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Relative performance of white clover (*Trifolium repens*) cultivars and experimental synthetics under rotational grazing by beef cattle, dairy cattle and sheep

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ABSTRACT

Assessment of the relative performance of white clover (*Trifolium repens* L.) cultivars, using multi-year and multi-location seasonal growth trials, is key to identification of material with specific and broad adaptation. This paper is based on a multi-year and multi-location study of 56 white clover entries comprising 14 commercial cultivars and 42 experimental synthetic lines evaluated for seasonal growth under rotational grazing across four locations in New Zealand over 4 years. The four locations (and animals grazing) were: Kerikeri (beef cattle), Aorangi (beef cattle), Ruakura (dairy cattle), Lincoln (sheep). Significant ($P < 0.05$) genotypic variation among the 56 entries, and genotype × year, genotype × location and genotype × season interactions, were estimated. We were able to identify cultivars and experimental synthetics with specific and broad adaptation to the three grazing management types. Cvv. AberDance, Apex, Demand, Prestige, Quartz and Riesling, with leaf size ranging from small to medium–large, showed highly above-average performance under sheep grazing. Synthetic lines 15 and 45 also had highly above-average performance under sheep grazing. Cvv. Legacy and Kopu II showed above-average performance under cattle and dairy grazing. Synthetics 15, 48, 49, 44, 22 and 18 and cv. Quartz had above-average performance under all three grazing managements. Synthetics 27, 33 and 38 had highly above-average performance across all three grazing managements and were superior to all 14 cultivars evaluated. Several of these superior synthetics are being tested across multiple grazing environments. Among the 14 cultivars evaluated, Legacy and Quartz showed superior seasonal growth performance across the three grazing managements. Quartz is being evaluated in several on-farm trials across temperate regions of the world.

Keywords: broad adaptation, forage breeding, genotype-by-environment interaction, grazing management, leaf size, multi-location testing, pattern analysis, vegetative persistence.

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Introduction

Pastures based on white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.) form the main feed base for the milk, meat and wool production industries in Australasia. Superior feeding value, high acceptability by stock and fixation of atmospheric nitrogen make white clover a significant perennial forage legume in temperate New Zealand and Australian farming systems. Reviews by Ulyatt (1973) and Thomson (1984) highlight the importance of white clover for improved animal production. The annual contribution of white clover to New Zealand's economy is more than NZ$3 billion, considering its value to the grazing and seed industries, nitrogen fixation and honey production (Caradus *et al.* 1996*b*). In a survey of gross value of production from wool, sheep and beef from pastures with a significant proportion of white clover in the dryland temperate zone of Australia, Jahufer *et al.* (1996) estimated values for the individual states of AU$202 m (New South Wales), $256 m (Victoria), $18 m (Tasmania), $0.6 m (South Australia) and $6 m (Western Australia).

For white clover to enhance pasture production, stable seasonal herbage yield and plant persistence are two important criteria (Gramshaw *et al.* 1989). However, white clover performance in Australasia is constrained by a range of abiotic and biotic factors. Factors such as summer moisture stress, plant parasitic nematodes and clover root weevil (*Sitona lepidus*) (Barbour *et al.* 1996; Eerens *et al.* 2001; Knowles *et al.* 2003; Mercer *et al.* 2008) limit the genetic potential of white clover. Summer moisture stress is considered a major constraint on vegetative persistence and herbage production in temperate dryland environments of Australia (FitzGerald and Clark 1993; Hutchinson 1993). In New Zealand, moisture stress during summer and autumn can deplete the white clover content in pastures (Brock *et al.* 2003; Knowles *et al.* 2003). Intensification of New Zealand farming systems is changing pastoral environments, resulting in less favourable conditions for the traditional clover/ryegrass pastures (Clark *et al.* 2001). Another significant challenge to these pastures is the increasing climatic variability associated with global warming (Newton and Edwards 2007).

Several commercial white clover cultivars are available to farmers across the cattle, dairy and sheep grazing industries (Caradus *et al.* 1996*a*; Woodfield 1999). However, there is a need for continuous genetic improvement of white clover not only to overcome environmental constraints but also to improve production by grazing animals. New livestock breeds with greater genetic potential and nutritional demands require even more productive and reliable perennial pastures (Kemp *et al.* 2010).

Multi-location evaluation of elite breeding lines, together with commercial cultivars, across key target environments is a vital phase in cultivar development programs. This phase enables the identification of superior breeding material with specific or broad adaptation relative to commercial check cultivars, and provides valuable information on the relative performance of these cultivars across environments. We report a study of 56 white clover entries consisting of 14 commercial cultivars and 42 experimental synthetics, evaluated for seasonal growth across four locations in New Zealand for a period of 4 years. We examine relative performance of the cultivars and experimental synthetics at Kerikeri, Ruakura, Aorangi and Lincoln, under beef cattle, dairy cattle and sheep rotational grazing.

Materials and methods

Entries

In total, 56 white clover entries including 14 commercial cultivars and 42 experimental synthetic lines were evaluated. Here, an experimental synthetic is an F_2 developed from an isolated polycross of elite parental genotypes. The commercial cultivars and their leaf sizes (VS, very small; S, small; SM, small–medium; M, medium; ML, medium–large; L, large) were: AberAce (VS), AberDance (M), Bounty (M), Apex (M), Demand (SM), Huia (M), Klondike (L), Kopu II (L), Legacy (L), Prestige (S), Quartz (ML), Riesling (ML), Sustain (ML) and Tribute (ML). Breeding methods and pedigree descriptions of the experimental synthetics are not provided for reasons of intellectual property.

Trial sites

Field trials were established in 2014 at four locations: AgResearch, Lincoln Research Farm in Canterbury; AgResearch, Aorangi Experimental Farm; Ruakura Research Centre; and Plant and Food Research Station, Kerikeri, Northland. Key location characteristics are presented in Table 1.

Field trials

All four locations were planted according to row–column experimental designs (John 1987) with three replicates. The trials at Kerikeri, Ruakura and Aorangi were sown in autumn 2014. At all three locations, seed of perennial ryegrass cv. Ceres150 (AR37) was direct drilled into the experimental area at the rate of 20 kg ha^{-1}. This was followed immediately by hand sowing seed of the white clover entries at 5 kg ha^{-1}. Each of the three locations had

Table 1. Key information on trial locations Lincoln Research Farm, Aorangi Experimental Farm, Ruakura Research Centre, and Plant and Food Research Station, Kerikeri.

