

final report

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Prepared by: Associate Professor Max Shelton
School of Agriculture and Food
Sciences, Faculty of Science

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Meat & Livestock Australia Limited
Locked Bag 991
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Breeding a psyllid-resistant *Leucaena* hybrid for northern Australia - Phase 2

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Abstract

The psyllid insect greatly reduces the productivity of leucaena (*Leucaena leucocephala*) in tropical and subtropical Australia during periods of humid weather when psyllid challenge is greatest. Control of the insect is possible by aerial spraying but breeding for resistance is the desirable approach.

In 2003, a breeding program was commenced to develop a psyllid resistant leucaena based on the hybridization of *L. leucocephala* (highly susceptible species) with *L. pallida* (a highly resistant species). By 2008, a backcrossed heterozygous phenotype was achieved which needed two cycles of progeny testing and selection to obtain a self-fertile variety that was homozygous for psyllid resistance and therefore relatively uniform and stable. Plants were also selected for their leafiness, branchiness, moderate flowering and seeding, and high digestibility.

The program successfully achieved its objective and three breeding lines are now available for commercialization and release to industry. Plant Breeders Rights will be sought over the period 2013-2015. The benefits of the new variety will be far reaching and will accumulate with time in this long-lived plant as more and more of the new variety is planted thus avoiding the severe loss of productivity when psyllid challenge is severe.

Executive summary

Introduction

Leucaena (*Leucaena leucocephala*) pastures for beef cattle production are productive and sustainable but susceptibility to the psyllid insect (*Heterospylla cubana*) has limited expansion of current commercial cultivars into more humid areas (>800 mm/yr) (Shelton & Dalzell 2007). Psyllids also cause intermittent damage in lower rainfall regions during humid periods.

The psyllid, which arrived in Australia in 1986, is a leaf sucking insect specific to the *Leucaena* genus, feeding on the growing tips of susceptible cultivars (Bray 1994). Psyllid damage can reduce production by 50-70% in humid regions and 20-50% in sub-humid environments (Bray 1994, Mullen and Shelton 2003). Control of the insect is possible by aerial spraying but breeding for resistance is a more desirable approach. Work on psyllid resistance in the *Leucaena* genus through the 1990s showed that several *Leucaena* species including the tetraploid *L. pallida* were resistant (Mullen *et al.* 2003).

A breeding program to develop psyllid resistant varieties began in 2002 at The University of Queensland (UQ) based on the F1 inter-specific hybrids between *L. leucocephala* and *L. pallida* (KX2) developed at the University of Hawaii in the 1980s and 1990s. Between 2002 and 2005, UQ initiated a program of recurrent selection in an attempt to produce stable outcrossed KX2 lines but inbreeding depression for yield and poor forage quality led to a change in the breeding strategy, and a backcrossing program was implemented between 2005 and 2008. Two cycles of backcrossing to elite *L. leucocephala* ssp. *glabrata* material were completed.

Phase 2 of the breeding program required two cycles of progeny testing and selection for self-compatibility to achieve stability and uniformity (2009 to 2013). The objective was to select the best line (s) for release to industry.

Methods

The work was conducted at the Redlands Research Station (27°53'S, 153° 25' E), 30 km east of Brisbane. The station receives an annual rainfall of 1322 mm and was an ideal location to conduct psyllid resistance trials due to the high challenge from the insect for significant periods of the year. Each contiguous plot contained plants spaced 0.5 m apart in a randomised block design. Buffer and border rows of the highly susceptible cv. Peru were included to ensure even psyllid pressure across the site. Psyllids were initially controlled with dimethoate until plants were well established.

In cycle 1, 240 individual trees from 9 breeding lines were established and evaluated. In cycle 2, 40 breeding lines selected from cycle 1 were evaluated. In both cycles the breeding lines were compared to the commercial cultivars Peru, Cunningham, Tarramba and Wondergraze.

The selection criteria:

- Psyllid damage rating of growing tips (PDR) (1=no damage, 9=dead) (Wheeler 1988).
- Yield index based on basal diameter² x height (Stewart *et al.* 1992)
- Floral development rating (FDR) (1=vegetative, 5=mature seed pods)
- Leafiness and branchiness rating (1= low, 5=high)
- In vitro dry matter digestibility (IVDMD) of the first fully expanded leaves from 10 plants per plot

In cycles 1 and 2, Excel was used to sort and compare data means and standard deviations for each parameter. In the second cycle, Minitab was used to create 'box and whisker' plots for each parameter. The breeding lines were then compared with the commercial lines by comparing the median, lower and upper quartiles, and degree of spread and skewness of the data.

Results & Discussion

At completion of the two cycles of testing and selection, all breeding lines were superior in psyllid damage rating (PDR) compared to the commercial cultivars but had a higher variability for this parameter. The median PDR was 2.9 for the breeding lines compared to ~7.7 for commercial cultivars. This difference and their low variability were good evidence that the breeding program was successful as a PDR <3 indicates minor damage under psyllid attack while a PDR of >8 indicates significant damage and large productivity losses. The digestibility and associated spread were slightly lower for the breeding lines (median of 68.3%) compared to the commercial cultivars (median of 69). High forage quality is one of the most important characteristics of *leucaena*. Whilst a lower digestibility was expected due to the much lower DMD of *L. pallida*, selection for psyllid resistance was possible with little impact on digestibility.

Yield index was similar for the breeding lines (median of 2.8) compared to the commercial cultivars (median of 2.6). Since the yield index was measured at a time when psyllid challenge was minimal, yield differences due to the psyllid damage were minimised. However, the data range for this character was large reflecting the genetic diversity of the breeding lines. The breeding lines were similar to the commercial cultivars in leafiness, branchiness, and floral development ratings with the data range again higher for the breeding lines. Regarding floral development, it is important to select lines that seed adequately to meet seed production demands, but not excessively so as to become a weed risk. When all parameters were considered together with minimum selection criteria established for each trait including seed

availability, eight of the 40 breeding lines were considered for the commercialization process. Of these, it is recommended that 3 lines (24, 34 and 39) will be carried forward. These lines were chosen as they met all criteria, they showed excellent regrowth vigour following cutting and there was sufficient seed available without showing excessive seediness.

Conclusions

The breeding program was a success. All lines showed superior psyllid-resistance compared to the commercial cultivars. The selected lines had similar digestibility and agronomic parameters (yield, leafiness, branchiness, floral development) compared to the commercial cultivars. Plant Breeder's Rights will be sought over the period 2013-2015 for eventual release of one of these lines to industry. It is anticipated that the new variety will make a significant impact of the productivity of leucaena pastures, especially in humid environments and at humid times of the year when the negative impact of the psyllid on growth is severe. The benefits will be substantial and will accumulate with time in this long-lived plant as more and more of the new variety is planted.

Table of Contents

1	Background	7
2	Project objectives.....	8
3	Methodology	8
3.1	Location	8
3.2	Experimental design	8
3.3	Trial management.....	8
3.4	Measurements and observations.....	9
3.5	Dry matter digestibility.....	9
3.6	Statistical analysis	10
3.7	Progeny test and selection – cycle 1	10
3.8	Progeny test and selection – cycle 2.....	10
4	Results and discussion	13
4.1	Progeny test and selection - cycle 1.....	13
4.2	Progeny test and selection - cycle 2.....	13
5	Success in achieving objectives	25
6	Impact on meat and livestock industry – Now and in five years time.....	25
7	Conclusions and recommendations	26
8	Acknowledgements.....	27
9	Bibliography	27
10	Appendices	28
10.1	Appendix 1- Pedigree and forage quality of parents of PT2 elite lines....	28
10.2	Appendix 2 – Data from second progeny test (PT2).....	30
10.2	Appendix 2 – Data from second progeny test (PT2) (continued)	31

1 Background

Leucaena (*Leucaena leucocephala*) pastures for beef cattle production are productive and sustainable but susceptibility to the psyllid insect (*Heterospylla cubana*) has limited expansion of current commercial cultivars into more humid areas (>800 mm/yr) (Shelton and Dalzell 2007). Psyllids also cause intermittent but serious damage in lower rainfall regions during humid periods.

