and we underscore here the fire maintenance of these two species at a low density.

The present work supports the previous observations on the dynamics of this forest[6]. Currently, the tree density of the Ainurmarigudi Reserve Forest is decreasing, which is a clear threat to the sustainability of the ecosystem. A further step will be to know whether this trend is prevailing over the whole Bandipur–Mudumalai area. However, it may be necessary to adopt a better fire management policy.

Frequent and uncontrolled fires, as in the present situation, endanger the ecosystem. Total protection is not possible because of the traditions of the local people, nor is it advisable. Even when attempted, it leads to a more closed forest, which is not advantageous for the grazing herbivores. During the course of plant succession, the biomass increases, and when the fires do occur they are generally violent, killing a large number of plants and animals[4]. If there are no fires, weeds may invade and block the succession process, reducing the forage availability[28]. An intermediate solution could be fire control. Late burning reduces tree population by killing young trees[10], but early burning does not drastically affect the tree community[24] and could be applied safely. Setting early fires of lower frequency (once in two or three years) has the advantage of preventing catastrophic fires, stopping the spread of weeds and maintaining the tree cover. As a measure to safeguard the immense floral and faunal wealth of this ecosystem, it is advisable to determine carefully the optimal burning rate and frequency by experimental work.

1. Gadgil, M. and Guha, R., *This Fissured Land, An Ecological History of India*, Oxford University Press, 1992.
2. Puyravaud, J. P., Pascal, J. P. and Dufour, C., *J. Biogeogr.* to be published.
3. Stott, P., *Prog. Phys. Geogr.*, 1991, **15**, 18–28.
4. Gillon, D., in *The Fire Problem in Tropical Savanna* (ed. Bourlière, F.), Elsevier, Amsterdam, 1983.
5. Moreno, J. M. and Oechel, W. C., *Ecology*, 1991, **72**, 1993–2004.
6. Gaulier, A. and Planchais, I, Engineer dissertation, ENGREF, Nancy, France, 1993.
7. Trabaud, L. and Oustic, J., *Seed Sci. Technol*, 1989, **17**, 589–599.
8. Archer, S, *J. Biogeogr.*, 1990, **17**, 453–462.
9. Menaut, J. C., Gignoux, J., Prado, C. and Clobert, J., *J. Biogeogr.*, 1990, **17**, 471–481.
10. Hopkins, B., *Forest and Savanna*, Heinemann, London, 1974.
11. Phillips, J., in *Fire and Ecosystems* (eds Kozlowski, T. T. and Ahlgren, C. E), Academic Press, New York, 1974, pp. 24–33.
12. Gautier, L., *Candollea*, 1992, **47**, 179–193.
13. Gautier, L., *Candollea*, 1993, **48**, 179–193.
14. Goldammer, J. G., *Fire in Tropical Biota. Ecosystem Processess and Global Challenges*, Springer, Berlin, 1990.
15. Pascal, J. P, *Wet Evergreen Forests of the Western Ghats of India*, French Institute, Pondicherry, India, 1988.
16. Sharatchandra, H C. and Gadgil, M., *J. Bombay Nat. Hist. Soc.*, 1975, **72**, 623–647.
17. Nair, S. S., Nair, P. V., Sharatchandra, H C and Gadgil, M, *J. Bombay Nat Hist Soc*, 1977, **74**, 401–435.
18. Ranjit Daniels, R. J., *Curr. Sci*, 1993, **64**, 706–708
19. Sukumar, R., Dattaraja, H. S, Suresh, H. S., Radhakrishnan J, Vasudeva, R., Nirmala, S. and Joshi, N. V., *Curr. Sci.*, 1992, **62**, 608–616.
20. Prasad, S. N. and Hegde, M., *Proc. Indian Acad. Sci. (Plant Sci.)*, 1986, **96**, 121–133.
21. Prasad, S. N., and Sharatchandra, H. C., *Proc. Indian Acad. Sci. (Plant Sci.)*, 1984, **93**, 83–97.
22. Murali, K. S. and Sukumar, R., *Oecologia*,, 1993, **94**, 114–119.
23. Bourgeon, G., *Explanatory Booklet on the Reconnaissance Soil Map of Forest Area, Western Karnataka and Goa*, French Institute, Pondicherry, India, 1989.
24. Rose Innes, R., in *Proceedings of Annual Tall Timbers Fire Ecology Conference*, 1971, pp. 147–173.
25. Devineau, J. L., Doctoral thesis, University of Paris VI, Paris, 1975.
26. Spichiger, R., Doctoral thesis, University of Geneva, Geneva, 1977.
27. Bowman, D. M. J. S. and Fensham R. J., *Aust. J. Ecol.*, 1991, **16**, 111–118.
28. Sukumar, R., *The Asian Elephant: Ecology and Management*, Cambridge University Press, 1989.