Location characteristics	Lincoln (43°38′S, 172°30′E)	Aorangi (40°38′S, 176°19′E)	Ruakura (37°46′S, 175°18′E)	Kerikeri (35°13′S, 173°56′E)
Soil type	Wakanui silt loam soil (Hewitt 1993)	Kairanga silt loam (Cowie 1978)	Horotiu sandy loam soil (Lowe 2010)	Okaihau gravely clay (Taylor and Pohlen 1968)
Long-term mean annual rainfall (mm)	680	970	1072	1775
Long-term av. max. temperature (°C)	17.2	17.8	18.9	19.7
Long-term av. min. temperature (°C)	7.3	8.5	8.7	11.8
Altitude (M)	10.0	17.0	37.0	72.0

plots of size 1 m by 2 m. At Lincoln, the trial was planted in early spring 2014. Ceres150 (AR37) perennial ryegrass was direct drilled together with the white clover entries at the same sowing rates as used at the other three locations. The experimental plots at Lincoln were 1.5 m by 4 m in size. Post-planting hand weeding and herbicide spot spraying were used to control broadleaf weeds and white clover from buried seed. Superphosphate fertiliser (150 kg ha^{-1}) was applied across the trials in late autumn in Years 1 and 3.

Grazing and trait measurements

Rotational grazing was implemented at all four locations. At Kerikeri and Aorangi, the trials were grazed by Angus cattle when the estimated sward dry matter (DM) yield reached 2200–2500 kg ha^{-1}. Grazing was conducted down to sward residuals of ~1200 DM kg ha^{-1}. Low annual nitrogen (N) inputs of 20–50 kg ha^{-1} were applied. At Ruakura, the trial was grazed by Holstein–Friesian dairy cattle when the estimated sward DM yield was ~2500–3000 kg DM ha^{-1}. Post-grazing residuals were ~1500 kg DM ha^{-1}. Under dairy grazing, high annual inputs of N were applied: 50 kg ha^{-1} five times per year, twice in spring (early and late), once in summer (mid to late), once in mid-autumn, and once in winter (mid to late). At Lincoln, grazing was done by Romney ewes. Under sheep grazing, low annual inputs of N were applied: 15–20 kg ha^{-1} two or three times per year, twice in spring (early and late) and once in mid-autumn. At each location, an F400 FARMWORKS Electronic Rising Plate Meter (www.farmworkspfs.co.nz) was used to estimate the quantity of herbage and provide a rough guide on when to graze. The plots were grazed when the estimated sward DM yield was at ~2000–2200 kg ha^{-1} down to a grazing residual of 1100 kg DM ha^{-1}. At each location, the trial was grazed for 2–3 h, season dependent, to ensure rapid and uniform defoliation. After grazing, the animals were immediately removed from the trial area.

Seasonal growth was scored at each location prior to grazing. The score scale ranged from 1 (poor) to 9 (high) (Ford *et al.* 2015). Leaf size was also measured before grazing. Leaf size was scored using a scale of 1 (very small) to 5 (very large) (Ford et al. 2015). Leaf size score data from Ruakura were not included in the analysis owing to error.

Data analyses

Variance component analyses

Seasonal growth score data were analysed: (*i*) on an individual location basis across 4 years, and also across all years, seasons and locations; (*ii*) within each season across all 4 years and locations; (*iii*) within each grazing type (beef cattle, dairy cattle, sheep) and combinations of grazing types (beef cattle/sheep, beef cattle/dairy cattle), across all 4 years; and (*iv*) within Years 2–4 within each individual grazing management type. Leaf size score data were analysed across all seasons, 4 years and three locations (Kerikeri, Aorangi and Lincoln). Variance component analyses were conducted by using the residual maximum likelihood (REML) procedure (Patterson and Thompson 1971, 1975; Harville 1977) in DeltaGen software (Jahufer and Luo 2018; https://deltagen.agresearch.co.nz/app/deltagen). Linear mixed models were used in the analyses, using the REML algorithm. The estimated genotypic means were based on best linear unbiased predictors (BLUPs) (White and Hodge 1989).

The linear mixed model was used for analyses within individual locations across seasons and years:

$$Y_{ijklmn} = M + g_i + y_i + (gy)_{ij} + s_k + (gs)_{ik} + (gsy)_{ijk} + b_{jkl} + r_{jklm} + c_{jkln} + \varepsilon_{ikjl}, \quad (1)$$

where Y_{ijklmn} is the value of an attribute measured from entry i in row m and column n of replicate l nested in season k in year j. Here $i = 1, \ldots, n_g$; $j = 1, \ldots, n_y$; $k = 1, \ldots, n_s$; $l = 1, \ldots, n_b$; $m = 1, \ldots, n_r$; and $n = 1, \ldots, n_c$; where g, y, s, b, r and c are entries, years, seasons, replicates, rows and columns, respectively. M is the overall mean; g_i is the random effect of entry i, $N(0,\sigma_g^2)$; y_j is the fixed effect of year j; $(gy)_{ij}$ is the effect of the interaction between entry i and year j, $N(0,\sigma_{gy}^2)$; s_k is the fixed effect of season k; $(gs)_{ij}$ is the effect of the interaction between entry i and season k, $N(0,\sigma_{gs}^2)$; $(gsy)_{ijk}$ is the effect of the interaction between entry i, season k and year j, $N(0,\sigma_{gy}^2)$; b_{jkl} is the random effect of replicate l within season k in year j, $N(0,\sigma_b^2)$; r_{jklm} is the random effect of row m within replicate l within season k in year j, $N(0,\sigma_r^2)$; c_{jkln} is the random effect of column n within replicate l within season k in year j, $N(0,\sigma_c^2)$; and ε_{ijklmn} is the residual effect of entry i in row m and column n of replicate l during season k in year j, $N(0,\sigma_\varepsilon^2)$.

For the within individual season analysis across locations and years the linear model was similar to Eqn 1, except that s_k, season, was replaced by l_k, location.

The linear mixed model was used for analysis across all years, seasons and locations:

$$Y_{ijklmno} = M + g_i + y_i + (gy)_{ij} + s_k + (gs)_{ik} + l_l + (gl_{il}) + (gsy)_{ijk} + (gly)_{ijl} + (gsl)_{ikl} + b_{jklm} + r_{jklmn} + c_{jklmo} + \varepsilon_{ikjlmno}, \quad (2)$$

where $Y_{ijklmno}$ is the value of an attribute measured from entry i in row n and column o of replicate m nested in location l in season k in year j. Here $i = 1, \ldots, n_g$; $j = 1, \ldots, n_y$; $k = 1, \ldots, n_s$; $l = 1, \ldots, n_l$; $m = 1, \ldots, n_b$; $n = 1, \ldots, n_r$; $o = 1, \ldots, n_c$; where g, y, s, l, b, r and c are entries, years, seasons, locations, replicates, rows and columns, respectively. M is the overall mean; g_i is the random effect of entry i, $N(0,\sigma_g^2)$; y_j is the fixed effect of year j; $(gy)_{ij}$ is the effect of the interaction between entry i and year j, $N(0,\sigma_{gy}^2)$; s_k is the fixed effect of season k; $(gs)_{ij}$ is the effect of the interaction between entry i and season k, $N(0,\sigma^2_{gs})$; l_l is the fixed effect of location l; $(gl)_{il}$ is the effect of the interaction between entry i and location l, $N(0,\sigma^2_{gl})$; $(gsy)_{ijk}$ is the effect of the interaction between entry i, season k and year j, $N(0,\sigma^2_{gsy})$; $(gly)_{ijl}$ is the effect of the interaction between entry i, location l and year j, $N(0,\sigma^2_{gly})$; $(gsl)_{ikl}$ is the effect of the interaction between entry i, season k and location l, $N(0,\sigma^2_{gsl})$; b_{jklm} is the random effect of replicate m in location l within season k in year j, $N(0,\sigma^2_b)$; r_{jkln} is the random effect of row n within replicate m in location l within season k in year j, $N(0,\sigma^2_r)$; c_{jklo} is the random effect of column o within replicate m in location l within season k in year j, $N(0,\sigma^2_c)$; and $\varepsilon_{ijklmno}$ is the residual effect of entry i in row n and column o of replicate m in location l during season k in year j, $N(0,\sigma^2_\varepsilon)$.

