nikon, Tokyo, Japan) with 20x and 40x magnifications.

Soil Preparation

Two hundred kg of soil was taken from the field and mixed well and then spread for one week for drying in the green house. After one week, the soil was still having about 32% moisture when used for planting the seedling. Before planting, ecto-mycorrhizae growing in different flasks having liquid ½ MMN medium were combined in 4x500 ml flasks and homogenized. Then in a large tub, 15 kg air-dried earth was then mixed with 75 g Hydrogel and in addition added the ecto-mycorrhizae and mixed well. Then each seedling except control was planted with this one kg of soil preparation in the field.

Biometric and physiological analysis

Shoot and root length, collar growth, seedling weight before and after air exposure, relative moisture loss, number of buds, vitality and carbohydrates in fine roots and buds were determined in spring. Shoot and root lengths were measured from the collar region to the tip of shoot and root, respectively. Collar diameter was recorded from the collar region of the seedling with the help of Digital vernier calipers. The whole seedling weight was recorded before and after each air exposure. During the growth season, bud break and the quantum yield of the chlorophyll fluorescence were measured; and towards the end of the growing season, the seedlings were harvested and used for the determination of the shoot and root length, collar diameter, no. of buds (new + old buds), no. of leaves, leaf area, fresh biomass of the fine and coarse roots, leaves, plants with and without leaves as well as their dry biomass after drying the plant tissues at 60^0C for one week in oven, degree of ecto-mycorrhization, vitality in fine roots and buds, and carbohydrate concentrations in fine roots and leaves. Bud break was noted, when the first leaf drove out from the bud. Ecto-mycorrhization was determined after counting the ecto-mycorrhizal and non-mycorrhizal root tips from a homogenous root sample of each seedling, which were stored at the time of harvest in cold room at 4°C in polythene bags, and the ecto-mycorrhization for rest of the seedlings recorded in this manner. Ecto-mycorrhizal percentage was calculated as:

$$\frac{\text{No. of ecto-mycorrhizal root tips}}{\text{No. of ecto-mycorrhizal + Non-mycorrhizal root tips}} \times 100$$

After counting the ecto-mycorrhizal and non-mycorrhizal root tips, the roots of the seedlings were divided into fine and coarse roots, for measuring their fresh and dry biomass. Plant survival was determined at the end of the growing season. Plants with no leaves or live buds were considered as dead. Per unit mass leaf area was determined by dividing the sample leaf area by the mass of sample leaves, and the leaf area of 5 leaves per seedling was measured by LI-3000 Area Meter (Licor Ltd., Nebraska, USA).

Chlorophyll fluorescence measurements

The measurement of chlorophyll fluorescence from Photosystem II (PS II) has become a useful method for the determination of mechanisms of photosynthesis and to study the effects of various environmental conditions on the photosynthetic reactions (Bolhar-Nordenkampf *et al.*, 1989). Chlorophyll fluorescence measurement is rapid, extremely sensitive and can be performed on intact as well as isolated chloroplasts. It has become an important tool in the study of photosynthesis, in particular the functioning of PS II (Schreiber *et al.*, 1995).

Absorbed light energy may follow one of three paths in leaves: most is used in photosynthetic electron transport; however, beyond the saturation and closure of centres, excess light energy can be dissipated as heat and/or re-emitted as fluorescence (Walker, 1987). When measuring fluorescence, the two alternative paths of energy dissipation are known as two mechanisms of fluorescence quenching, q_P and qN. Photochemical quenching (q_P) refers to photosynthetic electron transport and non-photochemical quenching (qN) to thermal dissipation of excitation energy as heat.

The potential quantum yield of PS II (Fv/Fm), in the dark-adapted state, was assessed pre-dawn in the field using a portable pulse-amplified modulation fluorometer MINI PAM (Walz GmbH, Effeltrich, Germany). This ratio represents the activity of PS II, which is used to access the functional damage to the plants. Minimum fluorescence yield (F_0) was obtained upon excitation with a week measuring beam from a pulse light emitting diode. Maximal fluorescence yield (F_m) was determined after exposure to a saturating light pulse to close all reaction centres. F_v is the variable fluorescence (F_m-F_0). It provides a quick and useful estimate of light reaction activity and photosynthetic rate. Photochemical quenching (q_p) and non-photochemical quenchings (qN, NPQ) were also conducted by MINI PAM by the saturation pulse method.

Vitality assessment

2,3,5-triphenyl tetrazolium chloride test (TTC) was used to measure the physiological status of the fine roots, lower and upper buds before and after root air exposure, of the seedlings selected for this purpose in the spring (March before bud break, 10.3.2004); as well as measured the physiological health of the fine roots and middle buds after the growing season at harvest on 18.8.2004. For determining the vitality, a solution of 60 ml phosphate buffer (KH_2PO_4) with pH 5.8 was prepared and mixed well with 0.608 g TTC salt. Then weighed about 40 mg fine roots in 2 ml e-cups and added with 1 ml above prepared TTC solution and then all the samples were incubated in dark on shaker at 25^0C over 24 hours. After incubation, the samples were washed thrice with 800 µl double distilled water. Then added in each sample 1.5 ml 96% ethanol and heated in 70^0C water

bath for 30 minutes and shaken well twice during heating after taking the samples from the water bath. Then cooled rapidly the samples by putting in ice water and centrifuged at 4^0C, 15000 rpm for 15 minutes. Then measured the absorbance at 485 ηm in photometer. The blank was used with only 96% ethanol. If absorbance readings were more than 1.00, then diluted the sample with the same quantity of 96% ethanol and measured again the absorbance at 485 ηm.

For determination of extinction per gram of fresh sample, the following calculations were used:

$$\text{Extinction/g of fresh sample} = \frac{\text{Absorbance reading (485 ηm) x dilution}}{\text{Weight of sample}} \times 1000$$

Fine root samples were taken after pooling the fine roots from two plants and 5 replications were taken for each air exposure. Similarly the lower and upper buds were also pooled from two plants. The buds were scaled off. Before putting the buds in the TTC solution, the scaled buds were cut halves. Lower buds were too small, so used more halves as compared to the upper buds after scaling.

Carbohydrate measurements

Samples were frozen (-80^0C) until processed. For each treatment, all the tissues were ground separately with mortar and pestle in liquid nitrogen. Soluble sugars (glucose, fructose, sucrose) and starch were analyzed enzymatically (Beutler, 1978; Schopfer, 1989) in buds and fine roots of the beech lot taken for this purpose in spring, and in leaves and fine roots at the final harvest. Starch was quantified after degradation in glucose equivalents. Extraction was carried out on a powdered sub-sample of 75 mg in 2 ml e-cups by incubating with 1.5 ml

extraction medium (DMSO/HCl 25%, 80:20 volume) at 60^0C for 30 minutes in water bath. After rapid cooling by ice water, centrifuged at 4^0C, 5000 rpm for 5 minutes. Then 200 µl aliquots of this solution was added with 1.2 ml cooled 0.2 M Citrate buffer (pH 10.6) and centrifuged again at 4^0C, 5000 rpm for 5 minutes after mixing well. For glucose measurements, 200 µl supernatant in e-cups was mixed with 200 µl 50 µM Citrate buffer (pH 4.6) and put on ice; and for starch 200 µl supernatant was mixed with 200 µl Amyloglucosidase solution and incubated in the water bath at 57^0C for 20 minutes and then put on the ice for measurement. The blank was used without plant material after adopting the other procedures same and thus separate blanks were used for glucose and starch. For carbohydrate photometric measurements, the 250 µl solution 1 (4 mM NADP + 10 mM ATP + 9 mM $MgSO_4$ + 0.75 triethanolamine + 6M HCl, pH 7.6) was mixed with 100 µl sample and 400 µl double distilled water and then absorbance (E1) was read at 340 ηm. Then again added 10 µl Hexokinase, Glucose-6-phosphate and after mixing well, again absorbance (E2) was read after 5 minutes incubation. Then again after adding 5 µl PGI, absorbance (E3) was measured after 5 minutes incubation. In the next step absorbance (E4) was measured after adding 10 µl Fructosidase solution (0.5 µl 0.32M Citrate buffer pH 4.6 + 0.01 g Fructosidase) having a incubation period of 10 minutes. All the absorbances were read at 340 ηm. For determination of the glucose concentration, the following calculations were used

$C = Vassay*MW/(\varepsilon*d*x)*Ecorr$ (g/L)

Where

C: glucose concentration of the extract in the test system,
Vassay: volume of test system (1.5 µl),
MW: molecular weight of glucose (180.16 g/mol),

Ecorr: calculated absorbance of samples solution,
ε: mole absorbance of NADPH at 340 ηm (6300 $L*mol^{-1}*cm^{-1}$),
d: thickness of cuvette (1 cm),
x: volume of sample solution (200 µl).

Whereas

$Ecorr_1$ = Blank (E2-E1); $Ecorr_2$ = Blank (E3-E2),
$Ecorr_3$ = Blank (E4-E3),
Glucose: E2corr = sample (E2-E1)- $Ecorr_1$
Fructose: E3corr = sample (E3-E2)- $Ecorr_2$
Sucrose: E4corr = sample (E4-E3)- $Ecorr_3$

All carbohydrate data are expressed on a tissue fresh weight basis.

Water relations measurement

The relative moisture loss in the soil and seedlings was calculated after air exposure, on the basis of difference in fresh to dry weight compared to fresh weight. [(Fresh weight-Dry weight) x Fresh weight $^{-1}$ (%)].

Soil elemental composition

Elemental composition and pH of the field site (Table 5) were determined at the end of the experiment with the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Spectro Analytical Instrument, GmbH, Boschstrasse, Kleve, Germany).

C/N measurement

On 18.8.2004 i.e. final day of harvest, leaves from the seedlings selected for biomass measurements were harvested and dried in oven at 60^0C for one week. After measuring the dry weights, the leaves were machine grinded and a homogenous combined sample per plant was made. Three replications per sample, each comprising 650 to 950 µg in 5 x 9 mm size tin cartridge (Hekatech, Wegberg, Deutschland) were analyzed for C/N with the CHNS-O-Elemental Analyzer

(CHNS-O-EA 1108-Elemental Analyzer, Carlo Erba, Milano, Italy). The analytical method is based on the complete and instantaneous oxidation of the sample by "flash combustion" which converts all organic and inorganic substances into combustion products. The resulting combustion gases pass through a reduction furnace and are swept into the chromatographic column by the carrier gas (helium) where they are separated and detected by a thermal conductivity detector, which gives an output signal proportional to the concentration of the individual components of the mixture. The standard used was acetanilide (71.09% C; 10.36% N; Carlo Erba, Milano, Italy).

Experimental design and statistical analysis

Treatments:

(i) No. of duration of air exposure: Three (0h, 2h, 6h)

(ii) Presence or absence of mycorrhiza and hydrogel: Two (inoculation, non-inoculation)

Design : Factorial RBD

Replications : 15

There were 30 seedlings in each air exposure, consisting of 15 each for inoculated and non-inoculated. Out of 15 seedlings from inoculated and non-inoculated, 5 seedlings each were used for carbohydrate measurements and rest for biomass measurements at the harvest. So a total of 90 seedlings were used in the field planting.

Analysis of variance (ANOVA) followed by Tukey's HSD test ($P<0.05$) was used for the effects of air exposure on biometric and physiological parameters. Statistical procedures were carried out with the Software Package SAS (SAS

Institute Inc., Cary, NC, USA, ©1989-2003). Significant differences were based on $P \leq 0.05$.

