The measurement of perennial ryegrass persistence

M.B. DODD[1*], D.F. CHAPMAN[2], C.I. LUDEMANN[1], W. GRIFFITHS[1], K.N. TOZER[3] and L. DONNELLY[4]
[1]*AgResearch Ltd, Grasslands Research Centre, Private Bag 11008, Palmerson North 4410, New Zealand*
[2]*DairyNZ, PO Box 85066, Lincoln University, Lincoln 7647, New Zealand*
[3]*DairyNZ, Private Bag 3221, Hamilton 3240, New Zealand*
[4]*AgResearch Ruakura, Private Bag 3123, Hamilton 3240, New Zealand*
[5]*SeedForce Ltd, Christchurch, New Zealand*
mike.dodd@agresearch.co.nz

Abstract
Poor persistence in perennial ryegrass has been identified as a major limitation to pasture productivity, particularly in the upper North Island. Persistence can best be defined as the continuity of forage yield relative to a cultivar's potential. Though there is limited evidence of differences in persistence between cultivars, there is interest in including persistence in the DairyNZ Forage Value Index. This requires an agronomically robust metric of persistence, measured over a suitable time frame and connected to economic value. Five candidates are evaluated: plant populations, tiller populations, basal cover, ground score and annual dry matter yield. Scarcity of long-term data is a major limitation to development of performance values for persistence, and must be addressed. The four abundance-based measures also lack a clear connection to economic values, from the limited data available. A persistence metric is proposed, that relates medium-term dry matter yield to short-term dry matter yield, for which perennial ryegrass functional type and cultivar differences are demonstrated.

Keywords: basal cover, dry matter yield, Forage Value index, ground score, tillers

Introduction
Poor persistence in perennial ryegrass has been identified as a major limitation to pasture productivity, particularly in the upper North Island. The reasons for this are not known but likely involve a combination of factors such as climate, soil, pest pressure and grazing management. Some farmers believe that modern genotypes are less persistent than older ecotype-based perennial ryegrasses (Tozer *et al.* 2011). While there is now good evidence to indicate that there is no inherent difference between 'new' and 'old' ryegrass cultivars in their ability to survive in pastures grazed by dairy cows (Lee *et al.* 2017), differences have been reported among cultivars in persistence of dry matter (DM) yield (Chapman *et al.* 2015), and ability to survive in physically stressful environments (Thom *et al.* 1998).

It has been proposed that persistence be included as a trait in the Forage Value Index (FVI, Chapman *et al.* 2017) to enable farmers to make better choices between cultivars and to give plant breeders direction for germplasm selection. A fundamental requirement for this development is the identification of a suitable measure of cultivar performance values for persistence. This is challenging since, by definition, persistence is a long-term outcome which is expensive to measure directly. An additional challenge is to define what it is about pastures that should persist, or conversely what is not persisting. Operationally, this can be either an entity (the grass itself) or a characteristic of the entity (e.g. a yield or forage quality trait, Parsons *et al.* 2011).

Working definitions of persistence vary in the agronomic literature. While they have a common focus on the species sown (rather than the whole sward), they diverge on the matter of persistence of the entity (e.g. tiller and plant populations, Camlin & Stewart 1978) versus persistence of the trait (e.g. yield, Chapman *et al.* 2015).

To develop a robust metric for persistence that can be incorporated into the FVI, the following are necessary:

a. Define an ecologically robust and agronomically relevant measure of persistence that can reliably differentiate between cultivars (or at least functional types such as ploidy and heading date). The metric should be readily measurable on large numbers of plants and be heritable;
b. Define a relevant time frame;
c. Identify existing long-term datasets, or collect such datasets, that include the selected measure across a range of germplasm;
d. Demonstrate a clear link between the persistence measure and the performance value of the germplasm that can then be assigned an economic value.

The purpose of this paper is to use these criteria to explore the utility of some potential candidates for the measurement of persistence, and to propose a suitable metric.

