Irish Journal of Agricultural and Food Research **48:** 227–242, 2009

Influence of testing procedure on evaluation of white clover (*Trifolium repens* L.)

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This study examined data sets derived from the white clover cultivar evaluation programmes of AFBI (N. Ireland), and DAFF (Republic of Ireland) to determine whether elite performing genotypes are identifiable, independent of test procedure and leaf size factors. Genetic variation in yield and persistency, independent of the leaf size continuum effect, was observed. Identification of elite cultivars by breeders or testers therefore required readjustment of assessment standards to account for the mostly curvilinear relationships between performance and leaf size. The different testing procedures, involving cutting or grazing at different heights, frequencies and nitrogen rates changed the relative performances between the cultivars, making it difficult to predict performance potential beyond specific test conditions. The underlying causes for these changes in rankings was considered, including sensitivity to season and location, the antagonistic affects of defoliation pressure and companion grass competition, the independence of different seasonal profiles and the probable role of other morphological characteristics. In is concluded that testing authorities must calculate the management by leaf size relationships to adjust pass/fail standards if elite performing cultivars are to be correctly indentified.

Keywords: cutting; grazing; persistency; white clover; yield

Introduction

The ability of white clover (*Trifolium repens* L.) to fix atmospheric N, plus its high nutritive value and intake characteristics have made it the most important perennial legume in temperate climates (Abberton and Marshal, 2005). Due to relatively low fertilizer N prices, white

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clover use declined across Ireland in the 1970s and 1980s. Recent work showing how white clover can offset increased feed/energy/fertilizer costs (Crosson *et al*., 2006) as well as assisting in compliance with regulations restricting the usage of fertilizer N (EU nitrate Directive 91/6/76/EEC and the EU Water Framework Directive 2000/60/EC, http://ec.europa.eu/), (Andrews *et al.*, 2007), has contributed to a resurgence in interest. This has focused attention on the need for genetically superior cultivars to improve performance, and Chapman and Caradus (1997) and Woodfield *et al.* (2006) have provided clear evidence that this is being achieved. In Ireland, government, farmers, breeders, advisors and the seed industry support independent evaluation systems that seek to only promote elite performers through annual "Recommended Lists". As virtually all white clover seed sold in Ireland over the last 25 years was recommended for use in the year it was sold (Gilliland, Johnston and Connolly, 2007; Culleton and Cullen, 1992), the testing schemes are extremely influential on farmer choices and so must correctly identify elite cultivars suited to current farm practices.

A priority in white clover breeding programmes has been to improve yield and persistency (Abberton and Marshall, 2005). However, yield and persistency are strongly related to the management conditions under which they are tested (Swift *et al.*, 1992; Evans and Williams, 1987). While Evans and Williams (1984) have shown that where management conditions strongly influence cultivar performance this can determine the relationship between leaf size and performance, these effects may be manipulated through breeding (Chapman and Caradus, 1997). It has been concluded, therefore, that providing an evaluation and classification of white clover cultivars for yield and persistency, comparable to the performance at farm level, requires testing under different defoliation pressures. Consequently, it is vital that the testing programmes make evaluations of cultivars that relate to their relative agronomic merits on farm. Failure to do so could impede the genetic improvement of white clover and the sustainability of farm enterprises.

Sixteen cultivars of white clover are currently recommended for use in Ireland (Gilliland, 2009; DAFF, 2009) and these represent a pool of elite genotypes adapted to Irish growing conditions from within an EU common catalogue of 135 cultivars. The objective of the current study was to determine the effect of defoliation treatment, including the method (i.e., cutting or grazing), height and frequency of defoliation, and N fertilizer application rate on the relative and absolute yield and persistency characteristics in white clover cultivars under the evaluation systems currently used in Ireland. The effect of management conditions on the evaluation and classification of white clover was examined as was the adaptability among the current pool of elite performing white clover cultivars in Ireland. The overall objective was to determine whether current Irish testing systems sufficiently assess the true agronomic potential of white clover cultivars for their grazing and conservation use on farms. Although based entirely on data from trials conducted in Ireland the findings may be applicable to many other countries, particularly in Europe, that utilise similar testing procedures.

Materials and Methods

The data used in this study were generated from the cultivar evaluation trials run by the Agri Food Biosciences Institute (AFBI) for Northern Ireland at Crossnacreevy, Co. Down (54°32'N, 5°52'W) and the Department of Agriculture, Fisheries and Food (DAFF) for the Republic of Ireland at Athenry, Co. Galway (53°18'N, 8°45'W), Backweston, Co. Kildare (53°22'N, 6°30'W), Fermoy, Co. Cork (52°08'N, 8°17'W), Kildalton, Co. Kilkenny (52°21'N, 7°20'W) and Raphoe, Co. Donegal (54°52'N, 7°36'W). The data comprised results from the 16 cultivars currently recommended in Ireland, plus six candidates currently in evaluation trials and two cultivars that had been listed but were recently removed. The cultivars were AberAce, AberAtom, AberGuard, Aber S184, Grasslands Demand: (small leaf sized); AberDai, AberHerald, AberVantage, Avoca, Chieftain, Crusader, Excalibur Grasslands Bounty, Grasslands Chalice, Grasslands Huia, Grasslands Tribute, Menna (medium leaf sized); AberJet, Alice, Barblanca, Trinity (large leaf sized); AberSpring, Aran and Triffid (very large leaf sized).

