

## ECTOMYCORRHIZAL EFFECT ON NET PHOTOSYNTHESIS OF *PINUS BRUTIA* var. *ELDARICA* SEEDLINGS

Shad K. Khalil<sup>1</sup>, and John G. Mexal<sup>2</sup>

### Abstract

*Pinus brutia* var. *eldarica* seedlings were inoculated with two ectmycorrhizal fungi i.e. *Rhizopogon vinicolor* and *Hebeloma crustuliniforme*, to determine how different mycorrhizal and non mycorrhizal (NM) fungi control affect the response of photosynthesis, dry matter, shoot-root partitioning and photosynthetic water use efficiency (PWUE). Containerized seedlings were grown in greenhouse for about 9 months under well-watered and nitrogen fertilized conditions, and then transferred to a growth chamber where measurements were made three days latter. All seedlings were watered to field capacity the evening before measurements were made. Net photosynthetic rate and PWUE over one day period were measured, followed by harvesting and determination of dry matter of foliage. Both mycorrhizal fungi caused substantial increase in net photosynthesis and PWUE over non mycorrhizal (NM) control, but none of the mycorrhizas resulted in significant change in total dry matter or shoot-root allocation of dry matter. Net photosynthesis measured over time increased from morning then it declined to the minimum at mid-day, thereafter, net photosynthesis slightly increased towards the end of the day. Seedlings inoculated with *Hebeloma crustuliniforme* mycorrhizal fungi resulted in maximum net photosynthesis compared with seedlings either inoculated with *Rhizopogon vinicolor* or non mycorrhizal (control). It is proposed that net photosynthetic rate is correlated with the rate of export of photosynthates to the sink generated by mycorrhizal growth. Strong mycorrhizal demand for photosynthates stimulates photosynthesis to the mycorrhizal fungi.

Key words: Ectomycorrhizas, *Rhizopogon vinicolor*, *Hebeloma crustuliniforme*, photosynthesis

---

<sup>1</sup> Associat Professor, Department of Agronomy, Agriculture University Peshawar, Pakistan.

<sup>2</sup> Professor, Agronomy & Horticulture Department, New Mexico State University, Las Cruces, USA.



## Introduction

Higher rates of net photosynthesis have been observed in plants correlated with presence of ectomycorrhizas on the roots (Dosskey et al., 1990; Rousseau and Ried, 1990; Dosskey, 1991; Lehto, 1992; Panwar, 1991; Brown and Bethlenfalvay, 1987; Nylund and Unestam, 1987; Reid et al., 1983; and Allen et al., 1981). However some researchers observed little or no effect on net photosynthesis due to mycorrhizal colonization (Reinhard, 1992; Syvertsen and Graham, 1990). It is not clearly identified which mechanism causes this effect. Mycorrhizal fungus stimulate photosynthetic rate when carbohydrates are diverted (Reinhard et al., 1992; Reid et al., 1983).

It is hypothesized that rate of photosynthesis is related to the demand for carbohydrates (Dosskey et al., 1991; Sweet and Wareing, 1966). The increase in demand for carbohydrates by fungal association increases the rate of photosynthesis (Herold 1980). Koch and Johnson (1984) observed more translocation of labelled  $^{14}\text{CO}_2$  to mycorrhizal roots than to non mycorrhizal roots. These translocated photosynthates are utilized for fungal growth. The mycorrhiza improves mineral nutrients uptake especially phosphorus and may alter hormonal balance (McArthur and Knowles, 1993; Paul et al., 1985; Harley and Smith, 1983; and Allen 1985). The present experiment was designed to study the effect of mycorrhizal inoculation on net photosynthesis, photosynthetic water use efficiency and dry matter partitioning of *Pinus brutia* seedlings.

