Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI - 10.26479/2017.0205.08**

EFFECT OF AM FUNGI WITH ADDITIONAL PHOSPHATE TO IMPROVE PLANT GROWTH, BIOMASS YIELD AND P-UPTAKE IN *MELIA AZADIRACHTA* L. (GAEORTNER.) ROXB

Sharanappa Jangandi[1], Narayan J[2], H.C Lakshman[3], Chaitra B. Negalur[3]

1. Zonal Agricultural and Horticultural, Research Station, Babbur Farm Hiriyur. Chitradurga

2. Department of Environmental Science. Kuvempu University. Shankarghatta - Shimoga India

. 3.Department and Studies in Botany, Microbiology Laboratory, Karnatak University, Dharwad. India.

ABSTRACT: Green-house experiments were undertaken to study the effect of the two AM fungi inoculation with three levels of K_2HPO_4; 50mg/kg, l00mg/kg and 150mg/kg on *Melia azadirachta* L. The results showed that plants received AM fungal inoculation showed increased P content and significantly increased plant height, root length, leaf area and chlorophyll content with l00 mg/kg compared to non-inoculated plants after l80 days. However, the percent root colonization and AM fungal spore number drastically reduced in Gm+Gl+3P level treated plants. It can be concluded that there is need of pre-inoculation in AM fungi with low balanced level of phosphorous treatment to seedlings at nursery level, before they transplanted into the field. And the significant utilization of P source with AM fungi of *Melia azadirachta* L. has been discussed.

KEYWORDS: *Melia azadirachta* L., *Rhizophagus fasciculatus, Glomus macrocarpum*, Phosphorous treatment, per cent root colonization, Arbuscular mycorrhizal (AM) fungi

***Corresponding Author: Dr. Chaitra B. Negalur** Ph.D.

Department of Studies in Botany, Microbiology Laboratory, Karnataka University, Dharwad, India.

* Email Address: chainegalur@gmail.com

1. INTRODUCTION

In recent years researchers on mycorrhiza have had tremendous opportunities to develop new ideas and open new avenues of endeavor in this rapidly developing field of biological sciences. At the same time, the benefits of their applications are becoming increasingly apparent in a number of countries. The productivity of tropical forests is decreasing to a considerable extent which poses manifold problems to restore the ecosystems. Increasing pressure of human and livestock population, indiscriminate extraction of forest products, regular fire and mining activities developed many issues in forest areas for reclamation and restoration of forest ecosystem. Some beneficial microbes are present in soil, which promote plant growth by providing access to the nutrient absorption, nitrogen fixation or synthesizing some growth promoting substances to the plants by different processes. Among these arbuscular mycorrhizal (AM) fungi play an important role in nutrient uptake and P absorption. Mycorrhiza colonized plants have resistance against disease, they are equally important in reclamation of mined soils [Jamaluddin, 2002; Lakshman, 2005; Romana and Lakshman, 2009].

Agricultural and horticultural crops have been shown to benefit from AM fungi on a wide range of plant roots [Mosse, 1973; Howeler *et al.,*1987; Lakshman, 1996; Katiyar *et al.,* 1995] have demonstrated that the effect of AM fungus (*Glomus fasiculatum*) along with phosphate level on the growth of *Morus alba.* Similarly, Karthikeyan *et al.,* [2009] have showed the effect of *Glomus mosseae* along with phosphate levels on the growth and alkaloid content in *Melia azadirachta* L. *Melia azadirachta* L., (Geortner.) Roxb. is an important timber yielding tree. These are cultivated in most of Indian forests. *Melia azadirachta* L., has purgative properties used Indian Ayurvedic system of medicine. There is no report of AM fungal studies on this plant. The present study was carried out to understand the effect of AM fungi on *Melia azadirachta* L., with respect to growth, biomass production, per cent root colonization and phosphorus content in shoot fewer than three different phosphate levels over non-inoculated plants.

