# The 8th International Herbage Seed Conference

# Proceedings & Abstracts

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# The 8th International Herbage Seed Conference

## Herbage Seed: Environment & Life

## Proceedings & Abstracts

MP Rolston, YR Wang (eds)

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State Key Laboratory of Grassland Agro-ecosystems (SKLGAE), College of Pastoral Agricultural Science and Technology (CPAST), Lanzhou University, China

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# Welcome Letters

Dear colleagues

It is our great pleasure to welcome you to the 8th International Herbage Seed Conference to be held in Lanzhou. The theme of the conference is “Herbage Seed: Environment and Life” and will include following five topics:

(1) Genetics and breeding;

(2) Seed production: achieving high yield and environmental sustainability;

(3) Endophyte utilization and pest control;

(4) Seed quality: from field to storage; and

(5) Seed production in developing countries, and extension, technology transfer and training.

The conference is divided into two phases. The first is oral and poster presentations related to the above five topics. Then, we will visit seed production farms and companies, and diverse agricultural systems in the Hexi Corridor, Gansu Province, the most important seed production region in China. We hope this conference not only to provide a unique platform for discussion of seed science and technology, but also to give you a whole picture of the herbage seed industry in China.

Our Local Organizing Committee will try its best to prepare a good program and to make good arrangements for your visit.

We look forward to welcoming each of you to Lanzhou.

Yours sincerely

The Local Organizing Committee

Dear colleagues

The International Herbage Seed Group executive welcomes delegates to the $8^{th}$ IHSG Conference in Lanzhou, China. Our previous conferences have been held in Europe (Denmark, Germany, Italy and Norway), the USA (Oregon and Texas) and Australia. Coming to China was a move to include developing countries as hosts and to give local researchers in host countries exposure to international herbage seed researchers from around the world. Our $9^{th}$ Conference in 2017 will meet in Pergamino, Argentina to continue the theme of reaching out to research colleagues in developing countries and to bridge the divide between temperate and tropical seed research. We thank the local organizing committee for their preparation work and for Lanzhou University and Lanzhou City for being our hosts. To the delegates we wish you an enjoyable conference where you can make new or renew acquaintances, establish research collaborations or challenge our thinking on the science questions we pursue.

Phil Rolston

President

International Herbage Seed Group

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Plenary paper

# Overview of the Chinese Herbage Seed Industry

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**Abstract**

The Chinese herbage seed industry began in the 1980's when China started to implement policies of reform, opening-up to the world, and the national economy started to boom. Compared to the major agricultural crop seeds, the herbage seed industry has had less investigated. But significant progress is evident. This paper reviews the development and current situation of the Chinese herbage seed industry.

**Demand, production and trade of herbage seed**

Demand for herbage and turf grass seed in China is large as it is required for both grassland restoration and animal production. A series of national policies has been implemented to encourage plantings of herbage seed during the last 15 years. Statistics show that an average of 150,000 tonnes of herbage seed has been sown annually during recent 5 years (2009-2013), but domestic production is only about 110,000 tonnes. Thus, nearly one fourth of the total herbage and turf grass seed sown in China is imported from other countries. The primary regions for herbage seed production are Gansu, Inner Mongolia and Qinghai. These 3 provinces located in northwest of China produce about 68% of the total seed produced nationally. Lucerne, oats, sudangrass, milkvetch, *Elymus nutans* and common vetch are the primary species produced, with lucerne and oats ranked the top of domestic seed production. Some native species such as *Caragana, Artemisia*, and *Leymus chinensis* have performed very well for producing animal feed and/or improving degraded grassland in northwest China. But most of the native seeds are harvested from natural grassland, and low yield and poor quality have limited their utilization.

The main imported herbage species are ryegrass, fescue and *Poa pratensis,* followed by lucerne and white clovers. These seeds are mainly imported from America, Canada, several European countries, Australia and New Zealand. China exports only a few thousand tonnes of herbage seed to other countries annually with lucerne as primary species exported.

**Quality control of herbage seeds**

A seed testing scheme has been in place in China since 1982 but no seed certification scheme has been established yet. There are 11 herbage seed laboratories with adequate facilities and trained staff. Five of these are nationally accredited and two are ISTA members. Seed testing has played a significant role since it started by providing seed quality information for farmers, seed companies and quality control organizations. However, the total number of seed samples tested by official seed testing laboratories is only about 5,000 a year in the whole country which represents a very small proportion of what it should be.

A Seed Control Regulation was issued in 1989 which was replaced in 2000 by the Seed Law, and is under revision currently. The Seed Law covers all aspects of the seed industry, while strengthening the law enforcement as necessary.

**Evaluation and registration for new herbage variety**

The China National Herbage Variety Registration Board (CNHVRB) was established in 1987, and China has been a member of the International Union for Plant Variety Protection (UPOV) since 1999. For a long time, the evaluation for new cultivar registration was mainly based on agronomic merit like yield, nutritional value and adaptability to ecological conditions. However in recent years DUS testing has been required for new variety registration in addition to agronomic performance.

In total 484 herbage varieties have been registered from 1987 to 2014, an average of 17.2 annually. The registering varieties are divided to four categories according to the breeding origin: bred, domesticated, introduced or local variety. In addition they are further divided into 3 categories according to their usage: forage, turf or ecological use. Significant achievement has been made through the work of CNHVRB. For instance, the chaos in herbage variety management that persisted in China for a long time has gradually changed. But the absence of a seed certification scheme makes it virtually impossible to control variety purity and other seed quality components. In addition there are insufficient numbers of new herbage varieties released to meet the requirements of livestock production and environmental improvement under diverse ecological conditions

**Research support**

Over 1000 research papers have been published relating to seed production and seed testing technology since the 1980's. However there is a large gap between research knowledge and what is being applied in commercial seed production.

In conclusion, the Chinese herbage seed industry was initiated in the late 1980s, and although it is still developing considerable progress is evident. Limitations for the development of the herbage seed industry in China could be outlined as: weak support from herbage plant breeding programs, difficulties is maintaining variety purity, lack of scale and mechanization, and limited application of research results to seed production practices.

# Session 1. Genetics and Breeding

# The plant breeders' dilemma: forage versus seed

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**Abstract**

High seed yields are crucial for forage cultivars and failure to achieve this can lead to the cultivars commercial demise. The primary focus for breeding forage cultivars are those that determine forage yield, disease and pest resistance, quality and persistence. Seed yield is often at best a secondary consideration although poor seed yielding lines are usually discarded. Crop management has a very large impact on seed yields and in recent years large gains have been made through improved crop understanding. However, even with excellent crop management cultivars are known to vary in their seed yielding ability with some more suited to some crop management systems than others. Adding seed yield as an additional selection trait into a breeding program also is likely to reduce the rate of genetic gain in major traits such as forage yield.

Nevertheless, breeders should not be complacent as there is considerable room to select for increased seedset, a tighter flowering period, non-shattering, delayed or no lodging, resistance to seedcrop diseases and selecting for these parameters is unlikely to reduce pasture or turf quality.

In many species however germplasm is not available with seed retention or a short stiff architecture and priority should be given to searching for appropriate germplasm with these traits.

## Introduction

High seed yield is a crucial factor in determining availability of grass cultivars to end users and it often influences seed prices which can be a crucial factor in the uptake of cultivars. Seed production must also be profitable when compared to other land use options including high value activities such as vegetable seed production and in some cases even more diverse activities such as dairying or vineyards. Seed companies also require reliable and consistent seed production to supply markets and increasingly this means producing in regions with reliable spring rainfall or irrigation.

Seed production of grasses is carried out under a range of climates and management systems around the world and it is important to understand that cultivar ranking of seed yield may differ under different systems. This means that breeders will have to breed cultivars which suit a particular seed production system. Seed yields of forage grasses vary enormously across regions and management systems with perennial ryegrass yields and annual ryegrass yields traditionally only achieving 1000kg/ha, or less, but commercial yields higher than

3000 kg/ha are now regularly achieved in New Zealand when best practice management procedures are followed (Rolston *et al.* 2007).

Breeding for seed production is seldom a key objective of forage or turf plant breeders. Usually forage yield or turf performance takes absolute priority and seed yield is secondary. The only time most forage breeders get concerned about seed yield is when difficulties arise. Most cultivars will progress to market when they exhibit improved forage yield or turf performance and "an adequate" seed yield. There is no question that breeders would like to see improved seed yields but not at the expense of improved forage yields or turf performance.

The question is often asked as to whether there is any technical reason why we can't breed cultivars with both improved seed yields and forage yield or turf performance. In general for grasses the answer is that high seed yield and high forage yield or high turf performance are not mutually exclusive.

There is potential for an interaction between high seed yield and forage quality in situations where seed yield is driven by number of seedheads but in most intensive seed production systems, number of seedheads in seed crops is seldom limiting. This is in contrast to legumes such as white clover where an axillary bud of a stolon may develop into either a flower or a branched stolon and selecting for number of flowers may impact vegetative development (Baker & Williams 1987).

In the proprietary seed industry where seed companies own a cultivar and require consistent supplies of seed for marketing, it is common to work closely with seed growers on crop management to maximize seed yields. This management often lifts seed yield well above what would be expected from the cultivar without a management package. Some late flowering perennial ryegrass cultivars may yield less than 800kg/ha seed when first grown without an understanding of management requirements; however, when the cultivar's management is optimized by researchers' seed yields of over 2500kg/ha can be expected.

It is interesting to compare the seed yield of perennial grasses such as *Lolium perenne,* and *Festuca arundinacea* and even annual *L. multiflorum* with that of annual cereals such as wheat barley and oats. Why do forage and turf grasses lack the seed yields of annual crops when there are so many more florets available?

## The flowering induction process

Many of the important forage grasses such as *Lolium perenne, L. multiflorum* and *Festuca arundinacea* have a dual requirement to induce flowering which involves vernalisation in winter followed by a daylength requirement for stem elongation and emergence. Short days can substitute for vernalisation in some grasses, including perennial ryegrass, colonial (*Agrostis tenuis*) and creeping bentgrass (*Agrostis stolonifera*). In this sense there appears to be no major differences between the flowering induction responses of perennial grasses and annual graminaceous crops (Heide 1994).

Cultivars within these species vary considerably in vernalisation requirement, often depending upon the climatic origin of the germplasm. As vernalisation requires metabolism to occur above approximately 5-7°C this means that southern European germplasm usually has a lower vernalisation requirement than northern European material, but interestingly much of the material in the far north of Europe has evolved where winter temperature are too cold for metabolism and these cultivars can have lower vernalisation requirements than cultivars from lower latitudes. In New Zealand cultivars with a high vernalisation requirement appear to respond positively to early sowing while low vernalisation cultivars appear to have the flexibility to be sown late, a valuable feature for seed growers in some situations. Selecting for low vernalisation in a cultivar should allow considerable flexibility in sowing date for seed growers.

