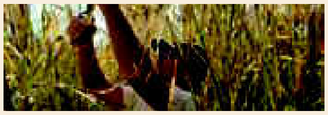

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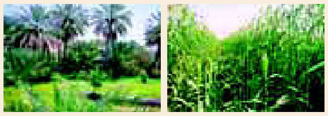

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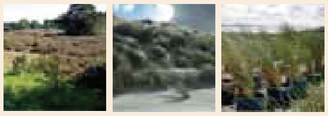

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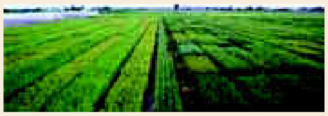

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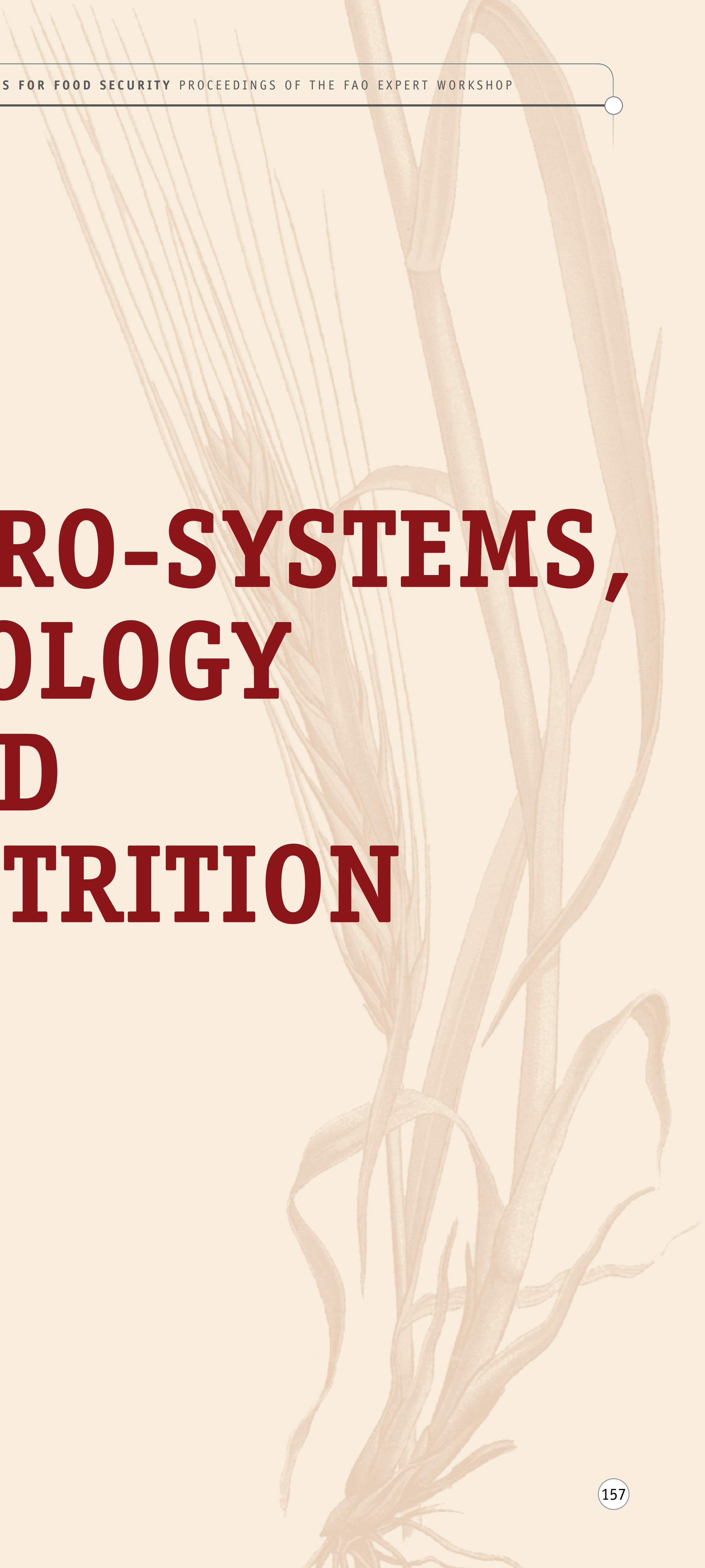

AGRO-SYSTEMS, ECOLOGY AND NUTRITION

12

FROM GENETICS AND BREEDING TO AGRONOMY TO ECOLOGY

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ABSTRACT

With annual grain monocultures, there is frequent clear-cutting of crop root systems; as a result, ecosystem processes below the soil surface cannot sustain themselves in a healthy state. Each year, we disrupt this rich ecological resource, which otherwise could have been contributing to high, stable grain yields. The science of agronomy arose out of the necessity to cope with such a compromised landscape and make up for lost ecological processes. But we are severely limited in what we can accomplish with the "software" we call agronomy as long as we are saddled with the deficiencies of the annual crop species that have always served as the "hardware". Once perennial grain-producing species become available, however, soil ecosystems can contribute much more to food production, while much of the landscape-repair work that we now call upon agronomy to provide will not be necessary. Perennial hardware will open up innumerable possibilities for bringing new ecological software into agriculture. An example is provided by the potential development of perennial sorghum for tropical regions. To date, breeding of winterhardy grain sorghum has occurred solely in North America. When those breeding populations begin to be evaluated in diverse tropical environments, selection for proper adaptation will be essential. Once that is accomplished, an interim objective may be to develop a "super-ratooning" sorghum. But in the longer term, a true perennial growth habit will make possible whole new farming systems that combine sorghum with perennial food legumes and other crops. We can make such systems productive and ecologically sound without resorting to many of the agronomic interventions that annual grain crops require.

Keywords: crop root system, soil ecosystem, grain sorghum, perennial food legumes, agriculture and ecology

INTRODUCTION

In recent years, the potential benefits of perenniality for agriculture have come to be widely recognized. For example, in 2009, a group of researchers assembled by the Royal Society in the United Kingdom endorsed the idea of perennial grains. They noted, "Perennial crops would store more carbon, maintain better soil and water quality and would be consistent with minimum till practice. These crops would also manage nutrients more conservatively than conventional annual crops, and they would have greater biomass and resource management capacity" (Royal Society, 2009).

In a further discussion of perennial grains' potential, the National Research Council of the National Academy of Science in the United States went into more detail with the following statements (National Research Council, 2010):

- Perennial plants reduce erosion risks, sequester more carbon, and require less fuel, fertilizer, and pesticides to grow than their annual counterparts.
- Perennials have greater access to resources over a longer growing season.
- Perennials have greater ability to maintain the health and fertility of a landscape over longer periods of time.
- Recent advances in plant breeding... provide new opportunities for plant breeders to select for desired characteristics.

In a policy paper in the journal *Science*, twenty-eight United States and international scientists called on plant breeders and geneticists worldwide to launch an all-out effort to develop perennial grain crops. (Glover *et al.* 2010). Then in August 2013, perennial grain researchers from around the world met in Rome for their first global meeting: the FAO Expert Workshop on Perennial Crops for Food Security (this volume).

These and other recent events have been prompted by the recognition that substituting perennial cereals, grain legumes, and oilseeds for their annual counterparts would constitute a major change of crop "hardware", enabling agriculture to support a vast new range of cropping-system “software.” The transformation will make possible food-producing ecosystems that function as well as healthy, fully intact natural ecosystems. Previous efforts to create such systems have been limited by our dependence on the problematic software of annual crops. Perennial grains will provide a solid foundation on which necessarily complex food-producing ecosystems can be built.

In doing this work, it will be essential to draw upon the extensive research that has been carried out by ecologists over more than a century. Embedded within wild ecosystems are answers needed in the design of current and future agricultural ecosystems. Processes that have run for many millions of years, some known and some yet to be discovered, are of paramount interest if we are to develop a sustainable, resilient agriculture. Moreover, successful use of the ecosystem as a conceptual tool can be expected to provide a bonus: greater social and economic justice for farmers, their communities, and the wider society.

Were we to stop with the development of perennial monocultures, we would miss the opportunity to realize the robust potential that biological diversity has to offer. To achieve sustainable nutrient management, stabilize or prevent insect and disease damage, optimize water use, take advantage of complementary growth periods, and ensure other important features of low-input farming will require information drawn from diverse natural ecosystems. Fortunately, the principles of ecosystem function derived from studies of grasslands, forests, and other non-cultivated landscapes are available for adoption (Jackson and Piper, 1989).

FROM AGRONOMY TO ECOLOGY

Starting with the invention of agriculture, annual grain monocultures have by definition required removal or disturbance of entire vegetative structures and communities. Ecosystem processes below the soil surface, opaque to the farmer, were crippled. This annual “clear-cutting” of the soil, which rendered a vast array of ecological processes unavailable to the food producer, has come at a heavy cost in degraded and eroded soil, water, and biodiversity.

The science of agronomy arose out of the necessity to mitigate and substitute for disrupted ecological processes in order to ensure a consistent food supply. Agronomy is a discipline forced to cope with a compromised landscape and make up for lost ecological processes through management. It can be viewed as a ten-thousand-year-old success story accomplished first by farmers alone, and in more recent times, by highly trained experts working with technologically astute farmers, backed up by fossil fuel subsidies and an industrialized, growth-oriented economy. But it is an unsustainable success story. With perennial cereals, grain legumes, and oilseeds, however, diverse ecological processes formerly denied to agricultural landscapes will have a chance to be reactivated and sustained over the long term.

The notion that native perennial ecosystems constitute the best model for agriculture is an acknowledgement and appreciation of the complexities and efficiencies in ecological relationships that have come about through community succession and natural selection operating on individual organisms over very long periods of time. As Jackson and Piper (1989) noted, “Nature is ill-defined, and natural ecosystems are dynamic. But the patterns and processes discernible in natural ecosystems still remain the most appropriate standard available to sustainable agriculture.”

Deciding which aspects of non-agricultural ecosystems should inform the design of food-producing systems remains an open question. Denison *et al.* (2003) asked to what extent the outcomes of natural selection in native systems are congruent with the goals of agriculture:

> *We present two main hypotheses. Our first hypothesis is that natural selection had ample opportunity, before the wild ancestors of our crops were domesticated, to test alternative solutions to problems that limited individual fitness under preagricultural conditions... further improvement of these traits is likely to be difficult. Instead, opportunities for further genetic improvement of crop yield will mainly involve tradeoffs between plant adaptation to agricultural versus natural conditions, or between the competitiveness of individual plants and the collective performance of plant communities. Our second main hypothesis is that natural selection is the only reliable source of improvement (by any definition relevant to agriculture) in natural ecosystems that operate on a time scale longer than the lifetime of individual plants. Natural selection acts at the level of genes, individuals, and family groups, not communities and ecosystems. Therefore, our second main hypothesis is inconsistent with the suggestion that agricultural ecosystems whose structure is based on natural ecosystems will be consistently more efficient, sustainable and productive. Although this hypothesis rejects mindless mimicry, natural ecosystems, properly understood, are nonetheless a valuable source of ideas for agriculture.*

The perspective of Denison and colleagues has been valuable in motivating a deeper analysis and greater clarity in describing the value of natural ecosystems as conceptual tools for grain-producing agriculture. One crucial realization that has become clearer than ever is the extent to which their arguments rest implicitly on the assumptions that 1) progress in crop development equates to increased yield alone, and 2) the continued protection and facilitation of high yields with purchased inputs is both possible and desirable. An agriculture that requires minimal inputs and is resilient to changing environmental conditions is very different from the agriculture underlying Denison and colleagues’ critique.

Natural selection acting on genes, individuals, and family groups can result in consistent properties that are manifest at the community and ecosystem level. This is different from selection happening at the community and ecosystem level. Consider, for example, the ability of members of the legume family to host nitrogen fixing bacteria in their roots. This mutualism is clearly the outcome of natural selection acting on legume plants and rhizobia bacteria; however, the entire ecosystem benefits from this mutualism as the N fixed by it is released for other organisms to appropriate upon legume senescence (Hooper and Dukes, 2004, DeHaan *et al.*

2010). The same is true for other nutrient acquisition strategies (e.g. Vance *et al.* 2003). In an agricultural system in which all nutrients are not provided in the form of mineral fertilizers, this type of community-level interaction becomes not only relevant but essential.

The life history strategy of perennials and their near-universal dominance of land ecosystems is another example of natural selection acting on the level of genes, individuals, and family groups and in turn predictably influencing community and ecosystem-level functions. While perenniality confers numerous advantages to individual plants existing in communities, it also prevents nutrient loss, soil erosion, weed invasion, and other ecological outcomes that benefit the ecosystem as a whole, whether native or agricultural (Picasso *et al.* 2008, Glover *et al.* 2010).

A contrasting example is provided by diversity. It is a reliable, naturally occurring characteristic of almost every native ecosystem, not because of natural selection, but more because of limits on the ability of natural selection to produce organisms that perform optimally under all environmental conditions in time and space. A diverse native plant community necessarily includes diversity of root architectures, leaf tissue chemistries, and other features. And when functioning together, these diverse plant attributes help prevent nutrient loss, explosion of insect herbivore populations, invasion of noxious weeds, and other ecological outcomes highly beneficial to agriculture (Ratnadass *et al.* 2012, Vandermeer, 2012). If insects and weeds are not to be controlled with pesticides, and prevention of soil erosion is to be independent of herbicide use, and if nutrient losses are not made up by application of non-renewable fertilizers, the ecological derivatives of diversity become even more important.

Perennialism per se, as suggested above, does constitute a significant mimicry of nature, and likely will bring with it a range of beneficial ecosystem functions. But it is important that we do not settle for the degree of improved ecosystem function that would be conferred, for example, by single-species stands of grain-producing perennial grasses. There is a reason why such an ecosystem is never seen in nature: it is not sustainable over time. Therefore, two strains of perennial grain research—breeding and ecology—must be carried out jointly.

BREEDING PERENNIAL GRAIN SORGHUM

Researchers aiming to develop food-producing ecosystems based on perennial grain crops face a chicken-and-egg situation: the perennial crop breeder would like to know more about the system into which the new species will be incorporated, but to study alternative systems, the ecologist needs the new crop species that do not yet exist. Nevertheless, much can be learned from experimental systems that incorporate the intermediate products of perennial grain breeding. Perennial sorghum provides one of many examples.

Sorghum is a tropically adapted genus. To survive and produce grain for more than one season in a temperate climate, a sorghum plant must not only produce rhizomes; those rhizomes must also be cold-tolerant enough to survive through the winter at between 2 and 20 cm below the soil surface and produce ramets that emerge in the spring. The Land Institute launched its research on perennial sorghum in the early 1980s by making hybrids between induced tetraploids of *Sorghum bicolor* and various rhizomatous, winterhardy accessions of the tetraploid grass *S. halepense*. Populations derived from those crosses, as well as from backcrosses to the *S. bicolor* parent, were evaluated in the field in Kansas, and the results were published by Piper and Kulakow (1994). They concluded that breeding of a perennial grain sorghum was feasible but would require repeated cycles of selection for perenniality and backcrossing to improve yield and other traits. Meanwhile, using RFLP mapping, Paterson *et al.* (1995) showed that genomic regions associated with rhizomatousness and perenniality in a diploid population derived from a *S. bicolor* x *S. propinquum* cross, when evaluated in a south Texas field environment, were scattered across at least nine of sorghum's ten linkage groups.

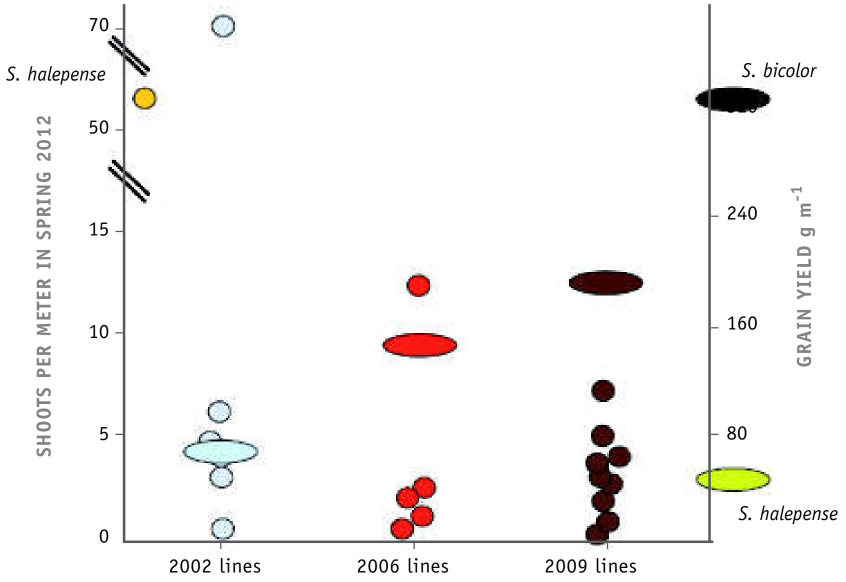
These basic studies were followed by the initiation, in 2001, of a perennial sorghum breeding programme at The Land Institute. The initial crosses were made between perennial selections from Land Institute populations and diploid grain sorghum inbred lines, to produce tetraploid segregating populations. Each year, we have selected perennial plants with improved cropping traits from annual x perennial or perennial x perennial populations and then backcrossed them to a range of annual lines, to capture more diversity from the *S. bicolor* gene pool. We have also introduced additional *S. halepense* accessions as parents.

In these populations, degree of perenniality is not associated with grain yield but is negatively correlated with kernel weight. Furthermore, most perennial selections tend to have traits that are generally undesirable in a crop plant: often excessive tillering and branching, non-synchronous flowering and maturity, excessive plant height, and small, hulled, brown kernels. Some of these associations, such as between rhizomatousness and aboveground tillering, may result from pleiotropy. Others, such as between winterhardiness and low kernel weight, may result from a low frequency of recombination between chromosomes of the two species in regions surrounding the genes required for perenniality. With Andrew Paterson at the University of Georgia, we have studies underway to elucidate more fully the basis for these negative associations (Paterson, this volume).

Despite the negative trait associations, selection and backcrossing have succeeded in increasing productivity while maintaining perenniality. In 2011-13, we ran a field trial comparing perennial families selected at three stages of The Land Institute's breeding program: 2002 (the original perennial parents), 2006, and 2009. Grain yield and kernel weight have increased with backcrossing and selection, but have not attained the levels of commercial grain sorghum (Figure 1). Perenniality is not as strong as that of *S. halepense*, but here, moderation is desirable; for a crop plant, the aggressive rhizome development displayed by the weedy parent would be an unnecessary and unproductive waste of resources. Among these selected lines, there was no correlation between degree of perenniality and grain yield; however, perenniality was associated with low weight of individual kernels (r=-0.46**).

FIGURE 1. NUMBER OF SHOOTS EMERGING IN SPRING PER METRE OF ROW IN SPRING, 2012, FOR 'GYPSUM 9' (*SORGHUM HALEPENSE*) AND 27 PERENNIAL SORGHUM FAMILIES SELECTED FROM THREE STAGES (2002, 2006, AND 2009) OF THE LAND INSTITUTE'S BREEDING PROGRAMME (CIRCLES AND LEFT Y-AXIS), TOGETHER WITH MEAN GRAIN YIELDS OF THE THREE GROUPS OF SELECTIONS (OVALS AND RIGHT Y-AXIS, WITH YIELDS OF GYPSUM 9 AND A COMMERCIAL GRAIN SORGHUM HYBRID, PHILLIPS 664, INDICATED ON THE AXIS.)

Yields are from first-year harvests of Salina, Kansas, retrospective trials in 2011 and 2012, with each year's trial in a randomized complete block design with four replicates. The experimental unit was a 3 m row.



Recently, we have selected lines potentially superior to those that were evaluated in the retrospective trial. These have come from crossing the *S. bicolor* inbred line BTx623 with plants from 'Gypsum 9', a *S. halepense* population collected near the town of Gypsum, Kansas in 2009. This cross was initiated to create populations for mapping loci associated with perenniality and other traits and monitoring recombination frequencies in interspecific hybrids of tetraploid sorghum (Paterson, this volume). But these populations have also become a valuable source of perennial breeding parents that have a more domesticated plant type and higher grain production. In these populations, associations between perenniality and deleterious wild traits appear to be weaker. Forty-nine partially winterhardy F_2-derived F_3 families derived from a BTx623 × 'Gypsum 9' cross and evaluated in 2011-2012 did not differ in mean height, flowering date, grain yield, and threshability from their winter-tender siblings (Table 1). However, strength of perenniality, expressed as the proportion of plants in a family that re-emerged in spring, had loose negative correlations with grain yield per plant and threshability.

TABLE 1. MEANS OF SIX TRAITS FOR GROUPS OF SORGHUM FAMILIES

49 families that had a percentage of spring emergence greater than zero, and 122 families with zero emergence, in a 2012-2013 field trial in Salina, Kansas. The families were F_3 progenies of 171 F_2 plants from a BTx623 (*Sorghum bicolor*) × Gypsum 9 (*S. halepense*) hybrid. They, together with parents, were evaluated in randomized, nonreplicated three metre rows with 1 metre between rows. Standard errors of means were based on variances of families within groups. Free-threshing index was the ratio of the weight of an approximately 5 g dehulled grain sample to the weight of the sample before dehulling; therefore, it has a possible range of 0 to 1. Correlations are rank correlations over 50 data pairs: 49 pairs of means of the families containing surviving plants, plus a single pair of means computed over all families that had zero survival.

		MEAN					
GROUP, BY SPRING EMERGENCE	No. OF FAMILIES IN GROUP	PROPORTION OF PLANTS EMERGED, SPRING	No. RAMETS, AUTUMN	PLANT HEIGHT	FLOWERING, DAYS AFTER 30 JULY	GRAIN YIELD PER PLANT, DEHULLED	FREE-THRESHING INDEX
No plants emerged	122	0	0.75	236	25.9	42	0.71
Some plants emerged	49	0.28	1.38 a	247	24.4	45	0.68
Correlation versus proportion of plants emerged			0.25	0.10	0.10	-0.34*	-0.31*

a Significantly different from the mean of the “no plants emerged” group ($P<0.05$)

* Significantly different from zero ($P<0.05$)

We still struggle to select for perenniality independently of other wild traits; however, among perennial BTx623 × ‘Gypsum 9’ selections, and among rhizomatous but cold-sensitive selections from other crosses, it has been possible to identify much more croplike lines that prove winterhardy in the warmer southeastern United States. These selections presumably would be perennial in the tropics as well. Therefore, we have been generating a second gene pool as a base population for breeding perennial sorghum for the tropics and semitropics. Breeding for tropical adaptation, which would start with crosses between temperately adapted perennial lines and tropically adapted annual lines, has not yet started. When it does, a medium-term outcome along the way to developing a rhizomatous perennial sorghum could be an improved rattooning sorghum that grows more vigorously and produces higher yields in the second (and possibly subsequent) season than do current annual cultivars (Paterson, this volume).

The current perennial sorghum gene pool bears only a loose resemblance to the annual grain crop. Dwarf plants are rare, and tillering is excessive. Ramet emergence, tillering, flowering, and maturity all are highly asynchronous, tending to extend over periods of weeks. Until these characteristics are adjusted through breeding, perennial sorghum in temperate regions will remain unsuitable for mechanized cultivation and harvest, whatever the degree of improvement in grain yield. However, in regions where hand harvesting and crop polycultures are the norm and where tall stover is harvested for fodder or other uses, tall plants and asynchronous maturity would not necessarily be obstacles to adoption. And perennial cultivars could have advantages beyond erosion prevention. In many parts of the tropics, one of the most dire problems facing sorghum farmers is poor stand establishment, thanks to adverse soil and moisture conditions. With perennial sorghum, there would be no need to achieve a good seedbed and precise seed placement under adverse conditions; furthermore, the shoots and roots of ramets emerging from deep rhizomes would be much more robust than those of seedlings.

But while presenting opportunities for adoption of perennial sorghum, tropical regions raise new challenges as well. Tropical environments are very diverse, and with seasonal variation in temperature and rainfall, most are not friendly to sorghum cultivation year-round. Research is needed to determine which environmental signals will be required to keep perennial sorghum plants alive but not actively growing during the off-season. In temperate zones, the onset of cold weather enforces several months of dormancy, after which new perennial plants emerge much earlier in the spring and grow more rapidly than do annual plants. But in the semiarid tropics, for example, would a perennial sorghum plant's rhizomes be able to survive deep in the soil through a long dry season and emerge with the return of rains? Answers to these and other questions have not yet been sought.

PROSPECTS FOR ECOLOGICALLY SOUND PRODUCTION

In trying to visualize agricultural ecosystems that include perennial sorghum and other perennial grains, the chicken-and-egg problem remains. How do we design ecologically sound production systems for new crops as long as the best of their germplasm is still half-wild? Will data gleaned from experiments using proto-perennial grains be applicable to cropping systems for fully domesticated cultivars once they are developed? We cannot know the answers to those questions in advance, but some insights could come from forging ahead with field research that employs today's crops-in-the-making.

Throughout tropical and semitropical regions, supplying grain crops with biologically fixed nitrogen, together with other nutrients, is an urgent need. If, as a first step toward a full-blown food-producing ecosystem, perennial sorghum is to be incorporated into a biculture, the obvious choice of partner species would be legumes—preferably perennial. The sorghum plant would derive the greatest fertility benefits from a non-grain legume that is included solely in a nitrogen-fixing role. But where increased food production per hectare and diet diversification are top priorities, an cereal-plus-grain legume intercrop is more appropriate. In Malawi, for example, Snapp *et al.* (2010 and this volume) have been investigating systems for growing pigeon pea (*Cajanus cajan*) as a perennial grain legume intercropped with annual maize (*Zea mays*). They have shown that "Semiperennial rotations provided twofold superior returns, whereas diversification of maize with annual legumes provided more modest returns" as compared with maize monocrops. These and other results provide an incentive, once perennial sorghum germplasm has been screened for adaptation to local conditions in areas where pigeon pea is well-adapted, to run small-scale pigeon pea intercrop experiments that include preliminary breeding lines of perennial or enhanced-rattooning grain sorghum. Such studies could proceed across a range of environments, in parallel with continuing breeding efforts until acceptable perennial sorghum cultivars are developed.

We are also investigating whether in this or other systems, perennial sorghum might supplement its nutrition through endophytic nitrogen fixation. It has been found, for example, that *S. halepense* harbours in its rhizomes nitrogen-fixing endophytic bacteria that also can make phosphorus more available and chelate iron (Weiman, 2012). As a genus, *Sorghum* also has a strong tendency to form associations with arbuscular mycorrhizal fungi and thereby increase its phosphorus uptake under certain conditions (Neumann and Eckhard, 2004); perennial sorghums for low-input systems should be evaluated for their ability to gain access to phosphorus and other nutrients through mycorrhizal associations.

With perennial sorghum, the development not only of farm-ready cultivars but also of ecologically-based cropping systems will probably be achieved first in the tropics. In many regions of Africa, Asia, or Latin America, a biculture of a perennial or rattooning cereal like sorghum with a perennial pulse could be a highly effective system for providing staple foods while reducing soil degradation, during the transition to more ecologically durable systems. Meanwhile, both resistance breeding and ecological research will be needed to address yield losses to pathogens, pests, and weeds, especially parasitic weeds like striga in the case of sorghum. More complex perennial polycultures involving a range of plant families will need to be evaluated, not only for productivity but for ecological functioning as well. For example, soil water and nutrient uptake efficiencies can be increased in polycropping arrangements where soil resources are partitioned in time or space.

Where agro-forestry is already being employed, integration with herbaceous perennial grain crops should be explored. Trees can improve crop microclimate conditions and provide nutrient inputs through biological N fixation and in some cases by bringing phosphorus and other rock-derived nutrients to the surface via roots from deep in the soil profile (Buresh, 1995). This could be particularly important in tropical regions with highly weathered, nutrient depleted topsoils. Some of this work can inform the eventual development of new cropping systems in the temperate zones, but much location-specific work will be required there as well.

The details will differ for other perennial crops, for other combinations of crops, and for every environment. But the ultimate goal remains constant: by adding perennial grain crops to the agricultural inventory, we can finally create opportunities for a deep synthesis of agriculture and ecology.

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ECONOMICS AND SYSTEM APPLICATIONS FOR PERENNIAL GRAIN CROPS IN DRYLAND FARMING SYSTEMS IN AUSTRALIA

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ABSTRACT

The development of perennial grains could provide a number of sustainability and farm management benefits for Australian dryland crop-livestock farming systems. Whole-farm bio-economic modelling has shown that perennial wheat would have greatest economic feasibility if it had dual-purpose attributes by providing additional forage post-harvest (during summer) and early in the winter growing season. This accrued from the ability to increase livestock numbers without a proportionate reduction in returns from grain production. Grain-only perennial wheat achieving similar prices would require yields of 60-100 percent of annual wheat to compare with current systems, while dual-purpose perennial wheat was still economically favourable with grain price AU$35/tonne less and grain yields 40 percent of annual wheat. In all cases perennial wheat would be most attractive on soils or situations where current annual cereal systems are most marginal. Cost-benefit analysis based on modelled increase in farm profit (AU$20/ha farm area), suggests that a 20 year investment in perennial wheat would result in a 10-fold return if it was adopted on 450 000 ha assuming 75 percent chance of success. While perennial wheat would have the largest impact in Australian farming systems, the development of perennial legumes for dual-purpose grain-grazing could also offer some potential. Several native Australian legumes have been assessed for their suitability and there are several short-lived perennial legumes that could have potential in such a system both in Australia and elsewhere (e.g. *Lablab purpureus*). Finally, perennial grain crop development should consider the range of farming systems where they might be used including facultative perennial systems, phase rotations (e.g. 2-4 year long rotations), companion or relay cropping (oversowing them with other crops/pastures) or polycultures involving a range of perennial species.

Keywords: dual-purpose, modelling, rotations, polyculture, companion cropping, cost-benefit

INTRODUCTION

Annual cereal crops, mainly wheat, in rotation with annual pastures have dominated grain production systems in Australia. This reliance on annual species has caused environmental problems such as dryland salinity, soil erosion and degradation, nutrient leaching and eutrophication. Reintroduction of productive and profitable perennial plants into agricultural landscapes to more closely mimic the original vegetation by increasing ground cover and annual water use can address many of these problems (Hatton and Nulsen, 1999). Farming systems incorporating agro-forestry and perennial forage plants are being utilised in many areas (Bell *et al.* 2013), but perennial grain crops could also provide a major opportunity to improve the sustainability of agricultural systems without the need to discontinue cropping activities (Glover *et al.* 2010; Bell *et al.* 2010b).

Perennial grain crops might be developed from either domestication of promising wild species or via hybridization of current annual crops with their perennial relatives (Cox *et al.* 2002). Both these avenues hold promise for developing perennial grain crops suitable for Australian conditions, but significant breeding effort would be required (Larkin, 2013). While breeding a genetically stable, productive and persistent perennial crop holds many challenges it is vitally important to consider how a perennial crop might be used in a farming system. This can provide insights that will guide the characteristics required in the crop and their relative importance for breeding efforts. While a number of sustainability benefits from perennial wheat are predicted and cost saving such as reduced tillage, fertiliser requirements and energy inputs are anticipated, the relative profitability of perennial grain crops compared with conventional annual systems needs to be analysed to justify investment in perennial crop development. This can also potentially expose the economic trade-offs between different attributes and help identify diverse crop ideotypes that might be valuable in different farming systems. This paper will provide a summary of some whole-of-system economic analysis conducted in Australia and consideration of diverse options for integrating perennial grain crops into farming systems. This provides some useful insights into priorities and strategies, and identifies opportunities for perennial grain crop development more widely. In particular, much attention has been applied to perennial wheat hybrids, yet there may be opportunities for complementary perennial grain legumes or other cereals either from direct domestication of native species or targeted breeding of other species.

