

# Use of single cross hybrids to measure heterosis for yield in diverse lucerne genotypes growing in a subtropical environment

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**Abstract.** Yield stagnation is a worldwide issue for lucerne breeding, and reasons for the yield plateau include emphasis on disease and pest resistance and not yield *per se*, and the broad-based synthetic approach to lucerne breeding which is generally used. In this study, an incomplete diallel was made between 50 lucerne clones with representatives from the 3 hypothetical heterotic groups, *Medicago sativa* subsp. *falcata*, dormant subsp. *sativa*, and non-dormant subsp. *sativa*. Male sterile clones were also included among the dormant group. The single crosses were compared in a subtropical environment at Gatton, Queensland, for yield and other relevant agronomic traits against the adapted synthetics Sequel (dormancy group 9), UQL-1 (group 7), and a highly non-dormant experimental synthetic (line B) derived by introgression of highly non-dormant Arabian germplasm into Sequel. The trial was conducted in a known low-disease-pressure site for Phytophthora root rot, and anthracnose was managed by regular application of prophylactic treatments. The best single cross outyielded Sequel and line B by 13% and 8%, respectively. In this environment, yield was very much influenced by the dormancy group of the test material, with group 9 material significantly outyielding more dormant material. General combining ability (GCA) effects were more important determinants of cumulative yields than specific combining ability (SCA) effects, with these effects being significantly greater than zero for only 4 of the 236 crosses tested over the 15-month period. Similarly, GCA effects were more important for determining autumn height and persistence. The research did identify a small number of clones with good GCA for yield *per se*, and it would appear that future work should focus on developing more narrow-based synthetics with 4–8 parents which have been selected on the basis of their GCA for yield *per se*. DNA markers would appear to have a role in selecting clones carrying multiple resistances, and in establishing marker pedigrees for high-yielding parental clones such as we have identified, which can be traced through subsequent generations of recurrent selection in cultivar improvement.

## Introduction

Lucerne (*Medicago sativa*) is one of Australia's most important forage legumes, with around 3.5 Mha being grown either for hay production or for grazing (Pearson *et al.* 1997). Uses of lucerne, other than as forage, include crop rotation with cereals to increase soil nitrogen levels, improvement of water retention properties of soil, and reduced dryland salinity through lowering of the watertable (Irwin *et al.* 2001). Hill (1996) estimated that an additional 86 Mha in eastern Australia, and 9 Mha in Western Australia, could be further sown to lucerne, indicating room for expansion of area sown in Australia, with most of this being in New South Wales and Queensland.

It is generally accepted that lucerne yields have plateaued, both in North America since the 1940s (Lamb *et al.* 2006) and in Australia after 1985 (Lowe *et al.* 2000), as have yields of a number of other forage species (Casler 2008). In both of these continents, productivity increases have come through breeding for disease and pest resistance, giving a subsequent increase in persistence. In the absence of disease and pest pressure leading to lowered persistence, there has been no increase in yield *per se*, subsequent

to these periods. This yield stagnation is largely due to: lucerne being a perennial, the diversion of breeding effort from yield to disease and pest resistance, and the poor exploitation of non-additive gene action in current lucerne breeding schemes based on synthetics (Hill 1971, 1983; Hill *et al.* 1988), leading to outbreeding depression in advanced generations of the synthetic (Bingham 1998).

All cultivated lucerne is autotetraploid, and the plant is outbreeding and subject to severe inbreeding depression (Stanford 1951; Busbice 1969). These factors have influenced lucerne breeding methodologies, where lucerne cultivars are generally commercialised as genetically broad-based synthetics, developed by randomly mating elite  $S_0$  clones and advancing through several generations by open pollination (Hill *et al.* 1988). Thus, these lucerne breeding methodologies, based on recurrent selection and polycrossing to produce synthetic varieties (Tysdal *et al.* 1942), have changed little over the last 60 years.

The development of commercial hybrids in lucerne has not become mainstream methodology, even though self sterility,

cytoplasmic male sterility, and female sterility are known to occur in lucerne (Viands *et al.* 1988). The first report of cytoplasmic male sterility in lucerne was from Davis and Greenblatt (1967). Generally, production of hybrid seeds using cytoplasmic male sterility, which is the most efficient pollen control method of hybrid production (Barnes *et al.* 1972), has not been economic due to the difficulty in locating maintainers of male sterility, and as a result of poor seed production, particularly when the male sterile and polleniser plants are seeded in separate rows (Viands *et al.* 1988). Sun and Yen (1984), cited in Viands *et al.* (1988), studied forage yield from single cross, 3-way, and double cross hybrids from non-inbred parents. After examining >1000 hybrids they concluded that genetic background was more important to forage productivity than the type of hybrid. Dudley (1964) identified that the double cross will seldom be superior in performance to the single cross when non-inbred parents are used. This generally confirmed earlier work of Demarly (1963) who reported yield increases over the check varieties of 38, 39, and 45% in single, 3-way, and double cross hybrids, respectively.

The interest in lucerne hybrids has been partially driven by the work of Kehr and Gardner (1960) and Dudley *et al.* (1969) who reported that up to two-thirds of the genetic variance for forage yield in the lucerne populations they investigated was non-additive, suggesting that vast improvements could be made quickly by capitalising on non-additive gene action. The current breeding methods, based on synthetics, make little use of specific combining ability (SCA) that results from non-additive gene action as a result of superior epistatic combinations of favourable interacting non-homologous loci in linkage blocks (linkats) (Demarly 1979). This phenomenon is also termed 'maximum complementary gene interaction', which is greater in autotetraploids than in diploids (Bingham *et al.* 1994). Multi-locus epistasis also plays a major role in the superior performance of elite hybrids of many outcrossing species (Allard 1999). Intra-allelic interactions are also important to maximising yield, where the tetra-allelic state gives maximum heterozygosity and vigour (Bingham 1998); however, over-dominance is thought to be less important than complementary gene interactions in conditioning yield in lucerne (Bingham *et al.* 1994). These non-additive effects cannot be effectively captured in later (syn 2 onwards) generations of a synthetic, which is where commercialisation occurs. The potential to increase lucerne yield in southern Queensland through utilisation of SCA effects was demonstrated by Mackie *et al.* (2005) where substantial SCA effects were recorded in unselected *sativa* × *falcata* single crosses. A prerequisite to increasing SCA effects in lucerne breeding is the identification of genetically diverse plants, or populations, which will express heterosis when hybridised. Genetic diversity within lucerne cultivars is known to be high. Studies on genetic diversity for RFLP markers on Italian lucerne cultivars (Labombarda *et al.* 2000; Pupilli *et al.* 2000) found that the within-cultivar variation explained 98–99% of the total observed variation. Flajoulot *et al.* (2005) found similar results for several French cultivars developed over the period 1991–2003.

Lucerne also varies widely in the level of expression of autumn dormancy, and maximum dormancy is expressed at 15.5°C and a 12-h photoperiod (Schonhorst *et al.* 1957). Winter dormancy is closely associated with winter hardiness, which is not as

significant an issue in determining lucerne survival in Australia as it is in North America. Cultivars are classified for winter dormancy level according to their relationship to the 9 recognised germplasm sources for lucerne, with *M. falcata* 1 (most dormant) and African 9 (least dormant) (Barnes *et al.* 1977). A tenth source, based on very non-dormant germplasm from Saudi Arabia, has recently been recognised, although it was first reported in 1931 (Westover 1931). Unless lucerne undergoes a dormancy period, the plant does not tend to persist, and yields decline (Peterson 1972). It could be expected, however, that germplasm from widely differing winter dormancy groups will express heterosis when inter-crossed due to the diversity in alleles that could be expected in material from wide geographic regions. K. F. Lowe (unpublished data) has shown that non-dormant (group 9) cultivars outyield more dormant (group 7 and less) cultivars in Queensland. Heterosis that might be exhibited when crosses are made between plants from different dormancy groups could also be important in overcoming the yield stagnation currently experienced in mainstream lucerne breeding.

