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The case for developing a sterile variety of leucaena

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Executive summary

Leucaena (*Leucaena leucocephala*ssp. *glabrata*) is one of the most widely sown pasture legumes in the tropical and sub-tropical areas of Queensland. Estimates of the total area established exceed well over 220,000 ha. Where suited, this leguminous tree is the basis of a highly productive and persistent pasture. Technologies have been developed to address issues with establishment, grazing management and the risk of toxicity.

As with most successful, introduced pasture plants, there are concerns about the weed risk associated with seed spread from stands of forage leucaena into non-grazed areas, especially areas of environmental significance such as waterways. Prior to its widespread use as a pasture plant, leucaena plants (most likely the so-called ‘common’ species of leucaena, *L leucocephala*ssp. *Leucocephala*) had already colonised pockets of ungrazed, non-agricultural land along urban and coastal locations of Queensland and some other areas of northern Australia. This has served to raise concerns about the potential for pasture plantings to exacerbate this problem.

Critical to the ongoing success of this species is the need to ensure that new initiatives such as the development of a sterile variety of leucaena are both technically feasible and likely to be accepted as addressing concerns regarding the weediness of this species such that they may be broadly adopted by producers. Concerns regarding the weediness or perceived weediness of leucaena have led to this aspect having prominence in the code of practice for leucaena growers promoted by the Leucaena Network (http://www.leucaena.net/codeofconduct.pdf).

This report evaluated the technical feasibility of developing sterile leucaena using two contrasting methodologies: genetic modification and hybrid breeding systems and the likelihood that these methodologies could develop cultivars that address the concerns regarding invasiveness in leucaena.

The following factors were considered critical in the development of a sterile leucaena cultivar:

- Sterility *per se* is unlikely to increase the area sown to leucaena
- Sterility is not likely to accelerate the replacement of areas already planted to leucaena
- All methods for developing a sterile variety are likely to require medium to long term research and development
- Sterility will need to be combined with other traits (Psyllid resistance, frost tolerance, palatability) to achieve commercial acceptance
- Sterile varieties are likely to require changes in industry practice that will require training and education, either through
    - Developing and applying methods for transplanting of live seedlings, or
    - Adoption of hybrid seed production systems by commercial seed producers, or
    - Development of industry and grower protocols for the commercial production of a genetically modified forage plant.

Based on the analysis in this report it is not recommended that a transgenic (GM) approach is undertaken unless the following factors can be addressed to satisfaction of the project partners:

- The potential of an international research partner (for example EMBRAPA in Brazil) is explored
- A commercial partner with the willingness to embrace the legislative and commercial challenges of releasing a sterile cultivar can be found.

The approach of developing triploid interspecific hybrids is a technically feasible alternative but should focus on the following issues:

- Maintenance of palatability and psyllid resistance
- Development of cost-effective mechanisms for the production of hybrid seed or, Development of cost-effective *in vitro* methods for the propagation of large numbers of seedling plants as used by the forestry industry

These elements should be identified as 'go/no go' points in any breeding program and regular consultation made with government and industry as to the need for sterility in Leucaena and revision of the cost:benefit of the program.

The analyses demonstrate a positive NPV for the successful development of a sterile leucaena cultivar/s as long as the following factors are maintained

- Industry continues to sow leucaena at rates equal to those of today
- The commercial advantage of leucaena systems over grass pastures is maintained

The value of the investment is quite sensitive to these factors and they suggest that continued investment in marketing of the value of leucaena will be required. This may not require investment from MLA if sufficient funds are invested from the private sector.

It is also clear that the sterile cultivar/s must also maintain the current agronomic performance of selected seeding cultivars. This will necessitate the correct choice of germplasm for crossing and regional agronomic testing and validation.

The project did not consider the relative importance or attractiveness of breeding for sterility against other traits in leucaena nor did it consider the relative importance and benefit:cost of leucaena breeding versus other tropical pasture species.

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Background

Meat and Livestock Australia has requested tenders to undertake an assessment of the case for developing a sterile variety of leucaena.

Leucaena (*Leucaena leucocephala* ssp. *glabrata*) is one of the most widely sown pasture legumes in the tropical and sub-tropical areas of Queensland. Estimates of the total area established exceed well over 220,000 ha. Where suited, this leguminous tree is the basis of a highly productive and persistent pasture. Technologies have been developed to address issues with establishment, grazing management and the risk of toxicity.

As with most successful, introduced pasture plants, there are concerns about the weed risk associated with seed spread from stands of forage leucaena into non-grazed areas, especially areas of environmental significance such as waterways. Prior to its widespread use as a pasture plant, leucaena plants (most likely the so-called 'common' species of leucaena, *L leucocephala* ssp. *Leucocephala*) had already colonised pockets of ungrazed, non-agricultural land along urban and coastal locations of Queensland and some other areas of northern Australia. This has served to raise concerns about the potential for pasture plantings to exacerbate this problem.

