

Restructuring Alfalfa through Introgression of *Medicago arborea* Traits

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Traits present in *Medicago arborea*, such as large seeds (400% over alfalfa), robust stature and drought tolerance would increase alfalfa's versatility. Work has been in progress in Wisconsin and Queensland Australia for the past ten years to introgress *M. arborea* genetic material into the *M. sativa* genome, through sexual reproduction using male sterile alfalfa as the seed parent in *M. sativa* X *M. arborea* crosses (1-4). Hybrids and their derivatives, are termed Alborea. Individual hybrids contain up to 8% of the *M. arborea* specific SSR markers; however, specific SSR markers are mostly different in respective hybrids. The molecular markers indicate that DNA from all *M. arborea* chromosomes was introgressed among the first ten hybrids. Thus far, 32 hybrids have been produced. Hybrids were crossed in various combinations, and recurrent selection used to pyramid *M. arborea* traits in Alborea lines. Alborea selections also have been crossed and backcrossed with alfalfa for breeding purposes. Alborea crosses have been identified in Australia and Wisconsin that are at least as vigorous and productive in the glasshouse and field as the best commercial control lines. In a glasshouse pot experiment, the progeny of an Alborea genotype X Sequel (Australian cultivar) cross yielded 147% of Sequel, whereas the progeny of the dormant alfalfa parent X Sequel yielded 66% of Sequel. Families deriving from this Alborea have consistently yielded well over 4 years in the field at Gatton and Ballarat (Australia). Similarly, in a non-dormant background, Alborea X alfalfa produced families that yielded at least as well as Sequel both in the glasshouse and field (Gatton). These results could be explained by genetic complementation between the two genomes in Alborea, leading to heterosis for vigour and yield. Several other traits have been transferred from *M. arborea* to Alborea. These traits include winter activity, yellow flower colour, larger seeds, single coil flat pods, and several morphological changes including bush-like erect plant architecture. Seeds per pod of Alborea from hand crosses averaged 4.3, with range 1.8-7.3, while three alfalfa checks averaged 4.7. The work to date indicates that with further breeding and selection, alfalfa can be restructured into a more versatile and productive plant.

1. Armour et al. 2008. Transfer of anthracnose resistance and pod coiling traits from *Medicago arborea* to *M. sativa* by sexual reproduction. *Theor. Appl. Genet.* 117: 149-156.
2. Bingham et al. 2005. Alfalfa says Hello to the Genome of *Medicago arborea*. *Medicago Genetic Reports* 5: 1-6 www.medicago-reports.org (See also: vol. 6-16)
3. Bingham et al. 2013. The hybridization barrier between herbaceous *Medicago sativa* and woody *M. arborea* is weakened by selection of seed parents. *Plants MDPI* (on line) 2: 343-353.
4. Irwin et al. 2016. Restructuring lucerne (*Medicago sativa*) through introgression of the *Medicago arborea* genome. *Agricultural Science* 28 (1):40-46.

Traits segregating in Alborea. Most traits are from *M. arborea*, but some traits are new. Traits can be selected for or against, as needed.

Morphological Traits	Comments
Large leaf size	These traits are often associated, but are not tightly linked. Traits can be selected independently.
Large flower size	
Large pods	
Large seeds	
Tallness	Structural traits are likely from <i>M. arborea</i> . However, some traits are likely due to new genetic interactions.
Bush-like growth	
Erect growth	
Lodging resistance	
Flower shape/colour	
Solid stems	New variation not present in either <i>M. arborea</i> or alfalfa.
Branching roots	
New root morphology	
New leaf morphology	
New crown morphology	

Traits Continued. Traits are segregating in Alborea populations; no plant has all the traits. Identification of traits is just beginning; the list will grow!

Physiological Traits	Comments
Winter activity	Winter active in Qld, AU. Survives in WI. How?
Summer dormancy	Probably select against.
Drought tolerance	Linked to summer dormancy?
Frost tolerance	<i>M. arborea</i> & Alborea stay green @ -10C.
Salinity tolerance	Awaiting selection.
Longevity	Looking good.
Seedling vigour	Large seed, cotyledons, & spade leaf ensure vigour.
Cross fertile with alfalfa	Yes, see fertility page.
Hybrid vigour with alfalfa	Specific combining ability important.