PRELIMINARY ECONOMIC ANALYSIS OF PERENNIAL CEREAL CROPS IN AUSTRALIAN FARMING SYSTEMS

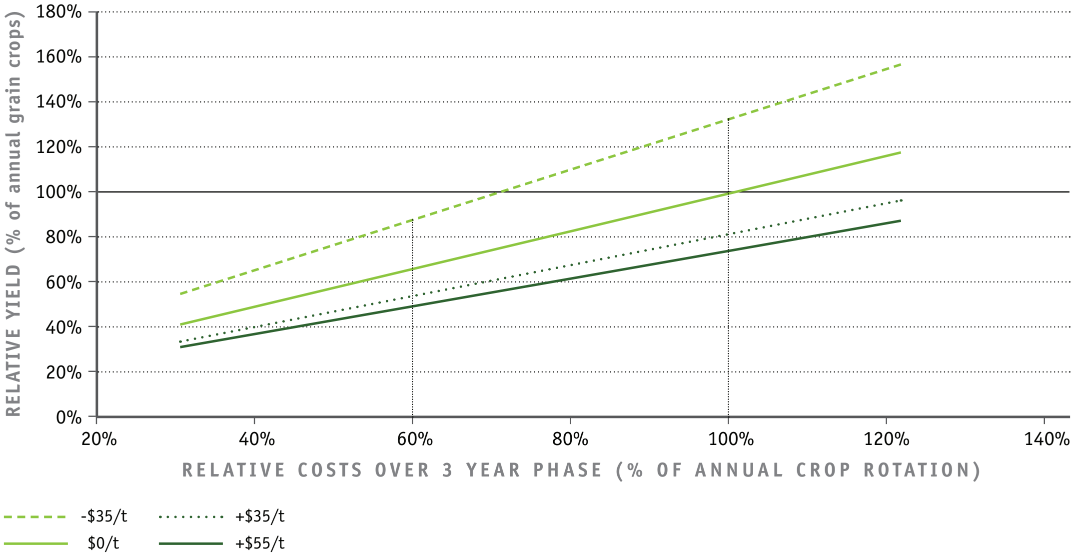
Grain-only production

Gross margins and whole-farm economics of a perennial cereal utilised for grain production were only compared with returns from conventional annual crop-based systems (based on a wheat-wheat-grain legume rotation). Yield, price and costs for the annual crop rotation were drawn from data for the medium rainfall regions of south-western Australia (350-500 mm mean annual rainfall) (more details are available in Bell *et al.* 2008). Because the income and costs for a perennial cereal system are uncertain, the sensitivity of break-even profitability was explored across a range of relative grain price, yield and variable production costs between the perennial and annual crop phases.

Figure 1 depicts the relationships between relative grain yield, growing costs and grain price on the relative profitability of a perennial crop compared with a typical annual crop rotation. This demonstrates the importance of the likely market for grain products on the cost-price requirements for a perennial grain crop. For example, if a grain-only perennial crop received a price premium or incentive payment of >US$35/tonne then it would achieve equal economic returns with grain yields 70-80 percent of annual crop yields at the same production cost per hectare. On the other hand, a lower quality grain product, similar to an animal feed grain receiving US$35 less per tonne than a milling grade product would require significantly higher grain yields than an annual grain crop system; an unlikely scenario for a perennial grain crop. One claim is that perennial grain crops will have lower production costs due to savings from less frequent sowing, herbicide applications and lower fertiliser replacement requirements (Crews 2005). Bell *et al.* (2008) estimate this could be 60 percent of an annual crop system. This would enable grain yields to be 50 percent and 65 percent of annual crops if a perennial cereal received a US$35/tonne price premium or the same price as an annual cereal crop, respectively. A perennial cereal receiving a lower price differential would require grain yields of >80 percent of an annual crop rotation to obtain a similar return.

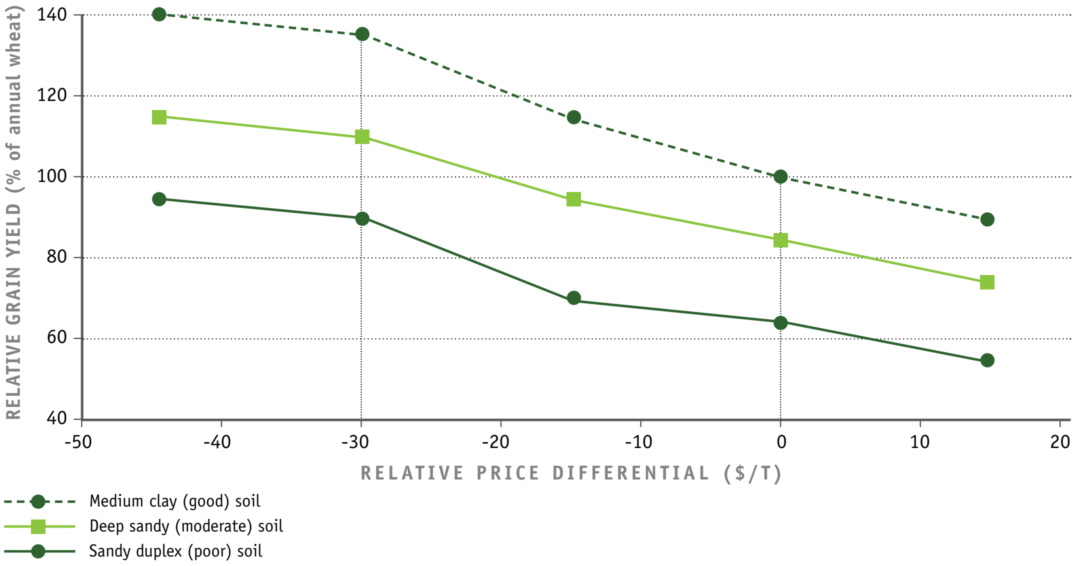
This analysis in Figure 1 was based on a 3-year phase of a perennial crop but the longevity of a perennial crop phase is also a factor that may impact on its relative profitability compared with annual crop systems. The yield required for a perennial crop to compare with an annual crop system declines with the duration of a perennial crop phase, as establishment costs are spread over more years. However, this reduction in grain yield required is small (<3 percent) once the duration of the perennial crop rotation is greater than three years because annual production costs (i.e. replacement fertiliser, harvesting costs) remain consistent. This suggests that unless perennial wheat yields are stable or increase with age of the stand, then there is little direct economic advantage in long-lived perennial crops. This is especially pertinent when considering potential trade-off that may exist between plant grain yield and longevity (see DeHaan *et al.* 2005).

FIGURE 1. GRAIN YIELD REQUIRED BY GRAIN-ONLY PERENNIAL CEREAL TO OBTAIN SIMILAR 3-YEAR GROSS MARGIN RETURNS TO AN ANNUAL CROP ROTATION (WHEAT-WHEAT-GRAIN LEGUME) ACROSS A RANGE OF RELATIVE GROWING COSTS AND GRAIN PRICE DIFFERENTIALS FOR PERENNIAL WHEAT COMPARED WITH THE ANNUAL CROPS



A perennial grain-only cereal crop that yielded 60 percent of an annual wheat crop with 60 percent of the variable costs but receiving US$35/tonne lower price was made available in a whole-farm bio-economic profit optimising model (MIDAS) (Morrison *et al.* 1986). The whole-farm model captures many of the biological and economic interactions that occur across a whole farm including variation in soil capabilities (denoted by different land management units with specific production and cost structures), rotational impacts and farm overheads (for more detail refer to Bell *et al.* 2008). Using the standard production/cost assumptions, a perennial grain crop producing only grain was not chosen on any land management units. The shadow or opportunity cost for a perennial cereal to be adopted on the farm was lowest on the less productive soil types (around US$25/ha), but was much higher on land management units where other annual crop and pasture systems were more profitable (US$40-100/ha). Subsidisation or other additional systems benefits would have to be larger than this to encourage adoption of a grain-only perennial cereal. Figure 2 demonstrates the relative grain yield and price required by a perennial cereal crop for it to be adopted across soil types differing in their productivity on a profit-maximising farm. This indicates that a grain-only perennial cereal is most likely to be adopted if it can be used on poorer or more marginal soil types for annual crop and pasture systems. On the most productive and profitable soil types grain returns similar to annual cereal crops would be required to displace current systems.

FIGURE 2. RELATIVE GRAIN YIELD REQUIRED FOR GRAIN-ONLY PERENNIAL CEREAL AT DIFFERENT PRICE DIFFERENTIALS COMPARED TO ANNUAL BREAD WHEAT TO BE PROFITABLY INCORPORATED ONTO DIFFERENT SOIL TYPES OF A DRYLAND FARM IN SOUTH-WESTERN AUSTRALIA



Dual-purpose graze and grain option

Because of the longer growing season, the potential to maintain green leaf for longer than an annual crop and respond to out-of-season rainfall, a perennial crop is likely to provide some additional forage compared with annual grain crops. This opportunity is likely to be similar, but perhaps larger than where long-season wheats are currently grazed during their vegetative growth period early in the growing season and allowed to regrow to produce grain later in the season (Bell *et al.* 2013). Whole-farm bio-economic modelling was necessary to determine if such a dual-purpose perennial crop may offer advantages to livestock production and whole-farm productivity over annual crops in mixed crop-livestock farming systems. This approach enables many of the complex interactions between crop and livestock enterprises, timing of livestock feed supply and the economics of the whole farm system to be analysed concurrently. In addition to standard production and price assumptions described previously, high quality green forage was made available for grazing early in the growing season following the break of season (i.e. start of the rain prior to sowing annual crops) and/or additional green forage was available after harvest in addition to the crop residue or stubble from the annual crops. No yield penalty from grazing was assumed.

Additional grazing obtained from a perennial cereal crop greatly improved its profitability and resulted in 20 percent of the profit-maximising farm plan being allocated to the perennial crop under standard assumptions. Again this was mainly on the soil types where other crop and pasture systems were least profitable even though lower production of grain and forage was assumed on these soils for the perennial cereal crop. Hence a perennial cereal crop was found to be a profitable addition to a mixed crop-livestock enterprise in southern Australia when it provided an additional 900 kg/ha post-harvest forage and 700 kg/ha early season forage, a yield 60 percent of annual wheat at a AU$35/tonne lower grain price and 60 percent of the production costs.

Modelling suggested that the dual-purpose perennial cereal crop could increase farm profit by 38 percent or AU$21 per farm hectare (Table 1), which equates to a net gain of AU$105/ha of perennial cereal sown (i.e. change in farm profit per unit area sown to the perennial cereal in the optimal scenario). This mainly came about through the ability to increase livestock numbers by providing forage at key times of the year. This also brought about structural changes in farm allocation between crops and pastures by enabling improved utilization of pastures by deferring the use of supplements, and an increase in pasture area on the farm to support this higher potential stocking rate. The dual-purpose crop also reduced the grazing of crop residues or stubbles which may also have other environmental and production benefits on other parts of the farm.

TABLE 1. FARM PROFITABILITY, ALLOCATION OF LAND TO CROP AND PASTURE, AND LIVESTOCK NUMBERS AND SUPPLEMENTATION UNDER AN OPTIMAL FARM PLAN WITH AND WITHOUT THE INTEGRATION OF A DUAL-PURPOSE PERENNIAL CEREAL

	WITHOUT GRAZING	WITH GRAZING	CHANGE (%)
FARM PROFIT (AU$/HA)	$55.6	$76.5	+ $21 (38%)
PERENNIAL CEREAL AREA (%)	0	20	+ 20
CROP AREA (%)	55%	45	- 10
PASTURE AREA (%)	45%	55	+ 10
STOCKING RATE (DSE/HA)	7.6	8.9	+ 1.3 (17%)
SUPP FEED (KG/DSE)	59.4	58.4	- 1.0

Adapted from Bell *et al.* (2008).

Due to uncertainty about the amount and timing of additional forage that might be provided by a dual-purpose perennial grain crop, a sensitivity analysis to these factors showed that a perennial grain crop providing even less additional forage could still be valuable and there is capacity to trade-off between forage and grain yield. Table 2 shows that forage provided early in the growing season before other feed sources are available is particularly valuable. Even small amounts of forage, as little as 175 kg/ha, provided at this time would increase farm profit and see 10 percent of farm allocated to perennial wheat. Providing forage after harvest was less valuable, but perennial wheat was still a profitable addition to the farm when only 500-1 000 kg of additional forage was provided after harvest only.

TABLE 2. SENSITIVITY OF AREA OF PERENNIAL CEREAL SELECTED (% OF FARM) IN THE OPTIMAL FARM PLAN TO THE TIMING AND AMOUNT OF FORAGE SUPPLIED FROM A DUAL-PURPOSE PERENNIAL CEREAL

EARLY GROWING SEASON		AFTER HARVEST ONLY		BOTH AFTER HARVEST AND EARLY SEASON	
Additional forage (kg/ha)	% perennial wheat	Additional forage (kg/ha)	% perennial wheat	Additional forage (kg/ha)	% perennial wheat
700	12	900	11	1600	20
525	10	675	13	1200	16
350	13	450	0	800	13
175	11	225	0	400	11

Adapted from Bell *et al.* (2008).

Table 3 also shows a further sensitivity analysis showing the area of the farm that would be sown to a perennial cereal under the optimal farm plan where lower levels of additional forage are supplied and lower relative grain yields were provided by a perennial cereal crop. This demonstrates that there is potential to trade-off between the grain yield and forage provided by a dual-purpose perennial grain crop. For example, if 800 kg/ha of additional forage was provided (350 kg/ha early in growing season and 450 kg/ha after harvest), perennial grain yields could be as low as 40 percent of annual wheat and still make a positive economic impact and would be included in an optimal farm plan on mixed crop-livestock farms. This shows that grain yields as low as 40 percent of an annual wheat might be feasible in a perennial cereal crop if it provides modest levels of additional green forage at key times of year. This also has significant implications for the attributes that might be targeted in developing perennial grain crops; revealing that forage production may be a vital attribute to consider and that lower grain yields could be profitable if additional forage for livestock at key times could be obtained from a perennial grain crop.

TABLE 3. SENSITIVITY ANALYSIS TO LOWER AMOUNTS OF ADDITIONAL FORAGE SUPPLY AND LOWER RELATIVE PERENNIAL CEREAL GRAIN YIELD ON THE AREA OF PERENNIAL WHEAT (% OF FARM AREA) UNDER THE OPTIMAL FARM PLAN

Standard assumptions were a relative grain yield of 60 percent and 1600 kg/ha of additional forage supplied.

FORAGE SUPPLIED AFTER HARVEST AND EARLY GROWING SEASON (kg/ha)	RELATIVE PERENNIAL WHEAT GRAIN YIELD		
	60%	50%	40%
1600	20%	19%	14%
1200	16%	16%	14%
800	13%	13%	12%
400	11%	0%	0%

Source: Bell *et al.* 2008.

Residual indirect production and environmental implications

It is important to point out that the above economic analyses considered only the direct production implications of a perennial cereal in a conventional dryland farming systems. This did not consider the implication of other technologies such as perennial pastures or dual-purpose annual crops which might diminish the relative benefit obtained from forage provided by perennial wheat. There are also several other indirect and sustainability factors are also important. No value was attributed to environmental benefits that a perennial crop might provide, whether this is an impact on the long-term condition of the land where a perennial crop is grown (e.g. soil carbon, soil erosion) or off-site external impacts (e.g. water eutrophication, sediment flow, greenhouse gas emissions). While the maintenance of land condition may directly influence a farmer's intention to adopt a perennial crop, without policy or economic incentives it is unlikely the off-site externalities would play a major part of farm decision making. However, a perennial crop may also offer several whole-farm management benefits that might prove attractive to farmers (Bell *et al.* 2010b). Firstly, because seeding frequency would be reduced, farmers could adjust or reduce their capital investment in seeding and spraying equipment, or alternatively farm a larger area without the need for more machinery. This would also be beneficial by reducing labour requirements at peak times of the year such as sowing. Secondly, the possibility of utilising perennial wheat for either grain or grazing purposes would enable greater enterprise flexibility where producers could delay their decision beyond the time of planting in response to climatic and economic conditions. Because of lower external inputs such as crop establishment costs, fertilizer and herbicides (as discussed previously), the risk exposure to climatic and market fluctuations is also reduced.

INTEGRATING PERENNIAL CEREALS INTO FARMING SYSTEMS

In addition to the economic and agronomic characteristics outlined above, perennial cereals could be utilised in a range of different ways in a farming system which may require different attributes (Bell *et al.* 2010b). One of the most important of these is the persistence or stand longevity of a perennial cereal which will influence the permanency, or length of time the perennial crop remains in the system. Hence, depending on the capacity of a perennial crop to persist or maintain productivity in different agro-climatic zones or conditions, different uses of perennial wheat may be appropriate. Below some likely systems are discussed where a perennial cereal could conceivable fit into a farming system

Perennial grain polycultures

In North America, the ultimate system for perennial grain production proposed by some would involve a permanent ‘polyculture’ mixture including warm- and cool-season perennial grasses, perennial legumes and composites that mimic their natural prairie systems (Piper, 1998)(Figure 3a). Perennial mixtures are thought to be more sustainable than pure stands, and have been shown experimentally to produce more grain yield and biomass from mixtures compared with monocultures of each species (Piper, 1998). This is because there were synergies between the functional groups, such as legumes compensating for low N supply. However, realizing successful perennial crop polycultures would require species that complement one another spatially, seasonally or in nutrient requirements, so that (a) land, labour or resources are used more efficiently; (b) yield is increased; (c) losses to insects, diseases, and weeds are reduced; or (d) yield variation is reduced (Piper, 1998). The development of such a system is an ambitious undertaking and its complexity would bring challenges. Water limitations during the wheat growing season in many Australian cropping systems, may impose significant competition between components of a mixture, especially among competitive perennial species. Many Australian environments also have short growing seasons and hence it would be difficult to time the growth, development and harvest of diverse grain crops into such a short period.

Companion or relay cropping

It is likely that in Australian systems, much simpler mixtures of perennial cereals would be more suitable. Companion or relay of other crops (e.g. grain legumes or cereals) into existing perennial crop stands may provide several of the benefits of mixtures (e.g. N inputs, enable competition to be managed and increased system productivity). Inputs of nitrogen might be obtained by growing an annual pasture legume (e.g. medic or clover) under a perennial cereal to provide a cheap source of N, as well as to compliment grazing from a perennial cereal (Figure 3b). Similar systems involving perennial pasture grasses in mixtures with annual legumes already exist in many environments (see Hayes *et al.* 2013, this conference). In more arid environments, where lower densities of perennial plants persist, companion cropping during periods favourable for growth could be a good strategy for increasing productivity (Figure 3c). In higher rainfall regions of southern Australia, a companion crop could be grown at the same time as the perennial cereal during the moist winter-growing period without excessive competition for water. For example, in some regions in the uniform rainfall zone of southern NSW, annual cereal grain crops are sown into permanent perennial grass pastures (pasture cropping) which is being found to increase water utilization, and enable fertilizer inputs that benefit the productivity of the perennial system (Miller and Badgery, 2009). Where longer growing seasons or summer rainfall is higher, relay systems might enable a crop or forage to be sown or regenerate after the harvest of the perennial wheat. Annual decisions on whether or not to companion or relay crop the perennial wheat could be made tactically based on seasonal prospects, the requirements for nitrogen inputs, disease pressures and perennial crop densities.

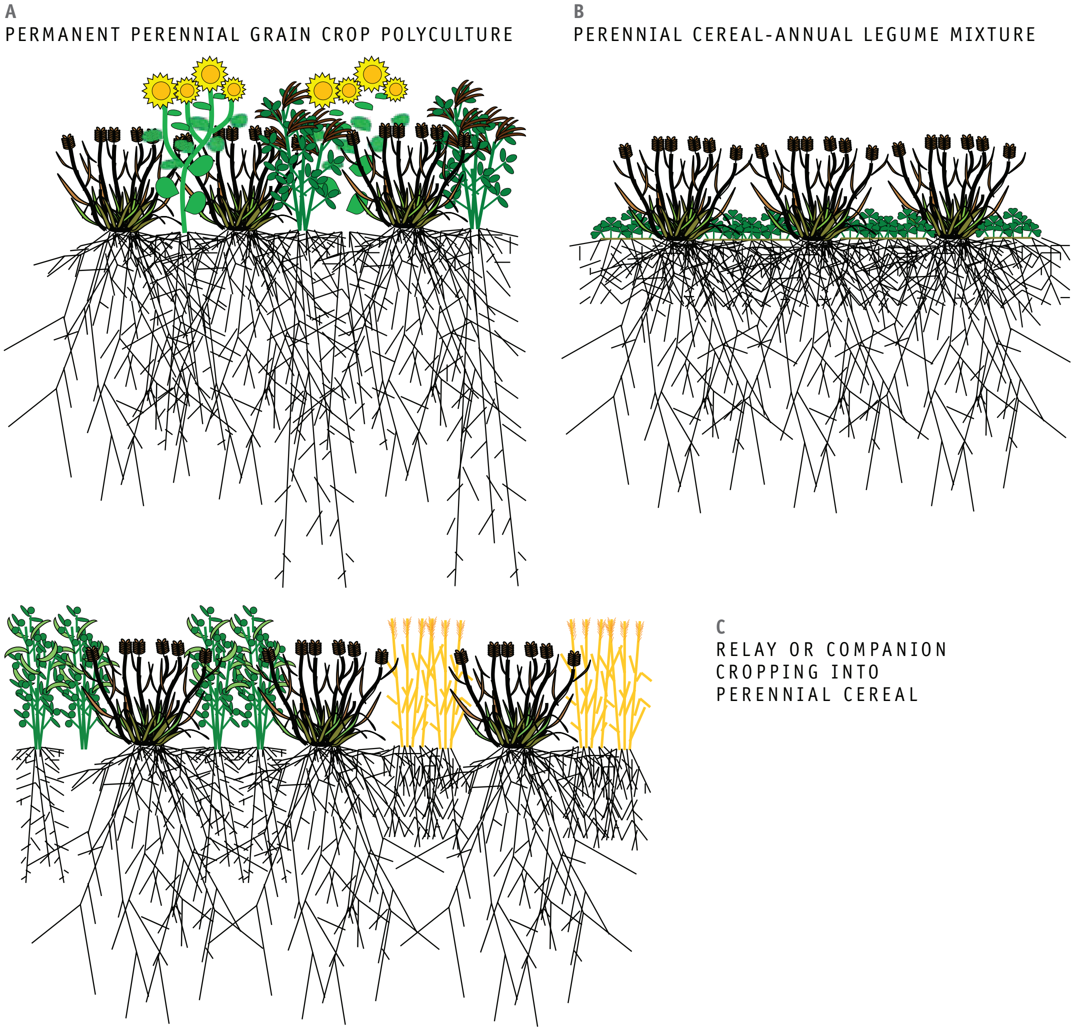
Phase rotations

A perennial crop might be used in a similar way to perennial pasture legumes and some grasses (e.g. alfalfa) as a phase of 2-4 years followed by a phase of annual crops or pastures (Figure 4). In this system, a perennial crop does not need to be long-lived and the perennial crop could be removed once plant populations or productivity decline. Alfalfa and other perennial pastures used in this way provide hydrological benefits by depleting subsoil water content and then allowing this dry soil buffer to refill during subsequent years of annual crops or pastures (Ridley *et al.* 2001; Ward 2006). The annual crop and pasture phase would also enable soil nutrient reserves to be replenished, provide disease break benefits should soil borne or foliar diseases build up and enable weed control options to be diversified.

Such a system is also self-regulating. In low rainfall environments, the length of the perennial phase would be shorter because subsoil water reserves are depleted and productivity of perennials decline more quickly, but in these environments drainage events below the annual crop root zone are less frequent, and longer phases of annual crops can be accommodated before dry subsoils are refilled (Ward, 2006).

FIGURE 3. DEPICTIONS OF ALTERNATIVE FARMING SYSTEMS INVOLVING PERMANENT PERENNIAL CEREAL CROPS

(a) polyculture mixture with perennial legume and composite as proposed by Piper (1998), (b) annual legume understory to provide N inputs and minimal competition for water during the growing season, (c) relay or companion cropping of annual grain legumes or cereals.



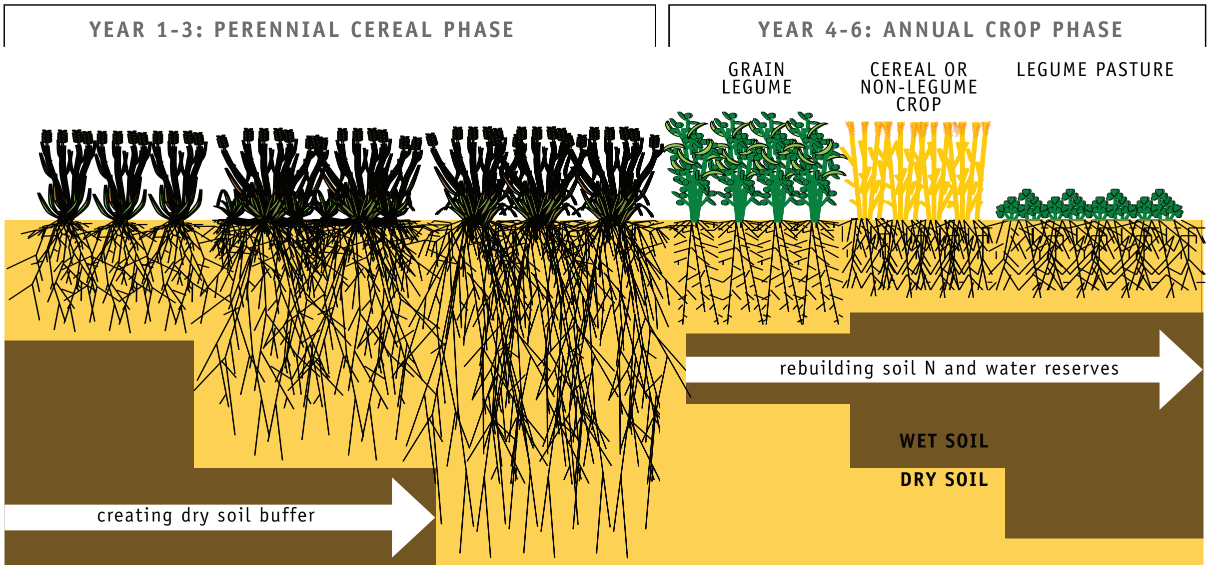
A
PERMANENT PERENNIAL GRAIN CROP POLYCULTURE

B
PERENNIAL CEREAL-ANNUAL LEGUME MIXTURE

C
RELAY OR COMPANION
CROPPING INTO
PERENNIAL CEREAL

On the other hand, like perennial pastures, perennial cereal persistence may be better in higher rainfall zones, and hence, allow longer rotations where a greater proportion of perennial is needed in crop rotations to reduce the higher drainage and runoff rates in these environments (Ward, 2006). Perennial wheat suitable for these phase rotation systems would need to produce grain in the first year, and be cheap and reliable to establish and remove.

FIGURE 4. DEPICTION OF A PHASE ROTATION INVOLVING A THREE YEAR PHASE OF A PERENNIAL CEREAL CROP WHERE SUBSOIL WATER AND MINERAL N IS DEPLETED FOLLOWED BY THREE YEARS OF ANNUAL CROPS AND PASTURES WHERE SOIL NUTRIENTS AND SUBSOIL WATER RESERVES ARE REPLENISHED



Facultative perennial crop systems

Even in situations where a perennial cereal has uncertain year-to-year persistence (e.g. low rainfall environments) there may still be a fit and advantages in farming systems. Such a perennial wheat genotype would require cost and ease of establishment and management, and grain yields similar to annual wheat and, in most years, would be analogous to using a long-season dual-purpose wheat. However, in favourable years when the perennial wheat was able to persist adequately, this may allow an opportunistic crop or may be used as a pasture in the subsequent year. By reducing the requirement for longevity and/or allocation of resources to survival strategies, higher grain yields might also be achievable more immediately.

PROSPECTS FOR OTHER DUAL-PURPOSE PERENNIAL GRAINS

While perennial cereals, based on wheat hybrids, would have the largest market opportunity in Australia, a range of other perennial cereals and non-cereal grain crops might have significant value in Australian farming systems. This is particularly the case if perennial crops have greatest opportunities on marginal or challenging soils to provide dual-purpose grazing and grain production opportunities.

Perennial grain legumes

The development of perennial legumes for dual-purpose grain-grazing could also offer some potential by providing high quality forage, in particular protein, for livestock at key times, provide inputs of N and disease management options in rotations, as well as potentially fitting on soil types where few grain legumes are well adapted. A perennial grain legume providing its own N needs would also reduce the need for the perennial crop to be grown in a mixture with other species, hence providing easier management of a monoculture.

A significant effort has been continuing in Australia to investigate the potential for a range of exotic and native perennial legumes as forage species but little attention has been applied to perennial grain legume options (Li *et al.* 2008). The native Australian legumes examined in this work were found to occur in areas with arid climates, and infertile and poor soils (Bennett *et al.* 2010; Pang *et al.* 2010) and hence were thought to be a good place to look for potential new grain crops adapted low-input agricultural systems in harsh growing conditions where other crop species may not be well suited. Australia's native legume flora is also largely unexplored for their potential as grain crops.

Examination of the natural distribution among a range of native legume genera found in semi-arid and arid regions of temperate Australia, together with information on traits linked to agronomic success as grain crops (i.e. harvestability, grain qualities and fecundity) revealed several species considered worthy of further evaluation for their grain production potential (Bell *et al.* 2010a). A range of these species have been grown under controlled conditions to compare their growth and reproductive traits, seed yield and composition with commercial annual grain legumes (Table 2; Bell *et al.* 2012). Seed yields of seven native perennial legumes were >40 percent of chickpea (*Cicer arietinum*), with grain protein, fat and fibre similar to the commercial grain legumes in the range desirable in food and feed industries. In several species the reproductive allocation were also similar to the annual commercial grain legume cultivars and much higher than might be expected from perennial species. These results are quite exciting for several reasons. Firstly, growing conditions were favourable to the annual species (well watered and fertilised in greenhouse), while under lower fertility and moisture limited conditions the relative productivity of the native perennials would be expected to be improved. Secondly, these results were based on only one accession of each taxon; undoubtedly there is substantial capacity to explore germplasm with greater productivity, larger seed size and phenological development. These species are also those that have been identified as having potential as forage plants and hence could have potential as dual-purpose options.

