Intraspecific and interspecific crossing of Desmanthus yields new and novel plants

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Abstract
Desmanthus was first introduced into Australia from the Americas decades ago as a potential forage legume with much to offer Australian agriculture. There are more than 24 species of Desmanthus but the key species exploited for pastures are *D. virgatus* and *D. leptophyllus* and more recently *D. bicornutus*. Elite varieties of these pasture species not only have the ability to survive in low input low rainfall conditions, but provide valuable high protein forage with leaf typically being in the order of 20% crude protein. Agrimix Pasture Concepts funded a breeding program which commenced in 2012, to develop new and novel varieties combining enhanced productivity while maintaining inherent drought tolerance. A surprising outcome has been some selections from interspecific crosses showing quite a different plant type, with much softer and erect growth, later maturity and indications of greater cold tolerance. These crosses are now being evaluated in the field in a number of diverse locations and have the potential to add valuable new legume genetics to pastures in temperate to tropical environments.

Keywords
Pasture legumes.

Introduction
The legume genus *Desmanthus* has long been touted as having potential as a pasture legume particularly for Australia's northern clay soils which are vast and until recently lacked an adapted sown pasture legume (Burt et al. 2016). The genus originates in the Americas and contains 24 species with Mexico having the greatest number of species (Luckow 1993). *Desmanthus virgatus* and *D. leptophyllus* and more recently *D. bicornutus* are the main species used or under development as pasture legumes. In the USA *D. illinoensis* is native and is also being developed (Muir and Pitman 2004).

Recently five new cultivars sold as a blend named *Progardes* have been released and some 20,000 ha have been sown mainly in Queensland (Gardiner 2016; Loch 2016). The target land types for these cultivars are the northern clay soil plains, Brigalow, Blackwood and Gidgee land types typically in the 450 - 1000 mm rainfall zones. However there is also considerable interest in seeking new summer growing legumes in more temperate environments such as northern NSW.

The diversity in the Desmanthus gene pool in terms of plant habit (herbaceous prostrate plants to large erect shrubs) is very considerable as is the range of land types and environments that they originate from. The good animal production data in earlier commercial pastures, combined with the natural genetic variation within this genus has led to this breeding program, with the aim of developing new genotypes with enhanced pasture performance and suitability to new geographic regions.

Methods
A range of genotypes were selected, some with known field performance and others chosen on the basis of the region and soil types from where they were originally collected. The lines were from three species, *D. leptophyllus*, *D. virgatus* and *D. bicornutus*. Plants were grown in a glasshouse with natural light. Controlled cross-pollination was achieved by emasculating and hand crossing. F_1 plants were carefully observed to check for phenotypic differences from their female parent. Seed from F_1 plants was bulked to provide seed for the F_2 generation. The F_2 generation was established by growing seedlings in a greenhouse and then transplanting to the field. Selections were chosen based on a range of phenotypic characteristics. The F_3 generation was sown in December 2015. Where sufficient seed has allowed, F_4 selections are being grown in multi locations to further assess their agronomic performance.

Results and Discussion

One of the initial tasks was to develop a suitable crossing technique. This involved careful observation of flowering behaviour in the various species in order for emasculations and cross-pollinations to be quickly performed. Initially, one of the issues was pollinated flowers aborting, partly through lack of available pollen to make the crosses. Many growth processes in *Desmanthus* occur surprisingly quickly. From pollination to the young pods forming only takes three days. Upwards of 80 crosses were made, some between lines of the same species and others between different species. Of these 80+ crosses, 45 produced pods and seed.

In the F_1 generation, some interspecific F_1 plants grew very well, with impressive foliage and vigour, but failed to produce seed. Fortunately, some interspecific plants did produce sufficient seed to enable progressing to an F_2 field population. In addition to the interspecific populations, a number of intraspecific populations are being progressed. In the F_2 and subsequent field nurseries, the original parent lines have also been sown, enabling comparisons with the breeding selections to be made. The phenotypic variation between the parent lines and selections in the F_2 and F_3 populations has been significant, in terms of growth habit, vigour, maturity and cold tolerance. Significant variation has also been recorded between plants in different populations. Figure 1 shows typical F_3 plants in two populations, one derived from a *D. virgatus* x *D. virgatus* cross (left) and the other from a *D. leptophyllus* x *D. virgatus cross* (right). The pollen source was the same for both these populations. Key features of this *D. virgatus* x *D. virgatus* cross are semi-prostrate growth habit and early maturity. The plant on the right from an interspecific cross is showing a very different growth habit with more leafy bright green foliage, and is much later maturing.

Figure 1. Three month old plants of a *D. virgatus* x *D. virgatus* cross (left) and plants from a *D. leptophyllus* x *D. virgatus cross* (right), showing differences in growth habit, foliage type, plant colour and maturity.

Significant variation for maintaining green foliage into late autumn and growth after winter has also been observed between the various populations. Figure 2 shows F_3 plants in two populations taken on January 6, 2017, showing differences in regrowth and recovery after winter. On the left are plants derived from a *D. virgatus* x *D. virgatus* cross and on the right is a plant from a *D. leptophyllus* x *D. virgatus cross.* The softer more leafy foliage seen in the growth before winter is again a significant feature in the mid-summer growth of this *Desmanthus* interspecific cross plant.

Drought tolerance is of major importance in the breeding and selection for new *Desmanthus* cultivars. Even in the nurseries, it is beneficial to provide a degree of selection pressure for heat and drought tolerance, and establishing in high soil temperatures. The recent heatwave conditions experienced throughout much of Eastern Australia have provided ideal conditions for such selection pressure. The F_4 field nursery was sown near Kingaroy in the South Burnett region of Queensland (26.5° S, 151.8° E) on January 10, 2017, in a red ferrosol type soil. Seed was sown into a dry seedbed at a depth of approximately 1 cm and the average soil temperature at this depth at 12:30 pm was found to be 51°C (Figure 3 left image). As the ground was very dry, irrigation was required, but it was not practical to start watering until January 16. When inspecting the site for germination and emergence on January 25, the average soil temperature at 3:15 pm at 1 cm was 55° C. Seedling establishment counts have not been recorded, but for the overall site, establishment has been quite satisfactory. The hot weather continued as evidenced by the average daily maximum air temperature for the 22 days after sowing being 34.0° C and average daily minimum air temperature for that period

20.7° C. Soil surface temperatures were measured on February 2, to assess the heat load the young seedlings were under. At 1:45 pm that day, the soil surface temperature was found to be 63° C (Figure 3 right image). The official maximum air temperature that day was 37.6° C. The young *Desmanthus* seedlings were coping very well with this quite extreme soil surface temperature.

Figure 2. F_3 plants from two Desmanthus breeding populations taken on January 6, 2017, showing differences in regrowth and recovery after winter. On the left are plants derived from a *D. virgatus* x *D. virgatus* cross and on the right is a plant from a *D. leptophyllus* x *D. virgatus cross.*

Figure 3. The Kingaroy Qld, F_4 Desmanthus nursery. The soil temperature at 12:30 pm on the day of sowing, at 1 cm on January 10, 2017 was 51° C (left). On the right are young Desmanthus seedlings at the same site on February 2, with soil surface temperature at 1:45 pm of 63° C.

Conclusion

As typical in plant breeding programs, the outcomes can't always be predicted. Certainly careful planning and selection of initial material from which to breed is essential, but the results can be surprising and provide new and novel outcomes. This *Desmanthus* breeding program is demonstrating this, with indications of new material with unique features, which may in the future assist in further expanding the benefits from this pasture plant, through more sustainable and productive pastures, leading to improvements in animal production.

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