This paper reports studies conducted to assess the potential to increase lucerne forage productivity in southern Queensland through measuring the heterosis expressed in single cross hybrids, and to benchmark their yields against commercial and experimental synthetics. An incomplete diallel was generated, with vacuum emasculation, between 50 lucerne clones from diverse genetic backgrounds and winter dormancy groups, generating 236 single cross hybrids which were subjected to genetic analyses. Included within the 50 clones was clone D, a known high-yielding and winter-active clone, for which quantitative trait loci (QTLs) for yield have been mapped (Musial *et al.* 2006), and clones W116 and W126 which have been used to map resistances to *Phytophthora medicaginis*, *Stagonospora meliloti*, and *Colletotrichum trifolii* (races 1, 2, and 4) (Musial *et al.* 2005, 2007; Irwin *et al.* 2006; Mackie *et al.* 2007). A high proportion of the group 9 clones tested were from cv. Rippa, which was based on 636 S<sub>0</sub> plants with representatives from each of the 9 recognised germplasm sources for lucerne (W. Bunn, Cal/West Seeds, pers. comm.), and which has consistently performed well in low-disease-pressure sites in Queensland (K. F. Lowe, unpublished data). Several cytoplasmic male sterile clones from dormant backgrounds were also used as parents (Bingham 2002). The single cross hybrids were tested for yield and other agronomic traits over 15 months in rows in the field at Gatton Research Station, and 2 high-yielding and adapted synthetics (Sequel and UQL-1) were used as comparisons, along with the superceded cv. Hunter River. Two experimental synthetics (line B and UQL-6), bred by the authors, were also tested. Line B was a syn 2 generation of a cross between clones from the group 10 Saudi Arabian cv. Hejazi (Smith *et al.* 1995) and Sequel, and UQL-6 contained 50% *M. sativa* subsp. *falcata* in its genetic background.

## Materials and methods

### *Genetic material used and generation of populations*

The clones used in this investigation and their origins are listed in Tables 1 and 2. With the exception of clones W116 and D previously used in genetic mapping (Musial *et al.* 2006), no *a priori* knowledge existed about the performance of individual

**Table 1. The effect of female parentage on total and seasonal yield, autumn height and persistence of elite lucerne clones grown in rows at Gatton over a 15-month period**

| Female parent | Origin of clone                                   | Dormancy of parent cultivar | Dormancy of crosses | No. of crosses | Yield (g/m of row)           |                 |                 | Autumn          |                 | Final              |             |      |
|---------------|---|-----------------------------|---------------------|----------------|------------------------------|-----------------|-----------------|-----------------|-----------------|--------------------|-------------|------|
|               |   |                             |                     |                | Total <sup>A</sup> (14 cuts) | Winter (2 cuts) | Spring (3 cuts) | Summer (4 cuts) | Autumn (3 cuts) | Autumn height (cm) | density (%) | Mean |
| WA1131        | W1643 <sup>B</sup>                                | 4                           | 7,9                 | 5              | 847                          | 85.7            | 258.5           | 230.7           | 140.5           | 27.8               | 70.1        | 3.4  |
| WA1131        | W1643   | 4                           | 7,9                 | 4              | 853                          | 72.3            | 258.8           | 265.3           | 153.4           | 28                 | 78.3        | 3.7  |
| WA1121        | 6-4 ms <sup>C</sup> × B <sub>2</sub> <sup>D</sup> | 4                           | 7,9                 | 2              | 915                          | 87.2            | 290.4           | 264.3           | 150.1           | 24.8               | 82.5        | 4.1  |
| WA1136        | W1643   | 4                           | 9                   | 2              | 929                          | 98.2            | 290.8           | 254.3           | 159.6           | 31.7               | 84.3        | 4.3  |
| WA1132        | W1643   | 4                           | 9                   | 1              | 945                          | 87.3            | 297.7           | 297.7           | 169.8           | 31.1               | 78.5        | 4.7  |
| WA1107        | 6-4 ms × OP <sup>E</sup>                          | 4                           | 7,9                 | 8              | 978                          | 84.2            | 288.8           | 271.9           | 171.8           | 26.9               | 82.8        | 2.8  |
| WA1134        | W1643   | 4                           | 9                   | 1              | 1018                         | 85.9            | 289.1           | 290.7           | 192             | 29.2               | 84.2        | 4.7  |
| WA1135        | W1643   | 4                           | 7,9                 | 5              | 1043                         | 98.9            | 326.7           | 277.6           | 151.4           | 31.1               | 74.9        | 3.4  |
| WA1124        | 6-4 ms × B <sub>2</sub>                           | 4                           | 7,9                 | 7              | 1064                         | 96.5            | 324.8           | 334.6           | 177             | 29.4               | 82.6        | 2.9  |
| WA1130        | 6-4 ms × W16040 <sup>F</sup>                      | 4                           | 7,9                 | 2              | 1099                         | 96              | 325.6           | 364.3           | 180.5           | 27.9               | 85.1        | 4.3  |
| WA1125        | 6-4 ms × W16040                                   | 4                           | 9                   | 1              | 1104                         | 101.2           | 338.3           | 326.7           | 168.3           | 27.6               | 83.9        | 4.7  |
| WA1120        | 6-4 ms × W16040                                   | 4                           | 7,9                 | 3              | 1105                         | 94.9            | 322.5           | 343.1           | 181.4           | 27.4               | 85.8        | 4    |
| WA1123        | 6-4 ms × OP                                       | 4                           | 9                   | 2              | 1110                         | 88.2            | 340.9           | 348.9           | 189.1           | 30.1               | 82.5        | 4.3  |
| WA1127        | 6-4 ms × B <sub>2</sub>                           | 4                           | 7,9                 | 4              | 1110                         | 97.4            | 327.4           | 309.4           | 184.8           | 29.8               | 79.7        | 3.6  |
| W126          | Trifecta  | 7                           | 7,9                 | 17             | 1123                         | 113.9           | 311.6           | 278.8           | 178.4           | 27.7               | 80.2        | 2.1  |
| WA1104        | 6-4 ms × OP                                       | 4                           | 7,9                 | 4              | 1138                         | 100.6           | 323.4           | 360.7           | 186.9           | 31                 | 86.1        | 3.6  |
| WA1103        | 6-4 ms × OP                                       | 4                           | 7,9                 | 15             | 1158                         | 103.5           | 338.9           | 320.1           | 163.3           | 31.8               | 77.1        | 2.1  |
| WA1122        | 6-4 ms × B <sub>2</sub>                           | 4                           | 7                   | 1              | 1159                         | 104.2           | 320.8           | 352.9           | 206             | 27                 | 83.9        | 4.7  |
| WA280         | UQL-1   | 7                           | 7,9                 | 5              | 1164                         | 105.2           | 335.9           | 320.6           | 198.6           | 29.7               | 81.1        | 3.1  |
| WA1128        | 6-4 ms × B <sub>2</sub>                           | 4                           | 7,9                 | 8              | 1168                         | 93.7            | 333.9           | 350.9           | 197.1           | 25.7               | 86.8        | 2.6  |
| WA1119        | 6-4 ms × B <sub>2</sub>                           | 4                           | 7,9                 | 11             | 1181                         | 94.1            | 347.4           | 368.5           | 202.9           | 30.1               | 87.9        | 2.3  |
| WA281         | UQL-1   | 7                           | 9                   | 1              | 1181                         | 111.1           | 334.3           | 356.4           | 188             | 30.8               | 84.9        | 4.3  |
| WA327         | Rippa   | 9                           | 7                   | 1              | 1196                         | 109             | 317.2           | 308.5           | 190.8           | 30.1               | 79          | 4.4  |
| WA268         | UQL-1   | 7                           | 7,9                 | 3              | 1220                         | 110.2           | 345.8           | 350.1           | 204.1           | 27.8               | 87.5        | 3.7  |
| WA324         | Rippa   | 9                           | 7,9                 | 5              | 1277                         | 97.3            | 341.2           | 377             | 224.9           | 32.6               | 87.8        | 3.3  |
| WA322         | Rippa   | 9                           | 9                   | 1              | 1290                         | 99.1            | 342.1           | 360.2           | 229.6           | 32.9               | 82.2        | 4.2  |
| WA382         | Rippa   | 9                           | 7,9                 | 15             | 1303                         | 117.2           | 352.3           | 330.5           | 216.7           | 34.9               | 82.4        | 2.1  |
| WA272         | Aquarius  | 9                           | 7,9                 | 3              | 1328                         | 114.5           | 352.8           | 360.3           | 223.4           | 32.4               | 84.8        | 3.7  |
| WA326         | Rippa   | 9                           | 7,9                 | 9              | 1333                         | 120.7           | 382.6           | 333.4           | 198             | 31.3               | 81.2        | 2.8  |
| WA329         | Rippa   | 9                           | 7,9                 | 3              | 1341                         | 112.8           | 355.8           | 352.7           | 211.6           | 32.9               | 82.4        | 3.8  |
| WA265         | Sequel HR   | 9                           | 7                   | 1              | 1345                         | 105.4           | 357.6           | 386.4           | 206.1           | 31.2               | 84.2        | 4.7  |
| WA325         | Rippa   | 9                           | 7,9                 | 8              | 1353                         | 103.7           | 360.2           | 377.1           | 226.5           | 33.3               | 83.3        | 2.7  |
| WA321         | Rippa   | 9                           | 7,9                 | 4              | 1373                         | 116.2           | 382.8           | 386.1           | 218.8           | 30.4               | 83.5        | 3.3  |
| WA383         | Rippa   | 9                           | 7,9                 | 5              | 1375                         | 129.2           | 360.5           | 353.5           | 220.4           | 32.3               | 86.1        | 3.4  |
| WA333         | Rippa   | 9                           | 7,9                 | 3              | 1391                         | 110             | 373.7           | 404.9           | 236.5           | 34.1               | 86.1        | 3.7  |
| WA335         | Rippa   | 9                           | 7,9                 | 6              | 1430                         | 112.3           | 349             | 401.5           | 260.8           | 35.1               | 87.6        | 2.8  |
| WA332         | Rippa   | 9                           | 7,9                 | 7              | 1434                         | 112.8           | 378.7           | 398.3           | 252.2           | 36.4               | 85.1        | 3    |
| W116          | Sequel  | 9                           | 7,9                 | 19             | 1481                         | 116.4           | 378.3           | 404.7           | 269.4           | 35.1               | 89.8        | 1.8  |
| WA323         | Rippa   | 9                           | 7                   | 1              | 1492                         | 117.1           | 418.9           | 420.2           | 214.7           | 32.4               | 83.8        | 4.7  |
| WA334         | Rippa   | 9                           | 7,9                 | 7              | 1522                         | 116.5           | 374.2           | 427.7           | 278.8           | 35.2               | 89.2        | 2.8  |
| D             | Demnat  | 9                           | 7,9                 | 19             | 1526                         | 114.6           | 400             | 453.6           | 265.9           | 39.9               | 84.3        | 1.8  |
| WA381         | Rippa   | 9                           | 7,9                 | 7              | 1552                         | 118.6           | 402.2           | 425.1           | 254.8           | 35.1               | 85.5        | 2.8  |