The psyllid, which arrived in Australia in 1986, is a leaf sucking insect specific to the *Leucaena* genus, feeding on the growing tips of susceptible cultivars (Bray 1994). Psyllid damage can reduce production by 50-70% in humid regions and 20-50% in sub-humid environments (Bray 1994, Mullen and Shelton 2003). While it is possible to control psyllids by regular spraying with dimethoate, control using biological methods is desirable. Work on psyllid resistance in the *Leucaena* genus through the 1990s showed that several *Leucaena* species including the tetraploid *L. pallida* were resistant (Mullen *et al.* 2003).

A breeding program to develop psyllid resistant varieties began in 2002 at The University of Queensland (UQ) based on the F1 inter-specific hybrids between *L. leucocephala* and *L. pallida* (KX2) developed at the University of Hawaii in the 1980s and 1990s. Between 2002 and 2005, UQ initiated a program of recurrent selection in an attempt to produce stable outcrossed KX2 lines but inbreeding depression for yield and poor forage quality due to the high proportion of *L. pallida* genes led to a change in the breeding strategy; accordingly, a backcrossing program was implemented between 2005 and 2008. Two cycles of backcrossing (BC) to elite *L. leucocephala* ssp. *glabrata* material were completed producing BC2 breeding lines that are now approximately 87.5% *L. leucocephala* ssp. *glabrata*. The backcross parent used was a superior intraspecific *L. leucocephala* ssp. *glabrata* hybrid (K584 × cv. Tarramba).

The BC2 breeding lines had excellent psyllid resistance and biomass yield coupled with the branchy/bushy habit and the excellent forage quality of *L. leucocephala*. However, these lines were now heterozygous for the psyllid resistance genes (i.e. they contained both resistant and susceptible alleles for each resistance gene) and additional breeding and selection work was required to remove the susceptible alleles. We believe there are 2-4 genes responsible for conferring psyllid-resistance; however, they do appear to act in a simple way. We anticipated approximately <10% of progeny to be homozygous for psyllid resistance. We estimated that two cycles (approximately 18 months per cycle) of selfing and selection of homozygous highly psyllid-resistant individuals would be required to ensure the resultant variety was relatively uniform and bred true-to-type.

It was expected that a short list of elite lines would be selected from which 1-2 lines would be selected for release as the new variety. This process was expected to take 3 years.

2 Project objectives

The objectives of the program were:

By 30 March 2013:

- 1) Complete 2 cycles of self-fertilization, progeny testing and agronomic evaluation of the BC2 (twice backcrossed to *L. leucocephala*) breeding lines.
- 2) Produce 10 kg of seed, if a genetically stable BC2 breeding line has been identified.

3 Methodology

3.1 Location

The breeding program was conducted at the DAFF Redlands Research Station, Cleveland, Queensland, Australia (27°53'S, 153° 25' E), 30 km from Brisbane. The elevation of the experimental site is approximately 22m above sea level. The climate is primarily subtropical with a widely dispersed but summer dominant rainfall pattern. The station receives an annual average rainfall of 1322 mm, two thirds of which falls between November and April. Maximum temperatures rarely exceed 32°C and minimums rarely fall below 5°C. The soil at the experimental site is a friable and deep krasnozem (oxisol).

3.2 Experimental design

A randomised block design was used for all experiments. Treatments were the breeding lines and 4 commercial cultivars arranged into blocks (replicates). There were 5 replications in progeny test 1 (PT1) and 6 replications in progeny test 2 (PT2). Each replicate comprised 4 rows 2 m apart arranged in 6 m contiguous plots. Each plot contained 12 plants sown 0.5 m apart. Rows of the highly psyllid-susceptible cv. Peru were planted as borders around the edge of the trial and in every 3rd row throughout the trial to ensure an even distribution of psyllid pressure.

3.3 Trial management

Seed was initially scarified by individual clipping, pre-germinated in germination trays, and planted into grow-tubes in the glasshouse. Plants were watered, fertilized with osmocote, inoculated and transplanted when approximately 30 cm high. Dead seedlings were replaced as necessary during the first 4 weeks after transplanting. Weeds were controlled using pre-emergent applications of 400 mL/ha Spinnaker® (240 g/L imazethapyr) (Nufarm Australia Ltd). Post-emergent weeds were controlled using a mixture of 1 L/ha Fusilade® (212 g/L fluazifop-butyl) (Crop Care Australasia Pty Ltd) and 1.5 L/ha Basagran® (480 g/L bentazone) (Crop Care Australasia Pty Ltd). Weeds were controlled in the intra-row by cultivation and manual chipping. Plants were irrigated when weekly rainfall did not exceed 25 mm. Hares and kangaroos were

excluded from the trial site by a mesh fence. Psyllids were controlled by spraying regularly with dimethoate at recommended rates until plants were fully established.

3.4 Measurements and observations

The following measurements, observations and calculations were made:

- a) Psyllid damage ratings (PDR) of plant growth tips. Young growth was observed twice during the growing season. Damage was ranked on a scale of 1-9 according to Wheeler (1988) (Table 1).
- b) Branchiness ratings (BR) were recorded using a scale of 1-5 which accounted for the number of primary and secondary branches. A score of 1 indicated the tree was arboreal with little/no branching, while a score of 5 indicated the plant had branched profusely (Table 1).
- c) Leafiness ratings were recorded using a 1-5 scale where 1 indicated poor leafiness, while 5 represented excellent leafiness (Table 1).
- d) Dry matter (DM) yield index (YI) was estimated from plant height (cm) and stem diameter (cm) using the equation: $YI = h \times d^2$ (Stewart *et al.* 1992). Plant height (to nearest 5 cm) was measured from soil level to stem apex. Basal stem diameter (cm) was measured within 5 cm of soil level. This enabled the non-destructive approximation of biomass yield as there is a strong correlation between estimated ($h \times d^2$) and DM yield of plants (Mullen & Gutteridge 2002).
- e) Floral development rating (FDR) was assessed twice during the growing season using a rating scale of 1-5 (Table 1).

3.5 Dry matter digestibility

Leaf tissue samples (first fully expanded leaves) from several plants from each plot were collected. These samples were placed in packets and immediately frozen on dry ice. The samples were then freeze-dried and ground ready for analysis. The *in vitro* dry matter digestibility (IVDMD) analysis was performed at the University of Queensland Gatton Laboratories according to a method modified from Minson and McLeod (1972).

In vitro dry matter digestibility was selected to estimate forage quality of the breeding lines because it integrates the effects of protein, fibre and condensed tannin content on the digestibility of a forage in the rumen and small intestine.

3.6 Statistical analysis

Excel was used to rank data, calculate means and standard deviations. Minitab was used to create 'box and whisker' plots for each parameter. The breeding lines were compared with the commercial lines by comparing the median, lower and upper quartiles, and degree of spread and skewness of the data. Excel was used to create graphs of key parameters.

3.7 Progeny test and selection – cycle 1

Progeny testing and selection of breeding lines for cycle 1 (PT1) commenced in September 2009. Seed from 11 elite trees representing 9 breeding lines developed in Project NBP.307 V2 (*Psyllid-resistant Leucaena hybrid for northern Australia*) (including 1 composite line comprising seed from 3 different trees) were planted in grow tubes in the glasshouse. Two hundred and forty individual trees of each breeding line were established. All commercial leucaena cultivars were included for benchmark comparisons. There were >5000 seedlings planted including border rows of cv. Peru.

Data were collected from the entire population on two occasions. The first observations of psyllid damage rating (PDR) and phenological stage of development were taken from 29 April – 6 May 2010. The second set of observations (PDR, stem height, basal stem diameter, branch and leaf development rating and phenological stage of development) were made from 2 - 5 August 2010. A total of >23,500 data entries were collected and analysed from the first progeny test trial.

Forage quality assessment (*in vitro* dry matter digestibility) was completed on a subset of these elite trees to ensure that only those with quality equal to or better than the current commercial varieties were selected.

3.8 Progeny test and selection – cycle 2

Forty elite PT1 lines were chosen for the PT2 trial. The BC2 PT2 lines were established in the glasshouse (Plates 1b, 1c and 1d) and seedlings transplanted into the field, with cv. Peru border rows, in the last week of October 2011 (Plates 1e and 1f). A total of 5254 seedlings were planted.