Candidate measures of persistence
This section considers the advantages and disadvantages of four measures of abundance (plant

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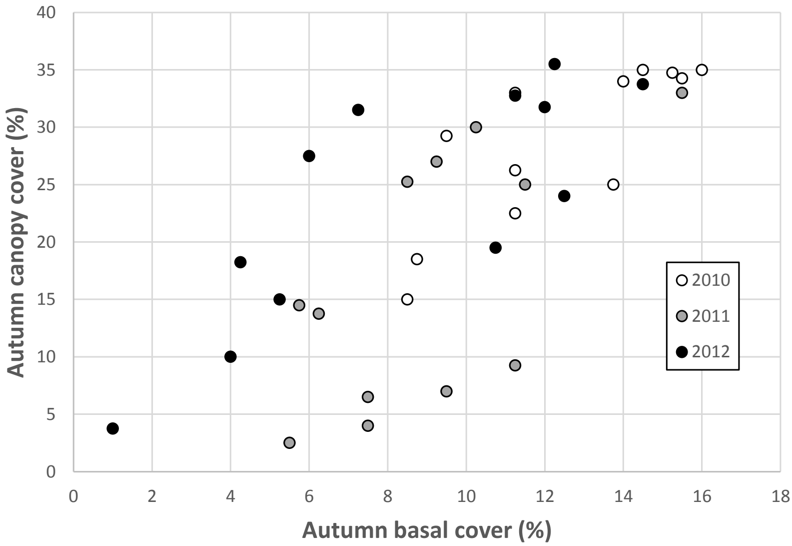

Figure 1 Basal and canopy area of perennial ryegrass, in small plot mixed species swards in the Waikato study of Tozer *et al.* (2017). Open circles = April 2010, Grey circles = April 2011, Filled circles = April 2011.

populations, tiller populations, basal area/cover and ground score) and one measure of performance (DM yield), as potential candidates for development of a persistence metric.

Plant populations

While relatively simple to measure in the early stages of sward establishment, measurement of individual plants becomes problematic beyond 2 years, due to the clonal nature of grass plants undergoing continual tillering and fragmentation (Brock *et al.* 1996). It is difficult to delineate clusters of tillers as belonging to discrete plants in mature swards. For this practical reason alone, plant population variables are a poor choice for measuring persistence. In addition, simple population counts do not account for variation in plant size, which has a substantial effect on individual plant longevity (Lee *et al.* 2017).

Tiller populations

The tiller is the fundamental unit of vegetative growth in grasses and thus is probably the most agronomically robust measure of persistence (Matthew *et al.* 2000). However, tiller development has considerable morphological plasticity in grasses, mediated through the inverse relationship between tiller density and size (Gastal & Lemaire 2015). This relationship allows swards to attain maximal leaf area (within prevailing environmental and genetic limits) by multiple combinations of tiller density and size (Matthew *et al.* 1996). Thus, while lack of persistence will usually be associated with a long-term decline in tiller numbers; short-term declines over a non-critical range in tiller densities (4000-8000 tillers/m^2) may not necessarily reflect persistence failure (Matthew *et al.* 2013).

A key advantage of using tiller variables to measure persistence is the ability for plant breeders to select for this trait. While cultivar marketing material often includes qualitative information on relative tiller density, it appears to have been of low priority in previous cultivar development, perhaps because the evidence on its contribution to persistence is equivocal (Lee *et al.* 2012). However, as with plant populations, tiller populations are time-consuming to measure accurately in the field.

Basal cover

Basal cover is a measure of the abundance of plants according to the coverage of live tissue at ground level, usually expressed as a proportion or percentage of the total area surveyed. The precise details of measurement vary between studies, such as point intersect (Rhodes & Collins 1993) or presence within defined horizontal grid cells (Virgona & Bowcher 2000). As with plant and tiller populations, measurement is necessarily manual and time consuming in the field. No published examples of the use of basal cover for comparisons of cultivar persistence are obvious, although Tozer *et al.* (2017) assessed both basal and canopy cover of ryegrass within a range of pasture species mixes sown in small plots. Their data from 3 consecutive years indicate only a moderate positive correlation between the two (r = 0.73, Figure 1). Canopy cover is likely to be strongly correlated with visual ground cover estimates, the fourth abundance measure considered here.