The experimental design of each trial was a randomised complete block with four replicates. The white clover cultivars were sown in a mixture with an intermediate heading perennial ryegrass at a seeding rate of 3.5 kg/ha of white clover and 18 kg/ha of perennial ryegrass at the AFBI site and 5.0 kg/ha of white clover and 18 kg/ha of perennial ryegrass at the DAFF sites. Plot size was 5 × 1.5 m and 7 × 1.5 m at the AFBI and DAFF sites, respectively. Four different trial systems were compared at Crossnacreevy and a fifth system at the DAFF sites was used to examine aspects of location and season on relative clover performance. These differing systems were as follows:

1) Simulated Cattle Grazing at high N (Crossnacreevy only): Plots were cut at a target height of 30 mm using the reciprocating cutter-bar of a Haldrup plot harvester. An annual total of 200 kg/ha N was applied in split applications through the growing season. Harvesting began in spring at an estimated average herbage yield of 1,500 kg/ha DM and continued on an approximately monthly cycle to give seven to eight cuts from April to the end of October.

2) Simulated Sheep Grazing at Low N (Crossnacreevy only): Plots were cut at a target height of 20 mm at intervals of 10 days from 31 March to 30 June and 15 days from 1 July to 31 October using a rotary blade mower. An annual total N of 80 kg/ha was applied in two applications in spring and mid-summer.

3) Cattle Grazing at high N (Crossnacreevy only): Plots were grazed with a suckler beef herd from mid-March to the end of October on an approximately monthly rotational basis, timed when plots had reached an estimated average herbage DM yield of 1,500 kg/ha. An annual N total of 200 kg/ha was applied in split applications throughout the grazing season. Dung pats were removed from the plots after each grazing to avoid damage and plots were then topped to remove any rejected herbage.

4) Cattle Grazing at Low N (Crossnacreevy only): Plots were grazed with a suckler beef herd from mid-March to the end of October on a rotational basis, timed when plots had reached an estimated average herbage DM yield of 1,500 kg/ha. An annual total N of 80 kg/ha was applied in two applications in spring and mid-summer. Dung pat removal and topping were performed as in the High N grazing management.

5) Simulated Cattle Grazing at Low N (DAFF sites only): Plots were mowed to a target height of 30 mm from mid-April to the end of October using the reciprocating cutter-bar of a Haldrup plot harvester. An annual total N of 55 kg/ha was applied in one application in spring. Harvesting began in spring at an estimated average herbage DM yield of

1,500 kg/ha and continued through the season, on an approximately monthly cycle, to give a maximum of eight cuts per year, depending on site and length of growing season in any year.

Adequate phosphate and potassium was applied in all trials in compliance with best practice nutrient management guidelines (e.g., RB209 regulations, Anon., 2007). Herbage yield was measured on the entire plot of the Simulated Grazing at High N treatments. A subsample of the herbage harvested was hand separated into grass and clover. Grass and clover samples were dried at 105 °C for 24 h in a forced air circulation oven to determine dry matter concentration. Plot yields from multiple harvests within a year were summed to give the annual, spring (up to 30 April), early summer (1 May to 30 June), late summer (1 July to 31 August) and autumn (1 September to 31 October) yields. It was not feasible to measure yield on the other treatments. Clover content was determined by botanical separation of a 300 g sub-sample into grass and clover on a dry weight basis (no broad leaf weeds present). Leaf size was measured at Crossnacreevy as the area of the middle trifoliate leaflet from 60 spaced plants of each cultivar, as described by Gilliland (2009). This was used to classify the cultivars according to leaf size, as used in all cultivar testing programmes. Ground cover, defined as ground area covered by live white clover plant tissue, was used as an index of persistency. All management systems were visually scored for ground cover in 5% unit increments in June and October.

All data used were derived from cultivar trial data sets generated over the past 10 years (1999–2008) and comprised second and third trial year data. All plots were managed as described above in the first year but no data were recorded. Analysis of variance and regression analysis were performed using Genstat (Genstat Release 3.2, Lawes Agricultural Trust, Rothamsted), with the over-years data sets undergoing fitted constant analysis. The biplots were analysed using pattern analysis, a combination of cluster analysis and principal component analysis (Watson *et al*., 1995; Kroonenberg, 1994; Gabriel, 1971). Pattern analysis was used to provide a graphical summary of the information on the performance of the cultivars across different management systems or seasonal periods.

Results

Significant differences in performance existed between the cultivars with respect to measured variables (Table 1). A 56% range in white clover yield (relative to maximum) produced only a 15% range in total herbage production. The majority grass component (supported by an applied N of 200 kg/ha) was less variable (12%) but compensated for much of the white clover yield variation. Differences in seasonal yield between the cultivars were also significant ($P < 0.001$) in each period, with the range in differences increasing to a maximum 71% in the autumn. Significant differences ($P < 0.001$) were also found between white clover cultivars with respect to ground cover score under differing grazing and simulated grazing regimes, at differing levels of N fertilizer. With the exception of the simulated cattle grazing system at high N, the range for all the other systems was similar at between 24 to 30%. The simulated cattle grazing at High N was the least differentiating whereas actual cattle grazing was the most differentiating. The greatest selection pressure, however, appeared to be applied by the cattle grazing at low N which had the lowest ground cover scores recorded.