<sup>A</sup>Data for final two winter harvests are presented in an Accessory Publication. <sup>B</sup>W1643 is a large seeded line derived from 6-4 ms. <sup>C</sup>6-4 ms is a male sterile clone from Saranac. <sup>D</sup>B<sub>2</sub> is a male sterility maintainer clone from Blazer. <sup>E</sup>OP is open pollination with North American adapted material, mainly Vernal. <sup>F</sup>W16040 traces to a polycross of clones from Saranac and Vernal and other north American adapted material.

Table 2. The effect of male parentage on total and seasonal yield, autumn height and persistence of single crosses of elite lucerne clones grown in rows at Gatton over a 15-month period

| Male parent      | Origin of clone | Dormancy of parent cultivar | No. of crosses | Total (14 cuts) |       | Winter (2 cuts) |      | Yield (g/m of row) |      | Summer (4 cuts) |      | Autumn (3 cuts) |      | Autumn height (cm) |      | Final density (%) |      |
|------------------|-----------------|-----------------------------|----------------|-----------------|-------|-----------------|------|--------------------|------|-----------------|------|-----------------|------|--------------------|------|-------------------|------|
|                  |                 |                             |                | Mean            | s.e.  | Mean            | s.e. | Mean               | s.e. | Mean            | s.e. | Mean            | s.e. | Mean               | s.e. | Mean              | s.e. |
| WA1 <sup>A</sup> | WISFAL          | 4                           | 4              | 945             | 107.2 | 99.7            | 7.8  | 318.6              | 22.6 | 273.8           | 37   | 143.6           | 25.5 | 23.2               | 2.3  | 86.1              | 6.5  |
| WA267            | UQL-1           | 7                           | 4,7,9          | 970             | 38.6  | 89.2            | 4.6  | 297.4              | 10.7 | 309.3           | 13.6 | 148.9           | 8.6  | 27.2               | 0.8  | 82.7              | 2.2  |
| WA225            | WISFAL          | 4                           | 4              | 982             | 107.6 | 104.2           | 7.8  | 313.8              | 22.6 | 295.4           | 37.1 | 166.8           | 25.6 | 26.4               | 2.3  | 83.1              | 6.5  |
| WA126            | Trifecta        | 7                           | 4              | 1006            | 49.7  | 102.2           | 5.5  | 296.6              | 12.9 | 257.6           | 18.1 | 156.5           | 11.6 | 26                 | 1.1  | 77.8              | 3    |
| WA327            | Rippa           | 9                           | 4,9            | 1034            | 60.9  | 100             | 6.1  | 312.9              | 15   | 263.5           | 22.1 | 147.1           | 14.5 | 28.9               | 1.3  | 58.1              | 3.7  |
| WA263            | Sequel HR       | 9                           | 9              | 1053            | 108   | 101.1           | 7.8  | 326.3              | 22.7 | 304             | 37.2 | 167.1           | 25.7 | 28.8               | 2.3  | 83.6              | 6.5  |
| WA643            | 6-4 ms          | 4                           | 4              | 1068            | 108.2 | 100.1           | 7.8  | 320.6              | 22.7 | 311.8           | 37.3 | 175.1           | 25.7 | 30.4               | 2.3  | 83.5              | 6.5  |
| WA280            | UQL-1           | 7                           | 4,7,9          | 1101            | 43.9  | 95.7            | 5    | 303.8              | 11.7 | 327.5           | 15.7 | 201.5           | 10.1 | 29.7               | 0.9  | 89.9              | 2.5  |
| WA281            | UQL-1           | 7                           | 4,9            | 1150            | 48.8  | 104.5           | 5.4  | 320.4              | 12.7 | 331.8           | 17.8 | 192.9           | 11.3 | 30.6               | 1    | 86.4              | 2.9  |
| WA258            | UQL-1           | 7                           | 4,7,9          | 1175            | 36.4  | 99.9            | 4.4  | 324.8              | 10.2 | 329.8           | 12.6 | 197.8           | 8    | 28.7               | 0.7  | 87.1              | 2    |
| WA268            | UQL-1           | 7                           | 4,7,9          | 1176            | 39.3  | 101.2           | 4.6  | 331.9              | 10.8 | 362.1           | 13.8 | 210.4           | 8.9  | 28.4               | 0.8  | 87.7              | 2.2  |
| WA324            | Rippa           | 9                           | 4,7,9          | 1182            | 44.4  | 95.2            | 5.1  | 334.2              | 11.8 | 361.3           | 16   | 203.9           | 10.3 | 30.6               | 0.9  | 87.9              | 2.6  |
| WA262            | UQL-1           | 7                           | 7,9            | 1185            | 71.6  | 101.3           | 6.7  | 331.8              | 17   | 343.1           | 25.8 | 195.4           | 17.4 | 32.3               | 1.6  | 83.3              | 4.5  |
| WA265            | Sequel HR       | 9                           | 4,7,9          | 1198            | 35.8  | 107.2           | 4.3  | 348.3              | 10.1 | 308.4           | 12.3 | 194.8           | 7.8  | 33.7               | 0.7  | 82.3              | 1.9  |
| WA326            | Rippa           | 9                           | 4,7,9          | 1239            | 50.7  | 111.1           | 5.5  | 368.6              | 12.9 | 318             | 18.1 | 183.7           | 11.9 | 31.8               | 1.1  | 76.1              | 3.1  |
| WA335            | Rippa           | 9                           | 4,7,9          | 1246            | 56.7  | 102.7           | 5.9  | 331.9              | 14.3 | 344.8           | 20.7 | 228.8           | 13.5 | 33.8               | 1.2  | 89.5              | 3.5  |
| D                | Demnat          | 9                           | 4              | 1246            | 74.1  | 106.1           | 6.8  | 356.5              | 17.4 | 355.2           | 26.8 | 180.1           | 17.9 | 34.6               | 1.6  | 58.7              | 4.6  |
| WA383            | Rippa           | 9                           | 4,7,9          | 1256            | 61.7  | 112.5           | 6.2  | 339.2              | 15.2 | 342.4           | 22.4 | 198.1           | 14.7 | 34.4               | 1.3  | 77.5              | 3.8  |