Critical to the ongoing success of this species is the need to ensure that new initiatives such as the development of a sterile variety of leucaena are both technically feasible and likely to be accepted as addressing concerns regarding the weediness of this species such that they may be broadly adopted by producers. Concerns regarding the weediness or perceived weediness of leucaena have led to this aspect having prominence in the code of practice for leucaena growers promoted by the Leucaena Network (http://www.leucaena.net/codeofconduct.pdf).

There are several precedents in Australian agriculture where species of potential agricultural importance such as the temperate grass species tall wheatgrass (*Thinopyrum ponticum*) and lovegrass (*Eragrostis curvula*) have adaption to saline and dry environments respectively, have improved cultivars that are more digestible and likely to be less weedy than original ecotypes but are still listed as weedy species by some states or agencies which has greatly reduced their commercial uptake. In many cases the weediness assessments are on the species *per se* as opposed to the improved cultivars. However, in both of these cases the improved cultivars are not sterile as proposed for leucaena.

For any leucaena breeding strategy to be successful it must:

- target relevant technologies/practices
- provide an economic justification of the value of these technologies/practices against both productivity and weediness/sustainability indicators
- engage all relevant sectors of the supply chain including investors, researchers, commercial companies, processors and producers

The leucaena network (www.leucaena.net) has previously surveyed its members (Appendix 1) as to their support or otherwise for the development of a sterile forage variety. It is interesting to note that work on a sterile variety was supported by >90% of respondents and 80% indicated that they would plant this variety if it were available at a commercially viable price. However, 50% of respondents indicated their desire to see research focus on developing a variety that combines both cold/frost tolerance and sterility. It is unfortunate that this survey did not rank sterility against other traits of agronomic importance, nor indicate what trade-offs if any producers were willing to make to adopt a sterile variety. Methodologies to pursue this sort of data now exist and have been used to rank the importance of traits in other forage breeding programs (Smith and Fennessy 2012) and avoid the limitations of these surveys which can lead to a large number of traits being prioritized. Notwithstanding the limitations of the survey the results do show a level of support for the development of a sterile variety among leucaena growers.

Objective 1. Describing and evaluating the most promising breeding strategies and their feasibility, advantages, disadvantages, likelihood of success, and their likely time and resource requirements.

In this task data has been accessed from the published literature and through direct consultation with scientists involved in leucaena genetics. This data, and experience from other species, was used to assess a range of technologies including (but not necessarily limited to):

- ploidy manipulation in combination with interspecific hybridization
- genetic modification of floral development genes
- cytoplasmic male sterility

with respect to their technical feasibility, likelihood of success and cost-effectiveness. The team is aware that interspecific hybridization is possible in leucaena and that in some cases the resultant hybrids have been at least partially sterile but have often had lower palatability than elite lines. We also have experience with the genetic manipulation of flowering traits in forage species and the practical use of GM technologies in forages more generally including logistical and regulatory requirements for GM forage development through to the evaluation of GM forage plants in trials managed by commercial seed companies.

The team have made an assessment of these contrasting breeding methodologies with recommendations addressing the following points as outlined in this Objective:

- feasibility
- advantages
- disadvantages
- likelihood of success
- likely time and resource requirements

Genetic modification of floral development genes

The generic steps in a transgenic breeding program are illustrated below in Figure 1 (from Strauss *et al.* 1995). This process can take between 5 and 15 years depending on the knowledge of the genes and species involved; it should be assumed that the timeline for leucaena will be at the upper end of the scale.