Table 1. Diversity in leaf size, dry matter (DM) yield (t/ha) and ground cover (%) among white clover cultivars under different management conditions at Crossnacreevy

Variable and system	Mean	Max	Min	Significance[1]
Leaf size (mm^2)	940	1527	392	***
Herbage annual DM yield under simulated cattle grazing at 200 kg/ha N				
White clover	4.0	5.2	2.3	***
Companion grass	8.7	9.3	8.0	***
Total herbage	12.7	13.6	11.5	***
White clover seasonal DM yield under simulated cattle grazing at 200 kg/ha N				
Spring	0.43	0.85	0.12	***
Early summer	1.33	1.73	0.94	***
Late summer	1.47	1.92	0.94	***
Autumn	0.89	1.29	0.38	***
White clover ground cover under different grassland systems				
Simulated cattle grazing (200 kg/ha N)	51.5	58.9	44.7	***
Simulated sheep grazing (80 kg/ha N)	54.0	69.3	42.2	***
Cattle grazing (200 kg/ha N)	56.7	67.1	37.6	***
Cattle grazing (80 kg/ha N)	45.1	57.5	33.8	***

[1] For differences among cultivars.

Examination of the seasonal growth patterns of the white clover cultivars when grown in mixture with perennial ryegrass at three test sites, showed the typical seasonal distribution curve of production (Figure 1). Clover comprised only a minor component of the harvested herbage in spring but rose to around 50% of the total herbage mixture by mid August before falling rapidly at the end of the growing season.

Principal component analysis of individual seasonal cultivar distributions

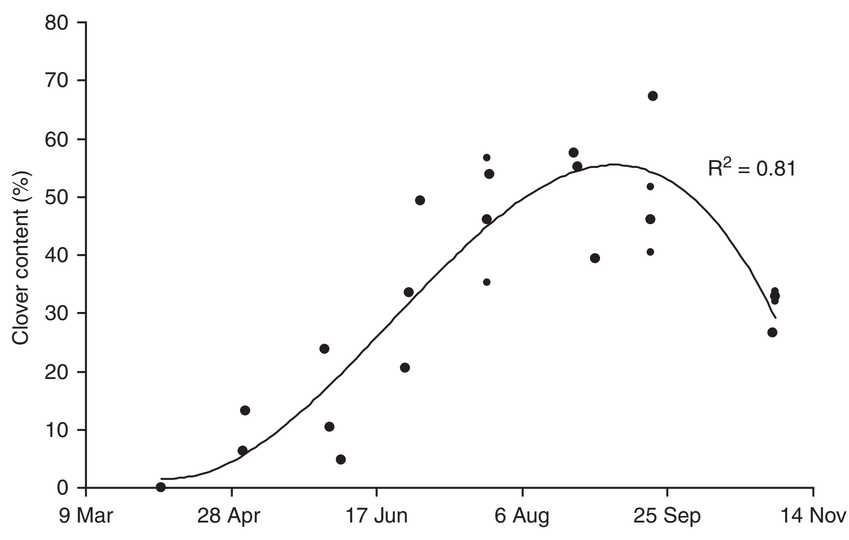

Figure 1. Seasonal distribution of clover content in grass-clover mixtures at Athenry, Co. Galway, and Raphoe, Co. Donegal.

(Figure 2), successfully attributed 94% of the variation into the two components on the biplot. This showed that early and late summer periods had similar vectors, indicating that relative cultivar performance remained unchanged from the early to the late summer period. From a cultivar differentiation perspective, therefore, there were only three discrete growth periods (spring, summer and autumn) when cultivars differed in their relative performance. The angles between the 4 directional vectors indicate a positive relationship (angle <90°) among the 4 seasonal assessments of clover yield. It was also notable that cultivars could be subdivided into several discrete groups along the primary component (representing 83% of the variation). Total productivity potential had a significant impact on this distribution, as 5 of the 6 largest leafed cultivars (Aberjet, 1131 mm^2 (= leaf area); Barblanca, 1207 mm^2; Triffid, 1221 mm^2; Aberspring, 1271 mm^2; and Aran, 1527 mm^2) were found clustered in Group 4, and these were also among the highest yielding of all the cultivars examined. Chieftain (1006 mm^2) was not in the largest group but is at the large end of the medium sized group and is high yielding for its leaf size, under Irish conditions. Furthermore, the position of these cultivars in the biplot, relative to the directional vectors, indicated that Aberjet, Aran and Chieftain were more productive in summer than Aberspring, Crusader, Barblanca, and Triffid, which showed highest spring performances.