Experiment II: Drought and re-watering cycle under controlled conditions in Poplar

Tissue cultured seedlings of *Populus canescens* were used in this experiment at the Institute of Forest Botany, Georg August University of Goettingen. These seedlings remained in the hydroponics from July 20, 2004 to August 28, 2004. The nutrient solution composition used in hydroponics culture was as below:

Salt	**Conc. (g/l)**	**Conc. (µM)**
KNO_3	50.55	0.2
$Ca(NO_3)_2.4H_2O$	Three alternatives (see below this composition)	
$MgSO_4.7H_2O$	37.00	
KH_2PO_4	40.82	0.6
K_2HPO_4	3.60	0.04
$MnSO_4.H_2O$	0.169	
H_3BO_3	0.309	
$Na_2MoO_4.2H_2O$	0.846	
$CoSO_4.7H_2O$	0.0056	
$ZnSO_4.7H_2O$	0.0288	
$CuSO_4.5H_2O$	0.016	
Fe.EDTA	1.8355	
$FeCl_3.6H_2O$	0.675	

Alternative	**1**	**2**	**3**
Ctr. (g/l)	0.2mM	2mM	8mM
$Ca(NO_3)_2.4H_2O$	-	106.27	460.49

After well growth in the hydroponics, they were then planted in the sand peat medium in the green house with the 4 treatments as below:

1. Control means only in sand peat medium
2. *Paxillus involutus* (Maj strain) with sand peat medium
3. Hydrogel with sand peat medium
4. *Paxillus involutus* (Maj strain)+hydrogel with sand peat medium

Soil preparation

Sand peat material was prepared in bulk quantity in the ratio of 75% sieved peat and 25% sand. Then equally divided this material into four parts and autoclaved twice each part separately. Autoclaved sand peat material for planting control plants was used without any *Paxillus involutus* (Maj strain) inoculation and hydrogel mixing. For ecto-mycorrhizal plants, autoclaved sand peat preparation was inoculated with liquid culture of *P. involutus* one week before planting of Poplars in the plastic boxes. Third autoclaved preparation was mixed with 5 g hydrogel per kg of sand peat medium; and the last preparation was inoculated with *P. involutus* and also mixed with hydrogel at the rate of 5 g per kg of autoclaved material, this preparation was also made one week ahead of planting. Then each separately prepared material was filled in 24 plastic boxes; means a total of 96 plastic boxes were used for planting the plants for all treatments.

Then took Poplar seedlings from hydroponics and planted one in each box with 40 ml of *P. involutus* liquid culture on 28.08 2004 and as these Poplar seedlings were very much sensitive to the external environment, they were then placed in automated climatic chamber having 16 h light and 8 h dark, after covering with plastic bags. The plastic bags remained on the plants for one week and removed slowly after exposing the seedlings slowly and slowly to the external environment from top.

Mycorrhizal Preparation

Paxillus involutus (Maj strain) was grown in agar medium having low sugar content over cellophane for 4 weeks and then grown in 100 ml MMN medium of low sugar content (2 g glucose without malt extract and agar)) for liquid preparation of ecto-mycorrhizae for 2 weeks. Then prepared the homogenized liquid material of ecto-mycorrhizae. The composition used for the ½ MMN medium were the same as for the experiment 1.

Hence, a total of 4 treatments (control, with *Paxillus involutus* only, with hydrogel only, with both *Paxillus involutus* and hydrogel) were used in this experiment and 24 plants were planted in the plastic boxes for each treatment and then from each treatment again divided the plants into 3 watering regimes i.e. 8 plants with normal watering, 8 plants for drought and 8 plants for re-watering.

Preparation of microscopic slides for ecto-mycorrhizal cross sections

The microscopic slides were prepared according to the method described in experiment 1.

Styrol-Methacrylate embedding

Ecto-mycorrhizal as well as non-mycorrhizal poplar roots were stored in FAE solution in e-cups at the time of harvest (Plate 3). Then the procedure explained in experiment 1 was adopted to fix the poplar roots.

Growth measurements

The plants were measured for growth parameters (collar diameter, height and leaf no.) for the first time on 22.9.2004 and second time on 09.10.2004 before

the start of drought treatment and finally on 20.10.2004 (drought). The second harvest was done on 22.10.2004 after re-watering, for the determination of physiological parameters. Height was measured from the collar region to the tip of shoot and collar diameter was recorded from the collar region of the seedling with the help of digital vernier calipers. For the whole cycle of experiment, the plants were supplied with nutrients liquid medium three times only. The composition of the nutrient liquid medium was same, as used at the time of hydroponics culture. For the rest of the period, they were watered with only tap water. After this 2nd time measurement on 9.10.2004, the last watering was given to all the plants (watering stopped only after looking seepage of water from the bottom of each box) selected for drought and re-watering treatments; whereas the plants selected for controls in each treatment were watered daily in the morning. The seedlings were harvested, before and after drought, for measuring the physiological observations as well as for the biometric parameters viz. shoot length, collar diameter and no. of leaves; and after re-watering for the physiological observations. The number of leaves per plant was noted by counting the total leaves of all the plants of each treatment and dividing by number of plants in each treatment. Fresh biomass of whole plant and each organ (leaf, shoot, fine and coarse roots) was determined at the drought harvest and their dry weights were determined after drying them in oven at 60°C for one week. After counting the ecto-mycorrhizal and non-mycorrhizal root tips, the roots were divided into fine and coarse roots for measuring their fresh and dry biomass. Physiological parameters (vitality, sugar, ion composition in phloem and xylem, relative water

content, leaf water potential, C/N analysis), Photosynthetic parameters (chlorophyll fluorescence, photosynthesis), Wood anatomy, Volatile in leaf were determined.

The degree of ecto-mycorrhization was determined in the same manner as per the procedure adopted in Experiment-I. Ecto-mycorrhizal roots cross sections were taken to determine its effect on the root anatomy.

Leaf water potential

After the start of the drought, at six moments, three seedlings were taken at random from each treatment of the well watered and drought conditioned seedlings, and pre-dawn leaf water potential was estimated according to the method described by Scholander *et al.* (1965) using a pressure chamber. Thus measurements were taken on 3^{rd}, 8^{th}, 9^{th}, 10^{th}, 11^{th} (harvest day for drought plants) and finally on 12^{th} (after re-watering) day.

Water relations measurement

The relative water content in the plant material was calculated on the basis of difference in fresh to dry weight compared to fresh weight. [(Fresh weight-Dry weight) x Fresh weight –1 (%)].

Gas exchange measurements

Photosynthesis and transpiration rates of three seedlings from each treatment were measured on the 2^{nd} and 8^{th} day of drought. During these measurements, photosynthetically active radiation (PAR) was made constant at 700 μmol $m^{-2}s^{-2}$, and relative humidity of the air remained at an average of 31.6%. The leaf area used for gas exchange measurements in the cuvette was 5 cm^2, the

temperature of closed cuvette was fixed at 24^0C and the flow of air was made constant at 800 ml/minute. These gas exchange parameters of attached leaves were measured using a portable photosynthesis system HCM-1000 WALZ.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence (pre-dawn) was measured on drought day 0, 3rd and then daily from drought day 6th (15.10.2004) till harvest for drought and re-watering. The potential quantum yield of photosystem II (Fv/Fm), in the dark-adapted state, was assessed pre-dawn in the poplars as done in the first experiment 1 using a portable pulse-amplified modulation fluorometer. A new PAM fluorometer, the MINI-PAM (Walz GmbH, Effeltrich, Germany) was used in this study.

Carbohydrate measurements

Samples were frozen (-80^0C) until processed. For each treatment all the tissues were ground separately with mortar and pestle in liquid nitrogen. Soluble sugars (glucose, fructose, sucrose) and starch were analyzed enzymatically (Beutler, 1978; Schopfer, 1989) in fine roots of the poplar at the time of harvest as explained in experiment 1. All carbohydrate data are expressed on a tissue fresh weight basis.

C/N measurement

On 20.10.2004 and 22.10.2004 i.e. days of harvest after drought and re-watering, respectively, leaves were harvested from the seedlings. After taking their fresh weights, they were dried in oven at 60^0C for one week. After measuring the dry weights, then leaves were machine grinded and a homogeneously combined

sample per plant was made. Three replications per sample, each comprising 650 to 950 µg in 5 x 9 mm size tin cartridge (Hekatech, Wegberg, Deutschland) were analyzed for C/N with the CHNS-O-Elemental Analyzer (CHNS-O-EA 1108-Elemental Analyzer, Carlo Erba, Milano, Italy). The standard used was acetanilide (71.09% C; 10.36% N; Carlo Erba, Milano, Italy).

Vitality assessment

2,3,5-triphenyl tetrazolium chloride test (TTC) was used to measure the physiological status of leaf after harvest on 20.10.2004 and 22.10.2004. For determining the vitality, a solution of 100 ml phosphate buffer (KH_2PO_4) with pH 5.8 was prepared and mixed well with 1.0 g TTC salt. Then taken 3-4 pieces of leaf discs in 2 ml e-cups and weighed; and then added with 500 µl above prepared TTC solution and then all the samples were incubated in dark on shaker at 25^0C over 24 hours. After incubation, the samples were washed thrice with 800 µl double distilled water. Then added in each sample 1.5 ml 96% ethanol and heated in 70^0C water bath for 30 minutes and shaken well twice during heating, after taking the samples from the water bath. Then cooled rapidly the samples by putting in ice water, and centrifuged at 4^0C, 15000 rpm for 15 minutes. Then measured the absorbance at 485 ηm after taking 600 µl supernatant. Extinctions coming above 1.0 were measured again after diluting with 96% ethanol. Colour of the end product was green instead of red, as observed in the fine roots and buds of the beech. Calculations were used as described in experiment-I.

Energy Dispersive X-ray analysis at the Scanning Electron Microscope

The principle of the x-ray microanalysis is based on the interaction between the high-energy electrons of the jet of an electron microscope and the atoms of the biological preparations. Lower energy level electrons fired at the atoms are hurled from the inner orbits and replaced by electrons of high energy levels from the outer orbits. This energy difference is therefore emitted as x-ray. Since the atomic radii and the number of electrons in the orbits are dependent on the different nuclear charge of the respective elements, thus the freed x-ray is specific for the element concerned.

In order to accomplish the EDX analysis, stem samples were cut into 1 cm long pieces and rapidly frozen in a 2:1 mixture of propane: isopentane cooled with liquid nitrogen to –196°C in a mesh. Samples were freeze-dried at –45°C for three days and stored at -20°C in a desiccator over silica gel. For scanning electron microscopy (SEM), the semi-quantitative ion regulation of the samples took place at the scanning electron microscope (REM AMR 1200 B, Leitz) to which a energy dispersive x-ray analysis device (EDXA, KEVEX 4000) was attached. This EDX plant possibly makes a registration of all elements starting from order number 9 (fluorine).

The element analysis was accomplished under an accelerating voltage by 15 kV with 1000 times enlargement of the sample to be measured. After one analysis, duration of 200 seconds of the resulting x-ray spectrum was noted by an analog writer; for the determination of relationship of the element specific x-ray to the non-specific background (peak: background ratio), a relative scale for the mass

concentration of the respective ion could be determined. Every sample was measured in the young phloem and young developing xylem portion of the stem.

Preparation of microscopic slides for stem cross sections

The microscopic slides were prepared according to the method described in experiment 1.

Wood anatomy

For wood anatomical studies, three seedlings were taken from each stress stages of each treatment, i.e. a total of 36 stem cuttings were used for paraffin embedding. The stem samples were taken at equal heights from the collar region of each seedling. At the harvest stage of before and after drought, and at re-watering, 1 cm stem samples were placed in the FAE solution till the time of paraffin embedding, the concentration for which was as 90 Part 70% Ethanol, 5 part Acetic acid and 5 part Formaldehyde 37%. Then the following procedure was adopted for the paraffin embedding of stem samples for use in wood anatomical study.