2. MATERIALS AND METHODS

Seeds of *Melia azadirachta* L. were obtained from the department of forest Government of Karnataka, Sirsi, North Canara District. Pure cultures of AM fungi *Glomus mosseae* and *G. leptonicum* were maintained on Maize (*Zea mays* L.) as a host plant and kept in the poly-house, Department of Botany Karnatak University Dharwad-580003, India. Experimental pots measuring 30 cm × 30 cm diameter filled with 6 kg of soil (equal proportion of pure sand and sterilized garden loamy soil) with 15g mixed, inoculum containing rhizosphere soil of maize (host) and AMF colonized root pieces, hyphae and AM fungal spores (115-201 spores/ g of soil was used. The inoculum was placed 4cm below the surface of each pot. Healthy *Melia azadirachta* L. seeds were surface sterilized in 2% sodium

hypochlorite, soaked in water for 12 hrs and sowed in experimental pots. These pots were arranged in completely randomized block design (CRBD) with 4 replicates. One month old seedlings of *Melia azadirachta* L. were transplanted from nursery and with mycorrhiza *G. mosseae* and *G. leptonicum* was inoculated and non-inoculated plants were maintained. *Melia azadirachta* L. plants were treated with three phosphate levels (K_2HPO_4: 50mg, l00 mg and 150 mg in 100 ml of distilled water). P treatment was given at 7 days interval, and it was continued till the last observation was taken. Observations were made after 60, 90 and 120 days after AM fugal inoculation. Roots and rhizospheric soil sample were collected from plants after the treatment period. The AM fungal spores were recovered by wet sieving and decanting method [Gerdemann and Nicolson, 1963]. Roots were transferred to 10% KOH solution and heated at 90°C degree for one hour and the time period was adjusted according to root bit delicacy. KOH was poured off and roots were rinsed with tap water. These bits were taken out and acidified by placing in 2N% HCl solution, washed with distilled water, stained in 0.05 tryphan blue in lactophenol [Phillips and Hayman, 1973]. Horizontal and vertical grid lines were scanned and total number of roots bits intersecting grid line and total numbers of intersections involving infected roots bits were recorded. The percent of root colonization was calculated by the formula proposed by [Givannetti and Mosse, 1980] Phosphorous content of shoots was determined by Vandomolybolate phosphoric yellow color method [Jackson, 1973]. The other parameters such as plant height, root length, leaf area, shoot dry weight, was carried out after the plant materials were kept in 70^0c in autoclave uniformly for 48 hrs chlorophyll content of leaves, per cent colonization and accordingly AM fungal spores were measured [Arnon, 1949] on each harvest of 60, 90, and 120 days by adopting required scientific procedures.

3. RESULTS AND DISCUSSION

Factors that can contribute to their survival and performance of essential activities of microbial adaptability, adequate soil management and use of the minimum effective dosage of the fertilizers sustainability of soil and the plant systems require good development and function of mycorrhizal symbiosis. Applications of easily soluble fertilizers especially phosphorus fertilizers increase the fraction of easily phosphorus in soils, but this is negatively correlated with the presence of AM fungi.

Table 1: Effect of AM fungi *Glomus mosseae* and *G. leptionicum* and three phosphate level treatments on *Melia azadirachta* L. with respect to plant height, root length, leaf area and shoot dry weight for 120 days.

