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Domestication of new annual pasture legumes for resilient Mediterranean farming systems

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SUMMARY – Sown pastures in Mediterranean environments have historically been based on a limited number of annual legume species, the most successful being subterranean clover (*Trifolium subterraneum*) and barrel medic (*Medicago truncatula*). Although these species are widely established, there are many situations where they are not suited, due to biological, edaphic or climatic constraints. Over the past 10 years, a broad range of annual pasture legume species have been evaluated with a particular focus on the needs of emerging farming systems. Species such as biserrula (*Biserrula pelecinus*), gland clover (*Trifolium glanduliferum*), eastern star clover (*Trifolium dasyurum*), bladder clover (*Trifolium spumosum*), French serradella (*Ornithopus sativus*) and yellow serradella (*Ornithopus compressus*) have now been commercialized and are being adopted in mixed farming systems of southern Australia. Frequent resowing is a feature of modern pasture systems and this has directed research effort towards the development of cultivars that have a low cost of seed production. We contend that the development of new pasture legumes needs to consider traits that confer ease of domestication, as well as those delivering high productivity and persistence. Important traits include ease of seed harvest, hard seed and hard seed breakdown patterns, maturity and tolerance to pests and disease. Detailed analysis of some of these traits has revealed new opportunities to reduce the cost of pasture establishment such as under-sowing hard-seed of the pasture legume in a preceding grain crop. The hard-seed will not germinate and compete with the grain crop and the pasture will establish in the following season through natural *in situ* hard-seed breakdown. Other opportunities are identified for weed management. Pasture improvement in the Mediterranean basin may also benefit from a focus on these plant attributes and allow the development of locally adapted cultivars.

Keywords: Pasture legumes, hard seed, Mediterranean farming systems.

RESUME – *"Domestication de nouvelles légumineuses annuelles sur les parcours pour des systèmes agricoles méditerranéens résilients". Historiquement, les parcours ensemencés dans les environnements méditerranéens ont été surtout basés sur un nombre limité d'espèces de légumineuses fourragères annuelles dont les plus performantes sont le Trèfle Semeur (*Trifolium subterraneum*) et la Luzerne Tronquée (*Medicago truncatula*). Malgré le fait que ces espèces soient largement établies, elles restent inadaptées à de nombreuses situations à cause des contraintes climatiques, édaphiques, biologiques ou autres. De plus, les coûts élevés de réensemencement et les nombreux changements dans les systèmes d'exploitation ont créé d'autres limitations pour leur utilisation courante. Dans les 10 dernières années, une grande variété d'espèces de nouvelles légumineuses fourragères a été évaluée tenant compte particulièrement de la demande des systèmes agricoles émergents. Des espèces telles que l'Astragale Double Scie (*Biserrula pelecinus*), le Trèfle Gland (*T. glanduliferum*), le Trèfle Étoilé Oriental (*T. dasyurum*), le Trèfle Écumeux (*T. spumosum*), la Saradelle Française (*Ornithopus sativus*), la Saradelle Jaune (*O. compressus*) sont maintenant commercialisées et depuis peu adoptées dans des systèmes agricoles mixtes d'Australie du Sud. Les ensemencements fréquents sont une particularité des systèmes pastoraux modernes, ce qui a dirigé les efforts de la recherche vers le développement de cultivars à bas prix de production de semences. Nous pouvons affirmer que la sélection de nouvelles légumineuses pastorales doit tenir compte des caractères qui permettent aussi bien une domestication facile qu'une productivité et une persistance optimisées. La facilité de la récolte, la dureté de la semence et son mode de levée, la maturité et enfin la tolérance aux insectes et aux maladies sont des caractéristiques recherchées. Les analyses détaillées de certaines de ces caractéristiques ont révélé de nouvelles opportunités permettant de réduire les coûts d'établissement comme le fait de planter des semences dures de légumineuses dans un précédent cultural de céréales. Ces graines dures ne germeront pas et ne présentent aucune compétition pour la culture de grains et ne s'établiront qu'à la saison suivante suite à une lente rupture de leur dureté* in situ*. D'autres opportunités ont été identifiées quant à la gestion des mauvaises herbes. L'amélioration des parcours dans le bassin méditerranéen doit porter une grande attention aux attributs de ces plantes permettant ainsi le développement de cultivars adaptés à ces zones.*