The linear mixed model was used for analysis across the three grazing management types, all seasons and years:

$$Y_{ijklmno} = M + g_i + y_i + (gy)_{ij} + s_k + (gs)_{ik} + m_l + (gm_{il}) + (gsy)_{ijk} + b_{iklm} + r_{jklmn} + c_{jklmo} + \varepsilon_{ikjlmno}, \quad (3)$$

where $Y_{ijklmno}$ is the value of an attribute measured from entry i in row n and column o of replicate m nested in grazing management l in season k in year j. Here $i = 1, \ldots, n_g$; $j = 1, \ldots, n_y$; $k = 1, \ldots, n_s$; $l = 1, \ldots, n_l$; $m = 1, \ldots, n_b$; $n = 1, \ldots, n_r$; $o = 1, \ldots, n_c$; where g, y, s, l, b, r and c are entries, years, seasons, grazing management, replicates, rows and columns, respectively. M is the overall mean; g_i is the random effect of entry i, $N(0,\sigma^2_g)$; y_j is the fixed effect of year j; $(gy)_{ij}$ is the effect of the interaction between entry i and year j, $N(0,\sigma^2_{gy})$; s_k is the fixed effect of season k; $(gs)_{ij}$ is the effect of the interaction between entry i and season k, $N(0,\sigma^2_{gs})$; l_l is the fixed effect of grazing management l; $(gl)_{il}$ is the effect of the interaction between entry i and grazing management location l, $N(0,\sigma^2_{gl})$; $(gsy)_{ijk}$ is the effect of the interaction between entry i, season k and year j, $N(0,\sigma^2_{gsy})$; b_{jklm} is the random effect of replicate m in grazing management l within season k in year j, $N(0,\sigma^2_b)$; r_{jkln} is the random effect of row n within replicate m in grazing management l within season k in year j, $N(0,\sigma^2_r)$; c_{jklo} is the random effect of column o within replicate m in grazing management l within season k in year j, $N(0,\sigma^2_c)$; and $\varepsilon_{ijklmno}$ is the residual effect of entry i in row n and column o of replicate m in grazing management l during season k in year j, $N(0,\sigma^2_\varepsilon)$.

The linear models used in the analysis of entry performance within the beef cattle, dairy cattle and sheep grazing type managements, and across the beef cattle/sheep and beef cattle/dairy cattle management combinations, across locations and years, were a reduced form of Eqn 3.

Pattern analysis

Pattern analysis, a combination of cluster analysis and principal component analysis, was conducted by using DeltaGen to provide graphical summary of the performance of the 56 white clover entry-by-growth BLUP matrices generated from the variance component analyses: within individual locations; within individual seasons; within individual years; across all years, seasons and locations; grazing management type. Each of the entry-by-growth BLUP matrices, from the different variance component analyses, was summarised by using cluster analysis to generate cultivar groups. This was followed by principal component analysis (ordination) of the same white clover entry-by-growth BLUP matrices to generate biplots. In DeltaGen, each of the entry groups identified from clustering is assigned a different colour and superimposed on the biplot. This resulted in a graphical summary of information within each of the entry-by-growth BLUP matrices.

In DeltaGen, cluster analysis is performed using a hierarchical agglomerative classification procedure with squared Euclidean distance as a measure of dissimilarity (Burr 1968, 1970; Wishart 1969) and the Hartigan clustering algorithm (Hartigan 1975) is used as the grouping strategy. Principal component analysis was conducted according to Jolliffe (2002). Before conducting cluster analysis, the data were standardised to remove scaling effects (Cooper and DeLacy 1994), using the 'Standardisation' option in DeltaGen.

Results

Leaf size and mean seasonal growth

There was significant ($P < 0.05$) genotypic variation among the white clover entries for mean leaf size across all seasons, years and locations Kerikeri, Aorangi and Lincoln (Table 2). Genotype × location interaction for leaf size was also significant ($P < 0.05$). There was significant ($P < 0.05$) genotypic variation for mean seasonal growth across 4 years among the white clover entries at each of the locations Kerikeri, Ruakura, Aorangi and Lincoln (Table 2). Genotype × year interactions were significant ($P < 0.05$) at all locations. Significant ($P < 0.05$) genotype × season interaction was estimated only at Ruakura and Aorangi. There was significant ($P < 0.05$) genotype × season × year interaction at Lincoln. These interactions indicated that there was a change in the relative performance among the white clover entries across seasons and years, depending on the location. Analysis of mean seasonal growth of the 56 entries across 4 years and four locations indicated significant ($P < 0.05$) genotypic variation among them. The interactions of the entries with years, locations and seasons were all significant ($P < 0.05$).

Table 2. Variance components and associated standard errors estimated from analysis of the traits mean leaf size (1, very small; 5, very large) across all seasons, 4 years and three locations, and mean seasonal growth (1, poor; 9 high) evaluated at/across the four locations and across 4 years, of the 56 white clovers entries.

Source of variation	Mean leaf size across seasons, 4 years and three locations[A]	Mean seasonal growth across 4 years at:				
		Kerikeri	Ruakura	Aorangi	Lincoln	Four locations
σ^2_g	0.169 ± 0.038	0.282 ± 0.070	2.013 ± 0.399	1.275 ± 0.263	0.514 ± 0.113	0.713 ± 0.142
σ^2_{gy}	0	0.183 ± 0.040	0.404 ± 0.066	0.273 ± 0.041	0.360 ± 0.056	0.018 ± 0.018
σ^2_{gl}	0.063 ± 0.017	–	–	–	–	0.287 ± 0.045
σ^2_{gs}	0	0	0.039 ± 0.025	0.088 ± 0.020	0	0.052 ± 0.009
σ^2_{gly}	0.022 ± 0.011	–	–	–	–	0.299 ± 0.029
σ^2_{gsl}	0	–	–	–	–	0
σ^2_{gsy}	0	0	0	0	0.130 ± 0.032	0
σ^2_ε	0.408 ± 0.016	1.544 ± 0.053	2.070 ± 0.062	1.456 ± 0.034	1.427 ± 0.041	1.610 ± 0.022

Genotypic (σ^2_g), genotype × year interaction (σ^2_{gy}), genotype × location interaction (σ^2_{gl}), genotype × season interaction (σ^2_{gs}), genotype × location × year interaction (σ^2_{gly}), genotype × season × location interaction (σ^2_{gsl}), genotype × season × year interaction (σ^2_{gsy}) and pooled error (σ^2_ε) variance components.