Ground score

Camlin & Stewart (1978) used a 0-9 scale visual score of relative ground cover during winter to assess persistence of ryegrass cultivars in the second year after sowing, demonstrating that this was highly correlated to herbage yield in the fourth year. This measurement is now referred to as ground score (GS) and changes in GS over time have been used in the

Irish equivalent of the FVI as the basis for assessing persistence (Pasture Profit Index, PPI, O'Donovan *et al.* 2016). The economic value for persistence in the PPI is derived from the relationship between inter-annual changes in DM yield and GS (1683 kg DM/unit GS) as reported by Cashman *et al.* (2014).

New Zealand National Forage Variety Trial (NFVT) protocols originally included point analysis measurements after 3 years as a standard assessment of persistence, but in 2013 this changed to using visual ground score in winter each year (rating on a 1-9 scale at approximately 2 weeks post-grazing, where a '1' corresponds to 10% or less sown ryegrass presence and a '9' corresponds to 90% or greater sown ryegrass presence, M. McLeod pers. comm.). This shift was made in recognition of a poor relationship between the initial method and observations of persistence in other trials or on-farm (Kerr *et al.* 2012). Adoption of GS reflects its practical utility, scalability from small plot to paddock, and the inherent integration of plasticity in the main yield components (tiller or plant number and size).

Dry matter yield

Measurement of dry matter yield over time represents the most conclusive assessment of persistence, since dry matter yield is the primary driver of productivity (Parsons *et al.* 2011; Chapman *et al.* 2015). However, yield over time is highly dependent upon climatic conditions, and thus it is necessary to define persistence as the continuity of potential dry matter yield. This presents the challenge of defining what potential yield is. There are three possibilities: a) yield measured in the first few years of establishment, b) maximum yield of a range of genotypes of the species in each year of measurement, and c) yield estimated by a physiologically-based model simulating yield under a given weather pattern. Using yield in a given year or sequence of years after establishment as the potential yield allows for relativity to the potential of that genotype with likely minimal impact of weed ingress. However, it is problematic in that the difference between the potential yield benchmark post-establishment and the yield in later years could be interpreted as poor persistence, but actually be the result of other factors such as climatic conditions or increases in pest load (Farquhar *et al.* 2017). Using the maximum yield expressed by a range of genotypes during a period of measurement could be confounded by genotype inclusion, that is, it assumes that the genotype best-adapted to those climatic conditions is being used and measured. The potential issue in using a modelling approach is that most models are parameterised to field measurements of average yield rather than maximum yield. However, an approach that uses sensitivity analysis of variation in plant traits may be useful in quantifying potential yield as a benchmark (e.g. White & Snow 2012).

The best option appears to be to use yield measured on an individual cultivar during an establishment period of suitable length to minimise the effects of seasonal climate and weed ingress, this also has the advantage of expressing persistence in the context of the potential of that cultivar, independent of the performance of the cultivar relative to other cultivars.

Time scales and data limitations

While there is no agreed definition of the appropriate time scale for the assessment of persistence in perennial pastures, farmer surveys give some indication of a "user expectation" of the longevity of perennial pastures. Daly *et al.* (1999) surveyed sheep and beef farmers across New Zealand and reported that 45% of respondents expected pastures to last between 11 and 20 years, while 31% expected pastures to last longer than 21 years. Rijswijk & Brazendale (2016) surveyed dairy farmers in the upper North Island and noted an average expectation of 11 years before pasture renewal was necessary, with just over half the respondents selecting a 6 to 10-year time frame. Ideal pasture renewal time frames are strongly influenced by economic considerations (Malcolm *et al.* 2014), who highlight the dependence on the annuity derived. The PPI uses 12 years as a benchmark, citing the balance between persistence and genetic gain, O'Donovan *et al.* 2016).