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Movement of ^{32}P in sunflower plants inoculated with single and dual inocula of VAM fungi

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Sunflower plants were inoculated with single and dual inocula of *Glomus intraradix* and *Glomus mosseae*. ^{32}P-labelled phosphate was applied after sufficient infection was obtained in all treatments. Plants inoculated with dual inocula of the two VAM fungi showed significantly higher shoot weight than those inoculated with single inocula and control treatments. Of the ^{32}P transported to the plants, the percentage of ^{32}P transported to the shoot was 59.9 in *G. intraradix* + *G. mosseae*, 25.12 in *G. intraradix*, 33.31 in *G. mosseae* and 35.2 in uninoculated control. The present study reveals that the increased growth in plants inoculated with dual inocula compared to those inoculated with single inocula of VAM fungi may be due to the increased transfer of P to the shoot from the root.

It is now well-established that vesicular-arbuscular mycorrhizal (VAM) infection can enhance P uptake by

plant roots[1]. The enhanced P uptake by mycorrhizal plants has been found to be mainly due to the external fungal hyphae, which act as an extension to the root system, thereby providing a more effective absorbing surface. However, the effectiveness of VAM fungi and the length of the external hyphae have been found to vary with the host–fungus combination[2]. Evidence is also increasing that species of VAM fungi differ in their effectiveness with changes in soil pH[3], nutrient status of soil[4], and composition of soil microflora[5]. Studies have revealed that an ideal endophyte should possess the ability to infect the host plant early in the growth period, efficiently explore the soil for nutrients, transfer nutrients readily to the host, multiply readily, compete effectively with indigenous VAM fungi and infect a wide range of hosts under a variety of edaphic and environmental conditions. Since all these properties cannot be found in a single endophyte, a mixed inoculum containing more than one VAM endophyte may prove to be a more effective form of inoculum under a wide range of agro-climatic conditions[6, 7]. Moreover, a mixed inoculum could also provide for a better inoculum source throughout the growth season of the host plant, as each endophyte may be important at different times during the growth period. Studies on P uptake from isotopically labelled media have shown that VAM fungi are able to absorb P directly from the soluble P in the soil and translocate it to the host root[1]. Gray and Gerdemann[8] found that there was 160 times more radioactivity in the tops of mycorrhizal plants than non-mycorrhizal plants. It has also been observed that though plants infected with single inocula of VAM fungi clearly effected P uptake in roots, the rate of transport of P into the shoot was little affected[9]. Experimental evidence suggests that VAM fungi store very little of the absorbed P for a short period and so additional P absorbed by them should be rapidly transferred from the root to the shoot, to optimize the benefit of the mycorrhizal symbiosis to the host plant. Most of the studies on P uptake have been done in soils with low or moderate P status. In soils high in phosphate, mycorrhizal interactions with plant P uptake have received less attention[9], mainly because plants do not depend much on mycorrhizal association when soil P level is high. However, recent studies on the mycorrhizal dependence of crops have indicated that certain tropical crops are highly mycotrophic and will not grow or produce well in low-P soils, without a highly effective mycorrhizal association. It has also been noted that the critical soil P level and response of the plant to P application varies greatly according to the efficiency of native VAM fungi. Some studies on mixed inocula have shown that increased beneficial effect of the mycorrhizal association on the host plant, produced by a mixture of two VAM fungi as compared to that produced by a single inoculum, is due to the higher root colonization[10–12]. The present experiment was designed to investigate into the effect of single and dual inocula of VAM fungi on the movement of phosphate into roots and shoots of sunflower.