[A]Leaf size data for the location Ruakura and cv. AberDance not included in the analysis owing to error.

The range of mean leaf size scores, sorted from small to large, based on evaluation of the 56 white clover entries across 4 years and three locations is presented in Fig. 1; the means of only 55 entries are shown, with cv. AberDance excluded owing to error. Cvv. Huia, Prestige, Bounty, AberAce, Demand and Tribute showed leaf sizes at the lower end of the score scale (i.e. small). Cvv. Apex, Sustain, Klondike and Riesling showed small–medium to medium leaf size. Cvv. Kopu II, Quartz and Legacy had leaf sizes within the medium–large range of the score scale. Cv. Legacy had the largest leaf size among the 13 cultivars. There was a range of leaf sizes among the experimental synthetics from small (51, 10, 52, 56, 1, 4) to large (33, 37, 36).

Cluster analysis of the 56 white clover entry-by-multi-location growth BLUP matrix generated four entry groups (Fig. 2). Groups 1 and 2 had above-average performance across the four locations Lincoln, Aorangi, Ruakura and Kerikeri. Group 1 had a higher average performance than Group 2, and consisted of 11 experimental synthetics (8, 23, 25, 26, 27, 28, 33, 35, 36, 37, 38) and large-leaf cv. Legacy. Synthetics 27, 35, 37 and Legacy showed broad adaptation across the four locations. In Group 1, whereas

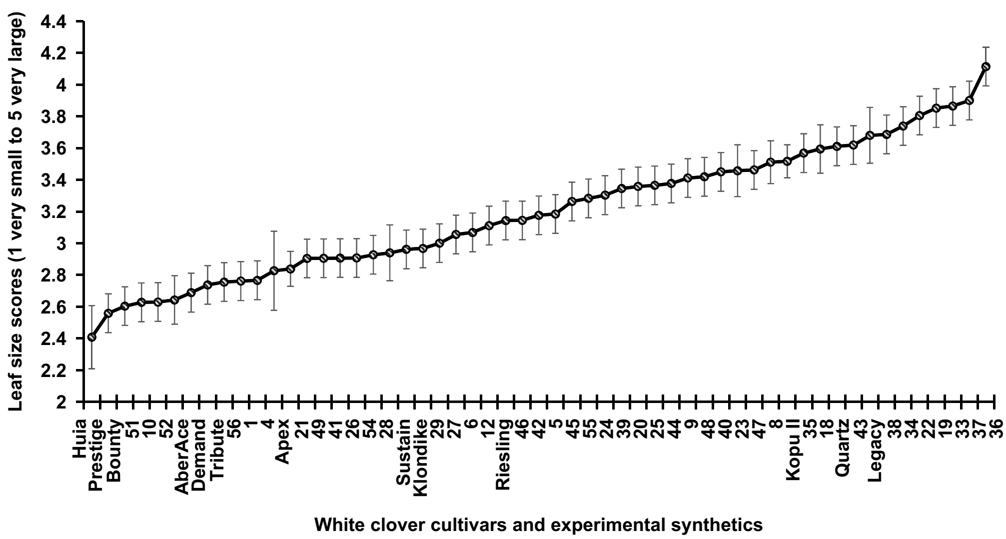

Fig. 1. Average leaf size best linear unbiased predictor values and associated standard errors (vertical lines) of 55 white clover entries evaluated across 4 years and three locations (Kerikeri, Aorangi, Lincoln). The entries are sorted from small to large leaf size; cv. AberDance excluded owing to data error.

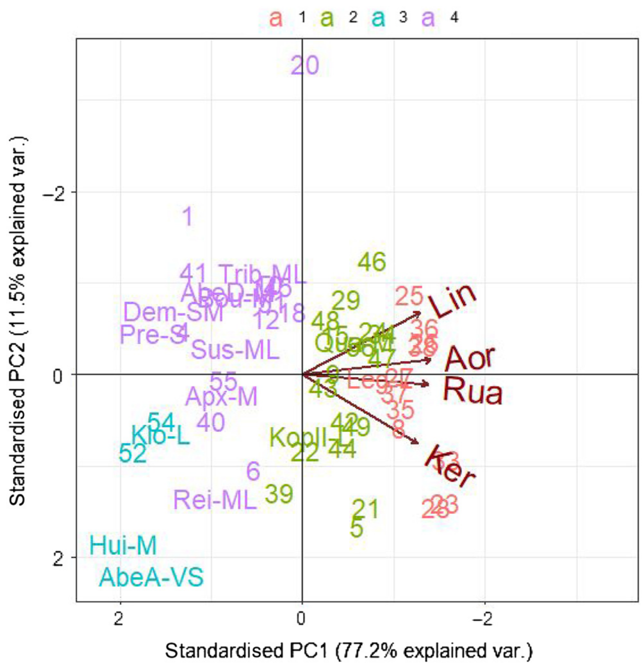

Fig. 2. Biplot based on standardised best linear unbiased predictor values of mean growth across seasons and years of the 56 white clover entries (14 cultivars and 42 experimental synthetics) evaluated at each site (indicated by the directional vectors): Ker, Kerikeri (beef cattle grazed); Aor, Aorangi (beef cattle grazed); Rua, Ruakura (dairy cattle grazed); Lin, Lincoln (sheep grazed). The different groups (1–4) are indicated by colours. Leaf sizes: VS, very small; SM, small–medium; M, medium; ML, medium–large; L, large. Cultivars (with leaf size): beA-VS, AberAce; AbeD-M, AberDance; Apx-M, Apex; Bou-M, Bounty; Dem-SM, Demand; Hui-M, Huia; Klo-L, Klondike; KopII-L, Kopu II; Leg-L, Legacy; Pre-S, Prestige; Qua-ML, Quartz; Rei-ML, Riesling; Sus-ML, Sustain; Trib-ML, Tribute.

synthetics 25 and 36 showed specific adaptation to Lincoln, synthetics 33, 23 and 28 had specific adaptation to Kerikeri.

Group 2 consisted of 17 experimental synthetics and two cultivars, Quartz and Kopu II. Quartz showed specific adaptation to the sheep-grazed location, Lincoln. This cultivar also had above-average performance across all four locations. Kopu II showed above-average performance across Kerikeri, Ruakura and Aorangi. Its performance under sheep grazing at Lincoln was below average. Based on the association among the directional vectors, location pairs Kerikeri and Ruakura, Ruakura and Aorangi, and Aorangi and Lincoln showed positive correlation (angle between the vectors <90°). There was a weak positive association between Kerikeri and Lincoln. Note that these relationships among the four locations are based on mean seasonal growth of the 56 white clover entries across the 4 years.

Growth within individual seasons across locations

Variance component analysis across locations and years within each season indicated significant ($P < 0.05$) genotypic variation among the 56 white clover entries for summer, autumn, winter and spring growth (Table 3). There was significant ($P < 0.05$) genotype × location interaction within each season. There was also significant ($P < 0.05$) genotype × location × year interaction within the summer, autumn, winter and spring seasons. There was no significant ($P > 0.05$) genotype × year interaction.