| WA272            | Aquarius        | 9                           | 4,7,9          | 1266            | 38.1  | 114.3           | 4.6  | 364.4              | 10.6 | 356.3           | 13.4 | 212.9           | 8.4  | 30.7               | 0.8  | 86.3              | 2.1  |
| WA382            | Rippa           | 9                           | 4,7,9          | 1274            | 49.5  | 110.4           | 5.5  | 347.9              | 13   | 347             | 18.2 | 216.6           | 11.6 | 31.6               | 1    | 79                | 3.1  |
| WA116            | Sequel          | 9                           | 4,9            | 1290            | 44.5  | 95.1            | 5.1  | 340                | 11.9 | 356.5           | 16   | 232.7           | 10.3 | 32.7               | 0.9  | 89.9              | 2.6  |
| WA325            | Rippa           | 9                           | 4,9            | 1293            | 44.4  | 103.4           | 5.1  | 346                | 11.8 | 363.4           | 15.8 | 215.5           | 10.3 | 31                 | 0.9  | 85.5              | 2.6  |
| WA321            | Rippa           | 9                           | 4,7,9          | 1300            | 42    | 108.8           | 4.9  | 367.2              | 11.4 | 374.9           | 15   | 207             | 9.6  | 31.3               | 0.9  | 84.3              | 2.5  |
| WA333            | Rippa           | 9                           | 4,7,9          | 1327            | 51.1  | 102.3           | 5.6  | 351.6              | 13.3 | 412.5           | 18.8 | 229.2           | 12   | 33.4               | 1.1  | 85.3              | 3.2  |
| WA322            | Rippa           | 9                           | 4,9            | 1342            | 53.8  | 101.5           | 5.8  | 357.4              | 13.7 | 402.5           | 19.7 | 238             | 12.7 | 33.1               | 1.1  | 87.8              | 3.4  |
| WA332            | Rippa           | 9                           | 4,7,9          | 1353            | 46.8  | 104.5           | 5.3  | 361.3              | 12.3 | 376.9           | 17   | 235.9           | 10.8 | 34.6               | 1    | 87                | 2.8  |
| WA323            | Rippa           | 9                           | 9              | 1373            | 83.3  | 111.2           | 7.2  | 378.7              | 19.3 | 396.7           | 30.7 | 225.1           | 19.7 | 32.5               | 1.8  | 86.1              | 5.4  |
| WA1101           | Rippa           | 9                           | 4              | 1382            | 110   | 105.8           | 7.8  | 355.6              | 22.9 | 393.1           | 37.7 | 229.8           | 26   | 32.8               | 2.4  | 83.7              | 6.6  |
| WA334            | Rippa           | 9                           | 4,9            | 1389            | 49.1  | 101.6           | 5.5  | 349.9              | 12.8 | 396             | 18   | 253.4           | 11.5 | 33.9               | 1    | 90.7              | 3    |
| WA329            | Rippa           | 9                           | 4,7,9          | 1394            | 53.6  | 108.1           | 5.8  | 352.6              | 13.6 | 387.5           | 19.4 | 243             | 12.8 | 32.6               | 1.2  | 90.3              | 3.3  |
| WA381            | Rippa           | 9                           | 4,7,9          | 1414            | 46.3  | 113             | 5.3  | 372.3              | 12.3 | 399.8           | 16.9 | 238.2           | 10.7 | 34.8               | 1    | 85.3              | 2.8  |

<sup>A</sup>WA1 is a clone from *M. sativa* subsp. *falcata* cv. WISFAL (Bingham 1993).

clones. An incomplete diallel was generated consisting of 236 single cross hybrids, the parents of which are listed in Tables 1 and 2. The clones tested had winter dormancy ratings ranging from 4 (dormant) through to 9 (highly winter active). Several cytoplasmic male sterile clones, all originating from seed supplied by Dr E. T. Bingham, University of Wisconsin, were also evaluated. The source of male sterility was clone 6-4 ms, derived from Saranac (E. T. Bingham, pers. comm.), which had been crossed by open pollination with: North American-adapted commercial material, designated OP in Table 1; B<sub>2</sub>, a male sterility maintainer clone; WI6040 which traces mainly to Saranac and Vernal; and WI643, a large seeded line derived from 6-4 ms. More detail on the origin of this material can be found at [www.medicago-reports.org/](http://www.medicago-reports.org/). The above 4 lines were the sources of the male sterile clones listed in Table 1, which were identified by J. A. G. Irwin at the University of Queensland. In addition, the commercial synthetic cvv. Sequel, UQL-1, and Hunter River were included as controls. Sequel is consistently the highest yielding commercial cultivar in evaluation trials at Gatton Research Station (Lowe *et al.* 2000). One experimental synthetic tested, Line B, had significantly more winter activity than Sequel (J. A. G. Irwin and K. F. Lowe, unpublished data), indicating that it belonged to dormancy group 10. The remaining experimental synthetic, UQL-6, was a syn 2 generation from crosses of clones from the Australian-grown and/or bred cvv. Hunter River, Aquarius, UQL-1, Sequel HR, Sequel, Hallmark and Rippa as females with 50 *M. sativa* subsp. *falcata* (WISFAL) (Bingham 1993) clones used as males. UQL-6 has a dormancy rating of 5.

#### Experimental design

The experiment was sown in June 2005 at Gatton Research Station (27°34'S, 152°20'E; 90 m a.s.l.). The soil type was an alluvial black clay (Ug 5.12, Northcote 1971). There were 236 single cross hybrids, 5 experimental synthetics, and 3 synthetic cultivars (Sequel, UQL-1, and Hunter River). Only data for 2 of the experimental lines are presented here because of commercial sensitivity relating to the other 3. The field layout consisted of 56 columns and 9 rows, which were divided into 3 blocks. Within the first block (row 1 to part of row 5), plots were allocated at random to all crosses and 10 duplicates of the check cultivars (Sequel and UQL-1). As all of the 236 single cross hybrids could not be replicated 3 times due to seed shortage, plots in the remaining blocks were allocated at random to those crosses with seed available and to the check cultivars and experimental synthetics as in the first block. The replication and commercial synthetic cultivar duplication were sufficient to provide a measure of site variability. Each plot consisted of a 1-m row into which 200 seeds were sown, with 50-cm spacing between plots, and an inter-row spacing of 1 m.