Days	**Phosphate level**	**Shoot length (cm)**	**Root length (cm)**	**Leaf area (cm^2)**	**Shoot Dry weight (g)**
60	C -50mg/kg soil	12.1±2.0	7.0 ±0.0	27.4 ±2.0	0.56±0.5
90	C -50mg/kg soil	14.2±0.0	7.9 ±1.0	31.2±5.0	1.13 ±0.1
120	C -50mg/kg soil	21.4±1.2	10.0 ±2.0	38.0 ±0.3	1.54 ±2.6
60	C+1P	15.3±5.0	8.01 ± 0.2	33.1±5.3	0.68 ±0.1
90		22.6±3.3	11.0 ±0.2	42.0 ±2.2	1.51 ±0.7
120		25.2 ±1.0	11.9 ±1.5	44.5 ± 0.7	1.63±0.5
60	C+2P	16.8 ±5.0	9.2 ± 0.4	36.1 ±0.1	0.91 ±0.4
90		23.1 ±2.4	12.0 ± 0.0	42.5 ± 0.4	1.52 ±1.6
120		30.0±0.0	13.7 ±1.3	46.1 ±2.6	2.61 ±0.4
60	C+3P	19.7±3.1	11.3 ±1.5	39.2±3.0 .	2.7 ±2.3
90		28.0±1.0	14.2 ±1.0	44.0 ±0.0	2.63 ±7.2
120		31.5±2.2	17.1 ±2.0	48.1 ±5.0	2.51 ±0.6
60	Gm+Gl	26.1 ±4.1	13.1 ±1.4	45.5 ±6.3	2.71 ±0.2
90		32.4±1.0	15.6 ±3.0	51.0 ±2.3	2.82 ±4.0
120		35.8±7.2	18.8 ±2.2	55.2 ±0.1	2.63 ±3.1
60	Gm+Gl +1P	25.7 ±2.0	1.5.1 ± 1.0	46.5 ±6.2	2.76±2.0
90		V 33.1±0.5	19.2 ±2.2	56.5 ± 3.2	2.55 ±1.5
120		37.51±3.1	21.2 ±0.5	61.1 ±5.0	2.99 ±2.3
60	Gm+Gl +2P	29.3±4.4	16.4 ±2.5	52.0 ±2.2	2.51 ±6.0
90		46.1±4.0	19.7 ± 2.0	60.0 ±0.5	2.93 ±0.7
120		49.8±1.5	21.1 ±0.3	62.6± 0.3	3.43 ±3.0
60	Gm+Gl +3P	31.2±7.1	17.3 ± 2.5	54.1 ±2.1	2.60±7.0
90		90.5±2.0	19.0 ± 0.4	62.6 ±0.8	2.62 ±3.3
120		39.0±0.2	18.4 ±1.0	64.0 ± 0.3	2.50±0.0

C= control ; C+1P= control with first phosphate level (50mg/kg soil) C+2P =control with second phosphate level (100 mg P kg soil), C+3P = control with third phosphate level (150mg P/ kg soil), Gm+Gl = *Glomus mosseae* + *G. leptonicum,* Gm+Gl+lP = *Glomus mosseae* + *G. leptonicum* with (50mg/kg soil) Gm+Gl+2P=*Glomus mosseae*+ *G leptonicum* with (100 mg/kg soil), Gm+Gl+3P = *G. mosseae* + *leptonicum* with (150 mgP/kg soil).

Table 2: Effect of AM fungi *G. mosseae and G. leptonicum* and three phosphate level treatment on *Melia azadirachta* L. with regard to chlorophyll content in leaves, phosphate content in shoot, per cent root colonization and spore number for 120 days.

Days	Phosphate level	Phosphate content	Total Chlorophyll Mg/g	Percent root-colonization	AMF spores 50g. soil
60	C -50mg/kg soil	0.34 ± 0.0	0.46 ±0.2	0.00	0.00
90	C -50mg/kg soil	0.71 ±0.1	1.72 ±3.1	0.00	0.00
120	C -50mg/kg soil	0.73 ±0.0	144 ±2.0	0.00	0.00
60	C+1P	0.56 ±1.0	0.96 ±0.4	0.00	0.00
90		1.22 ±0.8	1.36 ±5.3	0.00	14±0.5
120		1.48 ±0.2	1.54 ±4.7	0.00	19±1.3
60	C+2P	0.59 ±1.0	1.62 ±5.1	0.00	26±2.6
90		1.33 ±0.0	1.55 ±0.4	0.00	10±1.4
120		1.78 ±0.3	1.69 ±5.5	0.00	09±0.3
60	C+3P	0.66 ±0.9	1.29 ±4.3	0.00	11. ±5.0
90		1.57 ±0.1	1.62 ±6.2	0.00	14±2.0
120		1.81 ±9.0	1.73 ±7.1	0.00	13 ±1.1
60	Gm+Gl	0.77 ±1.1	1.51 ±8.0	53±7.0	79±5.0
90		1.10 ±0.0	2.14 ±6.2	56±4.0	62±9.2
120		1.42 ±0.7	3.62 ±4.5	59±0.5	61±5.0
60	Gm+Gl +1P	0.98 ±0.1	2.51 ±5.0	61±0.7	53±2.4
90		1.51 ±0.0	3.31 ±4.0	58±1.4	59±3.3
120		1.98 ±1.6	3.47 ±5.5	52±1.2	63±5.1
60	Gm+Gl +2P	1.16 ±7.0	2.85 ±6.7	48±4.0	61±0.2
90		1.63 ±0.0	3.38 ±0.8	43±3.2	58±1.1
120		2.11 ±0.5	3.64 ±4.1	42±5.1	53 ±1.3
60	Gm+Gl +3P	1.19 ±0.4	2.86 ±5.0	47±2.2	51 ±0.7
90		2.21 ±0.8	3.36 ±4.2	46±8.3	49±5.1
120		2.14 ±0.1	3.42 ±2.3	44±4.6	49±7.4

