Ryegrass in pastures – breeding for resilience

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Abstract
The suggestion that modern pasture cultivars persist less well than their predecessors is not supported by facts. However in some regions there is a crisis in farmer experience of persistence of ryegrass pastures, and ryegrass breeding can contribute to a resolution. This paper considers the turnover of tillers in a pasture, the population structure of a grass sward and the involvement of endophyte and companion clover. Knowledge gaps in key processes of pasture persistence are discussed. The plant breeding process involves access to relevant genetic variation, its assessment, and creation of improved populations and eventually cultivars based on selected superior plants. All these scientific processes use ever-evolving techniques. Breeding objectives evolve with industry needs and the changing environment. The methods employed in the breeding phases, the breeding objectives and the final evaluation of cultivars require ongoing revision. Plant breeding will make a growing contribution to modern farming if its developing capabilities are interfaced with other research disciplines providing critical information on key pasture processes.
Keywords: *Lolium perenne*, endophyte, persistence, tillering

Introduction
Perennial ryegrass (*Lolium perenne*) is the grass of choice for long-term pastures under fertile conditions in New Zealand (NZ) because it has proved to yield well, to be easy to establish and grow, tolerant of intensive grazing, and adapted to the range of management practices that characterise our livestock farming. It also has relatively high feeding value characteristics for a forage grass and is regarded as a good companion species for white clover (*Trifolium repens*).

Given its reputation as an easily manageable grass tolerant of our systems, how do we find ourselves addressing a crisis of confidence in the persistence of ryegrass-based pastures? Persistence of ryegrass pastures has been raised as an issue regularly in the past particularly in those warmer and drier environments which are more marginal for ryegrass (Fraser 1994; L'Huillier & Aislabie 1988; MacFarlane 1990; Thom *et al.* 1998), but it is clearly more acutely perceived as such at the present time. Grazing management, soil and other environmental factors are addressed elsewhere in these proceedings. What are the implications for ryegrass breeding?

Genetic variation for persistence and development of modern cultivars

That all ryegrass is not equal, with genetic variation for factors associated with persistence, has been known from the earliest research work with ryegrass in NZ (Cockayne 1912; Levy & Davies 1929). Ryegrass seed harvested from permanent pasture was shown to be superior in persistence and in year-round leafiness, compared with ryegrass sourced as a cash-crop by-product of arable farming. An initial aim of the certification system in NZ was to assure the provenance of the recognised superior types. Organised selection for genetic improvement came later.

Old populations of ryegrass known to be persistent have been the foundation for ryegrass breeding in NZ (Burgess & Easton 1986; Easton 1983; Stewart 2006; Widdup & Ryan 1992). In particular, old Hawkes Bay pastures and the Mangere population have been widely used, but collections from throughout the country have been important in different breeding programmes. Accessions from areas of north west Spain with a similar mild climate have made an important contribution in recent years (Easton *et al.* 1989a).

There is no evidence that modern cultivars as a group are less persistent than those that preceded them. Any trial comparing old with new cultivars under the same management and endophyte status has found that the new cultivars maintain their production and sward composition at least as well as the old (Crush *et al.* 2006b; Easton *et al.* 2001a). Plant breeders regularly compare their material with widely-used controls in trials across multiple environments and observe enhanced persistence of their best lines.

Further, there is little evidence of higher-producing plants being less tolerant of unfavourable conditions. The higher-producing plants remain so under most conditions, perhaps being more vulnerable to collapse under severe defoliation in extremely dry or impoverished environments (Hazard *et al.* 2001). Ryegrass plants show considerable plasticity, with tiller size and number responding to management in a compensating ratio (Matthew *et al.* 1996). Within given conditions, there is genetic variation for tiller size and number (Bahmani *et al.* 1997), and plants or cultivars with a genetic disposition to large tillers may be less able to adopt the habit required to survive intense defoliation or lack of resource. However, this failure applies at the limits of ryegrass's environmental adaptation. Within the usual adaptation range of ryegrass, higher producing plants suffer no persistence penalty.

Table 1 reproduces data from a report of cultivar performance in Poukawa, Hawkes Bay under a 750mm annual rainfall, summer dry environment (Muir 2009). Plots were sown in autumn 2005, and yield cuts taken regularly. Ground cover was scored annually. The table gives annual yield for the first year and ground cover in July 2009, 4 years after sowing, during which there had been severe drought.