#### Agronomic measurements

Yield was assessed 14 times, over the period August 2005 to September 2006, with a 4–5 week cutting interval. The entire row of each entry was defoliated to 2.5 cm with hand shears, oven-dried, and then weighed. Analyses were conducted on dry matter yield (g/plot). Height was measured 4 times according to accepted practice (UPOV 2005), at the mid-spring harvest in October 2005, at the autumn solstice in March 2006, in mid winter in July 2006, and at the end of the experiment in September 2006 by assessing

the maximum standing height at 3 points in the row and recording the average. Maximum stem length was assessed in August 2006 (with stem extended from crown to stem tip) along with average canopy width at time of harvest (i.e. both sides of row centre). Plant erectness was calculated by dividing maximum stem length by natural plant height (Boschma and Williams 2008). Initial plant numbers were assessed 6 weeks after sowing by counting all established plants in the row. Plant density was assessed in autumn (March 2006) and at the end of the experiment (August 2006) by estimating the % of live crown (basal) area remaining in the 1-m row. Foliage cover at the end of the experiment was calculated by multiplying row length (% final plant density × 1 m) and canopy width (expressed as m<sup>2</sup>). No allowance was made for any canopy extension at each end of the experimental row. Dormancy rating of each entry was assessed visually at the mid-July harvest in 2005.

In this paper, only data for total and seasonal yield, autumn height, and persistence are presented. The data for the other attributes mentioned above are lodged as an Accessory Publication with the Managing Editor, and will be made available on request.

#### Management

The experiment was fully irrigated by applying 25 mm every 2 weeks with overhead sprinklers in a solid set arrangement. Unsampled border rows were mown at the time of sampling. Because the purpose of the work was to assess genetic potential for yield *per se*, a spraying schedule using a combination of benlate and mancozeb, applied fortnightly in alternate applications, was instigated to manage endemic diseases such as anthracnose (*Colletotrichum trifolii*). Insect damage from leaf rollers and aphids was noticed on occasions and applications of insecticides were used to control these outbreaks. The experiment was sited on a well-drained area of Gatton Research Station where *Phytophthora* root rot was known not to manifest itself.

#### Statistical analysis of the data

The total yield over 14 harvests, seasonal yields, spring and autumn heights, row coverage, and final density were analysed by fitting general linear mixed models with rows, columns, and lines as random effects using residual maximum likelihood (REML) methods in GENSTAT (Payne *et al.* 2007). This model was determined to be adequate using sample variograms and plots of the residuals. Male and female parentage means of the crossed lines were derived from the model with random effects: rows, columns, class (crossed, 5 experimental synthetics, and 3 synthetic cultivars), and male and female parentage and their interaction within the crossed class.

Genetic analyses were undertaken on the means of the crosses. These means formed an incomplete diallel and were analysed using a GenStat procedure, based on Griffing's definitions (Griffing 1956) of combining abilities with the model

$$y_{ij} = \mu + g_i + g_j + s_{ij}$$

where  $y_{ij}$  is mean total yield for cross of line  $i$  with line  $j$ ;  $g_i$ , average performance of line  $i$  in hybrid combination, termed general combining ability (GCA),  $\sum g_i = 0$ ; and  $s_{ij}$  is average departure

in performance of cross from that expected from GCAs of parents, termed specific combining ability.

The variance matrix of the means from the REML analysis was used to estimate the variance of differences between general combining ability (GCA) effects and between specific combining ability (SCA) effects. The differences within GCAs were tested for significance. The SCA effects for total yield, autumn height, and density were tested to determine if they were significantly different from zero with *t*-tests. The expected means of selected lines were derived from the GCAs and plotted against observed means (Falconer and Mackay 1996).

## Results

### Agronomic determinations

#### Yield

The cumulative and seasonal yields (except for second winter, which are lodged as an Accessory Publication) of the identified synthetic cultivars and lines are shown in Table 3. The non dormant cv. Sequel outyielded the semi-dormant cv. Hunter River by 56% over the duration of the trial, with the very non-dormant experimental synthetic line B outyielding Sequel by 4.6%. These non-dormant synthetics also outyielded the dormant synthetics in all seasons in the Gatton environment. The mean performance of clones when used as females and males is shown in Tables 1 and 2, respectively. The same clone WA381 had the highest mean performance for total yield both as a female and as a male parent (Tables 1 and 2). The highest yielding single cross tested was W116 × WA381, followed by D × WA334, D × W116 and WA381 × WA322 (Fig. 1). While each produced >1700 g/m row compared with 1582 and 1654 g/m row for Sequel and line B, respectively (Table 3), none was different ( $P < 0.05$ ) from B and only the first 2 were different ( $P < 0.05$ ) from Sequel. For the best single cross (W116 × WA381), this represented a 13% yield increase over Sequel and 8% higher than the experimental synthetic Line B. All of the above-mentioned single crosses involved parents selected from the non-dormant (group 9) cvv. Demnat (D), Sequel (W116), and Rippa (WA322, WA334, and WA381) (Oram 1990). Clone D is known to be highly susceptible to *Colletotrichum trifolii* and *Phytophthora medicaginis*, and its good performance in single crosses indicates the effectiveness of fungicidal applications in managing *C. trifolii* and the absence of

*P. medicaginis* from the test site, allowing an accurate assessment of yield *per se*.

The influence of winter dormancy level on cumulative yield can also be observed in Fig. 1, where each cross is identified by its winter dormancy group(s), and the performance of crosses based on dormancy group has been circumscribed. The highest yielding crosses are the 9 × 9 group, and the lowest yielding are the 4 × 4, 4 × 7, and 4 × 9 groups, with the remaining crosses including group 7 clones showing intermediate total yields.

The seasonality of yield for each clone when used as a female or a male is presented in Tables 1 and 2 (except for second winter, which is lodged as an Accessory Publication), and while highly winter active group 9 clones generally yielded highest in winter, they also tended to outyield group 7 and group 4 clones and their crosses throughout the year, reflecting the trend described above for the synthetic cultivars and lines. The experimental synthetic line B was one of the highest yielders, irrespective of season, and outyielded Sequel substantially over summer (497 v. 419 g/m row).

#### Plant height, erectness, and persistence

There was little variation between clones in averaged height measurements (Tables 1 and 2), irrespective of whether the clone was used as the male or the female. As autumn height is used worldwide to assess winter dormancy, we will focus on that measurement in this paper. Autumn height showed that most of the group 4 crosses were markedly shorter than the other material. Clone D crosses were greater in natural plant height at the autumn measurement than most other clones tested, indicating very high levels of winter activity. The height values obtained for the synthetics (Table 3) generally agreed with that expected, based on their dormancy ratings. The more erect clones (those with a value close to 1) were generally the ones selected from the dormancy group 9 cultivars, whereas the more dormant material was less erect with values up to 1.25 (data lodged as an Accessory Publication). Persistence (% final density) was generally >70% for the relatively short duration of the trial, with crosses involving WA327 and D as the male being the least persistent (58 and 59%, respectively), and those involving WA334 as the male being the most persistent (91%) (Tables 1 and 2). All synthetics tested persisted well, including Hunter River (Table 3), providing further evidence of the low disease pressure at the site.

**Table 3. Total and seasonal yield, autumn height and persistence of elite lucerne synthetic lines or standard cultivars grown in rows at Gatton over a 15-month period**

Values are mean standard error

| Synthetic <sup>A</sup> | Winter dormancy rating | Yield (g/m of row) |                 |                 |                 |                 | Autumn height (cm) | Final density (%) |
|------------------------|------------------------|--------------------|-----------------|-----------------|-----------------|-----------------|--------------------|-------------------|
|                        |                        | Total (14 cuts)    | Winter (2 cuts) | Spring (3 cuts) | Summer (4 cuts) | Autumn (3 cuts) |                    |                   |
| Hunter River           | 5                      | 1012 ± 77.5        | 96.5 ± 8.4      | 263.2 ± 18.6    | 289.2 ± 27.6    | 198.2 ± 19.3    | 28.7 ± 1.71        | 88.1 ± 4.6        |
| UQL-6                  | 5                      | 1088 ± 77.8        | 95.7 ± 8.5      | 297 ± 18.7      | 311.6 ± 27.7    | 181.6 ± 19.3    | 28.7 ± 1.72        | 91.2 ± 4.6        |
| UQL-1                  | 7                      | 1263 ± 34.7        | 120.6 ± 4.3     | 332.9 ± 9.7     | 329.3 ± 11.5    | 213.4 ± 7.6     | 31.2 ± 0.71        | 86.8 ± 1.8        |
| Sequel                 | 9                      | 1582 ± 34          | 122.5 ± 4.3     | 398.4 ± 9.6     | 419.2 ± 11.4    | 295.9 ± 7.5     | 37 ± 0.71          | 92.2 ± 1.8        |
| B                      | 10                     | 1654 ± 77.6        | 122.4 ± 8.5     | 430.8 ± 18.7    | 496.7 ± 27.6    | 297.3 ± 19.3    | 39.5 ± 1.72        | 88.8 ± 4.6        |

<sup>A</sup>UQL-6 and B are experimental synthetics described in the Materials and Methods. The remaining cultivars are described in Oram (1990) and Irwin *et al.* (2001).