C= control ; C+1P= control with first phosphate level (50mg/kg soil) C+2P =control with second phosphate level (100 mg P kg soil), C+3P = control with third phosphate level (150mg P/ kg soil), Gm+Gl = *Glomus mosseae* + *G. leptonicum,* Gm+Gl+lP = *Glomus mosseae* + *G. leptonicum* with (50mg/kg soil) Gm+Gl+*2P=Glomus mosseae*+ *G leptonicum* with (100 mg/kg soil), Gm+Gl+3P = *G. mosseae*+ *leptonicum* with (150 mgP/kg soil).

Arbuscular mycorrhizal fungal inoculation had a significant positive effect, on *Melia azadirachta* L. plant growth with different parameters which is shown in Tables (1 and 2). The results revealed that plants grown with the inoculation of AM fungi have higher biomass than non-inoculated (control). Plants height and root length increased significantly in both AM fungi inoculation with l00 mg p/l phosphate treatments when, compared to control plants (Table 1). *Melia azadirachta* L. plants inoculated with phosphate treatment brought an increased shoot length and root length as compared to non inoculated plants as shown in (Fig 1). This may be attributed to either mechanisms of mycorrhizal infection and development in the host tissue [Kormanic *et al.,* 1982] or the improvement in phosphate uptake as a result of AM fungal infection [Jeffries, 1987; Lakshman, 1996]. Similar, observation were made by [Chiramel *et al.,* 2006] who have reported that plants inoculated with *Glomus tunicatum, G. leptonicum* and *G. mosseae* showed higher plant growth compared to non inoculated plants. The present findings are in agreement with [Elahi *et al.,* 2010; Man *et al.,* 2011], who have documented the effect of inoculation with AM fungi and growth and total biomass of *S.melongena* at high phosphate level treatment (Gm+Gl+3P). Similarly, *Melia azadirachta* L. plants showed decreased shoot and root length as compared to (Gm+Gl+2P) after 120 days of AM fungal inoculation. Plants that have received AM fungal inoculation showed increased total chlorophyll content in leaves and P level in shoots (Fig 2). After 120 days of AM fungal inoculation, dry weights of shoots drastically decreased in Gm+Gl+3P level treated to plants of *Melia azadirachta* L. as compared to non-mycorrhizae inoculated plants, because at high phosphate level plants growth slowly was suppressed due to which there is reduced accumulation of minerals in mycorrhizal plants shown (Table 1). Similar results were obtained by earlier workers [Rubio *et al.,* 2003; Lakshman and Kolkar, 2008]. Per cent mycorrhizal colonization increased with an increase in phosphate level in early stages of root colonization (Table 2). In the present investigation, there was significant increase in the percent root colonization at 60 and 90 days with *G. mosseae* and *G. leptonicum* inoculation. But, there was a decreased per cent root colonization after 120 days when, the plants treated with (Gm+Gl+3P level) because of high P concentration in the rhizosphere soil. It is reported that high soil P level reduces both intraradical as well as extraradical AM fungal development [Abbott and Robson, 1984; Liu *et al.,* 2000; Roopa and Lashman, 2009]. Total chlorophyll content consequently increased in AM inoculated plants than those of non-mycorrhiza inoculated (control) plants at first and second level phosphate treatment *i.e.,* 50-100 mg P/kg soil and after 120 days of AM fungal inoculation. However, at high phosphate level (150mgP/kg soil), the total chlorophyll content was decreased in AM fungi inoculated plants (Gm+Gl+3P) after 120 days of inculcation, which is mainly that mycorrhizal colonization or P Fertilization influenced the concentration of photosynthetic pigments