In the first step of Paraffin embedding, pipetted out the FAE solution from the sample's vial (container) and was being replaced with the same amount of 70% ethanol for 2 h. Then after 2 h, the 70% ethanol was replaced with the following different solutions step by step as:

80% Ethanol for 2 h (2 ml/each sample)
90% Ethanol for 2 h (2 ml/each sample)
96% Ethanol for 12 h (2 ml./each sample)
96% Ethanol / Iso-propanol (1:1) for 12 h (2 ml/each sample)
Iso-propanol for 12 h (2 ml/each sample)
Iso-propanol for 12 h (2 ml./each sample)
Iso-propanol/Roti-Histol (3:1) for 12h (1.5 ml Iso-propanol: 0.5 ml Roti-Histol)
Iso-propanol/Roti-Histol (1:1) for 12h (added to previous, 1 ml Roti-Histol to make the solution 1:1)

Iso-propanol/ Roti- Histol (1:3) for 12 h (0.5 ml Iso-propanol : 1.5 ml Roti-Histol)
Roti-Histol for 12 h (2 ml./each sample)
Roti-Histol for 12 h (2 ml./each sample)
Roti-Histol for 12 h (2 ml./each sample)

Then transferred the stem 1 cm samples (which were in vial with 2 ml Roti-Histol solution previously) to 2 ml e-cups and then the samples were covered again with 100 % Roti-Histol solution and after that cold Rotiplast granules were added in it and placed at room temperature for 12 h. Then these samples with Roti-Histol+Rotiplast granules were placed at 40^0C in oven for another 12 h. The Rotiplast melted.

The samples were taken from the oven after 12 h and were placed in 2 ml e-cups having already the melted Rotiplast. The Rotiplast was melted in the oven at 61-63^0C temperature for 12 h. The stem samples remained in the oven for 12 hours at this temperature. Then on the next day repeated the same procedure i.e. placed the samples into e-cups again into melted Rotiplast and in the oven for 12 hours at 61-63^0C temperature.

On the next day, took out the stem samples from the e-cups and were placed in the vertical position into the prepared aluminum bags and poured the melted Rotiplast in these aluminum bags and covered fully the stem sample with the melted Rotiplast. Then placed at room temperature for cooling.

Then, stem samples with solid Rotiplast were taken out and fixed on to the small wooden blocks, after scratching and heating the one side of wooden block for fixing the stem samples with Rotiplast. Then with a warm needle again fixed well the samples by removing the gaps between Rotiplast and wood surface. Then the samples were placed again at the room temperature.

Then 25 µm thick stem cross sections were made by sliding microtome (Reichert-Jung, Heidelberg, Germany). Sections were put on the glass slide on a drop of distilled water and were placed on hot plate at 40^0C for overnight for getting the section to expand and fixed on to the glass slides.

Then in the next step, the sections were de-paraffinized after passing the glass slides having sections on them, through several solutions shown below for 5 minutes in each, step by step.

1st step : 100 % rotihistol
2nd step : 100 % roti histol
3 rd step : 100 % roti histol
4th step : 100 % roti histol
5th step : 1:1 ratio of roti histol and Isopropanol
6th step : 100 % Isopropanol
7th step : 100 % Isopropanol
8th step : 96 % Ethanol
9th step : 70 % Ethanol
10th step : 50 % Ethanol
11th step : 30 % Ethanol
12th step : distilled water

These sections were stained at room temperature for 10 minutes in 0.05% Toluidine blue in 0.1 M phosphate buffer pH 7. The excess stain was removed by flowing gently the distilled water over these slides. Then the sections were put for drying at room temperature in dark condition. The glass slides were then mounted with cover slips after embedding the sections in Euparal.

Photographs were taken at different focuses after polymerization by putting lead blocks on the cover slips. Well-stained sections and a micrometer scale were photographed under a light microscope (Axioskop, Zeiss, Oberkochen, Germany) with a digital camera (Nikon Coolpix 4500, Nikon, Tokyo, Japan) with 10x, 20x and 40x magnifications. All the photographs of the young developing xylem

portion were taken, which developed during the normal, drought and re-watering period just adjoining the phloem portion. Then finally the stem cross sections were analyzed by Image J. Analysis Software for the following parameters:

Per cent vessel lumen area, Per cent fiber lumen area, Per cent Ray Parenchyma area, Per cent cell wall and middle lamella area, no. of vessels per mm^2, vessel lumen area and vessel diameter (µm). The area unit was in μm^2.

Volatile measurements

Volatiles in the above ground plant parts of the seedlings growing in boxes were measured on the 9^{th} day of drought for two plants each for control normal (CN), control drought (CD), *Paxillus* normal (PN) and *Paxillus* drought (PD); and again on the 11^{th} day of drought for two plants each for CN, CD, PN and PD treatments.

Sampling of volatiles

Samples for GC-MS/EAD analysis were collected using the method of closed-loop-stripping-analysis (CLSA)[Boland *et al*., 1984]. Above ground portion of the seedlings growing in the plastic boxes i.e. stem along with leaves of poplar were covered with the bags of polyethylene film, stainless steel capillaries (I.D. 1 mm) and a miniature pump (Fuergut, Tannheim, Germany) circulated the air in the bags through an adsorbent trap loaded with 1.5 mg charcoal (CLSA-Filter, Daumazan sur Arize, France). Sampling was performed for 2 h with a flow of 1 l/min. Volatiles were eluted from the charcoal with 75 µl of a mixture consisting

of methylene chloride (2 parts) and methanol (1 part) (both solvents Suprasolv-quality, Fa. Merck/VWR, Darmstadt, Germany).

GC-MS system

The GC-MS system consists of a 6890N gas chromatograph connected to a 5973N quadruple mass spectrometer (both Agilent, Palo Alto, USA). The GC is equipped with a type 7163 autosampler and a split/splitless injector. Data acquisition is done with the MS ChemStation Software (Agilent). A J&W Scientific HP-5MS column (Agilent) is used (length 30m, ID 0.25 mm, film thickness 0.25 µm).

One µl samples are injected into the injector in the pulsed splitless mode (pulse pressure 150 kPa until 1.5 min) at a temperature of 250°C. The GC is operated in the following temperature program: start: 50°C, hold for 1.5 min, increasing 6°C/min to 200°C, hold for 5 min. Helium (purity 99.999%) is used as carrier gas after passing through a combined adsorbent trap for removal of traces of water, oxygen and hydrocarbons ("Big Universal Trap", Agilent). The carrier gas flow is set to 1 ml/min resulting in a gas vector of 24 cm/s. The GC-MS interface is operated at a temperature of 280°C.

The mass spectrometer uses electron ionisation (EI) at 70 eV and is used in the scan mode with a mass range from 35-300 mass units at a scan speed of 2.78 scans per second.

For peak identification the NIST mass spectral library (National Institute of Standards and Technology, Gaithersburg, USA) and the Mass Finder 2.1 software

together with the library "Terpenoids and Related Constituents of Essential Oils" (D.H. Hochmuth, W.A. Koenig, D. Joulain, Hamburg, Germany) are used.

Experimental design and statistical analysis

Treatments:

Presence or absence of mycorrhiza and/or hydrogel	Four (Control, *Paxillus involutus* Maj strain, Hydrogel, *Paxillus involutus* Maj strain+hydrogel)
Stress stages	Three (Normal watering, drought, re-watering)
Design	Factorial CRD
Replications	Eight

There were 24 seedlings in each treatment, consisting of 8 seedlings in each watering regime. So a total of 96 seedlings were used in this study.

Analysis of variance (ANOVA) followed by Tukey's HSD test ($P<0.05$) was used to determine the effects of treatment and watering regimes on the biometric and physiological parameters. Statistical procedures were carried out with the Software Package SAS (SAS Institute Inc., Cary, NC, USA, ©1989-2003). Significant differences were based on $P\leq0.05$.

RESULTS

Experiment I: Drought stress of planting stock of beech and their survival in the field plantation

Bud swelling, bud break, seedling survival, mycorrhization and fluorescence

Bud swelling and bud break in the seedlings of 0 h exposure completed after 46 and 56 days, respectively, of the transplanting, however, these were completed after 52 and 70 days, respectively in 2 h exposure; and 95 and 123 days, respectively of the transplanting in 6 h exposure. There were no clear differences in bud break between the seedlings with the inoculation or absence of mycorrhiza and hydrogel, except that the bud break started earlier in the control plants irrespective of exposure compared to their respective inoculated plants. But longer exposure of bare rooted seedlings to air exposure delayed the bud opening compared to control (Fig.1).

Furthermore, planting stress for 6 h had adverse effects on the survival of the beech seedlings compared with 0 or 2 h. But it was also found that the survival in the hydrogel + mycorrhizal inoculated plants for 6 h root air exposure was slightly higher than that of its respective control. Also the planting stress for 6 h had significant adverse effect on mycorrhization compared with shorter exposure of 0 or 2 h. The effect of increasing air exposure on the anatomy of the

mycorrhizal beech root tip's cross-sections can be clearly seen in the Plate 4. Also the anatomical differences between the mycorrhizal and non mycorrhizal root tip's cross sections at 0 h exposure can be compared clearly in the Plate 5 at 20f and 40f magnification. The quantum yield of chlorophyll fluorescence (Fv/Fm) and photochemical quenching (q_p) parameters were not negatively affected by any treatment except that mycorrhizal plants had slightly higher values compared to their respective controls (Table 6).

Seedling development

Growth parameters were influenced by inoculation and air exposure. Exposure of all bare rooted beech seedlings to increasing air exposure, affected adversely all the biometric parameters compared to control in the period between planting to harvest. Mycorrhizal and hydrogel treatment of the air exposed bare rooted seedlings had more increased in root collar diameter, shoot length, root length and number of buds as compared to their respective non-inoculated control plants during the period from planting to harvest (Table 7).

Likewise, increasing air exposure negatively affected the periodic increment in whole plant fresh and dry weight as well as in the whole plant fresh weight without considering the fresh leaves mass between planting to harvest (Table 8). The plants which were exposed to lower duration of 0 and 2 h developed comparatively more new buds than the plants of 6 h exposure and also had significantly less old buds which could not develop into leaves till the end of the growing season. It was also observed that the inoculated seedlings had comparatively more emergence of new buds compared to their respective non-

inoculated seedlings (Table 9). Similarly, the inoculated seedlings accumulated more biomass of fine and coarse roots at all exposures compared to their non-inoculated counterparts. But increasing air exposure had adverse effect on the accumulation of fine and coarse root biomass (Table 10).

Seedlings inoculated with *Paxillus involutus* + hydrogel, developed more leaf fresh and dry mass as well as area at all air exposures compared to their respective non-inoculated plants. Furthermore, the leaf biomass was significantly more in case of 0 h; and leaf area in case of 0 and 2 h exposed inoculated plants compared to their respective non-inoculated plants. Number of leaves were also more in case of inoculated plants compared to non-inoculated controls at all air exposures. Likewise, the leaf area per unit mass was also slightly more in case of inoculated plants at all air exposures (Table 11).

Effect of exposure on vitality parameters

Increasing air exposure to bare rooted seedlings in spring had considerably negative effects on the vitality of top buds, whereas the health of lower buds and fine roots seemed not to be influenced (Table 12, Fig.2). Likewise it has also been found that the increasing air exposure before planting, did not significantly affect the vitality of fine roots and middle buds at harvest in the month of August (Table 13, Fig.3).

Effect of exposure on carbohydrate concentration

Allocation of metabolic solutes differed between plant organs following air exposure. Soluble carbohydrate (glucose and fructose) levels were higher in buds compared to fine roots following air exposure in spring, whereas the

concentrations of sucrose and starch were low in buds compared to fine roots (Table 14, Fig.4; Table 15, Fig.5). But the concentration of soluble carbohydrates (glucose, fructose, sucrose) and starch at harvest (August) were found more in fine roots compared to leaves (Table 16, Fig.6; Table 17, Fig.7).

Glucose accounted for the largest proportion of total soluble carbohydrates (69-72% and 65-67% in buds and roots, respectively) in spring following air exposure, whereas the glucose concentration in the inoculated and non-inoculated plants accounted for 58-62% and 50-75% of the total soluble carbohydrates in leaf and fine roots, respectively, at harvest.