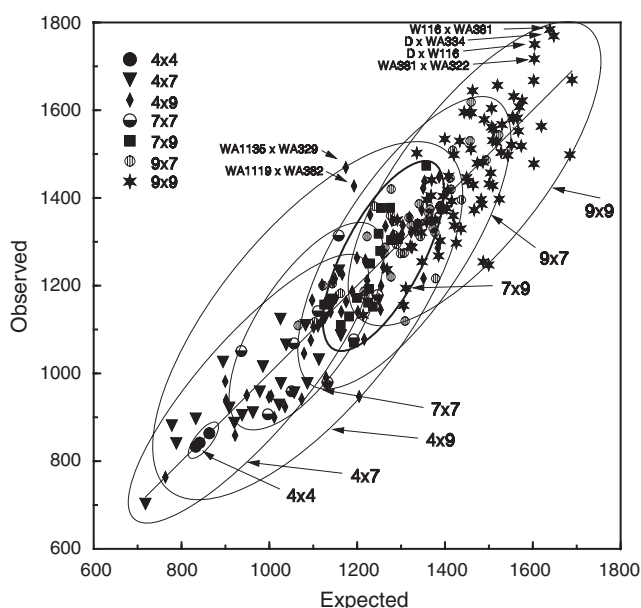


Fig. 1. Observed and expected means derived from parental general combining abilities (GCAs) for total yield (g/m row) over 14 harvests at Gatton. The line represents where observed and expected values are the same.

### Combining ability analysis

#### Total yield

On the basis of the performance of the single cross hybrids, we were able to separate additive yield effects (GCA) from non-additive total yield effects (SCA) (Griffing 1956). In autotetraploids, significant GCA indicates predominantly additive gene action (GCA in autotetraploids also includes a portion of dominance gene action due to diploid gametes), while significant SCA indicates non-additive gene action (Levings and Dudley 1963; Gallais 2003). The SCA effects were tested to determine if they were significantly different from zero with *t*-tests. To visualise SCA determinations (Fig. 1), we have plotted the expected yield for each cross against its observed yield. If the yield of an entry falls above the expectancy line, this indicates a positive heterotic response, and those below the line show a negative heterotic response. Significantly different from zero ( $P < 0.05$ ) and positive SCA effects were observed for the following 4 crosses: W116  $\times$  WA326 ( $181.1 \pm 86.2$ ), W116  $\times$  WA381 ( $145.0 \pm 72.7$ ), WA1119  $\times$  WA382 ( $233.7 \pm 106.8$ ), and WA1135  $\times$  WA329 ( $294.4 \pm 93.7$ ). The latter 2 crosses were between group 4 male sterile clones and group 9 clones from Rippa, indicating significant heterotic effects arising from interactions between different alleles in the genetically diverse parents. The remaining 2 crosses of the above 4 with SCA effects significantly different from zero (W116  $\times$  WA326 and W116  $\times$  WA381) involved group 9 clones from the adapted cultivars Sequel and Rippa. Six crosses (W126  $\times$  WA258, WA1128  $\times$  W116, WA1135  $\times$  D, WA329  $\times$  WA325, WA382  $\times$  WA334, and D  $\times$  WA381) showed negative heterotic effects significantly different from zero.

GCA effects (Table 4) were more important than SCA effects in determining yield among the set of clones we tested, and this has implications for future breeding methodologies for lucerne.

Clones which gave the highest positive GCA effects for yield and were not significantly different ( $P > 0.05$ ) from each other were WA1101 ( $391.6 \pm 125.0$ ), WA323 ( $349.7 \pm 69.3$ ), WA329 ( $234.5 \pm 35.9$ ), WA334 ( $262.7 \pm 28.3$ ), WA381 ( $299.25 \pm 27.9$ ) and D ( $264.9 \pm 22.3$ ). These clones all traced to group 9 clones from the cvv. Rippa and Demnat.

#### Plant height

The SCA effects for autumn height were also tested to determine if they are significantly different from zero with *t*-tests ( $P < 0.05$ ). To visualise the SCA derivations for autumn height, we have plotted the expected height for each cross against its observed height (Fig. 2). Those crosses where SCA effects for autumn height were significantly ( $P < 0.05$ ) different from zero, and which were positive, were: W116  $\times$  WA381 ( $4.2 \pm 1.6$ ), W126  $\times$  WA280 ( $5.2 \pm 2.4$ ), WA324  $\times$  WA267 ( $4.7 \pm 2.3$ ), WA325  $\times$  WA322 ( $4.2 \pm 1.6$ ), D  $\times$  W116 ( $6.7 \pm 1.9$ ), D  $\times$  WA321 ( $3.5 \pm 1.6$ ), and D  $\times$  WA335 ( $3.4 \pm 1.6$ ). All of these crosses were either between group 9 or group 7 clones. Significantly different from zero negative heterotic effects for autumn height were recorded for 2 crosses: WA1103  $\times$  WA335 and D  $\times$  WA324. Clone D, the winter active plant from cv. Demnat, has the largest positive GCA effects for autumn height (Table 4), which were significantly greater than all other clones except WA1101, while W1, from subsp. *falcata* cv. WISFAL, had the largest negative GCA effects.

#### Persistence

Only the cross WA1135  $\times$  WA329 showed positive SCA effects for persistence, which were significantly different ( $P < 0.05$ ) from zero by *t*-test (Fig. 3), this being a cross of a group 4 clone (WA1135) with a group 9 clone. Eleven of the crosses (W126  $\times$  WA326, WA1103  $\times$  W126, WA1103  $\times$  WA327, WA1103  $\times$  WA382, WA1128  $\times$  WA383, WA1131  $\times$  W126, WA1135  $\times$  D, WA280  $\times$  WA265, WA325  $\times$  WA321, WA382  $\times$  WA265, and D  $\times$  WA267) demonstrated negative SCA effects for persistence, which were significantly ( $P < 0.05$ ) different from zero by *t*-test. Several of the clones demonstrated large positive GCA effects for persistence [e.g. WA1101 ( $7.9 \pm 7.0$ )], whereas others demonstrated large negative effects [e.g. WA327 ( $-14.8 \pm 2.8$ )]. Several of the group 4 clones had positive GCA effects for persistence, indicating their potential use in recurrent selection programs for improving persistence determined by abiotic factors.