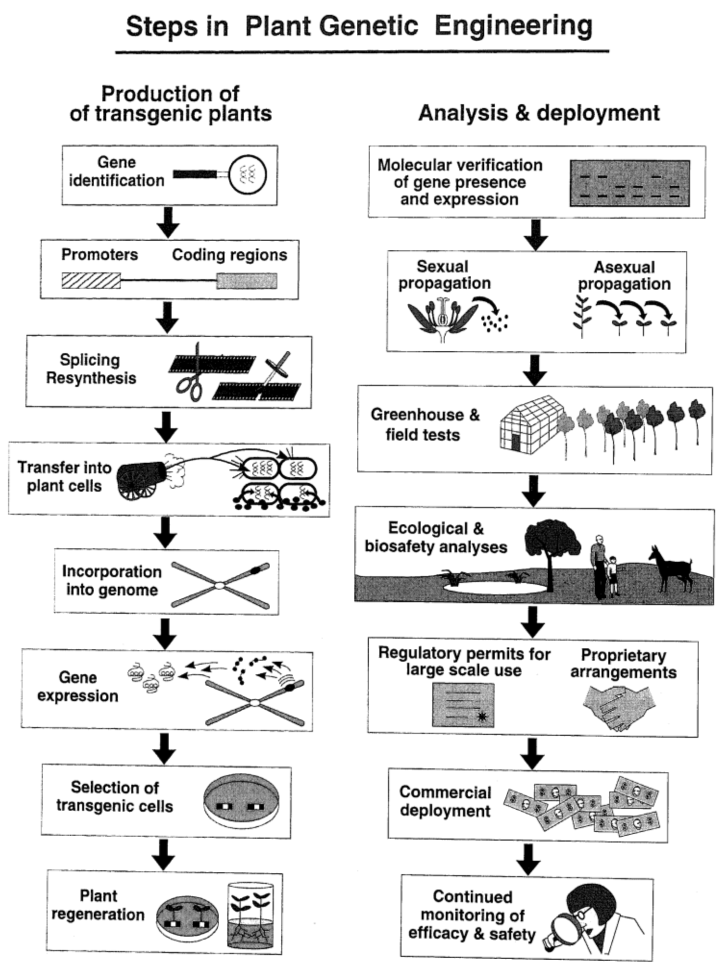

Figure 1. Generic process for GM plant production.

Choice of genes and likelihood of success

Most of the efforts to develop sterile plants through genetic modification have focused on the suppression of genes involved in reproductive development. There are a range of mechanisms through which these genes may be suppressed including antisense RNA, sense suppression, promoter based suppression (Strauss *et al.* 1995), RNA interference (Zhang *et al.* 2006) or zinc finger nucleases (ZFNs) (Shukla *et al.* 2009, Zuker *et al.* 2010, Kim & Kim 2011). The choice of method will depend on the gene being manipulated and IP and licensing arrangements. It should be noted that the ‘genome editing’ options offered by ZFNs may enable the loss of gene function without any residue of foreign DNA remaining in the plant which offers commercial appeal but plants developed using these processes have not yet passed through regulatory and commercialisation processes to test whether they will reduce the cost of commercialisation or ease the regulatory burden on GM plants.

The choice of genes for manipulation can be prioritised based on the following criteria

- specificity of expression (allows targeting to specific tissues in this case flowers)
- production of high amounts of RNA to allow strong expression of the transgenic product
- genes with known function in other species (homology between species allows similar genes to be found in leucaena and targeted for gene suppression)

A range of genes have been identified that disrupt floral development in model species such as *Arabidopsis* and *Antirrhinum* including genes such as *SQUAMOSA* and *APETALA* from the MADS-Box family of regulatory genes (Angenent 1992, Huijser 1992, Mandel 1992), the *LEAFY/FLORICAULA* (Weigel *et al.* 1992, Coen *et al.* 1990) family and *APETALA2* (Okamuro *et al.* 1993) which tend to control the transition of early inflorescence meristems into floral meristems and tend to result in altered and non-functional flowers or the development of extra leaves and stems at the expense of floral production.

Another approach would be to use genetic modification to induce cytoplasmic male sterility (CMS) to stop the production of functional male pollen (Ruiz and Daniel 2005). Naturally occurring CMS has been reported for maize (*Zea mays*; Kriete et al., 1996), oilseed rape (*Brassica napus*; Kriete et al., 1996), rice (*Oryza sativa*), and *Beta beets* (Kriete et al., 1996), but such a natural occurring system is not available for most other crops used in agriculture. A natural source of nuclear male sterility was identified in leek (*Allium porrum*; Smith and Crowther, 1995). The CMS phenotype is a maternally inherited trait caused by incompatibility between the nuclear and cytoplasmic genomes. CMS systems are used to produce commercial F_1 hybrid lines, but full advantage of this system may be obtained if a nuclear restorer gene is available to suppress the male sterility in the hybrid plant. A few such nuclear fertility-restorer (*Rf*) genes have been isolated, including the maize *rf*2 gene that encodes an aldehyde dehydrogenase (Cui et al., 1996; Liu et al., 2001) and the petunia (*Petunia hybrida*) *Rf* gene. This CMS and Rf mechanism has enabled the commercial development of sugarbeet hybrids that are triploid and do not produce fertile seed. Without fertility restorer mechanisms plants would likely need to be propagated in tissue culture and transplanted to the field.

A modern genomics breeding program would use a mixture of reference sequence and *de novo* sequencing to identify candidate genes for manipulation in the breeding program. As there is no reference sequence in leucaena it would be necessary to use the whole genome sequence of another legume species for this purpose and examples would be *Medicago truncatula* and *Lotus japonicas.* The targeted sequencing of the leucaena genome could then be used to identify the relevant gene sequences against the reference genome. This approach is now being widely used for species with a paucity of published gene sequence information and has recently been used to identify genes involved in lignin biosynthesis in tropical *Acacia* spp. (Wong *et al.* 2011).