## Materials and Methods

Seeds of *Pinus brutia* var. *eldarica* were soaked in water for 24 hours and planted in 175 cm<sup>3</sup> Ray -Leach tubes (containers) filled with a mixture of peatmoss and vermiculite in March, 1992. Seeds were covered with approximately one cm layer of vermiculite. The inoculum was prepared by using a mixture of 9 soil: 1 inoculum for each species i.e., *Rhizopogon vinicolor* and *Hebeloma crustuliniforme*. In non mycorrhizal (NM) control no inoculation was done. Seedlings were maintained in a greenhouse for about nine months and were watered when needed. Seedlings were fertilized approximately once every three weeks with Peter's Professional Conifer mix fertilizer at the rate of 150 ppm nitrogen. Temperatures in the greenhouse fluctuated seasonally and diurnally but were kept above 21°C during winter and below 35°C during summer.

15 containerized seedlings including five each mycorrhiza and non



mycorrhiza (control) were transferred to growth chamber set at day/night temperature of 30/20°C, two days prior to measurements. All seedlings were thoroughly watered the evening before measurements were begun. Within the growth chamber illumination was provided by incandescent and fluorescent white lamps to give a photon flux density (400-700 nm) of 800 to 900  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Plants in the growth chamber were watered twice daily. Two fans, attached at opposite corners inside the chamber, were used to insure a well-mixed internal atmosphere. Net photosynthetic rate and PWUE measurements were taken periodically during the course of one day on the top three youngest fully expanded needles using a portable LCA2 IR gas analyzer system (Analytical Development Co. Ltd., Hoddesdon, UK). Ten consecutive measurements were taken at 6-s interval on each seedling at 6 hours (one hour predawn), 8 hours, 10 hours, 12 hours and 18 hours of light, then lights were turned off and measurements were made one hour after dark (19 hours). Four plants from each treatment were measured. Gas exchange of the pine seedlings was measured by using the procedure described by Padilla et al. (1993).  $\text{CO}_2$  concentration in the air flow entering to the system was about 400 ppm. Air flow was regulated to give 900 cc minute<sup>-1</sup>. Leaf area of each seedling was measured by passing all needles through a portable LI-COR leaf area meter (Model LI 3000 A, LI-COR, Lincoln, NB). The leaf area recorded by the instrument was adjusted by multiplying the values with a factor  $\pi/2$  as described by Nowak and Caldwell (1984). After photosynthetic measurements each seedling was separated into shoot and roots, oven-dried at 65°C for 72 hours and dry weight was determined. Data were statistically analyzed according to completely randomized design with SAS (SAS Institute, 1990).

## Results

### Net Photosynthesis

*Rhizopogon vinicolor* and *Hebeloma crustuliniforme* significantly affected net photosynthetic rate of *eldarica* pine seedlings. Figure 1 reveals that predawn  $\text{CO}_2$  exchange rate was almost the same for non mycorrhizal (control) and mycorrhizal inoculated seedlings. Mycorrhizal inoculation stimulated net photosynthetic rate significantly ( $p < 0.05$ ) compared to non mycorrhizal control, two hours after lights were turned on. This higher net photosynthesis trend in mycorrhizal seedlings was maintained throughout the day, except for the observation recorded four hours after lights were turned on. Rate of photosynthesis increased during the early part of day, which decreased as less



mid-day time. Thereafter photosynthesis slightly increased again.

*Hebeloma crustuliniforme* fixed maximum CO<sub>2</sub> followed by *Rhizopogon vinicolor* while non mycorrhizal control fixed minimum CO<sub>2</sub>, Fig.2.

### Photosynthetic Water Use Efficiency (PWUE)

Mycorrhizal inoculation significantly affected PWUE at  $p < 0.05$ . Fig.3 reveals that PWUE was maximum for seedlings inoculated with *Hebeloma crustuliniforme*, followed by *Rhizopogon vinicolor*. The non mycorrhizal (control) resulted in low PWUE. The PWUE was highest in early part of the day, which gradually decreased and levelled off towards the end of the day (Fig.4).