At the end of 2012, it was decided that further yield and psyllid damage measurements should be undertaken to provide additional data to ensure that the best lines were selected for commercialization. Accordingly, half the plots (replications 4-6) were cut to 75 cm in early December 2012. Yield measurements (fresh weight only) were recorded in March 2013, and an additional psyllid damage rating was recorded in April 2013 (at a time when psyllid challenge was very high).

Table 1. Rating scales for psyllid damage, branchiness, leafiness, and floral development.

Psyllid Damage Rating Scale (Wheeler 1988)		Branchiness Rating Scale	
Rating	Damage observed	Rating	Description
1	No damage observed	1	1 primary & few secondary branches (apical dominance)
2	Slight curling of leaves	2	2-3 primary & few secondary branches
3	Tips & leaves curling and yellow	3	4-6 primary &/or many secondary branches
4	Tips & leaves badly curled, yellowish & covered in sap	4	6-10 primary &/or many secondary branches
5	Loss of up to 25% of young leaves	5	>10 primary & secondary branches (no apical dominance)
6	Loss of up to 50% of young leaves		
7	Loss of up to 75% of young leaves		
8	100% loss of leaves & blackening of lower leaves		
9	Blackened stem with total leaf loss		
Leafiness Rating Scale		Floral Development Rating Scale	
Score	Degree of leafiness development	Score	Floral development
1	Few leaves on stems	1	Vegetative growth only
2	Limited number of leaves on stems	2	Green buds present
3	Moderate number of leaves on stems	3	Open flowers present
4	Significant leaf development	4	Green seed pods present
5	Profuse leaf development	5	Mature seed pods present



a) Selfed seed developing on elite PT1 tree in March 2011 (Further 4-6 weeks until mature).



b) Planting germinating seed of elite breeding lines into grow tubes



c) Progeny of elite trees in glasshouse at the DAFF Redlands Research Station.



d) Progeny of elite trees in glasshouse at the DAFF Redlands Research Station.



e) Hand-planting of seedlings into field site



f) Seedlings of elite plants transplanted into field at Redlands

Plate 1. Photographs of process of establishing seedlings in glasshouse followed by transplant into field

4 Results and discussion

4.1 Progeny test and selection - cycle 1

The first progeny test was successfully completed and elite vigorous psyllid resistant individuals from all 9 breeding lines were identified (Tables 2 and 3).

Severe psyllid pressure occurred at the Cleveland site from first establishment of the leucaena; the commercial varieties (cvv Peru, Cunningham, Tarramba & Wondergraze) were badly damaged (Table 2) suppressing their biomass yield.

The BC2 progeny segregated for psyllid resistance (and hence yield) as expected. The proportion of progeny considered psyllid resistant (PDR = 1-3) ranged from 5-20% within breeding lines. Within each breeding line, elite individuals were found with good psyllid resistance, yield and tree form (Table 3; Plates 2 and 3).

While the mean IVDMD values were lower than for the commercial cultivars, many individual plants within the breeding lines had IVDMD similar to those of the commercial varieties (Table 4). Comparing lines of progeny, lines 6 and 9b had higher means, while line 8 had the lowest mean IVDMD and exhibited great variability (38-81 %DM) for this trait. Other lines were intermediate.

Two hundred elite trees were retained for seed production. However, only trees which were self-fertile were selected for the second and final phase of progeny testing and selection. Continuous wet weather from September 2010 to February 2011 delayed seed production by 3-4 months which delayed commencement of the second (and final) cycle of progeny testing.

Of the 200 elite individual trees selected from the first cycle of progeny testing (Table 3), selfed seed was collected from 40 trees for evaluation in the second cycle.

4.2 Progeny test and selection - cycle 2

While the PDR's were measured on three separate dates, only the first and last observations were utilised in the selection process due to unusually low psyllid pressure at the time of the second observation.

The average PDR (first measurement) for the breeding lines was 2.8 with a pooled STD of 1.04, while the commercial cultivars averaged a PDR of 7.5 and STD of 0.61 (Figure 1). All breeding lines (BL) had significantly better PDR than the commercial varieties. The best PDR for any BL was line 27 at 1.8, while the highest PDR observed was line 19 at 3.9.

The commercial cultivars had significantly higher PDR than any of the BL, with the best performing cv. (Tarramba) having a PDR of 6.1. The highly susceptible cv. Peru received the highest PDR average of 8.4. Twenty seven breeding lines (coloured green) met the minimum selection criteria of $PDR \leq 3$.

The third measurement of PDR occurred in April 2013 when only the elite BL were assessed (Table 5). Data confirmed the low level of damage on the breeding lines compared to the commercial lines.

The yield index (YI) was calculated for each tree based on the equation: basal diameter \times apical height². The mean YI for the BL was 3.4 with a pooled STD of 1.1 (Figure 2), while the commercial cv. had a mean YI of 2.7 and a pooled STD of 0.76. Sixteen BL outperformed the best yielding commercial cv. Tarramba. The YI of Cunningham was 2.1 with an associated pooled STD of 0.65. All breeding lines met the criteria of $YI > cv. Cunningham$. When replications 4-6 were cut and regrowth measured in March 2013 (Table 5), similar yield trends were obtained. There was good correlation between the two yield measurements even though they were obtained differently (the first was a yield index while the second was fresh weight of regrowth).

Floral development ratings were observed twice; the mean of both observations was used to represent the rate of reproductive development of each breeding line. FDR did not vary widely between the BL and commercial cultivars although there was variation within the breeding lines. FDR means ranged from 1.9 to 3.3 (Figure 3). The pooled STD also did not differ significantly with the lowest of 0.5 and highest of 0.9 belonging to lines 42 and cv. Wondergraze respectively. Using criteria $FDR \leq cv. Wondergraze (W)$ ($FDR = 3$), 22 BL (coloured green) met the FDR selection criteria.

For leafiness, the best performing BL was line 14 with a LR of 3.46 and pooled STD of 0.68, while the lowest performing BL was line 19 with a LR of 2.03 and STD of 0.51 (Figure 4). This compared with leafiness ratings for the commercial cultivars of 2.53 (STD 0.44), 2.56 (STD 0.54), 2.32 (STD 0.46) and 2.47 (STD 0.54) for Tarramba, Wondergraze, Cunningham and Peru respectively. Twenty-two breeding lines met the independent truncation criteria $LR \geq 2.4$ (coloured green).

The average branchiness rating of the BL was 2.0 with a pooled STD of 0.58, while the commercial cultivars had a BR of 2.1 and a pooled STD of 0.51. There was no significant difference between the BL and commercial cultivars for this particular agronomic parameter. The best performing BL was line 40, with a BR of 2.57, while line 37 had the lowest rating at 1.5 (Figure 5). Thirty-five BL met the criteria $BR \geq 1.8$ (coloured green).

Digestibility data are shown in Figure 6. Twenty three of BL had *in vitro* dry matter digestibility (IVDMD) equal to or above the lowest ranking commercial cultivar Wondergraze.

All data from the breeding lines and commercial cultivars were then compared based on a weighted total (Figure 7). The weights were assigned to each selection criteria relying on our assessment of the importance of each criterion. Data were scaled with psyllid damage rating and *in vitro* digestibility weighted at greater importance (weighting of 3) than yield index, branchiness,

leafiness or floral development rating (weighting of 1). Differences between breeding lines and the commercial lines were also demonstrated in box and whisker plots (Figure 8) which compared values for median, lower and upper quartiles.

An initial selection of the varieties to be carried forward was based on these weighted scores combined with our assessment of minimum thresholds for each character. Thirty BL achieved scores of 51 or greater (Figure 7). When seed stocks were assessed and all BL that were not self-fertile were removed, just 8 lines remained (Table 5). Of these, it is recommended that 3 lines (24, 34 and 39) will be carried forward. These lines were chosen as they met all criteria, they showed excellent vigour following cutting (Table 5) and there was sufficient seed available without showing excessive seediness. Breeding line 12 (very little seed) should also be considered.



Plate 2. Susceptible commercial cv. Cunningham has been badly damaged by psyllids and has poor biomass yield (PT1 plants).

Plate 3. One of the superior vigorous and psyllid-resistant elite progeny identified for advancement through the breeding program (PT1 plants).

Table 2. Mean (\pm standard error) psyllid damage rating (PDR) and yield index for whole populations of different commercial cultivars and BC2 breeding lines (PT1).