Table 3. Variance components and associated standard errors estimated from the analysis of seasonal growth scores (1, poor; 9 high) of the 56 white clover entries within individual seasons across the four locations across 4 years.

Source of variation	Mean summer growth	Mean autumn growth	Mean winter growth	Mean spring growth
σ_g^2	0.613 ± 0.131	0.932 ± 0.189	1.082 ± 0.202	0.655 ± 0.133
σ_{gy}^2	0.045 ± 0.032	0	0	0.051 ± 0.026
σ_{gl}^2	0.288 ± 0.056	0.358 ± 0.067	0.186 ± 0.054	0.227 ± 0.045
σ_{gly}^2	0.325 ± 0.049	0.110 ± 0.048	0.326 ± 0.052	0.170 ± 0.041
σ_ε^2	1.533 ± 0.040	1.437 ± 0.063	1.549 ± 0.052	1.849 ± 0.045

Genotypic (σ_g^2), genotype × year interaction (σ_{gy}^2), genotype × location interaction (σ_{gl}^2), genotype × location × year interaction (σ_{gly}^2) and pooled error (σ_ε^2) variance components.

Fig. 3 provides a graphical summary of mean individual seasonal growth of the 56 white clover entries across the four locations and 4 years. The directional vectors indicate a positive association among all four seasons (angle between vectors <90°); in particular, spring and autumn showed the 56 entries having a similar average performance across the four locations and 4 years. Cluster analysis of the 56 entry-by-four seasons growth BLUP matrix generated four entry groups. Group 1, which had above-average seasonal growth, consisted of 21 experimental synthetics and cvv. Quartz and Legacy. Some members, including Quartz, showed broad seasonal adaptation. Synthetics 23, 24, 28, 34 and 38 had highly above-average winter growth; and synthetics 20, 29, 33 and 56 showed highly above-average summer growth. Whereas cv. Quartz showed above-average performance across all seasons, cv. Legacy showed highly above-average summer seasonal performance. Cv. Kopu II, a member of Group 2, also showed above-average growth in summer, but had below-average winter growth.

Grazing type and seasonal growth

Analysis of mean seasonal growth across all years, seasons and locations within each grazing type and combinations of dairy cattle/sheep and beef cattle/dairy cattle indicated significant ($P < 0.05$) genotypic variation among the 56 entries (Table 4). There was significant ($P < 0.05$) genotype × year interaction estimated for all the grazing types and grazing combinations. Although there was significant ($P < 0.05$) genotype × grazing type (dairy cattle/sheep and beef cattle/dairy cattle)

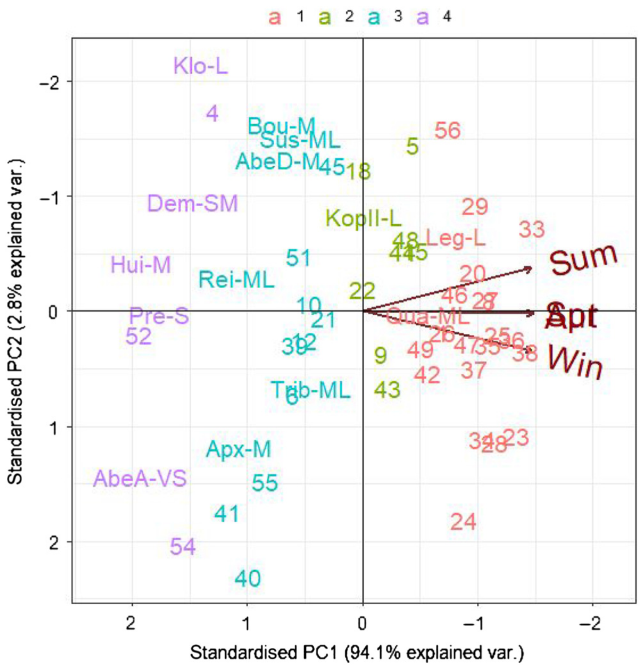

Fig. 3. Biplot based on standardised best linear unbiased predictor values of 56 white clover entries (14 cultivars and 42 experimental synthetics), based on within individual season growth across the four locations. The directional vectors indicate the seasons: Sum, summer; Aut, autumn; Spr, spring; Win, winter. The different groups (1–4) are indicated by colours. Leaf sizes: VS, very small; SM, small–medium; M, medium; ML, medium–large; L, large. Cultivars (with leaf size): AbeA-VS, AberAce; AbeD-M, AberDance; Apx-M, Apex; Bou-M, Bounty; Dem-SM, Demand; Hui-M, Huia; Klo-L, Klondike; KopII-L, Kopu II; Leg-L, Legacy; Pre-S, Prestige; Qua-ML, Quartz; Rei-ML, Riesling; Sus-ML, Sustain; Trib-ML, Tribute.

interaction, other contributors to this variance component could also be location-specific, abiotic and biotic effects.

Variance component analysis of mean seasonal growth in Years 2–4 within each of the grazing type managements indicated significant ($P < 0.05$) genotypic variation among the 56 white clover entries (Table 5). For the cattle grazing type management at two locations Kerikeri and Aorangi, there was significant ($P < 0.05$) genotype $\times$ location interaction in Years 2–4.

Performance of the 56 white clover entries under beef cattle, dairy cattle and sheep grazing, based on mean seasonal growth in individual Years 2, 3 and 4 of the multi-location trials is graphically summarised in the biplots (Fig. 4*a*–*c*). Cluster analysis of the entry-by-mean individual year growth score BLUPs, estimated from the beef cattle grazed data collected across two locations, Kerikeri and Aorangi, generated three entry groups (Fig. 4*a*). Group 1 with above-average performance across all years had 27 members and included cvv. Quartz and Legacy. Synthetics 23, 26, 29 and 33 showed highly above-average performance across the three years. Quartz showed above-average performance, especially in Year 4. Legacy had above-average in Years 2 and 3 but was average in Year 4. Cv. Kopu II, in Group 2, showed above-average performance in Year 4. There were positive correlations between the years; however, Years 2 and 3 had the closest association.

Under dairy cattle grazing (Fig. 4*b*), cluster analysis revealed six groups. Group 6, the highly above-average group, consisted of 10 experimental synthetics of which 33, 35, 36 and 42, along with cv. Legacy, had highly above-average performance across all years. Group 5, showing above-average performance, consisted of 14 experimental synthetics and cvv. Sustain, Quartz and Kopu II. Of the Group 5 cultivars, Kopu II had slightly above-average performance across Years 2–4, and Quartz, with a higher performance in Years 2 and 3, had lower performance in Year 4. Experimental synthetics

Table 4. Variance components and associated standard errors estimated from the analysis of mean seasonal growth scores (1, poor; 9, high) of the 56 white clover entries within individual grazing type management and dairy cattle/sheep, beef cattle/dairy cattle combinations across all seasons and years.