Vertisol soil of high P status (Olsen P-97.5 mg/kg; pH 7.2) collected from γ-field, Bhabha Atomic Research Centre, Trombay, India, and acid-washed sand were autoclaved twice, 2 weeks before use. About 100 seeds of sunflower (cv. Morden) were sown in steam-sterilized acid-washed sand to obtain sterile seedlings. The substrate for plant growth was prepared by mixing sterilized soil and acid-washed sand (1 : 1 w/w). Two kg of soil mix was filled in each of the plastic bags of size 30 × 18 cm. Approximately 3-month-old cultures of the VAM fungi, viz. *G. intraradix* Schenck and Smith and *G. mosseae* (Nicol. & Gerd.) Gerdemann and Trappe, multiplied on buffle grass (*Cenchrus ciliaris* L.) were used as inocula. Single (60 g) and double (30 + 30 g) soil inocula, containing chopped mycorrhizal root pieces, hyphae and spores were mixed about 6 cm below the soil surface in mycorrhizal pots. Uninoculated pots were mixed with 10 ml filtrate of mycorrhizal inoculum. One-week-old seedlings of uniform size were transferred to pots. There were three replicates for each treatment. A plant from each treatment was harvested after 7–15 days to observe for mycorrhizal infection. About 45% and 60% of mycorrhizal infection was observed in roots of plants infected with single and dual inocula of VAM fungi, respectively, after 14 days of inoculation. On the 15th day, 0.5 ml radioactive phosphate solution containing 3.0 μCi $H_3{}^{32}PO_4$ was diluted with 1500 ml distilled water and 25 ml of this solution was fed to each of the inoculated and uninoculated pot. After 17 days of ^{32}P application, the plants were carefully removed from the pots and carried to the laboratory in sterilized polythene bags. The roots were washed with several changes of sterile distilled water, dipped in 0.1 mM $H_3{}^{32}PO_4$ solution for 30 min and rinsed with two changes of sterilized distilled water. It is well documented that in crops different pairs of leaves contribute differentially towards seed development. In sunflower, the leaves of the lower half start senescing much earlier and, therefore, the seed filling primarily depends upon the assimilates contributed by the leaves at the upper half, since they remain green and active in photosynthesis for a longer period. Therefore, for measuring radioactivity, plants were divided into 6 different parts:

Part I – tip of shoot with small leaves and bud (L-1)
Part II – 1st pair of true leaves from the tip (L-2)
Part III – 2nd pair of true leaves from the tip (L-3)
Part IV – 3rd pair of true leaves from the tip (L-4)
Part V – stem (S)
Part VI – root (R)

Each plant part was kept separately in disposable kimble

scintillation glass vials. Plant material was dried in an oven at 60–70°C for 24 h and the dry weight was recorded. Each dried plant part was dipped in 10 ml solution of PPO/POPOP/Dioxan scintillation fluid and transferred to trays for ^{32}P counts. Radioactivity was determined for 1 min in a computerized liquid scintillation counter using Cerenkov radiation. The total ^{32}P was estimated from the counts after correcting for background and decay. Data were analysed using single-factor analyses of variance.

In general, there was higher root and leaf dry weight in plants infected with *G. intraradix* + *G. mosseae* compared to plants infected with single inocula (Table 1). The double inocula treatment had significantly higher leaf dry weight than *G. mosseae* in only L-4 and L-3 pairs of leaves, which indicates that *G. mosseae* possibly contributed most to the better performance of dual inocula. Performance of *G. mosseae*, which was better than *G. intraradix* in vertisol soil of high-P status used in the present study was, however, poor in sandy-loam soil of moderate P status used in earlier pot and field experiments[12], which indicates that effectiveness of VAM species varies much with edaphic conditions. Results obtained in the present study with double inocula treatment, however, are consistent with the observations made earlier in pot and field experiments[12]. Differences in growth of plants infected with single and dual inocula of VAM fungi may be attributed to the higher root colonization during early growth period of plants inoculated with dual inocula. The lower root colonization associated with reduction in root dry weight in single inocula treatments might have offset any promotion of P uptake by VAM fungi. The reduced dry weight of the mycorrhizal roots inoculated with single inocula was possibly due to the competition for P by the VAM endophyte. Similar observations have been made earlier during the early part of the mycorrhizal associations in other crop plants[13]. Koide and Li[14] suggested that host plant limits mycorrhizal infection through some mechanism when it is not benefited from mycorrhizal association.