Line	Number (n)	PDR 1 ¹	PDR 2 ²	Yield index ³
cv. Peru	120	8.5 \pm 0.06	8.1 \pm 0.04	625 \pm 43
cv. Cunningham	120	8.7 \pm 0.05	8.1 \pm 0.05	680 \pm 26
cv. Tarramba	120	7.4 \pm 0.05	6.9 \pm 0.06	1500 \pm 51
cv. Wondergraze	118	7.8 \pm 0.09	7.6 \pm 0.05	1289 \pm 70
Line 1	238	5.7 \pm 0.10	5.2 \pm 0.11	1297 \pm 68
Line 2	240	4.8 \pm 0.10	3.8 \pm 0.10	1403 \pm 59
Line 3	240	6.6 \pm 0.10	6.3 \pm 0.11	1684 \pm 69
Line 4	240	5.7 \pm 0.10	6.0 \pm 0.10	1581 \pm 61
Line 5	240	6.3 \pm 0.10	5.7 \pm 0.09	1569 \pm 49
Line 6	240	6.0 \pm 0.11	5.0 \pm 0.11	1688 \pm 54
Line 7	240	5.1 \pm 0.12	4.8 \pm 0.08	1610 \pm 60
Line 8	228	5.1 \pm 0.13	4.8 \pm 0.10	2489 \pm 78
Line 9a	61	5.8 \pm 0.22	5.7 \pm 0.20	2202 \pm 151
Line 9b	85	6.0 \pm 0.16	6.2 \pm 0.19	1668 \pm 99
Line 9c	94	5.7 \pm 0.16	5.7 \pm 0.16	1673 \pm 92

¹ Psyllid damage rating in April/May 2010 (1 = no damage; 9 = stem death)

² Psyllid damage rating in August 2010 (1 = no damage; 9 = stem death)

³ Yield index (total biomass) = height (cm) x [basal diameter (cm)]² in August 2010

Table 3. Mean (\pm standard error) psyllid damage rating (PDR) and yield index for 201 elite BC2 populations from different BC2 breeding lines (PT1).

Line	Number (n)	PDR 1 ¹	PDR 2 ²	Yield index ³
Line 1	9	4.3 \pm 0.50	3.8 \pm 0.28	3887 \pm 432
Line 2	47	3.3 \pm 0.12	2.7 \pm 0.13	2585 \pm 112
Line 3	18	3.8 \pm 0.19	3.6 \pm 0.23	3422 \pm 234
Line 4	14	3.9 \pm 0.13	4.1 \pm 0.27	2751 \pm 176
Line 5	7	4.1 \pm 0.46	4.1 \pm 0.26	2602 \pm 131
Line 6	23	3.9 \pm 0.25	2.5 \pm 0.12	2880 \pm 153
Line 7	36	3.4 \pm 0.21	3.8 \pm 0.13	2858 \pm 137
Line 8	31	3.5 \pm 0.20	3.2 \pm 0.17	3545 \pm 228
Line 9a	4	3.3 \pm 0.25	3.5 \pm 0.29	3336 \pm 420
Line 9b	5	4.4 \pm 0.51	3.8 \pm 0.58	3042 \pm 334
Line 9c	7	4.4 \pm 0.37	3.7 \pm 0.29	2807 \pm 318
Total elite pop.	201	3.6 \pm 0.08	3.3 \pm 0.07	2995 \pm 68

¹ Psyllid damage rating in April/May 2010 (1 = no damage; 9 = stem death)

² Psyllid damage rating in August 2010 (1 = no damage; 9 = stem death)

³ Yield index (total biomass) = stem height (cm) x [basal stem diameter (cm)]² in August 2010

Table 4. Mean (\pm standard error) and range of *in vitro* dry matter digestibility (IVDMD) observed in index tissue (youngest fully expanded leaves) for populations of different commercial cultivars and the progeny of BC2 breeding lines (PT1).

Genotype	Number (n)	IVDMD	
		Mean	Range
cv. Peru	10	77.4 \pm 2.02	68 - 83
cv. Cunningham	10	78.0 \pm 1.78	64 - 86
cv. Tarramba	10	73.9 \pm 1.75	62 - 80
cv. Wondergraze	10	77.5 \pm 1.32	68 - 84
Progeny from BC2			
Line 1	7	68.7 \pm 2.12	57 - 73
Line 2	16	68.6 \pm 1.63	59 - 77
Line 3	12	68.0 \pm 2.08	53 - 80
Line 4	4	69.6 \pm 2.65	62 - 73
Line 5	2	67.5 \pm 6.33	61 - 74
Line 6	10	74.4 \pm 1.20	68 - 81
Line 7	18	69.5 \pm 0.94	64 - 75
Line 8	22	64.4 \pm 1.78	38 - 77
Line 9a	3	68.4 \pm 3.63	64 - 73
Line 9b	3	73.2 \pm 0.97	72 - 75
Line 9c	2	68.4 \pm 3.63	65 - 72
Total elite population	99	68.5 \pm 0.67	38 - 81

Table 5. Summary characteristics for selected PT2 plants using additional data. (Green rows highlight lines selected for possible commercialization)

Breeding line	Combined weighted score	Yield ¹ (kg/plot)	IVDMD (%)	Psyllid ² damage rating (1-9)	Psyllid ³ damage rating (1-9)	Seed collected (g)
4	55	31	71	2.1	1.9	750
11	54	28	70	2.1	1.8	1590
12	52	36	71	3.4	2.8	126
22	52	32	67	2.6	2.4	1131
24	53	37	71	3.3	1.9	366
31	54	31	69	2.1	1.6	586
34	55	42	69	2.4	2.5	618
39	51	42	68	3.2	2.2	489
T	45	31	71	6.1	8.0	
W	37	28	68	7.5	8.6	
C	37	24	70	8.1	8.0	
P	34	21	68	8.4	7.9	

¹ Fresh weight yield of regrowth measured in March 2013 (kg/plot)

² Psyllid damage rating of regrowth in February 2012

³ Psyllid damage rating of regrowth in April 2013

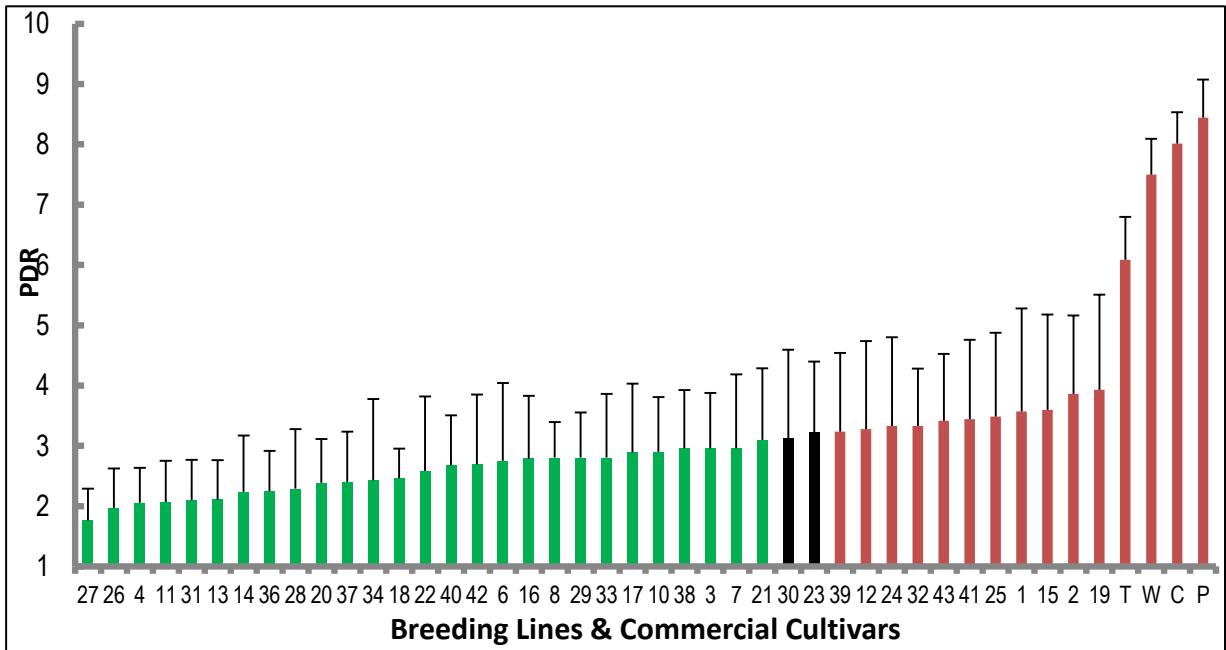


Figure 1: Mean PDR of each BL and commercial cvv. with pooled STD (PT2)