### Seedling Morphology

No significant differences in total dry weight, shoot or root weight were observed due to mycorrhizal fungi or non mycorrhizal control).

### Discussion

Mycorrhizal inoculation with *Hebeloma crustuliniforme* and *Rhizopogon vinicolor* significantly increased net photosynthetic rate. These findings support the concept that net photosynthetic rate is correlated with rate of export of photosynthates to the mycorrhizal fungi. Strong mycorrhizal demand for photosynthates stimulates photosynthesis (Dosskey, 1991; Herold, 1980; and Bagnall *et al.*, 1988). This high demand for photosynthesis is due to greater photosynthate sink in mycorrhizal roots than non mycorrhizal root system (Koch and Johnson, 1984; and Reid *et al.*, 1983).

Seedlings responded differently when inoculated with two different mycorrhizal fungi *Hebeloma crustuliniforme* showed a substantial increase in net photosynthesis over *Rhizopogon vinicolor*. Our results support the findings of Dosskey *et al.* 1990 and Dosskey *et al.*, 1991 who reported variation in net photosynthesis of different mycorrhizal fungi but significantly higher than non mycorrhizal control. However, Dixon and Hiol-Hiol (1992) reported similar rates of photosynthesis for different mycorrhizal fungi. This could be hypothesized that rate of photosynthesis by different mycorrhizal fungi is species dependent.

The data suggest that mycorrhizal root system developed a substantial sink for photosynthate. This production of additional photosynthate by the needles of



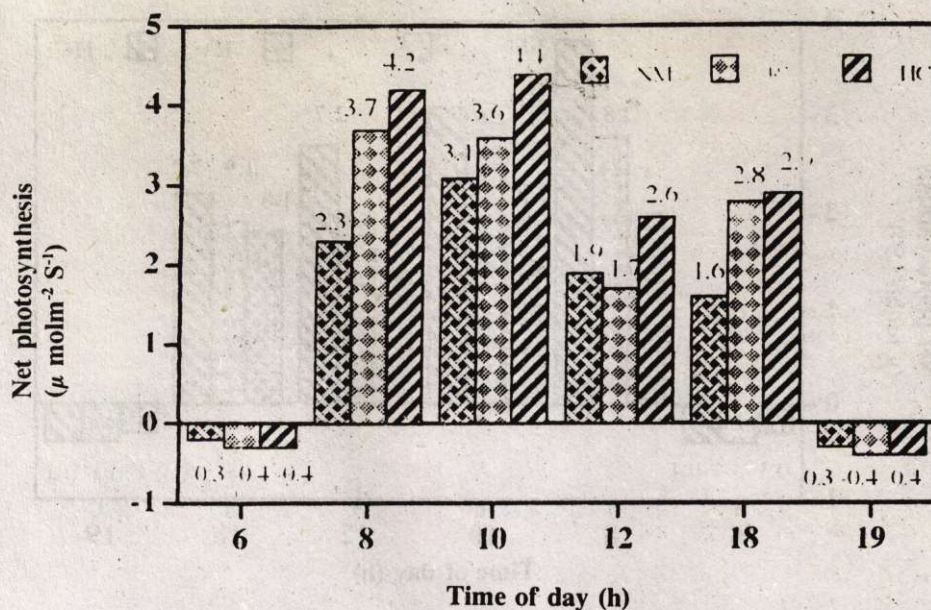


Fig.1. Rate of net photosynthesis of one year old *Eldarica pine* seedlings measured periodically over one day under irrigated condition: Non mycorrhizal (N.M.), *Rhizopogon vinicolor* (R.V.), *Hebeloma crustuliniforme* (H.C.)