Increasing air exposure to bare-rooted seedlings resulted in significant increase in soluble carbohydrates in buds and fine roots in spring, as a result of increase in glucose and fructose concentration. Sucrose and starch concentrations were not affected by the exposure treatment in buds whereas slightly increased in fine roots towards 6 h exposure in spring (Table 14 and 15). All the soluble carbohydrates and starch were not affected by air exposure treatment in leaf and fine roots following final harvest in August. But it was also found that the concentration of soluble carbohydrates and starch in leaf and fine roots were comparatively low in inoculated seedlings compared to their respective non-inoculated controls, following final harvest in August (Table 16 and 17). The concentration of soluble carbohydrates (glucose and fructose) and starch were higher in March than in August in fine roots (Table 15 and 16).

Moisture content

Exposure of bare rooted seedlings to the increasing air exposure, caused a decrease in moisture content. Relative moisture loss of the seedlings was 20.73% and 23.04% for 2 and 6 h, respectively following air exposure, before planting in the field. There was also a reduction in the moisture content to the tune of 14.84% and 19.54%; and 16.17% and 19.72%, respectively, for 2 and 6 h air exposure, for the plants selected for TTC and carbohydrates measurement in spring (Table 18).

However, air exposure could not lead similar effect on moisture at the time of harvest (August) in the inoculated and non-inoculated plants, though all the inoculated plants had slightly more relative moisture content compared to their non-inoculated controls. It has also been found that inoculation of seedlings with mycorrhiza + hydrogel reduced the detrimental effects of spring air exposure on root/shoot dry weight ratio at the end of growing season. This root/shoot ratio was 1.99-, 2.44-, 2.19- folds higher and leaf/root ratio was 1.64-, 1.85-, 1.2- folds higher, respectively, in the inoculated plants of 0, 2 and 6 h air exposure compared to their non-inoculated counterparts, however, the coarse/fine root dry weight ratio was 1.22-, 1.06-, 1.73- folds higher, respectively, in non-inoculated plants of 0, 2 and 6 h air exposure compared to their inoculated counterparts (Table 19).

Leaf C/N analysis

Exposure of bare rooted seedlings to increasing air exposure before planting, did not affect significantly the nitrogen per cent, carbon per cent and C/N ratio in leaf within each air exposure between the mycorrhizal and non-mycorrhizal inoculated plants at harvest. Per cent N in leaf was comparatively

more in 2 and 6 h exposed plants than that of 0 h. But the per cent carbon was slightly more in unexposed plants compared to the plants of increased exposure. The leaf C/N ratio of 0 h exposed plants was higher compared to 2 and 6 h exposed plants. The mycorrhizal inoculated plants yielded slightly more leaf C/N ratio in 2 and 6 h air exposed but not for unexposed plants (Table 20).

Experiment II: Drought and re-watering cycle under controlled conditions in poplar

Effect of water stress on mycorrhizal colonization and biometrics parameters

At the harvest of the experiment, mycorrhizal colonization of the roots was similar for both the treatments (*Paxillus involutus* + hydrogel; *Paxillus involutus*) under normal watering condition, with about 76% of the fine roots infected with ecto-mycorrhizal fungi. The mycorrhizal and non-mycorrhizal differentiation in poplar root's cross-section is shown in Plate 6. In all non-inoculated seedlings, there was also mycorrhizal colonization of the fine roots, but the percentage of the mycorrhization was below 3 per cent. At the end of the drought cycle, mycorrhizal colonization of the drought stressed seedlings in both the treatments (*Paxillus involutus* + hydrogel; *Paxillus involutus*), reduced significantly compared to the inoculated normal watering plants. Mycorrhizal colonization of the seedlings inoculated with *Paxillus involutus* + hydrogel showed high colonization under re-watering regime when compared with the *Paxillus involutus* re-watered seedlings (Table 21).

Biometric parameters were influenced by the *Paxillus involutus* and/or hydrogel treatments and water stress. Significantly increased growth under normal

watering condition before the start of drought, between two consecutive measurements from 22.9.2004 to 9.10.2004, was noted first time for the parameter collar diameter, in ecto-mycorrhizal seedlings compared to the non-inoculated control (Table 22).

At the end of the experiment, statistically significant differences ($P<0.05$) among different treatments were observed for collar diameter, height, number of leaves, plant fresh and dry weight; fresh and dry mass of leaves, shoots, fine roots and coarse roots. Non-stressed seedlings generally had greater biometric parameters than drought stressed seedlings. Drought stressed seedlings of the dual treatment with *Paxillus involutus* Maj strain + hydrogel generally had significantly more growth for collar diameter, plant fresh and dry weight, fresh mass of leaves; fresh and dry mass of shoots, fine roots and coarse roots than control water stressed seedlings (Table 23 and 24).

Effect of water stress on moisture content

Water stressed control seedlings displayed significantly lower relative water content (RWC) in whole plant, leaves and shoots than its control seedlings growing under normal watering condition. Drought also resulted in the decrease of RWC in whole plants, leaves and shoots of other stressed mycorrhizal and/or hydrogel inoculated poplar seedlings compared to their respective normal watering counterparts, but in such cases, the reduction in RWC was not significant. It was also observed that the drought-induced decrease in RWC was higher in leaves than shoots in all the treatments (Table 25). The coarse to fine root ratio on a dry weight basis was highest (2.25) in control stressed plants and was lowest (1.34) in both

ecto-mycorrhizal + hydrogel inoculated normal watering plants. The leaf to root dry weight ratio was decreased under the drought stressed seedlings compared to their respective normal watering plants. Among the stressed seedlings of all the treatments, it was highest in control stressed seedlings (2.31) and was lowest (1.71) in dual inoculated stressed seedlings (Table 25).

Effect of water stress on plant water relations

The effects of ecto-mycorrhizal and/or hydrogel inoculation on the pre-dawn leaf water potential during and after recovery from drought stress are shown in Tables 26 and 27. Pre-dawn leaf water potential values were high and constant in non-stressed plants throughout the experimental period. The decrease in pre-dawn leaf water potential on the 3rd day of drought was significant for all drought-stressed treatments except dual inoculation with ecto-mycorrhiza + hydrogel with respect to their normal watering plants. It was also significantly low on the 8th (-1.387 MPa) and 9th (-0.953 MPa) day of drought in the control stressed seedlings compared to their normal watering plants. On the day of harvest, all the stressed seedlings except dual inoculation, had significantly low pre-dawn leaf water potential, compared to the normal watering plants. The stressed seedlings with dual inoculation could maintain a significantly higher pre-dawn leaf water potential on the day of harvest compared to the control stressed seedlings (Table 26).

During recovery from drought, all the stressed re-watered seedlings at equal stem height except control re-watered seedlings (-1.280 MPa), recovered well their pre-dawn leaf water potentials and had the similar values to their respective normal

watering plants. Control re-watered plants showed signs of severe damage to leaf due to drought stress, when considered that leaf for the determination of water potential at the same height (from collar region towards apex). However, recovery in the pre-dawn leaf water potential was also equally good for the controlled re-watering plants as well, when taken the healthy leaf for the determination of the water potential; and this healthy leaf was present at more height compared to other treatments (Table 27). The after-effects of water stress on water potentials were more pronounced in the control seedlings.

Effect of water stress on gas exchange parameters

Plants infected with ecto-mycorrhizal strain had slightly higher assimilation rates than uninfected plants under normal watering condition. Water stress decreased the photosynthetic and transpiration rates in all the treatments on 2^{nd} and 8^{th} day from the start of drought. Two days after the start of drought, the photosynthetic and transpiration rates started decreasing in the stressed seedlings, compared to their respective normal watering plants, however a higher reduction in photosynthetic rate was observed in control drought plants than in ecto-mycorrhizal and/or hydrogel stressed seedlings, a behaviour that was maintained on 8^{th} day of drought as well. However, the reduction in photosynthetic capacity was significant in the control stressed seedlings on the 8^{th} day of drought compared to the normal watering seedlings. Transpiration decreased approximately 2-folds on the 8^{th} day of drought in all the stressed seedlings compared to their respective normal watering seedlings (Table 28).

Effect of water stress on chlorophyll fluorescence

The quantum yield of chlorophyll fluorescence (Fv/Fm) and photochemical quenching (q_p) parameters were not negatively affected by any treatment under normal watering condition. The chlorophyll fluorescence yield (Fv/Fm) exhibited only small fluctuations during the drought and recovery period of the dark-adapted leaves and did not cause significant change in the F_v/F_m parameters, indicating that photo-system II was undamaged. The fluorescence and photochemical quenching parameters started showing minor increase in the stressed seedlings, with the progress of drought compared to their respective normal watering plants; and then showed a decreasing trend of Fv/Fm at the extreme stage of drought, first for the control stressed plants. Mean F_v/F_m (0.795) of control stressed plants at harvest, was lower than the stressed ecto-mycorrhizal and/or hydrogel treated seedlings, although serious damage was not observed. From the initial stage of drought to the 8^{th} day, the dark-adapted leaves of all the stressed seedlings did not show the values of non-photochemical quenching (qN and NPQ). The non-photochemical quenching (qN= 0.001 for PD, 0.005 for CD; and NPQ= 0.001 for PD, 0.004 for CD) started showing their values from the 9^{th} day of drought and their values (qN= 0.004 for HD, 0.010 for PD, 0.020 for CD; and NPQ= 0.003 for HD, 0.008 for PD, 0.017 for CD) were on the increasing trend towards the hard stress of harvest (Table 29 and 30).

During recovery from drought, on the first day of re-watering, the values for Fv/Fm and photochemical parameters were even towards on the decreasing trend for HR, PR and CR; and the values for the non-photochemical quenching

parameters were towards increasing trend compared to the drought harvest day; however on the 2nd day of re-watering, all the values for the stressed re-watered seedlings reached towards normalcy (Table 31).

Effect of water stress on soluble sugars and starch

In fine roots collected at the time of harvest, before re-watering, there were more soluble carbohydrates in all the drought stressed plants than their respective normal watering plants, as a result of increase in glucose and fructose concentrations. Glucose accounted for the largest proportion of total soluble carbohydrates (53-60%) in fine roots of the drought stressed plants; whereas in re-watering plant roots, it was 51-66%. Sucrose and starch concentrations were not affected by drought in all the treatments. Only the concentrations of glucose and fructose were significantly increased in the control stressed plants compared to the normal watering control plants, though the concentrations of glucose and fructose increased in all the other treatments as well, but the effect was not significant. The glucose and fructose concentrations were even lower than their respective normal watering plants in all the treatments except in hydrogel re-watering treatment, during recovery from the water stress (Table 32, Fig.8).

Effect of water stress on leaf C/N analysis and vitality parameters

The vitality parameters of the leaves were not affected by drought and recovery in all the ecto-mycorrhizal and/or hydrogel inoculated and non-inoculated seedlings. Drought did not affect significantly the C/N ratio in leaves among the stressed ecto-mycorrhizal and/or hydrogel and non-inoculated seedlings compared to their respective normal watering plants. Per cent nitrogen in leaf was found

comparatively more in the normal watering plants with respect to their respective stressed plants and thus resulted in the higher C/N ratio of the stressed plants compared to the normal watering plants. It was also being observed that C/N ratio was comparatively higher in control stressed (19.866) and re-watering (19.051) plants compared to all other stressed treatments and was lowest in dual inoculated stressed (18.122) and re-watered (17.451) seedlings (Table 33).

Effect of water stress on ion composition in phloem and young developing xylem

The concentrations of phosphorus, potassium and calcium ions in the phloem of the poplar stems did not reduce significantly in the stressed re-watered plants compared to the normal watering plants. However, it was found that the phosphorus (0.826) and potassium (4.711) ion concentration reductions were more in control stressed re-watered plants compared to all the other stressed re-watered plants. The highest concentration of potassium was found in the phloem portion of the poplar stem followed by calcium and phosphorus ions (Table 34, Fig. 9a, b, c).