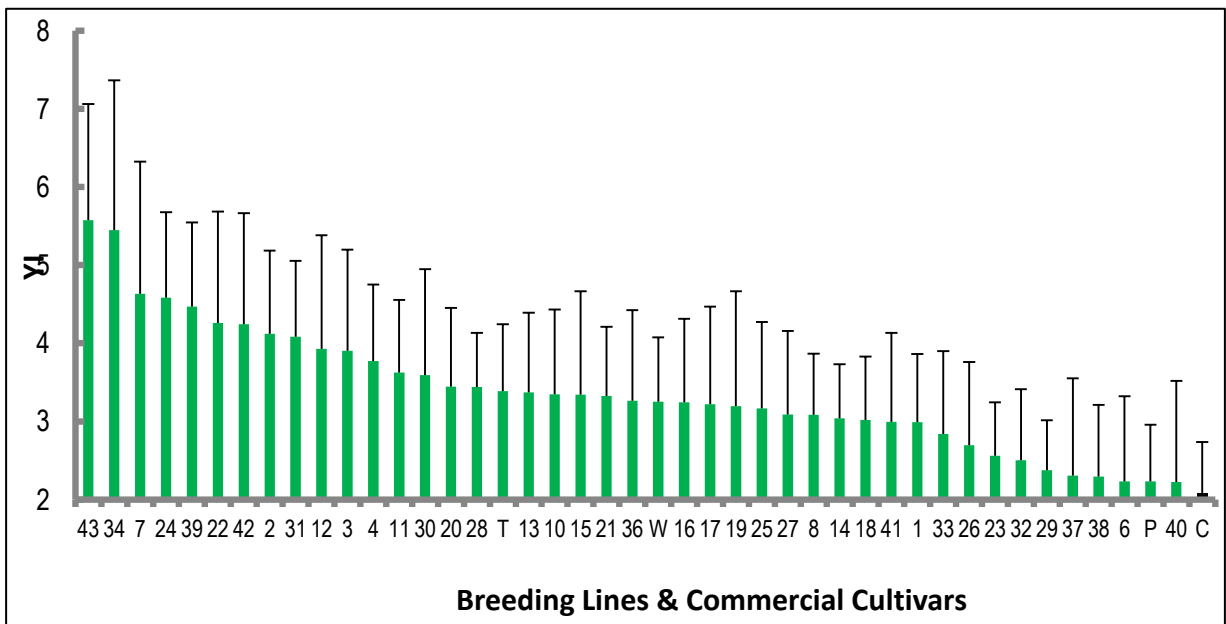


Figure 2: Mean YI of BL and commercial cvv. with pooled STD (PT2).

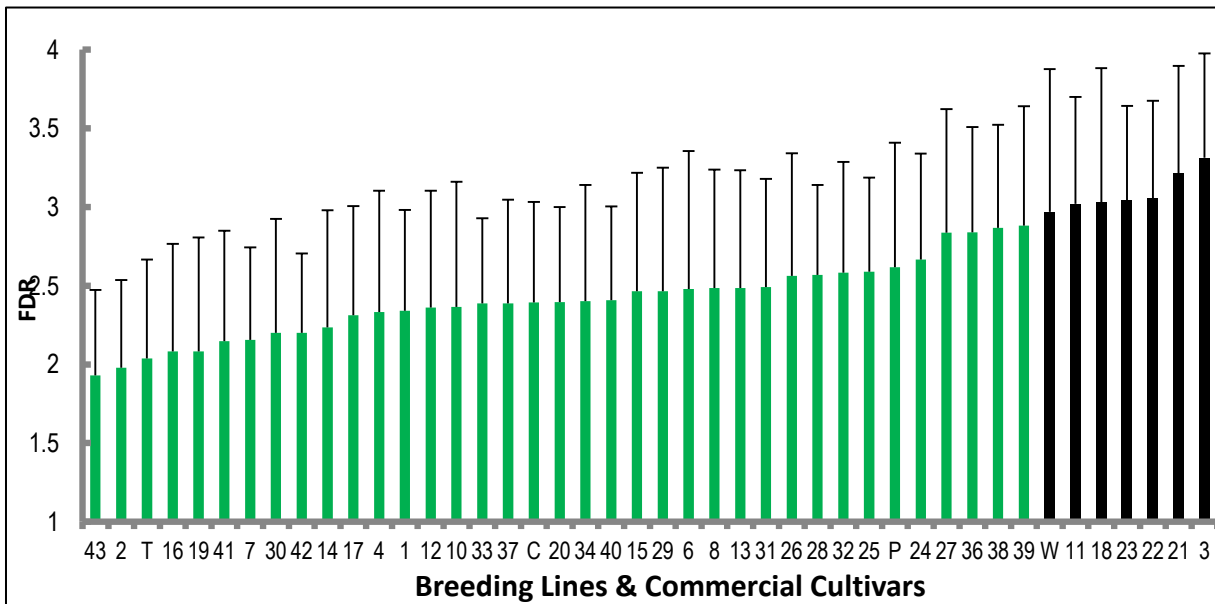


Figure 3: Mean FDR of breeding lines and commercial cvv. with pooled STD (PT2).

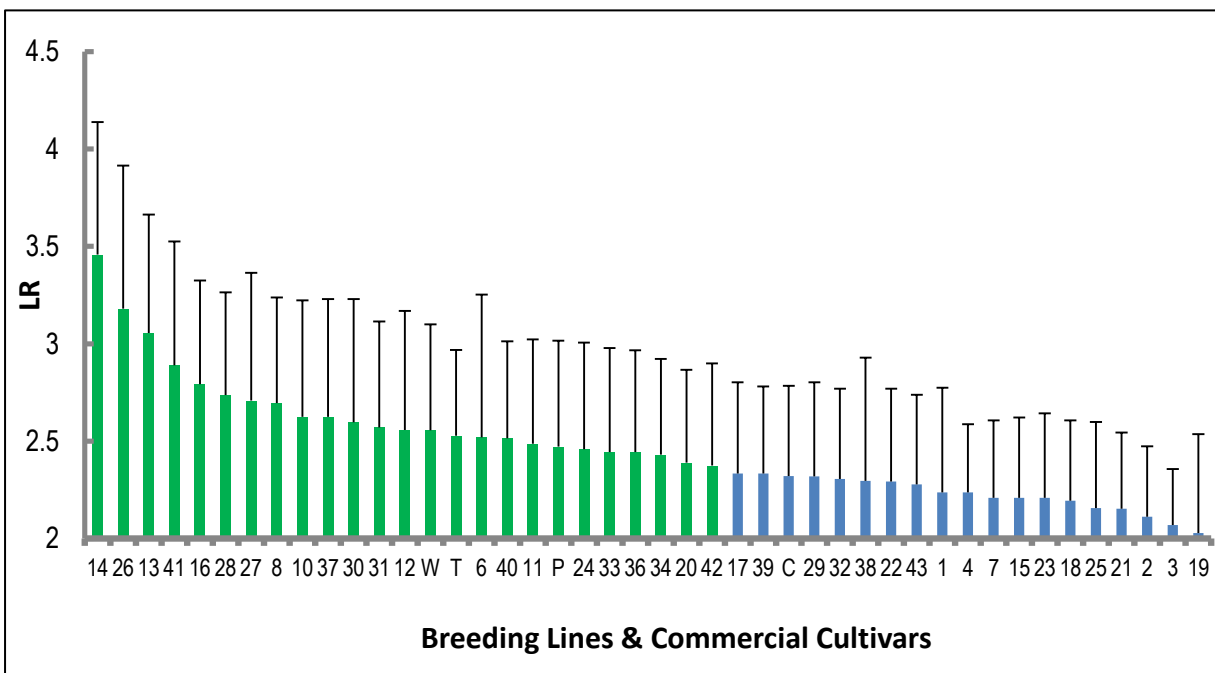


Figure 4: Mean LR of breeding lines and commercial cvv. with pooled STD (PT2).