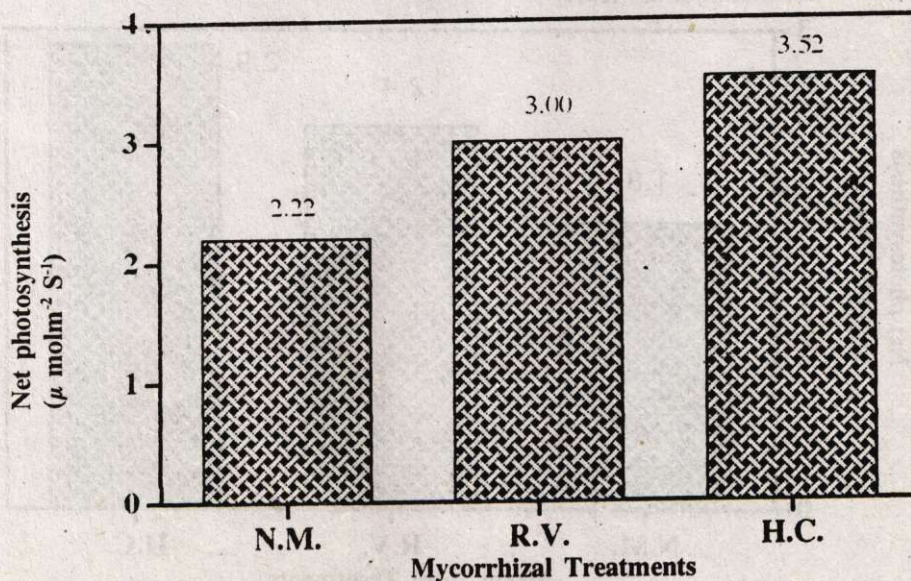


Fig.2. Rate of net photosynthesis of one year old *Eldarica pine* seedlings measured periodically over one day under irrigated condition: Non mycorrhizal (N.M.), *Rhizopogon vinicolor* (R.V.), *Hebeloma crustuliniforme* (H.C.)



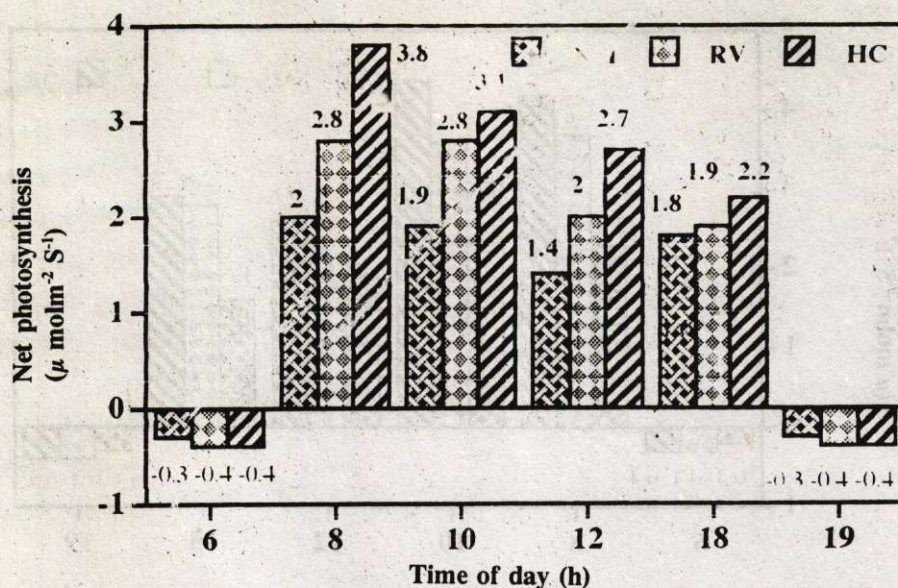


Fig.4. Photosynthetic water use efficiency (PWUE) of one year old *Eldarica pine* measured periodically over one day under irrigated condition inoculated with mycorrhizae: Non mycorrhizal (N.M.), *Rhizopogon vinicolor* (R.V.), *Hebeloma crustuliniforme* (H.C.)