TABLE 4. SEED YIELD, HARVEST INDEX, SEED SIZE, CRUDE PROTEIN (CP) AND FAT CONTENT OF SEVEN UNDOMESTICATED AUSTRALIAN NATIVE LEGUMES COMPARED WITH TWO COMMERCIAL ANNUAL GRAIN LEGUMES (CHICKPEA AND FIELD PEA; HIGHLIGHTED IN GREY) WHEN GROWN IN A GREENHOUSE UNDER THE SAME CONDITIONS

SPECIES	SEED YIELD (g plant^{-1})	HI (g g^{-1})	SEED MASS (mg seed^{-1})	CP CONTENT (%)	FAT CONTENT (%)
Pisum sativa	9.9	0.50	258.9	26.3	1.2
Glycine species	4.8	0.54	11.2	32.2	5.2
*Cicer arietnum**	4.6	0.60	188.7	22.9	4.5
Lotus cruentus	3.4	0.65	1.5	32.0	5.9
Cullen tenax	2.8	0.30	5.2	32.1	11.3
Glycine canescens	2.7	0.35	16.9	34.0	6.2
Swainsona kingii	2.2	0.47	2.7	34.3	2.5
Cullen cinereum	2.1	0.30	5.2	36.2	11.8
Swainsona colutoides	2.0	0.21	3.1	27.5	2.1

* actual species is unknown

Adapted from Bell *et al.* (2012)

While this analysis examined potential in Australia's native herbaceous legumes adapted to temperate environments, there is also a range of tropically adapted perennial legumes that might have potential. For example, Australia possesses a large diversity of perennial legumes in the genera *Glycine*, *Crotalaria*, *Canavalia* and *Vigna* all which have close relatives which are grown as annual grain legumes (e.g. *Glycine max* – soybean, *Crotalaria juncea* – sunn hemp, *Vigna radiata* – mungbean) (Bell *et al.* 2010a). These perennial relatives of grain legume crops could provide a useful source of perennial germplasm adapted to arid conditions and infertile soils. Several tropical species such as lablab (*Lablab purpureus*) and pigeon pea (*Cajanus cajan*) are already used as annual dual-purpose crops in some countries (particularly in smallholder settings), but have germplasm that are short-lived perennials.

Other perennial cereals

A diverse range of other perennial cereal crops could have advantages over wheat in some situations. For example, perennial triticale could be produced from hybrids between *Triticum* species and *Secale montanum* with advantages over wheat due to its greater tolerance of acid soils (and high aluminium levels), low nutrient availability, drought and temperature stress (Jessop, 1996). Annual triticale is currently grown in Australia where wheat performance is reduced by these stresses and it is also widely used as a dual-purpose graze and grain crop. Hybridisation of *Triticum* with *S. montanum* should also be easier than with *S. cereale* (used to develop existing triticale), because the former is thought to be more closely related to wheat (Appels, 1982). Perennial grain rye using *S. montanum* has also been the target of some efforts internationally, and could improve the rooting depth, drought and heat tolerance of rye, but past efforts have encountered problems maintaining both perenniality and fertility (Reimann-Philipp, 1995). In Australia, *S. montanum* has been breed as a forage grass and hence adapted and agronomically suitable germplasm is likely to be available and may provide a useful starting point for any efforts to develop either a perennial rye or triticale (Oram, 1996). However, the increases in grain size above the commercial *S. montanum* forage variety would be required to produce a useful grain product and to increase grain yields (Hayes *et al.* 2012).

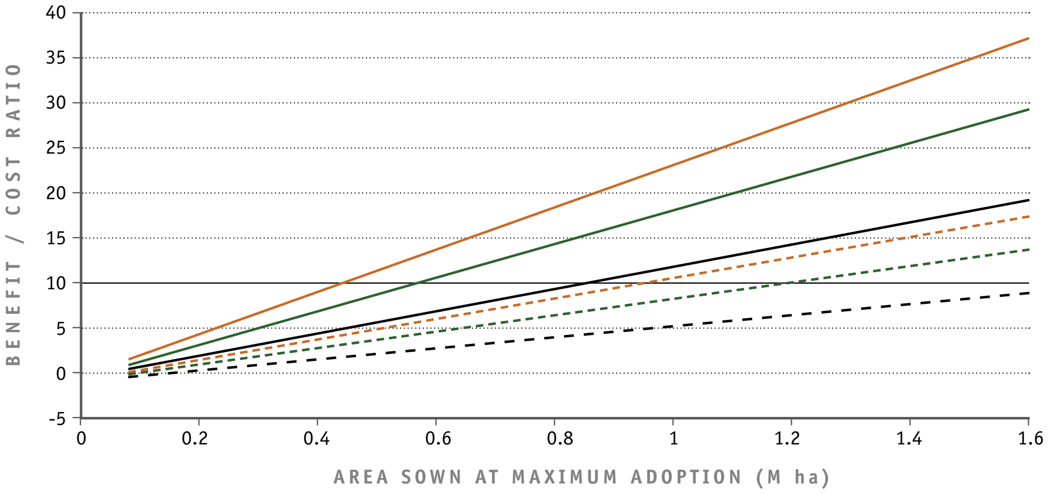
In addition, we should not discount direct domestication of already adapted native Australian grasses such as *Microleana stipoides* (Davies *et al.* 2005). This grass is an important forage species and has been shown to have many attributes suitable as a dual-purpose grain and graze crop. Warm season perennial cereals, such as sorghum and pearl millet, may also be better suited in Australia’s northern grain growing zone where rainfall is summer dominant. In these environments, commercial sorghum crops regularly ratoon after harvest and sometimes survive for more than one year, unless they encounter severe frost. Hence, breeding a perennial sorghum suited to subtropical farming systems may involve selection from within the range of pre-breeding material already available and hence avoid challenges with wide hybridization.

VALUE PROPOSITION FOR INVESTMENT IN PERENNIAL CROP DEVELOPMENT

Despite the significant opportunities and benefits that development of a perennial grain crop could provide it is important to consider and establish the value proposition for investment in their development. That is, would breeding a perennial grain crop pay off economically? Based on the economic outcomes predicted from a dual-purpose perennial cereal in the whole-farm modelling described previously (i.e. AU$20/farm ha (as shown in Table 1) and AU$10/ farm ha (assuming smaller areas are adopted on farms; e.g. Table 3), Figure 3 shows the benefit-cost ratio (i.e. calculated cumulative economic return over 20 years divided by the cost of development, with a discount rate of 5 percent) across a range of scales of adoption and assuming investments of AU$1 million per year over 10, 15 and 20 years. This demonstrates that the likely scale of adoption is a key factor influencing the likely return on investment in a perennial crop. Successful peak adoption on 0.4-0.5 million ha would achieve a 10:1 minimum benefit/cost on a AU$20 million investment over 20 years with 75 percent probability of success. Lower probabilities of success even over shorter time-frames and/or lower returns per farm hectare challenge the capacity for a perennial cereal to produce such high returns on investment unless it was suitable for a large proportion of Australia’s cropping zone.

FIGURE 5. RELATIONSHIP BETWEEN AREA ADOPTED AND BENEFIT/COST RATIO FOR AN INVESTMENT OF AU$1 M/YEAR IN THE DEVELOPMENT OF A PERENNIAL CROP ASSUMING 25 PERCENT LIKELIHOOD OF SUCCESS AFTER 10 YEARS (BLACK), 50 PERCENT LIKELIHOOD OF SUCCESS AFTER 15 YEARS (GREEN) AND 75 PERCENT LIKELIHOOD OF SUCCESS AFTER 20 YEARS (RED) ASSUMING A NET ECONOMIC ADVANTAGE OF AU$20/FARM HA (SOLID LINES) AND AU$10/FARM HA (DOTTED LINES)

For further details on assumptions refer to Bell *et al.* (2008).



CONCLUSION

This paper points out that it is important to consider the farming systems context into which a perennial grain crop might be introduced. This can help guide those qualities and attributes that might be most desirable and lead to greatest adoption and economic returns. In an Australian context is seems that a perennial grain crops with dual-purpose attributes providing grazing for livestock as well as grain yield and is adapted to the less productive parts of the landscape are likely to be the most advantageous in Australian farming systems. This can also offset initially lower grain yield and quality of a newly developed perennial cereal and provides an opportunity for a transitional genotype that might be developed based on forage grass that provides opportunistic grain production. While most of efforts so far have focussed mainly on wheat, there may actually be other perennial grain options which are easier to realize and could meet these requirements more easily (e.g. sorghum, triticale, perennial lablab or domestication of a native grass or legume). Similarly, a diverse range of farming systems could be developed in which a perennial crop might be used and wider consideration of these options should be taken in future breeding efforts.

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FROM FIELD TO TABLE: PERSPECTIVES AND POTENTIAL FOR FRUIT DOMESTICATION

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ABSTRACT

Domestication, the evolution of species in response to human selection, is the foundation upon which agriculture is built. Most contemporary crops are the products of evolutionary processes that began thousands of years ago, and that continue today as scientists harness emerging technologies to develop new crop varieties for a rapidly changing world. Current understanding of evolution under domestication is based primarily on annual plants, often self-compatible species that are propagated from seed each year. However, attention is refocusing on the development of perennial crops as concerns mount about a growing population, a changing climate, and sustainable agriculture. The domestication process in perennial fruit crops departs from that observed in annuals due primarily to differences in breeding systems (most perennial plants are outcrossing) and mode of reproduction (many perennial crops are clonally propagated). These differences have implications for two important aspects of perennial fruit crop domestication: 1) the extent and structure of population genetic variation in cultivated populations and their wild progenitors and 2) the genetic basis of agriculturally important traits. In order to better understand the hallmarks of perennial plant domestication, and to understand how perennial crop domestication might proceed in the future, we look to perennial fruit crops that have been evolving under domestication for thousands of years, including the apple (*Malus domestica*) and grape (*Vitis vinifera*) and their wild relatives. Using these examples, we explore the geographic and taxonomic mosaic of perennial crop domestication, the impact of genetic bottlenecks on variation in cultivated populations, crop-wild gene flow, and the genetic basis of phenotypic variation. We emphasize the importance of variation housed in wild-relatives for breeding fruits as well as rootstocks. These two iconic crops provide an important roadmap for exploring how best to conserve naturally occurring variation in perennial plant species and how to utilize it in plant breeding.

Keywords: perennial fruit crops, domestication, genetic variation, *Malus domestica*, *Vitis vinifera*, gene flow

INTRODUCTION

The global significance of plant domestication cannot be overstated, as all modern food plants are the products of domestication, and future improved or new crops will necessarily undergo this process as humans strive to meet the needs of a growing population and a changing climate. Crop populations originate with the transfer of seeds or cuttings from natural settings to agricultural landscapes. As farmers identify individuals with traits that enhance crop production, and remove individuals with undesirable traits, this selective cultivation over the course of many generations causes crop populations to diverge morphologically and genetically from their wild progenitors. Although the domestication process may have started thousands of years ago for many plant species, it is not merely a phenomenon of the past. Today, ongoing domestication efforts occur primarily through targeted plant breeding programmes guided by modern genetic and genomic approaches. Contemporary domestication includes both the continued improvement of crops that originated thousands of years ago (like wheat and beans), as well as attempts to domesticate species that have not previously experienced artificial selection (like some species being used for biofuels). Indeed, the evolution of crop plants under domestication is the primary pathway to improving nutrition, yield, and resistance to abiotic and biotic stress under current and future predicted climates in the world's food plants.

The origins of domestication trace back nearly 10 000 years to the transition of humans from hunter-gatherer populations to agricultural societies. Several lines of evidence support multiple, independent origins of agriculture in at least seven geographically distinct regions (Vavilov, 1992). These agricultural centres are also "centres of domestication", geographic regions where the majority of crops originated, and that today retain important genetically variable and agriculturally valuable wild progenitors of modern crops. Originally, it was thought that for a given crop species, wild plants from a single geographic region at a single time point were taken into cultivation, followed by subsequent rounds of selection on cultivated individuals to generate the domesticated lineage (Zohary, 2012). However, more recent analyses suggest domestication likely involved multiple origins of a crop from wild populations over the course of many years, and perhaps from different geographic regions within a domestication centre (Brown *et al.* 2009). Today, domestication is viewed as a dynamic evolutionary process that occurs across broad spatial and temporal scales. Founder effects associated with the establishment of crop populations, ongoing artificial selection, and other evolutionary processes such as crop-wild gene flow, continue to contribute to the pace of plant evolution under domestication.

Agricultural societies are based primarily on domesticated annual plants that are usually self-fertile, and are propagated from seeds (Glémin and Bataillon, 2009). Not surprisingly, much of our current understanding of plant evolution under domestication is based the effects of genetic drift and artificial selection on these annual plant species (Hancock, 2005). For example, scientists have described a suite of traits in members of the grass family that change in predictable ways under domestication, including loss of shattering, synchronous flowering, larger fruits/grains, and more numerous fruits/grain per inflorescence (Glémin and Bataillon, 2009). Surveys have also shown that annual plant domestication is often accompanied by a domestication bottleneck (i.e. a reduction in genetic variation in cultivated populations relative to their wild relatives) (Miller and Gross, 2011). Finally, a large body of work has identified the genetic basis of many domestication traits, and this work shows that some traits are the result of single or few loci of large effect, while other domestication traits result from myriad, interacting loci of small effect (Olsen and Wendel, 2013). The majority of the calories consumed by humans are derived from annual grains and legumes; without doubt, these plants will continue to form the foundation of agriculture. However, as concerns mount about a changing climate and the sustainability of modern agriculture, attention is focusing on the potential of perennial plants, which offer promising options for food production while decreasing environmental impacts, and will likely play an increasingly important role in food production in the future.

PERENNIAL PLANTS HAVE BEEN IMPORTANT COMPONENTS OF AGRICULTURAL SOCIETIES SINCE THEIR INCEPTION

Perennial species make up between 35 and 80 percent of the total number of plant species domesticated in each of the major centres of origin (Meyer *et al.* 2012; Zohary, 2012). Perennial crops include a variety of herbaceous and woody plants that live for more than two years. These crops represent a broad range of plant families, and generally fall into two categories: 1) perennial species that are grown for their roots or other below-ground vegetative components, and 2) perennial species that are grown for their fruits. Although perennial plants that produce edible roots, tubers, or fleshy fruits have been cultivated for thousands of years (e.g. apple, grape, horseradish, potato), to our knowledge perennial grains have been conspicuously absent from agriculture (Van Tassel *et al.* 2010). The wide diversity of geographic and phylogenetic origins of traditional perennial crops means that each domesticated perennial is likely to have some unique features. However, it is possible to identify a general domestication syndrome associated with the evolution of perennial plants in response to artificial selection, which will be informative as breeders look towards domesticating other perennial species, including grains.

Current understanding suggests that annual plants preceded perennials in domestication, with perennial plant domestication reaching its first peak of activity 4 000 years ago (Meyer *et al.* 2012; Miller and Gross, 2011). Evidence suggests that this first peak of perennial domestication coincides with the widespread adoption of vegetative propagation. Just as naturally self-compatible annual plants appeared to be predisposed to domestication, similarly, perennial species that could be easily vegetatively propagated were among the first perennials to enter the domestication process. Interestingly, it seems that perennial grasses and legumes may have been overlooked by early farmers because natural selection had not favoured high seed production and ability to self-pollinate in perennial species to the extent that it had in annuals. In other words, annual grains may have been favoured over perennial grains historically because of their wild ancestors' higher seed productivity and/or greater ease of propagation (Van Tassel *et al.* 2010).

How do perennial plant species evolve under domestication? This question lies at the core of contemporary research programmes aimed at developing perennial grains and legumes; however, compared with annual crops, relatively little is known about how perennial species change in response to human selection. For example, what are the main traits that are under selection during perennial crop domestication? What is the extent and impact of domestication bottlenecks and artificial selection on genetic variation in cultivated populations of perennial plants? What is the potential for crop-wild or wild-crop gene flow in long-lived species? What is the genomic basis of domestication traits in perennials? Understanding evolutionary processes associated with perennial crop domestication will inform conservation strategies aimed at preserving genetic variation in cultivated populations and their wild progenitors, and will facilitate breeding efforts that are based on targeted selection within existing domesticates as well as the development of new domesticates.

WHAT MAKES PERENNIAL PLANTS DIFFERENT FROM ANNUAL PLANTS?

Aside from living for more than one year, perennial plants have several attributes that differentiate them from annual plants and that play a significant role in their capacity for evolution (McKey *et al.* 2010; Zohary and Spiegel-Roy, 1975). Perennial plants often have longer juvenile phases and lengthy reproductive cycles; where it takes an annual one year to grow from a seed and produce another seed, it could take a perennial plant many years before a germinated seed is capable of producing viable offspring. This duration means that it can take several years or even decades for a single generation to be completed. In a one-hundred year time period, an annual plant will have 100 cycles of sexual reproduction on which selection can act; in contrast, a perennial plant could have half that, or much less. In practical terms, this means that evolution could take much longer in perennials than annuals because it takes many more years to achieve the same number of sexual cycles. All things being equal, under this scenario we might expect domesticated perennials to show less divergence from their wild progenitors over time relative to annual plants, because fewer cycles for selection have occurred. However, many perennial plants exhibit stark morphological contrasts compared with their wild progenitors, suggesting that evolution, although operating over fewer sexual cycles, results in clear morphological changes over relatively few generations. This suggests that there may be unique aspects to perennial plants that are not regularly observed in annuals, and that are contributing their evolutionary potential and trajectories.

There are two other features of perennial crops that stand in stark contrast to annual plants, and both are related to reproduction. The first distinguishing feature of perennial species is that they tend to be obligate outcrossers and exhibit a range of mechanisms that prevent self-pollination and/or self-fertilization, including dichogamy, dioecy, or self-incompatibility, among others (Zohary and Spiegel-Roy, 1975). This is in contrast to the self-compatible systems found in most annual crops, either the result of evolution of self-compatibility under domestication or because annual crops were domesticated primarily from wild populations with the capacity for self-fertilization (note that some annual crops are predominantly outcrossing; however, many of the outcrossing annuals, such as maize and pearl millet are self-compatible). Generally speaking, outcrossing functions to increase heterozygosity within individuals, increase variation within populations, and decrease differentiation among populations as individuals exchange genes with plants from nearby populations or wild relatives. Interspecific gene flow likely plays an important role in the origin and evolution of perennial crops (Hughes *et al.* 2007 and see below). In crop populations where reproduction is based solely on sexual reproduction by seed, obligate outcrossing may slow the breeding process because all individuals, including those with the most desirable combination of traits, must hybridize with other individuals to yield seed. This can lead to the dilution of favoured traits in the cultivated population and increase the rate of masking of recessive alleles. On the flipside, obligate outcrossing in crops produces a nearly limitless amount of variation on which natural and artificial selection can act.

The second distinguishing feature of perennial plant reproduction relative to annuals has to do with how the plants reproduce in nature relative to how they are propagated in cultivation. Some perennial plants in natural populations reproduce clonally. This is thought to be a mechanism to enhance the likelihood of long-term viability of a genotype by producing multiple ramets, increasing the probability that at least some part of a genotype could transcend negative stochastic events that occur over the course of an extended lifespan (Vallejo-Marín *et al.* 2010). In cultivation, the majority of perennial crops are vegetatively propagated through layering, cuttings, grafting, or some combination of these three. Vegetative propagation addresses breeding challenges associated with long juvenile phases by instantaneously replicating genotypes with favourable traits. Because perennial crops are outcrossing and individuals are usually highly heterozygous, clonal reproduction replicates those individuals, thus resulting in populations comprising largely heterozygous individuals. However, clonal reproduction can lead to a low level of population variation and a high degree of population structure as entire populations can consist of one or a handful of genotypes. Increasing clonality within populations may be associated with reduced sexual reproduction and/or reduced fertility due to trade-offs associated with increased allocation of resources to vegetative growth, inbreeding depression, or mate limitation (McKey *et al.* 2010). Indeed, mate limitation in clonally propagated perennial plants is consistent with observed shifts toward self-compatibility, or in dioecious species, to hermaphroditism or parthenocarpy (McKey *et al.* 2010; Zohary and Spiegel-Roy, 1975)

In short, evolutionary processes in perennial plants are unique due in part to the combination of long juvenile phases, obligate outcrossing, and clonal reproduction. Even though annual crops undergo yearly cycles of sexual reproduction, seed production in annuals results largely from self-compatible individuals that produce relatively homozygous offspring. In contrast, while perennial crops have to wait years or decades for each cycle of sexual reproduction, the heterozygous individuals produced via outcrossing may be immortalized through clonal propagation. These differences in reproduction have significant implications for the extent and structure of population genetic variation in cultivated populations and crop wild relatives, and also for the genetic basis of agriculturally important traits.

IMPLICATIONS FOR POPULATION STRUCTURE AND THE GENETIC RESPONSE TO SELECTION

Perennial wild species are the sources from which perennial crops are descended. Several properties of perennial plant populations, including an outcrossed breeding system, hybridization with sympatric congeners, and life history strategies, promote variation within natural populations and decrease differentiation among populations (Petit and Hampe, 2006). Evolutionary processes operating in nature establish the highly diverse genetic foundation on which the domestication process is based. Understanding natural genetic variation in crop wild relatives is important for characterizing resources for breeding. In addition, the same evolutionary processes that shape variation in natural populations (outcrossing, intra- and interspecific gene flow, clonal reproduction) have implications for the genetic basis of evolution under domestication.

The trend for wild perennial species, including many of the wild relatives of domesticated perennials, is low population structure and high genetic variation (Petit and Hampe, 2006). These patterns are products of the characteristic perennial life history traits and breeding systems discussed above. In terms of population structure, for example, tree species are predicted and observed to be resistant to founder effects during the colonization of new habitats. This is partially due to the long juvenile phase of most trees, during which time the population can only grow via the arrival of new migrants (Austerlitz *et al.* 2000), and partially due to the rapid restoration of genetic diversity via long-distance pollen dispersal (Hampe *et al.* 2013). Because the changes in allele frequency that occur during mild or severe genetic bottlenecks are an important source of population differentiation, and thus population structure, many tree species (especially wind-pollinated species) will not exhibit population structure. These processes continue in existing natural populations within a species range, and are combined with a pattern of loss of homozygous individuals in population cohorts (Jolivet *et al.* 2013) to contribute to the high levels of genetic diversity seen in many tree species, including crop progenitors. High levels of gene flow often extend to interspecific hybridization among closely related species. Gene flow among close relatives appears common in long-lived species, and has been well documented in systems such as the oaks and poplars (Petit *et al.* 2004; Stolting *et al.* 2013). Nonetheless, tree populations can be vulnerable to the effects of habitat fragmentation, and some studies show that trees in long-term fragmented habitats either show signs of inbreeding or increased genetic structure among younger cohorts (Vranckx *et al.* 2012). This may have important implications for the wild relatives of some crop species, especially those in areas with a long history of human habitation and high population densities.

Interestingly, the high levels of gene flow among populations of tree species do not prevent populations within those species from responding to geographically variable selective regimes. Studies in wild species consistently show that populations are locally adapted along biotic and abiotic gradients within a species range, some of which are quite extensive (González-Martínez *et al.* 2006). In cases where the genetic bases of these adaptive traits have been documented, the underlying loci appear to be numerous and of relatively small effect in terms of the percentage of variation explained (Eckert *et al.* 2010). The population structure, genetic variation, and currently documented genetic basis of adaptive traits in natural tree populations have important implications for the process and genetic basis of domestication in perennial plants. First, the lack of genetic structure means that genetic analysis of crop relatives might be less likely to result in false associations due to linked variation. Genetic structure has presented a major impediment to accurately identifying the genetic basis of selectively advantageous traits in many annual systems (wild and domesticated). Second, if wild perennial populations can adapt to different selective pressures via many changes of small effect, and in the face of gene flow, then it is possible perennial crops may respond to artificial selection in a similar manner. This would stand in contrast to the genetic basis of domestication documented in many (although not all) annual crops, in which domestication traits are controlled by loci or genes of major effect (Purugganan and Fuller, 2009). Progress and challenges in the effort to identify and characterize the genetic basis of adaptation in perennial crops are detailed below.

WHAT CAN BE LEARNED FROM PERENNIAL CROPS THAT HAVE BEEN UNDERGOING DOMESTICATION FOR SEVERAL CENTURIES?

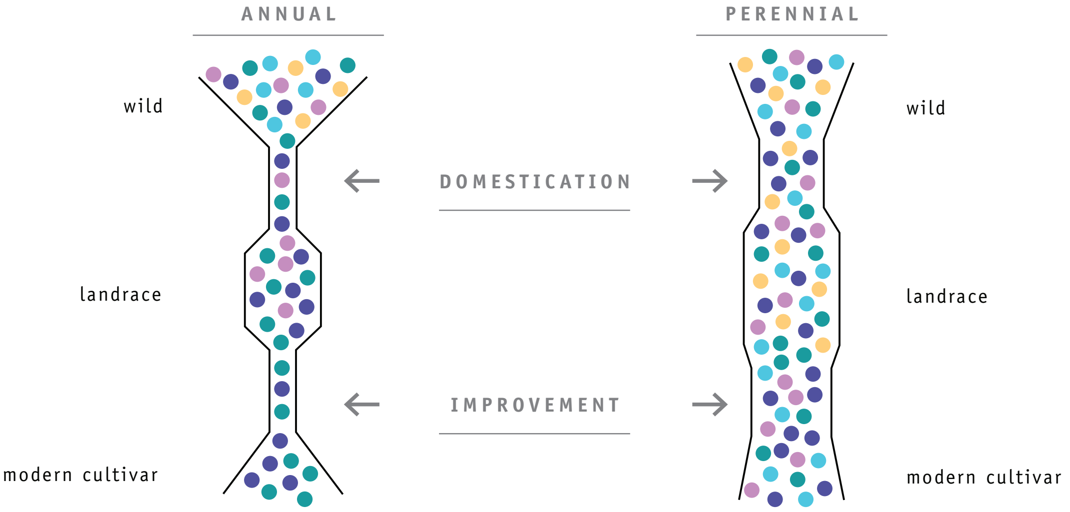
Research indicates that perennial crops originate and evolve in a fundamentally different way than annual crops, and these differences have important implications for crop breeding and improvement (McKey *et al.* 2010; Miller and Gross, 2011; Zohary and Spiegel-Roy, 1975). While more research on the topic is badly needed, current evidence indicates that multiple origins are the rule rather than the exception for perennials, with more than half of the perennial crops studied to date showing evidence of two or more origins (Miller and Gross, 2011). This is particularly interesting in light of the low levels of genetic structure detected in most of the wild relatives of perennial crops; the lack of structure should make estimates of multiple origins fairly conservative because there is not enough differentiation in most of the wild species' range to accurately detect distinct lineages. Moreover, the "more than half" estimate of multiple origins for perennial crops does not include the instances of multiple perennial crops of the same genus – at least six genera contain two or more domesticated species. Taken together, these trends may indicate that some species and genera of perennial crops should be considered good candidates for a new or re-domestication process, or simply for extensive improvement. It is certainly clear that desired traits in these polyphyletic crops can be assembled from a variety of starting points, so redeveloping these traits in a related species or from a new population within the same species should be feasible.

In addition, domesticated perennials appear to undergo crop-wild and wild-crop gene flow. Crop-wild gene flow is a broadly common phenomenon, and has been documented for annual and clonal crops (Ellstrand *et al.* 1999). In perennial crops, the best-documented cases are from some of the older domesticates (grape, olive, and apple), where gene flow has been demonstrated both from the crop into the wild species and vice versa. Gene flow from domesticated lineages into wild populations is an area of concern for conservation reasons; this scenario has been documented for apple and grape (Di Vecchi-Staraz *et al.* 2009; Gross *et al.* 2012). Hybridization between feral and domesticated olive may also be contributing to weed evolution in Australia (Besnard *et al.* 2007). However, there are also more positive outcomes in grapes and apples, were the wild species' contribution to the domesticated lineage may have been a key part of the domestication process, contributing to the development of unique varieties or facilitating the movement of a domesticated lineage into a new geographical region (Cornille *et al.* 2012; Lopes *et al.* 2009; Myles *et al.* 2011). Gene flow may well be more common than it appears to be based on existing studies, as it can be difficult to detect when there is insufficient genetic differentiation between the crop and its wild progenitor. Thus, it is likely that larger marker datasets (i.e. those resulting from next-generation sequencing approaches) will reveal more instances of gene flow in future studies. In light of the information in apple and grape, and in consideration of the propensity for multiple origins of several perennial crops, wild germplasm represents a powerful resource for perennial plant breeding. While the long juvenile phase of most perennial crops can justifiably make plant breeders cautious in the crosses they choose, the relative ease with which the domestication phenotype can be assembled (either from multiple starting points or in the face of gene flow from the wild species) suggests that the time investment may yield a high return.

Perennial crops appear to experience a relatively mild genetic bottleneck associated with domestication (Figure 1). While genetic bottlenecks vary in intensity from crop to crop, for annual species the average reduction in genetic variation during the domestication bottleneck is ~40 percent. This is in strong contrast to the average bottleneck in perennial crops, where the average reduction in diversity is only 5 percent (Miller and Gross, 2011). There are many factors that could contribute to the relatively mild genetic bottlenecks in perennial crops, including the aforementioned trends towards outcrossing (when sexual reproduction occurs), multiple origins of crop lineages, and crop-wild gene flow. Whatever the cause, the mild genetic bottlenecks mean that many domesticated perennials have high genetic variation, often close to what is seen in their wild relatives. The full extent of this variation has not yet been utilized in cultivation or breeding programmes (Myles, 2013; Myles *et al.* 2011). Ongoing efforts to preserve the range of variation under cultivation in living germplasm banks or repositories support an invaluable resource for crop improvement. It is also interesting to consider that the genetic bottleneck that accompanies annual plant domestication is one of the contributors to the phenotypic and genetic differentiation between the crop and the wild species. The loss of diversity during the bottleneck is random, and can lead to loss or fixation of alleles by chance alone; these changes in allele frequency allow the crop to be distinguished from the wild species based on genetic analysis. However, many domesticated perennials can also be effectively distinguished from their wild progenitors, *despite* a very mild bottleneck. Thus, the genetic and phenotypic differentiation must be attributed to other factors, including (both not limited to) artificial selection. Finally, from a plant breeding perspective, the mild genetic bottlenecks in perennial crops mean that genome scans designed to detect regions of low diversity (suggestive of genes under selection) will not be impeded by the confounding effects of a genetic bottleneck, as has been the case for some annual crops (e.g. Hamblin *et al.* 2006).

FIGURE 1. DOMESTICATION AND IMPROVEMENT BOTTLENECKS FOR ANNUAL VERSUS PERENNIAL CROPS

Different coloured circles represent variation in alleles or phenotypes present a given species undergoing domestication. The width of the funnel represents the relative population sizes during different stages of domestication. Variation is either lost during the domestication and improvement process in annual crops, or retained through these processes in perennial crops.



GENETIC BASIS OF DOMESTICATION TRAITS

Evolutionary biologists and plant breeders have pursued a variety of approaches to elucidate the genetic underpinnings of domestication traits in perennial crops. The primary tool used thus far has been QTL mapping, and it has been applied to a number of perennial crop systems (see Miller and Gross, 2011). When QTL maps are based on crosses between two cultivars, they are very useful for pinpointing the genetic basis of agronomically valuable traits that segregate within a domesticated lineage. However, they tell us little about the genetic basis of domestication traits – the traits that evolve during the domestication process and that differ between wild and domesticated plants. The general trend of the QTL mapping studies (either within the crop or in the few existing crop-wild crosses) indicates that domestication traits are underpinned by many QTL of small effect, although QTL of major effect have also been observed.

