National Climate Change Adaptation Facility
2010 Masters Research Grant Summary Report

Growth and nitrogen fixation of tropical *Acacias*

Introduction

Nitrogen is mainly lost from most Australian tropical savanna ecosystems via fire and replenished through symbiotic nitrogen fixation. The frequency and severity of bushfires in Australia, which are common and extensive in tropical savannas, are expected to increase under climate change. Thus the nitrogen loss from tropical savannas can be exacerbated. This research investigates how climate change can affect the other side of the nitrogen balance – nitrogen fixation (and growth) by tropical *Acacias* (family: Mimosaceae).

Methodology

The research mainly consisted of field surveys and two shadehouse experiments. The effects of tree properties (e.g. crown width) and abiotic factors (e.g. soil moisture and soil nutrients) on effective nodulation[1] were examined by random sampling of about 1700 samples of soil, roots and root nodules up to 24 cm deep, once in the wet and once in the dry season. In a shadehouse, seedlings of each *Acacia* species were also grown on soil (bacteria) sourced from the natural populations of its own and three other species to test for changes in symbiotic effectiveness due to varying sources of bacteria. In another shadehouse experiment with established seedlings, two watering treatments (regular vs absence) were included in addition to the factorial combinations of four species X two soil sources (inland vs coastal) X two nodulating conditions (nodulating vs non-nodulating). The responses of two inland (*A. elachantha* and *A. ramiflora*) and two coastal species (*A. crassicarpa* and *A. aulacocarpa*/*A. holosericea*) were compared in each experiment.

Major findings

Using the ^{15}N natural abundance method, it was found that symbiotic nitrogen fixation and/or mycorrhizal uptake of soil nitrogen was likely to be the primary mechanism(s) by which *A. crassicarpa*, *A. aulacocarpa*, *A. elachantha* and *A. ramiflora* acquire nitrogen in the wild. Abundant nodules were found on the roots of coastal *A. crassicarpa* in surface soil (to 24 cm depth) while few nodules were found on the roots of coastal *A. aulacocarpa*, and inland *A. elachantha* and *A. ramiflora*. Their nodules, if any, might have developed in deeper soil (>24 cm). In short, increasing soil depth, greater crown width and root biomass of the *Acacia* hosts, the change from dry to wet season, decreasing soil moisture and soil bulk density were positively related to the development of effective nodules of *A. crassicarpa*. Total soil nitrogen and phosphorus were, however, not correlated with effective nodulation.

In the provenance experiment, nodulation always enhanced seedling growth, but *Acacia* hosts did not necessarily grow best with sympatric soil rhizobia. In fact, three of the four tested *Acacia* species established and/or grew better with inland rhizobia.

Absence of watering for eight weeks had a negative effect on the biomass and nitrogen contents of *Acacia* seedlings. The inland *Acacias* were more adapted to drought than coastal ones by having smaller changes in foliage water content, foliage thickness (deduced from specific foliage weight), and higher water use efficiency under drought than under the normal watering regime (deduced from foliage ^{13}C values). Secondly, nodule nitrogen contents and the relative importance of symbiotic nitrogen fixation in acquiring nitrogen under drought were lower than those under the normal watering regime. The percentage reduction was found to be more severe in inland *Acacias* than coastal *Acacias* and might be of adaptive significance. Furthermore, the decreases in foliage and nodule biomass, stem to root ratio and foliage nitrogen content in all the four *Acacia* species under drought were smaller in the presence of inland rhizobia than in the presence of coastal rhizobia, suggesting symbiosis formed with the inland source of rhizobia was less affected by drought.

[1] Effective root nodules consist of bacterial symbionts which fix atmospheric nitrogen for the host plants in return for carbon as the energy source.

Significance to adapting and protecting Australia's terrestrial biodiversity

The number of dry days is predicted to increase under climate change (BOM 2010). Since the biomass of effective nodules of *A. crassicarpa* in the dry season was found to be lower than in the wet season in the field study, effective nodulation and hence nitrogen fixation of this species will probably be directly inhibited by climate change or indirectly through slower tree growth and hence smaller tree size (crown width is positively correlated with effective nodule biomass). This result reinforces the importance of matching the precipitation pattern of the existing range of a species with the new range in the case of assisted range shift under climate change because nitrogen is a key nutrient for seedling establishment in the wild.

From the provenance experiment, species expanding into inland areas might benefit from more effective mutualistic symbiosis. Adding inland soil as a source of rhizobia might benefit the growth of tropical *Acacia* seedlings in a nursery situation. The lack of local adaptation of symbiosis implies that tropical *Acacias* can grow at least equally well in soil outside their current ranges as in sympatric soil. Thus any climate-induced or assisted range shifts of tropical *Acacia* species under climate change might not be constrained by mutualism.

From the drought experiment, tropical *Acacia* species restricted to inland areas, or species growing on (or expanding into) inland soil, appear to have higher resistance to a single drought event. Inoculation with inland rhizobia might alleviate the effect of drought on some *Acacia* species. Under climate change, prolonged periods of drought interrupted by less frequent but more intense rainfall events are expected (BOM 2010). This can potentially result in drier savanna soil in which mineralization and nitrification could be inhibited (Holt and Coventry 1990; Schmidt and Lamble 2002; Richards *et al.* 2012). Plant-available soil nitrogen and direct uptake of soil nitrogen via roots would be expected to decrease. At the same time, reduced water availability can reduce the effectiveness of symbiotic nitrogen fixation. If symbiosis with inland rhizobia helps the host plant to alleviate the negative effect of drought, this trait would be adaptive. In the case of assisted range shift through revegetation, inoculating the soil around the planted seedlings with inland rhizobia might confer the host plant with extra drought-tolerance. However, the inoculated rhizobial population should be significantly greater than the local rhizobial population to avoid outcompetition (Lie *et al.* 1987; Thrall *et al.* 2005; Thrall *et al.* 2007; Heath and Tiffin 2008).

Further research

Soil up to 1 m should be sampled in the future because nodules might have developed on the deeper parts of of the root system where soil is less susceptible to drying out, particularly in the dry season. The potential correlation of nodule biomass with monthly soil temperature and rainfall, and the effect of these climatic variables on nodule senescence rate should be investigated by carrying out monthly field surveys which, however, requires substantial manpower. The plant-available nitrogen and phosphorus in the soil should be analysed instead of total plant nitrogen and phosphorus which were found to have no significant effect on effective nodulation in this study.

The lack of local adaptation of *Acacia*-rhizobia symbiosis found in the provenance experiment might be a result of the limited scope of this research. More populations of each of the four *Acacia* species should be studied and tests with individual soil rhizobial isolates should be undertaken to come to a more general conclusion. Future research should also investigate the size of soil rhizobial populations which was confirmed to be correlated with the height of the host plant in glasshouse experiments (Thrall *et al.* 2005; Thrall *et al.* 2007). The apparent advantage of inland rhizobial sources over coastal ones, to alleviate the effects of a single drought event on their host plants, should be further tested with more host and rhizobial populations of the four *Acacia* species.

Future cross-inoculation experiments should also be accompanied by studies examining other climatic effects (e.g. temperature and soil moisture) on the nodulation, symbiotic effectiveness and the growth and reproduction of the two sources of rhizobia. In addition, the examination of other *Acacia* species would help to confirm the trends identified in this study. Both the provenance and drought experiments should also be undertaken in the field to verify the findings of glasshouse/shadehouse experiments.