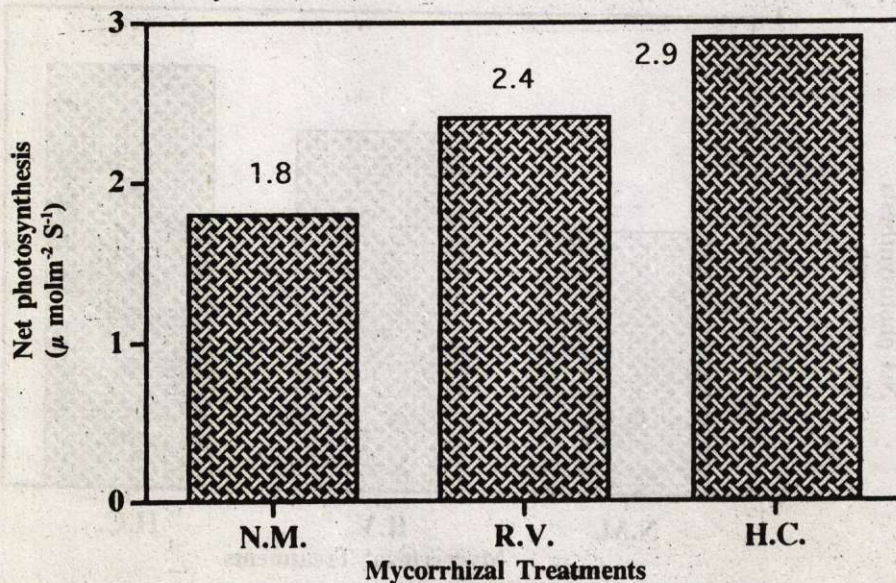


Fig.5. Photosynthetic water use efficiency (PWUE) of one year old *Eldarica pine* seedlings under irrigated condition inoculated with mycorrhizae: Non mycorrhizal (N.M.), *Rhizopogon vinicolor* (R.V.), *Hebeloma crustuliniforme* (H.C.)



mycorrhizal seedlings are exported to the roots which are utilized for the fungal growth. This increased demand for photosynthate by mycorrhizal root system is probably due to stimulation of photosynthesis by reducing the concentration of sucrose in the leaves (Herold 1980).

## References

- Allen, M. F. 1985. Phytochrome action: an integrative approach to understanding diverse mycorrhizal responses. Proceedings of the 6th North American Conference on Mycorrhizae (Ed. by R. Molina), pp.158-160. Forest Research Laboratory, Oregon State University, Corvallis, USA.
- Allen, M. F., W. K. Smith, T. S. Jr. Moore, and M. Christensen. 1981. Comparative water relations and photosynthesis of mycorrhizal and non mycorrhizal *Bouteloua gracilis* H. B. K. *Lag ex Steud.* *New Phytologist*. 88:683-693.
- Bagnall, D. J., R. W. King, and G. D. Farquhar. 1988. Temperature-dependent feedback inhibition of photosynthesis in peanut. *Planta*. 175:348-354.
- Brown, M. S., and G. J. Bethlenfalvay. 1987. Glycine-Glomus-Rhizobium symbiosis. VI. Photosynthesis in nodulated, mycorrhizal, or N and P fertilized soybean plants. *Plant Physiology* 85:120-123.
- Cairney, J. W. G., and I. J. Alexander. 1992. A study of ageing of spruce [*Picea sitchensis* (Bong.) Carr.] ectomycorrhizas. 11. Carbohydrates allocation in ageing *Picea sitchensis*/*Tylospora fibrillosa* (Burt.) Donk ectomycorrhizas. *New Phytologist*. 122:153-158.
- Dixon, R. K., and F. Hiol-Hiol. 1992. Gas exchange and photosynthesis of *Eucalyptus camaldulensis* seedlings inoculated with different ectomycorrhizal symbionts. *Plant and Soil*. 147:143-149.
- Dosskey, M. G., L. Boersma, and R. G. Linderman. 1991. Role for the photosynthate demand of ectomycorrhizas in the response of Douglas fir seedlings to drying soil. *New Phytologist*. 117:327-334.