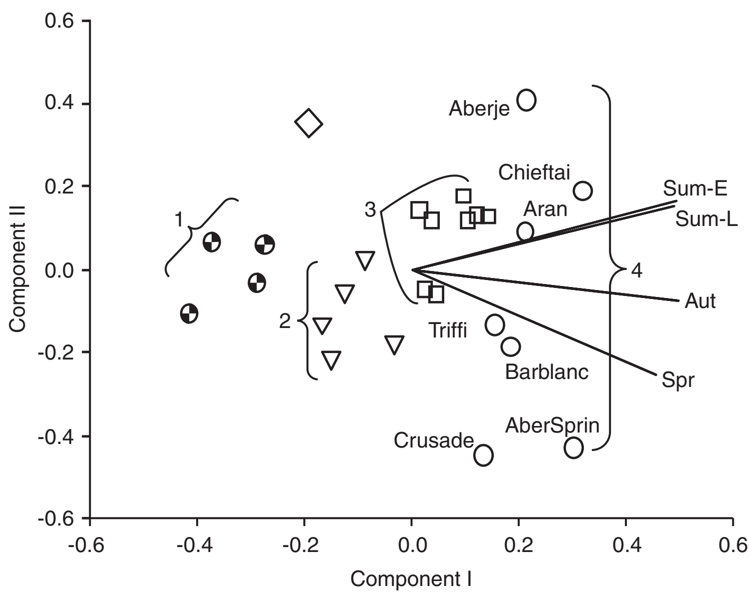

Figure 2. Biplot generated using standardized mean values of seasonal clover yield at Crossnacreevy, under simulated cattle grazing at 200 kg/ha N. Different symbols and values 1–4 indicate the cultivar groups generated from cluster analysis. The directional vectors represent the seasons (spring, Spr; early summer, Sum-E; late summer, Sum-L; autumn, Aut). Members of cultivar group 4 are indicated. Components I and II accounted for 83% and 11% of the total variation, respectively.

Although Crusader has a smaller leaf area (819 mm^2) than all the other members of Group 4, and has a lower total annual clover yield potential than all of the others, its production is significantly skewed in favour of high spring growth. This is why it was located within Group 4 in Figure 2.

The relationship between DM yield performance and clover leaf size across the white clover cultivars under simulated grazing at an N input of 200 kg/ha is illustrated in Figure 3. As leaf size increased from 400 to 1,500 mm^2, there was a corresponding curvilinear increase in DM production from 2 to 5.5 t/ha. Total herbage DM production followed a similar curve, tending towards a plateau at 13 t/ha. While there were significant yield differences between cultivar pairs of similar leaf size, overall only 30% of the yield variation among this elite group of cultivars could be attributed to any genotypic traits other than that predictable from leaf size.

A curvilinear relationship also best described the association between leaf size and ground cover percent for the combined data from all the management systems in this study (Figure 4). Within the range 400 to 1,500 mm^2 leaf size, ground cover declined as leaf size increased. This relationship attributed 70% of the variation in ground cover score to leaf size.

Figure 5 illustrates the individual relationships for the different management regimes combined in Figure 4. Simulated grazing at 200 kg/ha N and actual cattle grazing at 80 kg/ha N showed the previously observed changing relationship at the extremes of leaf size (Figure 5a and 5d). The influence of leaf size and cultivar were not, however, the same in both systems. Only 30% of the cultivar variation in ground cover under simulated grazing at an N input of 200 kg/ha was associ-

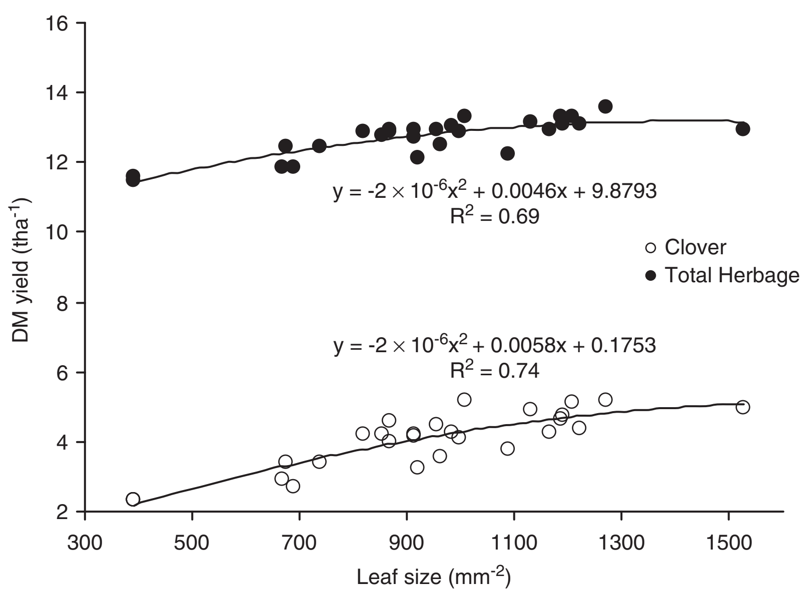

Figure 3. Relationship between annual herbage dry matter (DM) yield and leaf size for white clover cultivars under simulated cattle grazing at 200 kg/ha N at Crossnacreevy.

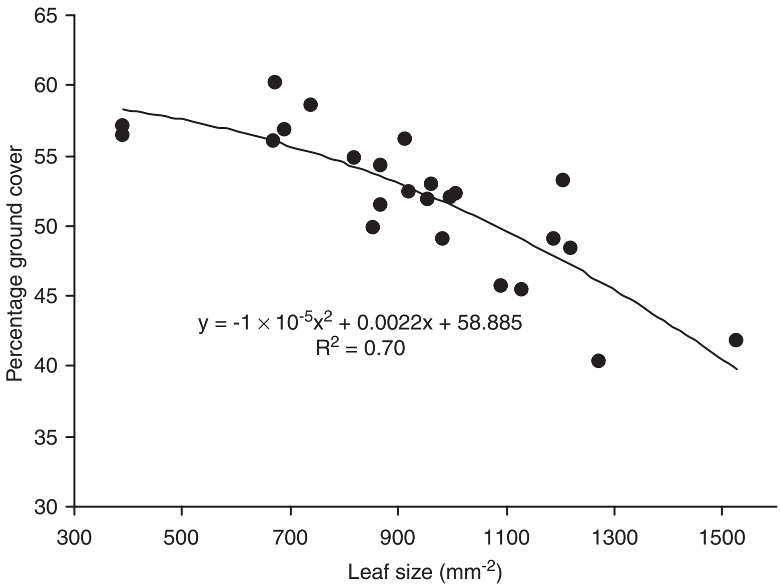

Figure 4. Overall relationship between clover ground cover (%) and leaf size for white clover cultivars across all management systems studied.