Mots-clés : *Semences dures, légumineuses de parcours, systèmes d'élevage méditerranéens.*

Introduction

Subterranean clover and annual medics have played a key role in the development and success of farming systems in Mediterranean climate regions of southern Australia. Plant improvement programs have expanded and enhanced the use of these species through the development of cultivars with a range of maturities, low anti-nutritional factors, improved symbiotic association and insect and disease tolerances. Even so, there remain situations where the success of these species is limited due to climatic and edaphic constraints and development of new challenges to cropping systems. These include the development of herbicide resistant weed populations, rising water tables, dryland salinity, loss of soil fertility and increased costs of production (Loi *et al.,* 2005).

The benefits of incorporating pasture legumes into Mediterranean farming systems remain as relevant today as when subterranean clover and annual medics were first adopted. Therefore, public breeding institutions have responded by developing alternative annual pasture legumes with adaptation to both existing and new farming systems (Nichols *et al.,* 2007). Several new species to Australian agriculture have been commercialised over the past 15 years (French serradella (*Ornithopus sativus*), biserrula (*Biserrula pelecinus*), sulla (*Hedysarium coronarium*), gland clover (*Trifolium glanduliferum*), arrowleaf clover (*Trifolium vesiculosum*), eastern star clover (*Trifolium dasyurum*) and sphere medic (*Medicago sperocarpus*) and hybrid disc medics (*Medicago tornata x Medicago littoralis*). These have been selected for deep root systems and extended growing periods, protection from false breaks of season, appropriate patterns of hard-seed breakdown, acid-tolerant root nodule symbioses, tolerance to pests and diseases and provision of lower cost seed, through ease of seed harvesting and processing.

In Western Australia the species with the greatest impact are proving to be French serradella, yellow serradella (*Ornithopus compressus*) and biserrula (Nichols *et al.,* 2007). In a survey of growers conducted in 2005, only 31% of all species sown were the traditional subterranean clover and annual medics. French serradella comprised 25% of all new sowings, biserrula 17% and yellow serradella 10%. These species are not only being incorporated into conventional cereal-ley farming systems (with much greater frequency of cropping than in the past), but are also used tactically in short term phase pastures.

The conventional cereal-ley farming system of southern Australia consists of a cropping phase followed by a pasture phase in which the pasture regenerates spontaneously. It has been traditionally based on annual medics (*Medicago* spp.) and subterranean clover (*Trifolium subterraneum* L.), both of which were introduced accidentally during the 19th century (Gladstones 1966). The self-regenerating capacity of these species is a key component of the success of this system. This is possible as the species possess high levels of seed dormancy (hardseededness), leading to the formation of a persistent seed bank in the soil. Medics generally have "harder" seeds than subterranean clover and persist in the soil for a longer period without fresh seed production. Medics are therefore better adapted to more frequent cropping than subterranean clover.

Phase farming systems are a modification of the cereal-ley system where pastures are grown between extended periods of continuous cropping. It is common in South Australia and New South Wales on acid soils where medics were poorly adapted and where subterranean clover often failed to regenerate after the extended cropping phase. Under these conditions farmers re-introduced subterranean clover at the beginning of each pasture phase. Therefore, phase farming differs from ley farming in the requirement to re-sow the pasture after each cropping phase: pastures in ley farming are expected to rarely require re-sowing (Reeves and Ewing, 1993).

Phase farming therefore needs a regular supply of legume seed. However recent pressures in the cereal-ley farming system have reduced the persistence of subterranean clover and annual medics in this system. These includes increased cropping intensity, residual herbicide use in the cropping system and weed seed set control in the pasture phase. Re-sowing has become more frequent in these systems, further increasing the requirement for pasture legume seed. The production of low cost pasture seed has therefore assumed much greater importance in plant selection programs, leading to particular emphasis on traits conferring ease of harvest (such as aerial seeding) and seed processing (such as threshability).

This paper describes the traits we consider to be relevant for the development of annual pasture legumes most likely to be successful for farming systems in Mediterranean climates. The importance of herbage and seed production has previously been described by Loi *et al.,* 2005 and is not considered in detail here as it is assumed that these are a given in most plant improvement programs.