Cultivars will vary in daylength requirement for head emergence, with late flowering cultivars having a longer day requirement than early cultivars.

The flowering of perennial grasses often extends over a considerable period of time from days to even months and the variation in flowering (Abel *et al.* 2015) and subsequent variation in seed maturity can be a serious issue. This variation in flowering can be caused by the genetic diversity among plants within a cultivar or population, developmental stage among tillers of a plant, differences in timing within a seedhead from middle outward in grasses, and even from bottom to top within a spikelet. This variation in development stage makes the timing of inputs and harvest very difficult. It may also mean that at harvest there is almost always considerable seed shattering, while other seeds are immature and not ready for harvest. Determining optimal timings of harvest in grasses can be difficult with the timing of windrowing or harvest in many grass crops usually determined by average seed moisture (Siberstein *et al.* 2007). Also cultivars flowering over a wide period of time also risk population shift during seed production (Hayward & Abdullah 1985, Floyd & Barker 2002).

Tropical grasses also respond to daylength and temperature in a similar manner but in general the seasonal triggers are more subtle with less seasonal variation in both temperature and daylength, and for this reason flowering can be extended over longer periods than temperate grasses (Loch 1980, Nada 1980).

Compared to annual cereal crops, perennial grasses usually have a wider age range of flowering tillers. Perennial grasses are mostly genetically diverse populations while cereals are often self-pollinating, this combined with the higher tiller number of forage grasses means that grasses express a significant amount of variation in flowering time across tillers in a field. Selecting cultivars with a tighter range of flowering should be very useful for seed production.

**Self incompatibility and genetic seed production issues**

During the development of cultivars care needs to be taken to ensure self-incompatibility alleles are not limiting as this may reduce seed set in some cultivars (Struder *et al.* 2008). An extreme form of this is used to develop near F1 hybrid populations (83% hybrids) by using different self-incompatibility alleles in the parental populations (Pembleton *et al.* 2014). Seed production of these F1 hybrids involves mixing the parental lines and sowing the crop as per a traditional seed production system; the resulting seed yield should not be different to "normal" outcrossing cultivars. However, in contrast the cytoplasmic male sterility system developed for perennial ryegrass in Germany (Wit 1974, Posselt 2010) involves planting and subsequent removal of pollinator rows with a loss of approximately 20% of the harvest area. This reduction of seed yield will need to be compensated for in seed price and a much improved forage performance.

Some cultivars of perennial species can have a low level of sterility. For example in some tall fescue cultivars, chromosome pairing can breakdown in hybrid combinations (Jauhar 1975) and up to 1% of plants may be sterile in early crosses of different continental tall fescues; however this problem can be selected against. Similarly in many long lived rhizomatous perennials, such as the widespread Chinese rangeland species, sheep grass (*Leymus chinensis*), the ability to reproduce via seed is less crucial for their survival and these often have low seed yields, seed dormancy and germination issues. Indeed some long lived clonally propagated perennial grasses can be surprisingly successful, yet sterile, as in vetiver grass (*Vetiveria zizanioides*). Likewise stoloniferous species like Bermuda grass (*Cynodon dactylon*) may be propagated by stolons and it becomes a challenge to develop seed propagated cultivars with equally high levels of turf performance.

**Breeding for herbicide tolerance**

With all grass seed crops it is essential to have a suite of herbicides available at economic prices to control broadleaf weeds and, if possible, problematic grass weeds, for example the annual grasses *Poa annua*, *Bromus* sp and hairgrass (*Vulpia* sp). Depending on the crop rotation and management, weeds may reduce seed yield and contaminate the resulting seed harvest. Historically in New Zealand up to 90% of crops were contaminated with other grass species, albeit at low or trace levels (Rowarth *et al.* 1998). However, better seed crop management today has reduced this considerably and having a suite of herbicides to control these problematic weeds is very useful. Unfortunately, the interest of agrochemical companies in developing herbicides for seed crops is limited due to the relatively small scale of the industry internationally and any new herbicides will be primarily for major crops such as cereal crops. Occasionally these can also be of specific value for grass seed crops.

The selection of herbicide tolerant cultivars can be of great value for both end users and seed producers. But this is a complex issue as the unintended development of herbicide resistant grasses through overuse of grass herbicides has caused problems in many parts of the world. In South America for example, *Lolium multiflorum* L. weed escapes have developed glyphosate resistance when growing under glyphosate tolerant soybeans and other crops (Preston *et al.* 2009).

Never-the-less, the deliberate breeding of grass cultivars tolerant to specific herbicides has potential if the unintended consequences can be carefully managed.

**Response to growth regulators**

In some countries and in some species, well timed applications of the growth regulators such as trinexapac-ethyl increases seed yield through delaying lodging and seeds per spikelet. This response is often 50% or more, but it can interact with the bulk of herbage present at application, a factor driven by grazing, early spring management and nitrogen application (Haldrup 2007, Young *et al.* 2007, Rolston *et al.* 2010, Chynoweth *et al.* 2010).

It is unclear whether breeders could breed cultivars which do not require growth regulators or which respond more effectively to the application of growth regulators. It is likely that breeding for delayed lodging in cultivars would interact with the effect of growth regulators.

The consequence of growth regulators is that harvest index is increased through both improved seed yields and reduced herbage mass. Moreover, there appears no reason why breeding efforts could not achieve similar improvements in harvest index.

**Components of yield**

The breeder has a choice of breeding for specific components of yield, the number of seedheads, seeds developing per head or 1000 seed weight.

The number of seedheads is generally not limiting under high fertility herbage seed crops as the numbers readily reach a limit for light interception. Some perennial grasses such as the fine fescues can have insufficient seedhead number in older stands and selection may be warranted to improve this. Where breeders attempt to improve seed yields simply by breeding for an increase in number of seedheads, or more specifically, the proportion of reproductive tillers, there is very likely to be an undesirable decrease in pasture or turf quality.

There seems little evidence that breeding for higher 1000 seed weight would be desirable in herbage cultivars as both small seeded turf cultivars and large seeded tetraploid cultivars can produce very high seed yields. There is however, little question that managing seed crops to fill the seed is desirable. It is clear that although sufficient seed heads are required to obtain good seed yields, a more effective strategy would be to breed for more efficient seedset within the seedhead (Boelt & Studer 2010).

In New Zealand perennial ryegrass seed crops, over half the developing seed fails to mature in the last two weeks of seed fill. At mid to late seed fill stage over 85% of florets of perennial ryegrass can contain a developing embryo but less than 20-30% may produce a harvested seed which can germinate and be sold (Rolston *et al.* 2006, Chynoweth 2012). In many situations only 2 or 3 seeds/spikelet are salable from the 12 potential florets on a spikelet (Elgersma 1990, Chynoweth *et al.* 2013).

Potentially seedset may be increased by selection as Bugge (1987) found that selecting seeds per spike in perennial ryegrass produced a selection response similar to that obtained by direct selection on high seed yield per plant, in the order of 5% improvement when the best 10% of plants were selected.

Forage grass seed yields are often low and variable with a harvest index, (the seed harvested as a proportion of above ground matter) of only 10–20% (Trethewey & Rolston 2009). This compares with annual graminaceous cereal crops where the harvest index of wheat, for example has increased from between 30-35% to almost 55% over a the last century, while barley and rice have shown similar trends (Atwell *et al.* 1999).

The breeding of cultivars with improved reproductive efficiency through more seeds per seedhead at harvest is unlikely to have an undesirable effect on forage yields or pasture or turf quality.

## Seed yield, forage yield and pasture quality

The development of seedheads in forage crops in spring provides a considerable boost to forage yield at this time of year but at the expense of quality, particularly as stems mature. The propensity for cultivars to develop seedheads in pastoral systems can be very different from the number of seedheads which develop from seed crops. The cultivars maintaining highest quality will be those with a low propensity to develop seedheads in regularly grazed (or managed) pastures but which can develop a full set of seedheads from an early closed seed crop under a seed crop management. Cultivars which develop high numbers of seedheads in regularly grazed pastures though may offer the potential for later closing for seed production – a factor which may be useful in some low cost seed production systems.

The tendency for many perennial grass species and cultivars to produce a second flush of seedheads in summer, often termed aftermath heading, is also likely to be reduced in cultivars when these tillers require a vernalisation requirement. It is unclear whether these vernalisation factors are controlled by the same genes as general vernalisation. It is also unclear whether cultivars which have many aftermath seedheads have greater seed yield potential than those lacking aftermath seedheads. However, it is possible that cultivars with greater propensity for aftermath heading do offer greater seed production in some seed production systems but at the expense of forage quality during summer.

## Seed shattering

In contrast to cereal crops where their domestication has led to non-shattering types with tight flowering and seed maturity, shattering is seen as a significant problem in forage seed crops and means that crops cannot be direct harvested like cereals unless harvested at high moisture and dried immediately. Most cereal crops such as wheat, barley, oats and rice have evolved from shattering wild grasses but contain a critical genetic mutation preventing shattering. This is a key mutation in the domestication of these crops allowing all heads to be harvesting on a single day. To date few grasses contain these type of genes and most are windrowed prior to shattering and left to sit gently on the ground undisturbed (hopefully) to dry before being be gently lifted with machinery and threshed. Shattering losses can be up to 50% in some crops and are seldom below 10% (Rolston *et al.* 2007).

There are reports in some species like cocksfoot of reduced shattering in southern Italian populations (Falcinelli *et al.* 1984) and this was found to be a single gene effect (Falcinelli 1991). Likewise, a seed retaining gene was used in 'Seedmaster' phalaris (Oram & Culvenor 1994) although most seed retaining cultivars today use a four gene mechanism (McWilliam & Gibbon 1981). However, in the *Lolium* genus a two gene model has been proposed for controlling shattering in *L. temulentum* and *L. persicum* (Senda *et al.* 2006). In some species the non-shattering trait may also reduce the ability to thresh the crop (Cunningham *et al.* 1994) and care would be needed to ensure non-shattering cultivars can be readily threshed.

Priority must be given to the discovery of non-shattering genes in each of the perennial forage grass species and the incorporation of these genes into commercial cultivars.

## Lodging

In most forage grasses the majority of tillers lodge and the timing of this can influence seed yield. It is desirable to maintain an erect crop for as long as possible to ensure pollination and maximum seed yields. Ideally a crop would only lodge prior to the crop becoming prone to seed shattering. Management of crops using growth regulators has shown a significant value in delaying lodging and lifting seed yields. Perennial ryegrass seed trials in New Zealand indicate that early lodging of perennial ryegrass will decrease seed yield by an average of 24 kg/ha per day with many crops losing more than this (Rolston *et al.* 2010).