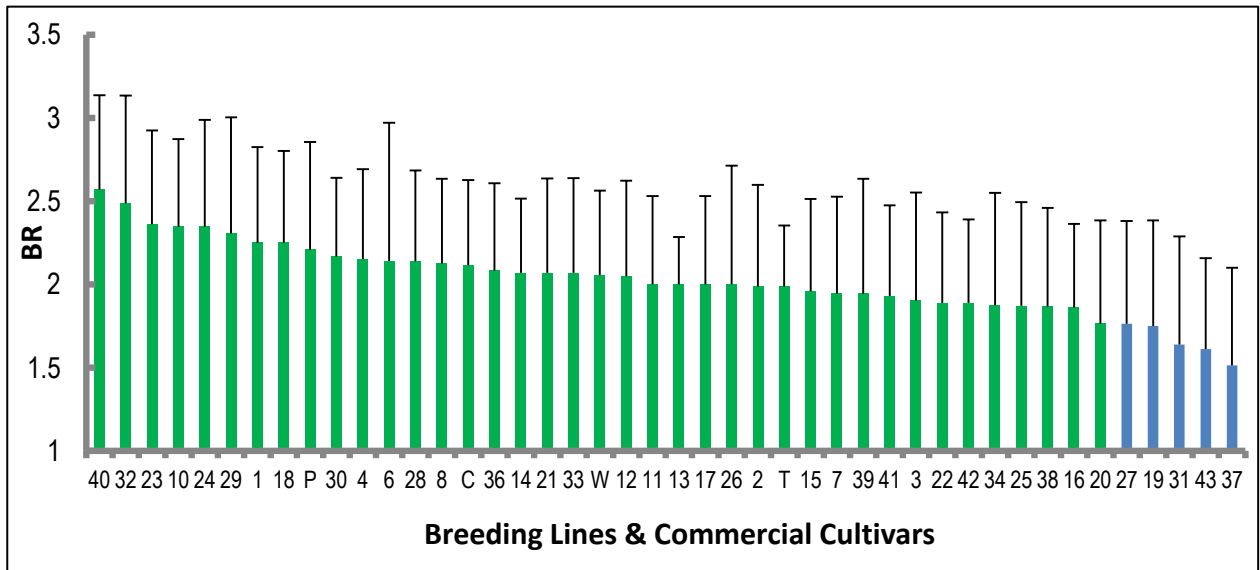


Figure 5: Mean BR of breeding lines and commercial cultivars with pooled STD (PT2).

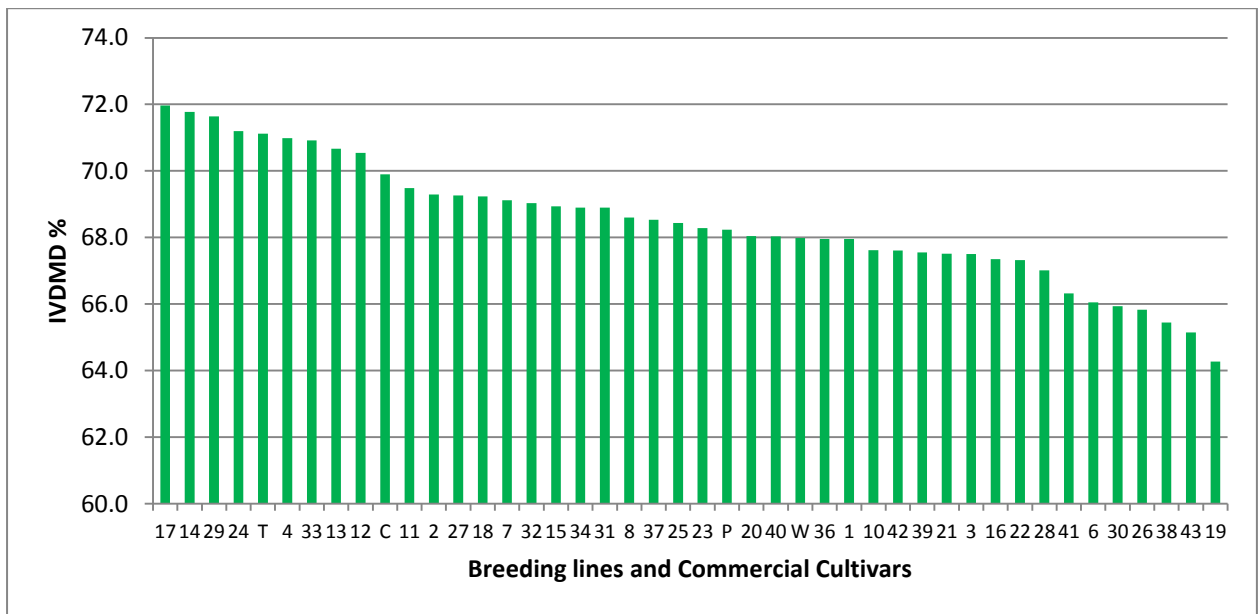


Figure 6: Mean IVDMD of breeding lines and commercial cultivars (PT2).

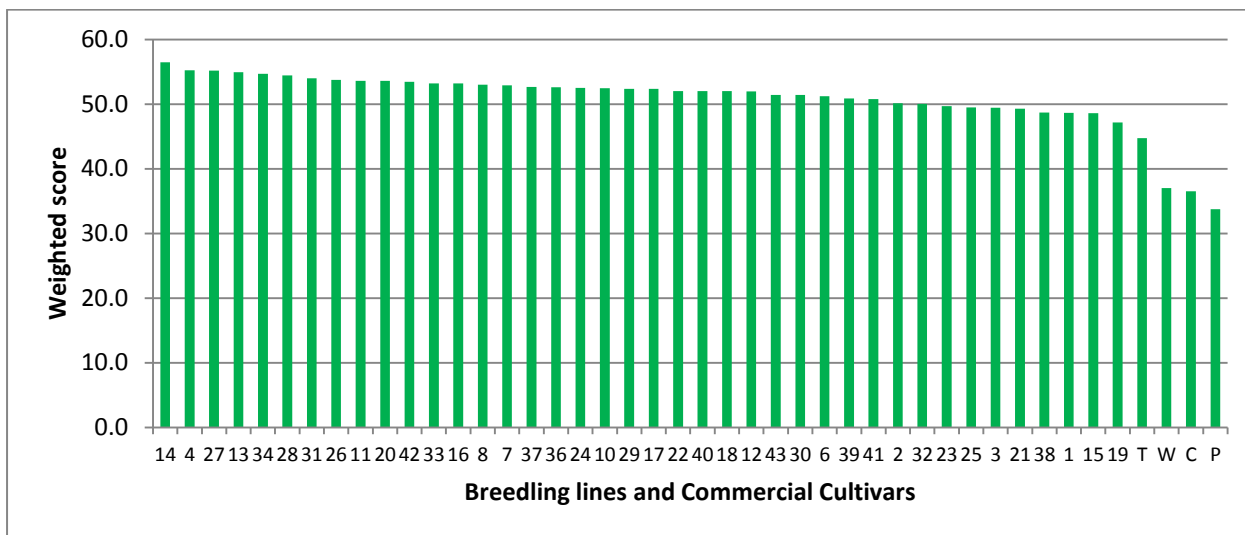


Figure 7. Weighted score of breeding lines and commercial cultivars (PT2).