Selection traits

Ease of seed harvest

Over time there has been increasing sensitivity to the economic and environmental costs associated with vacuum suction harvesting. This has been the traditional method used for seed production of subterranean clover and annual medics. It requires not only specialist machinery but also extensive knowledge and experience to be successful. As an alternative, species that can be seed harvested with conventional grain harvesters (headers) do not require access to specialist machinery. It is also considerably faster and does not lead to the potentially damaging effects of soil erosion common with suction harvesting.

Farmers that operate in low-input systems require a cheap source of seed, particularly when large areas of pasture need to be re-sown. Pasture legumes that can be harvested *in situ* by farmers with conventional harvesters or that can be purchased at very low cost are required. Consequently, the capacity for ease harvesting has become one of the most important selection traits for the development of new pasture species (Nutt and Loi, 1999).

Rose clover *(Trifolium hirtum)* is shown in Table 1 as an example of incorporating of seed harvest in the selection process. Similar dry matter and seed yields were achieved by genotypes CFD9.1a and 86906b, however 83% of the seed yield (approx. 900 kg/ha) was captured by header harvesting with CFD9.1a compared to only 19% (approx. 210 kg/ha) with 86906b. These results indicate that in some instances it is difficult to find superior genotypes to well adapted commercial cultivars, in this case cv. Hykon. Even so, P15015 shown an equivalent performance to cv. Hykon, in terms of the reported measurements, however there maybe additional advantageous characters that may justify its commercial release. These could include more robust hard-seed dynamics for target farming systems or insect tolerances.

Table 1. Total dry matter and seed yield and header harvest seed yield of 12 rose clover accessions and gland clover cv Prima

Genus	Species	Refline	DM (14/9/05) t/ha	Total Yield (a) kg/ha	Header Harvested Yield (b) kg/ha	Index of Harvestability % (b/a)
Trifolium	*hirtum*	Hykon	3.5	1445	1173	81
Trifolium	*hirtum*	P15015	4.2	1296	1170	90
Trifolium	*hirtum*	CFD30	4.4	1735	1165	70
Trifolium	*hirtum*	MCD114	4.0	1478	1058	73
Trifolium	*hirtum*	CFD9.1a	3.3	1089	892	83
Trifolium	*hirtum*	H1011b	3.3	1092	824	79
Trifolium	*hirtum*	H1011a	2.8	968	695	76
Trifolium	*hirtum*	95GCN39	2.2	898	592	66
Trifolium	*hirtum*	95GCN113	3.0	870	425	49
Trifolium	*hirtum*	SARDI Rose	3.0	874	363	44
Trifolium	*hirtum*	86906b	3.1	1047	206	19
Trifolium	*hirtum*	P5696	2.2	1003	141	14
Trifolium	*glanduliferum*	Prima	3.1	863	522	75
LSD (P=0.05)			0.8	364	219	

Our research experience has found the species most amenable to harvesting with conventional grain harvesters include the aerial seeding clovers such as gland clover, bladder clover (*Trifolium spumosum)*, arrowleaf clover, rose clover and purple clover (*Trifolium purpureum)*. Other species with a similar capacity are biserrula, French and yellow serradella, and *Lotus ornithopodioides* (Nutt and

Loi 1999). These species may also have potential in the Mediterranean basin where farmers may no longer need to be restricted to Australian varieties of subterranean clover and annual medics.

Hard-seed and hard-seed breakdown patterns

The survival of annual pasture legumes is strongly related to their capacity to build and maintain a persistent seed bank, from which pasture can regenerate after cropping or, in permanent grassland, after years of drought or overgrazing (Cocks 1993). This is achieved by the production of a high proportion (often greater than 90%) of hard-seed at maturity (seed coat impermeability). Seeds are prevented from imbibing and germinating until a set of physiological conditions relating to temperature and moisture availability have been met (Taylor 1993). Depending on the species and the prevailing conditions, this innate dormancy may last from a few months to decades. Hard-seed production and the dynamics of its breakdown have two important roles; first it ensures survival in the absence of seed production, during a crop rotation or adverse seasonal conditions; and second it prevents germination of the seeds after summer or early autumn rains (Taylor 2005).