Home of *Medicago arborea*



M. arborea flowers



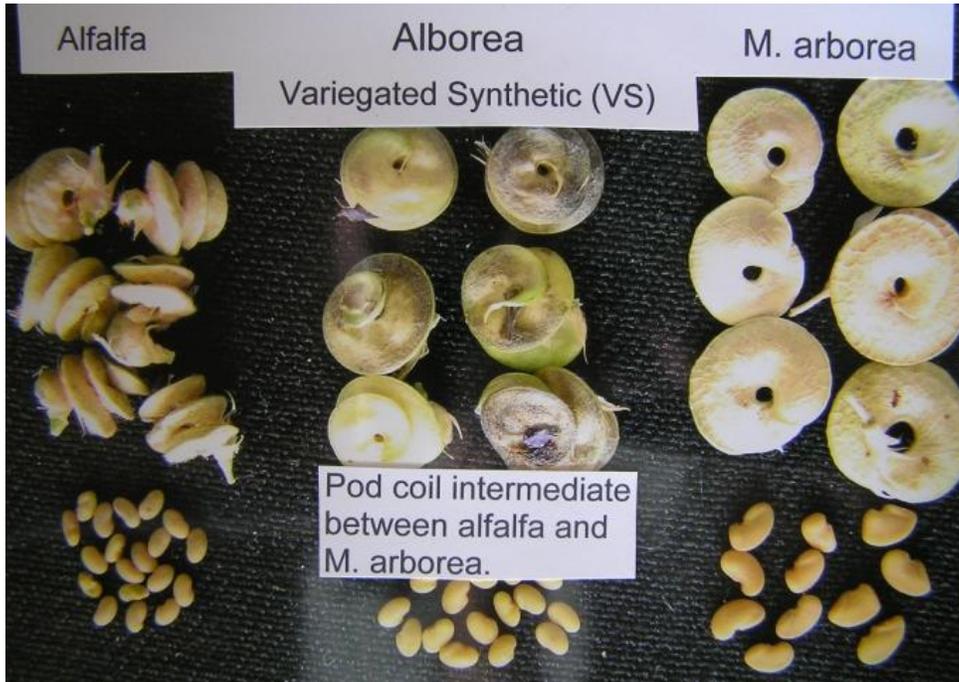
***M. sativa* (left) and *M. arborea* (right).**
M. sativa grows faster in summer.
M. arborea grows faster in winter.



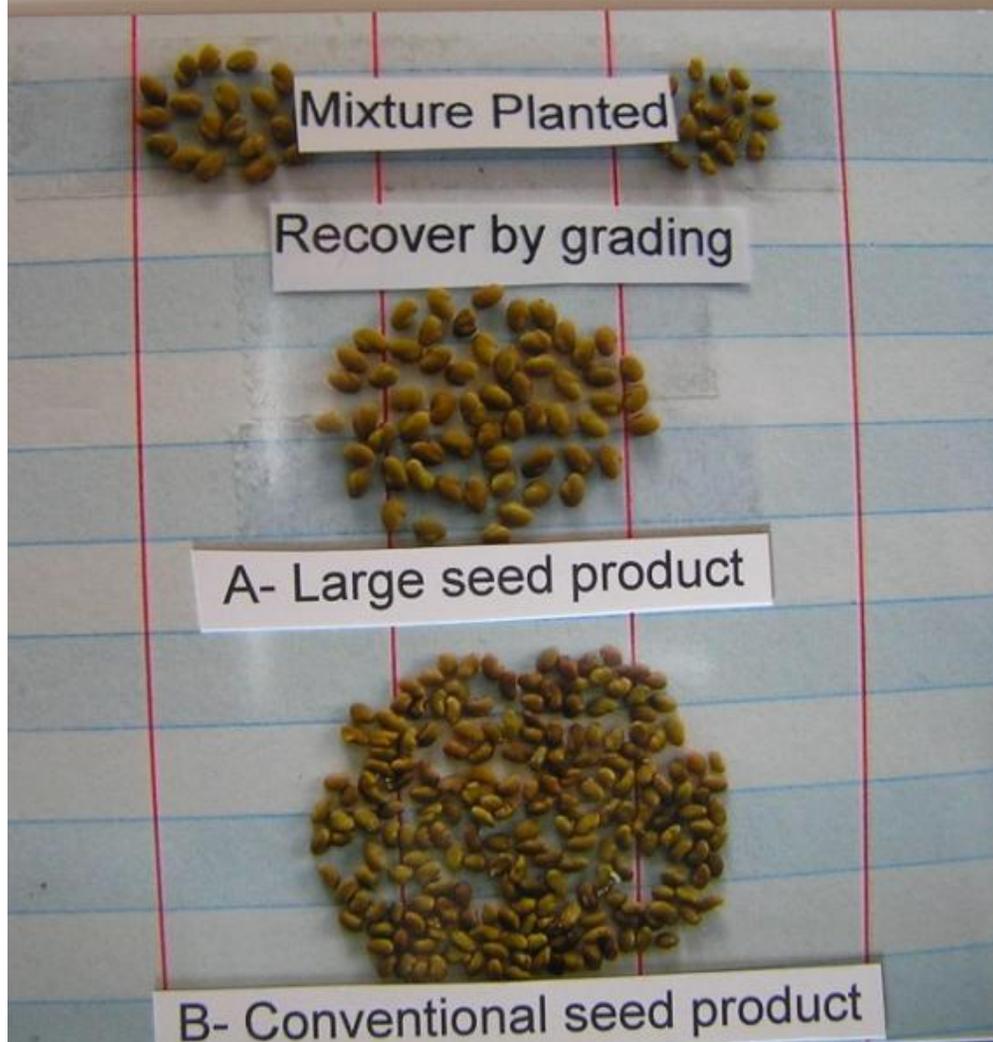


Leaf size and colour of alfalfa (left) and Alborea (right). Leaves sampled from hay stand before cutting on May 26, 2016. Each leaf from a different plant 6-7 nodes from base.