ated with leaf size, the vast majority of the variation being cultivar related. This is evident from the wide distribution of points on the graph. For cattle grazing at 80 kg/ha N, leaf size and cultivar differences were broadly equal in determining ground cover performances. The influence of leaf size as a determinant of ground cover under the cattle grazing at N input of 200 kg/ha (Figure 5c) was similar to that at N of 80 kg/ha, but in this case the curve was consistently steeper along its length. Consequently, a linear regression was almost as good a fit for the data ($R^2 = 0.58$). Furthermore, a linear regression was a substantially better fit than a curvilinear analysis ($R^2 = 0.18$) under simulated sheep grazing at N input of 80 kg/ha (Figure 5b). In summary, therefore, the predetermining influence of leaf size varied between the different management regimes – both in how strong an indicator of performance it was and also in how the relationship with ground cover remained constant or changed at the extremes of the leaf size range. Nonetheless, leaf size had a substantial influence on clover performance in all cases.

Following on from the above observations, potential relationships between different ‘grazing’ system managements were examined for clover cultivar ground cover performance (Table 2). Only weak correlations were found. Simulated grazing at N input of 200 kg/ha was poorly correlated with actual cattle grazing at the same applied nitrogen level (less than 40% predictable), and this association was further weakened when the cattle grazing was managed at the lower N input of 80 kg/ha. The strongest relationship existed between the two grazing systems (despite large differences in applied nitrogen use), though here again it was no more than a 50% association between the performance of the cultivars in each system. The simulated sheep grazing system was defoliated to a lower horizon and at much shorter

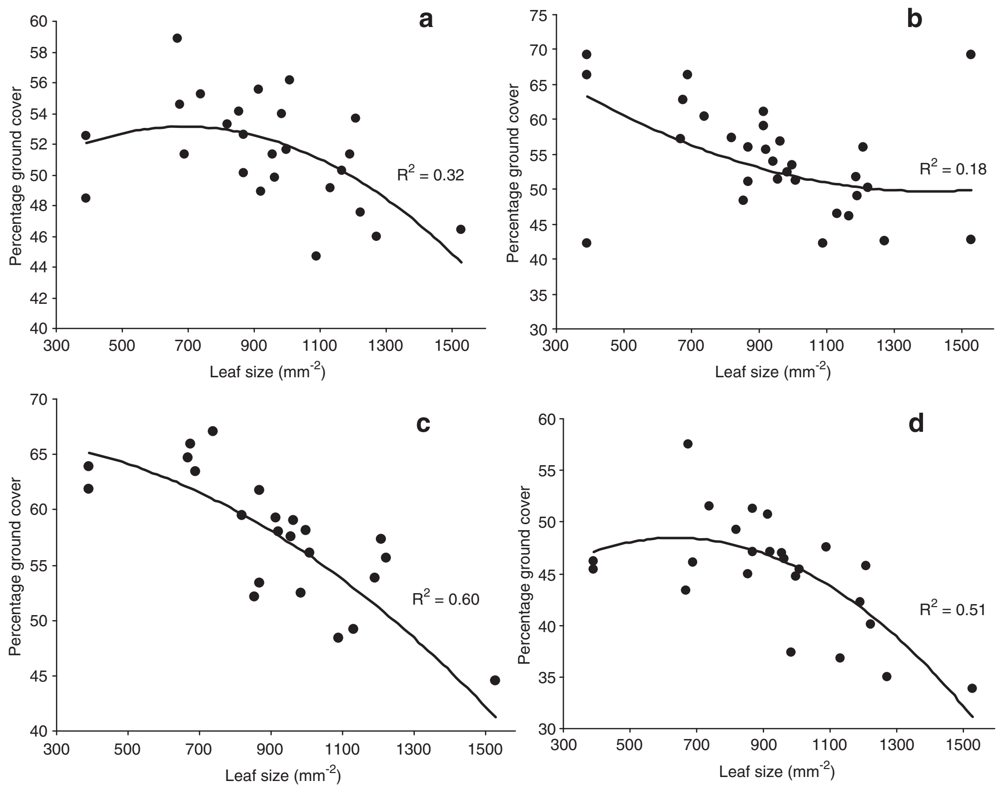

Figure 5. Comparison of the influence of white clover leaf size on the relationship between ground cover and different managements systems for cultivars.
a) Simulated cattle grazing at 200 kg/ha N. b) Simulated sheep grazing at 80 kg/ha N. c) Cattle grazing at 200 kg/ha N. d) Cattle grazing at 80 kg/ha N.