The pattern of seed softening (loss of impermeability) varies widely among legumes both within and between years (Taylor 2005). Taylor (1981) demonstrated that softening of subterranean clover seed takes place in two stages. The first is a pre-conditioning stage and is achieved by subjecting the seeds to high temperatures: the higher the temperature the more rapid the process. The second is a short period of fluctuating temperatures that appears to be specific to each species; for example 60/15 ^{o}C for subterranean clover and 35/10 ℃ for burr medic (Taylor 1993). Revell (1997) has demonstrated that yellow serradella is quite different to subterranean clover and the medics. He found that the softening process is actually accelerated by shallow burial, particularly in two accessions in which 95% of the seeds softened at 2 cm at the end of the first summer compared with only 6% on the soil surface. Softening at 6 cm was similar to that at the soil surface. It seems likely that, in the case of yellow serradella, light (or darkness) interacts with its temperature requirements.

In some species, seed becomes germinable during late summer or early autumn, while for other species such us subterranean clover a great proportion of the seed becomes germinable in the middle of summer. Therefore if subterranean clover is exposed to unseasonal rains the result is often high seedling death (false break of season) and loss of a large proportion of the seed bank. Many of the new species/cultivars selected in the last decade for Western Australian conditions have been selected for a pattern of hard-seed breakdown that avoids germination on summer rainfall events.

The wide range of seed softening characteristics that are now recognised provide opportunities for the selection and development of adapted pasture legumes to specific management systems including rotation with crops (Loi *et al.,* 2005). The recent selection of the first commercial cultivar of bladder clover is an example of the importance of hard-seed as part of the selection criteria for a cultivar.

Hard-seed breakdown from the start of the summer to autumn for 22 accessions of bladder clover, similar maturity to Dalkeith (105 days in Perth) can be clustered into 5 groups (Table 2). All accessions of bladder clover avoid possible germination with summer rainfall with a negligible amount of seed germinating after 90 days (middle of summer) compared to subterranean clover. The early winter values of hard-seed of bladder clover varied greatly within accessions. Accessions in group 1 are able to germinate with high numbers (60% germinating seed) compared to those in group 5 with only 10 % of seed germinating. A similar trend has been found between the groups when seed has been buried in early winter to simulate a crop rotation and then tested the following autumn to measure potential regeneration after the crop.

The selection of the first cultivar of bladder aimed to select a high seed and dry-matter yielding cultivar, with the ability to regenerate abundantly in both the second year and third year. Three accessions from three different groups were identified for the best agronomic performance; however, a second level of selection involving hard-seed dynamics was used to select the most suitable candidate from this group for cultivar development. The selected cultivar was within Group 1, has the potential for dense regeneration in both the second year (medium level of hardseed after 180 days) and third years (330 days). The enhanced softening of this cultivar observed after one year of burial may also be useful in the twin-sowing technique described below.

Table 2. Level of hardseededness of a range of accession of bladder clover and *Lotus ornithopodioides* growth and tested in Western Australia

Species	Accessions per group (No.)	Hardseed levels (%)			
		Surface			Buried
		Initial Average (0 days)	Mid-summer average (90 days)	Winter year 1 range (180 days)	Winter year 2 range (330 days)
T. spumosum					
Group 1	4	94	78	31-53	1-5
Group 2	5	94	84	66-77	2-19
Group 3	6	94	90	78-84	13-30
Group 4	7	93	91	85-88	26-51
Group 5	3	93	91	85-88	26-51
L. ornithopodioides	10	94	92	85-95	1-27
T. subterraneum cv Dalkeith	1	73	31	25	-

Maturity

In the last decade most emphasis has been on the selection of pasture legumes for medium to high rainfall areas. This has been largely driven by the wider genetic resource available with appropriate maturity from which to breed and select. Several cultivars of new pasture legumes species have been released and widely adopted particularly in the Western Australia wheatbelt (over 1 million ha sown each year) (Nichols *et al.,* 2007). However, there remains large areas in the low rainfall regions (250-300 mm) that do not have suitable cultivars.

Early maturity remains an essential trait to be considered when developing mixtures for Mediterranean climates, where variable seasons are the major constraint to pasture productivity and persistence. A combination of maturities may be the best strategy to exploit the potential of good seasons and persistence through adverse seasons. The availability of early maturing ecotypes of annual pasture legumes has been enhanced in recent times by collecting germplasm from arid parts of the Mediterranean basin and south-western Australia (Loi and Sandral 2000; Loi *et al.,* 2005 (pers. Comm.)). This new material will be developed in the near future to produce early maturing cultivars to expand the use of pasture legumes into low rainfall farming systems with a Mediterranean climate.