The statistical significant reductions were found for the phosphorus, potassium and calcium ion concentrations in the young developing xylem of the control re-watered plants compared to its control normal watering plants. In young developing xylem of the other re-watered plants of ecto-mycorrhizal and/or hydrogel treatments, statistically non significant reductions were there in the phosphorus and potassium ion concentrations compared to their respective normal watering plants except the higher concentration of phosphorus ion was found in the dual inoculated stressed re-watered plants compared to its normal watering plants. The calcium ion concentration (0.563) of the single ecto-mycorrhizal inoculated

seedlings, also reduced significantly in the xylem portion but the reductions were minor in the dual inoculation and single hydrogel treatment (Table 34, Fig.9a,b, c).

Effect of water stress on wood anatomical properties

Image analysis of the xylem portion of the poplar stem indicated an effect of water stress on the wood anatomical properties (Table 35, Fig.10a, b). Differences in per cent vessel lumen fraction and vessel lumen area were evident in the young developing xylem in response to water limitation and treatments (Plate 7, 8, 9). Per cent vessel lumen fraction and area was comparatively more in all the normal watering plants compared to the drought stressed and re-watered plants except the vessel fraction in the stressed hydrogel (35.49%) plants. The significant differences were not found for the fiber, ray parenchyma and cell wall fraction in the young developing xylem of all the treatments. However, the per cent cell wall fraction was highest in control stressed (38.21%) and re-watered (38.76%) plants among all the drought stressed and re-watered plants. Ray parenchyma fraction (approx. 3.5-6 %) contribution accounted for the least in the xylem wood (Table 35). Significant reduction in vessel size was observed for control stressed plants when compared to the single ecto-mycorrhizal inoculated stressed plants. Mean vessel lumen area and vessel diameter declined in all the stressed seedlings and there was a trend for increased vessel frequency in the water-limited plants (Plate 8) of each treatment. However, the differences in the vessel frequency were not significant but the highest numbers of vessels were present in the control stressed (168.61 no. mm^{-2}) plants (Table 35, Plate 8).

Effect of water stress on volatile release by the plants

The prompt release of volatile compounds was investigated from the above ground portion of the poplar following drought. These volatiles play a significant role in the atmosphere chemistry. Among all the volatile compounds, 2 Hexenal, Hexanal, .alpha Farnesene and 2 Nonen 1 ol were released only by the 50%, 25%, 50% and 25% of the plants observed respectively from the control drought treatment. 1,3,6 Octatriene 3, 7 diemethyl (Z) and nonanal were released from the drought stressed plants of ecto-mycorrhizal and control only, whereas Heptacosane was released by the stressed ecto-mycorrhizal plants. 3 Hexen 1-ol acetate (Z) was the only volatile compound which was released by the 100%, 50%, 25% and 75% of the plants observed respectively from control normal, Paxillus drought, control drought and Paxillus normal watering plants. Silicic acid (H4sio4) tetramethyl ester was released more by the stressed and non-stressed ecto-mycorrhizal plants compared to the non-inoculated plants (Table 36).

DISCUSSION

Experiment I: Drought stress of planting stock of beech and their survival in the field plantation

Bud swelling, bud break, seedling survival, mycorrhization and chlorophyll fluorescence

In the present study, no mortality was observed in beech for the shorter exposure of 0 h and 2 h, and the same has been observed by Girard *et al.* (1997a) in red oak seedlings. In accordance to the results obtained by Guehl *et al.* (1993) and Girard *et al.* (1997b) with Corsican pine; and by Bates and Niemiera (1997) with Washington hawthorn and Norway maple, it was found that exposure of bare rooted beech seedlings to air exposure delayed the bud opening compared to control (Fig. 1) and adversely affected the survival in the longer exposure of 6 h after outplanting (Table 6), indicating that broad-leaved tree species are prone to air exposure even during the spring season, when there are no leaves, the main transpiring organ of the plant. The adverse effect of longer duration of exposure (6 h), on survival and delay in bud break was to be expected as water loss from the plant tissue is considered a major stress (Table 18; Koslowski, 1985; SongZhu *et al.*, 2004). Previous studies have also shown the effect of desiccation in decreasing survival of Sitka spruce and Douglas-fir (McKay and White 1996; Tabbush, 1987),

loblolly pine (Feret *et al.*, 1985) and ash and birch (Insley and Buckley, 1985). In this present study on beech, critical levels of seedling exposure to drying conditions are shorter than 6 h. But, Hermann (1967) concluded that dormant hardwood plants could generally withstand prolonged root exposure without appreciable damage. Furthermore, Murakami *et al.* (1990) showed that *Crataegus phaenopyrum* L. was more sensitive than *Acer platanoides*, even though the two species lost water at the same rate, as species differ in the extent to which roots desiccate in a given treatment (Insley 1979; Tabbush, 1987; McKay and White, 1996). So a direct comparison of critical exposures even for identical stock is meaningless, without knowledge of the environmental conditions during desiccation treatments.

Planting stress for 6 h had significant adverse effect on mycorrhization compared with shorter exposure of 0 or 2 h (Table 6, Plate 4). This effect is in contrary to the effect of drought on mycorrhization by Shi *et al.* (2002). But unfortunately, similar work studying the effect of increasing air exposure on mycorrhization could not be found, so no direct comparison can be made.

Air exposure did not affect the quantum yield of chlorophyll fluorescence and photochemical quenching parameters (Table 6), as PS II is highly drought resistant, as found in investigations on the impact of various environmental stresses (drought, heat and strong irradiance), applied separately or in combination (Havaux, 1992); and also shown by Flagella *et al.* (1998) that quantum yield of PS II is reduced only under drastic water deficit.

Seedling development

Exposure of bare rooted seedlings to increasing air exposure in spring, after outplanting led to reduced growth parameters i.e. reduced biomass in different plant organs, leaf area, new bud formation as well as the reduced periodic increment in collar diameter, shoot length, root length and number of buds between planting to harvest in the field (Table 7-11). Similar results were obtained in different broad-leaved and conifers species (Englert *et al.*, 1993; Girard *et al.*, 1997a, b; Webb and von Althen, 1980). In the present study at all air exposures, the inoculation of beech seedlings with ecto-mycorrhiza and hydrogel has resulted in more periodic increment in the collar diameter, root length, shoot length, plant fresh and dry weights, number of buds; and also more accumulation of individual root, shoot and leaf biomass, and leaf area compared to their respective non-inoculated control plants at the end of growing season. This positive influence of ecto-mycorrhizal association on all the biomass parameters, could be attributed to the enhanced inorganic nutrition absorption in inoculated plants (Allen *et al.*, 1981; Cooper, 1984).

At all air exposures, the inoculated beech seedling had a higher root-shoot and leaf-root ratio (Table19), as compared to their respective non-inoculated plants, and this higher root-shoot and leaf-root ratios of the inoculated plants also could be attributed to the effect of mycorrhizal infection, which could have increased nutrients absorption, giving rise to a higher root, shoot and leaf biomass increment. Kucey and Paul (1982), Douds *et al.* (1988) and Wang *et al.* (1989) have reported the same effect on root-shoot ratio. Also the dry matter related ratio

of leaves to roots was diminished by 6 h air exposure compared to 0 and 2 h, though not significantly among the different treatments. These results are in accordance with those of Frank and Thomas (2000) in *Quercus* species. Beech seedlings with higher root to shoot ratio at 6 h exposure in inoculated plants, are able to have comparatively higher survival percentage than respective non-inoculated seedlings. Increasing air exposure also reduced the leaf area by reducing the biomass partitioning to the leaves at higher exposures. The same effect of reduced leaf area by drought was observed by Hees (1997) in pedunculate oak and beech. Based on the observation of growth parameters, this study also suggest that with the increasing air exposures, non-inoculated beech seedlings which also had a small ratio of root-shoot biomass were more affected by desiccation compared to the inoculated seedlings. As this study was limited only to one growing season, the more regeneration of new buds in the inoculated seedlings might lead to more significant advantages in the next growing season.

Effect of air exposure on vitality parameters

Damage to upper buds in spring, as indicated by the physiological plant quality measures of tetrazolium absorbance, increased rapidly over drying periods of 2 and 6 h, whereas the health of lower buds and fine roots seemed not to be influenced (Table 12, Fig.2). Similar to spring, air exposure treatment before planting to the seedlings, did not affect the health of the fine roots and middle buds when measured at the end of the growing season in August (Table 13, Fig.3). No effect of air exposure on beech fine root vitality in this study is in conflict with those of Symeonidou and Buckley (1999), and they reported damage to fine roots

in *Prunus avium* and *P. cerasifera* as a result of increasing exposure. The reason for the differences in vitality parameter of fine roots is owing to the intrinsic ability of withstanding planting stress of different species.

Effect of air exposure on carbohydrate concentration and C/N plant composition

Air drought stress of the bare rooted beech seedlings in spring, resulted in the significantly increased concentrations of glucose and fructose in buds and fine roots, whereas the effect was not significant on the sucrose and starch concentrations (Tables 14, 15, Figs. 4, 5). Six hour exposed treatment exhibited the highest concentration of soluble sugars, thus accumulated 69% and 31%, respectively glucose and fructose in the buds; and 67% and 31%, respectively glucose and fructose in the fine roots.

Air exposure to the un-inoculated seedlings at the end of growing season resulted in the increased concentration of soluble sugars in fine roots and leaves compared to their respective ecto-mycorrhizal treated plants (Tables 16, 17, Figs. 6, 7). This decreased concentration of soluble sugars in these parts may be attributed to mycorrhizal association, as the host provides the fungal partner with carbohydrates (Shi *et al.*, 2002).

Per cent N in leaf was comparatively more in 2 and 6 h exposed plants than that of 0 h. Similar results on N concentration but in shoots were presented by Pedro *et al.* (1999) in *Pinus halepensis*. The ecto-mycorrhizal inoculated plants yielded slightly more leaf C/N ratio in 2 and 6 h air exposure compared to their respective non-inoculated plants, but it was reverse for unexposed plants (Table 20) and this higher C/N ratio may be attributed to the efficiency of *Paxillus*

involutus Maj strain exploiting the soil surrounding the roots more under increasing stress conditions. Similar differences for nutrient uptake were reported by Mitchell *et al.* (1984).

From the physiological health point of view of the plant, these results are more important because field performance of the seedlings has been positively related to nitrogen (Van Den Driessche, 1991) and carbohydrate concentration (Puttonen, 1986). Soluble sugars play an important role in osmotic adjustments (Gebre *et al.*, 1994; Premachandra *et al.*, 1995) and in dehydration tolerance processes (Santarius, 1973). Significantly increased accumulation of soluble sugars in beech in spring suggest that osmotic adjustments may have occurred both in buds and fine roots. Previous studies have also reported a positive effect of drought on nutrient and soluble sugars (Morgan, 1984; Epron and Dreyer, 1996; Pedro *et al.*, 1999; Rehman *et al.*, 1996; Timmer and Miller, 1991).

Experiment II: Drought and re-watering cycle under controlled conditions in poplar

Effect of water stress on mycorrhizal colonization and biometric parameters

Paxillus involutus Maj strain formed ecto-mycorrhizae under both watering and drought regimes and the effect of drought on the anatomy of ecto-mycorrhizal root tips cross sections can be seen in Plate 10. The ecto-mycorrhization was formed by about 76% in adequate watering conditions and by about 65-66% under drought stress conditions. Drought stress had significant adverse effect on the ecto-mycorrhization compared to the inoculated normal watering plants (Table 21, Plate 10). Although, mycorrhizal colonization levels have sometimes been

reported to be unaffected by water stress (Morte *et al.*, 2001; Nelsen and Safir, 1982; Allen and Boosalis, 1983; Simpson and Daft, 1990); but, these results are in agreement with the work of Osonubi *et al.* (1991), Busse and Ellis (1985) and Mohammed *et al.* (1992). A reduction in mycorrhizal colonization by drought stress is dependent on root exudates (Graham *et al.*, 1982; Schwab *et al.*, 1983) which under drought stress would be limited due to reduced photosynthesis, as stomata most often remain closed to conserve water. In addition, water shortage in the soil can reduce and delay the spore germination (Tommerup, 1984), root growth and thereby subsequent mycorrhizal development.