Table 2. Relationships between ground cover performance of clover cultivars under different 'grazing' system managements

Dependent variable		Independent variable	
		Simulated cattle grazing (200 kg/ha N)	Cattle grazing (200 kg/ha N)
Cattle grazing (200 kg/ha N)	R^2	0.40	–
	y =	1.2581x – 8.16	
Cattle grazing (80 kg/ha N)	R^2	0.17	0.50
	y =	0.6426x + 11.97	0.5534x + 13.73

regrowth cycles. It was poorly correlated with both cattle grazing managements, and when compared against the other simulated management, the regression again accounted for no more than 20% of the variation (R^2 = 0.20). In all the comparisons, cultivar performances tended to be differentially responsive to each of the four imposed management regimes. When principal component analysis was performed on all these data (Figure 6), it was found that the vector for the simulated cattle grazing management was the most outlying of the four managements. This showed that similarities in relative cultivar performance existed between the two cattle grazing and simulated sheep grazing managements, but while the simulated cattle grazing was still positively associated (vector angle <90%), cultivar performance was much more specific to that management. Overall, the results showed that different pressures are being imposed on the cultivars under the different management regimes. The graph shows that the grazing managements and the simulated sheep grazing regime are all positioned at the defoliation end of the pressure arc, while the simulated cattle grazing regime is subject to greater companion grass competition. This indicated that the cutting procedure under the simulated cattle grazing at N input of 200 kg/ha imposed a dominant competition pressure from the rapidly growing companion grass, whereas the presence of animals or the close frequent mowed simulated sheep grazing, imposed a defoliation pressure on the clover.

The high sensitivity of clover cultivars, as observed when sward management was changed, was again evident when the relative performance between clover cultivars was compared between different sites and

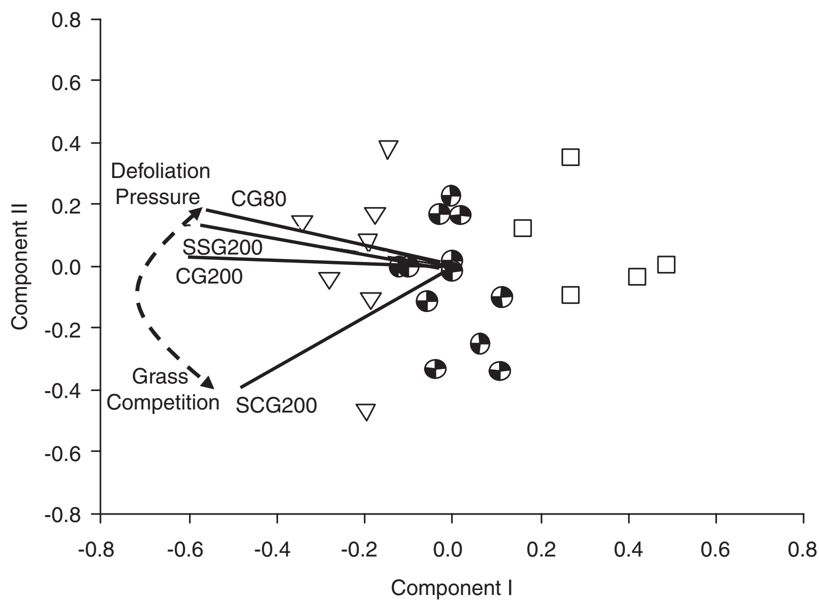

Figure 6. Biplot generated using standardized mean values of percentage of white clover ground cover of cultivars evaluated under different grazing systems. The different symbols indicate the 3 cultivar groups generated from cluster analysis. The directional vectors represent the different grazing systems (SCG200, simulated cattle grazing at 200 kg/ha N; CG200, cattle grazed at 200 kg/ha N; CG80, cattle grazed at 80 kg/ha N; SSG80, simulated sheep grazing at 80 kg/ha N). Components I and II accounted for 71% and 16% of total variation, respectively.

years (Table 3). Despite a constant management being imposed, mean yield across the cultivars differed significantly between sites and years, with significant site x year interactions. This also caused similar changes in individual cultivar ranking ($P < 0.001$) between sites and years.

It was not possible from the available cultivar performance data to directly assess breeding progress over years. Nonetheless, there was a large difference in the length of time the different cultivars had been in commercial use in Ireland. This ranged from Grasslands Huia and Kent Wild White, which were released prior to 1974 (when listing records began), through cultivars such as Alice and Aran from the 1980s, Grasslands Demand and AberDai from the 1990s to Grasslands Bounty and AberGuard in the current millennium. Therefore, performing regression analyses between clover cultivar performance and release dates would indicate whether the newest cultivars were the highest performers. Before doing this, it was necessary to determine whether there was a differiantial spread of leaf sizes across this time span. Otherwise the relationships already reported between leaf size and clover performance could corrupt such a comparison. No such relationship was found as the release of cultivars over time was not skewed for leaf size.

Examination of performance against clover cultivar age (Table 4) produced a slope for clover DM yield, from 3.6 to 4.4 t/ha, giving a rise of 22% from pre 1974 to 2008, or a maximum average rise of 0.65% per annum. This increase in magnitude is comparable with the best claims for ryegrass (Camlin, 1997). Despite this, the correlation with age was very poor. Similarly, when ground cover levels under the different management regimes were examined, the regression lines were virtually flat and almost always negatively correlated over time, albeit with regression coefficients of less than 10%. These analyses, therefore,

Table 3. Variation[1] in dry matter production (t/ha) by grass-clover swards at five sites in Ireland over 3 years

Year	Site[2]					Mean
	Cork	Kilkenny	Kildare	Galway	Donegal	
2008	9.71	9.45	10.38	9.52	6.88	9.19
2007	9.02	5.88	7.31	6.55	9.18	7.59
2006	8.10	6.96	8.44	7.26	7.39	7.63
Mean	8.94	7.43	8.71	7.78	7.81	

[1] Differences among years, site and year*site were all significant ($P < 0.001$); s.e. = 0.211, 0.272 and 0.471 for year, site and site*year means, respectively; L.S.D. (5%) = 0.434, 0.561 and 0.971 for differences among site, year and site*year means, respectively.
[2] These data were generated under the DAFF management system defined in the Materials and Methods as System 5.