The success or otherwise of a GM approach is not likely to be due to a lack of genes but rather the underlying technologies required to develop the system such as regeneration and transformation protocols etc. These steps and their respective timelines are listed in Table 1.

Table 1. Steps in the development of a transgenic breeding program.

Step	Likely timeframe	IP Considerations	Comments
1. Develop regeneration and transformation protocols	1 year	Published protocols for trees could be utilised.	Can commence with reporter type genes
2. Obtain full length sequences of target genes	6 months	Utilise partial sequences published and de novo sequencing .	Key genome sequencing step, use differential expression to confirm published targets.
3. Confirmation of function in transgenic plants	2 year	Should be able to provide patent protection	
4. Discovery of promoter sequences	6 months	Should be able to provide patent protection	Follows step2.
5. Production of transgenic plants	1 year		Require multiple events per gene or group of genes.
6. Screening of transgenic (GM) plants	3 years	Need secure nursery site	Needs OGTR approval

Costs of GM Commercialisation

Before embarking on a transgenic breeding program beyond the proof of concept phase there are several key factors that will greatly increase the cost of this approach relative to other models:

- Development and accreditation of containment facilities for all aspects of the breeding program, including laboratories, greenhouses and field trials
- Increased costs of database management to allow traceback and compliance
- Deregulation costs, the cost of deregulating transgenic events are estimated to be in the order of $5 – 20M (Kalaitzandonakes *et al.* 2007)

One potential new technology that may allow the benefits of transgenesis without some of the regulatory costs is genome editing through the targeted use of zinc finger nucleases (Shukla *et al.* 2009, Zuker *et al.* 2010, Kim & Kim 2011). These approaches have yet to be tested through international regulatory frameworks but multinational bioscience companies are working actively in this area, including DowAgrosciences who have partnerships in Australia and developing countries validating the technology in a range of species. One option in the development of a sterile cultivar of leucaena would be to wait until the development and commercialisation of major food crops using these new technologies and assess the impact on regulatory costs. This is likely to occur during the next 5 years.

In the development of GM technologies most companies have taken a multi-national approach and developed technologies that are adaptable across a range of jurisdictions to maximise market share and defray the costs of commercialisation. A sterile leucaena would be suitable for use in Australia, Brazil and Africa. The current status of GM crops has been summarised by James (2011; Appendix X)

Development of sterile triploid hybrids through interspecific hybridisation

Perhaps more promising than the use of genetic modification to develop a sterile variety of leucaena would be the use of the genetic diversity within the genus Leucaena (Brewbaker and Sorensen 1990; Brewbaker and Sorensson 1994; Shelton et al. 1998). Interspecific hybridization has been practiced in Leucaena to introduce traits such as psyllid resistance in the University of Queensland program. A major focus of existing programs has been the need to develop hybrids that produce seed to allow successful commercialization.

An alternative approach is to develop ‘triploid’ hybrids that do not produce fertile seed - this approach has been used to develop the sterile hybrid K1000 (L. esculenta x L. leucocephala). This sterile hybrid is not suitable for direct use in Australia due to its low palatability (even when compared to other hybrids). This low palatability has been attributed to the L. esculenta parent. However, like most early hybrids these appear to have been done with relatively limited germplasm bases and little selection for agronomic performance on either parental lineage. An alternative approach is described in Figure 2.

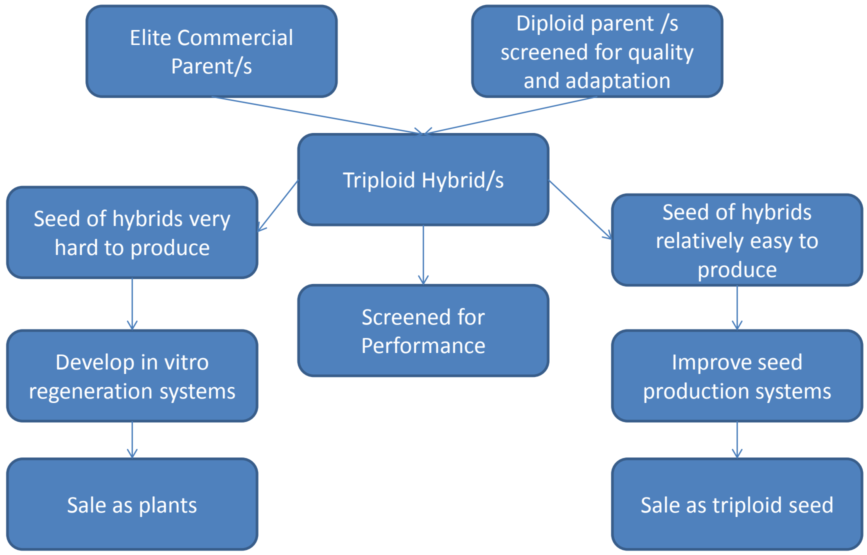

Figure 2. Schematic representation of the stages of a hybrid leucaena breeding program.