### Discussion

The research presented in this paper was predicated upon the findings in the US and northern Australia (Lowe *et al.* 2000; Lamb *et al.* 2006) that lucerne yields have plateaued, and that most of the previous gains in forage yield have come from increased levels of disease and pest resistance, not yield *per se*. Lucerne is generally commercialised as broad-based synthetic varieties, this being largely dictated by it being an outbreeder, highly subject to inbreeding depression, and its autotetraploid genetics (Bingham 1998). Possible breeding strategies to help overcome the constraints imposed by its biology include either hybrids, or synthetics based on far fewer parents than are currently being used, providing greater

**Table 4. Estimates of general combining ability (GCA) ( $\pm$  standard errors) for total yield, autumn height, and persistence for 50 lucerne clones from diverse sources**Effects, within attributes, followed by the same letter are not significantly different at  $P=0.05$ 

| Clone  | Total yield        |       | Autumn height  |      | Persistence     |      |
|--------|--------------------|-------|----------------|------|-----------------|------|
|        | GCA                | s.e.  | GCA            | s.e. | GCA             | s.e. |
| WA1101 | 391.6ghikl         | 125   | 3.9bcghij      | 2.8  | 7.9abc          | 7    |
| WA323  | 349.7il            | 69.3  | 3.3bcmDE       | 1.5  | 1.5abc          | 3.9  |
| WA381  | 299.3l             | 27.9  | 4.0bc          | 0.6  | 2.5adgi         | 1.6  |
| D      | 264.9gl            | 22.3  | 7.1j           | 0.5  | -1.6bj          | 1.3  |
| WA334  | 262.7gl            | 28.3  | 3.9bc          | 0.6  | 5.0as           | 1.6  |
| WA329  | 234.5ghil          | 35.9  | 2.6blE         | 0.8  | 4.3agm          | 2    |
| W116   | 219.0ghi           | 21    | 3.2bc          | 0.5  | 4.8a            | 1.2  |
| WA332  | 217.1ghik          | 30.5  | 4.6cF          | 0.7  | 2.3adgij        | 1.7  |
| WA333  | 190.7ghG           | 35.9  | 3.3bc          | 0.8  | 3.0afgij        | 2    |
| WA322  | 183.9ghkCFH        | 39.8  | 2.6clE         | 0.9  | 2.3acghij       | 2.2  |
| WA321  | 171.2hkvFH         | 26.7  | 1.0glmnxD      | 0.6  | 1.1acghij       | 1.5  |
| WA335  | 168.7hkvFH         | 32.5  | 3.9bc          | 0.7  | 4.7agr          | 1.8  |
| WA383  | 166.8hwF           | 38.3  | 2.7bnF         | 0.8  | 0.0bgs          | 2.1  |
| WA325  | 144.9kwFGH         | 26.7  | 1.1gnyDE       | 0.6  | 0.6cfghijr      | 1.5  |
| WA272  | 134.2uvwCEFGH      | 24.6  | 0.9glmnxD      | 0.5  | 2.1adgi         | 1.4  |
| WA326  | 123.4wxFGH         | 29.3  | 1.0gnyDE       | 0.6  | -2.5bh          | 1.6  |
| WA382  | 104.3twBH          | 23.8  | 2.7bw          | 0.5  | -1.3bi          | 1.3  |
| WA265  | 81.2stuwxAB        | 24.6  | 3.1b           | 0.5  | -1.1bcdef       | 1.4  |
| WA258  | 74.2stuxAB         | 27.1  | -1.1eituC      | 0.6  | 2.5adg          | 1.5  |
| WA324  | 66.9stxABEI        | 29    | 1.3gnwyDE      | 0.6  | 3.2agn          | 1.6  |
| WA268  | 52.3pstxABD        | 25.2  | -1.8euB        | 0.6  | 3.8agm          | 1.4  |
| WA262  | 39.5jmpstuvwABC    | 61.5  | 1.7bcghkC      | 1.3  | -1.5abc         | 3.3  |
| WA281  | 33.7mstAB          | 35.6  | 0.9ghklmn      | 0.8  | 2.4aeghij       | 2.1  |
| WA280  | -2.0jmDIJK         | 27.5  | -0.2ght        | 0.6  | 3.6agn          | 1.5  |
| WA327  | -10.7aAJ           | 49.9  | -0.2huD        | 1.1  | -14.8°          | 2.8  |
| WA1119 | -31.8ajm           | 31.5  | -1.0ehk        | 0.7  | 1.6acghij       | 1.8  |
| WA1128 | -32.7ajmp          | 36.2  | -3.9adopq      | 0.8  | 1.8acghij       | 2.1  |
| WA1103 | -35.8ajm           | 28.4  | -0.7ghklm      | 0.6  | -4.6b           | 1.6  |
| W126   | -62.1adj           | 23.8  | -2.6def        | 0.5  | -2.5bc          | 1.3  |
| WA1122 | -68.3abcdejmpstuvw | 117.4 | 1.8bceghv      | 2.6  | 1.3abckl        | 6.4  |
| W1643  | -69.0aozBFK        | 123.2 | 1.0bceghq      | 2.7  | 2.2abcdklpq     | 6.7  |
| WA1104 | -72.7abdjm         | 57.8  | -0.1eghklmn    | 1.3  | 2.2abc          | 3.1  |
| WA1127 | -80.1abdjm         | 56.2  | -1.0efghk      | 1.2  | -3.8bcdefl      | 3.1  |
| WA1130 | -81.7abdejms       | 83.2  | -2.8efkotux    | 1.8  | 2.8abc          | 4.6  |
| WA1120 | -89.7abdejms       | 68.1  | -3.1defstu     | 1.5  | 2.3abc          | 3.7  |
| WA1123 | -92.5abdejmsy      | 85.1  | -1.1defghklmnw | 1.9  | -1.9abckl       | 4.6  |
| WA267  | -121.8dnz          | 27.7  | -1.8ertu       | 0.6  | -0.6bcdefn      | 1.6  |
| WA1124 | -137.8bdez         | 40.7  | -1.3ehkv       | 0.9  | -1.2bcdefmn     | 2.3  |
| WA263  | -141.4abcdejmfs    | 118.6 | -1.3beghp      | 2.6  | -0.3abcdklpq    | 6.4  |
| WA1125 | -147.6abcdejmfs    | 121.8 | -4.0aefktuxy   | 2.7  | -1.0abcklo      | 6.6  |
| WA1135 | -180.6bceno        | 53.8  | -0.5eghkmv     | 1.2  | -11.3loq        | 3.1  |
| WA1107 | -210.2bceno        | 43    | -4.0afopqr     | 0.9  | -2.3bcdef       | 2.4  |
| WA1134 | -217.9abcdefj      | 119.9 | -3.0aeghgz     | 2.6  | 1.8abcklp       | 6.5  |
| WA225  | -221.4abcdefj      | 118.3 | -5.2aetuA      | 2.6  | 1.6abcklpq      | 6.5  |
| W1     | -254.9abcdef       | 119   | -9.5a          | 2.6  | 3.5abc          | 6.6  |
| WA1133 | -299.9cory         | 60.1  | -2.5deftu      | 1.3  | -5.1bceflp      | 3.3  |
| WA1136 | -303.5eor          | 85.7  | 0.4beghv       | 1.9  | 2.0abc          | 4.7  |
| WA1121 | -321.6cfoqr        | 76.4  | -7.1aops       | 1.7  | -2.0abckl       | 4.3  |
| WA1131 | -340.3fqr          | 50.3  | -3.7fpqrzAB    | 1.1  | -12.0kopq       | 2.8  |
| WA1132 | -346.6bcenoq       | 124.7 | 0.1bceghq      | 2.7  | -9.2bcdeflmnopr | 6.9  |

possibilities for making genetic gain for multiple quantitatively inherited traits such as forage yield, and drought tolerance. The hybrid strategy relies on identifying material which when crossed exhibits high SCA, and the latter strategy depends on identifying clones with good GCA. A diverse array of clones, representing dormancy groups 4–9 and encompassing the

hypothetical heterotic groups of dormant and non-dormant germplasm identified by Riday and Brummer (2002) were crossed in an incomplete diallel to investigate the best future approach to lucerne improvement in northern Australia. Their performance was compared with the best commercial and experimental synthetics available for that environment.