While QTL mapping has been very useful in the quest to identify loci associated with traits of agricultural importance with the goal of food improvement, it is also subject to several weaknesses. One of these is the high variability of QTL detection across populations and across years within populations (Crouzillat *et al.* 2000; Kenis *et al.* 2008). While this is expected to some extent (the expression of phenotypic traits in an orchard is dependent on environmental influences), the high variability makes it difficult to move forward to map-based cloning, a technique used to identify many domestication genes in annual plants. Efforts to clone the genes underlying these traits are also hindered by the fact that fine mapping requires analysis of hundreds of crossover events – the equivalent of a mapping population with at least 500 (and usually over 1 000) individuals. This is clearly not a realistic goal for every large-statured, perennial crop - the monetary and temporal investment required to maintain plants through their juvenile phase to maturity is beyond the reach of most individual researchers, and many institutions. Instead, investigations into domestication genetics and plant breeding are utilizing techniques that do not require mapping populations, but rely on existing variation and the power of massively parallel high-throughput sequencing techniques (i.e. "next generation sequencing" or NGS). Here we discuss the initial application of these techniques and their potential application to perennial crops in the future.

Genome-wide association studies (GWAS), also referred to as association mapping, is an excellent alternative to QTL mapping in perennial plants, because it takes advantage of the variation in existing populations (wild or domesticates), thus allowing breeders to skip the step of generating a new mapping population and simultaneously take full advantage of the large collections that exist for many perennial crops (Khan and Korban, 2012). However, there are two important considerations for the implementation of GWAS (Khan and Korban, 2012; Myles, 2013). One is that genetic structure or genetic differentiation within the surveyed population can lead to spurious associations. For example, the existing differentiation between wild and domesticated lineages means that a GWAS can be effectively carried out in *either* wild trees *or* domesticated trees – a GWAS conducted on a mixture of these two lineages would only reveal that all the wild phenotypes were in correlated with all the wild-specific markers. The second consideration is that linkage disequilibrium (LD) becomes a double-edged sword. Low levels of LD mean that significant correlations should be located in or (very close to) the gene controlling the trait of interest, but that it will require a very large number of markers to thoroughly cover the genome and actually capture these associations. High levels of LD can allow a large genome to be scanned using relatively few markers, but a significant association may still be very far from the functional gene or genomic feature. However, given the relatively low cost of generating SNP makers using NGS techniques, it seems unlikely that requiring a large number of markers will be a roadblock in the coming years. In light of this, it is fortunate that many perennial species have relatively low LD – this should make GWAS a powerful approach for elucidating the genetic underpinnings of phenotypic traits. In species with high LD, the targeted creation of QTL mapping populations that will generate recombinants in the genomic are of interest can complement the GWAS approach.

Association studies can also be carried out in a more targeted way if researchers have enough information to identify candidate genes that could contribute to the trait of interest. In this case, SNP markers can be genotyped in the region of interest, or the entire gene can be sequenced using targeted enrichment techniques (Cronn *et al.* 2012). This has been used to great effect in forest trees and fruit crops to confirm the association between candidate genes and traits of interest and to identify favourable alleles at these loci (Cevik *et al.* 2010; González-Martínez *et al.* 2007). This approach can be quite useful, but will ultimately be biased toward known genes. More 'agnostic' approaches like QTL mapping and GWAS still are necessary to identify genes that are not part of known families or have not been cloned in other species.

While both QTL mapping and GWAS are critical tools for dissecting the genetic basis of perennial crop domestication and improvement traits, they may not always be necessary to advance the crop domestication and improvement process from the perspective of breeders. Genomic selection (GS), the cousin of marker assisted selection (MAS), uses markers from across the genome to predict the phenotype of the plant in question (Heffner *et al.* 2009). This technique could be applied to the same types of populations used for GWAS studies, and utilizes the same type of markers, so the approaches are complimentary (Kumar *et al.* 2013; Myles, 2013). GS takes advantage of the fact that although many of the genes underlying traits are of small effect and difficult to map precisely, they still show detectable linkage with at least one marker. Once these patterns of linkage are established, it is possible to move forward to genotyping and phenotype prediction (based on a genotyped and phenotyped "training population"), allowing breeders to select seedlings for retention or removal long before they reach sexual maturity and set fruit for evaluation, thus speeding the process considerably.

All of these techniques have the potential to greatly advance the perennial crop improvement process, and can also increase our understanding of the genetic basis of perennial crop domestication. In particular, it is hoped that these techniques will allow crop breeders to take full advantage of the valuable genetic diversity present in most perennial domesticates. It should be noted, however, that while the high genetic diversity of most perennial crops will ultimately be an important resource to crop breeders, this same feature also presents a challenge for genomic approaches like GWAS and GS (discussed in Myles, 2013). Despite advances in sequencing technology and marker development, the option to start with inbred parents in crosses or in a population will greatly increase the power and accuracy of most NGS approaches. For example, SNP calls in heterozygous individuals are difficult because the heterozygous state has low support; more data is required to call these SNPs accurately. The overall diversity within perennials can also be difficult to accommodate, even if individuals are homozygous. This is because SNP data generated from NGS is too extensive to be checked manually, so its processing depends on the SNP and surrounding sequence matching a reference sequence almost exactly. This requirement is not always met in a species with high diversity, and results in many potentially variable sites being discarded. While analytical advances allowing for the imputation of missing data are being made, researchers and breeders working with these genetically diverse perennial crops must be prepared to generate an excess of data in order to have enough valid data to conduct their desired analyses.

WHAT CAN BE LEARNED FROM GRAPE AND APPLE?

In order to better understand the hallmarks of perennial plant domestication, and to predict how perennial crop domestication might proceed in the future, we look to two of the oldest and most economically important perennial fruit crops, apple (*Malus domestica*) and grape (*Vitis vinifera*), along with their wild relatives. This discussion follows on the heels of several recent papers that have drawn attention to these crops (Cornille *et al.* 2012; Meyer *et al.* 2012; Miller and Gross, 2011; Myles, 2013). Using these examples, we explore the geographic and taxonomic mosaic of perennial crop domestication, the impact of genetic bottlenecks on variation in cultivated populations, crop-wild gene flow, and the genetic basis of phenotypic variation. We emphasize the role of variation housed in wild-relatives for breeding fruits as well as rootstocks. These two iconic crops provide an important roadmap for exploring how best to conserve naturally occurring variation in perennial plant species and how to utilize it in plant breeding.

GRAPE

Cultivated grapevine (*Vitis vinifera* ssp. *vinifera*), the most economically important berry in the world, was domesticated from European grapevine (*Vitis vinifera* ssp. *sylvestris*) (This *et al.* 2006). The centre of diversity for *Vitis vinifera* appears to be in the Caucasus region, and multiple lines of evidence suggest that cultivated grapevines were domesticated from wild vines in this area (Grassi *et al.* 2006; Imazio *et al.* 2013; Myles *et al.* 2011; Pipia *et al.* 2012). Patterns of molecular genetic diversity point to a role for western European *V. vinifera* in the evolution of cultivated grapevine as well, either as a second source of cultivated materials (Arroyo-García *et al.* 2006), or as a participant in crop-wild gene flow in the area (DeAndres *et al.* 2012; Myles *et al.* 2011). The domestication process in grape is characterized by a shift from dioecious wild progenitors to hermaphroditic cultivars, the seedlessness resulting from parthenocarpy or stenospermocarpy (Cabezas *et al.* 2006), a broad domestication bottleneck with high levels of variation retained in cultivated populations, and rapid decay of LD (Myles *et al.* 2011). Extensive genetic variation in cultivated and wild grapevines have been confirmed in surveys of breeding collections (Aradhya *et al.* 2003; Myles *et al.* 2011), broad-scale analyses of natural grapevine diversity (Grassi *et al.* 2006) and regional analyses of wild populations in France, (Barnaud *et al.* 2009), Spain (DeAndres *et al.* 2012), and the Caucasus region (Pipia *et al.* 2012).

Although grapevine cultivation is based primarily on the European grapevine *V. vinifera*, other *Vitis* species play critical roles in grape production as well (Galet, 1979). Most *Vitis* species can be distinguished morphologically and genetically from one another (Aradhya *et al.* 2003; Miller *et al.* in revision; Péros *et al.* 2010; Zecca *et al.* 2012); however, the majority of subg. *Vitis* (the largest subgenus within *Vitis* and the one that includes the European grapevine) are interfertile. Interspecific hybridization has played an important role in the development of grapevine cultivars in some parts of North America (Ali *et al.* 2011): European grapevines do not grow well in the eastern and central United States due primarily to their susceptibility to native pests and pathogens. Early European colonists experimented with crossing *V. vinifera* and native North American *Vitis*, generating hardy hybrid grapevines that exist in vineyards in the eastern half of the United States today. In addition, North American grapevine species have contributed valuable rootstocks for the global grape industry (Galet, 1979; Peccoux, 2012). Widespread grafting of *V. vinifera* to North American species dates back to the mid-1800's when insects in the genus *Phylloxera* devastated the French grape industry (Sorensen et al. 2008). Starting with this crisis, North American grapevines have provided the foundation for rootstock development, and are the focus of research programmes working to elucidate molecular mechanisms and genetic underpinnings of abiotic and biotic stress resistance in rootstocks (Gong *et al.* 2011; Marguerit *et al.* 2012; Pavlousek, 2011; Polesani *et al.* 2012). Today, many vineyards consist of European *V. vinifera* grafted to North American *Vitis* species, including the river grape (*V. riparia*), the rock grape (*V. rupestris*), and Berlandieri's grape (*V. cinerea* ssp. *berlandieri*), and their hybrid derivatives (Galet, 1979).

Both grapevine scions and rootstocks are the focus of crop improvement efforts using molecular markers to facilitate selection, but both are the products of highly heterozygous, outcrossing populations that exhibit rapid LD decay. Given this, what is the genetic basis of phenotypic variation in grapevine? Traditional QTL analysis has been used to characterize genetic architecture of berry quality, yield, and pest/pathogen resistance. QTL studies identified a few loci of relatively large effect associated with variation in berry colour, berry weight, number of inflorescences per shoot, and seedlessness in table grapes (Cabezas *et al.* 2006; Costantini *et al.* 2008; Doligez *et al.* 2010), and phenological stages in wine grapes (Duchêne *et al.* 2012), also demonstrating that variation in a few regions of the genome is associated with traits of agricultural significance. In contrast, proanthocyanidin production has a more complex genetic basis with multiple loci of small effect contributing to phenotypic variation (Huang *et al.* 2012). An alternative to traditional QTL studies is GWAS, which makes use of existing germplasm collections, sidestepping the need to generate trait-specific mapping populations (Morrell *et al.* 2011; Myles *et al.* 2009). Given the extensive natural variation in *Vitis*, the outstanding germplasm collections that have been established for *Vitis* in Europe, North America, and elsewhere, and the developing genomic resources for this genus (Jaillon *et al.* 2007; Scalabrin *et al.* 2010), association mapping offers a promising approach for characterizing the genomic basis of phenotypic variation in grapes.

Another approach to exploring regions of the genome that are involved traits of agricultural importance involves transcriptomics, the analysis of the expressed portion of the genome. In grapevine, a growing body of work incorporates various methods of transcriptome analysis to identify genes that are active during key stages of fruit ripening, abiotic stress, or biotic stress. Pioneering work in this field analysed expressed sequence tags isolated from different plant organs, developmental stages, and cultivars to identify genes that were up- or down-regulated during various stages of grape growth (Goes Da Silva *et al.* 2005). Subsequent studies have characterized transcriptional activity during berry development (Ali *et al.* 2011), and have described genomic response to abiotic stress (Cramer *et al.* 2007; Liu *et al.* 2012; Tillett *et al.* 2011). Comparative transcriptomics studies across species have been particularly powerful in dissecting species or cultivar-level differences in pathogen susceptibility (Polesani *et al.* 2012) and flavonoid biosynthesis (Ali *et al.* 2011). Many of these studies used an Affymetrix gene chip that was developed for grapevine.

The transcriptome approach to identifying candidate genes associated with agriculturally important traits is only expanding with the advent of massively parallel sequencing of ribonucleic acid (RNA), (RNA-seq). RNA-seq offers an efficient, cost-effective way to access all species of transcript in a given tissue at a given time point, and can be used to describe both DNA sequence and transcript abundance (Wang *et al.* 2009). In grapevine, RNA-seq has been used to generate de novo transcriptomes for cultivars (Venturini *et al.* 2013), which can then be compared with reference genomes or transcriptomes of other cultivars facilitating the identification of cultivar-specific transcript. This promising approach is particularly powerful for clonally propagated perennial plants where individual genotypes live for multiple years and are replicated over diverse landscapes. For long-lived clones, RNA-seq offers the unparalleled opportunity to characterize temporal and spatial variation in a genotype's genomic response to whatever it may encounter.

APPLE

What is known about domesticated apple relative to the general features of perennial crop domestication? *Malus domestica* is one of the world's major fruit crops, is economically one of the most important, and is planted widely in the northern and southern hemispheres. As such, it has been the subject of intense study, and these studies show that apple demonstrates many of the major trends for perennial fruit crops detailed above. Genetic diversity in both wild and domesticated apple is very high, and individual plants are highly heterozygous (Cornille *et al.* 2012; Richards *et al.* 2009; Velasco *et al.* 2010). Wild populations of the progenitor, *Malus sieversii*, appear to have low population structure with high levels of outcrossing; this corresponds well with research on other wild tree species (Richards *et al.* 2009). Domesticated apple shows no sign of an improvement bottleneck, retaining the same high levels of diversity seen in its close wild relatives (Cornille *et al.* 2012). The results of one study indicate that this may be partially attributed to the influx of genetic variation from one of the close wild relatives, *Malus sylvestris* (Cornille *et al.* 2012). Gene flow from the domesticated species into *M. sieversii* has also been documented, which is an issue of possible conservation concern (Gross *et al.* 2012). As for many perennial crops, however, the broad genetic diversity of the crop and the wild species are not yet fully utilized or reflected in most modern breeding programme (other than introgression of disease-resistance genes) or in the active commercial orchards for this crop.

QTL mapping has been applied to several cultivar × cultivar crosses, and most domestication traits (mainly related to fruit quality) are controlled by loci of small effect (Kenis *et al.* 2008). Not surprisingly, these small-effect loci are inconsistent across populations and across years within a population, due to environmental effects and differences in genetic background. While these QTL studies have been enormously useful for MAS efforts dealing with disease resistance genes (which tend to have a larger effect) (King *et al.* 1999), a more targeted approach incorporating candidate genes has been necessary to identify and utilize alleles associated with fruit quality, and, more recently, growth habit (Baldi *et al.* 2012; Cevik *et al.* 2010). While these approaches are impressive, it is likely that important loci and genes remain undiscovered due to the lack of precision of QTL mapping and limited mapping population size used in most of these studies. GWAS approaches and genome scans for loci under selection offer promising avenues to identify these loci.

Both the breeding and genetics of apple were advanced with the resources building up to and culminating in the sequencing of the apple genome (‘Golden Delicious’ cultivar) followed by resequencing of 27 additional cultivars (Chagne *et al.* 2012; Velasco *et al.* 2010). While much work remains to curate the apple genome (a difficult task, due partially to the high heterozygosity), this has resulted in many advances. Analysis of SNPs developed based on these genomes indicate that LD in domesticated apples (outside of structured family populations) is low, which will make the link between significant markers in GWAS and the underlying gene more realistic than in a high LD species. Encouragingly, in the first GWAS approach for *Malus*, using a structured family population and 2 500 SNPs, several known candidate genes were recovered in the initial scans. Moreover, a GS approach to the same population indicate that fitting markers across the genome was effective in capturing phenotypic variation that is very difficult to track at the single-marker level (Kumar *et al.* 2013). This study also confirmed the difficulties inherent in working with a highly diverse species – the SNPs were based on an 8K SNP array, but only 2 500 were robust enough to be included in the final analysis. While even the number of SNPs used in this GS study may seem a daunting goal for non-model species, rapid advances in SNP generation technology and analysis are likely to level the playing field very rapidly. Instead, it is possible that the limiting resource for non-model crops might end up being the generation, maintenance, and phenotyping of large pedigreed populations such as nested association mapping (Kotoda *et al.* 2010) lines that are extremely useful for GWAS and GS studies. While these populations represent a substantial investment, they position researchers to immediately take advantage of developing technologies, and should be a priority for the research community.

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DEVELOPMENT AND MARKETING OF PERENNIAL GRAINS WITH BENEFITS FOR HUMAN HEALTH AND NUTRITION

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ABSTRACT

"*The Breeder's Dilemma - The Conflict Between Yield and Nutrition*" addresses the challenge of breeding for highly nutritious grains when yield is the predominant selection criterion (Morris and Sands, 2006). Perennial grasses, in particular those that have already been used as food sources by indigenous peoples, offer an opportunity to develop sustainable and nutritious grain crops from genetic resources that have not been subjected to rigorous selection for yield. To date, our team has developed and commercialized two perennial grass crops and evaluated their nutrition profiles. Indian Rice Grass (IRG, *Achnatherum hymenoides*) was used by indigenous people in the western United States. Grain from this perennial grass was consumed as a staple as early as 7 000 years ago, long before maize was cultivated. The grains are smaller, and much higher in protein and essential amino acid content compared to wheat. These seeds shatter and have a vernalization trait that suggests that they have not been domesticated in the modern agronomic sense. The grain can be ground into dark and flavourful, gluten-free flour that was marketed as Montina™. Another perennial grass product that has made it to market is Timtana™ flour, derived from Timothy grass seed (*Phleum pratense*). It is also high in protein, gluten-free and flavourful when used in baking. Both of these grains have a higher level of essential amino acids in their protein. With much of the world covered by perennial grains prior to agricultural development, there should be many more crops to develop as "new" emerging crops. A promising search strategy might be to focus on sites where baking ovens or ancient villages were once located. Collection of seeds of perennial plants from such locations may be particularly rewarding. Selection criteria might include several nutritional traits including high protein value, low glycaemic index, low phytic acid content, high omega-3 levels and absence of amylase-trypsin inhibitors.

Keywords: Indian rice grass, Timothy grass, nutrition, glycaemic index, phytic acid, omega-3, amylase-trypsin inhibitors

INTRODUCTION

A critical crop for the USA and world food production and nutrition is and will continue to be wheat. Valued for its superior bread making qualities, wheat is produced across the world and provides calories for a large number of people. It can be produced in dry climates with limited input. However, wheat is relatively low in protein nutritional quality, low in essential amino acids and has a high glycaemic index. In addition, wheat is closely associated with two emergent medical conditions: gluten intolerance (Sapone *et al.* 2011) and type 2 diabetes (Shulze *et al.* 2004; Gross *et al.* 2004). A long pressing problem in Montana is that there is no widely-used, profitable rotation crop for wheat (Chen *et al.* 2012). Legume production is increasing but the domestic and global market for pea and lentil is limited relative to the market for wheat. Rotation crops are important for optimum crop production because they break disease cycles and can greatly contribute to soil health and fertility. Development of alternate crops could increase rural and farm income, increase overall crop production, and have a significant impact on human nutrition and health. Our approach has been to search for high value crops that could serve as wheat alternatives, at a time when wheat prices have been high. To shift growers away from their traditional and subsidized crop and into planting an alternate crop, we needed to find a niche market where there was some value added advantage over wheat. This new market was the emergent gluten-free market (from US$200 million in 2007 to US$4.2 billion in 2013). Two of the four gluten-free crops that we have introduced into Montana agriculture are in fact perennial in habit.

Modern crop varieties have been often selected for high yield and transportation/storage stability. Increased yield equates to increased seed biomass. Increased biomass is more accurately described as increased carbohydrates (starch and fibre) and decreased protein (i.e. *The Breeders Dilemma*, Morris and Sands, 2006). Wheat, even soft white wheat, has also undergone extensive selection for increased gluten, valued for its superior bread- and pasta-making properties (Barro *et al.* 1997; Payne, 1987). A growing number of consumers are unable to eat gluten. It is estimated that 6 percent of the USA population is gluten intolerant (celiac disease) or gluten sensitive (Fasano *et al.* 2011). Additionally, gluten is increasingly connected to diseases such as arthritis and neurological disorders (El-Chammas and Danner, 2011). The expanding gluten-free industry has responded by crafting food products from low-protein flour blends of rice, potato, cassava, and sorghum flours. There was insufficient attention paid to protein content or quality, even though gluten intolerant (celiac) customers actually require even more nutritional foods due to poor absorption of nutrients.

There are a large number of alternative crop candidates that should be considered for intensive breeding programmes; however those that are now available with improved nutrition, sustainable production and rotation potential are rather rare. Our strategy was to look at ancient grains consumed by indigenous peoples. So far, we have concentrated on an ancient grain crop that was consumed by indigenous Americans, Indian Rice Grass (IRG, Montina™, *Achnatherum hymenoides*). Seeds of this grass were found in prehistoric dwellings in Arizona (Bohrer, 1973). In addition, we have found that a pasture grass, Timothy (Timtana™, *Phleum pratense*) also produces a quality food grain. As with most perennial grains, yearly yields are lower than wheat, but once established, these grasses can yield for an extended number of years. Both are than detectible gluten content (Table 1). The essential amino acid content of Indian Rice Grass protein is much higher in comparison to spring wheat. (Table 2) As with most perennial grains, yearly yields are lower than wheat (Table 3) but once established, these grasses can yield for an extended number of years, reducing input costs including annual seeding, ground preparation, etc.

TABLE 1. NUTRITIONAL ANALYSIS (100 G SERVING)

	WHITE WHEAT FLOUR	MONTINA™ FLOUR (INDIAN RICE GRASS)	TIMTANA™ FLOUR (TIMOTHY GRASS)
Total Calories	364	380	300
Calories from Fat	8	27	50
Total Fat (g)	1	3	7
Saturated Fat (g)	0	0	0
Total carbohydrate (g)	76	70	63
Dietary fibre (g)	3	24	17
Protein (%)	10-12	17	17
Gluten	>5%	<0.5mg	<0.5mg

TABLE 2. PERCENT ESSENTIAL AMINO ACIDS IN PROTEIN: INDIAN RICE GRASS (IRG) VS. WHEAT

	IRG	WHEAT
Lysine	3.2	2.4
Methionine	2.1	0.5
Threonine	3.7	2.8
Isoleucine	2.8	5.3
Valine	3.5	2.1
Leucine	7.9	4.6
Arginine	9.3	2.2
Histidine	3.9	1.2
Phenylalanine	5.8	4.7
Total % Essential Amino Acids in Protein	**42.2**	**26.8**

TABLE 3. ANNUAL SEED YIELD

SEEDING RATE	YIELD	TYPE
Wheat (60# seeded/acre)	2 000-4 000 lbs/acre	Dryland or irrigated
Timothy (5# seeded/acre)	400-500 lbs /acre	Irrigated
IRG (4# seeded/acre)	100-200 lbs/acre	Dryland or irrigated

PERENNIAL CEREAL GRAINS

The Palaeolithic to Neolithic shift about 12 000 years ago was a shift toward production agriculture from a more nomadic hunting and gathering lifestyle (Wade, 2006; Wells, 2010). Concomitant with this shift was an increase in population sizes and inhabitation of areas that could support agrarian populations based on domestication of plants and animals. If the adaptable Palaeolithic lifestyle was sustainable in one sense, the Neolithic lifestyle was sustainable in a very different way. With agriculture, larger, denser populations could be sustained; culture could be more robust with far more complex social interactions (Wade, 2006; Wells, 2010).

It is important to recognize the importance of the role that annual cereal grains played in the intensification of agriculture. Such grains could be stored in granaries to tide over long periods of drought, pestilence, floods and overt predation depending on how well they were protected. The increased yield of annual plants may have facilitated establishment of sizeable reserves of grains, enabling a rapid selection of annual plants that were palatable, predictable in harvest date (determinant floral type), non-shattering, and yield-responsive to water added through irrigation or by late rainfall (Wells, 2010). It is not known why perennial grains were excluded from this series of developments. One might surmise that a population of annual grains might have a higher rate of change under strong annual selection than would a population of perennial grains. Also, any selection for yield after only one season would tend to favour an annual growth type as perennial type plants would be conserving energy in their root and crown systems for the next season. This subject is extensively reviewed (Van Tassel *et al.* 2010).

The rapid change in selection was probably influenced heavily by certain “seed villages” where a culture developed around selection of a mixed population (landraces) of diverse plant phenotypes to reflect the variance in growing conditions, disease and pest predation from year to year. These seed villages, probably the source of landraces of crops, gave rise to selection of favourable plants in terms of agronomic characteristics including disease and pest resistance (Harlan, 1957; Berg, 1992). They have served as important sources of germplasm for modern pure line monoculture breeding efforts in many centres of origin. For example, in the horn of Africa, North Africa, and throughout the Near East, such landraces are still grown and are favoured probably due to their reliable mixture of genotypes locally adapted to pests and diseases, although the yields are often not as high as those of improved cultivars (Ceccarelli *et al.* 1987).

The genetic flexibility of landraces has been largely replaced with the genetic flexibility of plant breeding. Plant breeding programmes are highly effective in combining favourable traits and modern breeding programmes have led to the Green Revolution, touted as saving millions of lives from certain starvation throughout the world. While yield has increased dramatically, the mineral nutrition in wheat has gone down in the past 160 years (Fan *et al.* 2008) There were bound to be some trade-offs from this intensified yield-driven, large-scale monoculture of just a few staple annual crops, including loss of plant diversity and reduction in protein. Perhaps these trade-offs can now be mitigated with a greater mindfulness of sustainability through water utilization and nutrient recycling, integrated pest management, and greater attention to human nutrition (Sands *et al.* 2009). One approach, the turn to perennial crops, may reduce inputs including the cost of seeds and fallow ground erosion. Several factors need to be considered in selection of perennial crops with a priority on human nutrition. The longer a plant is in the soil, the more exposure it has to predation by insects and rodents. This can be a problem, needing a solution through biocontrol or management practices. However, it can also suggest why perennial grains could be a good source of resistance traits for annual plant breeding development. In terms of nutritional value, it takes considerably more metabolic energy for a plant to produce a gram of protein than to produce a gram of starch. These are some of the interconnected factors that probably lead to an inverse relationship between yield and nutritional value (Morris and Sands, 2006; Sands *et al.* 2009). Perhaps selection for agronomic traits has had minimal impact on most cultivated perennial grains and no impact in many ancient grains, leaving their nutritional attributes intact.

HIGH NUTRITIONAL VALUE IN CEREAL GRAINS: A GOAL CONSTRAINED BY PLANT BIOLOGY?

Seeds are perceived as rich and compact sources of nutrition. However, for seeds to meet the needs of their own survival and plant reproduction, they have trade-offs that result in traits that are incompatible with or antagonistic to human nutritional needs. Plant seeds evolved to survive and cycle to the next generation, carrying adequate supplies of energy and major minerals. They polymerize all small molecules. This strategy is based on the phenomenon that the colligative (osmotic) effect of a small molecule is the same as that of a large polymer. If the seed contained too many "free" small molecules, the embryo could not survive their osmotic effect. Oils, insoluble compounds such as phytic acids that tie up zinc and iron, and hemicelluloses, starches and proteins solve this problem for seeds, thereby providing energy, trace elements and nitrogen to the embryo upon germination. Plants need only an initial nitrogen source from storage proteins, as they have a complete retinue of amino acid biosynthetic enzymes to re-synthesize all 20 amino acids. In contrast, animals can only synthesize ten, hence non-essential amino acids (Block and Bolling, 1945). The essential amino acid biosynthetic pathways are totally absent from animals. The essential amino acids are synthesized in plants and microbes and must be consumed by animals. The essential amino acid families are the aspartate family (lysine, methionine, and threonine), the branched chain amino acids (leucine, isoleucine, and valine) and the aromatic amino acids (phenylalanine, tyrosine, and tryptophan). Arginine is also essential (Block and Bolling, 1945).

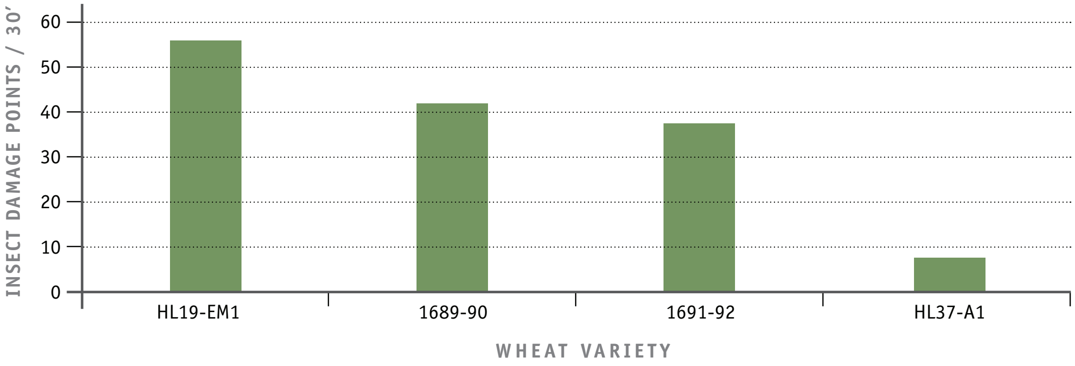
Throughout history, cereal grains have been regardwed as energy sources, (calories), and plant selection has proceeded accordingly. This view has resulted in selection of high yielding varieties (high starch i.e. calories) and lower protein. Furthermore the proteins in annual wheat, rice, barley, maize, sorghum and millet are imbalanced heavily in favour of non-essential amino acids (Ponter and Sauvant, 2004). Plants regulate the synthesis of these amino acids and have complex feedback systems to prevent overproduction. A case in point is lysine. From the standpoint of humans and animals; lysine is the most nutritionally limiting amino acid in cereal grains. To further complicate the nutritional picture, intensive breeding for pest and disease resistance may have resulted in selection of grains that are replete with families of small peptides that function as amylase trypsin inhibitors inhibiting digestive enzymes. These small peptides can drive intestinal inflammation and reduce nutrient absorption, especially in individuals afflicted with celiac disease (Junker *et al.* 2012). In our minds, the notable shortcoming of the aforementioned cereal grain intensification has been the lack of attention to human nutrition.

To remedy the nutrition crisis, we have identified several approaches outlined below that could be further developed: improving the nutritional quality of plants through intensified selection of specific amino acids, adding nutrients through fermentation of specific microbes, and identification of new perennial grasses that are high-protein, low-glycaemic and gluten-free.

IMPROVING THE NUTRITIONAL QUALITY OF FOOD STAPLES

To directly improve the nutritional quality of plants, we selected a series of high-lysine lines from a wheat population (18 years of selection) (Bright and Shewrey, 1983) and tested these lines for agronomic traits in plant breeders' field plots. A group of animals (aphids, grasshoppers, mice deer and antelope depending on location) devastated our cultivars in heavy preference over normal lines. Compared to their wild-type parent line (HL37-A1) they are favoured by insects and rodents, presumably because of their nutritional content (Figure 1) (Morris *et al.* 2006).