Table 4. Relationships between clover cultivar performance and cultivar release date (x)

Dependent variable	Regression equation	R^2
Total herbage yield (dry matter; t/ha)	0.00004x + 11.459	0.10
Clover yield (dry matter; t/ha)	0.00005x + 2.319	0.08
Clover ground cover (%) under		
Simulated cattle grazing (200 kg/ha N)	–0.0002x + 58.79	0.09
Simulated sheep grazing (80 kg/ha N)	–0.0002x + 62.37	0.02
Cattle grazing (200 kg/ha N)	–0.0003x + 68.57	0.06
Cattle grazing (80 kg/ha N)	0.0002x + 38.08	0.03

provided no evidence of breeding progress over time and suggested that each new cultivar had only filled alternative points along the leaf size range expected for the higher performing cultivars. These data do not, however, determine whether individual cultivars with exceptional performances had been developed. A complicating factor in this was that cultivars switched between above and below the regression lines depending on management, the newer cultivars were more specific in their management requirements than older cultivars. Therefore, they tended to peak in performance under a management that was favourable, but declined substantially under a regime for which they were not well adapted.

Discussion

In considering white clover characteristics, and adaptation to grassland farming, it is useful to reflect on current grassland production systems, and how they might evolve in the future. Livestock production systems in Ireland are largely based on grass, grazed *in situ*. As more and more farmers focus on 'low input' systems and cost reduction, increasing emphasis will be placed on grazing. In Ireland, whilst white clover is a component of virtually all sown mixtures (Culleton and Cullen, 1992), reliance on it to fix N and drive grass production is not commonplace (Abberton and Marshall, 2005). In part this stems from limitations in clover production potential in the first half of the year, and also the difficulty in budgeting grass growth when dependent on a variable N-source driven by temperature and solar radiation. Figure 1 illustrates a typical seasonal distribution curve for white clover in Ireland, where herbage production is limited in spring/early summer due to low sward clover content. Typically at farm level this is compensated by the application of early N to encourage grass growth. Breeding advances that modify the clover distribution curve to provide more clover in the early spring could enhance the role of clover and achieve the desired lower dependence on nitrogen fertilizer. Relevant to this was that principal component analysis of the seasonal data in this study clearly showed that high performance in spring was a distinctive seasonal trait with some exceptional spring performing cultivars already in commerce. Despite being a distinctive trait, high spring production was not found to be completely antagonistic to other seasonal periods. Thus the results indicated that breeding for improved spring growth, need not severely impede performance at other times of the growing season, though to achieve this would also require an increase in total clover yield, rather than simply a redistribution of production. These seasonal distribution results also show that the current practice on recommended lists of subdividing seasonal yields into two summer periods (Gilliland, 2009), may not be necessary as it does not provide any additional seasonal information.

The data on which this paper was based were derived from only three breeding programmes, located in Ireland, Wales and New Zealand. The cultivars examined were the best cultivars in official clover evaluation programmes in Ireland, including those on the current lists (Gilliland, 2009; DAFF, 2009). However, they still represent a wide range of types as indicated by the significant differences observed in leaf size and performance range. Table 1 suggests that within the seasonal windows, there is huge variation from minimum to maximum clover yield, e.g., in the spring, maximum clover yield is 198% of the mean. This again indicates the potential for breeders to manipulate this diversity and further enhance spring growth to meet the requirement of farmers. Similarly, an almost four fold increase in clover leaf size from minimum to maximum was matched by more than doubling in clover DM production (2.3 *cf*. 5.2 t/ha), with an increase in total herbage DM production of 2.1 t/ha. Similar levels of diversity existed in ground cover among the cultivars, regardless of which management was imposed. Therefore, an initial examination of the performance ranges among these elite cultivars would suggest significant plant breeding achievements. However, on examination of the relationships between leaf size and performance, it is evident that a considerable degree of this variation is locked into a predictable continuum associated with leaf size, rather than being entirely due to novel genotypes. This is consistent with the observations of other workers such as Morrison (1997) and Orr *et al.* (1990).

The curvilinear relationship observed between yield and leaf size appears to approach an optimum value at around 1,500 mm^2, with little or no additional gains in production above this level. Ignoring the 'extreme' cultivars, the bulk of the available material varied in size from 700 to 1,200 mm^2. When cultivars outside this range are excluded from the analysis, a linear relationship was the best fit for the resulting data set. Furthermore, below 700 mm^2, yield deficit increased more rapidly per mm^2 reduction in leaf size, and conversely above 1,200 mm^2, the rate of yield increase per mm^2 declined with greater leaf size. Similarly, the curvilinear relationship between ground cover and leaf size also indicated progression towards a threshold. In this case there was a limit to the ground cover improvement that could be expected by selecting among the small leaf cultivars. Similar to yield production, a linear regression again improved the description of the relationship between leaf size and ground cover for leaf sizes between 700–1,200 mm^2. Therefore, despite the significant yield and ground cover differences reported (Table 1), more than 70% of the yield diversity across these cultivars could be accounted for by leaf size. This relationship was largely fixed between 700 and 1,200 mm^2 but changed at the leaf size extremes as limiting thresholds were approached. The implication for breeding programmes is that genotypes that perform outside these parameters can be regarded as superior and that achieving progress in extreme leaf-sized genotypes may be more difficult. Alternatively, small gains at the extremes may be regarded as important as larger gains in the mid-range of leaf sizes. A further implication is that making an accurate measure of leaf size at an early step in a selection process, is essential to determine how new lines are performing against these expected regression profiles.