The effect of single and double inocula of VAM fungi on the uptake and transfer of ^{32}P is given in Table 2. Mycorrhizal roots had higher P concentration than roots of equivalent non-mycorrhizal plants, which was due to the presence of hyphae, vesicles and arbuscules inside the infected roots. Roots of plants infected with single inocula of *G. intraradix* and *G. mosseae* contained about 1.5 times the ^{32}P (74.88% and 66.90%, respectively) in roots infected with *G. intraradix* + *G. mosseae* (40.10%). In shoots, however, plants infected with *G. intraradix* + *G. mosseae* had higher ^{32}P (59.9%) than in plants infected with *G. intraradix* (25.12%) and *G. mosseae* (33.31%). The higher percentage of P in roots of plants infected with a single inoculum than that in plants infected with dual inocula of *G. intraradix* and *G. mosseae* indicates that the high P status in single-inoculum plants might have limited the growth of VAM fungi, thus reducing the plant growth response to mycorrhizal association[15]. Koide and Li[16] suggested that in sunflower, mycorrhizal infection is regulated by the P status of the root and not by the N or P status of the shoot. Cooper and Tinker[17] observed that onion roots infected with *G. mosseae* contained nearly twice as much of ^{32}P (44–53%) as the shoot (28–34%) after 4 days of ^{32}P addition. In the present study, it is evidently clear that there is little difference in the percentage of absorbed phosphate transported to the shoots among plants infected with single inocula of *G. intraradix* and *G. mosseae* and control. Similar observations have been made in other crop plants inoculated with a single inoculum[9]. The amount of ^{32}P transported to the shoots in *G. intraradix* + *G. mosseae* treatment was almost 2.5 times that in the uninoculated control. The data for distribution of ^{32}P in leaves show that ^{32}P was distributed differentially in different pairs of leaves, which was possibly due to the differential rate of transpiration in leaves. An increase in the proportion of ^{32}P appearing in the topmost portion of the plant (L-1) indicates that increased host transpiration moved P up from the root to the shoot. Cooper and Tinker[1]

Table 1. Dry weight (mg) of root (R), stem (S), tip of the shoot consisting of small leaves and bud (L-1), 1st pair of true leaves (L-2), 2nd pair of true leaves (L-3) and 3rd pair of true leaves (L-4)

Treatment	Dry weight (mg)					
	R	S	L-4	L-3	L-2	L-1
G. intraradix	171[c]	490[a]	122[a]	343[b]	351[c]	370b
G. mosseae	180[c]	433[b]	82[c]	352[b]	391[a]	460[a]
G. intraradix + *G. mosseae*	242[a]	498[a]	95[b]	382[a]	384[a]	396[a]
Control	200[b]	487[a]	60[c]	343[b]	371[b]	378[b]

[a, b, c]In each column the mean values superscribed with the same letter do not differ significantly ($P = 0\ 05$).

Table 2. Radioactivity in root (R), stem (S), tip of the shoot consisting of small leaves and bud (L-1), 1st pair of true leaves (L-2), 2nd pair of true leaves (L-3) and 3rd pair of true leaves (L-4)

Treatment	Counts/min/10 mg dry wt.					
	R	S	L-4	L-3	L-2	L-1
G. intraradix	17664[ab]	4633[b]	506[c]	105[c]	266[c]	415[b]
G. mosseae	22785[a]	6666[a]	3170[b]	621[b]	422[b]	396[b]
G. intraradix + *G. mosseae*	21700[a]	7232[a]	12141[a]	4072[a]	4139[a]	4819[a]
Control	16153[b]	4182[b]	3182[b]	542[b]	251[c]	616[b]

[a, b, c]In each column the mean values superscribed with the same letter do not differ significantly ($P = 0\ 05$).

found that at full transpiration, the amount of ^{32}P in shoots in mycorrhizal plants was almost 2.3 times that at low transpiration. The higher ^{32}P activity in L-4 pairs of leaves in all treatments may be due to their presence close to the roots.

The study confirms the hypothesis[7] that the use of a mixture of two compatible species of VAM fungi can prove to be a better alternative to a species of VAM fungi universally adapted to different agro-climatic conditions. The results also indicate that the increased growth in plants inoculated with dual inocula compared to those inoculated with single inocula of VAM fungi may be due to the increased transfer of P to the shoot from the root.

1 Cooper, K. M. and Tinker, P. B., *New Phytol.*, 1981, **88**, 327–339.
2. Azcon, R. and Ocampo, J A., *New Phytol.*, 1981, **87**, 677–685.
3. Hayman, D. S. and Tavares, M., *New Phytol.*, 1985, **100**, 367–379.
4. Abbot, L. K. and Robson, A. D., *New Phytol.*, 1978, **81**, 575–585.
5. Schwab, S. M., Menge, J. A. and Leonard, R. T., *Can J. Bot.*, 1983, **70**, 1225–1232.
6. Mallesh, B C. and Bagyaraj, D. J, *Leucaena Res Rep*, 1990, **11**, 56–57.
7. Daft, M. J. and Hogarth, B. G., *Trans. Br. Mycol. Soc.*, 1983, **80**, 339–345.
8. Gray, L. E. and Gerdemann, J. W., *Plant Soil*, 1969, **30**, 415–422.
9. Smith, S. E., *New Phytol.*, 1982, **90**, 293–303.
10. Ross, J. P. and Ruttencutter, R., *Phytopathology*, 1977, **67**, 490–496.
11. Simpson, D. and Daft, M. J., *Plant Soil*, 1990, **121**, 179–186
12. Mehrotra, V. S., Ph D thesis (Botany), Allahabad University, Allahabad, India, 1993.
13. Bethlenfalvay, G. J., Pacovsky, R. S. and Brown, M. S , *Phytopathology*, 1982, **72**, 894–897.
14. Koide, R. T. and Li, M., *New Phytol.*, 1989, **111**, 35–44.
15. Menge, J. A., Munnecke, D. E., Johnson, E. L. V. and Leonard, R. T., *New Phytol.*, 1978, **80**, 575–578.
16. Koide, R. T. and Li, M., *New Phytol*, 1990, **114**, 59–74.
17. Cooper, K. M. and Tinker, P. B., *New Phytol.*, 1978, **81**, 43–52.