- Dosskey, M. G., R. G. Linderman, and L. Boersma. 1990. Carbon stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizas. *New Phytologist*. 115:269-274.
- Harley, J. L., and S. E. Smith. 1983. *Mycorrhizal symbiosis*. Academic Press London.
- Herold, A. 1980. Regulation of photosynthesis by sink activity the missing link. *New Phytologist*. 86:131-144.
- Koch, K. E., and C. R. Johnson. 1984. Photosynthate partitioning in split root citrus seedlings with mycorrhizal and non mycorrhizal root system. *Plant Physiology*. 75:26-30.
- Lehto, T. 1992. Mycorrhizas and drought resistance of *Picea sitchensis* (Bong) Carr. 1. In conditions of nutrient deficiency. *New Phytologist*. 122:661-668.
- McArthur, D. A. J., and N. R. Knowles. 1993. Influence of vesicular-arbuscular mycorrhizal fungi on the response of potato to phosphorus deficiency. *Plant Physiology*. 101:147-160.
- Nowak, R. S., and M. M. Caldwell. 1984. A test of compensatory photosynthesis in the field: implications for herbivory tolerance. *Oecologia*. 61:311-318.
- Nylund, J. E., and T. Unestam. 1987. Ectomycorrhiza in semi- hydroponic scots pine: increased photosynthesis but reduced growth. *Proceedings of the 7th North American Conference on Mycorrhizae* (ed. by R. Molina), p. 256. Forest Research Laboratory, Oregon State University, Corvallis, USA.
- Padilla-Ramirez, J. S., V. M. Guerrero, C. H. Rivera, and R. D. Burghage. 1993. Photosynthetic response to temperature of eldarica pine seedlings. (personal communication).
- Panwar, J. D. S. 1991. Effect of VAM and *Azospirillum brasilense* on photosynthesis, nitrogen metabolism and grain yield in wheat. *Indian J. of Plant Physiology*. 34:357-361.



Parke, J. L., R. G. Linderman, and C. H. Black. 1983. The role of ectomycorrhizas in drought tolerance of Douglas-fir seedlings. *New Phytologist*. 95:83-95.

Paul, E. A., D. Harris, and A. Fredeen. 1985. Carbon flow in mycorrhizal plant associations. *Proceedings of the 6th North American Conference on Mycorrhizae* (Ed. by R. Molina), pp.165-169. Forest Research Laboratory, Oregon State University, Corvallis, USA.

Reid, C. P. P., F. A. Kidd, and S. A. Ekwebelam. 1983. Nitrogen nutrition, photosynthesis and carbon allocation in ectomycorrhizal pine. *Plant and Soil*. 71:415-432.

Reinhard, S., P. Martin, and H. Marschner. 1992. Interaction in the tripartite symbiosis of pea (*Pisum sativum* L.), *Glomus* and *Rhizobium* under limiting phosphorus supply. *Journal of Plant Physiology*. 141:7-11.

Rousseau, J. V. D., and C. P. P. Reid. 1990. Effects of phosphorus and ectomycorrhizas on the carbon balance of loblolly pine seedlings. *Forest Science*. 36:101-112.

SAS Institute. 1990. SAS user's guide:Statistics. version 6, 4ed. vol. 2, SAS Inst., Carry, NC.

Sweet, G. B., and P. F. Wareing. 1966. Role of plant growth in regulating photosynthesis. *Nature*. 210:77-79.

Syvertsen, J. P., and J. H. Graham. 1990. Influence of vesicular arbuscular mycorrhizae and leaf age on net gas exchange of citrus leaves. *Plant Physiology*. 94: 1424-1428.