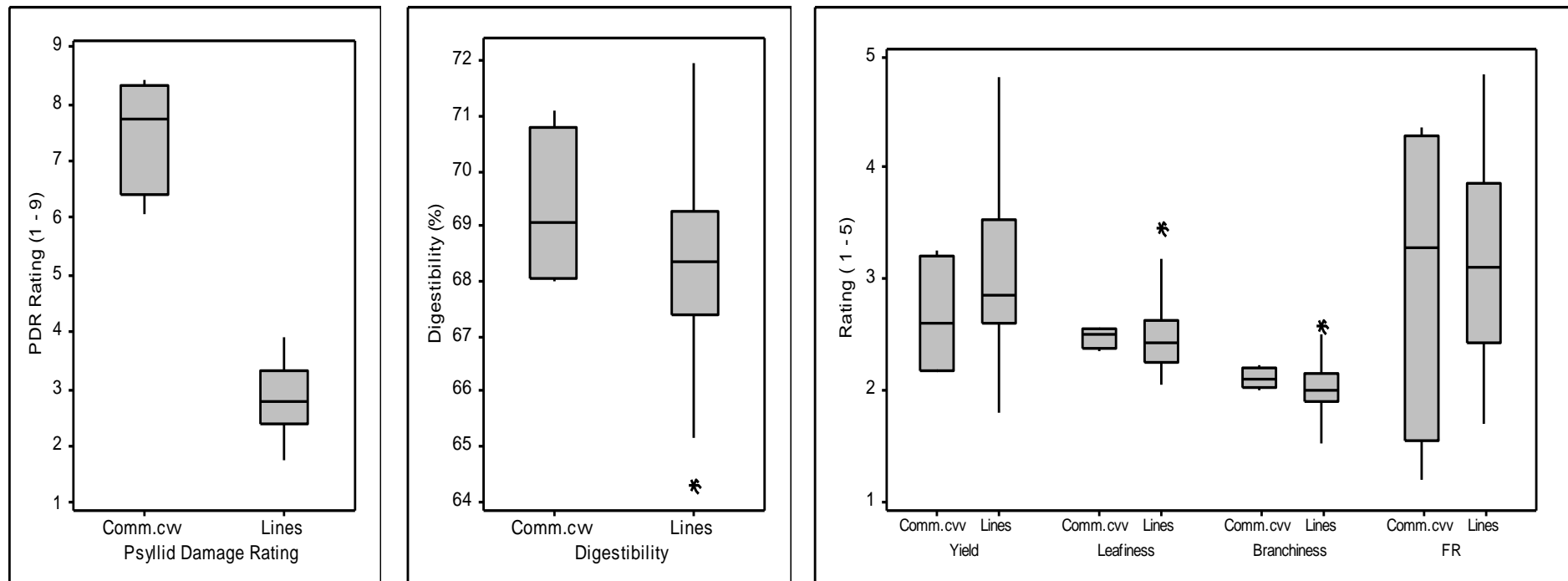


Figure 8. Box plot of psyllid damage ratings, digestibility and agronomic traits for breeding lines compared to commercial cultivars (PT2).

5 Success in achieving objectives

The breeding program was a success. All lines showed superior psyllid-resistance compared to the commercial cultivars. However, when other factors were considered (IVDMD, regrowth vigour, seed availability), just 4 elite breeding lines were selected for potential commercialization. The selected lines had similar digestibility and agronomic parameters (yield, leafiness, branchiness, floral development) compared to the commercial cultivars. There was also good uniformity among the breeding lines.

Plant Breeder's Rights will be sought over the period 2013-2015 for eventual release of one of these lines to industry. The reason that 4 lines were chosen was that it provides flexibility and scope for further selection during the PBR process. While it is desirable that just one variety be formally released, it is useful to have several available at this stage of the process.

While the total amount of seed produced from the breeding lines was 13.6 kg, it was not possible to achieve 10 kg, or even 5 kg, of seed any one of the selected lines. The plot size was just 6 m of row and there was a high level of competition among the trees as they matured thus limiting seed production potential.

Indeed, those lines which were most seedy will be a greater potential weed risk. It is generally agreed that the new variety should have a moderate seed production characteristic to ensure that commercial seed availability is not unduly limited but it should not be excessively seedy.

6 Impact on meat and livestock industry – Now and in five years time

Leucaena (*Leucaena leucocephala*) pastures for beef cattle production are productive and sustainable but susceptibility to the psyllid insect (*Heterospylla cubana*) has limited expansion of current commercial cultivars into more humid areas (>800 mm/yr). Serious psyllid damage also occurs in lower rainfall regions during humid periods. Yield losses have been estimated at 50-70% in humid regions and 20-50% in sub-humid environments (Bray 1994; Mullen and Shelton 2003).

The new psyllid resistant varieties will make a huge contribution in those many regions where psyllid damage is serious every year, such as in higher rainfall regions of north Queensland. However, the new varieties will also make a contribution in the main leucaena growing region of central Queensland. The impact will build over time as more of the psyllid resistant variety is planted. Benefits will be greater in 5 years and will accumulate over time in this long-lived plant as more and more of the new variety is planted.

7 Conclusions and recommendations

The varieties are now ready for commercialization. Discussions have been initiated with MLA to begin work on gaining Plant Breeder's Rights for the new variety(s). According to the PBR Office, this process may take up to 2.5 years. At the same time, the tendering process to gain expressions of interest from agency / company interested to grow and market seed of the new variety is underway. This will minimise delays in the process of release of the variety to industry.

It is noted that while our IVDMD analyses do reflect, in relative terms, the true digestibility *in vivo*, nevertheless it may be useful to compare the acceptability and response of the new psyllid-resistant breeding lines in a field grazing situation. It is therefore recommended an arrangement be negotiated with small areas of the new varieties, together with existing commercial *leucaena* cultivars, to be planted on two grazier's property under supervision from the University of Queensland. While feeding trials to establish liveweight gain potential will not be possible from such small plots, acceptability and resilience of the plants to grazing can be assessed.

8 Acknowledgements

The work of Dr Scott Dalzell in managing the program from 2002 until 2011 is acknowledged. His untiring efforts during this period were crucial to the subsequent success of the program.

The continuing advice of Professor James Brewbaker from the University of Hawaii is especially acknowledged, but also the advice and input of Dr Chris Lambrides and Dr Mark Dieters is acknowledged.

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10 Appendices

10.1 Appendix 1- Pedigree and forage quality of parents of PT2 elite lines.

2011 Planting Label	PT1 Description/ Rank	Parent IVDMD* (%)	Female Parent Pedigree				F1 hybrid female	
			Genotype from BC3	BC-2 parent	BC-1 parent	KX2 F3 parent	<i>L. pallida</i>	<i>L. leucocephala alabrata</i>
P	Peru	77.4	Tree no	Top 43 lines among SF	Elite among F3 lines from mass	Original KX2 cross		
C	Cunningham	78.0						
T	Tarramba	73.9						
W	Wondergraze	77.5						
1	1	71.9	8	BR26	BC3-4	15-93**	K748	K584
2	2	70.2	1	BC39	BC3-1	3-53**	K748	K481
3	3	64.6	7	BR22	BC3-5	12-124	K748	K584
4	4	75.0	6	BR21	BC3-5	12-124	K748	K584
5	6	61.4	2	BR9	BC3-13	15-93	K748	K584
6	7	76.1	8	BR26	BC3-4	15-93	K748	K584
7	9	63.4	8	BR26	BC3-4	15-93	K748	K584
8	13	72.7	3	BR15	BC3-5	12-124	K748	K584
9	20	72.5	1	BC39	BC3-1	3-53	K748	K481
10	22	64.7	9c	BR31	BC3-11	16-113	K748	K658
11	23	68.8	7	BR22	BC3-5	12-124	K748	K584
12	25	71.4	1	BC39	BC3-1	3-53	K748	K481
13	27	76.0	3	BR15	BC3-5	12-124	K748	K584
14	28	76.5	6	BR21	BC3-5	12-124	K748	K584
15	32	70.2	7	BR22	BC3-5	12-124	K748	K584
16	35	71.0	4	BR16	BC3-5	12-124	K748	K584
17	40	70.7	1	BC39	BC3-1	3-53	K748	K481
18	43	71.0	7	BR22	BC3-5	12-124	K748	K584
19	45	57.4	1	BC39	BC3-1	3-53	K748	K481
20	49	68.5	7	BR22	BC3-5	12-124	K748	K584
21	56	73.0	7	BR22	BC3-5	12-124	K748	K584
22	68	61.2	5	BR17	BC3-5	12-124	K748	K584
23	71	67.4	2	BR9	BC3-13	15-93	K748	K584
24	74	74.8	2	BR9	BC3-13	15-93	K748	K584
25	80	76.7	8	BR26	BC3-4	15-93	K748	K584
26	90	73.1	2	BR9	BC3-13	15-93	K748	K584
27	91	63.9	7	BR22	BC3-5	12-124	K748	K584
28	97	67.1	2	BR9	BC3-13	15-93	K748	K584
29	106	-*	2	BR9	BC3-13	15-93	K748	K584

* IVDMD forage quality analyses were only undertaken on the top 100 elite PT1 plants.