Source of variation	Mean seasonal growth across 4 years					
	Under cattle grazing	Under dairy grazing[A]	Under sheep grazing[A]	Under dairy cattle and sheep grazing	Under beef cattle and dairy grazing	Across all three grazing types
σ^2_g	0.502 $\pm$ 0.138	2.013 $\pm$ 0.399	0.514 $\pm$ 0.113	0.573 $\pm$ 0.121	0.992 $\pm$ 0.226	0.823 $\pm$ 0.165
σ^2_{gy}	0.147 $\pm$ 0.023	0.404 $\pm$ 0.066	0.360 $\pm$ 0.056	0.150 $\pm$ 0.023	0.145 $\pm$ 0.021	0.125 $\pm$ 0.018
σ^2_{gl}	0.297 $\pm$ 0.068	–	–	–	–	–
σ^2_{gs}	0.040 $\pm$ 0.011	0.039 $\pm$ 0.025	0	0.041 $\pm$ 0.009	0.046 $\pm$ 0.011	0.047 $\pm$ 0.009
σ^2_{gsy}	0	0	0.130 $\pm$ 0.032	0	0	0
σ^2_{gt}	–	–	–	0.142 $\pm$ 0.032	0.403 $\pm$ 0.081	0.306 $\pm$ 0.045
σ^2_{ε}	1.536 $\pm$ 0.029	2.070 $\pm$ 0.062	1.427 $\pm$ 0.041	1.739 $\pm$ 0.026	1.909 $\pm$ 0.029	2.146 $\pm$ 0.027

Genotypic (σ^2_g), genotype $\times$ year interaction (σ^2_{gy}), genotype $\times$ location interaction (σ^2_{gl}), genotype $\times$ season interaction (σ^2_{gs}), genotype $\times$ season $\times$ year interaction (σ^2_{gsy}), genotype $\times$ grazing type interaction (σ^2_{gt}), and pooled error (σ^2_{ε}) variance components.
[A]Only one location.

Table 5. Variance components and associated standard errors estimated from the analysis of mean growth scores (1, poor; 9, high) of the 56 white clover entries within individual grazing type management in Years 2–4.

Source of variation	Mean seasonal growth under beef cattle grazing			Mean seasonal growth under dairy cattle grazing[A]			Mean seasonal growth under sheep grazing[A]		
	Year 2	Year 3	Year 4	Year 2	Year 3	Year 4	Year 2	Year 3	Year 4
σ^2_g	0.503±0.16	0.731 ± 0.214	0.416 ± 0.206	2.088 ± 0.410	3.147 ± 0.645	3.033 ± 0.632	0.409 ± 0.128	0.327 ± 0.097	0.203 ± 0.079
σ^2_{gl}	0.49 ± 0.11	0.565 ± 0.135	0.921 ± 0.270	–	–	–	–	–	–
σ^2_{gs}	0	0	0	0	0	0	0.263 ± 0.109	0.007 ± 0.133	0
σ^2_ε	1.75 ± 0.055	1.273 ± 0.047	1.739 ± 0.120	2.272 ± 0.055	2.178 ± 0.178	2.003 ± 0.159	2.900 ± 0.145	2.273 ± 0.160	1.755 ± 0.117

Genotypic (σ^2_g), genotype × location interaction(σ^2_{gl}), genotype × season interaction (σ^2_{gs}) and pooled error (σ^2_ε) variance components and their associated standard errors (±s.e.).
[A]Only one location.

8, 36, 37 and 45 from Group 6 and 9, 20 and 21 from Group 5 showed highly above-average performance in Year 4.

Under sheep grazing (Fig. 4*c*), three entry groups were observed from cluster analysis. Group 1 consisted of 19 experimental synthetics and seven cultivars: AberDance, Apex, Demand, Legacy, Prestige, Quartz and Riesling. This group comprised entries that had highly above-average performance across all three years, and entries with high performance specific to Years 3 and 4. Synthetics 15, 23, 33 and 48 had highly above-average performance across all years. Cv. Quartz had the highest above-average performance across all years. Cv. AberDance also had highly above-average performance across years but lower than Quartz. Cvv. Apex, Demand, Legacy, Prestige and Riesling showed above- to highly above-average performance in Years 3 and 4. There was a slight negative correlation (angle between the vectors >90°) between Year 2 and Years 3 and 4, whereas Years 3 and 4 were highly positively correlated (angle between the vectors <90°).

The combination of cluster and principal component analysis of the 56 white clover entry-by-seasonal growth across all years BLUP matrix within each grazing management type generated a graphical summary (Fig. 5). There was a strong similarity in performance of the 56 entries under beef cattle and dairy cattle grazing, as indicated by the close association (angle between the vectors <90°) between the two directional vectors Dairy and Cattle (Fig. 5). As indicated by the directional vectors, response of the 56 entries to sheep grazing was different to that under dairy and cattle grazing. Cluster analysis revealed five entry groups. Group 1 consisted of five small to medium–large leaf cultivars, Prestige (S), Demand (SM), AberDance (M), Apex (M) and Riesling (ML), and experimental synthetic 54. This group of entries showed highly above-average performance specific to sheep grazing. Group 5 consisted of eight synthetics and medium–large leaf cv. Quartz. In Group 5, synthetics 18, 22, 39, 44, 48 and 49 had above-average performance under sheep grazing, whereas Quartz and synthetics 15 and 45 showed highly above-average performance. Cv. Quartz and synthetics 15, 48, 49, 44, 22 and 18, also had above-average performance under dairy and cattle grazing. All members of Group 4 had highly above-average performance under both dairy and cattle grazing management. Experimental synthetics 27, 33, and 38 showed highly above-average performance under all three grazing managements. The members of Group 3 showed specific adaptation to both dairy and cattle grazing management. This varied from above-average to highly above-average performance. The large-leaf cv. Kopu II had above-average performance. Cv. Bounty, a member of Group 2, showed average performance under sheep grazing and below-average performance under both cattle and dairy grazing management.

Discussion

Analysis of the performance of the 56 white clover entries consisting of 42 experimental synthetics and 14 cultivars, evaluated for seasonal growth across four locations over 4 years, showed significant genotype × environment interaction at different levels: genotype × year interaction (σ^2_{gy}), genotype × location interaction (σ^2_{gl}), genotype × season interaction (σ^2_{gs}), genotype × location × year interaction (σ^2_{gly}) and genotype × season × year interaction (σ^2_{gsy}), depending on the season, location and year combinations. There was also significant genotype × location interaction estimated for leaf size. These results clearly emphasise the importance of conducting multi-year–season–location evaluation trials in white clover breeding programs, especially under different grazing management types, as reported in this study: beef cattle, dairy cattle and sheep, under rotational grazing. Trials across multiple locations, years and seasons are especially important when selecting for broad adaptation (Cooper and DeLacy 1994; Cooper and Byth 1996). There have been several reports on genotype × environment interaction for a range of traits in white clover. Jahufer *et al.* (2013) reported significant genotype × year interaction for mean seasonal yield in white clover. Jahufer