Although direct harvesting of tall fescue at high seed moistures is practiced in regions where facilities are available to enable immediate drying, most herbage cultivars lodge and require windrowing. Short and stiff cultivars would allow direct harvesting as seen in many cereals. In order to achieve direct harvesting, crops would also need to combine a short, stiff growth habit with an appropriate non-shattering genetic composition and a more tightly synchronized flowering and ripening period. The short stiff growth habit would also improve harvest index as seen with the shift from tall to semi-dwarf wheat cultivars. Care may be required in breeding short stiff cultivars to ensure quality of pasture or turf is not compromised.

Priority must be given to the selection and/or discovery of shorter stemmed perennial forage grasses which when combined with non-shattering genes which would allow the direct heading of grass seed crops.

**Seedhead architecture**

One strategy attempted to improve seed yield in perennial ryegrass was to develop plants with branched seedheads rather than a spike (Rumball & Foote 2008). Although plants were developed with branches and considerably more potential florets for seed set the resulting seed yield was considerably less than normal spike types (Rolston *et al*. 2005).

**Soluble carbohydrate and seed fill**

Studies on carbohydrate metabolism in grasses shows that there is proportionately large concentration of water soluble carbohydrate (WSC) present in the stems of perennial ryegrass post-anthesis; moreover, that these concentrations continue to increase through to harvest. When harvesting a 2950 kg/ha seed crop, Trethewey & Rolston (2007 & 2009) have shown there was 2200 kg/ha of WSC remaining in the internodes at harvest. It is likely that perennial grasses maintain surprisingly high reserves of WSC in their tillers at harvest as a strategy for perenniality, while annual grasses utilize more of these reserves for seed production. More research is required to clarify whether this is tightly linked to perenniality.

The seed head itself may be more important than the flag leaf in contributing to seed weight and determining tiller seed yield (Trethewey *et al.* 2010, Trethewey & Rolston 2010). This is similar to the situation in annual cereals where spike and even awn photosynthesis is now being considered for selection to improve yield (Molero *et al*. 2013).

Knowledge of the mechanisms that underlie carbohydrate partitioning to the seed could in future result in significantly higher yields.

**Irrigation**

The production of seed crops under dryland conditions can dramatically reduce seed yields and often there is little the breeder can do to enhance the situation. Matching a cultivars maturity to the environment though is crucial as early flowering cultivars have the ability to develop mature seed prior to a drought. Irrigation's role is crucial in many environments in the last few weeks prior to harvest as it maximizes seed fill. Good moisture during seed fill and maturation has allowed late flowering wheat and barley cultivars with a slow maturing process to achieve world record yields in New Zealand.

**Pests and diseases**

It is clear that plant diseases can reduce seed yield and that breeding cultivars resistant to the diseases present in seed crops is very important. Stem rust (*Puccinia graminis*) and crown rust (*Puccinia coronata*) are two very common diseases of ryegrasses but many other diseases may occur which often require the expense of fungicide applications (Rolston *et al.* 2006). Certain fungicides can enhance seed yields even in the absence of leaf pathogens (Hampton & Hebblewaith 1984) by delaying leaf senescence in ryegrass. In a perennial ryegrass trial in New Zealand a reduction of 20% green leaf area from 70% to 50% reduced seed yield by 50% (Rolston *et al.* 2006). Breeding for disease resistance to maximize total green area retention would be a desirable trait to maximize seed yields.

In some circumstances seed yield may be reduced by insect pests and developing resistant cultivars either through genetic mechanisms of through incorporating endophytes will be beneficial for seed production, particularly during crop establishment.

Endophyte transmission may be a further complication for seed production in many pasture and turf grasses. In cultivars containing endophyte, it is important that the highest transmission levels possible are achieved. Breeding for compatibility between endophytes and the plant host has become a very important objective in some breeding programs. Cultivars which fail to consistently transmit high percentages of endophyte to their progeny seed are of little use to the industry and must be discarded.

## Conclusion

Herbage breeders clearly have technical ability to breed for improved seed yields but the "plant breeders' dilemma" is that an increased focus on seed yield will mean less progress on forage yield. However, there is no inherent technical reason why breeding for high forage yield is incompatible with high seed yield. A balanced approach to lift seed yields along with forage yield or turf performance is warranted and it is clear that there is considerable room to improve seed yields.

Breeding for seed yield should include selecting cultivars for:

- Focusing on the yield component of seed set and reduced abortion, rather than simply an increase in reproductive tillers or seed size. This improvement in reproductive efficiency and harvest index should be able to be achieved with little consequence for pasture or turf quality.

- Selection for non-shattering and seed retention will be desirable to reduce harvest losses taking care that the ability to thresh the crop is not compromised.
- Selection for short stiff plants with delayed or no lodging should increase seed yields, and combined with non-shattering should enable direct harvesting of a standing crop; analogous to wheat semi-dwarfs.
- Cultivars should benefit from a tighter range of flowering to minimize losses from shattering of early seed and undeveloped late seed.
- Cultivars should have excellent disease resistance on leaf, stem and seedhead with the ability to retain a high total green area.
- In New Zealand selection for low vernalisation appears to enable flexibility to sow a seed crop late, if and when necessary.

In many grass species germplasm may not be known which can be used as a source of these traits and a planned search for the traits will be required, particularly for seed retention and short stiff straw.

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# Can molecular techniques help solve the plant breeders' dilemma of forage versus seed?

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**Abstract**

Forage crops are essential for animal production and play important roles in sustainable agriculture. Forage yield and quality are the main target traits for improvement in a breeding program. To effectively propagate and successfully commercialize a cultivar, seed yield is a vital aspect of a new cultivar. An ideal cultivar should have desirable forage traits and good seed yield.

The use of molecular techniques offers new opportunities to develop cultivars with balanced forage productivity and seed yield. Genetic engineering allows the creation of novel variations to improve grasses and legumes. Tremendous progress has been made in genetic manipulation of forage crops in the past decade. The rapid advancement of cellular and molecular biology in forages provides novel methods to accelerate or complement conventional breeding efforts. It is fairly straightforward to improve specific forage traits without affecting seed yield by downregulation of endogenous genes or overexpression of alien genes. In some cases, introduction of certain DNA coding or non-coding sequences into non-stolon grasses led to the production of transgenic plants that can be easily propagated like stolon-forming species. A major obstacle for the utilization of transgenic technology is government regulation. Despite the potential economic and environmental benefit, it has been very difficult to deregulate and commercialize new transgenic cultivars. The situation is even more complicated in transgenic forages because many widely cultivated species are outcrossing and polyploid species.

Molecular markers have been regarded as powerful tools for analyzing genetic diversity and for the selection of parents in cultivar development. Marker analysis can measure the allelic effect of an individual locus of a quantitative trait, thus allows pyramiding the desirable alleles controlling a complex or intractable trait into an adapted genetic background.

Recent developments in genetic modification and improvement of forage crops will be reviewed and discussed.

# Flowering dynamics of a diverse perennial ryegrass population grown for seed

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**Abstract**

Although seed yield is a complex trait affected by numerous agriculturally applied and environmental factors, seed production traits reveal considerable genetic variation between ecotypes. Ecotype selections form the basis for many breeding programs, where by understanding seed yield parameters will allow possible seed production traits to be included in early selection/breeding cycles. The confounding contradiction between increasing regulatory restrictions imposed on agronomic practices (i.e., nitrogen restrictions), limitations in advances in agronomic understanding, and declining production areas, have seen limited increases in seed yields. Therefore, breeding for increased seed yields will be the next revolution for realising potential.

Here, "flowering" and seed production, unlike many so-termed agronomic traits are often over looked within standard breeding programs, leading to difficulties for seed production. With genetic improvement in mind, it is the aim of seed production researchers and producers to understand all aspects of seed production, including flowering behaviour within perennial ryegrass. Therefore, breeding for flowering homogeneity is considered important for sustainable breeding efforts to realised high seed yields.

In the research fields of Aarhus University, Flakkebjerg Research Centre 1560 individual plants (520 lines in 3 replications) were established. These 520 lines represent breeding material, cultivars, and ecotypes collected from all over the world. Plants were grown under limited agronomic inputs to express their natural variability within the population. During the 2014 growing season, plants were individually assessed for heading date and daily flowering during the respective periods. Heading data was a single determined time point when a respective plant had three tillers at BBCH growth stage 49 – 51 (first spikelet exposed). Flowering was recorded successively during the period BBCH growth stage 60 (start of flowering) to BBCH growth stage 69 (end of flowering) for all individuals.

For this study, heading date was spread over 10 weeks; moreover, heading date was shown to have influence on the synchronicity of flowering, with later heading varieties having a more consolidated flowering period. While these later heading varieties had a consolidated flowering period, many individuals within this population had prolonged flowering periods – of up to 30 days. However, the consolidation of flowering period is believed to be a key component of determining high realised seed yields. The results have important implications for seed production research as they will allow seed researchers and breeders alike to identify population groups with increased seed production potentials.

# Seed trait variation and correlation analysis of different *Leymus chinensis* germplasm

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**Abstract**

*Leymus chinensis* (Trin.) Tzvel is a perennial grass species, with good quality and high nutrition value, and is an important forage species for livestock in the steppes of northern Eurasia and China. We analyzed seed morphological characters, caryopsis proportion, and the 1000-seed weight of 28 *L. chinensis* accessions from six different geographical areas. The results showed that the length and width of lemma and caryopsis were differed significantly between the six areas, and the variation of length was greater than width. Caryopsis color was divided into pale-yellow, blue and intermediate. We found the lemma and palea of the majority of lines were not easy to peel off, and the lemma easier to peel off than palea. Correlation analysis indicated that lemma length and width, caryopsis length and width, caryopsis width and grain weight, caryopsis width and proportion were significantly positive correlated at 0.01 level, while significant correlations between caryopsis length and width, lemma width and 1000-seed weight were found at 0.05 level. The results are helpful for understanding geographic variation within *L. chinensis*, and for understanding factors of value in breeding for seed yield.