FIGURE 1. INSECT PREDATION ON 3 HIGH-LYSINE LINES COMPARED TO THE NORMAL LYSINE LINE HL37-A1



Source: Morris *et al.* 2006

This observation corroborates with our earlier work on chicken nutrition where we discovered that three-day old baby chicks discriminate against a zero lysine diet in favour of the same diet formulation with added lysine (Newman *et al.* 1984; Newman and Sands, 1983). The similar type of finding was reported (Osborne and Mendel, 1914), a century ago. They reported that rats did not grow on a wheat gliadin diet unless lysine was added. We know of a plant breeder who has simplified selection of nutritional traits simply by letting barn dwelling rodents select preferentially (i.e. eat) for nutritious lines. The basic concept is that a limiting factor (see Liebig's law of the limiting factor (Hardin, 1995) is still an operating paradigm in the animal feed industry. One important note, with respect to high-lysine wheat lines and probably high vitamin A rice lines, is that they are not yet commonly found in production agriculture (Morris *et al.* 2006). The increased predation on high-lysine lines will be very problematic unless the predation problem can be resolved. We speculate that such high nutrition lines might be used as trap crop loci to draw pests away from the desired crop. The best chance for high-lysine wheats and other similarly selected grains, if they are ever to reach the consumer, might be if they are crossed with high yielding advanced lines that have as a driver some particularly needed selection trait such as herbicide or rust resistance.

A decidedly different approach to plant based nutrition was tried by our group at Montana State University in the early 1980s. We constructed a DNA sequence designed to code for a highly nutritious protein that could be used to balance cereal grain diets (Jaynes *et al.* 1985). This synthetic protein was very high in lysine (22 percent), methionine (16 percent), and 10 percent each threonine, isoleucine and tryptophan. The DNA sequence was used to transform potato and the protein quality of the resulting transgenic potato was improved (Yang *et al.* 1989). We would hope that at some future point in time, the seed storage proteins of staple crops will be replaced with a new generation of designed, highly nutritious proteins as first demonstrated and described above by Jaynes *et al.* 1985.

Currently, we have selected and developed varieties of oat with higher levels (18-22 percent versus 12-13 percent) of protein. We have further selected these varieties for short stature to facilitate rapid visual identification and rogueing out of wheat and barley volunteer plants that contain gluten. This system has enabled production and commercialization of high-protein, gluten-free oatmeal and oat products.

APPROACHES TO IMPROVING NUTRITION FROM PLANT-BASED FOODS BY FERMENTATION WITH MICROBES SELECTED FOR EXCRETION OF SPECIFIC NUTRIENTS

As plant scientists, our strong interest in human nutrition has led us down several different avenues of research and development, including fermentation, forced selective breeding, review of undeveloped Palaeolithic grains, and high through-put selection of mutants. With regard to perennial grains, use of selected traditional fermenters can overcome the shortcomings of a particular grain. In our efforts to improve the nutritive value of both perennial and annual cereals, we have identified high phytic acid (binding zinc and iron), low quality protein and high glycaemic acid carbohydrates as high priority challenges. Our first approach to improving human nutrition did not actually involve plants directly. Fermentation has been a traditional means to preserve foods (wine, pickles, etc.) or to enhance flavour and texture (breads, yogurt, etc.). Foods can either be fermented with a known inoculum (e.g. yeast or sourdough starter) or with airborne inocula. In either case, the fermentation conditions are set up to favour the desired fermenting microbe.

Given that lysine is a limiting amino acid in many cereal based diets (Osborne and Mendel, 1914; Ponter and Sauvant, 2004), we selected two different high lysine-excreting bacteria (*Lactobacillus plantarum* and *Lactobacillus fermentum*). These bacteria are used for the fermentation of vegetables, dairy products and sourdough breads. We used an intensive selection procedure exposing these wild-type bacteria to higher and higher concentrations of toxic lysine analogues and selecting survivors (Sands and Hankin, 1974; Megeed and Sands, 2002). The survivors overcame the toxic analogues by overproducing lysine. When the lysine-overproducing *lactobacilli* were used to ferment dough, they continued to overproduce lysine, significantly increasing the lysine content of the resulting bread. The microbes could also be used to increase the lysine content of fermented vegetables or animal feed (e.g. silage). This strategy enabled fermented vegetables and cereal-based foods to be enriched in lysine regardless of the food or grain variety. The technology was also used to select lysine-excreting strains of yeast for bread production. It takes less time to select for such microorganisms than to improve lysine content of plants via breeding, with an estimated time of intensive repeated selection of 8 months for bacteria. A similar selection for either an enhanced annual or perennial plant would take years. These microbial strains and the methods are and have been available, but there has not yet been any widespread adoption. Commercial bacterial products used for food fermentation are generally touted for their organoleptic and probiotic properties, and not their excellent nutritional quality. Similarly, commercially available bread yeasts are promoted for their reliability and fast action, and price, not for the boost of lysine content or other important nutrients that they could deliver.

A HISTORICAL APPROACH TO HUMAN NUTRITION

As stated above, maybe we really need to step back and look at ancient grains and ancient peoples. Migrant populations depended upon what they could find. If meat was available, it was consumed. But if it was scarce, other sources of nutrition, primarily plants, were found. We tried to identify the ancient plants and to determine how to produce them. As mentioned earlier, the first plant that we worked with was IRG. The meal ground from seeds of this grass is high in protein, fibre, and flavour, with no trace of gluten. Grown as a perennial grass in the absence of gluten containing grains, the seed has been ground into flour and sold as Montina™, a gluten-free high-protein product for baking. The use of added gums (xanthan or guar) gives the bread the lift normally provided by gluten. The lesson learned here, with an admitted sample size of only one, is that ancient food grains, from before the plant breeding revolution, may be a worthwhile source of nutrition. We observe two types of evidence that this plant is not domesticated: the seeds require vernalization and seed shattering has not been eliminated. These two traits are not associated with domesticated grains (Wells, 2010). On the basis of this experience we strongly suggest that the search for unexploited grains is a productive strategy for identifying new annual and perennial grain crops.

Our second entry into the high protein gluten-free market niche was Timothy grass seed, trade marked as Timtana™. Timothy (*Phleum pratense*) was introduced into North America where it is established as a highly desired pasture grass. It is not known if grain from this grass was traditionally collected and consumed by people. There is a strong market for this small seeded perennial plant in the equine industry. To our surprise, no one had attempted to grind the seed into flour for human consumption. Timothy seed produced in isolation from gluten-containing cereals delivers excellent stand-alone or mixing flour for all manner of bread products, again is high in protein, flavour and fibre and gluten-free (Table 1, 2, and 3). It is the latter trait that has established this product in a high value niche market. Both Timothy grass and Indian rice grass are perennial and once established they have reduced water and fertilizer needs as compared with their annual counterparts.

Glycaemic Index

Protein malnutrition is a problem in much of the world (de Onis and Blossner, 2003). Additionally, an ever growing segment of the world is obese. Overall, of the world's adult population in 2005, 7.7 percent of men and 11.9 percent of women were obese and these percentages are projected to be increasing through 2030 (Kelly *et al.* 2008). Obesity is not in itself indicative of nutrition. It is indicative of over-consumption of calories especially in the form of starch. Most of our modern crops are selected for yield and the most efficient way for a plant to increase seed size is to increase storage starch relative to storage protein. In energetic terms, carbohydrates are less expensive to synthesize than protein. This is one reason why high protein wheat demands a premium price over lower protein wheat. In particular, plumper seed has a higher ratio of branched starch or amylopectin. Amylopectin, the branched form of starch, is rapidly digested, quickly releasing glucose (high glycaemic index), leading to that notable afternoon slump (Berti *et al.* 2004). This rapid spike in glucose is a real problem for diabetics. In contrast, amylose or straight starch is digested more slowly and the glucose spike is flattened. We suggest that we need to develop staple crops with lower glycaemic indexes (perhaps by reducing the GI to 50 percent of what they are now). This niche market could be even larger than the gluten-free market. Perennial grasses, with smaller seed sizes and less starch would be a good place to look for inherent low-glycaemic traits.

Overview

The requirements for the proper balance of essential amino acids needed for optimal nutrition have been known for nearly a century (Osborne and Mendel, 1914). It is time for a more proactive nutrition approach from plant science. There is evidence that valuable ancient food sources included perennial grains (Bohrer, 1973). Both Montina™ and Timtana™ are small seeded perennial grains. Perhaps the small seed size, relative to the major staple crops, is important in that the grain has to provide the plant with more nutrients per gram, and small seeds might offer a reduced target for predation. There are numerous molecular diagnostic products of basic research in plant genetics and biochemistry and tools available to implement improvement of crops relative to human nutrition. Given the advances in human biochemistry and physiology, we expect to see multidisciplinary linkages established to improve human nutrition relative to dietary components. Protein malnutrition should be a major target of plant geneticists. Plant breeders, by addressing these essential aspects of human nutrition, can fulfil the true needs of some populations that are not currently attaining their potential.

Perennialization as an approach to more sustainable agriculture might, in certain instances, turn the tide. However, there will be an uphill battle if yields are the principle "sine qua non" measure of success. Pests, weeds, and disease build-up in perennial systems will have to be addressed, perhaps with marker-assisted breeding, with multiline (mixed genotypes) approaches, with genetic engineering, and perhaps with pesticides either synthetic or biorational based measures. Perennial grains have their intrinsic sustainability values and advocates, in that they might reduce input costs. For example, in places where there are two rainy seasons, as in East Africa, the rattoon cutting of maize and/or sorghum after the long rains might lead to lower input costs and more erosion control and a real jump-start for the ensuing short rainy season, if weeds can be controlled. Perennial crops might be more sustainable in terms of soil holding, preventing bare ground wind and flood erosion, and lower input costs. They might need borrowed traits for disease and insect resistance from the existing intense annual plant breeding efforts. The strong suit of perennial crops might be that they could provide an input of enhanced human nutrition in addition to the environmental advantages that perennial crops can render.

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INTERCROPPING OF LEGUMES WITH CEREAL CROPS IN PARTICULAR WITH THE PERENNIALS TO ENHANCE FORAGE YIELDS AND QUALITY

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ABSTRACT:

Intercropping offers farmers the opportunity to engage nature's principle of diversity on their farms. Spatial arrangements of plants, planting rates, and maturity dates must be considered when planning intercrops. Intercrops can be more productive than growing pure stands. Many different intercrop systems are discussed, including mixed intercropping, strip cropping, and traditional intercropping arrangements. Pest management benefits can also be realized from intercropping due to increased diversity. Harvesting options for intercrops include hand harvest, machine harvest for on-farm feed, and animal harvest of the standing crop.

Since landholdings in Pakistan's mountainous Northern Areas are minute, farmers aim to maximize production per unit of area per season. An integrated approach that complements rather than competes with the existing farming system was needed. Forage production and availability have been affected by sole cropping vs. intercropping of forage legumes with cereals. To obtain early and good yields on small holdings under severe winter conditions, compatible fodder crops can be planted in mixtures to produce high fodder yields with good quality. Leguminous dwarf fodders like berseem can be mixed with taller species such as oats, ryegrass, brassicas etc. Lucerne is considered one of the most important leguminous fodder crops in Pakistan's Northern Areas.

Important priorities for future research include evaluating the potential for suitable cash cropping, promoting intercropping of potential fodder crops that might provide a more ensured/continuous supply over the winter, and improving the nutritional content of animal diets with, for example, the introduction/evaluation of improved alfalfa and fodder oats.

Keywords: intercropping, berseem, lucerne, alfalfa, fodder oats, soil fertility, sorghum

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INTRODUCTION

Historically, intercropping has commonly been practiced throughout the developing world. Due to limited land holdings, farmers usually practice an integrated and subsistence type of farming system that is not very flexible. For example, in Africa, corn (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), or millet (*Panicum* and *Pennisetum* spp.) are grown with pumpkin (*Cucurbita* spp.) cowpea (*Vigna unguiculata* (L.) Walp), pigeon pea (*Cajanus cajan* (L.) Millsp.), or beans (*Phaseolus* spp.). Cocoa (*Theobroma cacao* L.) is grown with yams (*Dioscorea* spp.) or cassava (*Manihot esculenta* Crantz). In the tropical Americas, maize (*Zea mays* L.) is grown with beans and squash (*Cucurbita* spp.). In both Africa and Latin America, beans or peas (*Pisum sativum* L.) climb tall cornstalks while pumpkins or squash cover the ground below. In these countries, many farmers have limited access to agricultural chemicals and equipment so prevalent in the developed world. Besides, intercropping is much less risky in that if one crop fails others may still be harvested (Machado, 2009).

Intercropping to reduce risk was a common practice in the United States and Europe before the 1940s, (Kass, 1978; Andersen, 2005), but the practice faded from significance as advances in mechanization and the availability of relatively cheap inorganic fertilizers and pesticides made monocropping more attractive. Paralleling the development of high-yielding varieties and production of cheap fertilizer that brought about the Green Revolution to feed rapidly growing populations, the practice of monocropping proved effective and economical (Horwith, 1985).

On the other hand, with fertilizer shortages developing and costs escalating, intercropping with legumes is again becoming desirable. The composite fertilizer price increased 113 percent between 2000 and 2007, led by gains in nitrogen prices (Huang, 2007). Meanwhile, environmental problems associated with heavy fertilizer use, e.g. surface- and groundwater pollution, soil acidification, and ammonia volatilization are becoming well known, and as synthetic fertilizer is a petroleum-based product, prices will continue to increase. Hence, fodder legumes such as alfalfa or lucerne (*Medicago sativa*), berseem (*Trifolium alexandrinum*), shaftal (*Trifolium resupinatum*), vetch (*Vicia sativa*), and cowpea (*Vigna unguiculata*) can be grown in association with fruit trees, providing fodder for livestock as well as improving soil fertility through biological nitrogen fixation. Oats might be a good choice for mixed planting with berseem or lucerne to maximize yields per unit area. There are several options available to enhance productivity through intercropping of several different crops. Some of these options are summarized below.

LEGUMES INTER-PLANTED IN ORCHARDS

The majority of the region's farmers rear livestock and also grow fruit trees. Therefore, an integrated approach that complements rather than competes with the existing farming system is required. In order to obtain superior quality fodder, improve soil fertility, and subsequently enhance fruit yields and quality, farmers intercrop lucerne, red clover, berseem, shaftal, or vetch in the orchards. Lucerne is considered one of the most important leguminous fodder crops that provide high quality hay for winter feeding.

Three improved winter active lucerne cultivars i.e. 'Sundar', 'Sequel', and 'Aquarius' were evaluated with a local cultivar in five to seven year old apple orchards in Chilas and Gilgit. 'Sundar' excelled over all cultivars in the double crop areas. With it, farmers have been able to harvest lucerne throughout the year on land protected from uncontrolled grazing.

Multicut forage sorghums (sorghum/Sudan grass hybrids), which were unknown in the area, provided an excellent means of increasing summer fodder production by producing three to four times as much fodder as the local maize. Local maize yields on average 39 tonnes/ha of green fodder, whereas the sorghum hybrid yields ranged from 110 to 138 tonnes/ha with an average of 127.7 tonnes/ha of air-dry material.

ENHANCED FODDER YIELDS, QUALITY AND SOIL FERTILITY PER UNIT AREA PER SEASON

In order to obtain early and good yields on small holdings in winter, compatible fodder crops may be sown in mixture to produce higher fodder yields and better quality per unit area per season. Short-statured leguminous fodders such as lucerne, berseem, and vetch can be mixed with oats, barley, ryegrass, brassica etc. Lucerne + oats, berseem + oats and shaftal +oats produced greater yields of green forage than did monocultures of the respective crops (Table 1).

TABLE 1. YIELDS OF SOME LEGUMINOUS FORAGE CROPS (TONNES/HA)

VARIETY	GREEN FORAGE	AIR DRY MATTER
LOCAL LUCERNE	55	19
SUNDER LUCERNE	120	45
SUNDER + OATS	190	58
SHAFTAL	45	14
SHAFTAL+OATS	80	25
BERSEEM	89	28
BERSEEM + OATS	130	47
OATS	90	38

Source: Dost, 1997

Oat + vetch and barley + vetch combinations produced 132-135 and 73-76 tonnes/ha of green fodder compared to 100 -105 and 56 -59 tonnes/ha pure oat and barley stands at both locations respectively in the 1 260 to 1 490 m altitude band (Table 2).

TABLE 2. GREEN AND DRY MATTER YIELDS (TONNES/HA) OF OATS, BARLEY, AND VETCH AT TWO SITES IN 1994-1997

CROPS	GILGIT (1490 m asl)		CHILAS (1260 m asl)	
	GREEN	DRY	GREEN	DRY
Oats	100	22	105	23
Oats + vetch	132	26	135	29
Barley	56	12	59	14
Barley + vetch	73	16	76	17

Source: Dost, 1997

A deep-rooted crop like lucerne can be intercropped with shallow-rooted crops like oats, rye, barley or a brassica; the annuals are usually sown between the rows of perennial fodder. Intercropping has a number of advantages over monocultures: more than one crop per season per unit area; easier weed control; higher yields than in pure sown crops; and fodder of better quality. Oats were intercropped in winter active lucerne and red clover in rows 30 cm apart at several sites. The intercropping of lucerne with oats produced greater green and DM yields than those of sole crops of either legume (Table 3).

Intercropping of oats with berseem clover provided earlier and greater fodder yields, and increased milk production by as much as 20 litres per cow per month on average compared with traditional practices. At the same time, the demand for purchased concentrates was reduced by 20 kg per month per animal and lactation period was extended by an extra two months (Dost, 1995).

TABLE 3. GREEN AND DRY MATTER YIELDS (TONNES/HA) OF LEGUMES AND OATS AT GILGIT IN 1993-1994

TREATMENT	GREEN YIELD	DRY MATTER
Lucerne	70	18
lucerne + Oats	115	30
Berseem	80	17
Berseem + Oats	135	30
Redclover	63	16
Redclover + oats	94	26

Source: Dost, 1995

Multiple cropping or mixed sowing techniques were carried out in North Pakistan by Dost (1997). The details are presented in Table 4.

TABLE 4. EFFECT OF MIXED SOWING ON GREEN AND DRY MATTER YIELDS (TONNES/HA) OF LUCERNE, RED CLOVER, AND OATS AT THREE SITES IN 1996-1997

TREATMENT	SULTANABAD		RAHIMABAD		SALING	
	GREEN	DRY	GREEN	DRY	GREEN	DRY
Lucerne cv. Sundar	110	30	105	26	68	20
Red clover	60	16	62	18	50	13
Oats	100	32	95	30	80	26
Lucerne + oats	140	39	136	37	102	30
Red clover + lucerne	115	32	105	26	70	22
Red clover + oats	90	26	93	28	75	23

Source: Dost. 1997

Oat has been used as a companion crop for sowing forages since the early 1990s in western Canada. In central Saskatchewan, oat was used at rates from 18 kg/ha to 72 kg/ha with 17 kg/ha sweet clover (*Melilotus officinalis*, *M. alba*) (Tinline, 1924). In southern Saskatchewan, Jefferson and Zentner (1994) sowed oats as a companion crop with lucerne on irrigated land. Lucerne sown alone produced much less than oat intercropped with lucerne or oat sown alone in the establishment year.

In Minnesota, Hartman and Sturtman (1983) recommended a seeding rate of 54-72 kg/ha for oat when used as a companion crop, compared with 72-90 kg/ha when sown alone for grain. Peter (1961) reported that oats cut for forage at the late dough stage plus a cut of intersown lucerne yielded more than lucerne established with or without herbicides and harvested twice in the establishment year. In contrast, Brink and Marten (1986) showed that oat as a companion crop to lucerne had inferior forage quality compared with barley when the mixture was harvested in the sowing year. In California, Lanini *et al.* (1999) reported that oat intersown into an established (but declining) lucerne stand was comparable to using paraquat herbicide for weed control, with the advantage of increasing first harvest forage yield. Marshal, McDaniel and Cregger (1992) suggested that growers planning to use oats as a companion crop should use early maturing, lodging-resistant cultivars, and remove the oat forage early to favour the establishing perennial forage crop.

NON WINTER-DORMANT VERSUS WINTER-DORMANT LUCERNE VARIETIES

One of the most important questions is whether we need perennials, winter-dormant or non-winter-dormant cultivars as they might affect the biomass as well as seed yields in the long run. Several perennial non-winter-dormant and winter-dormant varieties of lucerne were evaluated at three sites during 1993-1995. The details are presented in Table 5. Non-winter-dormant lucerne (Sundar being the main cultivar) has been extremely successful at all three sites. They may suffer some frost damage at high altitudes but grow throughout the year and yield more than twice as much as the winter dormant landrace in double-crop areas below 2 000 m. They also provided maximum green feed in the critical December-January period.

TABLE 5. GREEN AND DRY MATTER YIELDS (TONNES/HA) OF LUCERNE VARIETIES

VARIETIES	SITES					
	CHILAS		GILGIT		SKARDU	
	Green fodder	Dry matter	Green fodder	Dry matter	Green fodder	Dry matter
SUNDAR	165	50	174	52	90	26
MISASIRSA	98	32	117	34	74	20
PIONEER	92	29	95	30	86	22
SANORA	90	27	84	24	73	20
ILLUNICO	71	22	68	22	70	19
TYPE 8/9	100	32	96	30	74	21
POWERA	58	19	61	19	68	18
LOCAL	55	16	60	18	57	17
AVERAGE	91.13	28.38	94.38	28.62	73.25	20.38

Source: Dost, 1995

SINGLE CUT VERSUS MULTICUT FORAGE VARIETIES

Oat provides multiple cuts, tillers profusely, and yields more than wheat and barley in northern Pakistan. Standing oats can be cut progressively, releasing land earlier than normal for follow-on crops or relay cropping. Any remaining oats can be dried as hay. This coincides with optimum soil moisture for land cultivation and sowing of the following crop, and also allows small areas or peripheral lines on terraces to be saved for seed. In many, but not all instances, more recently bred cultivars outyielded older ones (Dost *et al.* 1994).

HYBRID VERSUS VARIETIES:

Local maize and millet are dual purpose crops that are extensively grown in North Pakistan. Multicut hybrid sorghum could ensure maximum tonnage of green as well as DM well distributed throughout the summer growing period.

Overall hybrid sorghums provided four cuttings in Gilgit and Chilas and two in Skardu and Khaiber. At all the locations, all hybrids produced two to three times more green fodder and DM yields well distributed over the entire growing period as traditional local maize and millet cultivars. Due to higher temperatures in Gilgit and Chilas, maximum forage yields were recorded as compared to Skardu and Khaiber.

CONCLUSIONS

It was observed that the multi-cut hybrid sorghums which were scarcely known in the area produced 100-125 tonnes/ha green fodder yields as compared to 25-30 tonnes/ha fodder yields by local maize. The improved varieties of maize were superior in grain, stover, and green fodder yields as compared to local landraces. However, improved varieties were 20 to 30 days late in grain maturity. Also the improved oats and lucerne varieties produced two to three times greater yields than local varieties.

The improved berseem clover varieties produced 132-140 tonnes/ha green fodder yields in six cuts as compared to 80-85 tonnes/ha by shaftal clover in three cuts. Although there is no tradition of applying chemical fertilizers to the forage crops in the region, maximum forage yields were obtained through application of 150-75 N-P kg/ha at most sites. However, increased use of fertilizer could not be justified in many instances for economic and environmental reasons.

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DEVELOPMENT OF CONTINUOUS LIVING COVER BREEDING PROGRAMMES TO ENHANCE AGRICULTURE'S CONTRIBUTION TO ECOSYSTEM SERVICES

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ABSTRACT

Over the last half century, 'Green Revolution' technologies have dramatically enhanced crop yields, but because of the emphasis on annual row cropping systems these increases have often come at the expense of food security and sustainability. Globally, many fear that agriculture is nearing a tipping point, with concerns that population pressure, declining natural capital, and diminished ecosystem service delivery will reduce global food security. As a result, a new Green Revolution is needed – a 'Forever Green Revolution' – that embraces continuous living cover on working lands through the development of a new suite of high yielding perennial (intermediate wheatgrass, sunflower, hazelnuts) and winter annual (pennycress, winter rye, winter barley) crops that provide economic return and improve multiple ecosystem services. By adding such crops to agricultural systems we can: enhance agricultural productivity, support rural economic development, and provide major environmental benefits to all citizens. Because these systems have longer growing seasons, they are able to capture more solar energy, water, and nutrients than purely annual systems and may be able raise crop yields, produce new high-value commodities (food, feed, and biomaterials), enhance soil quality, provide wildlife habitat, increase species biodiversity, and improve water resources. Additionally, agricultural systems that include perennial and winter annual crops may show greater resilience to climate change, as well as to weed, disease, and insect pressures. To accomplish a 'Forever Green' landscape, we propose three significant shifts in thinking: 1) focus public plant breeding programmes on development of crops that provide continuous living cover and high-value commodities 2) diversify and enhance agricultural stakeholder engagement in sustainable enterprise development, and 3) re-evaluation of concepts of production and efficiency in agricultural systems.

Keywords: agro-ecosystem, economic valuation, ecosystem service, RUSLE, tradeoff analysis yield

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INTRODUCTION

Over the past half century, Green Revolution technologies have dramatically enhanced crop yields (Baulcombe *et al.* 2009) while simultaneously reducing other ecosystem service outputs (Tilman *et al.* 2002). Globally, many fear we are nearing a tipping point (Garnett *et al.* 2013), and that given increased population pressure (Foley *et al.* 2011; Runge *et al.* 2003), declining natural capital (Jordan *et al.* 2007), and overall diminished ecosystem service delivery (Tilman *et al.* 2011) a new green revolution is needed – a "Forever Green Revolution" – that embraces continuous living cover on working lands through a new suite of perennial grain and biomass crops, and winter annual crops. Conceptually, this is related to the concept of evergreen agriculture that has been discussed as a way to improve food security across the world (Garrity *et al.* 2010). These crops must not only enhance profit for landowners, commodity groups, and agribusiness, but also ecosystem services for society. We propose that a sustained focus on developing continuous living cover is an essential avenue for sustainable intensification of agriculture (Garnett *et al.* 2013).

The potential benefits of continuous living cover have long been touted (Teasdale *et al.* 2007, Scheinost *et al.* 2001): decreased autumn tillage during the multi-year lifetime of a crop stand, leading to reduced input costs and soil erosion; reduced herbicides from spring weed suppression; increased habitat for beneficial insects (pollinators and predators), providing a biological control that reduces inputs and increases pollination services; decreased surface and subsurface water pollution. However, despite these benefits, relatively little has been done to include these crops in rotations or improve them. Nationally, perennial grains and winter-cover crops constitute less than 7 percent of all cropland (Wallander, 2013) in the United States. This is likely due to the limitations of current plant material to improve environmental quality and simultaneously increase economic viability of agricultural operations. However, it is possible to breed with multiple benefits in mind – benefits not only including high yield, but also increased ecosystem service delivery. The starting and ending point of sustainable intensification is land management, which primarily focuses on the questions, "What plant material is available?" and "Where should plant material be placed on the landscape?" To implement continuous living cover in current temperate-zone agro-ecosystems, there are two major options: winter-hardy annuals, and perennial grain and biomass crops.

Historically, winter-annual crops have provided multiple values to landowners not just as a winter cover, but also as livestock feed. Today, the increased segregation of animal and plant agricultures (Godfray *et al.* 2010) and the shift in animal rations toward maize and soybean derivatives, has meant that winter and cover crops are no longer as relevant to producers. Forage legumes and grasses are still important parts of the landscape, but they are disappearing due to this increased separation between animal and crop agriculture. While current winter annual cover crops such as winter rye (*Secale cereale* L.) can mitigate the off-site nutrient transport, soil erosion, and loss of soil organic matter that occurs under a maize (*Zea mays* L.) and soybean (*Glycine max* L.) rotation without jeopardizing landowners' livelihoods (Creamer *et al.* 1996; Strock *et al.* 2004; Kaspar *et al.* 2012), they offer little other value to farmers. Additionally, farmers commonly find current cover crop options difficult to establish and terminate without increasing risk to the subsequent cash crop (Leavitt *et al.* 2011). These concerns largely explain the small area devoted to cover crops in the United States. In response to cover crops' lack of economic viability, new winter annuals are being evaluated and developed, such as pennycress (*Thlaspi arvense*) and camelina (*Camelina sativa*). Both produce valuable oilseed in addition to their other ecological benefits (Phippen and Phippen, 2012).

The second form of continuous living cover is perennial grains and other herbaceous perennial crops, including high-yielding biomass crops. Perennial grains are less well-developed than other perennial crops; initial attempts to produce a perennial grain have been met with mixed results, with yields ranging from 10-70 percent of annual check cultivars (Scheinost *et al.* 2001; Sacks *et al.* 2003; Sacks *et al.* 2006). These mixed results have led some to question whether it is possible to breed a high-yielding perennial grain. This debate centres on whether it is physiologically possible for a plant to allocate resources to both sexual and asexual production in a way that would allow for yields comparable to sexual grains. Additionally, it has been argued that high yielding perennial grains do not occur in nature, have not already been domesticated, and therefore, are likely impossible to develop.

Counter to this, perennial plants introduced to novel environments where consumers are absent can experience rapid evolutionary change and allocation of resources to increased seed and biomass production (Evolution of Increased Competitive Ability Hypothesis) (Bossdorf *et al.* 2005). Further, Cox *et al.* (2002) and DeHaan *et al.* (2005) developed a framework suggesting that because of a longer growing season, perennial grains could capture more sunlight resources resulting in greater total biomass, which could be allocated to seed production. Indeed, many of the arguments against high-yielding perennial grains have drawn information from what is possible or observed in natural systems. But, in the novel environment of an agricultural system, it may be possible to develop new life strategies by changing the selective constraints the plants experience. For instance, insect herbivory, soil nutrients, water availability, and the degree of group selection all can be varied in an agricultural system. Barnes *et al.* (2013) explored some of these possibilities by developing a physiologic model of plant resource allocation that showed perennial seed production equaled or surpassed that of annuals under certain conditions, implying that high-yielding perennial grains may be bred for in the real world, and may offer a competitive alternative to annuals. Additionally, Bell *et al.* (2008) has shown that, under certain conditions, even if a perennial grain crop produces 30 percent less yield than an annual system, decreased input costs can make up the difference in profit, even as the perennial crop provides additional ecosystem services.

Major questions remain regarding perennial grains such as how they will respond to domestication. Will perennial grains transition similarly as annual crops and undergo "domestication syndrome" (the development of a series of traits related to domestication, which have been altered in a similar way in many species across many taxa) (Harlan, 1992; Vaughn *et al.* 2007; Weeden, 2007)? Even more uncertain is whether the annual domestication syndrome phenotype is the ideal phenotype (ideotype) for a perennial grain domesticate. For example, does the ideotype of the perennial *Helianthus* seed crop have a single inflorescence or multiple inflorescences that flower simultaneously (Kantar *et al.* 2014)? The above findings and questions simultaneously reinforce the need for continued research investment in perennial grains and provide cautious hope surrounding their potential success.

In order to rapidly develop continuous living cover as a strategy for sustainable intensification of temperate-zone agro-ecosystems, we call for interrelated paradigm shifts in two areas – plant breeding and stakeholder engagement. In essence, we argue that breeding must be situated in an integrative and systemic approach to sustainable intensification. Below, we describe a new approach to development of plant germplasm for sustainable intensification of agriculture. We term this approach the '*Reflective Plant Breeding Paradigm*' and we are developing it in the context of an ongoing research and development programme for continuous living cover and sustainable intensification at the University of Minnesota.