Current performance values in the FVI are calculated based on 3-year field assessments of DM yield in the NFVT. Three years is generally considered to be "short-term" for perennial pastures, and hence there is currently no systematic data collection incorporating a range of genotypes over time scales more relevant to the question of persistence.

Indeed, in New Zealand there are few agronomic field assessments of forage grasses longer than 3 years. Most studies of greater than 3 years have compared different grass species (e.g. Barker *et al.* 1993), and only four have incorporated comparisons of multiple genotypes/cultivars within species (Allan & Keoghan 1994; Chapman *et al.* 2015; Black & Moir 2015; Lee *et al.* 2017). Except for Allan & Keoghan (1994), who used a plant survival score, these studies have used DM yield and/or botanical composition as the main measure of persistence. Lee *et al.* (2017) observed no difference in the persistence of four perennial ryegrass cultivars under substantial climate and pest stress in the Waikato, based on DM yields, and this is the only study to have reported long-term measurements of tiller density (over 6 years). That data set shows a similar long-term decline for all four perennial ryegrass cultivars.

Only two studies incorporating long-term ground score assessments appear to be available. The study of Chapman *et al.* (2015) included unpublished persistence scores conducted in May of Years 2 and 3, July of Year 4 and September of Year 5. These data are explored further in the following section. The second data set comes from an unpublished field evaluation of 18 perennial ryegrass cultivars conducted from 2006-2014 at a dryland Canterbury site (733 mm mean annual rainfall). The cultivars were sown in spring 2006 as pure swards in small plots (1.5 x 8 m) on a Wakanui silt loam, with each cultivar replicated four times in a randomised complete block design (L. Donnelly unpubl. data). Plots were grazed by beef cattle and received the equivalent of 20 kg N/ha as fertiliser after every second grazing. Herbage accumulation was assessed by capacitance probe measurements pre- and post-grazing. Visual GS measurements using the NFVT method (NZPBRA unpubl. report) were conducted in February of years 6, 8 and 9. However, an analysis of variance found no significant differences between cultivars or heading date groups in the linear trend of ground scores over time.

It seems clear that there are insufficient data currently available to a) support the choice of one candidate measure over another, or b) constitute a robust cultivar comparison for discriminating on persistence. The design and implementation of relevant data collection over periods much greater than 3 years is therefore a vital step in resolving this situation, and generating performance values for persistence in the FVI.

Persistence measure and performance value

One of the key issues with all four abundance-based measures of persistence (plant population, tiller population, basal area and GS) at this stage is that, in contrast to the case for GS within the PPI (O'Donovan *et al.* 2016), no clear connection exists between any of them and a performance value for persistence in grazed pastures in New Zealand. A distinct advantage of the type of indirect measures discussed here is that they could be deployed early in the life of a pasture to predict long-term performance (the 'ex-ante' approach), avoiding the 'drag' on genetic progress imposed by waiting for 6+ years for information ('ex-post' approach). Thus, further investigation of connections to performance values is warranted.

If successful, two important linkages to FVI methodology could be established: 1) the connection between the persistence metric and the on-farm decision to ryegrass, which will be reflected in the annualised cost of pasture renovation; and 2) the connection between the persistence metric and forage yield over time, which will be reflected in the opportunity cost of foregone DM yield. The first of these is likely to be highly subjective, based on an individual's threshold for performance, though this may still be amenable to objective economic analysis (Malcolm *et al*. 2014). The second linkage suffers from a dearth of data from field studies that include long-term concurrent measures of species abundance and forage yield for multiple genotypes.

The trial described by Chapman *et al.* (2015) comes closest to meeting these requirements. Including a visual measurement of ground cover using a 1-10 scale (similar to the GS technique) in May 2008 (beginning Year 3) and September 2010 (mid-Year 5). Regression of these scores against measured herbage yield in the subsequent July-June growing seasons shows a weak positive relationship (Figure 2). The slope of the two regression equations is remarkably similar at ~700 kg annual DM yield for every unit score even after taking account of the different seasonal timing of the ground cover scores, and the difference in the interval between scoring and the subsequent total annual DM yield assessment periods. However, it must be recognised that this value is highly site and season specific and the variance is high, likely driven by tiller size-density compensation (Matthew *et al.* 2010, 2013). Figure 2 indicates a similar yield is achieved by cultivars scoring up to 5 GS units apart. A more robust approach uses the relationship between the inter-annual change in DM yield associated with an inter-annual change in GS (Cashman *et al.* 2014).