**Key words**: *Leymus chinensis*; seed traits; germplasm; correlation analysis; variation

# Screening of low seed shattering germplasm of Siberian wild rye (*Elymus sibiricus* L.) and implication for future genetic improvement

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**Abstract**

Siberian wild rye (*Elymus sibiricus* L.) is an important native grass in Qinghai-Tibet Plateau of China. It is difficult to grow for commercial seed production, since seed shattering cause yield losses during harvest. Progress in cultivar development with desired traits has occurred slowly for this species in the past years. One reason for the slow progress may be a lack of study and effective utilities of genetic resource and phenotypic variation. In this study, we present a molecular marker assessment of 24 accessions, cultivars, and breeding lines originated from the northeastern Qinghai-Tibet Plateau, and evaluated the variation of seed shattering. Of the 120 individual plants evaluated for genetic diversity, 160 bands were generated from 30 EST-SSR markers, 143 bands of which were polymorphic with the percentage of polymorphic loci (PPB) of 89.4% at the species level. The PPB within accessions ranged from 2.5 to 56.25 %, indicating high level of genetic diversity consistent with the variability observed in seed shattering (21.3-56.1%). The genetic diversity analysis reported here has identified potential heterotic groups, or resources to generate improvement through crossing with cultivated materials. This will facilitate genetic improvement and cultivar development with desired traits in future *E. sibiricus* breeding programs.

**Key words**: Siberian wild rye (*Elymus sibiricus* L.); seed shattering; genetic diversity; conservation and implication

# Targeted mining Cleistogamy genes in *Cleistogenes songorica* by Transcriptome Sequencing

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**Abstract**

*C. songoria* possesses a mixed breeding system which exists the coexistence of both cleistogamy (CL) and chasmogamous (CH) on the same individual. Here, transcriptome sequencing could be used to specifically reveal molecular mechanism of CL and CH. A total of 69,331 unigenes were obtained by *de novo* assembly. We got 18,336 different expression genes candidates with 8,219 up-regulated and 10,117 down-regulated. Then 9 unigenes related to cleistoganmy were selected to study their expression in different organs with quantitative PCR. In total, 329 SSRs distributed in 7,323 sequences were identified with 902 unigene sequences.

**Key words**: Cleistogamy, *Cleistogenes songorica*, transcriptome Sequencing

# Effects of salinity on germination of populations of *Panicum coloratum* var. *makarikariense*

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**Abstract**

Variability in response to salinity in a collection of *P. coloratum* var. *makarikariense* with the final aim of breeding was evaluated. The purpose of the study was to compare seed germination of four populations (DF, ER, UCB and TS) to increasing saline conditions corresponding to 0, 100, 200, 300 and 400 mM of NaCl. Germination at 400 mM almost never occurred and data was disregarded. Interaction between populations and treatments was significant ($p<0.05$). TS had significantly higher germination rate than all the other populations at 0 and 100 mM NaCl while UCB showed always the worst performance. Nonetheless, the reduction in proportion of germinated seeds caused by salinity was smaller in ER (32%) than in TS (45%) at 100 mM. DF and UCB germination decreased 58% and 52%, respectively. Again, at 200 mM the reduction in the proportion of germinated seeds was lower in ER (84%) while all the other populations decreased almost 90%. Seed germination at 300 mM was negligible. In conclusion, although ER is the population suffering a lower reduction in proportion of germinated seeds with increasing salinity, TS is the one showing the better performance, even under salt conditions. Therefore, ER might be considered a potential material for studying mechanisms of salinity tolerance at a germination stage whereas TS could be chosen to obtain better stand yields.

**Keywords:** Breeding, germination, *Panicum coloratum* var. *makarikariense*, salt tolerance.

# Genome-Wide Analysis of Late embryogenesis abundant (LEA) Gene Family in *Medicago truncatula*

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**Abstract**

Late embryogenesis abundant (LEA) proteins are expressed abundantly at the late stage embryonic development of plant seeds, and play important roles in seed desiccation tolerance, longevity and in responding to various environmental stresses, such as drought, low temperature and high salinity. At present, the phylogenetic studies of *LEA* gene family in *Medicago truncatula* have not been reported yet. In this study, with the application of bioinformatics methods, the *LEA* family genes of *Medicago truncatula* were identified through the whole genome, and the system evolution, gene structure, evolutionary pressure, chromosomal location and gene expression patterns were further analyzed. Based on BLAST searches and Pfam family domains identifications in the protein sequences, a total of 23 *LEA* family genes systematically identified from *Medicago truncatula* and classified into 8 subfamilies. All of the gene-encoded proteins predicted to be highly hydrophilic except *LEA2-2*. Gene location results showed that 23 *LEA* genes were distributed unevenly on 7 chromosomes except chromosome 6; the exon numbers of all the genes were no more than two, indicating a simple gene structure of this gene family. The expression profiles of *Medicago truncatula LEA* genes showed a characteristic of temporal and tissue specific, and regulated by drought stress. These results are helpful for understanding the seed development and functional analysis of *LEA* genes in legume species.

# Development and evaluation of a near infrared reflection model to predict carbohydrates in grass samples

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Near Infrared Reflectance (NIR) spectroscopy is a well-known and widely used method for quantification of water, protein, carbohydrates and fats in agricultural products. The aim of this Poster is to develop partial least-squares (PLS) and interval PLS (iPLS) regression models based on NIR spectra to predict total carbohydrates in grass samples. The results are preliminary and are based on 37 NIR spectra with known carbohydrate concentration (% $DM^{-1}$). The NIR spectra were obtained on dried and ground plant samples using an NIR spectrometer (Q-interline Spectroscopic Analytical Solutions, QFAFlex, Tølløse, Denmark) in reflectance mode. Log (R/1) was recorded within the wavelength range from 1100 to 2498 nm using 64 scans at 16 $cm^{-1}$ resolution. PLS and iPLS regression models were developed and validated using venetian blinds (six data splits and five blinds per sample). The PLS regression model had a root mean square error of cross validation (RMSECV) of 1.48 using four latent variables. Forward interval PLS regression models usually have a lower errors in specific wavelength regions which was also the case in the current study (RMSECV=1.2).

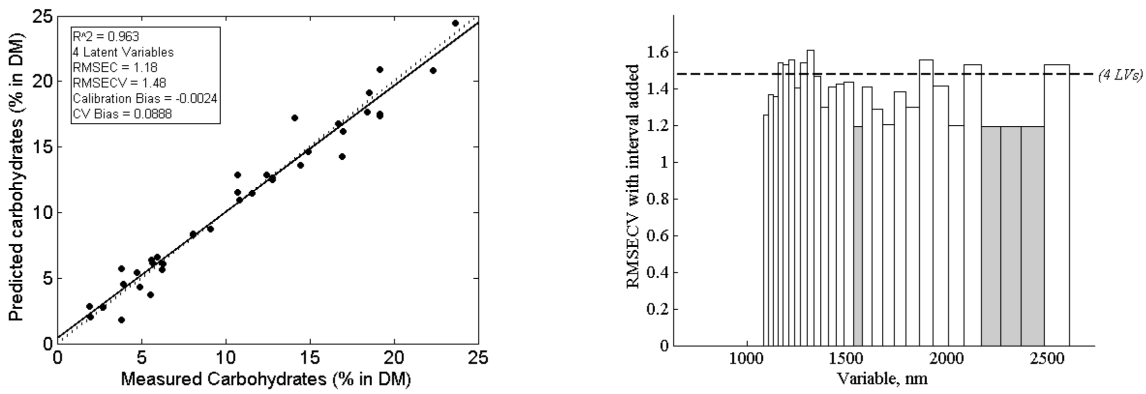


**Figure 1.** Predicted carbohydrates vs. measured carbohydrates for PLS regression model using the full spectra (1100 to 2498 nm) (left) and RMSECV for different wavelength regions using iPLS regression model (right).

The error of the wet chemical method was calculated as the standard deviation (STD) based on the average STD of four samples each measured three times and STD calculated for the four samples, average of these four STD values was 1.44 % carbohydrates in DM. The error of the PLS regression model was therefore comparable with the error from the wet chemical method, while iPLS were able to show a lower error. Errors from laboratory and NIRS methods can be calculated in many different ways giving different results, but based on our preliminary results we have shown that iPLS on NIR spectra is an interesting method to predict the concentration of carbohydrates in grass samples.

# Effects of salt and water stress on the seed germination and seedling growth of five *Melilotus* accessions

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**Abstract**

*Melilotus officinalis* and *M. albus* are two most important cultivar species in *Melilotus* spp, which have been used as forage crop, promotion soil stabilization and as a nectar source for honey bees. However, study on effect of seed germination and seedling growth under salt and drought stress had rarely reported. In this study, three *M. officinalis* accessions (PI 595335, Mo-LX 04 and PI 595388) and two *M. albus* (Ames 19257 and PI 593234) were selected as elite accessions from more than 100 accessions. Seeds of five *Melilotus* accessions were treated with different concentrations of NaCl and PEG 6000. Furthermore, a pot experiment was conducted to investigate the effect of Sustained drought stress on growth of two species. The results showed that: 1) as concentrations of NaCl increasing, germination index and seedling length for both species declined and a slight salt stress improved the germination rate. The salt tolerance of these five accessions decreased in the order of PI 593234＞Ames 19257 ＞ PI 595388＞ Mo-LX 04＞ PI 595335 under high concentrations (200 mmol/L and 300 mmol/L) of NaCl. Compared with *M. officinalis*, the accessions of *M. albus* have a good performance in high salt stress. 2) as the drought intensity becoming increased, the relative germination rate and germination index of both species tended to decline. Under low concentration, PEG 6000 treatment exert good inductive effective on the seed germination of all accessions in two *Melilotus* species except PI 595335, and increased the germination index and seedling length of *M. albus*. When the osmotic pressure reached -1.2MPa, Mo-LX04, Ames 19257 and PI 593234 still germinated. However, no germination occurred at -1.5MPa. 3) the five accessions grow best in 50% of field moisture capacity. And the growth of PI 595388 was superior to the other accessions under three different soil moisture conditions (70, 50 and 30% of field moisture capacity).

# Evaluation of variability of seed yield-related morphological trails in alfalfa (*Medicago sativa* L.)

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**Abstract**

Alfalfa (*Medicago sativa* L.) is one of the most important forage legumes worldwide. Breeding to improve seed yield will enhance the value of alfalfa. There are many genetic and environmental factors affect seed yield. The present study was conducted to determine the extent of genetic variation among 48 alfalfa cultivars using 4 seed yield-related morphological traits during 2013-2014. Forty-eight cultivars were collected from China, America, Holland, Australia, Mexico, France and Switzerland and planted in a randomized block design with 3 replicates in Lanzhou, Gansu province. Sixty plants from each cultivar were used to characterize the time of beginning of flowering, flower color, seedpod type and leaf area. The results showed that the time of beginning of flowering across all cultivars ranged from May 30 to June 6. There are three different flower colors, very dark blue violet variegated flowers, variegated flowers and cream, white or yellow flowers found in all cultivars, and the very dark blue violet variegated flowers showed the highest frequency. Most of cultivars showed two-coiled or three-coiled seed pod type, only 2 cultivars and 1 cultivar showed one-coiled and four-coiled seedpod type, respectively. Across all cultivars, the average leaf area was 254 $mm^2$, ranged from 177 $mm^2$ to 313 $mm^2$. These results are helpful for the selection, promotion and high yielding cultivation of alfalfa cultivars.