Tolerance to pests and diseases

Natural insect tolerance is a valuable characteristic, particularly when the cost of insecticide or fungicide control is high or control options are non-existent. Several of the new species have been selected with improved resistance to some of the major pests found in Southern Australia. Gland clover is a good example of the combination of complete resistance to red-legged earth mite (*Halotydeus destructor*), with other traits such as ease of seed harvesting and a delayed pattern of hard-seed breakdown. In general terms there has been greater success in selecting pasture legumes with resistance to pests amongst new species rather than within existing species. Selection for disease tolerance, however, has been more successful; for example, several cultivars of subterranean clover and purple clover have been developed with high tolerance to clover scorch (*Kabatiella caulivora*) through an intensive screening for tolerance within species (Nichols *et al.,* 2007). Improved tolerance to pests and diseases is particularly relevant for organic production systems and opportunities to exploit new species in this regard may be important for Mediterranean countries of the European Union.

Duty of care

An emerging issue for pasture improvement in recent times is the issue of risk management.

Developing new species for existing or new farming systems will inherently carry new risks, and these risks may be less defined at the start of the selection and breeding program than with "conventional" species. We need to ensure that addressing one set of problems does not introduce another, often equally significant, set of problems. Pasture scientists and research providers have an obligation to ensure that an appropriate standard of care is demonstrated at the time of commercialization of new species and cultivars to minimize the risk of harm, damage or economic loss for farmers, community and the environment.

Revell and Revell (2007) suggest that there is an obligation to follow a minimum set of tests across four main categories to adequately demonstrate appropriate duty of care. These are: (i) consequences for animal productivity or health; (ii) potential to become an environmental weed or an unmanageable crop weed; (iii) potential for problems in managing the agronomic performance or health of the plant and; (iv) the capacity to maintain the "integrity" of the cultivar. They suggest a framework validated with field experience within which to work. A set of minimum measurements supplemented with additional animal health studies where necessary aims to achieve a balance between meeting legal, environmental and social obligations without unduly jeopardizing the timely release of new, improved pasture cultivars. Such a process requires skills from a range of disciplines and invariably will increase the cost of plant development. It therefore needs to be appropriately resourced and should preferably occur in parallel with the cultivar development, rather than at the end of the process.

New opportunities with alternative pasture legumes

Summer sowing

The Mediterranean environment is generally characterized by a cool, wet winter and hot, dry summer with a shortage of green feed during long periods of the year. However, sporadic rainfall around 50-70 mm can be experienced during the dry season. Sowing a pasture legume after such events can be a useful tool to improve the quality of feed availability to animals and control troublesome summer weeds. This can only be achieved if farmers have access to low cost seed and sow the pasture legumes immediately after the rainfall event. Under high summer temperatures (30-35 C) and with plant available soil moisture, the legume will grow rapidly and compete with summer weeds. It also will provide a valuable high quality green feed and fix significant amounts of nitrogen for crops or pastures in the following growing season. In some cases, drought tolerant species such as biserrula and serradella, may survive the rest of summer and grow rapidly again at the normal break of the season, filling the feed gap generally experienced by farmers in autumn.

Twin-sowing

The twin sowing is a concept to help reduce the cost of pasture establishment. Using hard-seeded French serradella as an example, pods produced on-farm can be undersown with a cereal crop. Initially, seeds in the pod are almost 100% hard, therefore little germination will occur under the crop. During the summer and autumn after the crop, the hard-seed of the legume will breakdown and our studies suggest that up to 60% of this species will become germinable. This avoids a separate sowing operation for the pasture phase and provides a longer potential growing season. This combined with on farm production of seed allows the pasture to be established at a much reduced cost.

Bladder clover and *L. ornithopodioides* are further examples of legume species that may also have application for the twin sowing system. They are both prolific seed producers that are easily harvested with conventional grain harvesting machinery (Loi *et al.,* 2002; Loi *et al.,* 2003). They also have hard-seed breakdown patterns that suit this system because the larger the breakdown, the lower the rate of seed that is required to achieve adequate plant density in the pasture phase (Table 2).