This program should have 4 research phases:

1. Development of crossing strategy including pre-screening of putative diploid parents (1-3 years depending on existing data)
2. Hand crossing (1 year)
3. Evaluation of hybrid nurseries (3-5 years)
4. Development of seed production/regeneration systems (3 years) can run concurrently with 3. once preferred method of propagation is determined.

Objective 2. Evaluating likely adoption (area planted on a regional basis) of a new sterile leucaena variety given the time lag for development and adoption, likely commercial cost of the new variety, the likely distribution and total area of plantings with seeding varieties by that time, and the potential for sowing in environments now avoided due to weed risk.

The estimates of the area suitable for sowing to leucaena in Queensland have been estimated to be 13.5M ha, with an expected 300,000 to 500,000 hectare sown to leucaena by 2017 (Shelton and Dalzell 2007), based on the following regions of adaptation: Burdekin (2.8M ha), Fitzroy (4.7M/ha), Burnett (0.9M/ha), Mary (0.3M ha), Brisbane (0.5M ha), Condamine (3.6M ha) and Border Rivers (0.7M ha) with a further 1.2M ha in coastal Queensland suited for sowing to an adapted psyllid resistant cultivar. These estimates of potential area based on zones of adaptation are often higher than the true potential due to variation in the suitability of land for cultivation and other factors. However, even if this estimate is 30% higher than the true potential the analysis still suggests that leucaena is still only predicted to be cultivated on 5% of the adapted area by 2017. Based on this analysis it is clear that whilst there has been widespread adoption of leucaena the availability of suitable land is not the limitation to adoption but rather the willingness of producers to introduce the technology.

We were not able to access more detailed data on the regional distribution of leucaena plantings therefore the models developed were based on aggregating the target environments into those where leucaena is currently planted and those which would be suited for production should a psyllid resistant variety be released. Sterility will not change the geographic adaptation of the background cultivar that the trait is developed in, so will not alter the range of adaptation of leucaena *per se.*

Analysis Scenario 1.

What is the effect of alteration in seed price on the value of investment in leucaena?

Assumptions:

- Hybrid seed or GM seed will be more expensive
  - Current prices $15 - $40/kg
  - Sowing rate 2.5kg/ha
  - Modelled seed prices
    - $40, 60, 80, 100 (based on the assumption that the new cultivar will be competing with elite genetics - a $40 start price has been assumed)
- Leucaena based pastures have benefit over grass pastures
  - Modelled at $100, 150 and $200 /ha per annum to cover environmental variation in leucaena value
- Productive stand life of leucaena (currently 20 years)
  - Modelled at 10, 15 and 20
- Discount rate for NPV analysis at 10 and 20% after tax (discount rate is the return that could be expected should the funds be invested elsewhere with consideration of the ‘riskiness’ of the investment)

Outputs

- NPV for each of the scenarios listed above
- ‘payback’ period estimated as the year in which the NPV of the investment becomes positive

Scenario 1 Results - Effect of Seed Price on Value of Leucaena Establishment

Table 1. NPV ($ per ha) of leucaena establishment at variable seed prices and stand life

Seed Price ($/kg)	$40	$60	$80	$100
Stand Life (years)				
	Discount rate 10%, $100/ha advantage			
10 years	$160	$142	$124	$105
15 years	$306	$288	$270	$251
20 years	$396	$378	$360	$342
Payback (year)	7	7	8	8
	Discount rate 10%, $150/ha advantage			
10 years	$422	$403	$385	$367
15 years	$641	$622	$604	$586
20 years	$777	$759	$741	$722
Payback (year)	5	5	5	5
	Discount rate 10%, $200/ha advantage			
10 years	$683	$665	$647	$629
15 years	$975	$957	$939	$921
20 years	$1157	$1139	$1121	$1102
Payback (year)	4	4	4	4
	Discount rate 20%, $100/ha advantage			
10 years	$3	-$14	-$31	-$47
15 years	$51	$34	$18	$1
20 years	$70	$53	$37	$20
Payback (year)	10	12	13	15
	Discount rate 20%, $150/ha advantage			
10 years	$171	$154	$137	$120
15 years	$243	$226	$210	$192
20 years	$272	$255	$239	$222
Payback (year)	6	6	6	7
	Discount rate 10%, $200/ha advantage			
10 years	$338	$322	$305	$288
15 years	$425	$418	$402	$385
20 years	$473	$457	$441	$424
Payback (year)	4	4	5	5