# Common vetch (*Vicia sativa*) multi-podded mutants for enhanced commercial seed production

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**Abstract**

In comparison to other annual forage legumes, such as pea (*Pisum sativum* L.), advances in breeding vetches (*Vicia* spp.) are rather modest. One of the main obstacles in increasing the cultivation area under vetches is uncertain seed production, mostly due to their indeterminate stem growth and non-uniform maturity, with the genes controlling these important traits still unattested. In contrast in wild populations of common vetch (*V. sativa* L.) the genes have been identified, isolated and mutant plants with more than usual two pods per node tested. Crossing these mutant genotypes with wild-type ones demonstrated that the number of pods in these two vetch species is controlled by two genes, orthologs to *FN* and *FNA* in pea. If both genes are recessive, a plant will have more than two flowers per each node and, depending on still not clarified environmental factors, more than two pods per node. Developing vetch cultivars with more than two pods per node may be one of the solutions for enhancing seed production in this crop.

**Key words:** breeding; common vetch; multi-podded mutants; seed production

## Seed morphological diversity in 18 Melilotus species

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**Abstract**

Melilotus (sweetclover) belongs to the legume family of the tribe Trifolieae and comprises 19 annual and biennial species. Except for *M. macrocarpus*, plant samples of 48 populations of 18 *Melilotus* species were collected to study their seeds morphological traits. Five characters were measured: seed length, seed width, seed width-to-length ratio, seed circumference and the total weight of 100 seeds. The morphological traits of seeds exhibited considerable variations among species. Seed color is yellow or gray and varied among species. The coefficients of variation (CV) between species of seed length and seed width were high. With the exception of the width-to-length ratio, all the variance of seed traits showed a significant difference among source populations. According to the value of seed width-to-length ratio, all the species can be divided into three groups

**Key words:** diversity; *Melilotus*; morphological; seed

# The selection for low-shattering common vetch (*Vicia sativa* subsp. *sativa* L.)

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**Abstract**

The common vetch (*Vicia sativa* subsp. *sativa* L.), a self-pollinating, annual and diploid species, is one of the most important annual legumes in the world due to its short growth period, high nutritional value and multiple usages. Pod-shattering or loss of seeds from mature pods of vetch is one of the most important defects limiting its culture and utilization in field. In this study, we employed multiple experiments to evaluate for shattering characteristics of vetch to improve the seed production. Totally, 541 vetch accessions were selected including 5 common vetch subspecies. The germplasm showed a high degree of morphological diversity, such as the pod-shattering rate, the seed shape and other agronomic traits. In 2013 summer, we preliminarily selected 38 accessions showing less than 10% pod-shattering rate, and 45 accessions showing more than 90% pod-shattering rate. The phenotypes of these accessions were retested in the glasshouse at winter in 2013. After a second round of screening, we selected 9 accessions with less than 5% pod-shattering rate. In 2014, we continued to evaluate the pod-shattering characteristics of the 541 accessions in field. After three rounds of evaluation, we have gained 12 low-shattering vetch accessions and 12 high-shattering vetch accessions.

# Stylosanthes spp. in China: a Successful Way of Introduction, Breeding and Utilization

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**Abstract**

Stylosanthes spp., a genus of tropical forage legume, contains 48 species, is naturally distributed in the tropical, subtropical, and temperate regions of the Americas, Africa, and Southeast Asia. Of these, S. guianensis is the most widespread species and is one of the most important tropical forage legumes. It is used for grazing cattle, making leaf meal for livestock, for improving soil fertility in fruit-tree and rubber plantations and for cover crops in Australia, South America and south China. A range of Stylosanthes species was introduced from Australia, Africa, and South America into China in the late 1960. S. gracilis was the first introduced as a cover crop for young rubber plantations in 1962, and then S. guianensis cv. Cook and Graham introduced in the 1970s, and largely responsible for the stylo revolution in China in 1980s. A series of Stylosanthes spp. cultivars have been developed by selective breeding and mutation breeding successively, of those, S. guianensis cv. 'Reyan No. 2', S. guianensis cv.' Reyan No. 5', S. guianensis cv. 'Reyan No. 7', and S. guianensis cv. 'Reyan No.13', which selected from CIAT 184, S. guianensis cv. 'Reyan No.10' originated from CIAT1283, S. guianensis Sw.cv.907 has been developed through Cr60-γ radiation technology, S. guianenesis Sw.'Reyan No.20' and S. guianenesis Sw.'Reyan No.21' induced by space flight. Stylosanthes was used for improving tropical grassland and artificial grassland, for leaf meal production as feed sources to feed pigs and poultry, for water and soil erosion control, and as green manure in Southern China. Stylosanthes spp. were found to be well adapted to the environment in much of southern China and are now grown more than 200,000 ha.

**Key words**: *Stylosanthes* spp.; introduction; breeding; utilization

# De novo sequencing, assembly, and analysis of the tall fescue leaf transcriptome in response to nitrogen stress

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**Abstract**

Tall fescue (*Festuca arundinacea*) is one of the most important pasture grasses in the world. Understanding how genes respond to nitrogen starvation is essential for formulating approaches to manipulating genes for improving nitrogen use efficiency. However, little is known at the transcriptome and genomic level about nitrogen starvation in Tall Fescue. Here, we obtained more than 60 million sequencing reads from tall fescue seedlings under nitrogen starvation using Illumina sequencing technology, with a total of 299,761 Contigs representing 72,666 unigenes (with 676 nt mean length). Expression differential analysis revealed that a total of 13112 unigenes were detected to be responsive to nitrogen starvation with 2346 unigenes up-regulated and 10766 unigenes down-regulated. GO annotation and KEGG pathway mapping revealed that the unigenes participated in diverse biological functions and processes, including metabolic process, proteasome, transcription, apoptosis, and immune-response. The differentially expressed genes selected from transcriptome profiling were confirmed by quantitative real time PCR (qRT-PCR) analysis. Our data provided comprehensive gene expression information at the transcriptional level and the first insight into understanding of the molecular mechanisms of nitrogen starvation in tall fescue, which might be helpful for high nitrogen use efficiency tall fescue breeding.

# Construction of finger printing and analysis of genetic diversity with SSR markers for sorghum

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Abstract

In this study, 20 pairs of SSR primers were screened from 108 pairs to explore the DNA fingerprints, constructing the genetic diversity by analyses using 10 varieties of Sorghum Sudanense and 7 varieties of Sorghum bicolor ×Sorghum sudanense. The results showed that 20 pairs of primers totally amplified 121 bands, in which 117 bands were polymorphic and the average value of polymorphic information content (PIC) was 0.703. The number of varieties which could be identified by using single pair primer ranged from 2 to 14 and the number of primer mix which could be used to identify 17 varieties ranged from 2 to 5. It is valuable for fingerprint constructing. The genetic diversity analysis of 17 varieties showed that the average Nei's genetic diversity index (H) was 0.480, the average information Shannon diversity index (I) was 0.820, and the similarity text between 17 national varieties ranged from 0.58 to 0.96 as well. The result showed that the national varieties of *Sorghum Sudanense* and *Sorghum bicolor ×Sorghum sudanense* were rich in genetic diversity.

# Effects of colchicine and $^{60}$Co-γ-rays on the seed germination of a xerophytic grass

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**Abstract**

Plant mutagenesis is using external factors to accelerate the species genetic variation and get valuable mutants in a short time, which for creating conditions of cultivating new species, varieties and studying gene function. This experiment studied seed germination rate, germination index and the growth of seedlings of *Cleistogenes songorica* under different doses of colchicine's and $^{60}$Co-γ-rays treatments.

**Key words**: *Cleistogenes songorica*; colchicine; $^{60}$Co-γ-rays; germination.

# Session 2. Seed production: achieving high yields and environmental sustainability

# The Next Steps to Achieve Higher Seed Yields

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**Abstract**

Low reproductive efficiency and other factors cause low and variable seed yields in cool-season herbage seed crops. Despite serious limitations in seed yield, good progress has been made in the last 40 years in the advancement of seed yield of herbage seed crops. Increases in seed yield over the period have ranged from 17 to 213% in Oregon, USA and have been the result of enhancements in crop management practices and technological advances in crop production machinery. Seed yield components provide insight into the plant's utilization of inherent seed yield potential and illustrate deficiencies that must be overcome in order to attain future improvement in seed yield. The next steps to achieving higher seed yield in herbage seed crops will be predicated on greater involvement with plant breeders in the endeavor, and more widespread adoption of emerging crop management technologies.

**Key words**: seed yield, yield components, yield potential, reproductive efficiency

**Introduction**

Why increase seed yield in herbage seed crops? The primary rationale for increasing seed yield is twofold; the desire to improve upon current biological and economic inefficiencies that are evident in these species, and environmental considerations. Unlike cereal grain and pulse legume crops, herbage seed crops are biologically inefficient in the production of seed. Many flowers are produced by herbage seed crops yet relatively few of the flowers become seed, thus the potential seed yield may be many times greater than the actual seed yield harvested. Losses due to inadequate pollination and fertilization, abortion during seed development, and seed shattering all contribute to the relative low numbers of seed that are harvested compared to the crop's yield potential.

Greater seed yields are essential for the long-term financial stability of herbage seed production systems. While there have been gains in economic efficiencies for herbage seed production enterprises, the cost of producing the crops continues to rise. More seed must be harvested in order to remain economically competitive with other potential crops vying for the same land. In addition, the need for greater environmental protection as a motivating factor in increasing seed yield cannot be overlooked. The production of more seed with the same or fewer inputs, or more smartly applied inputs, will yield dividends for the environment.

This paper examines progress and future opportunities for advancement in seed yield in six of the most widely-grown cool-season herbage seed crops: tall fescue [*Schedonorus arundinaceus* (Shreb.) Dumort.], perennial ryegrass (*Lolium perenne* L.), annual or Italian ryegrass [*L. perenne* L. ssp. *multiflorum* (Lam.) Husnot], red clover (*Trifolium pratense* L.), white clover (*T. repens* L.), and alfalfa (*Medicago sativa* L.).