THE FOREVER GREEN INITIATIVE

The Forever Green initiative lays out a cohesive vision for how to accomplish "sustainable intensification" of the Upper Midwest agro-ecosystem. The initiative grew out of Minnesota's history with cover crops and perennial grains as well as the obligation of a Land Grant University to engage with multiple stakeholders: farmers and their advisors, agricultural industry, and the general public. Realizing this obligation resulted in the merging of traditional plant breeding focused on farmer needs with a diverse array of disciplines (Table 1). We are approaching this task from the ideological point of view that germplasm must be developed to create both economically and ecologically profitable crops. The initiative involves more than 15 disciplines ranging from ecology and agronomy to plant breeding and food science to economics and sociology, all focused on two interconnected questions: 1) What plant material? and 2) Where is the material best placed on the landscape? These two questions form two continuous, synergistic feedback loops where enterprise development and stakeholder engagement interact with the plant breeding process in the Reflective Plant Breeding Paradigm (Figure 1). The Reflective Plant Breeding Paradigm includes robust engagement of many different disciplines in order to define the agro-ecological performance of germplasm, and define the trade-offs and synergies that are present as part of the germplasm being tested under different enterprise development scenarios (Figure 1). The 'Forever Green' initiative is an attempt to empirically develop crops that when strategically placed on the landscape will fit new ecological niches and provide environmental services while simultaneously providing economic benefits through a commercial product. In essence, it is an empirical attempt to test "sustainable intensification". Specifically, the 'Forever Green' initiative is examining a wide range of crops including winter-annuals, short-rotation woody species, perennial grains, and perennial plants for natural products (individual projects are outlined in Table 1).

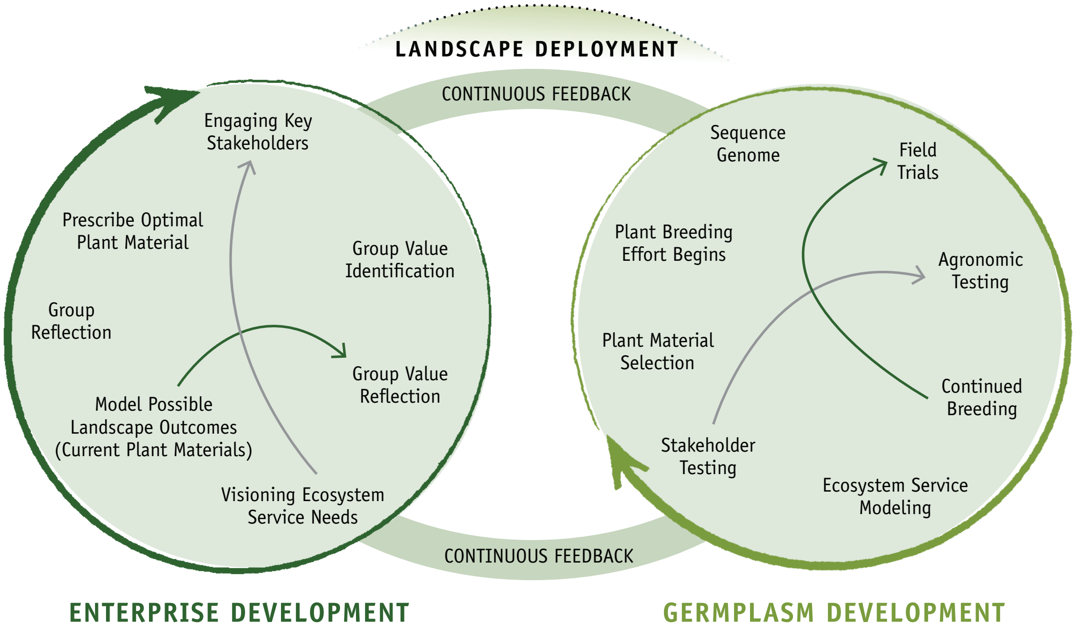
TABLE 1. BRIEF DESCRIPTION OF SOME OF THE CROPS THAT THE UNIVERSITY OF MINNESOTA IS WORKING ON TO INCREASE YEAR-ROUND GROUND COVER

CROP	DESCRIPTION OF PROGRAM	UNIVERSITY OF MINNESOTA DEPARTMENTS INVOLVED
INTERMEDIATE WHEATGRASS	A perennial grass crop that can produce many different high-value products, providing economic opportunities that in turn support the environmental benefits that perennials provide. It produces large yields of seeds that are a high-quality substitute for wheat, while its dense root system and rapid regrowth after harvest build soil carbon, store water for later use, and prevent soil erosion. It can also be harvested for hay or biofuel and is highly tolerant of weather extremes, including droughts and intense storms.	Agronomy and Plant Genetics Applied Economics Soil, Water, and Climate Ecology, Evolution, and Behaviour Food Science and Nutrition Plant Pathology Public Policy

CROP	DESCRIPTION OF PROGRAM	UNIVERSITY OF MINNESOTA DEPARTMENTS INVOLVED
FIELD PENNYCRESS	A new winter-annual cover crop for corn/soybean farmers. It is planted after harvest of maize or soybean and resumes growth in early spring after winter dormancy. It provides crucial protection for soil during autumn, winter and spring, and produces high-value oil and protein meal from unused fertilizer and water that would otherwise be wasted. As well, pennycress suppresses weed growth, reducing herbicide costs, and supports honeybees and other endangered pollinators.	Agronomy and Plant Genetics Applied Economics Soil, Water, and Climate Ecology, Evolution, and Behaviour Plant Biology Plant Pathology Bioproducts and Biosystems Engineering Animal Science
WINTER MALTING BARLEY	A potentially high value cover crop that could be double cropped with soybeans. Current winter barley varieties do not consistently survive winters in northern climates.	Agronomy and Plant Genetics Animal Science Plant Pathology
WINTER CEREAL RYE	A winter-annual cover crop that has been shown to provide many environmental services without impacting the soybean yields in a corn/rye/soybean rotation.	Agronomy and Plant Genetics
PERENNIAL FLAXSEED	An excellent source of omega-3 fatty acids, whose value as a dietary supplement is widely recognized, while offering the soil protection, habitat, and resource-use benefits of perennial crops. An emerging natural products industry is interested in sourcing key ingredients for many products from native and sustainably-grown crops.	Agronomy and Plant Genetics
PERENNIAL SUNFLOWER	An emerging perennial crop that can produce food oils that are highly valuable because they are free of trans fats, while also providing all of the benefits of perennial crops, including use of otherwise-wasted resources, soil protection, reduced costs, and better tolerance of droughts and floods, which are predicted to become more common in coming years.	Agronomy and Plant Genetics Ecology, Evolution, and Behaviour Horticulture Law School Entomology American Indian Studies
HAZELNUTS	A new food and energy crop. Hybrids between native and European hazelnuts combine beneficial qualities of each. As a long-lived shrub, hazelnuts can fit profitably into many niches in the agricultural landscape. For example, farmers could gain significant revenue from hazelnuts grown as windbreaks, shelterbelts, and living snow fences. In addition to valuable nuts, mature hazelnuts can produce large yields of edible, heart-healthy oils or biofuel oils.	Agronomy and Plant Genetics Horticulture Plant Biology Forestry
WILLOWS	A rapidly growing woody perennial crop. As a small tree, this crop can provide many options for improving the habitat value of Minnesota landscapes, while providing all of the advantages of perennial crops and providing new bio-products, including sustainably produced construction materials and bioenergy. Grown and harvested on a three-to-five-year cycle, willows can bring substantial revenue streams to farms that can support the environmental benefits that they provide.	Agronomy and Plant Genetics Horticulture Plant Biology Forestry
ALDERS	Woody trees and shrubs with the capacity to be grown on sites that cannot support traditional row-crop agriculture. Due to the symbiotic relationship alders form with the nitrogen-fixing bacterium Frankiia alni, the trees can be gown on low-nutrient soils without the need for additional nitrogen inputs. The species naturally occur on wet margins and saturated soils, areas that are not typically farmed. As such, alders represent a potential bioenergy crop that will not compete with food crops for growing space on the landscape.	Agronomy and Plant Genetics Horticulture Plant Biology Forestry
KURA CLOVER	A crop with unique application in soil conservation and as a living mulch crop. We propose to promote use of Kura clover as a living but suppressed perennial sod into which maize or other grain crops are planted into strips killed with an herbicide. When the crop is harvested, Kura clover, which has underground- spreading rhizomes, can regrow into the space where the maize was grown. The Kura clover can then be grazed in the late autumn and following year.	Agronomy and Plant Genetics Horticulture Plant Biology Soil, Water, and Climate
NATIVE PERENNIAL SPECIES	Native species across the Upper Mississippi River Basin have been examined for antimicrobial, antifungal, and antioxidant activity. Promising species have been examined and selected for larger-scale production.	Agronomy and Plant Genetics Horticulture Plant Biology Law School American Indian Studies

FIGURE 1. SHOWS SYNERGISTIC RELATIONSHIP AMONG STAKEHOLDER ENGAGEMENT, BREEDING, AGRONOMICS, AND MODELING THAT ARE A PART OF THE FOREVER GREEN INITIATIVE'S ATTEMPT TO DEVELOP A REFLECTIVE PLANT-BREEDING PARADIGM

The illustration shows Enterprise Development focusing on stakeholder engagement on the left, and research goals focusing on Germplasm Development on the right, with a permeable membrane connecting the programmes. Lines within each programme indicate an example of feedback relationships among the various parts; in theory all nodes are interconnected. The Reflective Plant-Breeding Paradigm is built upon the traditional plant-breeding paradigm, which is primarily focused on enhancing crop yield and disease resistance. The new paradigm attempts to bring the traditional strengths of plant breeding into contact with other disciplines such as ecology and public policy in order to effectively identify and select plant material and characteristics that will maintain yield and simultaneously provide the greatest number of other environmental services that are required for a truly sustainable system. The University of Minnesota's programme incorporates perspectives from 15 different disciplines to more effectively address the challenges of new crop and enterprise development, while acknowledging that plant material will only be adopted across the landscape if it is economically profitable. The integrative approach helps identify the appropriate plant material, landscape position, and end use for a plethora of plant material.



The Forever Green initiative is engaging with enterprise development and stakeholders as part of the plant breeding process to answer the "what" and "where" from communities' perspectives. Research in social learning shows that often people react in unexpected ways to newly developed scenarios depending on their perspective (Johnson *et al.* 2012). By including the public in discussions through social learning processes, there is a greater sense of ownership where potential social and scientific solutions can be more easily understood and imagined

(Johnson *et al.* 2012). Recent calls for more system-based approaches (Power, 2010) require that the public perception of new technologies be included in any assessment of their potential use. Specifically, landowners need to be shown the direct benefits of new plant materials for any changes in management practices to occur. Ultimately, short of heavy-handed legislation, landowners will be the ones to implement land cover change, so it is critical to include their input and values in the design of new plant material. In addition, it is essential that the process be transparent, equitable, and inclusive of all stakeholders in land management.

Many landowners perceive that one or more high-yielding crops mean the most profits. Our goal is to change that perception to one that is more holistic, so that landowners are concerned with net economic output (including ecosystem services) per hectare over time. This could incentivize double cropping and justify the potential yield reduction of one crop in favour of new practices that could increase the overall profitability of and reduce the risk to the agricultural system. This would require a dramatic culture shift among farm communities. As Warner (2007) stated, “the greatest obstacle to ecologically informed alternative practices has not been a shortage of ideas; it has been the dearth of practical educational initiatives.” The process would need to be conducted iteratively over a long period of time to allow social learning to take place (Dana and Nelson, 2012).

Accordingly, a pivotal feature of the Reflective Plant Breeding Paradigm is the developing concept of ‘*Landlabs*’ (Jordan *et al.* 2013). These are place-based, coordinated efforts to design and implement new agricultural enterprises that meet high performance standards in economic, environmental and social terms. Landlabs engage a wide range of local and regional stakeholders and innovators. The goal is to engage these actors to develop and coordinate novel land-use configurations, supply chains, and policies necessary for the emergence of new sustainable enterprises. In essence, Landlabs serve as active “incubators” for coordinating technological, economic and policy innovations in enterprise development, and thereby reduce the economic and environmental risks and uncertainties faced by farmers, entrepreneurs, and public and private investors. Thus, Landlabs provide a social and institutional context for the coupling of germplasm and enterprise development (Figure 1) that is essential to the Reflective Plant-breeding Paradigm.

Simultaneously, multiple academic disciplines are working together to respond to the findings in the Landlabs to further refine the genetic resources required by the public. This is being done through an iterative process of breeding and then modelling landscape scale performance. These findings are being provided on an ongoing basis in Landlabs to inform the innovation needed for sustainable enterprise development (Jordan *et al.* 2013). Ideally, the Reflective Plant Breeding Paradigm will engage stakeholders by identifying new plant material that fits changing values and production needs. Farmers will then play an integral role in testing new material and providing feedback to make sure that the shifting target of “sustainable intensification” is met without compromising the values of people or the researchers. The process involves iterative stages allowing for simultaneous enterprise and germplasm development (Figure 1). Incorporating a process of value identification and testing into germplasm development may facilitate adoption once the material is developed (Jordan *et al.* 2011).

New production systems that combine summer annual crops, winter annual crops, and perennials can optimize use of limited land, water, and nutrient resources more efficiently than current systems do. For this reason, we call these systems high-efficiency agriculture. These systems are arguably the most promising vehicle by which we can rapidly and sustainably intensify agriculture and enhance its ability to withstand climate variability. In a spirit similar to that of the Reflective Plant-Breeding Paradigm, two areas need further research and development to realize the great potential of these high-efficiency systems: 1) genetic improvement of plant materials, and 2) development of new strategies to integrate perennial crops into the landscape in ways that provide environmental benefits and economic opportunities. Current work at the University of Minnesota on high-efficiency agriculture systems—as part of the Forever Green initiative—focuses on a portfolio of highly promising options for improving Minnesota agriculture's productivity, efficiency, and adaptability to variable climates (Table 1). Although each individual programme has its own unique challenges, all are being evaluated based on the Reflective Plant-Breeding Paradigm (Figure 1). The Forever Green initiative represents an empirical attempt to put into practice the theory of sustainable intensification whereby systems are created that can successfully increase ecosystem service delivery and economic profitability.

ANALYSIS OF TRADEOFFS AND SYNERGIES AS THE LENS OF SUSTAINABLE INTENSIFICATION

While the Reflective Plant-Breeding Paradigm encompasses both enterprise and germplasm development, germplasm development and landscape deployment are both explicitly and implicitly involved in economic and ecological tradeoffs and synergies. Analysis of tradeoffs at the plant and landscape scale frames the process of enquiry in terms of what is biophysically and politically possible. At the plant scale, we are actively working to explain and model the tradeoffs between length of life and annual seed production. Theoretically, it is possible for a perennial to be high yielding (Barnes *et al.* 2013), however several potential constraints merit further consideration. In *Helianthus*, for example, the advancement of yield is being weighed against with other essential agronomic characteristics such as synchronous flowering time and shattering. In intermediate wheatgrass, the interaction between nutrient treatments and baking quality and post-harvest processing is being examined. In pennycress, the interaction between yield of the cover and yield of the subsequent soybean crop is being investigated. Emerging results suggest that old and new breeding techniques can either entirely overcome the initial tradeoffs or significantly mitigate their severity in many cases.

Implementation of perennial crops at the landscape scale suggests several areas where synergies or tradeoffs occur among ecosystem services. Four major ecosystem services – sediment retention, carbon sequestration, pollinator services, and biological control – are examined qualitatively below. First, it has long been observed that an increased reliance on the corn-soybean rotation has led to increased sediment and nutrient loss with small critical landscape positions contributing disproportionally more sediment and phosphorous to waterways (Galzki *et al.* 2011). However, implementing current best management practices, which do not target landscape positions for conservation practices, would lead to only incremental reductions in nutrient export (Vache *et al.* 2002). Identifying fine scale differences in terrain could allow for better temporal and landscape position of management practices to ensure maximum conservation benefits (Galzki *et al.* 2011). Further, nutrient and sediment loadings in waterways can have significant adverse effects on humans and ecosystems (Jones *et al.* 2001). Strategic development and landscape placement of new perennial plant material could lead to disproportionately large reductions in sedimentation at the watershed scale (Parish *et al.* 2012) while producing economically competitive yields, an example of synergism among ecosystem services and agricultural productivity.

Second, increasing soil carbon is an important ecosystem service to mitigate climate change and can be accomplished by land use changes (Powlson *et al.* 2011). Recently it has been shown that reductions in carbon emissions from reduced tillage are not as large as previously thought (Luo *et al.* 2010; Mishra *et al.* 2010), however the reductions from changing annual vegetation to perennial vegetation still have the potential to decrease atmospheric CO_2 (Collins *et al.* 2010). Therefore, perennial crops can potentially increase the amount of carbon that is sequestered in stable forms in agricultural soils.

Third, another significant benefit of continuous living cover cropping systems is their potential to attract and support beneficial insects for pollination and biological control. For example, there is widespread evidence showing that diversification of cropping systems enhances biological control of insect pests (Letourneau *et al.* 2011). Provisioning resources, such as floral nectar and pollen, in a diversified planting can attract and enhance predator populations leading to greater biological control (Hogg *et al. 2*011). Particular perennial plants and plant breeding programmes have the potential to contribute germplasm that enhances biological control. For example, *Helianthus* species are known for producing extra-floral nectaries, a nectar source excreted primarily from the petioles. Such nectar can provide an early pre-flowering, alternative resource for such beneficial predators as coccinellid beetles, which are shown to perform equally on sugar versus prey-only diets (Lundgren, 2009). Further, sunflowers have been shown to increase the density of these beetles in adjacent annual crops (Jones and Gillett, 2005). Given the importance of coccinellids as a beneficial predator (Gardiner *et al.* 2009), there is potential for strategic integration of perennial sunflowers to enhance biological control. This further illustrates the value of breeding for multiple benefits including nectar production for biological control while producing seed for oil production.

There are many and varied perspectives on what is considered highly productive. Productivity is intertwined with cultural values and, in practice, incorporation of values is accomplished through stakeholder engagement. Tradeoffs and synergies between ecosystem service phenotypes and traditional phenotypes for breeding programmes provide new targets for plant breeders; these phenotypes are inherently based on a different scale than traditional measures of productivity. These considerations are necessary to define the set of ecosystem goods and services that are valued by stakeholders in any given situation, and to define goals for breeding in the context of developing new sustainable agricultural enterprises.

A CASE STUDY: WATONWAN COUNTY, MINNESOTA

To demonstrate how new plant material could potentially function in a highly productive region of the United States, we conducted a case study involving the fertile landscape of southern Minnesota. Specifically, our analysis of Watonwan County, Minnesota, illustrates the *Germplasm D*evelopment side of the Reflective Plant-Breeding Paradigm, where breeding, agronomic testing, and continued breeding feed into ecosystem service modelling (Figure 1).

Minnesota has 10.93 million hectares of farmland, occupying nearly half the 22.5 million hectares in the state. Two highly productive and profitable crops, maize (3.52 million hectares planted in Minnesota in 2012) and soybean (2.87 million hectares) are the foundation of the state's agriculture, together with other important production systems such as animal agriculture, small grains, and horticultural crops. Most of Minnesota's current cropping systems consist of summer annuals. Considering Minnesota's strong cropping system base and the in-development plant materials of pennycress and intermediate wheatgrass, we began to ask: How do current Minnesota agro-ecosystems compare with the native prairie ecosystem in terms of ecosystem service delivery? How will these new crops potentially alter the delivery of ecosystem services when compared with current cropping systems and the native prairie?

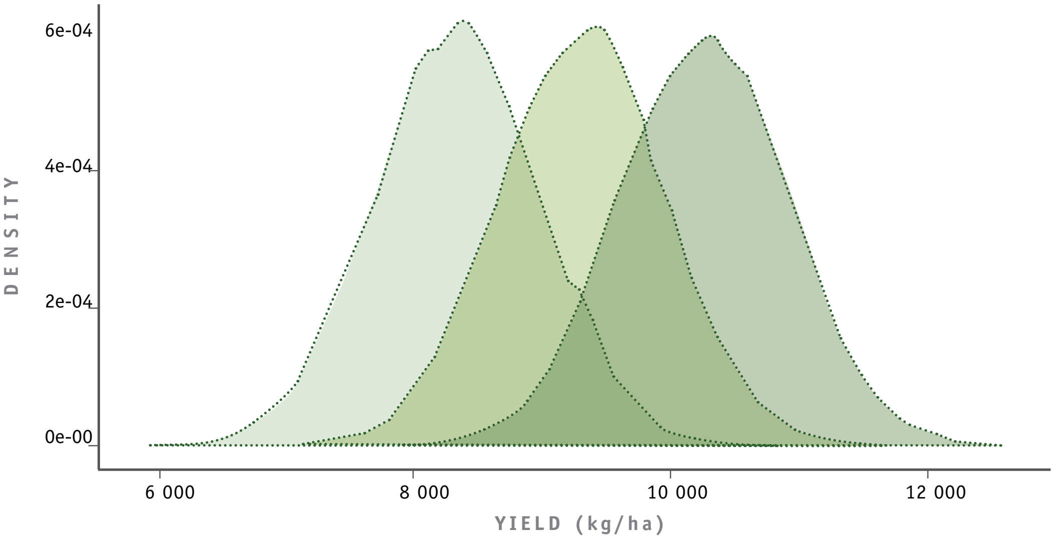
We performed a preliminary analysis that examined the tradeoff between the ecosystem services of sediment retention and total net return in the county given seven crop rotations – continuous maize (C), continuous soy (S), maize/soybean (CS), maize/rye/soybean (CRS), maize/pennycress/soybean (CPS), soybean/spring wheat (SW), and continuous intermediate wheatgrass (IWG). *We hypothesized that the new crops would enhance sediment retention and net economic output of Watonwan County, when compared with currently existing cropping practices.*

EXPERIMENTAL PROCEDURE FOR WATONWAN COUNTY, MINNESOTA CASE STUDY

For a full description of methods see Appendix 1. To summarize, we modelled sediment retention with the Revised Universal Soil Loss Equation (RUSLE) altered slightly to be applied in a geographic information system (GIS). We downloaded the baseline digital elevation model of Watonwan County from the Minnesota Department of Natural Resource's data warehouse. The 2006 National Land Cover Dataset (NLCD) for Watonwan County was downloaded from the Multi-Resolution Land Characteristic Consortium website (Fry *et al.* 2011) in order to differentiate between cropped and non-cropped land. To calculate the net economic return for each cropping system over the period of 2001-2010, we utilized crop production economic data containing average yield, production costs, gross return per acre (converted to gross return per hectare), net return per acre (converted to gross return per hectare, and price per bushel from the USDA-Economic Research Service (ERS) (retrieved July 2013).

Yield for each crop within each grid cell (100 m^2) was determined by creating three random normal distributions – a high, average, and low (Figure 2). Crop yield for each grid cell was then multiplied by the average value of the crop over a ten year period, and then adjusted to represent the respective value in rotation with other crops. To explore the comparative delivery of ecosystem services offered from the different cropping systems compared with the native prairie, we developed a series of landscape change scenarios. The scenarios consisted of transitioning the cropped area of Watonwan County from 100 percent native prairie to 100 percent agro-ecosystem for each crop rotation listed above. Land was placed into a rotation in 10 percent increments by soil erosion decile. Soil erosion and net return were summed across the landscape for each cropping system scenario. Graphs were all created using ggplot2 (Wickham, 2009) in R version 3.0.1 (R Core Development Team, 2013).

FIGURE 2. CORN-YIELD DISTRIBUTIONS CREATED AT RANDOM FROM EMPIRICAL DATA, USED TO MODEL YIELD ACROSS THE LANDSCAPE

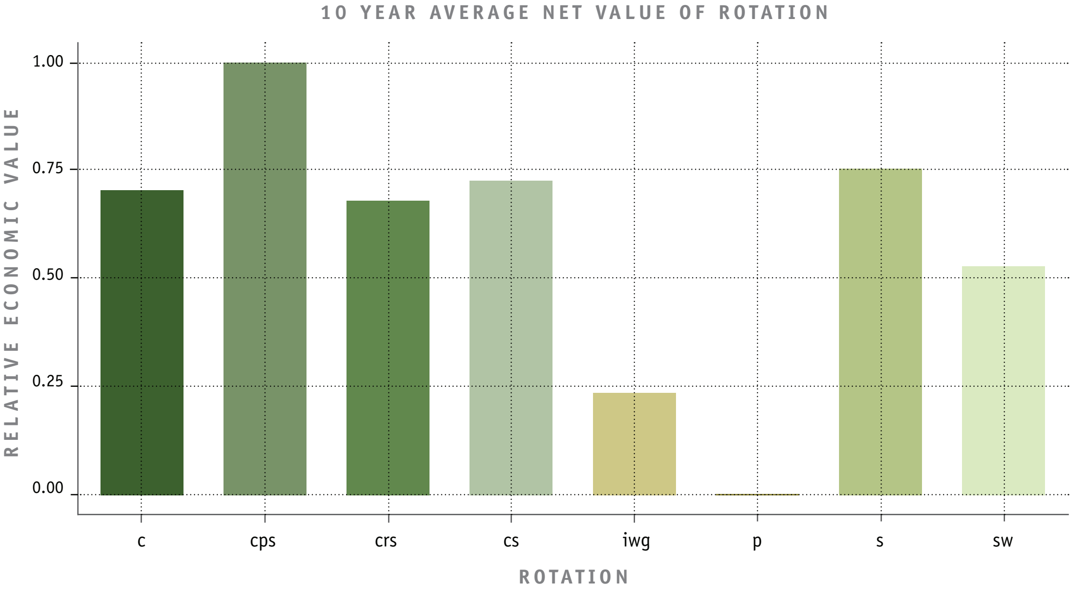


CASE STUDY RESULTS AND DISCUSSION

By modelling the effect of rotations on the potential for soil loss in Watonwan County; with RUSLE, we observed several trends. The seven rotations examined over a ten year time frame resulted in the following projections for soil-loss risk per crop rotation, ordered from greatest to least: SW, C, CS, S, CPS, CRS, IWG, and P (native prairie) (Figure 3). Comparatively, the C, S, and CS rotations, and the CRS and CPS rotations were similar. Intermediate wheatgrass had the least soil erosion potential compared with the other cropping systems analysed. SW had the greatest erosive potential likely due to the short amount of time spring wheat covers the landscape. P had essentially no erosion, which is verified in the literature (Kort *et al.* 1998). In Watonwan County ~15 percent of the land is at risk for sediment loss (greater than 5.5 Mg/ha/yr potential soil loss), so while the county in general is not at risk certain landscapes are, and different rotations could be used on these landscapes. For example, an intermediate wheatgrass planting reduced the risk of soil loss by approximately threefold compared with a corn/soybean rotation (Figure 3). Our data suggest that there is a great benefit from going to continuous cover on any landscape position; however, the greatest benefit will be seen on marginal lands.

FIGURE 3. RELATIVE SEDIMENT LOSS RISK DERIVED FROM THE REVISED UNIVERSAL SOIL LOSS EQUATION (RUSLE) OVER A 10-YEAR CROPPING SYSTEM OF EIGHT DIFFERENT CROPPING ROTATIONS

C = continuous corn, CPS = corn/pennycress/soybean, CRS = corn/rye/soybean, CS = corn/soybean, P = prairie, IWG = intermediate wheatgrass, S = continuous soybean, SW = soybean/wheat.



The modelled economic return from greatest to least was CPS, S, CS, C, CRS, SW, IWG, and P (Figure 4). Native prairie (P) was valued at zero because our interest was in comparing an unmanaged ecosystem to an agro-ecosystem, though we acknowledge that prairie mixtures could potentially be harvested and sold for biomass. Over the ten-year period, C, S, CS, and CRS produced similar net economic returns. If we had done the analysis over a shorter time period – say from 2008 to 2012 – we would have likely seen different economic outcomes because of the high value of maize and soy starting in 2008 caused partially by the United States' ethanol mandate (Zilberman *et al.* 2013) and an increased demand for soybean as animal feed in China (Godfray *et al.* 2010). The IWG rotation performed at approximately a third of the value of the CS rotation. The CPS rotation produced the greatest net return economically. This likely resulted from the ability of the CPS rotation to capture the high productivity and value of the CS rotation while simultaneously adding an additional cash crop half of the years, whereas rye does not offer the same economic benefits. Our analysis corresponded with USDA-ERS national average data for the general economic trends where data was available (Figure 5).

FIGURE 4. RELATIVE TOTAL NET ECONOMIC RETURN FOR A 10-YEAR CROPPING SYSTEM OF SEVEN DIFFERENT CROP ROTATIONS

C = continuous corn, CPS = corn/pennycress/soybean, CRS = corn/rye/soybean, CS = corn/soybean, P = prairie, IWG = intermediate wheatgrass, S = continuous soybean, SW = soybean/wheat.

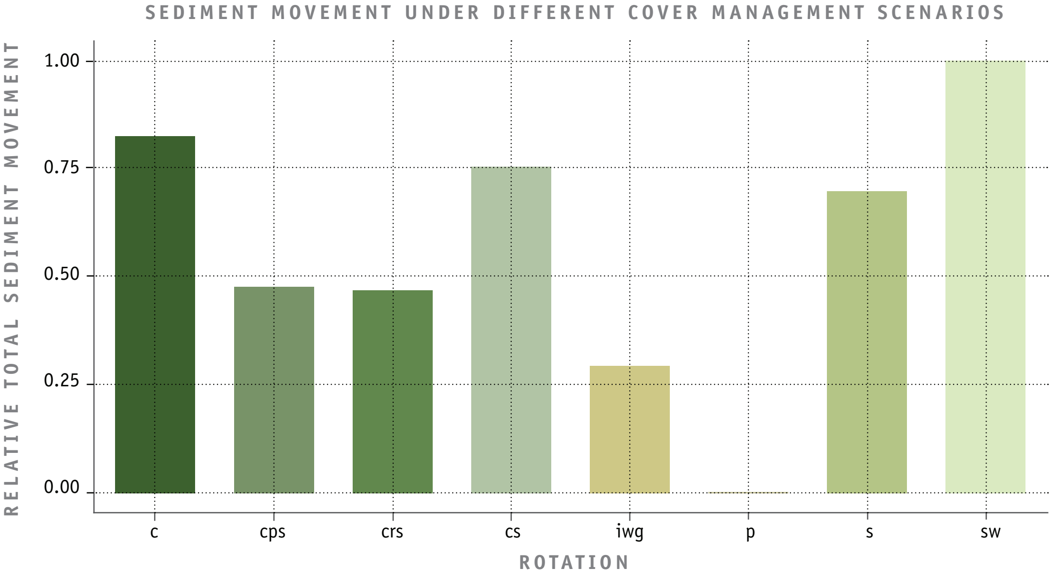


FIGURE 5. TEN-YEAR MEAN FOR NET RETURN PER HECTARE FROM USDA-ERS, 2001-2010

Intermediate wheatgrass return was calculated by discounting the value to 85 percent of wheat and modifying the input costs to account for decreased seed and field pass cost. Pennycress value was calculated as 50 percent of the value of soybean with the input costs being discounted, as it is only in the rotation for half of the years. Rye was not given an off-farm value, but additional costs were added for growing the cover crop after corn.