Strategy to recover hybrids of large seed Alborea. Study in progress.



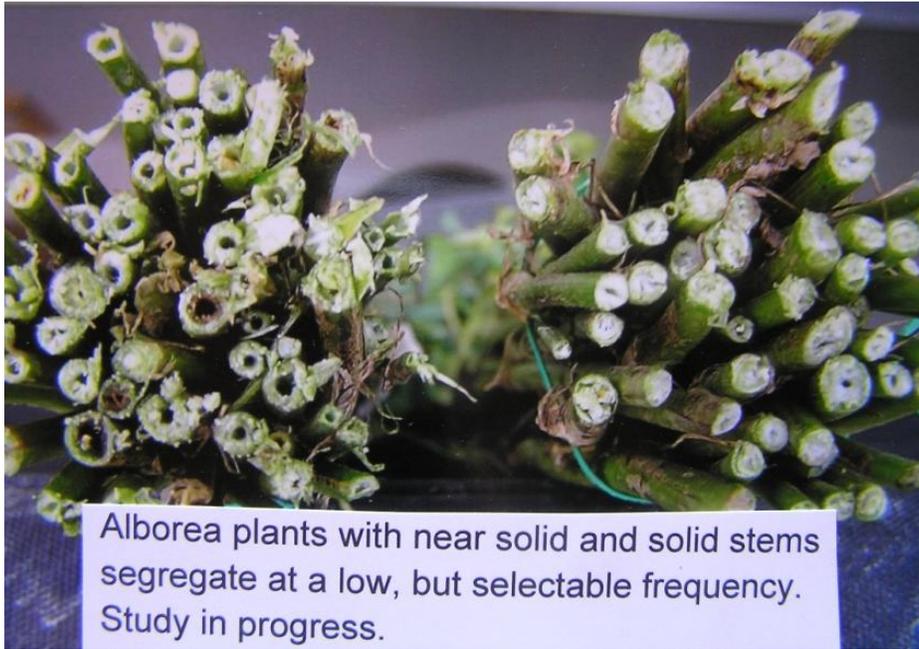
An experiment was planted in June 2016 involving yellow flowered Alborea lines in a mixed planting with alfalfa to select for Alborea lines with the best hybrid combining ability, and frequency of hybrids. The frequency of hybrids will be determined by the frequency of variegated plants.

Dry Leaf Differences – Room temp. 24 hrs.
(Will differences correlate with quality factors, drought tolerance, etc. ?)



As alfalfa leaves dry, they coil into distorted shapes within 24 hours. It was discovered by accident that the leaves of some Alborea plants wrinkle, but retain their basic shape as they dry, especially at 24 hours. Leaves of *M. arborea* (above right) retain their shape when dry. In the larger samples above, clusters of leaves of three different alfalfa cultivars are on the left. The three vertical columns in the middle are leaves of three different Alborea plants representing the range of differences in the present sample. Vertical columns on the right are *M. arborea*. Interestingly, *M. arborea* leaves turned yellow as they dried.

Will differences correlate with quality factors, drought tolerance, etc.?



Frost tolerance in Wisconsin of *M. arborea* and some Alborea segregates is greater than that of alfalfa and *M. falcata* (WISFAL).

M. arborea > Alborea > WISFAL > Alfalfa



Clone of one of our first Alborea hybrids showing green fall growth among drying summer growth, in same field, on same day as above. Many non-dormant Alborea plants survive Wisconsin winters. How? What's going on?



Statement about Fertility

Alborea reported below was selected for fertility each generation of development (YS=4 gens. VS & PS= 2gen.)

Data below based on hand pollinations in GH, 2016; ten pods per plant sampled.

Material	Seeds per pod	
	Mean	Range
Alfalfa cv. FD 4. 8 plants/80 pods	4.7	4.4 – 5.3
Alfalfa cv. FD 9. 8 plants/80 pods	4.55	3.5 – 6.0
Alborea YS, VS, PS 15 plants/150 pods	4.3	1.8 – 7.3
Alborea X alfalfa 5 crosses/5 plants of each cross/25 plants 250 pods	5.1	3.7 – 6.0
Backcross line: 87% alfalfa; 12% Alborea. 100 pods	4.6	3.8 – 5.2

Conclusions:

Seeds per pod of Alborea in hand crosses was near that of alfalfa. Alborea plants ranged from fewer seeds per pod than alfalfa, to more seeds per pod.

Seeds per pod of Alborea X alfalfa hybrids was the same as alfalfa, as was the backcross line.

Alborea seed production observed in the field, in isolation, with no competing bloom from alfalfa, is about 80% of alfalfa, with bees (wild and domestic). If competing bloom of alfalfa is present, seed production of Alborea YS is scant. VS and PS have not been tested.

End of presentation.