**Progress in Seed Yield Advancement**

Considerable progress has been made in the improvement of seed yield in cool-season herbage seed crops since 1975 (Fig. 1 and Fig. 2). The average seed yield harvested by Oregon, USA seed growers has increased nominally by 330 kg $ha^{-1}$ for white clover and by 1126 kg $ha^{-1}$ for tall fescue over the past 40 years (Table 1). Expressed as percentage gains over the time period, seed yield advancement has ranged from a low of 17% for annual ryegrass to a high of 214% for red clover. The amount of progress in yield advancement has varied among herbage crops. White clover and annual ryegrass had the lowest rate (slope) of improvement, while tall fescue and perennial ryegrass experienced the greatest rate of seed yield increase. Similar incremental increases in grass seed crop yields over time have been observed in New Zealand (Rolston et al., 2007).

The underlying reasons for these advancements in seed yield include development and adoption of superior agronomic practices, introduction of new pesticides and implementation of timely pest control strategies, and the use of more powerful and efficient equipment for harvest and other management operations. The role of genetic improvement in these increases cannot be discounted although breeders of herbage crops consider forage and turf quality rather than seed yield as a primary goal of their efforts.

The introduction of triazole fungicides in the 1980s had a considerable effect on seed yields of grasses; for example, perennial ryegrass seed yield was increased by up to 90% with propiconazole under severe incidence of stem rust (Welty and Azevedo, 1994). More recently, trinexapac-ethyl (TE) plant growth regulator (PGR) has been widely adopted for use as a lodging control agent in grass seed crops. Application of TE resulted in up to 45% increase in perennial ryegrass seed yield (Rolston et al., 2010; Chastain et al., 2014a). Tall fescue seed yield was increased by 40% with TE (Chastain et al., 2014b; Chastain et al., 2015). Less is known about TE in forage legume seed crops but Andersen et al. (2015) reported that red clover seed yield was increased by up to 15% in New Zealand and Oregon.

Spring irrigation increased seed yield in perennial ryegrass by up to 26% (Chynoweth et al., 2012). Huettig et al. (2013) found that spring irrigation strategically timed to coincide with peak anthesis increased seed yield by as much as 47% in tall fescue. Red clover seed yield increases averaged 13% with irrigation in Oregon (Anderson et al., unpublished). Irrigation in alfalfa seed production is a standard practice for Oregon and elsewhere. More effective nitrogen management timing and adoption of application rates that are more appropriate for seed yield targets have led to greater seed yields in grasses (Rolston et al., 2007; Chastain et al., 2014b).

The widespread adoption of seed moisture content as an aid in harvest timing has contributed to greater seed yields in herbage seed crops (Silberstein et al., 2010). Despite these improvements, seed yield losses in perennial ryegrass as a result of harvest activities still average 24% (Rolston and Chynoweth, 2010). Better harvest timing and improvements in harvest equipment have reduced seed yield losses, but new methodologies are needed to further reduce seed yield losses just prior to and during harvest.

Will we be able to sustain the increases in seed yield of the past 40 years into the future? What will be the contributing factors responsible for seed yield increases in the next four decades? The key to identifying the next steps in achieving higher seed yields in herbage seed crops is to understand the nature of the current limits on seed yield.

**Seed Yield Components**

A better understanding of the limits to seed yield can be elucidated through an examination of the seed yield components and their contribution to the expression of seed yield. Seed yield is the biological and mathematical product of the components of yield. Yield components vary in their relative contributions to yield, but fundamentally, seed yield is most influenced by two components: seed number and seed weight.

Seeds $m^{-2}$ is the yield component most strongly related to seed yield in cool-season grasses (Young et al., 1998; Rolston et al., 2010; Chastain et al., 2014a; Zapiola et al., 2014). Seed weight in the grasses depends mainly on the position of the seed within the spikelet (Rolston et al., 2007; Chastain et al., 2014a). Seed yield is the product of seed number and seed weight; however, the number of inflorescences $m^{-2}$, spikelets inflorescence$^{-1}$, florets spikelet$^{-1}$, and seed set can collectively or individually have an influence on seeds $m^{-2}$ (Chastain et al., 1997; Young et al., 1998).

Seed weight plays a role in grass seed yield (Boelt and Gislum, 2010; Zapiola et al., 2014); but when compared, the contribution of seeds $m^{-2}$ to seed yield is greater than that of seed weight (Chastain et al., 2011; Huettig et al, 2013; Chastain et al., 2014a; Chastain et al. 2014b). The variation in seed number attributable to environment, management and pests is typically greater than the variation observed in seed weight, which usually varies in a more narrow range.

The greatest opportunities to enhance seed yield through management is by increasing seed number. Seed number and consequently, seed yield, has been reported to be increased by PGR applications, spring nitrogen, spring irrigation, rust control and other management practices (Rolston et al, 2007; Chastain et al. 2014b). Irrigation, PGRs, and nitrogen affect seed weight to a small extent (Boelt and Gislum, 2010; Huettig et al, 2013; Chastain et al. 2014b). Variation in seed size and weight (non-uniformity) within seed lots results in part from the position within the inflorescence where the seed was formed (Rolston et al, 2007). This non-uniformity in seed size/weight contributes to harvest losses in the field (small seed) and to post-harvest cleaning losses.

Increased heads $m^{-2}$ in red clover contributed to greater seed yield, but seed weight was inversely related to yield (Anderson et al., 2015). Seed yield in white clover also seems to be related to heads $m^{-2}$. Heads in white clover arise in the leaf axils of stolons and to maximize head production, the stolons need unshaded space to grow into so manipulation of the canopy is often required. Multiple seed yield components have an impact on seed yield in alfalfa, including seeds pod$^{-1}$ and seed weight inflorescence$^{-1}$ (Bolanos-Aguilar et al., 2002).

Analysis of seed yield components illustrate the deficiencies that must be overcome in order to attain future improvement in seed yield. Systematic investigations of seed yield components in herbage seed crops and their relationships with each other as well as seed yield is needed to further improve our understanding of seed yield limitations.

**The Next Steps**

What are the constraints or limitations to further advances in seed yield? One of the biggest constraints is biological in nature - herbage seed crops produce only a fraction of their potential seed yield (Table 2). Reproductive efficiency, here defined as ratio of seed harvested to ovules present during flowering, is low and variable in herbage seed crops and seldom does this value reach 50%. In contrast, reproductive efficiency values for well-managed wheat grain crops rarely fall below 60%. Among grasses and legumes, tall fescue and red clover have the highest upper end in their range of reproductive efficiencies and have also shown the highest rate (slope) of seed yield advancement (Table 1). At the current rates of advancement shown in Table 1, projected seed yields of herbage seed crops will increase impressively over yields presently attained by Oregon farmers, but they will still fall well short of the potential yield (Table 2). Future advances in seed yield in herbage seed crops will be dependent on our ability to improve reproductive efficiency.

Manipulation of source-sink relationships has provided a greater insight into the physiological nature of seed yield limitations in herbage seed crops. The source (inflorescence, leaves, stems) appears to be more than adequate for achieving higher seed yields, but the mechanism for preferential partitioning of assimilates to sinks (seeds) is poorly understood at this time (Trethewey and Rolston, 2009). Utilization of seed yield potential in forming seed is determined by events taking place at and after flowering, but the overriding genetic limitations on the sink place the ability to make significant increases in seed number or size uniformity of harvested seed outside of our grasp with current knowledge.

Both seed yield and yield potential decline with increasing age of the stand in cool-season perennial grasses. The underlying reasons for age-related losses in yield of perennial grasses are largely not known at present and need to be further investigated. While some management practices can increase seed yield in the latter years of a stand, none of these are known to completely ameliorate the loss in vigor of stands. The only management option that can offset stand-age induced yield losses is limiting the life of the stand to one or perhaps two years. But this approach increases the cost of production per unit of seed produced by greater frequency of stand establishment.

Plant growth regulators have been a major contributor to the past observed increases in seed yield in herbage seed crops, but will new PGRs be as effective and economical as the previous generation of these beneficial chemicals? Flowering and seed development are spread out over wide time frames in herbage seed crops and are limitations to advances in seed yield. Better synchronization of flowering and subsequent seed development in herbage seed crops might be achieved through PGRs and other practices. Pollinator efficiency limits yield in forage legume seed crops and manipulation of the canopy by PGRs could enhance pollinator access.

Seed growers need a better understanding of crop development for improved timing and efficacy of management with the nascent technologies collectively known as precision agriculture. Concomitant with that need is a marked improvement in the ability to manage herbage seed crop fields for landscape non-uniformity and to smartly apply valuable inputs. Benchmarking is a tool that can be employed in the pursuit of higher seed yields in herbage seed crops. By using data analytics to compare farmer seed yields with benchmark yields obtained from similar environments, a path to higher seed yields and improved efficiency of production can be charted. The gaps between current yields and benchmarked yields can be identified and practices implemented to increase seed yields on a field, farm, or regional basis.

What are the opportunities to not only maintain the current rate of seed yield increases but to have a major breakthrough? A greater role for plant breeders in the advancement of seed yield in herbage seed crops will be necessary to significantly alter the present trajectory of seed yield improvement and perhaps to maintain the current rates of seed yield increase. Substantial increases in the uniformity of seed size/weight within harvested lots is likely only possible through plant breeding. Seed shattering is one of the most vexing of all of the limitations to seed yield in herbage seed crops and ameliorating this problem would result in major contributions to seed yield advancement. Breeding efforts in herbage seed crops might be aided by QTL mapping of traits associated with shattering reduction and higher seed yields.

**Conclusion**

Much progress has been made over the past 40 years in the advancement of seed yields in herbage seed crops. The key to identifying the next steps in achieving higher seed yields in herbage seed crops is to understand the nature of the current limits on seed yield. Future seed yield improvement in herbage crops is likely to come from concerted efforts in agronomy and plant breeding, and increases will be mainly through increases in seeds $m^{-2}$ rather than seed weight or size. More widespread adoption of still developing crop management technologies will aid seed production enterprises in their quest to increase seed yield of herbage seed crops.

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# Cytokinin: a key driver of seed yield?

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**Abstract**

The cytokinins have been implicated in many facets of plant growth and development including cell division and differentiation, shoot growth, root growth, apical dominance, senescence, and fruit and seed development. Cytokinin levels are regulated by biosynthesis, inactivation and degradation. During leaf, fruit and seed development the genes for biosynthesis (isopentenyl adenine; IPT), inactivation (*0*-glucosidase), and degradation (cytokinin oxidase/dehydrogenase; CKX) are all expressed. During the early stages of fruit and seed development cytokinin levels are transiently elevated and coincide with cell division. Exogenous application of cytokinin or ectopic expression of *IPT* have, on occasions, led to increased seed yield, leading to the suggestion that cytokinin levels may in fact be limiting yield.