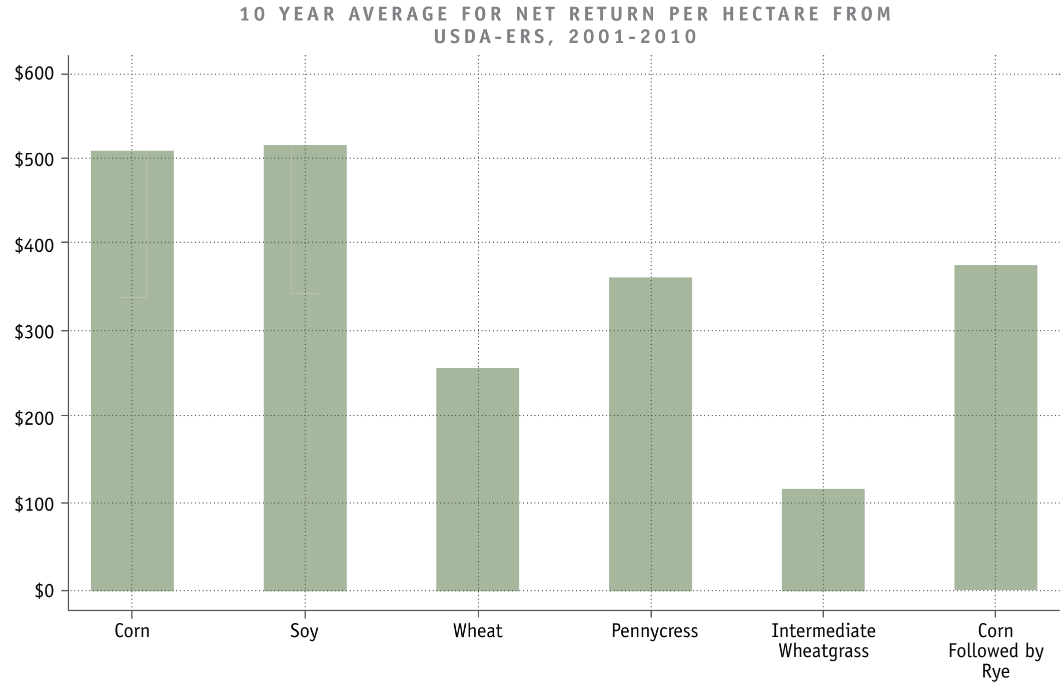
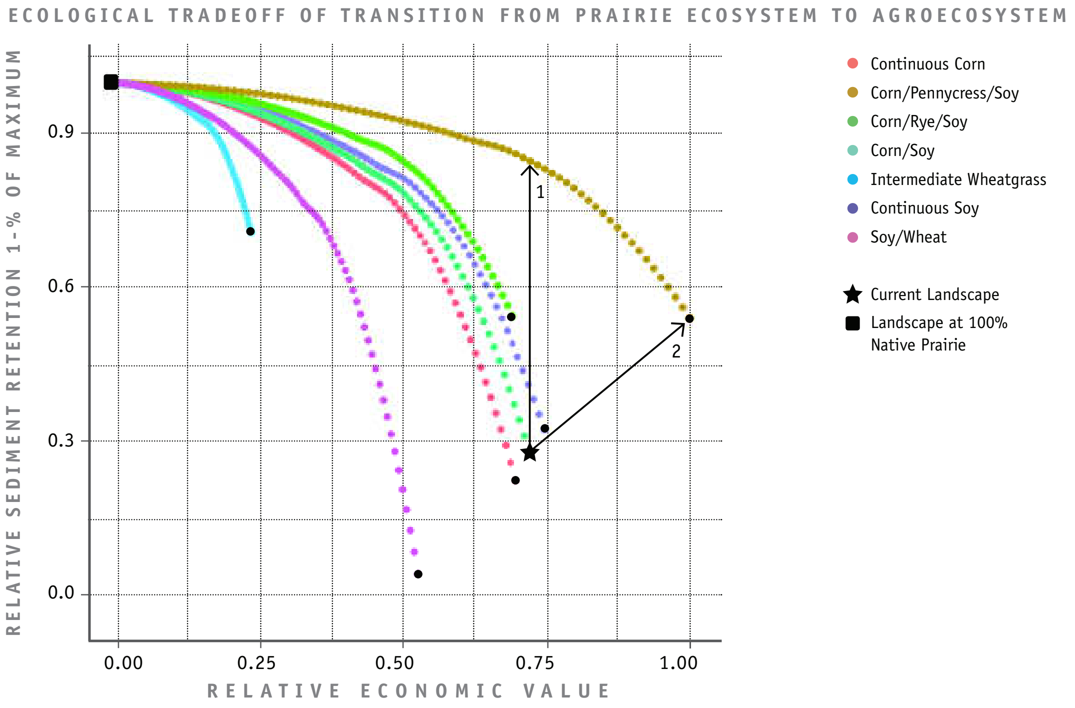


Figure 6 illustrates modelled changes in ecosystem delivery in Watonwan County from 100 percent native prairie to 100 percent managed agro-ecosystem. The relative loss of the ecosystem service of sediment retention was significantly reduced under certain rotations, even though economic output was greatly increased. For example, in the CPS rotation, there is a substantial increase in the delivery of ecosystem services when compared with CS, C, and S. Additionally, while the CRS rotation offered a similar level of sediment retention, the economic output from the CRS system was substantially less than with the CPS rotation. Both SW and IWG underperformed economically compared with corn- and soybean-based rotations; however, the ecological productivity of the IWG was much closer to prairie than any other rotation.

In Figure 6, the star represents an approximation of Watonwan County's current ecosystem service delivery. Black Arrow one shows the sediment retention service gain that could be made without losing any economic output at the county level by switching to a CPS rotation with approximately 15 percent of the landscape remaining in native prairie. Black Arrow two shows the potential economic and ecosystem service gains that would be possible by shifting 100 percent of cropped land from the existing rotation to 100 percent CPS rotation. This situation illustrates one of the major goals of the Forever Green initiative: to develop new material that positively alters both the economic and environmental output of a landscape. Our examination of perennial wheatgrass showed an increase in sediment retention and a reduction in profit compared with other crops. Nevertheless, the difference between intermediate wheatgrass and its closest relative, wheat (Figure 5), is relatively small. Economically, neither performs well against maize or soybean, however.

FIGURE 6. ECOLOGICAL TRADEOFF FOR SEVEN DIFFERENT CROP ROTATIONS AS CROPPED LAND IN WATONWAN COUNTY, MINNESOTA, IS CHANGED FROM 100 PERCENT PRAIRIE TO 100 PERCENT OF EACH OF THE DIFFERENT CROP ROTATIONS

Curves indicate the tradeoff between relative sediment loss and relative economic value of each rotation. The black dot at the end of a curve represents the maximum potential loss, and the star represents the position of the current landscape. The square represents a landscape that is entirely native prairie. Black arrow one shows the sediment retention service gain that could be made without losing any economic output at the county level by switching to a CPS rotation. Black arrow two shows the potential economic and ecosystem service gains that would be possible by shifting 100 percent of cropped land from the existing rotation to 100 percent CPS rotation.



The Forever Green crops that we modelled fit both ends of the spectrum. Intermediate wheatgrass provided excellent environmental benefits, but in its current form did not produce the required profitability. The corn/pennycress/soybean rotation provided an increase in ecosystem services (though not as great as with intermediate wheatgrass) and an increase in profit compared with the current system. This shows that we have existing technologies that can be applied to the landscape, as well as technologies that are on their way to being developed that may have greater environmental benefits.

IMPLICATIONS FOR THE FUTURE

The continued development of new plant material through integrated approaches such as the Forever Green initiative's Reflective Plant-Breeding Paradigm could provide win-win scenarios that deliver the profitability and the ecosystem services that stakeholders desire. In the current plant-breeding paradigm, now largely driven by markets for crops that support profitability, the breadth of ecosystem services desired by society are often overlooked because they lack sufficient profitability. Fortunately, universities and other non-profit organizations can take long-term views and greater risks, and produce outcomes from cropping systems once thought unimaginable. Perennial grains show promise, but at current levels of yield, their adoption by farmers is highly unlikely. While intermediate wheatgrass remains under development, other continuous cover crops such as pennycress appear to be nearly ready for landscape deployment. In the short term, increasing continuous landscape cover though the use of winter annual covers offers a promising avenue to deliver ecological and economic services. Eventually, as these cropping systems and associated supply and value chains (Jordan *et al.* 2013) are made less risky, growers and supply-chain firms may see these "alternative" crops as reasonable for investment. Given the preliminary results of the Watonwan County case study and other research being done at the University of Minnesota, the time of "reasonable for investment" may be close at hand.

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APPENDIX 1. **METHODOLOGY FOR WATONWON COUNTY, MINNESOTA, CASE STUDY.**

We chose Watonwan County in southern Minnesota to explore our cropping system scenarios because it represents highly productive land similar to that across much of the United States Corn Belt. The data for RUSLE was easily accessed from public sources of information. RUSLE is an empirically derived model that estimates rill and inter-rill erosion in tonnes/ha*yr (A) as a function of flow length in metres (L), slope in dimensionless units (S), rainfall and runoff erosivity index in MJ*mm/ha*yr (R), inherent soil erodibility in dimensionless units (K), cover type in dimensionless units (C), and supporting conservation practices in dimensionless units (S) (Renard *et al.* 1991; Desmet and Grovers, 1996) so that:

$$A = L*S*R*K*C*S.$$

We downloaded the baseline digital elevation model of Watonwan County from the Minnesota Department of Natural Resource's data warehouse in order to calculate the slope length and steepness (LS factor). The DEM was derived from Light Detection and Ranging (LiDAR) data captured in the spring of 2010 and downloaded orthorectified at a 1 metre spatial resolution in Nicotinamide adenine dinucleotide (NAD) 83 Universal Transverse Mercator (UTM) 15 coordinate system (retrieved July 2013). For further details on the creation of the DEM, refer to the online metadata (Minnesota DNR, 2010). The K factor was taken from the gridded Soil Survey Geographic (gSSURGO) database downloaded from the Natural Resources Conservation Services (NRCS) Data Gateway *(***Soil Survey Staff, 2013***)*. Data to calculate the R factor was found in the Agricultural Handbook (AH) 537 for Watonwan County (Wischmeier and Smith, 1978). The C factor was derived using the method found in AH 537 (Wischmeier and Smith, 1978), and because no supporting practices are being assessed for this study, the S factor was determined to be 1. All data was cropped to the area of interest and reprojected in the NAD 83, UTM zone 15 coordinate system in the Esri Geographic Information System software (ArcGIS) 10.0 (ESRI, 2011). The digital elevation model was resampled by interpolation to a 10 m^2 spatial resolution to correspond to the gSSURGO database. The LS factor was calculated entirely in ArcGIS 10.0 (ESRI, 2011) by first calculating the slope from the DEM using the slope tool. Flow direction was calculated from the DEM using the flow direction tool, and from the flow direction raster, flow accumulation was calculated. Following the recommendations established in Desmet and Grovers (1996) and Mitasova *et al.* (1996), flow length was replaced with flow accumulation, and then the LS factor was calculated using the equation:

$$LS = \left(\frac{A}{a_o}\right)^m \left(\frac{S}{s_o}\right)^n$$

where A is flow accumulation, a_0 is 22.13 metres based on the length of original test plots, S is slope, s_0 is 0.09 based on the slope of the original tests plots, and m and n are 1.4 and 0.5 – constants determined by empirical testing or the literature (Rabia, 2012). The equation was calculated using the raster calculator tool resulting in an LS raster. The K factor was then isolated from the gSSURGO database, and multiplied by the LS raster resulting in an LSK raster.

The C factor for each of the seven rotations and native prairie was calculated by the method established in AH 537 (Wischmeier and Smith, 1978) for a ten-year period. Utilizing the data table (Dowle *et al.* 2013), plyr (Wickham, 2011), and stats (R Development Core Team, 2012) packages in R version 3.0.1 (R Development Core Team, 2013), the C factors were each multiplied by the LSKR factors and then divided by 1 000 to give sediment movement (A) under the different cropping systems for each 100 m^2 grid cell. A was then put on a relative to maximum scale across all rotations.

In order to isolate the cropped land, the NLCD data layer was reclassified using the raster reclassification tool where classes 81 (Hay/Pasture) and 82 (Row Crops) were one and all else was 0. Using the raster algebra tool, the reclassified NLCD layer was multiplied by the LSK raster and the R factor from AH 537 to result in a cropland LSKR raster. This raster was then resampled to a 100 m spatial resolution and exported as a CSV file with a key field, the LSKR calculation, and the Crop Productivity Index (CPI) for each grid cell derived from the gSSURGO database.

To calculate the net economic return for each cropping system from 2001 to 2010, we utilized crop production economic data containing average yield, production costs, gross return per acre (converted to gross return per hectare), net return per acre (converted to gross return per hectare), and price per bushel from the USDA-ERS (retrieved July 2013). We calculated the net profit per kilogram of yield. The value of pennycress was calculated as 50 percent of the value of soybean, and intermediate wheatgrass as 85 percent the value of wheat. Input costs were modified to represent pennycress being in the rotation five of ten years, and intermediate wheatgrass having seeding costs only twice in the ten year period. We assumed intermediate wheatgrass would develop with the first year for establishment resulting in full input costs without any grain produced. Subsequent years were assumed to have reduced input costs and full yield until year six when it would need to be reseeded. Rye was not given an off farm value, but additional costs were added for growing the cover crop after corn.

Yield for each crop within each grid cell was determined by creating three random normal distributions – a high, average, and low (Figure 2) - built from the USDA-ERS 2001 to 2010 data and empirical data collected from 2006 to 2012 in Minnesota for pennycress and intermediate wheatgrass as a part of the Forever Green program, additional data for biomass value was gathered from Barnhart *et al.* (2012). The average distribution for each crop was based off of the mean and standard deviation of yield for the respective crop. The high and low distribution means were determined as the mean of the average plus or minus 1.5 times the standard deviation. The standard deviation from the average distribution was used in the high and low. Using the CPI for each grid cell in Watonwan County, yield was chosen at random from the appropriate distribution for each crop. This process resulted in a spatially informed yield for each grid cell.

Crop yield for each grid cell was then multiplied by the average value of the crop over a ten year period, and then adjusted to represent the respective value in rotation with other crops. The valuation resulted in a net rotation return per grid cell. Net value of a rotation was chosen because it captures what landowners would gain for themselves after the costs of production were met, and gives a sense of what type of livelihood can be made from the landscape under a given cropping system.

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ARE PERENNIAL CROPS MORE ADAPTED TO MAINTAIN LONG-TERM RELATIONSHIPS WITH SOILS AND, THEREFORE, TO SUSTAINABLE PRODUCTION SYSTEMS, SOIL RESTORATION AND CONSERVATION?

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ABSTRACT

Developing perennial crops involves many challenges, such as combining perenniality with high yield. However, attention also needs to be given to the sensitivity of perennial crops to tolerance for or resistance against pests and pathogens. Here, I discuss why it is important to consider soil-borne enemies and propose three avenues for further research.

Keywords: learning from nature, succession, plant traits, soil food webs, pathogens, ecosystem processes

Most major food and feed crops in the world have been derived from annual early successional plant species (Diamond, 1997). Traits that characterize early successional plant species are ephemeralism, preference for disturbed sites, low nutrient use efficiency, and pathogen sensitivity, however, such trait combinations in crops require crop rotation, land tillage, fertilization, and biocide use to control belowground and aboveground pests and pathogens. These requirements are a major constraint for sustainable agriculture, as they result in production of greenhouse gasses, loss of organic matter, nutrient leaching to ground- and surface water, and pollution of the environment with toxic biocides. Developing perennial crops could be a solution for circumventing intensive soil disturbance. However, early successional perennials also have adverse trait combinations that require intensive management practices. The question is what may be learned from nature when aiming at producing sustainable perennial crop production systems. I will discuss some research highlights on secondary succession following land abandonment in order to elucidate how soil food webs and soil ecosystem processes may respond to both changes in management and plant trait characteristics of early, mid, and late successional annual and perennial plant species.

Reducing land tillage and fertilization results in a development of the soil biodiversity and soil food web composition, which affects the mineralization and cycling of nutrients in ecosystems (De Vries *et al.* 2013). Such ecosystems could also be more resistant to extreme events, such as drought stress during the growing season (De Vries *et al.* 2012). Changes in soil food web composition and functioning are to some extent related to the presence of plant species with specific traits (Bezemer *et al.* 2010), whereas in part they are due to successional developments that are the result of reduced intensity of land use practices (Holtkamp *et al.* 2011). Insights from (semi-) natural ecosystems may stimulate thinking about how perennial crops could be developed in such a way that they will further enhance the sustainability of agriculture.

In a series of studies on the contribution of soil biota to vegetation development on abandoned ex-arable land, it was shown that early successional plant species had negative feedback interactions with the soil biota, both with soil fauna (De Deyn *et al.* 2003) and soil microbes (Kardol *et al.* 2006). Negative plant-soil feedback means that plants stimulate pathogenic components in the soil community more than symbiotic or mutualistic components, such as arbuscular mycorrhizal fungi (Bever *et al.* 1997). These negative feedback effects were not only evident in annual plant species, but also in (short-lived) perennials (Van de Voorde *et al.* 2011). Some plant species had positive feedback with the soil community, but those effects were more confined to later successional, slow growing plant species (Kardol *et al.* 2006). These species appeared to be promoted by the soil biota that were developing in their rhizosphere. However, this trait turned out to be combined with slow growth, which will be less desirable for plant species that are targeted for primary production.

Whereas these results are based on studies on non-cultivated (wild) plant species and mostly limited to temperate habitats, an increasing amount of studies is showing that negative plant-soil feedbacks occur in many early successional plant communities, independent of climate and soil type (van der Putten *et al.* 2013). Therefore, if annual crops are being developed into perennial crops, the advantage of perenniality, which will undoubtedly benefit the sustainability of soils, soil biodiversity, and counteract soil erosion due to reduced soil tillage, might be counteracted by the fact that crop ageing may go hand in hand with yield declines due to increasing exposure to soil-borne enemies, such as pathogens, root-feeding nematodes and herbivorous insect larvae.

There are several possibilities for counteracting these unwanted side effects of perenniality in crops, which may be accounted for in crop developing programmes. Thus far, there is little attention for these aspects and the question is how they may be accounted for. Here, I will provide three suggestions, which may need to be explored in subsequent studies. First, perennial crop varieties may vary in their susceptibility for negative plant-soil feedback development. Testing this would require screening of potential crop varieties in soils while allowing negative plant-soil feedback to occur. Recording effects of ongoing growth on temporal yield development and repetitive sowing in these soils may provide insight in the development of growth reducing soil biota. Second, the rhizosphere microbiome (Mendes *et al.* 2011) may be examined in order to test effects of perennial crop varieties on the development of a microbial community that may be antagonistic to major soil-borne pathogens and herbivores. Third, perennial crops may need to be grown in rotation, just as is being done with annual crops, in order to reduce the potential of soil-borne enemies between subsequent growth cycles. The main difference with current agriculture would be that crop rotations take many years, as each crop will be grown for several years in a row.

In conclusion, I wish to emphasize that the development of perennial crops may require prevention of negative side effects, such as the development of soil-borne pathogens. Perennial crops still have characteristics of early successional plant species, which make them sensitive to soil-borne enemies and, possibly, also aboveground pests and pathogens. Perennial crop development programmes, therefore, need to account for these unwanted side effects and I have proposed three avenues, but there will be clearly more possibilities to explore. The main point is that perennial crop development not only has to pass the hurdle of developing perennial varieties, but also of testing these varieties for resistance against, or tolerance of belowground and aboveground pests and pathogens. When accounting for these additional requirements, screening programmes may avoid future problems with e.g. yield declines in later years of perennial crop growth cycles.

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PERENNIAL GRAIN SYSTEMS: A SUSTAINABLE RESPONSE TO FUTURE FOOD SECURITY CHALLENGES

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ABSTRACT

Although conventional agricultural systems have provided growing supplies of food and other products, they have also been major contributors to global greenhouse gases, biodiversity loss, natural resource degradation, and public health problems. Concerns about the long-term sustainability of agriculture, especially in light of a growing population, have promoted interest in new transformative approaches to agriculture. Transformative approaches meet FAO's multiple goals of sustainable intensification: increasing crop production per unit area and enhancing environmental, economic, and social sustainability. Perennial grain systems are examples of such innovative systems but perennial grains, such as wheat and maize, will not be commercially operational for at least 15 to 20 years. For any perennial grain to be commercially available by 2030, more resources are needed to (i) accelerate plant breeding programmes with more personnel, land, and technological capacity; (ii) expand agro-ecological research on improved perennial germplasm; (iii) coordinate global activities through germplasm and scientist exchanges and conferences; (iv) identify global priority croplands; and (v) develop training programmes for scientists and students in the breeding, ecology, and management of perennial crops. In addition, farmer involvement, public-private collaborations, and significant changes in markets and policies will be necessary. Large investments have been committed to developing technologies for biofuel conversion of perennial crops because of their ecological advantages compared to annual sources, despite their potential to displace food crops. With similar commitments for developing food-producing perennial grains, commercially viable perennial grain crops could be available by 2030.

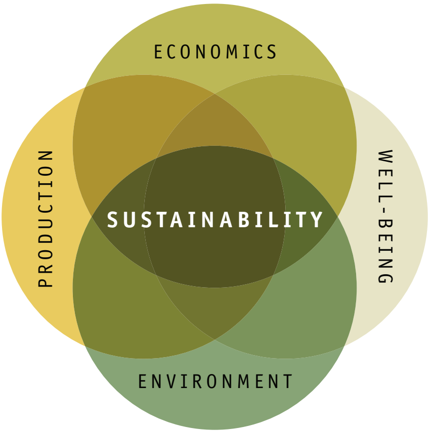
Keywords: agricultural research investment, ecosystem services, perennial grains, sustainability indicators, sustainable agriculture, transformative farming systems

THE MULTIPLE GOALS OF SUSTAINABLE AGRICULTURE

With increasing population pressure and finite resources, is it possible to meet both global food security needs and sustainability needs? According to Foley *et al*. (2011), tremendous progress could be made by (i) halting agricultural expansion, (ii) closing “yield gaps” on underperforming lands, (iii) increasing agricultural resource efficiency, (iv) shifting diets, and (v) reducing waste. Together these strategies could double food production while greatly reducing the environmental impacts of agriculture. Perennial grains could directly address (ii) and (iii).

To do so requires transformative farming systems to address global food security challenges. Why transformative? Because so many serious problems in agriculture exist as a result of not addressing multiple sustainability goals. According to a National Research Council report (2010) from the U.S. National Academy of Sciences, the multiple goals of sustainable agriculture are to (1) provide abundant, affordable food, feed, fibre and fuel; (ii) enhance the natural-resource base and environment; (iii) make farming financially viable, and (iv) contribute to the well-being of farmers, farm workers and farm communities. The National Research Council definition has similarities to that of FAO’s “sustainable intensification”, which is defined as increasing crop production per unit area and improving environmental, economic and social sustainability via management of biodiversity and ecosystem services (FAO, 2008). Sustainability is thus the intersection among economics, well-being, production, and environment (Figure 1).

FIGURE 1. THE FOUR COMPONENTS OF AGRICULTURAL SUSTAINABILITY



INCREMENTAL AND TRANSFORMATIVE APPROACHES TO SUSTAINABLE AGRICULTURE

The National Research Council report (2010) criticised mainstream, conventional farming for not addressing multiple sustainability goals. It identified numerous examples of innovative farming systems and practices that contribute to multiple sustainability goals, but noted they are not widespread. In order to improve the sustainability of U.S. agriculture, the National Research Council Report proposed both incremental and transformative approaches.

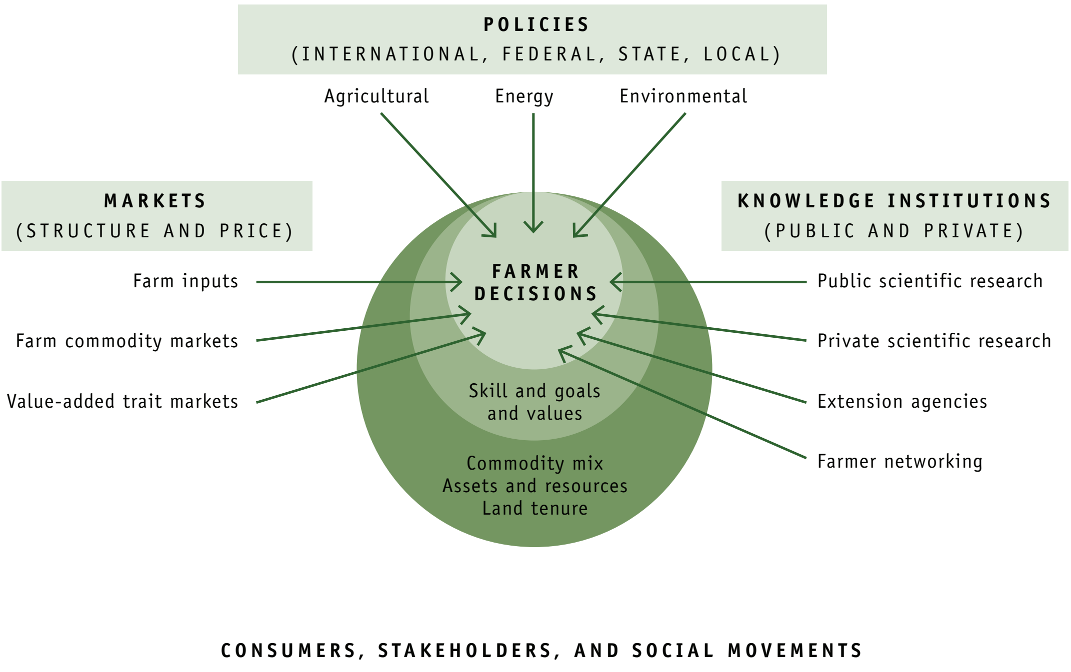
Incremental approaches are practices and technologies that address specific production or environmental concerns associated with mainstream conventional farming systems. Examples include two-year rotations, precision agriculture, classically bred or genetically engineered crops, and reduced or zero tillage. Incremental approaches offer improvements and should continue, but individually, are inadequate to address multiple sustainability concerns.

Conversely, transformative agricultural systems integrate production, environmental, and socioeconomic objectives and reflect greater awareness of ecosystem services on large, mid-size, and small farms. Examples include conservation agriculture, organic farming, mixed crop/livestock farming, integrated (hybrid) systems, agroforestry, and perennial grains.

COEXISTENCE OF DIFFERENT FARMING SYSTEMS

The future requires a coexistence of different farming systems that are sustainable. No one farming system will safely feed the planet, but rather a blend of farming systems will be needed. Proper alignment and coexistence of different farming systems at the landscape level will likely play a key role in future food and ecosystem security. The existence of innovative agricultural systems suggests that technical obstacles are not the greatest barrier. Rather, change is hindered by market structures, policy incentives, and uneven development and availability of scientific information that guide farmers' decisions (Reganold *et al*. 2011) (Figure 2).

FIGURE 2. DRIVERS AND CONSTRAINTS AFFECTING FARMERS' DECISIONS



An illustration of farmers embracing this decision-making process and striving for sustainability is Shepherd's Grain, a marketing label and alliance of a group of farmers in the U.S. Pacific Northwest, who use sustainable production practices and market differentiated wheat products together. Shepherd's Grain was founded by Karl Kupers and Fred Fleming, two U.S. direct-seed farmers from the large commercial grain-producing Palouse region in the states of Washington and Idaho. It has drawn growing attention from agrifood researchers and activists as an example of new "value chains" that can help support an "agriculture of the middle." Shepherds' Grain growers tend the soil and harvest wholesome wheat from farms across the Palouse but have to meet certain sustainability criteria, as defined and certified by the Food Alliance in Portland, Oregon. Shepherd's Grain wheat flours are sold in local health food stores throughout the U.S. Pacific Northwest and northern California and purchased by consumers for their quality, localness, and sustainability certification brand.

THE NEED FOR FARMING SYSTEMS RESEARCH

Unfortunately, most federal research grant programmes in the U.S. and globally still primarily support incremental research. For example, the bulk of public and private agricultural science in the U.S. is narrowly focussed on productivity and efficiency, particularly on technologies that fit into existing production systems and lead to private benefits (Reganold *et al*. 2011). We need to reallocate public funds to support transformative farming systems and systems research that measures multiple sustainability indicators at field, farm, and landscape scales.

Specifically concerning perennial grain systems, we need more studies as only relatively few have been conducted on perennial grains (e.g. Bell *et al*. 2008; Snapp *et al*. 2010; Hayes *et al*. 2012; Jaikumar *et al*. 2012). Moreover, we need farming system comparison studies, with replicates on a commercial farm or experiment station, or with commercial farms as replicates, in which early varieties of perennial grains are grown by themselves, in polycultures with other perennial grains, or in rotation with annual grains.

Such farming system studies require metrics for evaluating and measuring quantifiable components of a farming system. Since we would like a farming system to achieve multiple sustainability or ecosystem service goals, we can measure sustainability indicators or ecosystem services. Measuring a suite of sustainability indicators yields valuable results of a farming system's performance and health. Examples of indicators that can be used for measuring a farm's sustainability are listed in Figure 3. Of the four legs of sustainability – economics, well-being (social), production, and environment – the social sustainability indicators have been the least evaluated in comparison studies (Reganold, 2013).

FIGURE 3. EXAMPLES OF SUSTAINABILITY INDICATORS

SUSTAINABILITY INDICATORS

CROP/ANIMAL YIELD AND QUALITY
yield; plant nutrients; protein content; storage potential; vitamins; weed populations; insect pressure; disease

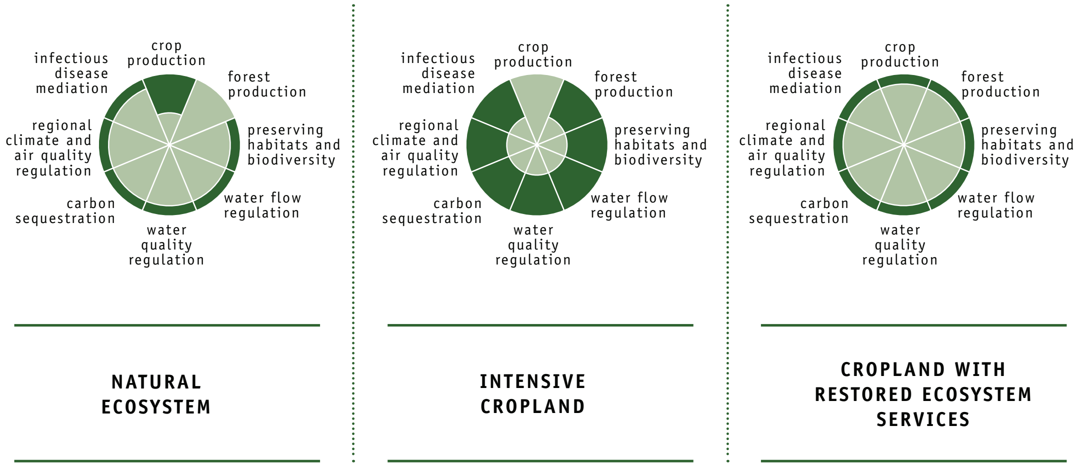
FINANCIAL PERFORMANCE
benefit/cost ratios; net returns; breakeven points; externalities

ENVIRONMENTAL QUALITY
soil health; pesticide and fertilizer impacts; soil erosion; surface and ground water pollution; energy efficiency

SOCIAL JUSTICE
worker wages, benefits, and safety; community well-being

In ecosystem studies, scientists have used ecosystem services as metrics. Examples of ecosystems services that can be measured on farms or plots are crop production, preserving habitats and biodiversity, water flow regulation, water quality regulation, carbon sequestration, air quality regulation, and infectious disease mediation. Figure 4 provides a good example by Foley *et al*. (2005), who illustrate ecosystem services under three contrasting land-use regimes: natural ecosystem, intensive cropland, and cropland with restored ecosystem services (Fig. 4).