During the initial stage of poplar seedling development, collar diameter was the first parameter, among all the morphological parameters, in which significantly increased growth was observed for the ecto-mycorrhizal seedlings compared to the non-inoculated control (Table 22), most likely as a result of improved nutrition through greater ecto-mycorrhizal infection (Dickie, 2000).

Ecto-mycorrhizal efficiency can be measured in terms of host plant growth under different water stress regimes. In this study, the drought stressed *Paxillus involutus* Maj strain+hydrogel treatment maintained the significantly higher ($P<0.05$) growth of collar diameter; plant fresh and dry weight; fresh mass of leaves; fresh and dry mass of shoots, fine and coarse roots than control water stressed seedlings. The effect was more pronounced in fresh shoots biomass than the fresh roots biomass (Tables 23, 24), which may be because ecto-mycorrhizal colonization caused a proportionally greater allocation of carbohydrates to the shoots than to the root tissues (Schwob *et al.*, 1998). Similar benefits of

mycorrhization were reported earlier by Jesus *et al.* (2004) in *Rosmarinus officinalis*, Ortega *et al.* (2004) in *Pinus radiata* and of hydrogel by Viero *et al.* (2002) for *Eucalyptus grandis*, Huettermann *et al.* (1999) for *Pinus halepensis*, Davies and Castro Jimenez (1989) for *Lagerstroemia indica* L. cultivar Natches.

The dry matter related ratio of leaves to roots was decreased in all the treatments under the drought-stressed seedlings, compared to their respective normal watering plants. This diminished ratio of leaves to roots in stressed plants is in agreement with those of Mazzoleni and Dickmann (1988) in *Populus* clones and Frank and Thomas (2000) in *Quercus petraea* and *Q. robur* in moderate stress. But when compared among the stressed seedlings of all the treatments, this ratio was highest in control stressed seedlings (2.31) and was lowest (1.71) in dual inoculated stressed seedlings, and this can be explained by the cessation of root growth at the extreme stage of drought in the non-inoculated plants. On contrary to leaf root ratio, the dry weight ratio of coarse root to fine root increased under the drought stressed plants compared to their respective normal watering plants. This ratio was highest (2.25) in control stressed plants and was less and comparable in all the stressed ecto-mycorrhizal and/or hydrogel inoculated seedlings (PHD=1.45, HD=1.79, PD=1.40, Table 25) and this can be due to even larger impact of drought on the poplar fine root biomass.

Drought had promoted leaf shedding in poplar, in all the stressed ecto-mycorrhizal and/or hydrogel inoculated, and non-inoculated seedlings. But leaf shedding was comparatively more in control stressed plants. Increased leaf shedding in moderately and severely stressed poplar in response to drought

acclimation has been reported earlier by Dickmann *et al.* (1994), Kelliher *et al.* (1980), Kelliher and Tauer (1980) and Schulte *et al.* (1986).

Effect of water stress on moisture content and plant water relations

The poplar seedlings were exposed to 12 days drought treatment. The relative water content of the plants, leaves and shoots were clearly reduced by drought in all the treatments and to a significant amount in the control stressed seedlings. Leaves were more strongly affected than shoots (Table 25). The reduction in relative water content of the plant organs are in agreement with the findings of Peuke *et al.* (2002) for identifying drought sensitive beech ecotypes. The loss of water from tissues may lead to an increase in the concentrations of osmotic solutes and result in a lower osmotic potential of the plants (Heuer, 1994).

The pre dawn leaf water potential is a common indicator for drought stress and water status in the plants (Fort *et al.*, 1997, 1998; Picon *et al.*, 1997; Cellier *et al.*, 1998; Tardieu and Simonneau, 1998; Thomas and Eamus, 1999; Schraml and Rennenberg 2000; Fotelli *et al.*, 2001). The leaf water potential during the drought cycle was similar in all the treatments of well water conditions. Drought stress decreased leaf water potential but this decrease was larger in control stressed plants than the ecto-mycorrhizal and/or hydrogel inoculated stressed plants. All the stressed ecto-mycorrhizal and/or hydrogel inoculated and non-inoculated seedlings except dual inoculation, exhibited significantly low pre dawn leaf water potential at the extreme stage of drought compared to their respective normal watering plants. Also the stressed seedlings with dual inoculation could maintain a significantly higher pre-dawn leaf water potential on the day of harvest even,

compared to the control stressed seedlings (Table 26). During recovery from drought, ecto-mycorrhizal and/or hydrogel treated seedlings recovered well compared to the control stressed plants. (Table 27). The after-effects of water stress on the water potential were more pronounced in the control drought seedlings. Amelioration of the effect of drought was observed on *Quercus velutina* seedlings colonized with *Pisolithus tinctorius* (Dixon *et al.*, 1983). A greater osmotic adjustment has also been reported in leaves of mycorrhizal basil plants than in non-mycorrhizal ones during a lethal drought period (Kubikova *et al.*, 2001). In the same way, mycorrhizal inoculated plants had postponed declines in leaf water potential during drought stress (Davies *et al.*, 1992; Subramanian *et al.*, 1997; El-Tohamy *et al.*, 1999) and normal leaf water status returned more quickly in mycorrhizal than non-mycorrhizal maize plants after the relief of drought (Subramanian *et al.*, 1997). In contrast, leaf water potential was similar in mycorrhizal and non- mycorrhizal plants when water was not limiting (Ebel *et al.*, 1996; Bryla and Duniway, 1997; Goicoechea *et al.*, 1998). The mycorrhizal contribution to plant drought tolerance might have occurred through drought avoidance mechanisms such as hyphal water uptake (Hardie, 1985; Ruiz-Lozano and Azcon, 1995; Marulanda *et al.*, 2003) or increased water uptake related to mycorrhizal changes in root morphology (Kothari *et al.*, 1990). Such mycorrhizal effects could allow plants to remain more hydrated than non-AM plants as soil dries (Auge *et al.*, 2001). In this present study, the higher pre-dawn leaf water potential of ecto-mycorrhizal and/or hydrogel stressed plants and the lower accumulation of soluble sugars in their fine roots than in control stressed plants,

suggest the possible beneficial effects of ecto-mycorrhiza and hydrogel on the plant physiology.

Effect of water stress on gas exchange parameters and chlorophyll fluorescence

Net photosynthesis and transpiration rates declined in the stressed plants in response to water limitation on the 2nd and 8th day from the start of drought (Table 28) and the photosynthetic reductions for the stressed plants, under the extreme stage of drought were significant for the control stressed plants (-0.07 μmol $m^{-2}s^{-1}$). Net photosynthesis and transpiration rates in the stressed plants were lower on the 8^{th} day of drought than at the beginning of the drought. Drought reduced transpiration to about 3.45-, 1.88-, 2.24- and 2.22-fold; and net photosynthesis to about 54.7-, 1.38-, 1.16- and 1.86-fold, respectively in the CD, PD, PHD and HD treatments from their controls, which could be related to the maintenance of aerial biomass (Morte *et al.*, 2001), the increase of root biomass, and the increase of the hydraulic conductivity observed in stressed mycorrhizal and/or hydrogel plants (Dell'Amico *et al.*, 2002). Many authors (Nelsen and Safir 1982; Auge *et al.*, 1986) have reported that colonization of the root systems by mycorrhizal fungi can affect the stomatal behaviour of host plants under well-watered conditions, with these effects being often associated with an altered root or whole plant hydraulic conductivity (Allen *et al.*, 1981) or an increased water uptake by extra radical hyphae (Faber *et al.*, 1991). Stomatal response is probably the most important factor controlling carbon fixation. Cornic and Briantais (1991) found in the cultivars of *Phaseolus vulgaris* that stomatal conductance declined before RWC was affected and photosynthesis was largely dependent on stomatal

aperture. These results are in agreement with those reported by Duan *et al.* (1996) and Auge *et al.* (1986), who found high values of transpiration, and leaf water potential in the inoculated water-stressed plants due to a higher rate of water absorption. On the other hand, Subramanian *et al.* (1995) found higher values of photosynthetic rate and leaf water content in mycorrhizal water stressed plants than in non-mycorrhizal stressed plants. In this present study, the high photosynthetic rate observed in ecto-mycorrhizal and/or hydrogel plants (Table 28) could be explained by non-stomatal factors.

Water stress did not cause significant changes in the chlorophyll fluorescence (F_v/F_m) and photochemical quenching parameters of the dark adapted leaves; and exhibited only the small fluctuations during the drought and recovery period in all the stressed treatments except the mean values of F_v/F_m (0.795) of control stressed plants was lowest at harvest than the ecto-mycorrhizal and/or hydrogel stressed seedlings; although, serious damage was not being observed in the efficiency of PS II in the control stressed plants as well, indicated that PS II quantum efficiency did not decline during stress; and further suggested that it is highly resistant as found in investigations on the impact of various environmental stresses (drought, heat, strong irradiance), applied separately or in combination (Havaux, 1992). Flagella *et al.* (1998) showed that quantum yield of PS II, as related to Calvin Cycle metabolism, is reduced only under drastic water deficit. Similar results were also reported by Munne-Bosch and Alegre (1999) that water deficit did not cause an additional decrease in F_v/F_m ratio. Although water stress reduced slightly the efficiency of PS II in control stressed seedlings (expressed as

F_v/F_m) with respect to their well-watered seedlings, the effect of mycorrhizal and hydrogel treatments on this relationship was positive, indicating a higher stability of the photosynthetic apparatus under water stress (Tables 29, 30).

One of the principal mechanisms employed by the plants to prevent or alleviate damage to the photosynthetic apparatus is the non-photochemical chlorophyll fluorescence quenching (qN and NPQ) (Krause and Weis, 1991; Ruban and Horton, 1995). In this mechanism excess radiant energy is dissipated as heat in the light-harvesting antenna of PS II. The non-photochemical quenching (qN, NPQ) values at the time of drought harvest were observed more in the control stressed plants, less in the ecto-mycorrhizal plants compared to the dual inoculation and hydrogel treated plants. These values were even increasing after 24 hours of re-watering and were highest in the order of CR (qN=0.245, NPQ=0.644), PR (qN=0.115, NPQ=0.326) and HR (qN=0.045, NPQ=0.051); and no values for these non-photochemical quenchings were observed for dual inoculated plants. Later after 48 hours of re-watering, a good recovery in the photosynthetic apparatus was there for all the stressed re-watered ecto-mycorrhizal and/or hydrogel plants with a poor recovery in control stressed re-watered plants (qN=0.122, NPQ=0.465, Table 30). Nevertheless, despite the largely unchanged activity of fluorescence yield, the stressed poplar seedlings were characterized by an increase in the non-radiative energy dissipation during hard stress, suggesting that the thermal dissipation of the excitation energy is protecting the photosynthetic apparatus from the oxidative stress. Decline in the photon yield of photosynthesis during desiccation can be either due to the damage of the

photosynthetic apparatus or to the PS II down-regulation (Calatayud *et al.*, 1997). Similar to the recovery of the photosynthetic apparatus in the stressed re-watered poplar seedlings, Deltoro *et al.* (1998) also found a fast recovery of chlorophyll fluorescence parameters when *Frullania dilatata* was re-hydrated which suggest that the decline in PS II efficiency is regulatory, serving a photo-protective role. Enhanced non-photochemical fluorescence quenching (qN) suggests that the observed photo-inhibition (decrease in the PS II efficiency) is due to photo-protective energy dissipation processes (He *et al.*, 1996). Increased energy dissipation may help to protect PS II from over excitation and photo-damage. However, it brings about a decline in the effective quantum yield of PS II photochemistry (Mattos *et al.*, 1999).