The unpublished data of Donnelly noted above, included both GS and DM yield assessments for two periods over the 8-year study: Year 6-7 and Year 7-8. A correlation analysis of the inter-annual change in DM production versus inter-annual change in GS (after Cashman *et al.* 2014) of the 18 cultivars showed no clear relationship.

The poor relationship between abundance measures and yield performance, albeit based on limited data, emphasises that the criteria for an indirect persistence metric with any utility for the FVI are far from being met. Until this situation is resolved, an alternative approach is proposed, which uses the more reliable variable of DM yield data to develop a scaling factor to modify the yield performance values currently used within the FVI.

A proposed persistence scalar

The persistence scaling factor proposed here is based on herbage yield measurement of the cultivar of interest, incorporating the following assumptions:

1. Herbage DM yield in the first few years following establishment best represents the yield potential of a particular cultivar. Three years is considered

a minimum period for setting this benchmark, to account for climatic variation between years.

2. The DM yield of a cultivar over the long-term (>3 years) relative to DM yield in the short-term (<3 years) best represents persistence from a farm systems perspective, can be measured in the field, and readily translates into economic value. Three years beyond the initial benchmark period is considered a minimum for assessing long-term yield, again to adequately account for the impact of inter-annual climatic variation.
3. Data used should exclude the contribution to yield of other species/cultivars within the measured area (e.g. white clover, unsown species.)

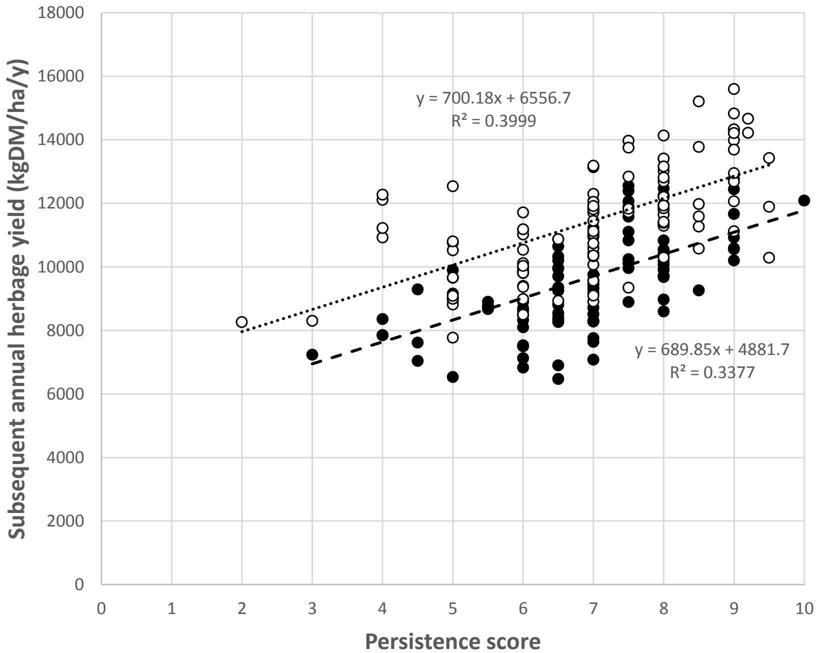

Figure 2 Regression of annual herbage yield versus persistence score for 25 cultivar/endophyte combinations each replicated in four plots, from the study of Chapman *et al.* (2015). Filled circles, dashed line = persistence score in May 2008 (Year 3 after sowing) versus total annual DM yield for July 2008-June 2009; open circles, dotted line = persistence score in September 2010 (Year 5 after sowing) and total annual DM yield for July 2011-June 2012.