The analysis (Table 10) suggests that the value of leucaena re-establishment with a sterile variety is relatively insensitive to changes in seed price compared to changes in stand longevity or discount rate. For current estimates of a 20 year stand life and $150 advantage per hectare of leucaena over grass pastures at a discount rate of 10% this equates to a reduction of only $30 in NPV if seed price were to double from $40 to $80 per hectare. Conversely if the new cultivars only persisted for 15 years then the NPV would be reduced by around $130 at all seed prices.

The discount rate in the NPV analysis was chosen to be 10% after tax to reflect current rates and also at 20% to reflect the scenario where the renovation was perceived to be more risky and thus the investor would seek a greater rate of return on ‘alternative’ investments with this capital. Both are realistic figures used in financial planning analyses.

It is also interesting to note that when the advantage of leucaena over grass was $100/ha per annum then the NPV was reduced by around 50% at 10% discount rates and to very marginal values at 20% discount rates. A reduction in the average advantage of leucaena could come about through 3 main scenarios

- *New cultivars are poorly adapted to target environments* – this can be mitigated by ensuring that the new technologies are incorporated into regionally adapted germplasm
- *Genetic gain in other forage germplasm negates some of the advantage of leucaena* – this analysis is outside of the scope of this project but the value of the leucaena project should be reassessed if the investors become aware of other alternatives.
- *Increases in leucaena adoption alter the supply function for beef to change prices* – this is considered unlikely as the adoption of leucaena has been relatively consistent and conservative with an upper area of 3.8% of potential area sown by 2017 at current forecasts relative to the potential area sown (300-500,000/13.2M ha by 2017). However, should current rates of renovation increase dramatically then a more detailed equilibrium displacement model be required to assess likely beneficiaries and whether the extra beef would lead to lower prices or alternatively producers would seek to produce the same amount of beef from smaller areas of improved pastures.

Therefore the analysis shows that there is scope for seed prices to increase and still provide significant benefits to pastures as long as stand-life, primary production, nutritive value, palatability, psyllid-resistance, and other attributes are maintained.

Analysis Scenario 2.

What is the likely benefit of a sterile cultivar to industry either in the absence of competition or in competition with seeded cultivars?

It is difficult to get accurate data on leucaena seed sales by region so in the following analysis the market is split into two regions

- The existing area adapted to leucaena production
- The area suited to a psyllid resistant cultivar

Other assumptions of the model are

- Sterile cultivar enters market in 2023 (10 year research and development)
- Based on current areas of 250,000ha sown in total and forecasts of 300,000 to 500,000 by 2017 then sowing rates are in the order of 8,000 to 40,000ha per annum are forecast, a mid estimate of 26,000 ha/annum will be used for this analysis
- Market saturation for leucaena is 5%, 15% and 25% of adapted area (experience with all pasture species is such that few actually are sown in the majority of the area of adaptation rather there are other alternatives used or producers choose not to renovate)
- At current rates of resowing the further 160,000 ha to reach market saturation would be sown in the adapted area by 2023 for 5%, such that at 5% market saturation only replacement of existing stands would occur by the time the new cultivar was released
- Stand life is 20 years
- Sterile cultivar represents 25%, 50% or 100% of seed sales
- Benefits calculated over a 20 year cultivar life

- Value of leucaena over grass is $150/ha
- Discount rate for NPV analysis is 10%

Table 2. Predicted sown area and net present value of sterile leucaena cultivars with market penetrance in 2013 in areas currently sown to leucaena.

Scenario	New area sown (ha/year)[2]	Area resown at end of stand life (ha/year)[3]	NPV ($)
5% Market Ceiling			
. 100% market share	0	33,000	$316M
. 50% market share	0	16,500	$158M
. 25% market share	0	8,250	$79M
> 5% Market Ceiling[1]			
. 100% market share	26,000	33,000	$566M
. 50% market share	13,000	16,500	$283M
. 25% market share	6,500	8,250	$142M

[1]at potential market ceilings above 5% the model predicts that market saturation will not be reached during the life of the cultivar (20 years) therefore the analysis for each of these scenarios 15%, 25% is the same, as the trajectory towards meeting the market ceiling is the same.

[2] the new area sown is a conservative figure and reflects current rates of sowing it does not assume that new cultivars will increase rates of sowing new areas. Whilst new cultivars and marketing may achieve an increase it is more likely that this will not be the case as the market is increasingly trying to influence those who have been recalcitrant to adopt the technology to date.