Points to note are:

Plots infected with standard endophyte have on average persisted better than those infected with AR1 (see below), but the variation within each group is great so that the mean difference is not statistically significant, especially if the hybrid ryegrasses, 'Sterling' and 'Supreme,' are removed from analysis.

There is no indication that older cultivars have persisted better than more recent ones.

Tetraploid cultivars, which generally have fewer, larger tillers than diploid, show a range of persistence, some being amongst the most persistent.

Within the endophyte groups, there is a modest positive correlation between yield in the first year and ground cover after four years. There is no indication that higher yielding cultivars persist less well.

Likewise, a trial of new breeding lines (Figure 1) in the Waikato showed no significant relation between yield in the first 8 months and ground cover in the third summer, and while the poorest lines were infected with AR1, some lines infected with AR1 were among the most persistent.

Recent discussion (Popay & Hume 2011, this volume) of the effect on pasture persistence of ryegrass endophyte strains covers the trade-off between persistence and other traits that will become an increasingly acute issue for plant breeders and farmers. Unselected wild-type (or 'standard') ryegrass endophyte strains, naturally present in old NZ pastures, produce alkaloids that protect the grass plant from attack by invertebrates (Easton *et al.* 2001b) and from over-grazing by livestock (Edwards *et al.* 1993). Some of these compounds also cause livestock toxicoses. Ryegrass hosting the AR1 endophyte strain that does not cause ryegrass staggers or heat stress, form swards that are less resilient to severe insect attack (Popay & Baltus 2001), and require more care in summer grazing management. Experienced Waikato dairy farmers who regularly assess pasture cover have reported to one of us (GAK) that post-grazing residual pasture cover on AR1 pastures in dry summers can reduce to 1100 kg DM/ha, whereas for wild-type endophyte pastures under the same conditions residual cover is 1400 kg DM/ha. AR37 endophyte that does cause some ryegrass staggers but not the other toxic effects associated with the wild-type endophyte offers excellent protection against insects (Hume *et al.* 2007) but may leave the plant open to over-grazing.

It is suggested that successful breeding against 'stemminess' (aftermath heading) in modern cultivars, in improving palatability and utilisation, may have had a similar effect in exposing plants to over-grazing. A possible negative association between persistence and forage quality has been a concern over a long period (Clements 1971).

The trade-off discussed above is characteristic of plant domestication - the more a plant is developed to meet the needs of agriculture, the more management support it requires for survival. As noted above, for forage plant breeding this has not been a major issue to date, but is likely to become more so. As plant breeders address the forage quality requirements for high-producing animals, and pursue opportunities to address greenhouse gas issues such as nitrous oxide and methane generation, such trade-offs are likely to arise - either directly as in the case of the endophyte alkaloids, or simply because attention to new traits inevitably reduces the attention that can be paid to persistence.

Populations and genetics

Ryegrass in a sward is a population at several levels. The population is comprised of tillers, each with its own roots, producing successive leaves and subtending daughter tillers. Tillers mature and die, some after becoming reproductive (Hunt & Field 1978; Korte 1986; Matthew *et al.* 1993). The ongoing health of the sward requires new tillers to replace those that die. There is a strong seasonal element of tiller death and replacement by new cohorts (Bahmani *et al.* 1997; Hunt & Field 1978; Matthew *et al.* 1993), with major loss in mid spring-early summer during the reproductive period and build-up through the subsequent months. Any factor which reduces the replacement rate of tillers is likely to cause reduced persistence.

Tillers derived from the same original seedling are genetically identical. A ryegrass sward is also a population of genotypes. A seeding rate of 20 kg/ha gives approximately 1000 seeds per m^2. The number of seedlings surviving to make a contribution to the established sward is very much lower, indicating

Figure 1 DM yield, kg.ha^{-1} over 8 months in Year 1 (vertical axis) and ground cover score after 3 years (horizontal axis) of perennial ryegrass breeding lines in Waikato 2008 – 2011

