FINAL REPORT ON THE AUSTRALIAN FLORA FOUNDATION FUNDED PROJECT
MICROBIAL SYMBIONTS OF STURT'S DESERT PEA
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The grant of $1,737.60 was expended on employing casual assistance for several small experiments and one larger experiment. The small experiments were pilot studies whilst I tried to develop the procedures and techniques needed to conduct experiments with Sturt Peas, *Rhizobia* bacteria and mycorrhizal fungi. The larger experiment was a study of the effects of different native soils and heat sterilisation treatments on the levels of microbial infection and growth of Sturt Pea seedlings.

Preliminary studies by a B.Sc.(Hons) student (Mr David Chinnery) and myself showed that carefully controlled inoculation of microbial symbionts onto seedling Sturt Peas was not going to be as easy as initially envisaged. The mycorrhizal fungi were Vesicular-Arbuscular Mycorrhizae (VAM) and could not be easily brought into pure culture. Inoculation of seedling roots with chopped Sturt Pea roots carrying VAM led to a very high death rate of seedlings (presumably due to transfer/proliferation of root pathogens on the dead roots) and an uncontrolled inoculation of *Rhizobia* bacteria. Pilot experiments on *Rhizobia* inoculation from pure cultures isolated from nodules on Sturt Peas growing in native soil worked erratically. The nutrient solution recommended for *Rhizobia* inoculation studies on Soya beans gave only poor growth of Sturt Pea seedlings growing in propagating sand. The poor growth of the seedlings was probably responsible for the poor levels of infection by *Rhizobia*. However, David Chinnery in his studies on mycorrhizal fungi found that diluted hydroponics solution ("Top" brand) gave adequate (but not excessive) growth of seedlings in sand.

Hence I decided that a different experiment was needed to try to answer some simpler questions about the behaviour of the Sturt pea/*Rhizobia*/VAM system:

1) Would 1/10th strength hydroponics ("Top" brand) give good levels of microbial infection on seedlings exposed to microbial inoculum ?
2) Could partial heat sterilisation of native soil give sufficient variation in microbial inoculum to enable study of the effects of different levels of inoculum on seedling growth?
3) Could different native soils (collected from Sturt Pea colonies in different places in outback SA) give the same results?

The experiment consisted of 4 soils, each receiving 3 treatments (control, 3 hours dry heat at 80°C, 2 hours dry heat at 100°C) with 8 seedlings for each combination of soil and treatment. The seedlings were grown in tubes (50 mm diameter by 100mm high) with a layer of autoclaved sand above and below the soil to minimise the risk of cross-contamination. Four tubes of the same soil and treatment were placed in a sealed pot to prevent cross-contamination from runoff. Each tube received 5 ml of 1/10th strength hydroponics per day whilst the seedlings were small, increasing later when seedlings became larger. The seedlings were kept in a constant temperature room at 25°C with 16 hrs light per day. Harvest took place after 8 weeks: each seedling had the root nodules :counted; the wet weight of the top measured and the roots placed in 50% alcohol for later observations on mycorrhizal fungi. After staining, the roots were examined for fungal structures and records were made of the frequency and amount of VAM structures within roots and the extent of external hyphae on the root surface.

The results can be summarised as follows :

1) Good growth and nodulation of some seedlings occurred, so the nutrient solution was not so weak that growth was impeded too much nor was it so rich in nitrogen that nodulation was discouraged.
2) There were no statistically significant effects of heat sterilisation on the number of nodules (P ~ 0.15) or the wet weight of seedling tops (P ~ 0.9), nor did the appearance of mycorrhizal fungi differ between the treatments within soils (visual appraisal only). Clearly, the two heat treatments failed to reduce the levels of microbial inoculum sufficiently to have an impact on the results. These microbes appear to be fairly resistant to dry heat - perhaps as an adaptation to long term survival in arid zone soils where high temperatures can occur in surface regions on summer days. A follow up experiment with two soils autoclaved for 30 min at 120°C again failed to show any differences from the controls in seedling growth and nodulation (visual examination only).
3) there were highly significant differences between soils in the number of nodules (P << 0.001) and the top weight of seedlings (P ~ 0.002)

Table : Mean *rhizobium* nodule count and top weight in 4 soils

	Soil			
	1	2	3	4
Nodules/seedling	5.12	5.57	0.55	4.85
Top weight gm (wet)	0.27	0.43	0.32	0.34
Correlation	0.65	0.51	0.55	0.36

The Pearson correlation between nodule count and wet weight of seedling top for each soil is significant in 3 out of 4 soils and has a mean of 0.51. Surprisingly, when comparing the 4 soils there is no correlation between mean nodulation and mean wet weight: soil 3, with only about 1/10th the number of nodules found in the other soils does not have the lightest seedlings. Thus the results of different soils were not always the same.

Another relevant observation was that the shape and size of nodules differed between the soils, suggesting that each soil carried a different *Rhizobia* strain.

The observations on mycorrhizal fungi were difficult to quantify: most root systems had VAM and external hyphae around the roots. Taking the observations from the smallest plants (wet weight < 0.2gm) and largest plants (wet weight > 0.5 gm) shows that 8 of the 17 small plants lacked any VAM structures whereas all of the 10 large plants had VAM. There did not appear to be any differences in the abundance of external hyphae between small and large plants. Thus there does appear to be a correlation between seedling size and the presence of VAM but not external hyphae (which may well be saprophytic fungi rather than mycorrhizal fungi).

The correlation between *Rhizobia* nodules and seedling size and the possible correlation between VAM and seedling size cannot be used to infer causation in this experiment. The variation in size of seedlings could well be the normal variation between seedlings and hence the higher numbers of nodules and VAM on larger seedlings may simply reflect the larger root systems on more vigorous seedlings. I have not shown in this experiment that nodulation and VAM infection causes more growth of Sturt Pea seedlings. However, the results are not in conflict with. the hypothesis that the microbes are symbiotic and assist the growth of seedlings.

I now plan to do some more work on *Rhizobia* and mycorrhizal fungi separately in order to improve the inoculation and cultivation procedures so that the original experimental design will become feasible. A possible solution to the problem of a suitable inoculum of VAM is a commercial source: Dr Sally Smith of the Waite Institute gave me a small sample of commercial VAM that she had imported and I tried it under a few plants and they grew larger (but not statistically significantly so) than control plants grown only in sand.

I should like to thank the Australian Flora Foundation for their support for my research on Sturt Peas and associated microbes. Progress has been slow and at times seemed elusive, but in the end I feel confident that knowledge and experience has been gained that will be very useful in guiding further developments in this area.