[2] the model assumes that rates of resowing will be high as those who have used leucaena for 20 years

The analysis shows for the area already served by adapted leucaena cultivars that, in the absence of a marked increase in the rate of establishment of new areas, a major source of value in 2023 will be the replacement of existing stands. Obviously if the stands were to last longer than 20 years (say 30 years) this would have a marked impact on the value. Whilst outside the scope of this report the model also suggests that further efforts to promote the establishment and increase both the rate and area sown are likely to lead to further economic benefits.

Table 2. Predicted sown area and net present value of sterile leucaena cultivars with market penetrance in 2013 in areas targeted by new leucaena cultivars with psyllid resistance.

Scenario	New area sown (ha/year)[2]	Area resown at end of stand life (ha/year)[3]	NPV ($)
5% Market Ceiling			
. 100% market share	0	3,000	$5M
. 50% market share	0	1,500	$2.5M
. 25% market share	0	750	$1.25M
> 5% Market Ceiling[1]			
. 100% market share	6,000	3,000	$63M
. 50% market share	3,000	1,500	$32M
. 25% market share	1,500	750	$16M

[1]at potential market ceilings above 5% the model predicts that market saturation will not be reached during the life of the cultivar (20 years therefore the analysis for each of these scenarios 15%, 25% is the same, as the trajectory towards meeting the market ceiling is the same). In this scenario it is assumed that this ceiling will be reached 10 years after the release of the psyllid resistant cultivar which is faster than that with conventional cultivars but reflects the work that has been done promoting the value of leucaena *per se.*

[2] the new area sown is a conservative figure and reflects current rates of sowing it does not assume that new cultivars will increase rates of sowing new areas. Whilst new cultivars and marketing may achieve an increase it is more likely that this will not be the case as the market is increasingly trying to influence those who have been recalcitrant to adopt the technology to date.

[2] the model assumes that rates of resowing will be as high as those who have used leucaena for 20 years and in this model 20 year stand life will not be reached for psyllid resistant cultivars until 2033.

The analysis shows that in the area targeted by psyllid resistant cultivars the potential value of the new cultivar will be influenced by the percentage of the zone of adaption that has been established to the new psyllid resistant cultivar due to be released in 2023. In simple terms the more effective promotion of that cultivar is, the longer it will take to get returns from a psyllid resistant and sterile cultivar. Ideally if psyllid resistance and sterility could be combined into a cultivar to target the broader zone of adaptation this would improve the economic analysis but if the broad adaption was to compromise economic advantage this would not be a realistic solution.

Objective 3. Assessing the likely impact on the perception of leucaena as a weed risk on a regional basis

There is evidence that increases in sterility would be viewed favourably by government regulators when developing legislation with respect to the weediness potential of leucaena. Despite contact with several groups involved in setting environmental regulations we were not able to assess what a ‘cut off’ level for seed production would be as most applications are assessed on a case by case basis. Therefore emphasis should be placed on developing cultivars with a high degree of sterility tending towards 100% sterility.

It should be noted that any cultivar developed using genetic modification will also be subject to risk assessment by the Office of the Gene Technology Regulator according to the Australian Government Gene Technology Act (2001). The object of the Gene Technology Act 2000 "is to protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with genetically modified organisms (GMOs)" (OGTR 2012). One part of this process is the development of a species biology document that is used as part of the Risk Assessment and Risk

Management Framework by the OGTR (http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1). The pest status review for Leucaena developed by the Queensland Government (Walton 2003; Appendix Y) is similar in scale and scope to one of these documents.

To date only the Whitsunday regional council has placed restrictions on the use of leucaena and these restrictions appear to allow the use of leucaena as long as the industry-endorsed code of practice is followed.

Objective 4. Evaluating the industry level risks and benefit:cost of R&D investment options

In this Objective the following scenario was addressed.

- Assessing the benefit:cost of the likely R&D investment based on the outcomes of Objective 2.

The research project is likely to cost in the order of $500k per annum over 10 years to develop leucaena cultivar/s that are sterile and able to be used across all leucaena growing zones. The assumptions used to come to this figure are shown in Table 3.

Table 3. Indicative costs of developing a sterile leucaena cultivar

Item	Years	Cost (pa)	Total
Principal Investigator (0.25 FTE)	1-10	$50,000	$500,000
Breeder (1 FTE)	1-10	$150,000	$1,500,000
Molecular Biologist/Tissue Culture (1FTE)	1-5	$150,000	$750,000
Technical Support (1FTE)	1-10	$100,000	$1,000,000
Laboratory Costs	1-10	$50,000	$500,000
Field Trial Costs	1-10	$50,000	$500,000
Other Costs	1-10	$25,000	$250,000
			$5,000,000

These costs should be seen as indicative and may vary according to the group who tenders for the research and also the MLA investment may of course be reduced by in-kind investment by the research agency/ies. However, the costs are based on the skills involved and the need for the research program to focus not only on the trait in question (sterility) but also the need to put this trait into otherwise elite germplasm.