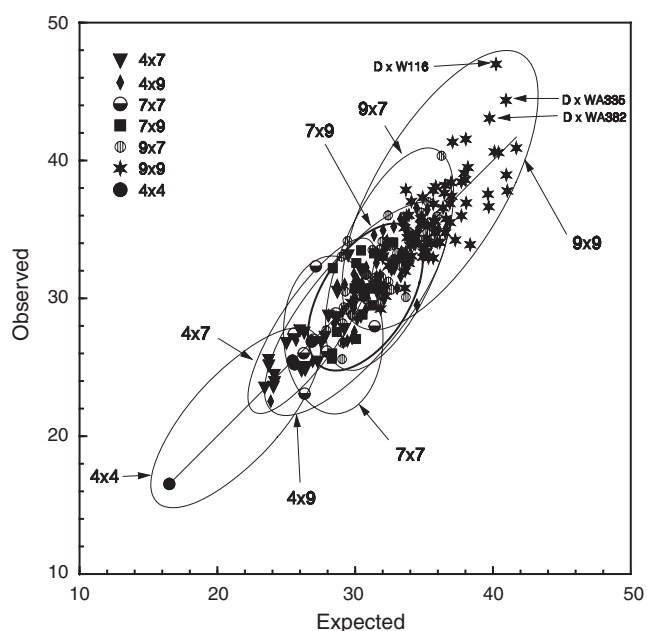


Fig. 2. Observed and expected means derived from parental general combining abilities (GCAs) for natural plant height (cm) recorded 2 weeks after the autumn solstice at Gatton. The line represents where observed and expected values are the same.

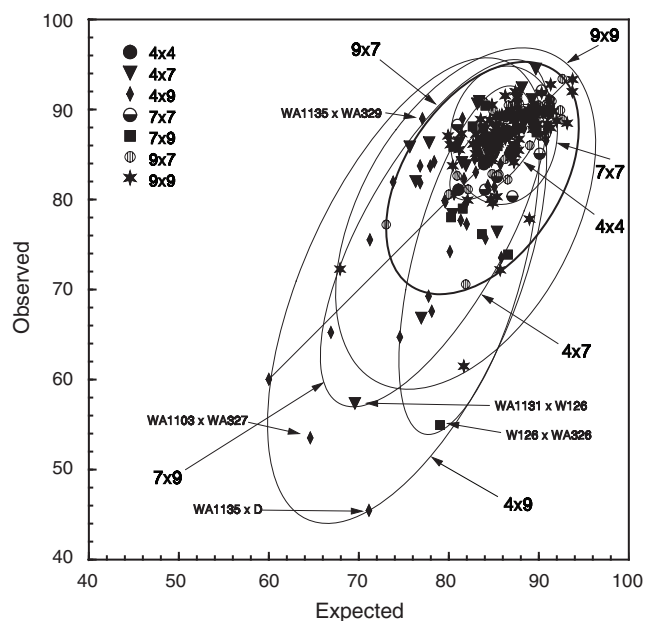


Fig. 3. Observed and expected means derived from parental general combining abilities (GCAs) for persistence (% of the row length remaining) over 15 months at Gatton. The line represents where observed and expected values are the same.

Generally, large and significant SCA effects were not observed, with the best single cross hybrid out-yielding the best commercial synthetic by 13%, and the best experimental synthetic by 8%. The results also clearly showed that in the Gatton environment, yield

was very strongly influenced by winter dormancy levels, with the highest yielding crosses being those between group 9 clones. Studies on the seasonality of yield showed this yield advantage of the non-dormant clones to be independent of season. GCA effects were again more important than SCA effects in defining dormancy levels of populations arising from single crosses between clones from a range of dormancy levels.

Based on the results we obtained, it would appear that future lucerne improvement for northern Australia should concentrate on the development of synthetics, using fewer parents which have undergone some form of progeny testing, and which have been derived from recurrent selection programs particularly where the traits being developed are quantitatively inherited. This is more likely to lead to commercial benefits more expeditiously than a hybrid or semi-hybrid approach (Brummer 1999), based on our findings. If a new source of non-dormant lucerne can be found, which exhibits heterosis with the non-dormant material we tested, then a semi-hybrid strategy could also still lead to significant improvement. Such a source could be material recently introduced from Oman by J. A. G. Irwin, this material being extremely non-dormant and large seeded (unpublished data). Busbice (1969) indicated that synthetics based on 4–16 unrelated  $S_0$  parents should not be adversely affected by inbreeding depression. It is important to maximise the frequency of favourable dominant or partly dominant genes in the  $S_0$  clones being used as parents, and DNA marker technology and recurrent selection have important roles in this process. Use of DNA markers to trace pedigrees of clones arising from recurrent selection programs will facilitate the selection of parent plants which carry desirable alleles, but are unrelated, thus minimising the effects of inbreeding depression. Brummer (1999) indicated the benefits of separately improving diverse populations by recurrent selection and then converging them in the final production of the synthetic or semi-hybrid, thus maximising the chances of increasing the frequency of favourable dominant alleles.

Essential to generating improved synthetics or hybrids of lucerne will be the identification of parental material which expresses differing favourable allele frequencies between the parents being crossed. Riday and Brummer (2002) identified 3 possible heterotic groups where different favourable allele frequencies could be expected, these being *M. sativa* subsp. *falcata*, dormant *M. sativa* subsp. *sativa*, and non-dormant *M. sativa* subsp. *sativa*. Musial *et al.* (2002) have demonstrated, using DNA markers, that subsp. *falcata* contains many different alleles to subsp. *sativa*, and Mackie *et al.* (2005) have demonstrated substantial SCA effects for yield in the subtropical environment from *sativa* × *falcata* crosses, some of which yielded at least as well as the best *sativa* × *sativa* crosses. Within-cultivar genetic diversity for selection-neutral DNA markers is known to be very high (Labombarda *et al.* 2000; Pupilli *et al.* 2000) so it could be expected that even within a single cultivar, very high-performing individuals could be identified, particularly if the cultivar traces to a broad-based parental population, such as exists with cv. Rippa. Rippa was based on 636  $S_0$  plants, with representatives from all known germplasm sources (W. Bunn, Cal/West Seeds, pers. comm.). Several dormant (group 4) subsp. *sativa* clones tracing to improved North American cultivars and exhibiting

cytoplasmic male sterility were tested as possible sources of heterosis in crosses with group 9 clones. Significant SCA effects for yield were generally not observed in this material in a subtropical environment, indicating that considerable recurrent selection would be needed to improve this material to a level where it could positively contribute to lucerne improvement for northern Australia on the proviso that it does contain genes that will contribute to higher yield in this environment.

We do not believe that the forage yield results we obtained have been biased by the differing levels of disease and pest resistance of the individual clones. While there was no *a priori* knowledge of the disease resistance levels of individual clones, except for D, W116, and W126, which have been used in previous mapping studies, the high yields observed for D crosses indicate that prophylactic treatments were effective in managing disease and pests which are endemic in eastern Australia. D is known to be highly susceptible to anthracnose, *Phytophthora* root rot, and spotted alfalfa aphid. Clone W126, which is known to be resistant to all identified *C. trifolii* races, yielded relatively poorly, providing further evidence that the results obtained are representative of the inherent yielding capacity of the material in this environment. Persistence levels for the 15-month duration of the trial were relatively high, and unlikely to have markedly affected yield.

The results presented in this paper showed that in the subtropical Gatton environment, a yield penalty was experienced if cultivars with a dormancy rating less than 9 (non-dormant) were deployed. The yield advantage of non-dormant material appeared to be independent of season, although the greatest advantage came during the winter months. More dormant cultivars would appear to have some positive attributes, however, e.g. persistence and quality traits, which can be introgressed into the non-dormant background by recurrent selection. Large SCA effects for yield were not identified in this work, indicating that the breeding of synthetic varieties, and focusing on identification of  $S_0$  parental clones with good GCA, would appear to offer the best short- to medium-term approach to lucerne breeding to improve yield *per se* in this environment. In our study, which tested clones from the 3 major potential heterotic groups identified by Riday and Brummer (2002) (subsp. *falcata*, and dormant and non-dormant subsp. *sativa*), large SCA effects for yield were not identified, thus making hybrid or semi-hybrid development appear an unviable option for overcoming yield stagnation at least in the short to medium term. The research did identify clones with good GCA for yield *per se*, and it would appear that future work should be directed towards developing more narrow-based synthetics with 4–8 parents which have been selected on the basis of their GCA. This approach would appear to have better prospects for overcoming the yield stagnation than the use of broad-based synthetics which currently predominate.

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