In this review, I will cover both classic and recent literature to introduce the complexity that arises not only because of the pleiotropic nature of the cytokinins but also because the genes coding for biosynthesis and metabolism belong to multigene families, the members of which are themselves spatially and temporally differentiated. Additionally, any increase in seed yield has to occur without affecting the quality of either the seed or forage, which means that increased cytokinin must be targeted both spatially and temporally. Finally, I will briefly cover some of the modern tools available to target and manipulate cytokinin levels to increase seed yield with the concurrent aim of maintaining quality. The seminal work by Ashikari et al. (2005) on yield of rice, showed that the cytokinins could be directly targeted by plant breeders.

**Keywords:** cytokinin oxidase/dehydrogenase; CKX; cell division; IPT; source; sink

## Introduction

Food security is reliant on seed production from many different species. Seed will need to be produced in increasing amounts to meet the demands for direct human and animal consumption, but also for the production of forage for animals and vegetables for humans. In dicot forages, such as forage brassica, yield may be determined by the number of pods set, and the number and size of seeds within the pod. In forage monocots, such as ryegrass, the number of spikelets and fertile florets within spikelets, and final seed size are all determinants of yield. Forages have been bred for optimised vegetative growth and biomass production while seed yield has yet to be optimised. However, optimising seed yield must be done against a background of maintaining both forage and seed quality. The seminal work by Ashikari et al. (2005) on yield of rice, showed that the cytokinins could be directly targeted by plant breeders.

## A brief overview of cytokinin biology

Cytokinins (CKs) are key hormones that regulate many developmental and physiological processes in plants. They play a crucial role in regulating the proliferation and differentiation of plant cells, and also the control of various processes in plant growth and development, including shoot and root development, fruit and seed development, delay of senescence, the transduction of nutritional signals, as well as a role in response to both abiotic and biotic stress (Werner and Schmülling, 2009). Cytokinin homeostasis is maintained through biosynthesis, activation, degradation and conjugation of the bioactive molecules. Cytokinins exist as nucleotide, nucleoside and nucleobase forms (Werner and Schmulling, 2009). The nucleobase forms (*trans*- and *cis*-zeatin (Z), and isopentenyl adenine (iP) are considered to be the active forms that bind to cytokinin receptors (Lomin et al. 2015). Transport of Z-type cytokinins via the transpiration stream and iP-types via the phloem is considered to provide root-to-shoot-to-root communication (Kudo et al. 2010).

The cytokinins are usually grouped into three categories: the isoprenoid cytokinins, exemplified by zeatin and isopentenyl adenine, are the most abundant type; the naturally occurring adenine derivatives with aromatic substituents, the topolins, are considered less abundant; and the highly active, synthetic diphenylureas, such as CPPU (4-chlorfenuron), are the third category (Mok and Mok 2001).

At the molecular level, cytokinin homeostasis is maintained by enzymes for cytokinin biosynthesis (isopentenyl transferase; IPT), activation (LOG), cytokinin degradation (cytokinin oxidase/dehydrogenase; CKX), reversible inactivation through conjugation by zeatin *O*-glucosyl transferases (ZOG), and reactivation by ß-glucosidase (GLU) (e.g. Song et al. 2012). These genes exist in multi-gene families. For example, *Arabidopsis* has nine *IPT* genes, seven *CKX* genes and three ZOG and GLU genes identified and functionally verified. Their orthologues have also been annotated and/or functionally verified in rice and maize. The individual gene family members are spatially and temporally differentially expressed (e.g. Miyawaki et al. 2004; Werner et al. 2003; Song et al. 2012; 2015).

## Seed development

Seed development begins with double fertilisation which leads to the development of the embryo and endosperm. However, the progress of development differs between monocots and dicots: in monocots the endosperm constitutes the major part of the mature seed, whereas in many eudicots the endosperm grows rapidly initially but is eventually consumed by the developing embryo, which occupies most of the mature seed (Sundaresan, 2005). The seed coat arises from maternal integument tissue whereas both the triploid endosperm and diploid embryo are filial tissue. In both monocot and dicot, the number of cells determined during the phase of free nuclear divisions in the developing syncytial endosperm is a determinant of final seed size, as the growth of the seed is primarily associated with the initial growth of the endosperm, and not with the later growth of the embryo (Sundaresan, 2005).

Seed development is often considered to occur in two distinct phases: the first, during which endosperm development, cell divisions, and embryo and cotyledon differentiation occurs, is often referred to as the morphogenesis phase; the second phase, referred to as maturation, includes embryo growth by expansion, the absorption of the endosperm by the embryo, and dry matter accumulation (Locascio et al. 2014).

**Cytokinins and seed development**

***Endogenous cytokinin changes correlate with the phase of cell division and establishment of sink size***

Following the identification of zeatin by Stuart Letham in *Zea mays* (Letham 1963), a tight positive correlation between cytokinin levels and the phase of cell division has been shown in developing fruits (e.g. Letham 1963; Letham and Williams, 1969; Lewis et al. 1996a) and seeds. Cereals (maize, wheat, rice, barley), have sharp, transiently elevated cytokinin levels immediately after anthesis (Morris et al. 1993). In wheat, for example, the changes in endogenous cytokinins occur during the phase of free nuclear division in the developing grain (Jameson et al. 1982; Bennett et al. 1973), the time when sink size is established. Similar conclusions were reported for maize (e.g. Brugière et al. 2008) and rice (Yang et al. 2003). In legumes, detailed GC-MS work on developing white lupin seed showed a high, transient peak of cytokinin in the liquid endosperm of developing seeds (Emery et al. 2000), There are suggestions, however, that the cytokinin may be involved in sink activity in addition to, or instead of, cell division (Emery et al. 2000; Brugière et al. 2008).

***Origin of the cytokinin in reproductive tissues***

Early work from the Letham lab tracked the movement and metabolism of cytokinin applied to the xylem of lupins and soybeans (Letham, 1994). It was clear from this work that, while adenosine applied to the xylem reached the developing embryo (Nooden and Letham, 1984), similarly applied cytokinin did not. While limited quantities reached the pod wall (Jameson et al. 1987) and even the seed coat (Singh et al. 1988), the cytokinin did not cross the apoplastic space between the seed coat and the embryo (Letham, 1994). Emery et al. (2000) estimated that the supply of cytokinin via phloem and xylem to developing fruit and seed of white lupin accounted for most of the cytokinin during early podset but could not account for the bulk of the cytokinin in the seed. Consequently, in contrast to the dependence of the flower/ovule on maternally supplied cytokinin, the developing legume embryo would appear dependent on the filial tissues for cytokinin biosynthesis (Singh et al. 1988, Emery et al. 2000).

***The developing seed is a site of cytokinin biosynthesis***

Early research attempting to show that seed tissue could biosynthesise cytokinin from applied substrates was unsuccessful (see Letham 1994). However, with knowledge that the supply of cytokinin entering seed tissues from maternal sources was limited, and in support of the endogenous measurements, analysis of gene expression shows expression of specific *IPT* gene family members in seed of both monocots and eudicots. For example, the rapid increase and almost equally rapid decrease in cytokinin post-anthesis in wheat (Jameson et al., 1983) is mirrored by the expression of specific *TaIPT* gene family members (Song et al. 2012). In maize, *ZmIPT2* expression occurred at the time when cytokinin levels were elevated, and cell division was occurring (Brugière et al. 2008).

In *Arabidopsis*, Day et al. (2008) showed, using laser-assisted micro-dissection of seed four days post-pollination, enrichment in genes associated with the cell cycle and with cytokinin biosynthesis and signal transduction in the syncytial endosperm. More recently, Belmonte et al. (2013) assessed gene expression in the sub-regions of *Arabidopsis* seeds during development and showed strong expression of both *IPT4* and *8* which was restricted to the chalazal endosperm during the morphogenesis phase of seed development. Most recently, Song et al. (2015 in press), monitored gene family members for both cytokinin biosynthesis and degradation in forage brassica, *Brassica napus. IPT* expression in the elongating silique and developing seeds indicated that these organs were capable of biosynthesising their own cytokinin.

***The problematic pleiotropic nature of the cytokinins***

It might seem logical to simply ectopically express an *IPT* gene to enhance the endogenous cytokinin levels within the plant. However, the cytokinins are involved in multiple facets of plant development in multiple organs, with contrasting effects on shoots and roots (Brenner and Schmülling, 2012). Constitutive over-expression of *IPT,* or the use of leaky promoters, has led to bushy plants with reduced or inhibited root growth (e.g. McKenzie et al. 1998 and references therein) and of no agronomic use.

***Cytokinin is limiting yield***

Two experimental examples support the contention that cytokinin is limiting to yield and that targeted increases in endogenous cytokinin might be of agronomic benefit. Ma et al. (1998) selected a chimeric vicilin-specific promoter that specified embryo-specific expression to drive expression of an *IPT* gene. The cytokinin levels were increased but only in the developing seeds and the transgenic tobacco plants were morphologically normal. Both cell division and dry weight of the vicilin-IPT seeds were enhanced (Ma et al. 2002). More recently, Aitkins et al. (2011) coupled an *IPT* gene to a flower-specific promoter, and increased the total number of pods formed (but did not increase seed size) in lupin.

***Enhancing yield by delaying senescence***

For many years it has been known that application of cytokinin or ectopic expression of *IPT* in leaves can delay senescence (Guo and Gan, 2014 and references therein). Guo and Gan (2014) summarise a wealth of experiments where an *IPT* gene has been linked to a promoter that is activated only as the plant begins to senesce – the *SAG-IPT* constructs. In tobacco, *SAG12-IPT* plants led to a 40% increase in seed yield under controlled conditions (Gan and Amasino, 1995). Under field conditions, increases in yield have been recorded for rice, peanut and tomato fruit (in Guo and Gan, 2014).

However, delaying senescence as a means to increase the longevity of the source can be problematic as this can establish competition between source and sink. This was shown clearly by Sýkorová et al. (2008) where the use of a *SAG-IPT* construct delayed senescence but had no impact on yield which was interpreted as the delayed senescence delaying the translocation of metabolites from leaves to developing grains (Sýkorová et al. 2008).

***Increased cytokinin levels are associated with increased cytokinin degradation***

A feature that needs to be considered when targeting an increase in cytokinin is the possibility of increasing cytokinin degradation, as the plant reacts to the increased cytokinin by activating homeostatic mechanisms. Key to this is cytokinin oxidase/dehydrogenase which catalyses the irreversible degradation of active cytokinin molecules (Werner et al. 2006). There are now numerous reports suggesting that whenever cytokinin levels are elevated by exogenous application of cytokinin or by ectopic expression of an *IPT* gene, that increased expression of *CKX* gene family members and /or CKX activity occurs (e.g. Motyka et al. 2003; Brugière et al. 2003), with Brugière et al. (2003) suggesting that endogenous cytokinin levels "dictate" *CKX1* expression in maize. Positive correlations have also been observed between expression of *IPT* and *CKX* gene family members in *Brassica rapa* (O'Keefe *et al.,* 2011), wheat (Song et al. 2012) and forage brassica, *B. napus* (Song et al. 2015).