It would be possible to cease this program at several stages along the research pipeline based on success in achieving intermediate aims, these include

1. Promising hybrid germplasm
2. Adequate seed yields of hybrid germplasm
3. Development of robust seed production systems for hybrid cultivars

And it is recommended that the project is managed in this way to avoid the ongoing cost of developing material that could be shown to be unlikely to have commercial success.

Unfortunately it is not possible to accurately assess the likelihood of success at each stage but intermediate outcomes 2 and 3 would seem to have the most technical risk and if seed yields were low then the research investors should work with industry to assess the likely commercial acceptance of seed at a range of price points given the analysis in Objective 2 of this report.

Based on an investment of $5M AUD the NPV of this research, given the likely benefits of the adoption of the new cultivars, was assessed using the scenarios described in Objective 2. A summary of these is given in Table 4.

Table 4. Predicted NPV of a $5M AUD research investment over the next 10 years given the adoption scenarios described in Objective 2.

Scenario	NPV ($)
5% Market Ceiling	
. 100% market share	$118M
. 50% market share	$59M
. 25% market share	$29M
> 5% Market Ceiling	
. 100% market share	$215M
. 50% market share	$108M
. 25% market share	$54M

From table 4 it can be seen that the value of project (minus costs) is in the order of $120 - $220M if there was no ability for the industry to use seeding cultivars, or if the industry willingly chose to use only the sterile cultivar, with commensurate reductions in value if the cultivar/s shared the market with other cultivars.

Objective 5. Recommendation on the case for proceeding with the development of a sterile leucaena variety along with defined breeding strategy/ies and risk management plan

All of the reading, consultation and analysis carried out as part of this report suggest that the following factors will be critical in the development of a sterile leucaena cultivar

- Sterility *per se* is unlikely to increase the area sown to leucaena
- Sterility is not likely to accelerate the replacement of areas already planted to leucaena
- All methods for developing a sterile variety are likely to require medium to long term research and development

- Sterility will need to be combined with other traits (Psyllid resistance, frost tolerance, palatability) to achieve commercial acceptance
- Sterile varieties are likely to require changes in industry practice that will require training and education, either through
    - Developing and applying methods for transplanting of live seedlings, or
    - Adoption of hybrid seed production systems by commercial seed producers, or
    - Development of industry and grower protocols for the commercial production of a genetically modified forage plant.

Based on this analysis it is not recommended that a transgenic (GM) approach is undertaken unless the following factors can be addressed to satisfaction of the project partners

- The potential of an international research partner (for example EMBRAPA in Brazil) is explored
- A commercial partner with the willingness to embrace the legislative and commercial challenges of releasing a sterile cultivar can be found

The approach of developing triploid interspecific hybrids is a technically feasible alternative but should focus on the following issues

- Maintenance of palatability and psyllid resistance
- Development of cost-effective mechanisms for the production of hybrid seed or,
- Development of cost-effective *in vitro* methods for the propagation of large numbers of seedling plants as used by the forestry industry

These elements should be identified as ‘go/no go’ points in any breeding program and regular consultation made with government and industry as to the need for sterility in Leucaena and revision of the cost:benefit of the program.

The analyses demonstrate a positive NPV for the successful development of a sterile leucaena cultivar/s as long as the following factors are maintained

- Industry continues to sow leucaena at rates equal to those of today
- The commercial advantage of leucaena systems over grass pastures is maintained

The value of the investment is quite sensitive to these factors and they suggest that continued investment in marketing of the value of leucaena will be required, this may not require investment from MLA if sufficient funds are invested from the private sector.

It is also clear that the sterile cultivar/s must also maintain the current agronomic performance of selected seeding cultivars. This will necessitate the correct choice of germplasm for crossing and regional agronomic testing and validation.

With these background points the following recommendations are made

- The development of a sterile cultivar of leucaena needs to be considered relative to other research and development activities in leucaena (which was outside of the scope of this report)
- Any future leucaena breeding program include the potential use of hybrids to develop sterile germplasm to mitigate the threat to industry through the possible reduction in area available to sow to seeding cultivars
- The balance between sterility and other traits in a breeding program be based on industry needs analysis and benefit:cost ratios.
- That consideration be given to exploring other research partners such as Embrapa Brazil to share the cost of investment and maximize the commercial return on any new cultivar/s
- Sterile lines with very poor seed production may have a role in countries where leucaena is vegetatively propagated but are unlikely to be successful in Australia.

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