Effect of water stress on carbohydrates, leaf C/N ratio and vitality parameters

Dehydration tolerance is often associated with osmotic adjustment (Morgan, 1984; Sinclair and Ludlow, 1986; Ludlow 1989; Gebre *et al.*, 1998). It has been suggested that the drought induced increase in soluble sugar concentration, decreases the solute potential thereby helping cells in maintaining turgor in an environment with low water potential (Venkateswarlu *et al.*, 1989; Irigoyen *et al.*, 1992). The glucose and fructose concentrations in fine roots increased significantly due to drought in the control stressed plants compared to the normal watering control plants; though, the concentrations of glucose and fructose increased in all the rest of drought stressed treatments compared to their respective normal watering plants; but, the effect was not significant among them. Accumulation of soluble carbohydrates in response to water stress has been

attributed to an imbalance resulting from growth being more sensitive than CO_2 assimilation to drought (Thomas, 1990). The decreased starch concentration in the roots suggested that some hydrolysis of starch to hexoses occurred. Such increased differential accumulation of glucose and fructose suggests that ecto-mycorrhizal+hydrogel treatment was more drought tolerant than the other treatments. In all the treatments after re-watering, the glucose and fructose concentrations were even lower than their respective normal watering plants except in the hydrogel re-watering treatment (Table 32). Similar results were obtained on the recovery of sugar concentrations to normal levels after re-watering (Nicolas *et al.,* 2002 in *Populus euramericana* clones; Gebre *et al.,* 1997 in *Populus deltoides* clones), confirming the role of soluble sugars as a contributor to osmotic adjustment.

Drought resulted in the significant increased concentrations of glucose and fructose in fine roots of the control stressed seedlings at the harvest compared to the stressed ecto-mycorrhizal seedlings. This decreased concentration of glucose and fructose in fine roots of the mycorrhizal seedlings may be attributed to the mycorrhizal association, as the host provides the fungal partner with carbohydrates (Shi *et al.*, 2002); and Schellembaum *et al.* (1998) suggested that the mycorrhizal fungus can be a strong competitor for root allocated carbon under conditions of limiting photosynthesis and less competitor under conditions of normal watering. However, the significantly lower concentrations of these sugars in stressed hydrogel plants compared to the control stressed plants, may be due to its contribution towards tolerance under water deficit conditions.

The increased per cent N and decreased C/N ratio (Table 33) in the stressed and non-stressed ecto-mycorrhizal and/or hydrogel plants compared to the stressed and non stressed control plants, may be due to the indirect effect of improved N nutrition by the ecto-mycorrhizal plants, as described earlier by Fitter (1988) and Harley and Smith (1983) and also may be because of encouraging results of hydrogel that has been shown earlier by Huettermann *et al.* (1999) for *Pinus halepensis*, Davies and Castro-Jimenez (1989) for *Lagerstroemia indica* L. cultivar Natches.

The effect of drought and re-watering in any treatment on the poplar leaf vitality could not be observed (Table 33). The direct observation on the leaf vitality through tetrazolium absorbance in the earlier studies could not be found, so comparison of this parameter on the basis of tetrazolium absorbance cannot be made. Also the red stable colour, which was the end product of tetrazolium absorbance in roots and buds of beech in the first experiment, could not be formed for leaves. Green colour of the solution at the time of measuring absorbance, was mainly because of chlorophyll in the leaves.

Effect of water stress on ion composition in phloem and young developing xylem

The phloem of poplar was characterized by high concentrations of P, K and Ca ions compared to the young developing xylem, in all the stressed re-watered and non-stressed seedlings. The concentrations of all these elements reduced in the stressed re-watered plants compared to the normal watering plants. However, the reduction of these ions among different treatments, were not significant in the phloem portion; but significant reductions were found for the phosphorus,

potassium and calcium ion concentrations in the young developing xylem of the control stressed plants compared to control normal watering plants (Table 34).

Nutrient availability declines with soil water content as mineralisation rate and mass flow in the soil decline (Nye and Tinker, 1977), both of which may result in decreasing element uptake and release into xylem conduits. The concentrations of P, K and Ca ions in the xylem and phloem portion of the poplar stem were positively correlated with the leaf water potentials at the initial stage, and also at the final stage of drought harvest. These relationships were even stronger for xylem ion concentration than the phloem ion concentrations (Table 37). Similar positive strong relationships were also reported by Osonubi *et al.* (1988) between element concentrations in the sap and plant water potential. Thus, based on the results of this study, this can be concluded that the decreased concentrations of elements in the xylem and phloem portion of stressed plants, is due to the result of limited supply of nutrients to the xylem from the soil, and decreased supply of photosynthates to the phloem under limited water supply.

Effect of water stress on wood anatomical properties

Drought influences several growth features of plant species such as xylem anatomy and radial growth (Carlquist, 1975; Fritts, 1976; Villar- Salvador, *et al.*, 1997). Several studies have found changes in mean vessel diameter, especially in species with diffuse-porous wood, along climatic gradients of water availability (Baas *et al.*, 1983; Baas and Schweingruber, 1987; Zhang *et al.*, 1992; Woodcock and Ignas, 1994; Sass and Eckstein, 1995). This is because of increased conductive efficiency provided by wider vessels and inter-conduit pits and the increased risk

of cavitation (Tyree and Sperry, 1989; Tyree and Ewers, 1991). In this study, the per cent vessel lumen fraction, vessel lumen area, vessel size reduced in response to drought more in the controlled stressed plants compared to all the other stressed treatments. The cell wall fraction and vessel frequency also increased more in the control drought stressed plants when compared to other stressed ecto-mycorrhizal and/or hydrogel inoculated plants, but this increment was non significant. However, a trend towards greater vessel frequency in water-limited poplar, is perhaps the balancing activity of the plant in maintaining the vessel lumen area. The mean vessel diameter decreased in control stressed plants because most vessels showed smaller diameters (Table 35, Plate 8). Similar effects of drought on the xylem anatomy were also found by Matthew *et al.* (2004) in *Eucalyptus*, Leyre *et al.* (2004) in *Quercus ilex*, February *et al.* (1995) in *Eucalyptus* and Lovisolo and Schubert (1998) in *Vitis vinifera*. The same effect on vessel diameter and frequency was shown in transgenic *Populus* hybrid (*Populus tremula* L. x *Populus tremuloides* Michx.) by Tuominen *et al.* (1995). There was a negative relationship between the per cent cell wall fraction and the vessel lumen area or the vessel diameter of stressed and non-stressed poplar seedlings, but, this negative relationship was stronger in the stressed seedlings (Table 38 and 39). However, the negative relationship was also reported by Matthew *et al.* (2004) in the well watered seedlings but in contrary to this study, their negative relationship broke down in the drought stressed seedlings, possibly because of the larger portion of the stem taken up by pith in the water limited seedlings and these contrary differences of Matthew *et al.* (2004) would be mainly because of application of

drought differently to the plants, as I withheld the water completely, whereas they maintained 50% field capacity even for the water limited plants.

Effect of water stress on volatile release by the plants

To date very little is known about the release of volatile compounds from plant tissues. Environmental factors such as light, temperature, and moisture status can greatly influence the emission of volatiles and the yield and composition of essential oils (Staudt and Bertin, 1998; Gershenzon *et al.*, 2000). Emissions of 2 Hexenal and .alpha Farnesene volatile compounds were triggered by drought and 3 Hexen 1-ol acetate (Z) was the only volatile compound which was released more under the normal watering conditions (Table 36). Effect of drought on Hexanal release pattern from ash-leaf maple (*Acer negundo*) was also reported by Li-JiQuan *et al.* (2000) and Jin-YouJu *et al.* (2004). During some of the experiments performed on poplar, developing a stress to the plant by cutting of leaves, resulted in the release of hexenal, and other C6 volatile organic compounds in the same temporal order as observed during light dark transition by Martin *et al.* (2004). So in light of the study conducted by Martin *et al.* (2004), what so ever the kind of stress, it can be concluded from this present study that differential pattern of release of volatiles under the conditions of moisture stress, play a significant role in affecting the atmosphere's chemistry.

SUMMARY AND CONCLUSION

Experiment I: Drought stress of planting stock of beech and their survival in the field plantation

Drought stress of planting stock of beech and their survival in the field plantation was planned to determine the threshold of air exposure before significant loss of planting stock occurs and to determine the indicators for the vitality of beech immediately after exposure to planting shock conditions and during the first season of seedling establishment in the field. This experiment was conducted on one-year-old bare rooted beech seedlings at the Institute of Forest Botany, Georg August University of Goettingen, Germany. The plants were lifted from the seedbed and exposed for 0, 2 and 6 h with bare roots to the air in the climatic chamber with 50% humidity and 20^0C temperature under full light illumination. Then these seedlings were transplanted with the presence or absence of ecto-mycorrhizal fungi and hydrogel. For each treatment, one batch of seedlings was harvested for the determination of vitality and carbohydrate contents in the roots and buds in the laboratory, and the other batch was transplanted randomly in multiple plots in an experimental area, after determination of morphological parameters. The experimental design was Factorial RBD.

No mortality was observed in beech for the shorter duration of exposure of 0 h and 2 h, but larger exposure adversely affected the survival after outplanting indicating that broad-leaved tree species are prone to air exposure even during the spring season, when there are no leaves, the main transpiring organ of the plant. The increasing air exposure to bare rooted beech seedling before planting, delayed the bud opening in the field; and longer air exposure of 6 h, adversely affected the mycorrhization compared with shorter exposure of 0 or 2 h. Air exposure did not affect the quantum yield of chlorophyll fluorescence and photochemical quenching parameters, as PS II is highly drought resistant.

Increasing air exposure to bare rooted beech seedlings, after outplanting led to reduced growth parameters. At all air exposures, the inoculation of beech seedlings with the ecto-mycorrhizal fungi and hydrogel has resulted in more periodic increment (between planting to harvest) in the collar diameter, root length, shoot length, plant fresh and dry weights, number of buds; and also resulted in more accumulation of root, shoot, leaf biomass and leaf area compared to their respective non-inoculated control plants at the end of growing season. Higher root-shoot and leaf-root ratio of the ecto-mycorrhizal fungi and hydrogel treated seedlings compared to their respective non-inoculated control plants at the end of growing season attributed the beneficial effect of ecto-mycorrhizal inoculation and hydrogel mixing, may be through increased nutrients absorption.

Damage to upper buds in spring, measured through tetrazolium absorbance, increased rapidly over drying periods of 2 and 6 h, whereas the health of lower buds and fine roots seemed not to be influenced. Similar to spring, air exposure

treatment before planting to the seedlings, did not affect the vitality of the fine roots and middle buds when measured at the final harvest.

The ecto-mycorrhizal and hydrogel inoculated plants yielded slightly more leaf C/N ratio in 2 and 6 h air exposure, but not in unexposed plants and this higher C/N ratio may be attributed to the efficiency of *Paxillus involutus* Maj strain, exploiting the soil surrounding the roots more under increasing stress conditions. Differences were found for carbohydrates immediately after treatment in spring. Thus, it can be speculated that planting stress increases respiration to provide carbon skeletons for repair and protection, thereby depleting the internal stores. This may lead to the influence on the bud break leading to the loss of top bud and also affected further growth. But the soluble carbohydrates and starch were not affected by air exposure treatment in leaf and fine roots following final harvest in August. Non-inoculated seedlings at the end of growing season resulted in the increased concentrations of soluble sugars in fine roots and leaves compared to their respective ecto-mycorrhizal treated plants. This decreased concentration of soluble sugars in ecto-mycorrhizal inoculated plant parts may be attributed to ecto-mycorrhizal association, as the host provides the fungal partner with carbohydrates.

Looking to the ecto-mycorrhizal and non-mycorrhizal anatomical cross sections of the beech roots, it can be clearly seen that the *Paxillus involutus* Maj strain, an ecto-mycorrhizal fungi that grew between root cortical cells of beech roots, formed a clear Hartig net, and thus increased the effective absorptive root surface area of the beech. Hence, it can be concluded from this experiment that, as

inoculation of seedlings with the ecto-mycorrhizal fungi in combination with hydrogel reduced the detrimental effects of air exposure on leaf area significantly and on other growth parameters with a less effect, it is suggested that using the ecto-mycorrhizal fungi in combination with hydrogel, the effect of air exposure on the plants can be minimized.