** Row number, plant number

- All back crosses were to cv Wondergraze
- BC = Best manual backcross progeny
- BR = Best of the rest of the back cross progeny
- BC3 = Back crossed onto elite F3 parent, None of the back crosses onto F1 parents were advanced into later evaluations as better psyllid resistance in the F3 crosses.

Appendix 10.1- Pedigree and forage quality of parents of PT2 elite lines (continued)

			Female parent pedigree						
2011 Planting label	PT1 Description/ Rank	Parent IVDMD* (%)	Genotype from BC3	BC-2 parent	BC-1 parent	KX2 F3 parent	<i>L. pallida</i>	<i>L. leucocephala glabrata</i>	
P	Peru	77.4	Tree no	Top 43 lines among SF trees		Elite among F3 lines from mass	Original KX2 cross		
C	Cunningham	78.0		9c	BR31	BC3-11	16-113	K748	K658
T	Tarramba	73.9		7	BR22	BC3-5	12-124	K748	K584
W	Wondergraze	77.5		2	BR9	BC3-13	15-93	K748	K584
30	113	-	9c	BR31	BC3-11	16-113	K748	K658	
31	121	-	7	BR22	BC3-5	12-124	K748	K584	
32	125	-	2	BR9	BC3-13	15-93	K748	K584	
33	134	-	9c	BR31	BC3-11	16-113	K748	K658	
34	135	-	2	BR9	BC3-13	15-93	K748	K584	
35	137	-	2	BR9	BC3-13	15-93	K748	K584	
36	142	-	7	BR22	BC3-5	12-124	K748	K584	
37	153	-	2	BR9	BC3-13	15-93	K748	K584	
38	156	-	7	BR22	BC3-5	12-124	K748	K584	
39	188	-	2	BR9	BC3-13	15-93	K748	K584	
40	300	-	1	BC39	BC3-1	3-53	K748	K481	
41	26	63.9	3	BR15	BC3-5	12-124	K748	K584	
42	44	72.0	9c	BR31	BC3-11	16-113	K748	K658	
43	33	75.1	9b	BC40	BC3-1	3-53	K748	K481	

Note: Eight selected breeding lines are highlighted. Top three in green, and next group in yellow.

* IVDMD forage quality analyses were only undertaken on the top 100 elite PT1 plants.

** Row number, plant number

- e. All back crosses were to cv Wondergraze
- f. BC = Best manual backcross progeny
- g. BR = Best of the rest of the back cross progeny
- h. BC3 = Back crossed onto elite F3 parent, None of the back crosses onto F1 parents were advanced into later evaluations as better psyllid resistance in the F3 crosses.

10.2 Appendix 2 – Data from second progeny test (PT2)

Breeding line	Yield index (,0000) (Feb 2012)	Fresh wt yield kg (Feb 13)	Branchiness (1-5)	Leafiness (1-5)	PDR (1st) (1-9)	FR (1st) (1-5)	FR (2nd) (1-5)	Mean FR (1- 5)	IVDMD %	Combined score	Total clean seed collected (g)
14	3.0	31.7	2.1	3.5	2.24	1.3	2.6	2.0	71.8	51.6	42.0
4	3.8	31.2	2.2	2.2	2.06	1.6	3.9	2.7	71.0	48.8	
27	3.0	24.2	1.8	2.7	1.76	2.1	2.9	2.5	69.3	48.0	398.2
13	3.4	18.6	2.0	3.1	2.11	1.4	3.8	2.6	70.7	48.2	473.5
34	5.4	41.8	1.9	2.4	2.43	1.8	3.9	2.8	68.9	50.1	618.6
28	3.4	27.8	2.1	2.7	2.29	1.3	2.3	1.8	67.0	49.7	
31	4.1	30.5	1.6	2.6	2.10	2.5	3.9	3.2	68.9	47.0	585.9
26	2.7	17.5	2.0	3.2	1.97	2.0	3.0	2.5	65.8	46.9	310.8
11	3.6	27.6	2.0	2.5	2.07	2.8	4.6	3.7	69.5	45.7	1587.3
20	3.4	25.0	1.8	2.4	2.39	1.4	2.2	1.8	68.0	48.0	406.9
42	4.2	40.4	1.9	2.4	2.69	1.3	3.0	2.2	67.6	49.2	84.0
33	2.8	24.1	2.1	2.4	2.81	1.5	2.1	1.8	70.9	47.8	27.2
16	3.2	28.9	1.9	2.8	2.79	1.0	1.7	1.3	67.4	49.1	12.4
8	3.1	24.8	2.1	2.7	2.81	1.2	2.4	1.8	68.6	48.1	35.5
7	4.6	35.0	1.9	2.2	2.96	1.2	3.1	2.2	69.1	48.7	66.0
37	2.3	20.0	1.5	2.6	2.40	1.7	2.3	2.0	68.5	45.7	251.9
36	3.3	21.3	2.1	2.4	2.24	2.7	3.9	3.3	68.0	45.0	918.0
24	4.6	37.0	2.3	2.5	3.33	2.0	3.6	2.8	71.2	48.3	366.6
10	3.3	27.2	2.3	2.6	2.90	1.4	2.8	2.1	67.6	47.8	156.5
29	2.4	14.6	2.3	2.3	2.81	1.8	2.7	2.3	71.6	45.6	38.9
17	3.2	21.9	2.0	2.3	2.89	1.9	3.3	2.6	72.0	45.8	173.8

Appendix 2 – Data from second progeny test (PT2) (continued)

Breeding line	Yield index (,0000) (Feb 2012)	Fresh wt yield kg (Feb 13)	Branchiness (1-5)	Leafiness (1-5)	PDR (1st) (1-9)	FR (1st) (1-5)	FR (2nd) (1-5)	Mean FR (1-5)	IVDMD %	Combined score	Total clean seed collected (g)
22	4.3	32.0	1.9	2.3	2.58	2.5	4.2	3.3	67.3	45.7	1131.0
40	2.2	18.6	2.6	2.5	2.68	1.9	3.1	2.5	68.0	45.6	132.3
18	3.0	19.8	2.3	2.2	2.46	2.9	3.8	3.3	69.2	44.2	465.5
12	4.0	36.0	2.1	2.5	3.38	1.8	3.1	2.5	70.5	47.7	126.0
43	5.6	37.6	1.6	2.3	3.42	1.1	1.8	1.4	65.1	49.2	45.5
30	3.6	25.7	2.2	2.6	3.13	1.3	2.4	1.9	65.9	47.2	135.5
6	2.2	20.8	2.1	2.5	2.75	1.8	2.5	2.2	66.1	45.1	78.5
39	4.5	42.2	1.9	2.3	3.24	2.5	4.1	3.3	67.5	46.0	489.3
41	3.0	26.8	1.9	2.9	3.44	1.1	1.8	1.5	66.3	47.0	30.4
2	4.1	34.8	2.0	2.1	3.86	1.4	2.5	2.0	69.3	46.5	52.7
32	2.5	24.2	2.5	2.3	3.33	2.4	3.4	2.9	69.0	43.8	339.5
23	2.6	19.3	2.4	2.2	3.23	2.5	3.3	2.9	68.3	43.0	108.2
25	3.2	25.6	1.9	2.2	3.49	1.9	3.1	2.5	68.4	43.7	86.0
3	3.9	23.7	1.9	2.1	2.96	3.3	4.8	4.1	67.5	41.5	1147.3
21	3.3	20.8	2.1	2.2	3.10	2.9	4.3	3.6	67.5	41.9	1061.1
38	2.3	12.9	1.9	2.3	2.98	2.3	3.6	3.0	65.4	40.9	420.2
1	3.0	22.1	2.3	2.2	3.57	2.2	4.0	3.1	68.0	42.3	150.7
15	3.3	20.6	2.0	2.2	3.60	2.6	3.7	3.2	68.9	42.0	969.3
19	3.2	23.9	1.8	2.0	3.93	1.4	2.4	1.9	64.3	42.5	79.0
T	3.4	31.3	2.0	2.5	6.08	1.1	1.2	1.1	71.1	43.8	
W	3.3	27.7	2.1	2.6	7.50	2.6	4.4	3.5	68.0	34.2	
C	2.0	23.6	2.1	2.3	8.05	1.4	2.6	2.0	69.9	35.0	
P	2.2	21.0	2.2	2.5	8.44	2.4	4.0	3.2	68.2	31.2	