The persistence scalar is expressed as the ratio of long-term to short-term herbage DM yield by the following equation:

Persistence scalar (Ps[4,5,6]) = Mean annual DM yield [Years 1,2,3,4,5,6]/ Mean annual DM yield [Years 1,2,3] (Equation 1, Table 1)

The calculation can be flexible to allow for other periods of long-term data collection, e.g. the mean of later years (Ps[6,7,8]) or non-consecutive years (Ps[5,7,9]) as long as this is expressed in the notation as indicated (Table 2).

A re-analysis of two recent perennial ryegrass cultivar field comparisons with this equation is presented in Tables 1 and 2. The first is from the Lee *et al.* (2017) study in the Waikato, which indicates that the two mid-season heading diploid cultivars 'Nui' and 'Commando' were significantly less persistent than the late-season heading cultivars 'Alto' and 'Halo' in that environment ($P<0.05$, Table 1). The second is from the Hawke's Bay data of Chapman *et al.* (2015), where the long-term yield data were from Years 7, 8, 10 and 11. The results showed both a significant difference between cultivars grouped by heading date and ploidy ($P<0.001$), as well as a significant cultivar effect within those groups ($P<0.05$). The very late tetraploid cultivars were significantly less persistent than the two diploid groups, with a large range within those groups (Table 2).

The proposed metric necessitates the collection of longer-term data from field evaluation trials than is

Table 1 Mean persistence scalar values (Ps[4,5,6] as per Equation 1) of four perennial ryegrass cultivars in the field experiment of Lee *et al.* (2017). Values followed by different letters are significantly different at $P<0.05$.

Cultivar (endophyte)	Ploidy	Heading	Ps[4,5,6] value (SED)
'Alto' (AR37)	Dip	Late	0.866 a (0.021)
'Commando' (AR37)	Dip	Mid	0.797 b (0.013)
'Halo' (AR37)	Tet	Very late	0.854 a (0.023)
'Nui' (SE)	Dip	Mid	0.800 b (0.006)

Dip = diploid; Tet = tetraploid.

currently the case. This potentially slows down the rate of development of new cultivars, although if there is sufficient demand for the improvement of persistence in future cultivars, this cannot be avoided since collection of long-term data is axiomatic for the trait. It is anticipated that the collection of such data may in future lend itself to development of short-term proxies with a higher level of confidence than is currently the case (viz. Chapman *et al.* 2015). Hence, measurements of parameters other than DM yield (particularly ground score) should also be included in such long-term trials. Ideally, genomic analysis of survivor plants would be conducted in parallel. A programme of this type may help elicit robust phenotypic or genetic indicators of long-term performance that will expedite trialling and genetic progress in the future.

Conclusions

There are very few multi-genotype comparative data sets of sufficient longevity relative to farmer expectations around persistence (6-10 years). The most agronomically robust measures (plant and tiller populations, basal cover) are highly variable in space and time and difficult and/or expensive to measure. For the simplest measure (ground score), sufficient long-term data and a verifiable connection to a performance value based on dry matter production are both lacking. Long-term DM yield is the easiest variable to translate to performance values, but the paucity of long-term studies means that clear conclusions regarding genetic differences among current perennial ryegrass cultivars in dairy pastures cannot yet be drawn. Trials of at least 6 years, that include yield, abundance and genomic data, should be established at representative locations to collect cultivar persistence performance values for the FVI.

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Table 2 Persistence scores (as per Equation 1) of three perennial ryegrass cultivar groups in the field study of Chapman *et al.* (2015) in the Hawke's Bay. Values followed by different letters are significantly different at P<0.05.

Heading date group	Ploidy	Ps[7,8,10,11] value Mean SED = 0.009	Cultivar range (Number of cultivars)
Mid	Dip	0.918 a	0.887-0.941 (11)
Late	Dip	0.921 a	0.897-0.944 (4)
Very late	Tet	0.878 b	0.870-0.889 (3)
Group effect		P<0.01	
Cultivar within group effect		P<0.05	

Dip=diploid; Tet=tetraploid

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