Experiment II: Drought and re-watering cycle under controlled conditions in Poplar

The effect of drought and re-watering was assessed on the tissue cultured poplar seedlings under controlled conditions in the green house, at the Institute of Forest Botany, Georg August University of Goettingen. The objective of the study was to investigate the effect of drought stress on various physiological parameters and also to find out the physiological parameters responding to drought stress. Initially, the tissue cultured poplar seedlings were grown in the hydroponics. They were then planted in the two times autoclaved sand peat medium in the green house with the four treatments as: sand peat medium, *Paxillus involutus* Maj strain with sand peat medium, Hydrogel with sand peat medium and *Paxillus involutus* Maj strain + hydrogel with sand peat medium. Sand peat material was prepared in the ratio of 75% sieved peat and 25% sand. For ecto-mycorrhizal inoculation, autoclaved sand peat preparation inoculated with liquid culture of *P. involutus*, one week before the planting of Poplars in the boxes and for hydrogel mixing, 5 g of it was mixed with 1 kg of autoclaved sand peat preparation. Water stress was induced by withholding water to the seedlings selected for drought and re-watering treatments, whereas the plants selected for controls in each treatment were watered daily in the morning. The experimental design was factorial CRD.

Paxillus involutus Maj strain formed ecto-mycorrhizae under both watering and drought regimes (Plate 10). Anatomical cross sections of the ecto-mycorrhizal and non-mycorrhizal root tips in this study under normal watering conditions, gives a clear indication of the adequate infection of ecto-mycorrhizae and of its influence on peripheral root cells of the poplar seedlings (Plate 6). Drought stress had significant adverse effect on the ecto-mycorrhization compared to the inoculated normal watering plants. Under normal watering conditions in all the treatments, collar diameter was the first parameter, increased significantly between two consecutive measurements, among all the growth parameters, under the influence of ecto-mycorrhization; most likely as a result of improved nutrition through greater ecto-mycorrhizal infection. *Paxillus involutus* Maj strain+hydrogel stressed treatment maintained the significantly higher ($P<0.05$) growth of collar diameter; plant fresh and dry weight; fresh mass of leaves; fresh and dry mass of shoots, fine and coarse roots than control water stressed seedlings. The effect was more pronounced in the fresh shoots biomass than the fresh roots biomass, which may be because, ecto-mycorrhizal colonization caused a proportionally greater allocation of carbohydrates to the shoot than to the root tissues. In all the treatments, the dry matter related ratio of leaves to roots was decreased under the drought stressed seedlings compared to their respective normal watering plants, but the dry weight ratio of coarse root to fine root increased under the drought stressed plants compared to their respective normal watering plants. Drought promoted the leaf shedding in all the stressed seedlings but leaf shedding was comparatively more in control stressed seedlings.

The relative water content in plant and different organs reduced by drought in all treatments, and to a significant amount in the control stressed seedlings. Leaves were more strongly affected than shoots. The leaf water potential during the drought cycle was similar in all the normal watering treatments. The stressed seedlings with *Paxillus involutus* Maj strain+hydrogel could maintain significantly higher pre-dawn leaf water potential on the day of harvest, compared to the control stressed seedlings. During recovery from drought, ecto-mycorrhizal and/or hydrogel treated seedlings recovered well compared to the control re-watered plants. The after-effects of water stress on water potential were more pronounced in the control seedlings.

Net photosynthesis and transpiration rates reductions increased with the progress of drought, but the photosynthetic reductions for the stressed plants, at the extreme stage of drought were significant for the control stressed plants, compared to the stressed *Paxillus involutus* +hydrogel or *Paxillus involutus* treatments.

Water stress did not cause significant changes in the chlorophyll fluorescence (Fv/Fm) and photochemical and non-photochemical quenching parameters of the dark adapted leaves, except the mean values of Fv/Fm of control stressed plants was lowest at harvest than the ecto-mycorrhizal and/or hydrogel stressed seedlings. Hence a serious damage was not being observed in the efficiency of photosynthetic apparatus. The non-photochemical quenching (qN, NPQ) values at the time of drought harvest and after 24 h of re-watering were observed highest in the control stressed and re-watered plants. Later, after 48 h of re-watering, a good recovery in the photosynthetic apparatus was there for all the

stressed re-watered ecto-mycorrhizal and/or hydrogel plants; and had a poor recovery in control stressed re-watered plants which suggest that the decline in PS II efficiency is regulatory, serving a photo-protective role.

The glucose and fructose concentrations in fine roots increased significantly due to drought in the control stressed plants compared to the normal watering control plants; though, the concentrations of glucose and fructose increased in all the rest of other stressed treatments too, but, the effect was not significant within the respective controls. Such increased differential accumulation of glucose and fructose suggests that the ecto-mycorrhizal+hydrogel treatment was more drought tolerant than the other treatments. In all the treatments after re-watering, the glucose and fructose concentrations were came to the normal level, even lower than their respective normal watering plants; except slightly high in the hydrogel re-watered treatment. The recovery of sugar concentrations to normal levels after re-watering, confirms the role of soluble sugars as a contributor to osmotic adjustment.

The per cent N increased and C/N ratio decreased in the leaves of stressed and non-stressed ecto-mycorrhizal and/or hydrogel treatments compared to the stressed and non-stressed control plants, may be due to the indirect effect of improved N nutrition by the ecto-mycorrhizal plants. The effect of drought and re-watering could not be established on the poplar leaf vitality in any treatment.

The high concentrations of P, K and Ca ions were observed in the phloem compared to the young developing xylem, in all the treatments. The reduction of these ions among different treatments, were not significant in the phloem; but,

significant reductions were found for the phosphorus, potassium and calcium ion concentrations in the young developing xylem of the control re-watered plants compared to control normal watering plants. The concentrations of these ions in both the xylem and phloem were positively correlated with the leaf water potentials at the initial stage and also at the final stage of drought harvest. These relationships were even stronger for xylem ion concentration than the phloem ion concentrations.

The results on wood anatomy showed that the per cent vessel lumen fraction, vessel lumen area, vessel size reduced in response to drought more in the control stressed plants compared to the other stressed treatments. The cell wall fraction and vessel frequency also increased more in the control drought stressed plants, when compared with other stressed ecto-mycorrhizal and/or hydrogel plants. However, a trend towards greater vessel frequency in water limited poplar, is probably the balancing activity of the plant in maintaining the vessel lumen area. Negative relationship was observed between the per cent cell wall fraction and vessel lumen area or vessel diameter of stressed and non-stressed poplar seedlings, but this negative relationship was stronger in the stressed seedlings.

Emissions of 2 Hexenal, Hexanal and .alpha Farnesene volatile compounds were increased by drought, mainly in the controlled stressed plants; 1,3,6 Octatriene 3,7 dimethyl (Z) and nonanal in drought stressed plants of ecto-mycorrhizal inoculation and control; Heptacosane in ecto-mycorrhizal stressed plants; whereas 3 Hexen 1-ol acetate (Z) was the only volatile compound which was released more under the normal watering conditions. It can be concluded from

this study that the differential release pattern of volatiles under the conditions of moisture stress, play a significant role in affecting the atmosphere's chemistry.

Hence, the present investigation on poplar brings out clearly that, the drought had significant negative effects on all the biometric parameters, degree of ecto-mycorrhization, pre-dawn leaf water potential, photosynthesis, sugar regulation in fine roots, xylem ion concentrations, wood anatomy of vessels as well as on the volatile release pattern. But, ecto-mycorrhizal and hydrogel inoculation greatly assisted the poplar plants, in making these adverse effects to a moderate level. There was no significant changes in the quantum efficiency of PS II (Fv/Fm) and photochemical (qP) quenching parameters in dark adapted stressed seedlings; despite the largely unchanged activity of fluorescence yield, the stressed seedlings were characterized by an increase in non-radiative energy dissipation during hard stress, suggests that the thermal dissipation of the excitation energy is protecting the photosynthetic apparatus from the oxidative stress. It can also be concluded from this study that the water potential is a good indicator of dehydration effects and chlorophyll fluorescence is comparatively less sensitive indicators of drought stress.

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Abstract

a)	Title of the thesis	:	**Studies on physiological parameters for drought tolerance in beech (*Fagus sylvatica* L.) and Poplar (*Populus canescens*)**
b)	Full name of degree holder	:	**RAJENDER SINGH BENIWAL**
c)	Title of degree	:	**Doctor of Philosophy in Agroforestry**
d)	Name and address of the Major Advisor	:	Dr. M. S. Hooda Professor & Head Department of Forestry CCS HAU, Hisar-125 004, Haryana (India)
e)	Degree awarding university	:	CCS Haryana Agricultural University Hisar –125 004 (India)
f)	Year of award of degree	:	2005
g)	Major subject	:	AGROFORESTRY
h)	Total number of pages in dissertation	:	178 (Text: 88, Bibliography: 39; Tables: 28; Figures:13; Plates:10)
i)	Number of words in the abstract	:	560

Key words: Beech, poplar, *Paxillus involutus* (ecto-mycorrhiza), hydrogel, drought, air exposure

In spring 2004, the experiment on drought stress of planting stock of beech and their survival in the field plantation was conducted at the Institute of Forest Botany, Georg August University of Goettingen, Germany, to determine the threshold of air exposure before significant loss of planting stock occurs and to determine the indicators for the vitality of beech immediately after exposure to planting shock conditions and during the first season of seedling establishment in the field.

Damage to upper buds in spring increased rapidly over drying periods of 2 and 6 h. Increasing air exposure to bare rooted seedlings, resulted in significant increase in soluble carbohydrates in buds and fine roots in spring; but, the soluble carbohydrates concentrations were not affected by air exposure treatment in leaf and fine roots following final harvest in August. No mortality was observed in beech for the 0 and 2 h exposure. Increasing air exposure adversely affected the plant survival after outplanting, delayed the bud opening in the field and also adversely affected the ecto-mycorrhization. At all air exposures, the inoculation of beech

seedlings with *Paxillus involutus* Maj strain and hydrogel has resulted in more periodic increment between planting to harvest in collar diameter, root length, shoot length, plant fresh and dry weights, number of buds; and also resulted in more accumulation of root, shoot, leaf biomass, leaf area, leaf C/N ratio, root-shoot and leaf-root ratio compared to their respective non-inoculated control plants at harvest.

The second experiment, drought and re-watering cycle under controlled conditions in Poplar was also conducted at the same institute, to investigate the effect of drought stress on various physiological parameters and also to find out the physiological parameters responding to drought stress. Drought stress in poplar also had significant adverse effect on the ecto-mycorrhization compared to the inoculated normal watering plants. Stressed seedlings of *Paxillus involutus* Maj strain+hydrogel inoculation, maintained the significantly higher ($P<0.05$) pre-dawn leaf water potential, photosynthesis, collar diameter; growth and biomass than control stressed seedlings. Visual effects of leaf shedding were more in control stressed seedlings than stressed seedlings of *Paxillus involutus* Maj strain+hydrogel treatment. Drought did not cause significant changes in the chlorophyll fluorescence (F_v/F_m) of the dark-adapted leaves, but these values were lowest in control stressed seedlings.

The glucose and fructose concentrations in fine roots increased significantly due to drought in the control stressed seedlings; but, the increased differential accumulation of glucose and fructose suggests that the ecto-mycorrhizal+hydrogel inoculated seedlings were more drought tolerant than the other treatments. Drought did not reduce significantly the concentrations of P, K and Ca ions in the phloem; but, significant reductions for these ions were found in the young developing xylem of the control re-watered seedlings compared to its control. Drought affected the xylem vessel anatomy greatly in control drought plants i.e. vessel lumen fraction (%), vessel lumen area and vessel size reduced in response to water stressed more in the control drought plants compared to the other stressed treatments. The cell wall fraction and vessel frequency also increased more in the control drought plants. Emission pattern of volatiles were also affected by drought. These results suggest that the *Paxillus involutus* Maj strain+hydrogel inoculation greatly assist the poplar plants under drought stress and helped in making these effects to a moderate level. These results are more important from the points of view of afforestation in drought prone conditions.

Major Advisor Signature of the Student

Professor & Head