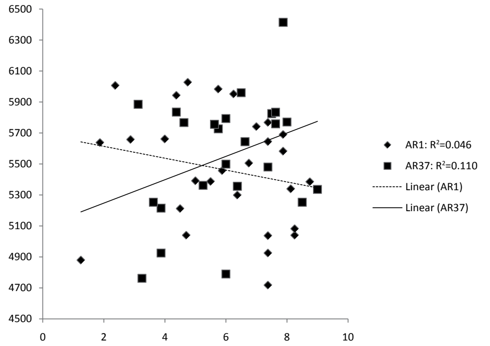

Figure 2 The plant breeding process

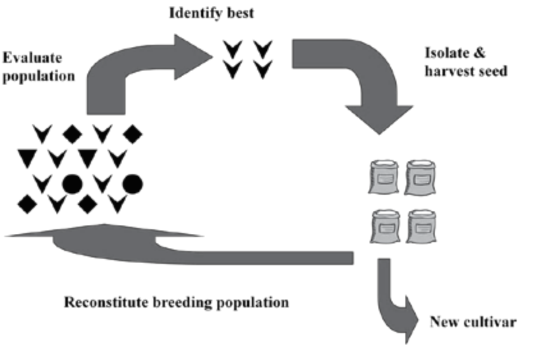

Table 1 Relative yield in first year (mean = 100) and ground cover score (Year 5) of ryegrass cultivars at Poukawa, Hawkes Bay (Muir 2009).

Cultivar	Description	Decade of release	Endophyte status[1]	Yield year 1	Ground cover, Year 5
Aberdart		2000s	AR1	99	107
Aries HD		2000s	AR1	97	104
Banquet	4N	2000s	SE	105	127
Bealey	4N	2000s	NEA2	108	118
Bronsyn		1990s	AR1	102	111
Bronsyn		1990s	SE	102	120
Cannon		2000s	AR1	93	97
Canterbury	ecotype		Nil	89	50
Commando		2000s	AR1	98	94
Extreme		2000s	AR1	100	85
Extreme		2000s	AR6	102	90
Hillary		2000s	AR1	95	94
Horizon		2000s	SE	105	97
Impact		2000s	AR1	107	118
Kingston		1990s	SE	100	108
Matrix		2000s	SE	103	118
Meridian		2000s	AR1	102	108
Nui		1970s	SE	99	108
Quartet	4N	2000s	AR1	98	113
Quartet	4N	1990s	SE	99	111
Revolution		2000s	AR1	102	111
Ruanui		1950s	Nil	93	63
Samson		2000s	AR1	103	111
Sterling	4N hybrid	2000s	AR1	99	80
Supreme	hybrid	2000s	AR1	100	57

[1]SE = 'standard endophyte,' unselected wild-type, Nil = no endophyte

competition and a high mortality rate during the establishment period, discussed by Brock & Thomas (1991). This phase represents an opportunity for natural selection to shift the mean characteristics of the population, but there is little evidence that such population shifts occur.

A low density of viable tillers can allow the establishment of new seedlings, of other species or of the sown species. In the case of new ryegrass seedlings within a ryegrass sward, these may result from germination of seed produced by the current population, or from buried seed that pre-dates the current sward. There is little evidence that seedling recruitment is important in maintaining a truly perennial ryegrass sward in NZ conditions, (but management to enhance its contribution has been suggested (Hume 1999; L'Huillier & Aislabie 1988)), however, apparently persistent ryegrass swards have been shown to be made up of hybrid ryegrass plants individually having a short lifespan, regularly replenished with seedling recruitment.

Ryegrass is not alone in the sward. It is usually infected with its fungal endophyte, and accompanied by a legume, most typically white clover. The proportions of ryegrass and white clover in the sward change with time, and stable set proportions can not be expected (Chapman *et al.* 1996). It is the resilience of this whole community that is our concern.

Parsons *et al.* (2010) pointed out that persistence, or lack of it, can be apprehended at several levels. Within a sward, resilience can be envisaged as requiring a viable, self-replacing population of tillers and plants that continue to represent the genetic integrity of the sown population within the limitations and challenges of the environment.

Most forage pasture cultivars are open-pollinated populations of genetically distinct individuals, similar in this respect to a herd or flock of livestock. They are unlike wheat, for which a cultivar is an infinitely multiplied line of genetically identical inbred individuals, or a maize cultivar which is set up by crossing inbred lines to produce innumerable copies of one or a small number of hybrids.

Traits that characterise a ryegrass cultivar (heading date, habit, seasonal growth pattern, disease tolerance) are expressed as the mean of the population, and a degree of variation about the mean is tolerated. The expression of traits is influenced by the environment, and there is interaction between genetic expression and the environmental influences.

Genetic variation for traits is the base material plant breeders work with, and to be useful it needs to be governed by genes that act consistently across environments and in different gene combinations. Genetic structures that are strongly influenced by interaction between genes, as described for ryegrass root morphology (Crush *et al.* 2006a; Crush *et al.* 2010) are more difficult to work with. Future developments may enable new cultivar structures that exploit advantageous gene interactions, as offered by the maize hybrids.