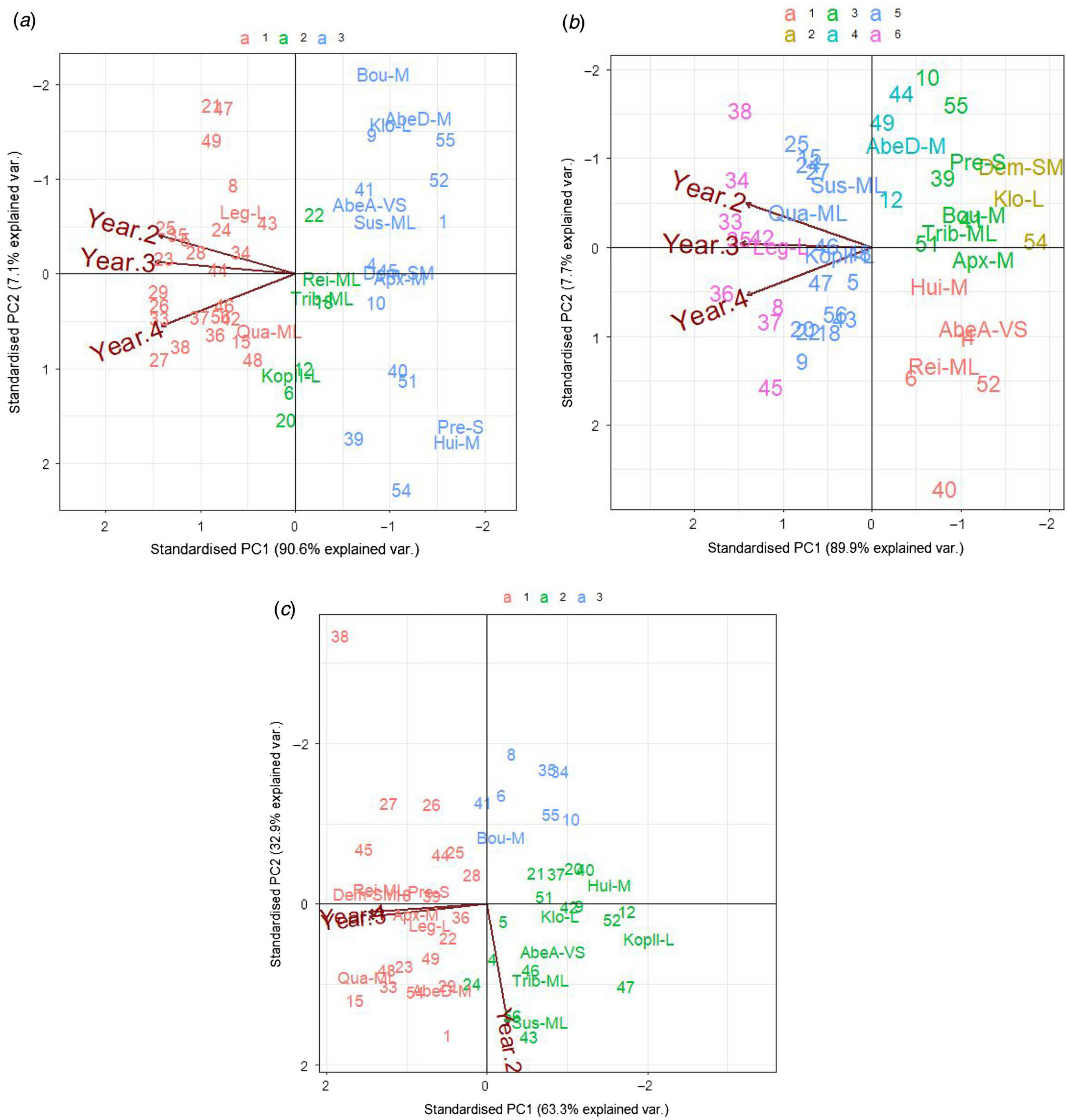

Fig. 4. Biplots based on standardised best linear unbiased predictor values of 56 white clover entries (14 cultivars and 42 experimental synthetics) based on mean seasonal growth within individual Years 2–4 (indicated by the directional vectors) under: (*a*) beef cattle grazing (across Kerikeri and Aorangi), (*b*) dairy cattle grazing (Ruakura), and (*c*) sheep grazing (Lincoln). The different groups (1–3, 1–6) are indicated by colours. Leaf sizes: VS, very small; SM, small–medium; M, medium; ML, medium–large; L, large. Cultivars (with leaf size): AbeA-VS, AberAce; AbeD-M, AberDance; Apx-M, Apex; Bou-M, Bounty; Dem-SM, Demand; Hui-M, Huia; Klo-L, Klondike; KopII-L, Kopu II; Leg-L, Legacy; Pre-S, Prestige; Qua-ML, Quartz; Rei-ML, Riesling; Sus-ML, Sustain; Trib-ML, Tribute.

et al. (1999) reported significant full-sib family × environment × year interaction for a range of morphological traits including stolon density, stolon branching, number of nodes, rooted node number and summer herbage yield among 80 white clover full-sibs. Significant genotype × environment interactions for a range of white clover

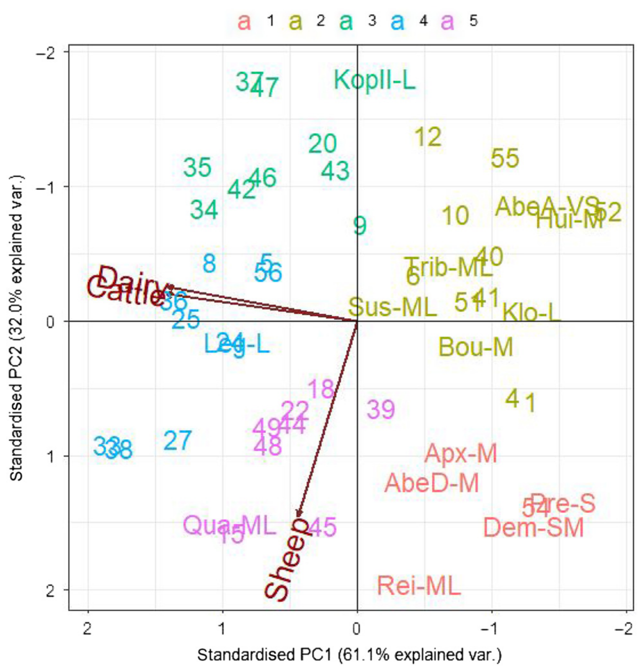

Fig. 5. Biplot based on standardised best linear unbiased predictor values of the 56 white clover entries (14 cultivars and 42 experimental synthetics), based on mean seasonal growth across years within each grazing management type Cattle, Dairy and Sheep, indicated by the directional vectors. The different groups (1–5) are indicated by colours. Leaf sizes: VS, very small; SM, small–medium; M, medium; ML, medium–large; L, large. Cultivars (with leaf size): AbeA-VS, AberAce; AbeD-M, AberDance; Apx-M, Apex; Bou-M, Bounty; Dem-SM, Demand; Hui-M, Huia; Klo-L, Klondike; KopII-L, Kopu II; Leg-L, Legacy; Pre-S, Prestige; Qua-ML, Quartz; Rei-ML, Riesling; Sus-ML, Sustain; Trib-ML, Tribute.

morphological traits such as internode length, leaf size, plant height, stolon density and dry matter yield have also been reported (Jahufer *et al.* 2009, 2016; Annicchiarico and Piano 2000; Finne *et al.* 2000). Significant line × year interactions for the proportion of clover in sward, clover dry weight and leaf size was estimated from evaluation of elite breeding lines in two trials rotationally grazed, one by sheep and the other by cattle, across 3 years (Caradus 1993).

