Report to the Australian Flora Foundation on work funded by a grant entitled:
Growth and mycorrhizal infection *of Atriplex vesicaria* **and** *Stipa nitida*

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Introduction

Mycorrhizas are associations between the roots of plants and fungi where the growth of the plant is usually increased. Mycorrhizal associations are widespread, with most plants- including all major crops - being infected by mycorrhizal fungi (Tester *et al.,* 1987). However, the status of infection of many Australian native plants is still unknown, particularly in the arid zone.

Members of the Chenopodiaceae, dominant shrubs in the Australian arid zone, are traditionally considered to be at most weakly mycorrhizal (Tester *ei al.,* 1987), despite work showing significant infection and growth responses to infection in *Atriplex canescens,* a chenopod shrub widespread in rangelands of the USA (Williams *et al.,* 1974). There was one published report of limited infection in *Atriplex vesicaria,* a shrub dominant in large areas of and Australia (Bevege, 1968). However, the extent of this infection in naturally growing plants was unknown; moreover infection by mycorrhizal fungi of other Australian chenopods **was** also unknown. It was proposed to investigate the extent and effects of infection in *Atriplex vesicaria* and also in a grass, *Stipa nitida,* which .is commonly found growing near *Atriplex vesicaria.* In preliminary experiments, *Stipa* had been found to be mycorrhiza1 in the field. *Atriplex* and the "companion plant" *Stipa* were to be grown separately and together, as the proximity of an infected plant has been reported to increase infection in weakly infected plants (Hirrell *et al.,* 1978; Miller *et al.,* 1983).

Methods

Work was in two main parts;

(a) A survey (after rains, when roots have recently grown) of the infection of roots of *Atriplex vesicaria* and *Stipa nitida* in the field: and

(b) Growth of plants in soil collected from the field which had been sterilized, then either left sterile or reinoculated with mycorrhizal fungi from pot cultures.

Although the intention was to grow plants *of Atriplex* with *Stipa nitida,* this proved to be difficult because (a) poor rains in the previous years meant seed of this grass was rare;
and (b) what seed we could find germinated at low frequency and over a long period of time, thus making controlled experiments difficult.

Nevertheless, we were interested in trying to assess the effects of a companion plant on the mycorrhizal infection of *Atriplex, so* "compromised" using onions. These were chosen as they are normally heavily infected with mycorrhizal fungi and had a growth rate and form which would not swamp the *Atriplex;* also, their roots are morphologically distinct, so could be easily distinguished when tangled with each other.

Soil was collected from the T.G.B. Osbome Reserve, Koonamore Station, S.A., sieved and used after mixing in a small amount of a soil-sand mix in which clover had been grown, heavily infected with the vesicular-arbuscular (VA) mycorrhizal fungus *Glomus mosseae.* This provided extra mycorrhizal fungi inoculum, as preliminary experiments had shown the soil to contain quite

low amounts of mycorrhizal inoculum.

Plants were grown in 1kg pots in the glasshouse at the Department of Botany, University of Adelaide, for three months, at which time they were harvested. Roots were washed from the soil, cleared in 10% KOH for three days, rinsed in 1M HC1, stained with trypan blue in lactophenol (Phillips & Hayman, 1970), and stored in glycerine. Percentage infection was scored using the line intersect method (Tennant, 1975).

In addition to the experiments with *Atriplex,* a second set of experiments was run using a very widespread chenopod, *Enchylaena tomentosa.* Seeds of this plant were collected from coastal dunes in Adelaide, and proved to be easily grown in coastal dune soil. In fact the ease of growth of *Enchylaena* enabled more detailed experiments to be done with this plant than with *Atriplex,* causing a slight change in emphasis of the project.

In all experiments, two plants per pot were grown, either two onion plants, two chenopod plants, or one chenopod and one onion grown together. The onions were grown separately from the chenopods to measure the infectivity of the soil, and helped account for the possibility that the chenopods repressed or killed the propagules.

Results

Field collections were only successful later in the year, as incorrect staining techniques were initially used. The problem was due to the very high tannin content of roots, making visualization of structures in the cortex very difficult. To try to alleviate this problem, roots were rinsed in bleach after being cleared in KOH. Although this procedure effectively removed tannins, it appeared to prevent the binding of the trypan blue stain to the chitin of the mycorrhizal fungal walls. New procedures were developed to remove the tannins, which avoided this problem - one involved a long series of alternating treatments in KOH and HC1 prior to staining; the other used the conventional staining procedure, after which roots were cleared in bleach and then restained. The former technique gave better staining but poorer clearing, whereas the second technique cleared roots better, but mainly stained just vesicles. These techniques were only developed towards the end of the project, and were thus only used on roots from about four field collections. Nevertheless it was found in all collections thai both *Atriplex vesicaria* and *Enchylaena tomentosa* were clearly infected with mycorrhiza1 fungi. Degrees of infection ranged from 4 to 20%, and primarily consisted of intracortical hyphae and large vesicles. Arbuscules were not seen. Unfortunately, due to the relatively small number of successful collections made, it was not possible to determine any effects of season; topography, neighbouring plants etc. on the mycorrhizal infection, at least for plants in the field.

In contrast the experiments on pot-grown plants showed clear effects of companion plants on the development of mycorrhizal infection. Plants of *Atriplex* grown alone were uninfected, but when grown with onions and/or *Stipa* were infected with vesicular mycorrhizal fungus, so 14 to 45% of their root length was colonized. (Infection of the onions ranged from 56 to 67%, of the *Stipa* from 16 to 33%.)

Plants of *Enchylaena* showed a similar stimulation of infection when grown with a usually mycorrhizal companion plant. When grown alone, no infection was observed in the roots of *Enchylaena;* but when grown in pots with onions, infection was regularly observed, with the fraction of root colonized ranging from 6 to 50%, depending on the type of soil in which the plants had been grown and the time for which the plants had been growing. Infection tended to increase with time, and was greater in a (very nutrient-poor) acid-washed sand than in sand collected from coastal dunes.

Conclusions

These results provide the most thorough documentation published to date of infection in Australian chenopods, a group of plants normally considered to be, at most, weakly mycorrhizal.

Plants from both arid and coastal habitats appear to harbour considerable degrees of infection. Experiments with plants grown in pots suggest that such infection is influenced by neighbouring plants, and that plants grown in isolation from commonly mycorrhizal plants will not be infected. Thus, the infection by mycorrhizal fungi of chenopod shrubs in the field may well be influenced by structure of the community in which those plants are growing.

It is now important to determine the effect of mycorrhizal infection on the growth and survival of chenopod shrubs, as alterations in community structure (such as caused by sheep and rabbit grazing) may affect the vigour of chenopod shrubs in more complex ways than previously realized.

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