shown in (Table 2). These results are in agreement with earlier contributors [Giri *et al,* 2003; Kapoor and Bhatnagar, 2007; Irfan *et al.,* 2011]. Present findings recorded that P content increased significantly on AM fungal inoculated *Melia azadirachta* L. as compared to non-mycorrhizal plants. P content in shoots increased after 60, 90 and 120 days after AM fungal inoculation in all the phosphated treatments (Table 2). The increased P uptake may be a result of increased phosphorylase activity on the surface of mycorrhizal roots. It may be also due to the increased absorbing area contributed by fungal hyphae [Pearson and Tinker, 1975]. The progress was observed to be greater in AM fungal inoculated plants than that of non- inoculated plants, it was found to be more pronounced in (Gm+Gl+3P) as compared to other treatments (Table 2).The results of the present work is in agreement with the findings of [Liu, 2000; Dhanda *et al.,* 2004, Irfan *et al.,* 2011] who have reported that plants which received AM fungal inoculation had higher phosphorus content in shoots than in non-AM fungal plants. The present study clearly revealed that the plants of *Melia azadirachta* L. which received AM fungal inoculation, exhibited a significant growth in plant heights, roots length and chlorophyll content in leaves, leaf area and P over the control plants [Chaitra *et al.,* 2016]. This suggests that AM fungi with balanced phosphate treatment uncertainly prove beneficial for increasing biomass production in agricultural and horticultural crops. Therefore, proper selection of indigenous mycorrhizal strains could play an important role in optimizing the growth of *Melia azadirachta* L.

4. CONCLUSION

The need for continued use of fertilizers in increasing the agricultural productivity to meet the growing demands of the people cannot be over emphasized. It is now becoming clear the ultimate "sink" of the fertilizers applied in agriculture and areas are soil. Soil being the storehouse of multitudes of microbes, in quantity and quality, receives the chemicals in various forms and acts as the scavenger of the harmful substances. The fertilizers reaching the soil in significant quantities alter the ecological balance. This may affect the overall microbial population, of which some of them may be selectively inhibited or killed. The fertilizers might alter the physiological conditions prevailing in the rhizosphere, which in turn alters the rhizosphere mycoflora both quantitatively and qualitatively. The chemicals used should avoid serious injuries to the great variety of microbes whose functions are vital to the crop-producing power of the soil. Finally, it can be said that higher concentrations of the fertilizers are not required in increasing plant growth as these cause greater degree of soil disturbance. The optimum benefit in terms of plant growth can be obtained from mycorrhizal symbiosis at a low fertilizer input. It is suggested that the low concentrations of the fertilizers must be applied which would have both an economic and environmental impact.

ACKNOWLEDGEMENT

Dr. H.C. Lakshman is very thankful to UGC DSA 1st phase, for financial support to the department research problem on diversity of microbes and higher plant from Western Ghats of Karnataka and their conservation.

CONFLICT OF INTEREST

The authors have no conflict of interest.

REFERENCES

1. Abbott LK, Robson AD. The effect of root density, inoculum Placement, and infectivity of inoculum on the development of VA mycorrhizal fungi. New Phytol. 1984; 97: 285-299.
2. Chairmel T, Bagyaraj DJ, Patil CSP. Response of *Andorgrophis paniculata* to different arbuscular mycorrhizal fungi.Journal of Agricultural Technology. 2006; 2(2): 221-228.
3. Chaitra BN, Lakshman HC, Suresh BG. Effect of AM fungus and Rhizobium inoculation to improve plant growth and biomass yield, N and P uptake in *Acacia nilotica* (L.) Will. Sci. Research Report. 2016; 6(1): 22-27.
4. Dhanda SS, Sethi GS, Behl RK. Indices of drought tolerance in wheat genotypes at early stages of plant growth. Journal of Agronomy and Crop Science. 2004; 190(l): 6-12.
5. Elahi FE, Mridha MA, Aminuzzaman U. Influence of AM fungal inoculation on growth, nutrient uptake, arsenic toxicity and chlorophyll content of eggplant grown in arsenic amended soil. Advances in natural and applied sciences. 2010; 4(2): 184-192.
6. Ferreira FS, Jarick J. Floral morphology of *Artemisia annua* with special reference to trichomes. Inter. J Plant Sci. 1995; 156:807-815.
7. Gerdemann JW, Nicolson TH. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Brit. Mycol. Soc. 1963; 46: 235-244.
8. Gir B, Kapoor R, Mukerji KG. Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *Acacia auriculiformis.* Biology and Fertility of Soil.2003; 38: 170-175.
9. Howeler RH, Siverding E, Saif S. Practical aspects of mycorrhizal technology in some tropical crops and pastures. Plant and Soil. 1897; 100:249-283.
10. Irfan A, Yoob M, Fite PK. Application of AM fungal inoculant on growth enhancement of *Solanum elangena* L. at different phosphorus level. Mycorrhiza News. 2011; 23(3) :9-12.