Leaf size is commonly used to classify white clover cultivars (Frame and Laidlaw 2005) and is also highly related to grazing system fit (i.e. beef/dairy cattle or sheep). Small leaf types are considered suitable for set-stocked sheep grazing, medium types for use under rotational sheep grazing, and medium–large to very large types for rotational beef cattle grazing (Abberton and Marshall 2005). Leaf size provides an indirect assessment of white clover plant type because of its positive and negative correlation with morphological traits associated with growth and vegetative persistence. For example, leaf size has a positive correlation with plant height and stolon diameter, and a negative correlation with stolon number (Jahufer *et al.* 1994; Abberton and Marshall 2005). Small leaf types are positively correlated with high stolon densities, associated with vegetative persistence (Caradus and Williams 1981; Piano and Annicchiarico 1995).

In our study, the mean leaf size scores measured from 13 cultivars across years and locations did not generally reflect those reported in the literature (Woodfield *et al.* 2003; Widdup *et al.* 2015; Gerard *et al.* 2017). For example, results from our experiment assigned cvv. Huia (medium leaf size), Bounty (medium leaf size), AberAce (medium leaf size), Demand (small–medium leaf size) and Tribute (medium leaf size) within the small leaf size range. Cv. Kopu II, considered as a large leaf type, scored as medium–large in our trials. This deviation of leaf size may be attributed to the plasticity of white clover under grazing (Caradus 1993; Brock and Hay 1996, 2001). The significant genotype × location and genotype × location × year interactions estimated for leaf size in our study further support these observations. There was a range of leaf sizes among the experimental synthetics, from small to large. Synthetics 51, 10 and 52 were in the small leaf category, and 33, 37 and 36 were in the large leaf category. This information is an important criterion for identifying experimental synthetics suitable for cattle, dairy and sheep grazing management systems.

Pattern analysis provided graphical summarises on performance of the 56 entries based on seasonal growth: (*i*) across the four locations Kerikeri, Ruakura, Aorangi and Lincoln; (*ii*) within seasons spring, summer, autumn and winter; (*iii*) during Years 2–4 within each grazing type; and (*iv*) across seasons and years within grazing type, beef cattle, dairy cattle and sheep, all under rotational grazing management. The biplot summarising mean seasonal growth of the 42 synthetics and 14 cultivars across the four locations clearly indicated broad adaptation of the synthetics 27, 35 and 37 and cv. Legacy. Cv. Quartz showed above-average specific adaptation at the sheep-grazed location, Lincoln. This cultivar also showed slightly above-average performance at Aorangi, Ruakura and Kerikeri. Cv. Kopu II showed broad adaptation across the beef cattle and dairy cattle grazed locations. Experimental synthetics 8, 27, 35 and 37, and cv. Legacy, showed superior broad adaptation across all three types of grazing management compared with cvv. Kopu II and Quartz.

Several experimental synthetics showed broad adaptation across all four seasons and were superior to cv. Quartz, which had above-average adaptation. Synthetics such as 23, 24, 28, 34 and 38 showed highly above-average winter growth. Synthetics 20, 29, 33 and 56, showed highly above-average summer growth. Compared with cv. Quartz, Kopu II showed low above-average growth in summer, spring and autumn. Kopu II had below-average winter growth. Cv. Legacy had highly above-average summer growth.

The perennial behaviour of white clover, an important selection criterion in cultivar development, is attributed to continuous vegetative growth of stolons (Westbrooks and Tesar 1955; Caradus and Williams 1989). During the first

12 months of growth, the white clover plant loses its primary seedling taproot and depends on nodal roots that support future stolon growth (Westbrooks and Tesar 1955). These significant changes in plant development make Year 1 performance an unreliable predictor of future vegetative persistence, and therefore, it was not included in the assessment of vegetative persistence across the 4 years in our study. Only years 2–4 were considered. Performance of the 56 white clover entries in Year 4 was of particular importance because it provided an assessment of overall vegetative persistence across the 4 years of evaluation within each grazing management type. Several experimental synthetics had above-average performance in Year 4 under the three grazing management types. Experimental synthetic 33 (large leaf size) showed superior performance in Year 4 under all three grazing managements. Synthetics 27 and 38, both with medium–large leaf size, had above-average performance in Year 4 under beef cattle and sheep grazing.

Our investigation enabled identification of cultivars and experimental synthetics with specific and broad adaptation to the three grazing management types. The small to medium–large leaf cultivars, AberDance (M), Apex (M), Demand (SM), Prestige (S) and Riesling (ML), showed highly above-average performance under sheep grazing. Some of these small to medium leaf cultivars with high stolon densities have been developed for sheep grazing (Caradus and Woodfield 1997; Woodfield et al. 2001). Quartz and synthetics 15 and 45 had highly above-average performance under sheep grazing. Synthetics 15, 48, 49, 44, 22 and 18 and Quartz also had above-average performance under all three grazing management types. Cvv. Legacy and Kopu II showed above-average performance under cattle and dairy grazing. There were also several synthetics with highly above-average performance under dairy and cattle grazing: 8, 25, 34, 35, 36, 37, 42, 47. Synthetics 27, 33 and 38 had highly above-average performance across all three grazing managements and were superior to the 14 cultivars evaluated. Some of these superior synthetics are currently being tested across multiple grazing environments to assess their merit.

Considering seasonal growth response of the 14 white clover cultivars evaluated in our study, Quartz and Legacy showed the best performance under all three grazing management types. However, Legacy showed better response to cattle and dairy grazing, whereas Quartz showed superior performance under sheep grazing and evidence of broad adaptation. Legacy and Quartz were superior to the other 12 cultivars evaluated: AberAce, AberDance, Apex, Bounty, Demand, Huia, Klondike, Kopu II, Prestige,Riesling, Sustain, Tribute.

Quartz was developed by combining material from the breeding populations of cvv. Saracen, Trophy and Tribute, with persistent ecotypes selected from dairy farms in the Waikato region of New Zealand that had known history of pasture renewal for >70 years. Cvv. Saracen and Trophy were developed from elite drought-tolerant breeding lines generated from a successful New Zealand/Australia trans-Tasman collaboration (Ayres et al. 2007; Jahufer et al. 2012). The development of cv. Quartz was based on combining a diverse range of genetic material to generate a cultivar with adaptation across New Zealand grazing systems. Results from our multi-location and multi-year field study under beef cattle, dairy cattle and sheep grazing have demonstrated the merit of Quartz for broad adaptation under New Zealand conditions. This cultivar is currently being evaluated in several on-farm trials across temperate regions of the world.

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