The successful establishment of legume species using the twin sowing technique is also reliant upon nodulation and effective symbiosis with rhizobia. The traditional legume inoculation by peat may not provide adequate survival of the rhizobium over the one year of crop and before the legumes establish. A new inoculant technology ALOSCA®, which is a clay based granule inoculant may offer the solution for rhizobium survival (Carr *et al.,* 2006). In other cases sufficient soil rhizobial populations may already exist that preclude the need for seed inoculation.

Strategic weed control

In the Mediterranean-type annual-cropping regions across southern Australia up to $1,033 million per annum is expended by growers to maintain crop weed populations at manageable levels (Sinden *et al.,* 2004). The evolution of herbicide resistant weed populations (especially in the two most economically damaging Australian crop weeds, annual ryegrass and wild radish) has made this task more difficult.

The number of herbicides (groups) available for weed control is becoming restricted with the development of multiple herbicide resistances in some weed populations and growers are becoming increasingly reliant on cultural and mechanical control options. Over the past 5 years multiple resistance in ryegrass populations has risen from 37 to 71% across the Western Australian wheat belt; 15% of populations being resistant to at least three herbicide groups (Owen *et al.,* 2005). This highlights the urgent need to develop weed control tools/systems not dependent on selective herbicides.

The evaluation of alternative pasture legumes has lead to the observation of delayed germination in some pasture species. This character is particularly pronounced in some genotypes of yellow serradella (cvv Santorini, Charano, Yelbini) and Eastern star clover (cv AGWEST®Sothis). This offers the opportunity for strategic weed control at the break of the season with non-selective herbicides and grazing. Because of the delayed germination of the legumes, this can achieve good weed suppression with out compromising pasture legume density.

For Santorini serradella, germination appears to be delayed by 10-14 days, compared to the emergence of weed species, and when germination does occur it is generally spread over four to five weeks. A research project is currently underway to provide farmers with guidelines to adopt this technique at a broad scale. Sothis is the first cultivar of eastern star clover released to Australian agriculture. It has good dry matter and seed production and its seed can be harvested with modified grain harvesters (Loi *et al.,* 2007; Norman *et al.,* 2005). It was selected primarily for its very pronounced delayed germination pattern, over 6-8 weeks later than weed species (Table 3). In this study, the density of Sothis was only moderately reduced by herbicide applications compared to subterranean clover (35% reduction compared to 97%). Weed densities were also substantially reduced by the herbicide application, particularly the herb component.

Table 3. Plant densities of eastern star clover AGWEST® Sothis, subterranean clover Dalkeith and weeds in herbicide-treated and unsprayed treatments (modified from Loi *et al.,* 2007)

	Sown legume plants/m^2	Grasses plants/m^2	Broadleaf plants/m^2
AGWEST® Sothis Unsprayed	722	6325	3548
AGWEST® Sothis Sprayed*	475	165	0
Dalkeith Unsprayed	5160	5295	3682
Dalkeith Sprayed*	161	145	4

*Plots were sprayed on the 27 of May 2004 (5 weeks after the first germinating rains) with a non selective herbicide (glyphosate 540 g a.i./ha).

Conclusion

For new annual pasture legumes to be successful, they need to be adopted by farmers. Plant improvement programs must recognize that seed supply and seed cost are critical determinants of successful adoption. Therefore, the development of new pasture legumes needs to consider traits that confer ease of domestication, as well as those delivering high productivity and persistence. Important traits include ease of seed harvest, hard seed and hard seed breakdown patterns, maturity and tolerance to pests and disease. The cost of seed is likely to be lowest when seed production occurs on-farm using existing farm machinery. It is likely that pasture improvement in the Mediterranean Basin could also benefit from a focus on these plant attributes and allow the development of locally adapted cultivars.

Detailed analysis of the seed ecology of new pasture legumes has revealed new opportunities to reduce the cost of pasture establishment (such as twin-sowing) and for weed management (when delayed germination is exploited). These opportunities need further validation at the farm level. The domestication of a new generation of forage legume species for southern Australian also offers a unique opportunity to increase pasture biodiversity in this region. Monoculture fields of pasture legumes are now recognised as ecologically unstable systems. It is vitally important to increase species biodiversity to overcome challenges posed by pests, disease, climate and farming practices. This needs to be conducted in the context of managing the risk of unforeseen problems from new species, requiring an appropriate level of duty of care for new cultivar releases.

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