These relationships between leaf size and various performance characteristics should not be interpreted as a simple cause and effect association. Large leaf size is not in itself a phenotypic characteristic likely to have such an extensive influence on performance responses to the defoliation systems studied. However, leaf size is a good indicator of a more general morphological ideotype. For example, large leaf cultivars normally have longer petiole and peduncal lengths than smaller leaf cultivars. They also normally produce fewer and shorter stolons and can have longer internode lengths (observable on single spaced plants of white clover in plant breeder's rights trials at Crossnacreevy, data not shown). It is these morphological characteristics that are more likely to be involved in the clover competition with grass and applied nitrogen, so giving the observed yield and ground cover performances under various grassland management systems. In support of this is the work by Davies *et al.* (1995), who showed that stolon length and growing point numbers were key morphological factors affecting clover performance under grazing.

Examination of the ground cover relationship across a range of different management regimes showed that it was not a fixed association, but was responsive, and so specific, to the defoliation regime being imposed. Most notable, was the finding that the simulated grazing management at N input of 200 kg/ha was the most distinctive of the systems tested, as indicated by principal component analysis. Although there was a positive relationship between this management and the others, it would not be expected to provide a strong predictor of cultivar performance under any of the other management regimes. This is similar to the findings of Evans and Williams (1987) when comparing rotational and continuous sheep grazing with a similar cutting system. The results of the current study suggest that different pressures are being imposed on the cultivars under the different management regimes. Interpretation of the principal component analysis was that the grazing managements and the simulated sheep grazing regime experienced greater defoliation pressures, while the simulated cattle grazing experienced more companion grass competition. The comparison between sites and years further exposed the high sensitivity of clovers, through the highly significant site by year interactions. This differential performance of clovers in response to such management pressures and conditions, poses a major difficulty for both plant breeders and evaluators. While it is undesirable that the simulated system did not accurately estimate ground cover under the imposed animal grazed regimes, the wider implication is that the highly sensitive nature of clover makes any estimate of relative clover performance specific to the conditions imposed or very similar there to. If the leaf size – performance relationships are determined by more specific morphological characters, as discussed above, then possibly a more generalised guide to clover performance across a diversity of on-farm management regimes could be gained by describing these morphological differences between cultivars. Such a novel approach to clover evaluation can be further justified from other morphological studies such as that by Davies *et al.* (1995).

Care needs to be taken in interpreting the regressions of performance against cultivar age, as only successful cultivars were examined. Cultivars released a considerable time ago and still listed today are likely to have been standard setters, well above the norm performance levels for their time. Consequently, as stated, the data from the current study is not well suited to revealing progressive cultivar improvements over time. The other observation that the newer cultivars were more sensitive to management than the older ones is, however, consistent with breeders producing more specialised cultivars. Notably, Evans, Williams and Evans (1996) reported specific adaptations for cultivars that were selectively bred for continuous grazing which manifested significantly different performances against the control cultivar Olwen when grazing or cutting was imposed.

Conclusions

This study clearly demonstrated that the performance expectation from a white clover cultivar is to a large degree predictable from its leaf size, most probably due to associations with various other morphological traits. An overview of all leaf size relationships indicated a degree of impediment for a clover cultivar with a leaf size phenotype at the outer regions of the range. This was because at the smallest end of the range, getting even smaller added progressively less ground cover but incurred increasing yield losses. Similarly at the upper end of the range, ground cover began to fall more rapidly, but without a compensating increase in yield performance. This would be expected from a biological viewpoint, as a purely straight line relationship indicates no threshold levels. The fact that the observed curves were notably shallow could be evidence that breeders have managed, at least partially, to suppress these basic trends when creating the elite cultivars studied. This possibility could not be confirmed in the current study as it requires comparisons with less improved clover genotypes.

Another significant finding was the high sensitivity of the clovers to varying management, site and year factors. Consequently, relative ground cover ranking between cultivars was very specific to the incident conditions. This made it difficult for evaluators or breeders to make confident predictions of performance beyond the conditions imposed in their testing and selection trials, especially to regimes where the balance between defoliation and companion grass competition was different. The results also show how difficult it may be to exceed the performance ceiling that the morphological ideotype, linked to leaf size, imposes, particularly at extreme leaf sizes. On both accounts, it would be interesting to investigate whether those cultivars that have exceeded the predicted performance for their leaf size, still had the 'expected' morphology consistent with that leaf size. It may be that breeding has modified this association to give elite cultivars a novel morphology for their leaf size. Such information may provide valuable traits for selecting elite genotypes in a breeding programme. Equally, an assessment of this aspect of white clover morphology could lead to more accurate estimates of a cultivar's potential adaptation to specific pasture management regimes, than currently achieved.

The immediate implications for testing authorities is that pass/fail standards need to comprise a progressive scale that takes account of changing expectation along the leaf size range. Similarly, breeders must be aware of the leaf sizes of their breeding lines in order to identify those genotypes that perform above the norm level consistent with a given size.

Acknowledgements

Thanks are due to Dr Zulfi Jahufer, AgResearch Limited, New Zealand for expert assistance with the principal component analysis and production of biplots.

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