



Zena's Retama sphaerocarpa Trial (Oct 2003-5)

Seeds were scarified, soaked and pre-germinated. 360 pre-germinated seeds were sown into forest pots on 31.10.03 (120 with natural soil plus live inoculum, 120 with natural soil and sterilized inoculum, and 120 with sterilized inoculum, sterilized soil and a filtrate of natural soil). The pots were filled 2/3 with soil, then a layer of 50 ml inoculum was added, the seeds were sown into this and covered with a light covering of soil. Slow release fertilizer was added at a rate of 20 ppm phosphorus to ensure an adequate but limiting supply of this nutrient.

Purpose of the controls: Sterile inoculum was added to the controls to standardize physical factors such as nutrient levels etc. Control 1 addresses the question, is natural soil just as good as pot-cultured inoculum, or has the pot-culturing increased beneficial mycorrhizal potential (as is intended)? Control 2, with no viable mycorrhizal propagules, but with a filtrate to replace any other soil microorganisms such as nitrogen-fixing *rhizobium*, should show how well the seedlings can grow without mycorrhiza.

The inoculum: starter soil was collected from topsoil (0-25 cm) under naturally occurring retama and mixed 50:50 with washed graded sand. Slow release fertilizer was added at the rate of 20 ppm phosphorus. Alfalfa and sorghum were sown into this mixture and grown for 3 months. A week prior to harvesting watering was stopped and the plants stressed to induce the production of spores by the mycorrhiza. The above-ground growth was discarded, as was the top and bottom cm of soil. The roots were cut into pieces about 1 cm long and mixed back into the remaining soil. This mixture then constitutes the inoculum.

Results:

	Nursery survivors/120		
Treatment	After 2 months	After 3 months	Mean height after 3 months (mm)
Inoculated	112	112	71
Control 1	110	110	69
Control 2	108	106	60

In March a sudden frost killed back most of the new growth that had been put on in the preceding mild spell. 80 seedlings were selected from each treatment by a random number table, and planted out into 1m² plots (randomised blocks), 16 plants per plot, 5 plots (80 plants) per treatment.

20 of each treatment were also selected, using the same random method, for a destructive harvest; mean fresh weight of root and shoot, and number of nitrogen fixing nodules were as follows:

	Root (g)	Shoot (g)	Nodules
Live inoculum	1.03	0.75	3.65
Control 1	0.72	0.58	1.45
Control 2	0.73	0.52	0

The trial was monitored monthly until January 2005, and again in April 2005 (Fig1), when average heights were:

Live inoculum 468 mm
Control 1 464 mm
Control 2 398 mm

There had been a recent sudden burst of new growth, often from near the base of the plant, so not reflected in the measurement for height. This was roughly assessed on a scale 0 – 3, but almost no difference in new growth was found between the three treatments.

The May 2005 measurements showed a loss in height for all treatments. Control 1 showed the smallest loss, and now had the highest average height:

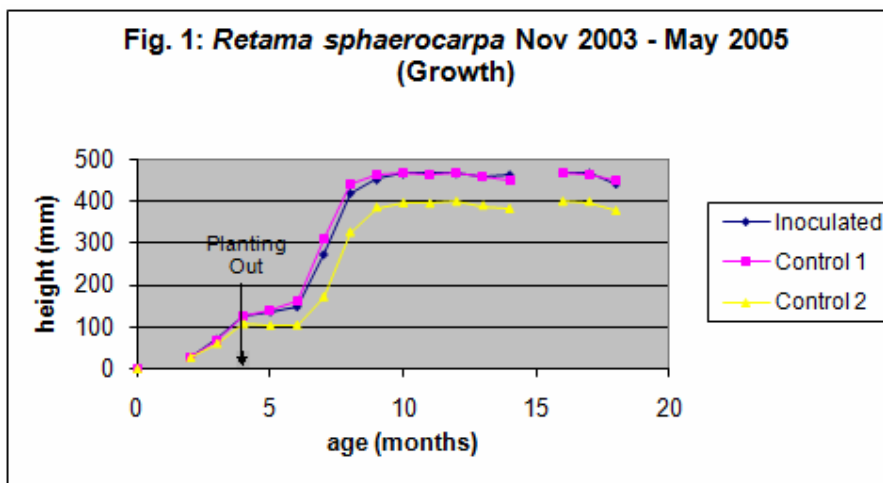
Live Inoculum 442 mm
Control 1 451 mm
Control 2 377 mm.