11. Jackson ML. Soil Chemical Analysis. Prentice Hall, India, New Delhi,.1973;498.
12. Jamaluddin. Bioinoulants for sustainable forestry. In: Bio inoculants for sustainable Agriculture and forestry. Edited by: S.M. Raddy, S. Rama Raddy. M.A Singarachary and S. Girisham. Scientific Publishers (India), Jodhpur. 2002; 21-25.
13. Jeffries D. Use of mycorrhizae in agriculture. Critical Reviews in Biotechnology. 1987; 15:319-358.
14. Kapoor R, Bhatnaga AK. Attenuation of cadmium toxicity in mycorrhizal celery (Apium *graveolens* L).World journal of Microbiology and Biotechnology.2007; 23: 1083-1089.
15. Karthikeyan B, Jaleel CAZ, Joe MM, Srimannaragan I, DeiveekaSundarm M. The effect of AM fungi and phosphorus level on the biomass yield and ajmalicine production in *Cartharathus rosea*. Eur Asian J Bioscience.2008; 2: 26-33.
16. Katiyar RS, Das PK, Choudhury PC, Ghosh A, Singh GB, Datta RK. Response of irrigated mulberry *Morus alba* L. to VA- mycorrhiza inoculation under graded doses of phosphorous plant. Plant and Soil.1995; 170: 331-337.
17. Kormanic PP, McGraw AC. Methods and principles of mycorrhizal research. Am. Phytopath. Soc. 1982; 34-45.
18. Lakshman HC. VA - mycorrhizal studies in some important timber tree species. Ph. D. Thesis, Karnataka University, Dharwad, India.1996; pp. 358.
19. Lakshman HC, Kolkar KP. Effect of AM fungus with additional P on the growth of *Hibiscus rosasinensis* L. Nat. J.L. Sci. 2008; 5(2): 196-199.
20. Lakshman HC. Response of *Albizzia lebeck* D C. to AM fungi and Rhizobium interaction.My Forest. 2005; 41(2A): 295-300.
21. Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL. Acquisition of Cu, Zn, Mn, and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels.Mycorrhiza.2000; 9: 331-336.
22. Mosse B. Advances in the study of vesicular arbuscular mycorrhiza. Ann Rev. Phytopathol. 1973; 11: 171-196.
23. Pearson B, Tinker PB. Measurement of phosphorus fluxes in the external hyphae of Endomycorrhiza. In: Endomycorrhiza. Edited by: F.E. Sanders, B. Mosseae, and P.B. Tinker,

Academic Press, New York. 1975; pp 277-287.

24. Phillips JM, Hayman DS. Improved procedures for cleaning and staining parasite and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 1970; 55(1): 158-162.
25. Romana M, Lakshman HC. Seasonal variation in three Leguminous tree seedlings colonized with AM fungi. Int. J. Plant. Sci. 2009; 46: 56-62.
26. Roopa KJ, Lakshman HC. Effect of AM fungus and additional phosphate on the growth of *Brassica juncea* L. I. J. Plant Sci. 2009; 4 (1): 52-55.
27. Rubio R, Borie F, Schalchli C, Castillo C, Azcon R. Plant growth responses in natural acidic soil as affected by arbuscular mycorrhizal inoculation and phosphorus sources. Journal of Plant Nutrition.2003; 25: 1389-1405.