Molecular biology is identifying genes responsible for controlling different plant processes. Different DNA sequences in specific genes can be associated with varying gene function. We expect such information to offer new opportunities in plant improvement. Natural variants occur and can be searched for. Variants can also be deliberately created. This too offers opportunities in the future but faces regulatory issues.

The breeding process

As for other open-pollinated perennial pasture plants, ryegrass breeding involves cycles of seed generation, evaluation and selection (Fig. 2). Genetic gain is accumulated through cycles. Extending the period of evaluation improves the precision and validity of data captured while reducing the number of cycles completed in a period. Three full years evaluation of breeding material imposes a duration of at least four years per breeding cycle. Evaluation of new cultivars begins when the second generation of composite seed is harvested. Typically it will require at least 6 to 9 years (and sometimes 12 to 15) from identifying a new target trait to developing an experimental cultivar to be tested.

The process begins with raw material, such as plants collected from old pastures, seed imported from overseas or recently completed crosses. Evaluation can focus on individual plants (in which case they will be in spaced plant nurseries), as families of siblings or half siblings (usually in sown rows), or as experimental populations, which really allows only acceptance or rejection with no opportunity to identify better sub-groups to form the basis of the next cycle. All the above methods are used in NZ programmes.

The future

What can breeders do to better meet industry expectations of resilient pastures with the characteristics that will support high-producing livestock? Clearly, management practices need to be optimised, and ultimately the farmer is responsible for the animals and pastures that are the farm's resource. However, plant breeders and other supporting professionals need to provide resources that are adapted to conditions likely to be met, and that impose the least constraints on farm management.

Assessment: The conditions under which breeding material is assessed need continued review. Information on critical processes of plant death or persistence and their timing will be critical for this. Breeders pay most attention to their plants when they are growing, and tend not to place pressure on them when they are not growing. On farms, pressure is maintained year-round.

Marker-assisted breeding (Barrett *et al.* 2006; Faville *et al.* 2004) is no substitute for careful observation and measurement, but will allow critical and expensive phenotyping of a limited number of plants to identify markers that can then be the focus of a screen over a much larger population. It will also allow the distinguishing and more focused selection of interacting processes that are confounded in simple phenotyping (Faville *et al.* 2006; Faville *et al.* 2007; Turner *et al.* 2008).

Other new developments in phenotyping include metabolomics, using high throughput chemistry and advanced computing power to analyse patterns of variation in the array of different compounds in herbage. Variation in combinations of molecules can be indicative of plant response to environmental and other stresses.

Traits: In terms of target traits, there will continue to be a combination of consultation with farmers and supporting professionals about priorities, and the development of new opportunities that arise from innovation. There was no cry from industry or consensus amongst professionals for later heading ryegrasses, but once offered by the plant breeders, these have become a major component of the NZ perennial ryegrass market, mean 30% by weight of all certification over 2007-2009, nearer 50% if Nui is discounted for the volumes exported for non-forage purposes (AsureQuality – NZ Seed Certification Statistics 2009/2010).

Genetic gain in any trait depends on the focus applied to it, and increasing the number of traits in a selection programme reduces the focus on any one trait. Cases for inclusion of a trait in a breeding programme need to show the likely value of its improvement.

The breeding of ryegrass is intertwined with the development of endophyte strains, and it is now understood that ryegrass genotype:endophyte strain differences exist, and can be large, adding complexity to the process. More attention needs to be paid to the interaction of ryegrass with selected endophyte strains for control of alkaloid levels (Easton 2007; Easton *et al.* 2002; Fletcher *et al.* 2006) and maintaining endophyte viability in seed, thus ensuring more reliable delivery of endophyte to specification. Direct effects of endophyte on tolerance of water stress or marginal nutrient levels have been described for tall fescue (Malinowski & Belesky 2000). Research has identified limited evidence of such effects for ryegrass (Barker *et al.* 1997; Crush *et al.* 2004; Spiering *et al.* 2006) and further work is warranted.

Attention also needs to be paid the critical points in the year, season or grazing cycle when sward viability is at risk. Cosgrove's analysis of old data (Cosgrove 2011, this volume) implies a strong case for greater attention to summer grazing, as earlier recommended by Matthew *et al.* (1993). These conclusions are in line with the classic data of Brougham (1960). Further research on the processes and points in the life cycle that are critical to persistence under modern and future conditions will enable a better focus.