The inoculated group had lost most height, but had the greatest density of branchlets. Branchlet density was rated on a scale 0 (low) to 5 (high):

Live Inoculum 3.18
Control 1 2.90
Control 2 2.66

Final survival rates were very similar for the three treatments:

Live Inoculum 80.8%
Control 1 82.9%
Control 2 79.5%



Discussion: There was no survival benefit from inoculation, but there was a growth benefit from both the cultured inoculum and the live natural soil, which was lost in the sterilized natural soil of Control 2; this benefit was particularly seen in the period immediately following transplantation into the field, when the plants of Control 2 suffered a marked transplant shock, which was less apparent in the other two groups. The mean heights of the Inoculated and Control 1 groups were very similar throughout, though the plants that had received the live cultured inoculum were on average sturdier than those in either of the control groups. This was shown in greater root and shoot biomass at 3 months, and greater branchlet density at 18 months. Plants of Control 2 group were significantly smaller in height, biomass and branchlet density.

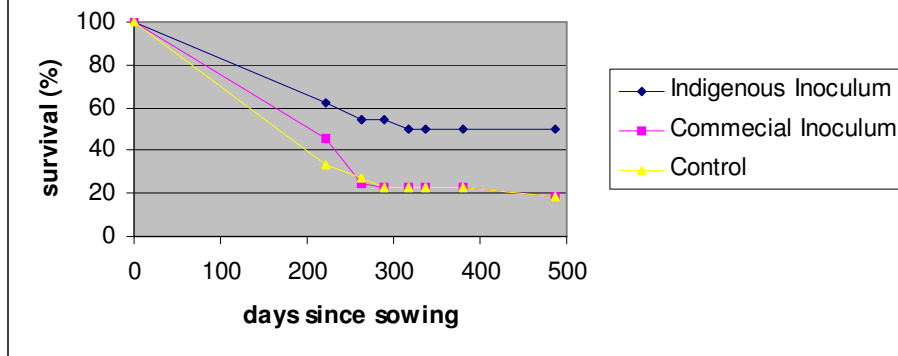
Retama is a legume, with the potential to fix atmospheric nitrogen in root nodules, providing certain rhizobial bacteria are present, and the nodulation rate of the trial plants at the end of the nursery stage of this trial is interesting: at age 3 months, the Inoculated group had more than twice as many nodules as Control 1; the plants of Control 2 had no nodules at all. Mycorrhizal fungi are known to facilitate nodulation, so it could be inferred that the fungi, multiplied by pot culture in the inoculum, but also beginning to be active in the natural soil gathered in autumn for use as substrate in Control 1, aided the early development of the retama seedlings partly at least by facilitating early nodulation. The filtrate applied to Control 2 would be expected to have contained the same *Rhizobium*, but in the absence of mycorrhizal fungi the *Rhizobium* had presumably not been activated.

A second Retama sphaerocarpa Trial was carried out by Tamas Keszler in 2007-09

This was on a much smaller scale. Seed was direct-sown in the field site in September 2007. There were 3 treatments: (a) received indigenous inoculum which had been trap-cultured on site, (b) received a commercial inoculum, Terravital Arid, supplied by Plantworks (www.friendlyfungi.co.uk), which contained the following fungal strains isolated in Mediterranean and N.African countries: *Glomus intraradices* (from Morocco), *Glomus aggregatum*, *Glomus deserticola*, *Glomus intraradices* (from Syria), *Glomus coronatum*, (c) was the uninoculated control. Each group was sown in a one metre square plot, with 22 sowing points in each case. Where more than one plant grew at a given sowing point these were left to grow on, and the tallest plant at each point was the one measured. This happened most in the inoculated treatments, meaning that these had a slightly higher germination rate (number of sowing points with extra plants – (a) 5, (b) 4, (c) 1.) This competition could of course be expected to restrict the growth of the tallest plant at these points.

Results: The indigenous inoculum gave 50% survival after 16 months; the commercial inoculum and the control treatments both gave only 18% survival (Fig.2.) However, mean height of survivors was similar in all treatments throughout the duration of this trial.

**Fig. 2: *Retama sphaerocarpa* Sept 2007 - Jan 2009
(Survival)**



Discussion: The plant roots were not examined for nodulation or mycorrhizal colonization, so no firm inferences can be drawn, but the results are consistent with the view that mycorrhizal fungi depend on the presence of other soil microorganisms for their beneficial effect on plant survival or growth. In this case, the missing factor in the commercial and control treatments may have been *Rhizobium* compatible with *Retama*.