FIGURE 4. COMPARING ECOSYSTEM SERVICES UNDER THREE LAND-USE REGIMES



Source: Reganold *et al.*, 2011

One could also measure a combination of sustainability indicators and ecosystem services. A good example of this is research by Glover *et al*. (2010), who evaluated sustainability indicators and ecosystem services between conventionally farmed grain fields and organically managed perennial grasslands at a range of spatial and temporal scales. First, they used commercial paired farm fields as replicates to evaluate ecosystem components of conventionally farmed grain fields and adjacent organically managed perennial grasslands. To make more refined determinations at smaller scales, they initiated replicated treatments on one of the farms. They also used watershed replicates in which the commercial farm replicates were embedded to make other larger-scale determinations.

THE CASE FOR PERENNIAL GRAINS

Farmers in this relatively young millennium face compounding pressures to meet the food needs of a growing, more demanding human population while reducing and reversing the extensive land degradation related to agriculture. Humans have more than doubled the yields of major grain crops over the past 60 years, and yet roughly one in seven people suffer from malnutrition (FAO, 2009). As the global population continues to grow, the demand for food, especially meat, also increases. Additionally, production of nonfood goods (e.g. biofuels) increasingly competes with food production for land and much of the land most suitable for annual crops is already in use (Godfray *et al*. 2010). Global food security largely depends on these annual grains—cereals, oilseeds, and legumes—that are planted on almost 70 percent of croplands and supply a similar portion of human calories. Three annual crops alone—maize, rice, and wheat—provide over 60 percent of human calories. Their production, though, often compromises essential ecosystem services, pushing some beyond sustainable boundaries (Cassman and Wood, 2005; Glover *et al*. 2010).

Current annual cereal crop production on large areas of marginal lands, particularly those on steeply sloping croplands, results in further land degradation and is unlikely to be sustainable over the long term (Cassman *et al*. 2003). These areas are much more suitable for perennial crops, such as forages and biofuels. Unfortunately, food security concerns and/or the greater market value of staple grains often pressure farmers to choose to plant annual grain crops instead of perennial crops even on lands poorly suited to their production. For these farmers, there are too few options to simultaneously meet their food, income, and ecosystems security needs. Meanwhile, the health of their farms continues to deteriorate at the very time that increased grain yields are critical. Facing the triple threats of climate change, land degradation, and a growing human population, business-as-usual approaches to transforming agriculture are no longer acceptable.

Perennial versions of the major grain crops, cereals, grain legumes, and oilseeds, would offer farmers more opportunities to meet their food and income needs while protecting their natural resources even on lands poorly suited to annual crop production. This is not an entirely new idea. Pioneering Russian scientists in the 1930s started perennial wheat breeding programmes and were followed by efforts in the United States in the 1960s (Cox *et al*. 2006). The technologies and resources of the time though limited the success of these programmes. The perennial wheat breeding efforts, for example, were abandoned in part because of plant sterility and undesirable agronomic characteristics (Cox *et al*. 2006). More recently, programmes have been initiated in Argentina, Australia, China, India, Nepal, Sweden, and the United States to identify and improve, for use as grain crops, perennial species and hybrid plant populations derived from annual and perennial parents: rice, wheat, maize, sorghum, pigeon peas, and oilseed crops from the sunflower, flax, and mustard families (Glover *et al*. 2010).

While perennial plant breeding programmes may not produce wide-scale impacts in farmers' fields for another 15 to 20 years, there is emerging evidence that novel perennial grain-based systems provide unique opportunities for protecting water and soil resources, while addressing the pressing problem of climatic variability. Even on the best croplands, perennial crops typically sequester more carbon, better protect soil and water resources, are more resilient to climatic changes, and are more productive above- and below-ground (Cox *et al*. 2006). Compared to annual crops, perennials have the potential to double sequestered carbon, and some can fix nitrogen. The extensive root systems and vegetative cover of perennial crops are the biological foundation to a 'climate smart' agriculture that captures and utilizes water resources, rehabilitates soil, and sequesters carbon. At the same time, food production must be a priority in the design of farming systems. This ensures immediate returns in the form of food security and economic benefits, in addition to environmental services from well-designed combinations of perennial, semi-perennial, and annual crops. Development of perennial grain crops has been termed the missing ingredient, as staple crops have historically been dominated by annual life forms (van Tassel *et al*. 2010).

RECOMMENDATIONS AND CONCLUSIONS

Large investments have been committed to developing technologies for biofuel conversion of perennial crops, despite their potential to displace food crops. With similar commitments for developing food-producing perennial grains, commercially viable perennial grain crops could be available by 2030. Public policies (e.g. the United States Farm Bill) and private funding are needed to support perennial grain systems. However, different strategies will be necessary to get funding for perennial grain development in specific countries, especially in developing compared to developed countries.

For any perennial grain to be commercially available by 2030, more resources are needed to do the following:

1. Accelerate plant breeding programmes with more personnel, land, and technological capacity;
2. Expand agro-ecological research on improved perennial germplasm; for example, we need perennial grain farming systems research on large plots and commercial-sized farm fields,

which in turn can generate confidence in further research investment. Such systems studies can be comparison studies with annual grain or mixed perennial/annual grain systems;

3. Support farmer involvement and develop public-private collaborations;
4. Coordinate global activities through germplasm and scientist exchanges and conferences;
5. Develop training programmes for scientists and students in the breeding, ecology, and management of perennial crops; and
6. Establish a World Perennial Grain Research Centre where resources can be focussed, priorities identified, and information and germplasm exchanged.

We need to change the discussion from annual versus perennial to complementary blends of the two. In addition, we need to better sell perennial grain systems based on their multiple sustainability benefits for global food security. Along these lines, including externalities and ecosystem services in economic studies would illustrate the financial viability of perennial grain systems. Perennial grains need to be more demand-driven by national governments, research institutes, and farmers and less supply-driven by institutions in developed countries. We need a systematic analysis of the highest potential perennial grain crops in development and the potential regions and global priority croplands where they are needed the most or can grow best.

Finally, we need to better communicate about perennial grains. If we want to reach farmers, producers, consumers, and extension agencies, social media utilities, such as YouTube videos, blogs, Facebook, and webpages, need to be used. Outreach events, such as field days and presentations, are also important. Perennial grain research findings from journals need to be reported in extension and outreach bulletins, articles in popular trade journals, and government technical guides and fact sheets.

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PERENNIAL GRAINS: BEYOND BOOTLEGGING, FEASIBILITY AND PROOF-OF-CONCEPT

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ABSTRACT

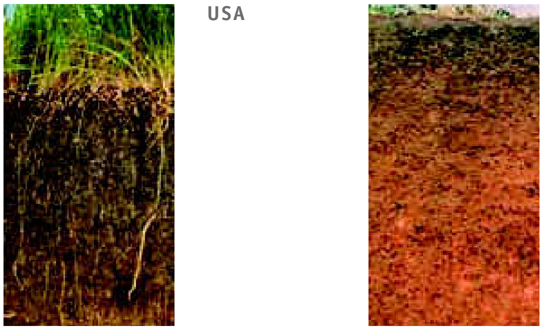
This paper examines the need for perennial grain development from a donor perspective. Why are perennial grains needed, especially in fragile and more remote areas? What kinds of investments does USAID currently make in perennial grain development? What lessons can be drawn to ensure the derivation of compelling cases for donor consideration? The paper concludes that: perennial grains must contribute to sustainable intensification and not extensification; must be demand-driven by scientists and farmers in target regions; priority targets should be identified based on priority regions and needs; with an emphasis on complementarity with current farming systems and research programmes; and short, medium and long-term benefits should be identified at different scales, to encourage staged investments.

Keywords: sustainable intensification, demand-driven, priority regions and needs, complementary systems, short, medium and long-term goals; staged investments

WHY PERENNIAL GRAINS

Food security is a major issue for the world. In the 1960s, the Green Revolution raised yields and grain supplies to defer the shortfall of food to a rising world population. Its successes, however, were not universal, as they focussed on narrow considerations of yield alone. High-yielding semi-dwarf cultivars were released which were responsive to alluvial and nutrient-rich soils, application of inputs, availability of irrigation. Consequently, benefits accrued in areas already productive, while those in more marginal situations of lower soil fertility and more remote locations generally missed out. For example, sub-Saharan Africa was largely bypassed, where 80 percent of soils have serious limitations, including low soil organic matter, short and variable wet seasons, and low if any investment in inputs.

FIGURE 1. CONTRASTING SOIL PROFILES IN USA (LEFT) AND SSA (RIGHT).



- >80% SOILS WITH SERIOUS LIMITATIONS
- LOW SOM
- 95% RAINFED
- HIGH VARIABILITY
- LOW INPUT INVESTMENT

Photo credit: Jim Richardson, Small World Gallery

Additional considerations needed to be taken into account, including broader considerations of yield (whole farm, livestock, nutrition), socio-economics (gender, cultural context) and natural resource management (soil, water, air, biodiversity). The likelihood is of further decline of the soil resource, with insufficient availability of organic matter (compost, manure, crop residues) to add to soil, leading to poor rainfall infiltration and retention as well as further decline and degradation of fragile soils. In this context, above-ground productivity will be low, with increased risk for investment in labour and inputs, and greater vulnerability to climate uncertainties. Farmer response is likely to require multiple planting operations, to address establishment and crop failure, requiring additional labour and necessitating lower yield potentials in the shorter seasons remaining. This may be partially compensated by extensification where additional land is available, but this in turn puts pressure on system sustainability via shorter or no fallow before the land is used again.

In this context, perennial grains could provide some relief by maintaining some stability of cover for land restoration, food and ecosystem security, nutrition and socio-economic considerations. For example, Dr Druba Thapa from the Nepal Agricultural Research Council sees potential for high-altitude perennial wheat on fragile soils in western Nepal "Perennial wheat may increase food and forage security significantly in the region, with deeper roots providing more stable grain and biomass yields. Deeper roots may increase uptake of selenium, zinc, iron and other minerals, and some of the 25 lines tested appear highly resistant to yellow rust."

FIGURE 2. HIGH ALTITUDE PERENNIAL WHEAT IN WESTERN NEPAL

Dr. Dhruba Thapa Nepal Agricultural Research Council Khumaltar Laitpur, Nepal

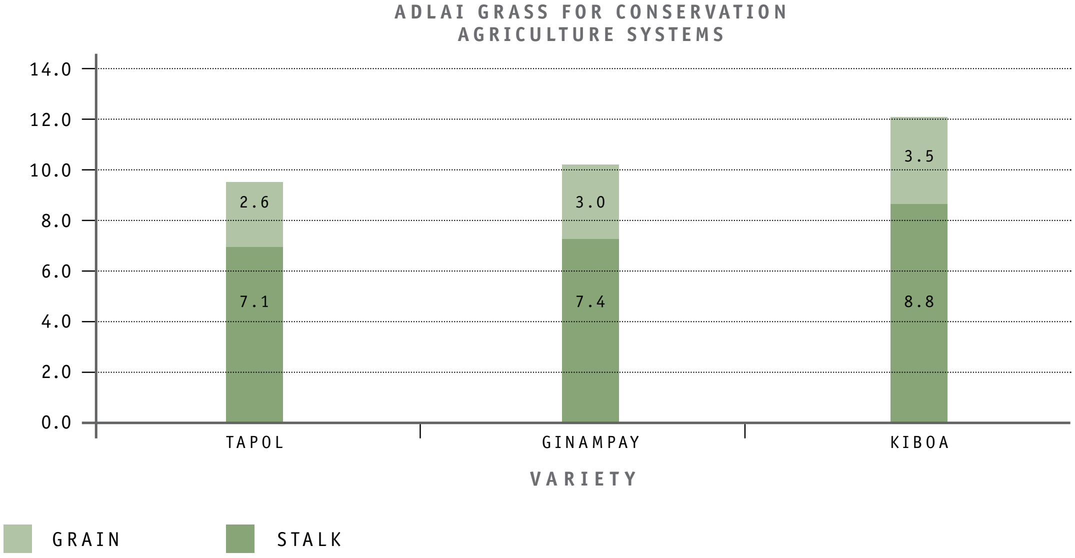
Photo credit: Dhruba Thapa

USAID PERENNIAL GRAIN INVESTMENTS

USAID is looking for opportunities where there is farmer demand for perennial systems that could assist their circumstances. These examples can be used to illustrate the criteria needed for priority to be assigned to such research investments.

Adlai grass has been identified for conservation agriculture systems in the Philippines. In diverse crop-livestock systems on acidic erodible hillsides, adlai grass has been shown to assist in providing a source of food, feed, resilience, ecosystem services complementary to farmer practice. Evidence is support of the concept is available from the World Agroforestry Centre and SANREM CRSP, where improved lines increased yield of both grain and stover.

FIGURE 3. ADLAI GRASS FOR CONSERVATION AGRICULTURE SYSTEMS. SANREM CRSP



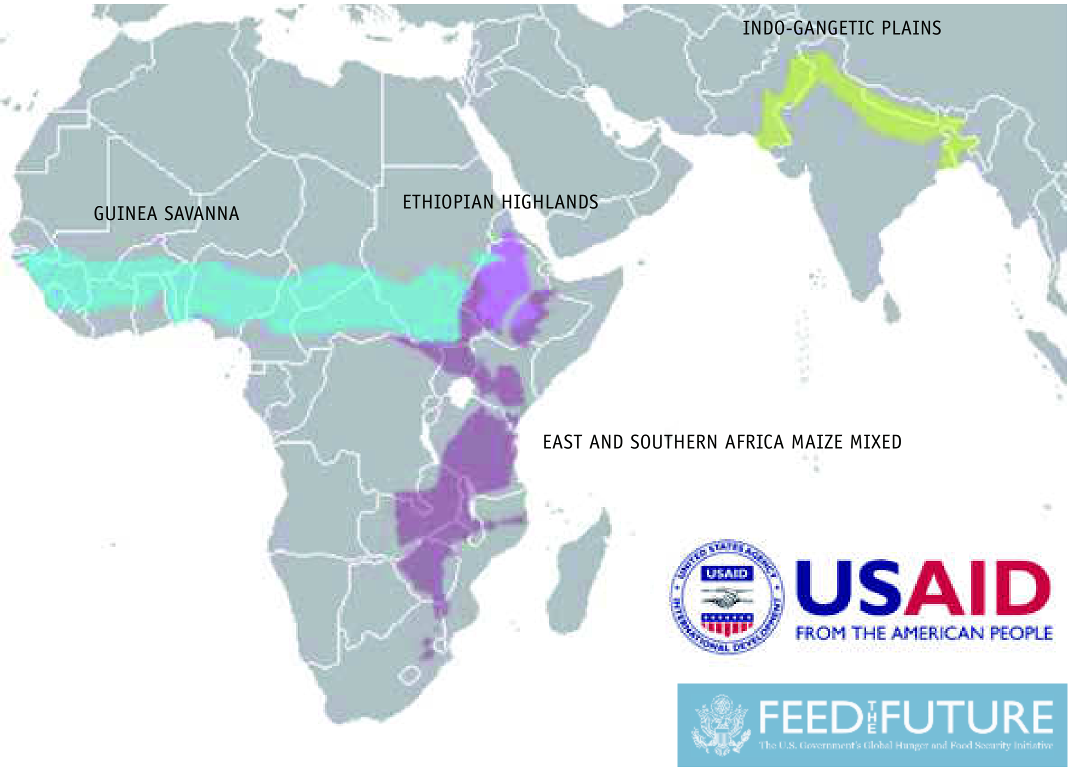
Likewise, the doubled-up legume systems presented by Snapp (this volume) allow diversification and intensification of traditional maize-dominated systems, using annual peanut, semi-perennial pigeon pea followed by maize, for substantial benefits in food, feed, resilience, ecosystem services, flexibility and complementarity with farmer practice. The system changes complement the role of fast maturing annuals along with the perennials.

FIGURE 4. INTERCROPPING OF PIGEON PEAS AND GROUNDNUT

Photo by Jim Richardson, Small World Gallery

A third example is the recent investment of US$5 000 000 over 5 years in the Feed the Future Innovation Lab for Climate Resilient Sorghum with University of Georgia and partners including West Africa and Ethiopia.

FIGURE 5. PROGRAMME FOR SUSTAINABLE INTENSIFICATION



Using these three case studies, it is possible to identify common features that encourage potential investment from a donor. These issues should be carefully considered by perennial grains researchers in proposing projects for donor support, noting that they require statements of interest and participation from target scientists and farmers in host countries.

ISSUES AND SOLUTIONS IN PERENNIAL GRAIN DEVELOPMENT FROM A DONOR PERSPECTIVE

1. Perennial grains are "supply-driven" solutions from developed country institutions

Issue: The development community seeks demand-driven solutions for targeted issues, regions, and farming systems. They can be identified by the international research community, national governments and farmers.

Solution: Constituency building is required: CGIAR, national research institutes, national governments, farmers.

FIGURE 6. PERENNIAL GRAIN BREEDING PROGRAMMES

Only the perennial wheat breeding programme in Nepal is located in a developing country that is of high-priority for development assistance (highlighted in green) for agencies like the United States Agency for International Development.

2. Focal crops and regions are based on scientist's interests and locations

Issue: No systematic analysis of highest-potential crops and regions, little overlap with high-priority regions already identified, and unclear how efforts will fit into or impact high priority farming systems.

Solution: Global survey and screening of high potential crops, "What is out there?" Opportunity analysis of high priority regions, crops, socio-economic conditions, "What is needed?" Modelling and early field trials in priority regions, "What is possible?"

3. Discussion remains focussed on annual vs perennial (or organic vs conventional, or polyculture vs monoculture)

Issue: "Either - or", "instead of" and "replace" narrow the possibilities, and suggest rotations are not possible. Annuals are and should be here to stay. Such language increases anxiety of all but full proponents, and blurs into "Low input vs Industrial systems".

Solution: "Both" and "Complementary" open up opportunities. "Perenniation," the integration of perennials into annual-based systems, with "complementary parallel breeding" and "Complementary parallel management".

4. Too much bootlegging; too little leveraging and coordination

Issue: Individual projects are not fully benefiting from other efforts. Information transfer is slow. Cost is presented in years; as time is required. Impairs production of international public good.

Solution: (This has equal responsibility with donors). Each programme needs to emphasise communication and coordination with the global community (beyond specific crops). Develop a professional society with formal lines of communication. Establish a World Perennial Grain Research Centre.

Use the perennial grains blog at Michigan State University for resources and announcements, pwheat.anr.msu.edu/index.php/about/, e.g. "Polyculture and Perennial Grains for Sustainable Agriculture" Symposium at the ASA-CSSA-SSA Annual Meetings in Tampa Florida in November 2013; e.g. Special Symposium "Perennial Grains for Food Security in a Changing World: Gene to Farm Innovations" at the AAAS Annual Meetings in February 2014.

CONCLUSIONS

To attract sufficient support for perennial grain research and development, we must better understand and communicate the multiple sustainability benefits of perennial grain systems, especially in the area of climate change adaptation and mitigation. The greater use of websites, blogs (e.g. the Michigan State University blog), professional conferences, and webinars can help communicate the scientific results to wider audiences, including scientific funding agencies and policymakers. Current efforts lack effective coordination and leveraging. A more formal framework for communicating information, transferring research results and germplasm, and identifying priorities needs to be developed via organized international organizations and professional societies.

To specifically address the needs of developing countries, particularly those regions most in immediate need of increased food and ecosystem security, perennial grain advocates should address additional issues as follows:

1. Perennial grains must contribute to sustainable intensification and not lead to extensification, which would require more land for production of the same amount of food;
2. Perennial grain development for international development contexts must include scientists, policymakers, and farmers in target regions. Currently, efforts are primarily ‘demand-driven’ by scientists working in developed countries (Figure 6);
3. The crops and regions on which current efforts are focused are primarily based on scientist interests and locations, not on systematic analyses of priority regions and needs. Computer modelling combined with on-the-ground trials can help identify priority farming systems and crops on which to focus limited resources;
4. Much of the discussion about perennial grains to date has been on the costs or benefits of annual crops versus perennial crops; most farmers in target priority regions rely on both. Greater attention must be paid to complementarity of annual and perennial crops and systems, from complementarity in plant breeding programmes to complementarity at the farm management level;
5. The timeline for widespread impact of perennial grains is long for traditional development funding streams. While some funding has been directed toward long term, high risk, high reward projects (“blue sky” projects), there are potential short and medium term benefits derived from investigating and/or developing perennial grains at multiple scales.

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A NEW SPECIES OF WHEAT THAT CONTINUES TO GROW AFTER HARVEST

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ABSTRACT

Crosses with hexaploid wheat and intermediate wheat grasses (*Thinopyrum intermedium*) were used to develop perennial wheat that exhibits post sexual cycle regrowth. These lines were bred to senesce fully after seed development and then regrown after a dormant cycle. Some plants however exhibited continuous growth in areas with mild winters and wet autumn months such as the Pacific Northwest areas of Washington State in the United States. Plants with continuous growth were at first discarded but are now being selected as a possible forage and grain multi-use crop for animal production. Forage quality is as high as wheat hay but the tonnage per acre is much greater. The chromosome constitution of the lines are stable at 56 chromosomes. Forty two are wheat and the other 14 are at this point unidentified. There are awned and awnless types and seed colour is red, white or blue. Height can exceed 2 metres. These lines seem to have great promise in short term rotations (2 to 3 years) where large amounts of organic matter is needed and flexibility on end-uses ranging from straw to hay to grain is desired.

Keywords: wheat, *Thinopyrum intermedium*, awn, Salish Blue

Beginning in 1995, crosses with hexaploid wheat and intermediate wheat grasses (*Thinopyrum intermedium,* -Host- Barkworth & Dewey) were used to develop perennial wheat that exhibits post-sexual cycle regrowth. These lines were bred to senesce fully after seed development and then regrow after a dormant cycle. Some plants however exhibited continuous growth in areas with mild winters and wet autumn months such as the Pacific Northwest areas of Washington State in the United States. Plants with continuous growth were at first discarded but are now being selected as a possible forage and grain multi-use crop for animal production. We have named an exemplary breeding line from this population “Salish Blue.” Salish Blue is an awnless, blue-seeded derivative of these breeding efforts. Forage quality is as high as wheat hay but the tonnage per acre is much greater. The chromosome constitutions of the lines are stable at 56 chromosomes. Forty two are wheat and the other 14 are at this point *Th. intermedium* of unidentified homoeology groups. There are awned and awnless types and seed color is red, white or blue. Height can exceed 2 metres. These lines seem to have great promise in short term rotations (2 to 3 years) where a large amount of organic matter is needed and flexibility on end-uses ranging from straw to hay to grain are desired.

In situ Hybridization

We performed fluorescent genomic *in situ* hybridization (FGISH) on root tip cells from Salish Blue using biotinylated genomic DNA from *Thinopyrum ponticum* Barkworth and Dewey as a probe. gDNA of *Th. ponticum* was used because our previous studies indicated that the 10n *Th. ponticum* genome is derived from each of the principal diploid ancestral genomes for all of the *Thinopyrum* species and thus is an ideal all-purpose probe for detecting *Thinopyrum* chromatin (Arterburn *et al.* 2011). Signal detection was accomplished using avidin-fluorescein and biotinylated anti-avidin. The FGISH probe bound strongly to the alien chromosomes, even compared to positive controls (metaphase cells of the *Thinopyrum* amphiploid AgCS). Fluorescent signals clearly indicate that 14 of the 56 chromosomes of Salish Blue are of alien origin, and the efficacy of probe binding indicates a member of the *Thinopyrum* species as the alien donor (Figure 1). Six replicates produced identical results, suggesting that Salish Blue is stable at 56 chromosomes. Because the wild parent of Salish Blue is the hexaploid *Th. intermedium*, FGISH is insufficient to determine which specific chromosomes of the parent have been retained in this amphiploid and which were lost during backcross breeding efforts. Because our previous investigations have confirmed that *Th. intermedium* is a descendant of *Th. elongatum* (2n = 14, EE), *Th. bessarabicum* (2n =14, JJ) and *Pseudoroegneria spicata* (2n = 14, StSt), we sought a means to use DNA evidence to indicate which specific homoeologous pairs from these donor genomes are present in Salish Blue (Arterburn *et al.* 2011).

MARKER ANALYSIS

We sought to identify polymorphisms in Salish Blue that correspond to known polymorphic loci on specific chromosomes from the E, J or St genomes. To accomplish this, we analyzed 24 DNA markers that have been localized to specific chromosomes in those diploid *Thinopyrum* species that are related to likely alien chromosome donors of Salish Blue (e.g. *Thinopyrum intermedium*). The markers analyzed were a combination of SSR polymorphisms detected on chromosomes of the E genome of *Th. elongatum*, and cleaved amplified polymorphic sequence (CAPS) polymorphisms detected on chromosomes of the St genome of *Ps. spicata* (Hu *et al.* 2012; Mullan *et al.* 2005). There are a further 20 SSR and CAPS markers available that we intend to assay. The results of this marker analysis can be seen in Table 1.

During this analysis, we identified five polymorphisms specific to Salish Blue. Curiously, only two of these amplicon/fragment size polymorphisms matched with a putative alien donor. A 315 bp polymorphism, amplified by SSR primers associated with chromosome 1E is shared between Salish Blue and the hexaploid *Th. junceum*. A 435 bp polymorphism, amplified with SSR primers associated with chromosome 3E, is shared between Salish Blue and the diploids *Th. elongatum* and *Th. bessarabicum*. While not conclusive evidence on its own, this suggests that two alien pairs in Salish Blue belong to homoeology groups 1 and 3 respectively. Two other polymorphisms detected in Salish Blue were amplified by primers associated with chromosome 7E, although the fragment sizes were subtly different from those detected in *Th. elongatum*, possibly due to additional microsatellite expansion in Salish Blue. A restriction cut-site polymorphism was detected in Salish Blue for a CAPS marker associated with chromosome 1St, although the fragment generated was distinct from the polymorphism associated with *Ps. spicata*.

This preliminary evidence indicates that alien chromosomes of homoeology groups 1, 3 and 7 may be present in Salish Blue. Additional marker and sequence work will be able to confirm this as well as elucidate the origins of the remaining four chromosomes pairs present in this line.

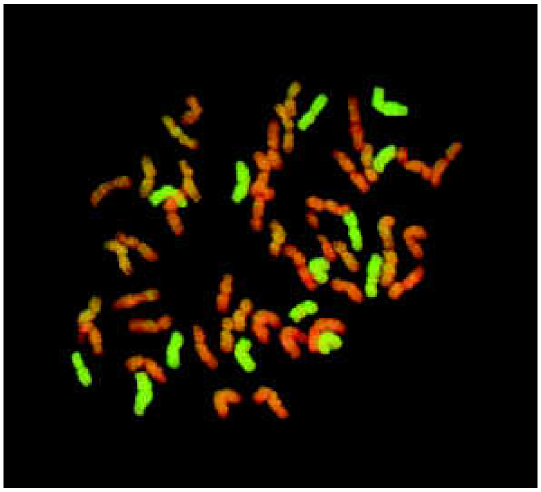
NUCLEAR GENE SEQUENCING EFFORTS

To provide further evidence of *Th. intermedium* chromosomes in Salish Blue, we are in the process of cloning and sequencing the various alleles of the beta-amylase I (bmyI) gene and the granule-bound starch-synthase (GBSSI) gene present in this amphiploid line. We have used this method successfully in the past to detect genome origins and have identified specific polymorphisms associated with distinct *Th. intermedium* (Arterburn *et al.* 2011). Identification of bmyI and GBSSI alleles matching those found in *Th. intermedium* will also confirm the presence of alien homoeology groups 4 and 7, respectively, in Salish Blue. This method is work-intensive in amphiploid samples such as Salish Blue because it requires sequencing of many clones from multiple PCR products to ensure that all alleles are detectable and free of background heterogeneous signal.

CONCLUSION

The genomic origin of the additional 14 chromosomes will lead to the naming of a new species of wheat. This new species and improved varieties within this species will have value in perennial wheat breeding programmes. Identification of the chromosomes will also lead to more efficient mapping and tagging of genes that control traits of interest such as regrowth and stay-green.

FIGURE 1. FLUORESCENT GENOMIC *IN SITU* HYBRIDIZATION (FGISH) OF SALISH BLUE



Identification of the chromosomes will also lead to more efficient mapping and tagging of genes controlling traits of perenniality in crops

TABLE 1. SUMMARY OF SSR AND CAPS MARKER ANALYSIS. MARKERS WHICH DETECTED NO *THINOPYRUM* POLYMORPHISMS ARE EXCLUDED

MARKER NAME	CHROMOSOME LOCATION	MARKER TYPE	POLYMORPHISMS DETECTED IN OUR STUDY
MWG634	4ES	STS	*Th. elongatum* = 450 bp
			Salish Blue = No polymorphic band
Xedm17	1E	SSR	*Th. elongatum* = 250 bp
Xedm28	2ES	SSR	*Th. bessarabicum* = 200 bp
			Th. elongatum = 200 bp

MARKER NAME	CHROMOSOME LOCATION	MARKER TYPE	POLYMORPHISMS DETECTED IN OUR STUDY
Xedm54	5ES	SSR	*Th. elongatum* = 185 bp
			Th. elongatum = 185 bp
			Salish Blue = No polymorphic band
Xedm74	1EL	SSR	*Th. bessarabicum* = 325 bp and 285 bp
			Th. junceum **= 315 bp and 285**
			Salish Blue = 315 bp
			Th. elongatum = 275 bp
Xedm105	7EL	SSR	*Th. elongatum* = No polymorphic band
			Th. bessarabicum = No polymorphic band
			Salish Blue = 340 bp
Xedm109	3E	SSR	***Th. elongatum*** **= 435 bp**
			Th. bessarabicum **= 435 bp**
			Salish Blue = 435 bp
Xedm149	6EL	SSR	*Th. elongatum* = 175 bp
Xedm156	7ES	SSR	*Th. elongatum* = 260 bp
			Th. bessarabicum = 270 bp and 295 bp
			Salish Blue = 280 bp
TNAC1001	1St	CAPS	Salish Blue = 275 bp
TNAC1102	2St	CAPS	*Th. bessarabicum* = 975 bp
			Th. junceum = 975 bp
			Th. intermedium = 1 000 bp
			Salish Blue = No polymorphic band
TNAC1178	2St	CAPS	*Th. bessarabicum* = 900 bp
			Th. intermedium = 900 bp
			Salish Blue = No polymorphic band
TNAC1248	3St	CAPS	*Th. elongatum* = 800 bp
			Th. intermedium = 750 bp
			Salish Blue = No polymorphic band
TNAC1408	4St	CAPS	*Th. intermedium* = 700 bp
			Salish Blue = No polymorphic band
TNAC1485	5St	CAPS	*Th. elongatum* = 1 000 bp
			Th. bessarabicum = 640 bp
			Th. intermedium = 640 bp
			Salish Blue = No polymorphic band
TNAC1674	6St	CAPS	*Th. elongatum* = 550 bp
			Th. bessarabicum = 775 bp
			Th. intermedium = 525 bp
			Salish Blue = No polymorphic band

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