Resistance to pests and diseases is a recurring theme in plant breeding. For ryegrass breeding in NZ, resistance to crown and stem rusts is a constant (Easton *et al.* 1989b; Lancashire & Latch 1970). In the last 20 years, pest resistance efforts have focused on endophyte research, but more recently work has begun on plant genetic resistance within populations infected with a known endophyte not offering resistance (Popay & Easton 2006; Popay & Gerard 2007). Nematodes (Mercer *et al.* 2008), bacterial wilt of ryegrass (Schmidt 1995) and viruses (Webster *et al.* 1996) are other organisms that have been considered as targets for resistance breeding.

Physiological traits such as tolerance of water stress (Crush *et al.* 2007; Wedderburn *et al.* 1992) and high temperature, and the form and function of roots (Crush & Nichols 2010) are currently under investigation. In modern agricultural conditions the plant's need to forage for nutrients in the soil's A horizon is less paramount than under natural conditions, and the ability to maintain access to water lower in the soil profile for continued growth assumes more importance. More radical options leading to greater efficiency of use of water are in the future. Tolerance to aluminium stress has been shown to be heritable (Bennet & Stewart 1999; Wheeler *et al.* 1992), but is not usually regarded as important in NZ.

Breeding material: As discussed above, the source populations from which cultivars have been bred in NZ are old pastures in this country, with north west Spain being an important exotic source (Stewart 2006). There has been successful use of other sources but, in general, material from elsewhere tends to be poorly adapted to our conditions. However, as breeders pursue new traits (physiological, forage quality, environmental mitigation), and as NZ farming faces a greater frequency of extreme weather events, genetic resources from other regions will become more important. These will not be immediately usable, but require crossing and back-crossing with locally adapted material, with critical experiments to identify valuable gene combinations and secure them in improved populations. Marker-assisted breeding will be essential in this process. Use of Festuloliums and introgression from tall and meadow fescue into perennial ryegrass has led to cultivars in overseas markets, and has been a feature of the Cropmark programmes in NZ (Cropmark 2011). At this point there is no documentation that the fescue genetics has provided a decisive advantage, except perhaps in cold and heat tolerance for climates more extreme than NZ's. Again though, the more ready availability of marker-assisted breeding will enhance the effective use of inter-species crosses.

Cultivar evaluation: New cultivars are typically evaluated at a number of representative sites over several years before they are commercialised. In-house evaluations are complemented by joint National Forage Variety Trials (NFVT) administered by the NZPBRA (Easton *et al.* 2001a), in which data for all entries can be scrutinised and critiqued by all participants. While the process is not transparent to the wider science community or user industries, it is governed by scientific norms and vigorous critique between the different breeding interests. Focus is on annual and seasonal herbage yield over the life of 3-year trials under controlled intermittent grazing or mowing. Breeding lines lacking persistence fail routinely after one or two summers and it is possible in many circumstances to predict persistence from data in these early years (Camlin & Stewart 1978).

The critical assessment of key processes in sward survival needs to extend to the evaluation of cultivars, and key pressure points that are typical on farm need to be simulated. That has always been the intent, but protocols need more regular examination in the light of evolving farm practice. Constraints needed for optimal performance of particular cultivars need to be communicated, and factored into value propositions. There is a tension between keeping a message simple and providing the necessary information for optimal use of a product.

Historically, most successful cultivars have been used throughout NZ (and in south eastern Australia). The future may require more targeted use of specific cultivars in different situations. In particular, the emphasis to be placed on persistence relative to other traits varies with the environment and the farming system. Further, the critical traits in persistence will vary with environment and management.

Confidence that the characteristics described and verified for a cultivar are delivered in advanced generation commercial seed crops is the rationale of the certification system. Concern that natural selection or contamination may drive advanced generations to drift from the descriptions based on early generation seed (Parsons *et al.* 2010) have been raised at various times, and earlier work suggested some drift of acceptable proportions (Easton & Barclay 1973; Rumball 1970). More recently developed traits will provide more critical data sets, and further experimental verification of the integrity of the system is warranted.

Conclusion

Expectations of pasture plant breeding in NZ have never been higher, and with the evolving demands placed on livestock farming and the pastures supporting them will only intensify. Competitive free-market activity has served NZ well in developing a range of well-performing cultivars. Meeting the challenges of the future requires a degree of coordination in that all serious players need to interface with research in pasture ecophysiology, environmental science, animal science and farm systems to identify key traits. Screening of diverse germplasm under conditions that match critical elements of on-farm management, and careful observation of key traits are essential if more radical progress is to be made. New developments in genetic and plant breeding methodologies will enhance our ability to deliver.

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