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Somatic tissues leading to embryogenesis in cumin

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Seeds of cumin (*Cuminum cyminum* L.) were germinated on Murashige and Skoog's (MS) medium supplemented with benzyladenine (BA) (8 mg/l) and kinetin (1 mg/l). From the germinated seedlings, various explants (roots, hypocotyls and cotyledons) were excised and inoculated on MS medium supplemented with 8 mg/l of BA. Out of all the explants, hypocotyls were found to be the source to somatic embryos. However, separation of embryogenic clumps was difficult on solidified medium; thus, on maturation root poles remained suppressed. Nevertheless, complete regeneration was observed in 10% of the cultures.

PLANT regeneration via somatic embryogenesis is preferred over organogenesis due to various advantages, one distinct advantage being that somatic embryos are bipolar structures bearing both root and shoot apices. Somatic embryogenesis is a pre-requisite in crop improvement. It was reported that embryogenesis has been observed in all the Apiacean spices, except in cumin[1]. Nevertheless, investigations in cumin led to rhizogenesis, caulogenesis and complete plantlet formation through callus[2].

Cumin is an important spice used as a flavouring agent for culinary purposes. Its oil has significant medicinal properties (antispasmodic, antihysteric, stomachiachic, astringent and cooling). The technique of tissue culture has applications in propagation and improvement of crops. The present investigation is the first report on somatic embryogenesis leading to regeneration in cumin.

Cumin seeds were surface-sterilized and inoculated on MS medium[3], solidified with 0.8% agar, incorporated with BA (8.0 mg/l) and kinetin (1.0 mg/l).

Explants from two-week-old seedlings were inoculated on MS medium supplemented with BA (8.0 mg/l).

From the mixed type of calli, simple callus (non-differentiating + embryo initials) and clumps of embryo initials were carefully separated and inoculated onto MS medium with the same BA concentration.

Seedlings obtained from seeds inoculated on BA-containing-medium differed from normal seedlings in their entire thickness. From the various organs, i.e. roots, cotyledons and hypocotyls, cotyledons showed necrosis, roots formed pale yellow callus which did not

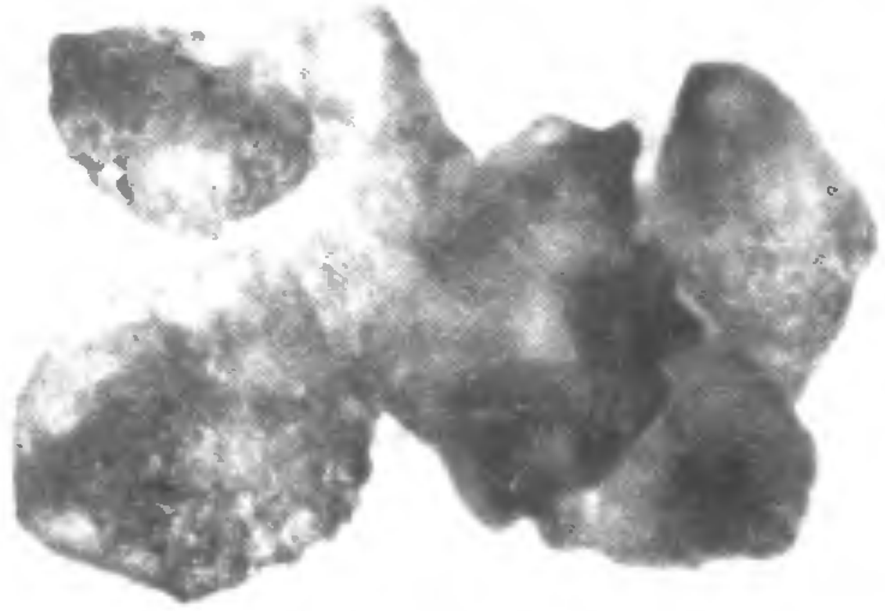

Figure 1. Group of proembryo initials