***Targeting CKX***

The seminal work by Ashikari et al. (2005) on yield of rice, showed that cytokinins could be directly targeted by plant breeders. In naturally occurring rice cultivars, Ashikari et al. (2005) showed that a QTL for increased grain number was a gene coding for *CKX*. A yield increase of over 20% was measured in the loss-of-function cytokinin oxidase/dehydrogenase, *OsCKX2*, mutant. Ashikari et al. (2005) suggested that the expression of *OsCKX2* in inflorescence meristems regulated the cytokinin level and thus controlled the number of flowers. Additionally, they showed that transgenic rice over-expressing *OsCKX2* had reduced grain numbers, whereas transgenic rice with antisense *OsCKX2* cDNA had reduced expression of endogenous *OsCKX2* and developed more grains. Bartrina et al. (2011) recorded a 55% increase in yield in a double *CKX* mutant of *Arabidopsis* and barley lines expressing RNAi targeted to *HvCKX1* and *9* showed increased yield (Zalewski et al. 2014).

**Strategies for enhancing endogenous cytokinin to increase yield**

Identifying an appropriate target can be problematic as many crop species are allopolyploids, with a concomitant increase in the number of gene family members. For example, forage brassica (*Brassica napus* cv Greenland), not only has the A genome from *B. rapa* and the C genome from *B. oleraceace*, there has also been a whole genome triplication of the brassica genomes since divergence from *Arabidopsis* (Lysak et al. 2005), followed by genome shrinkage (Mun et al. 2009). Additionally, homoeologous gene silencing and/or differential expression has been documented in a number of polyploid species including bread wheat (e.g. Song et al. 2012) and forage brassica (Song et al. 2015).

However, once a target gene family member has been identified, various plant breeding strategies can be invoked which tend to separate into those not involving genetic modification (e.g. marker-assisted selection (MAS), TILLING (targeting induced local lesions in genomes) and EcoTILLING) and those using various genetic modification techniques such as RNAi and the recent targeted genome modification techniques (Gaj et al. 2013). While EcoTILLING seeks mutations in naturally occurring accessions or cultivars of crop species, TILLING relies on populations developed following standard mutagenesis techniques. Both then rely on a sensitive DNA screening-technique that identifies single base mutations in a target gene. In the case of cytokinins, it is likely that a mutated structural gene such as a *CKX* gene is more likely to be detected than an over-expressed *IPT* gene. Ashikari et al. (2005) showed that reduced function and non-functional *CKX* genes exist in cultivars of rice.

RNA interference (RNAi) appears to have replaced anti-sense technology. An example of the use of anti-sense technology is described above for rice (Ashikari et al. 2005). The use of RNAi to enhance yield by knocking down specific *CKX* gene family members has been attempted in barley but not necessarily with consistent effects over time (Zalewski et al. 2014).

The most recent advances utilise sequence-specific nucleases (SSN) – techniques commonly referred to as "genome editing" (Gaj et al. 2013). An excellent review by Chen and Gao (2014) summarises the three approaches that utilise either zinc-finger nucleases (ZFNs), transcription activator-like nucleases (TALENS), or the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas-mediated RNA-guided DNA endonucleases. Their suggestion is that, in contrast to classical genetic engineering, the precise manipulation of genomes by SSN techniques may overcome some of the constraints associated with transgene-based plant breeding (Chen and Gao, 2014). Of the three techniques, the CRISPR/Cas9 technique is rapidly gaining a reputation as being relatively cheap and easy to implement with relatively low off-target activity (Belhaj et al. 2015). However, although the end-product is precise manipulation of a specific genome sequence, the underlying technology still requires an initial transformation event, and the resulting plant may still be considered to have been genetically modified in some jurisdictions.

At the time of writing this review I had not found any publications where the CRISPR technique had been applied to the regulation of *CKX* expression.

**Conclusion**

Cytokinin homeostasis within an organ is co-ordinately regulated by different multi-gene families whose individual gene family members provide precise control of organ development. Seed yield can be directly affected by disturbing the co-ordinate regulatory network, with CKX being a particular target of several research groups. To target CKX to seed production, it is necessary to identify the gene family members expressing specifically in processes leading up to and during seed development by elucidating their temporal expression patterns. Such information may then be applied in the identification of gene-specific, functionally-associated markers for MAS or for inducing and/or detecting valuable mutations using a TILLING strategy. Even the CRISPR technique will require such detailed knowledge. However, the techniques are now available to target the down-regulation of specific *CKX* gene family members, leading to the potential development of high performance commercial herbage seed production.

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# Climate change and herbage seed production: cause for concern?

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**Abstract**

The fact of climate change and its consequences on agricultural production has rightly received increasing attention. The Intergovernmental Panel on Climate Change in its fourth assessment report has projected change in all the climatic variables influencing plant growth, seed production and seed quality. Yet in the myriad of publications on climate change effects, very few have considered herbage seed production. The likely effects of climate change on herbage seed production and quality are reviewed, and possible mitigation strategies discussed.

**Key words:** elevated carbon dioxide; heat stress; mitigation strategies; seed quality; seed yield

# Un-realized seed yield potential: undersized seed and factors affecting seed loss

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**Abstract**

For herbage species, the gap between potential seed yield and actual seed yield is often large with the number of seed sites produced many times larger than the number of saleable seeds harvested. The causes of un-realized seed potential are varied and complex and include seed loss from undersized seed not making a saleable weight (20%), abortion (40%) and seed shattering both before and during harvest (20-40%). This is accentuated in species with an indeterminate flowering pattern. An extended period of flowering results in large differences in seed development, seed filling duration, rate of seed filling, seed quality and seed maturity. Unlike domesticated cereals, breeding programmes for herbage species such as ryegrass, clover and brassica, have concentrated on vegetative (forage) quality traits such as water-soluble carbohydrates (WSC), crude protein (CP), dry matter production and animal live weight gain. As a result, seed yield characteristics in conventional breeding programmes have received little attention. Seed production research has mostly been agronomic, focusing on crop management using a combination of inputs including nitrogen, plant growth regulators, closing date and irrigation to increase seed yields. The fundamental characteristics of species selected for herbage production are often in conflict with consistent and efficient seed production. This paper discusses the factors that limit realised seed production in herbage species. The issues highlighted are relevant to all herbage seed crops. Strategies and future research to reduce seed loss will be discussed.

# Irrigation and Trinexapac-ethyl Effects on Seed Yield in First-and Second-Year Red Clover Stands

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**Abstract**

Red clover (*Trifolium pretense* L.) is the most widely grown legume seed crop in Oregon. Previous work has shown that trinexapac-ethyl (TE) plant growth regulator (PGR) can increase seed yield of red clover under western Oregon conditions, as well as those of other production areas of the word. This study examined the effects of irrigation, TE, and potential interactions of irrigation and TE on seed yield and yield components in first- and second-year stands of red clover. Two plantings (2011 and 2012) were established near Corvallis, Oregon, USA and each were followed over a two-year period. Seven PGR treatments consisted of 140, 280, and 420 g ai $ha^{-1}$ TE applied at stem elongation (BBCH 32), at bud emergence (BBCH 50) and an untreated control. Approximately 100 mm of irrigation water was applied to irrigated plots at late bud emergence (BBCH 55) and was compared to a non-irrigated control. Averaged across first- and second-year stands, irrigation increased seed yield by 13% and seed weight by 5%. When applied at BBCH 32, the 140 g TE $ha^{-1}$ rate increased seed yields by 11% over the untreated control while higher rates (>280 g TE $ha^{-1}$) increased seed yield by an average of 18% in second-year stands. These seed yield increases were accompanied by an increase in inflorescences $m^{-2}$. Application of TE at BBCH 50 had mixed effects on seed yield: seed yield was modestly increased, not affected, or was reduced by TE in this application timing. Application of TE at both timings reduced seed weight, especially higher rates applied at BBCH 50. Seed number $m^{-2}$ was greatly increased by TE applications at BBCH 32 but not at BBCH 50. The results indicate that while both irrigation and TE can independently increase seed yield in red clover seed crops, there are no interactions between the two.

**Key words:** irrigation; plant growth regulator; seed yield; seed yield components; trinexapac-ethyl

# Spring defoliation dates on early flowering dryland perennial ryegrass in New Zealand

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**Abstract**

The effect of spring defoliation dates on seed yield of forage perennial ryegrass (*Lolium perenne)* was evaluated in three trials grown without irrigation; two with cv 'Commando' (head emergence Nui+1 day) and the other with cv 'Arrow' (Nui+7 day). For two trials there were five defoliation dates; for 'Commando' the dates were from the 12 September to 24 October; and for 'Arrow' no grazing to 20 October; while the second 'Commando' trial had three closing dates from 25 September to 15 October with either nil or 400 g/ha trinexapac-ethyl (TE) plant growth regulator (PGR). The optimum final defoliation dates were the 2 October for 'Commando' and between the 20 September and the 5 October for 'Arrow'. Closing both too early or late depressed seed yield. Commando seed yields increased from 2360 to 3010 kg/ha when final spring defoliation was delayed from 12 September to 2 October with the benefit of extra forage for livestock. TE delayed the optimum closing date from the 25 September (nil TE) to between 5 and 15 October (400 g TE/ha). Arrow seed yields were increased from 1650 kg/ha (no spring defoliation) to 2270 kg/ha when final defoliation was on the 5$^{th}$ October.

**Key words:** closing; defoliation; *Lolium*; seed yield; spring management

# Use of Precision Agriculture Tools in Conducting a large-scale Field Trial in Forage Kale Seed Production

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**Abstract**

Forage kale (*Brassica oleracea*) seed crops carry very high bulk that limits ability to conduct small plot trials with plot harvest equipment. Plant growth regulators (PGR) programs have potential to reduce this crop bulk and improve seed yields. A large plot trial was established in a grower's field using spray tramlines (36 m wide x 450 m long) and harvester yield monitors to compare three PGR treatments with 3 replications. Estimated yields were compared to physically measured plot yields. A treatment utilising paclobutrazol applied twice during stem elongation provided a significant 10% increase in seed yield over the standard treatment (1890 kg/ha cleaned seed) and a chlormequat program was just 3% increase over the control.

Harvester-estimated seed yields were slightly lower, but within 5% of measured yields and showed the same levels of significance between the treatments. The simple technique of replicating plots using GPS and yield monitors can be readily utilised by farmers and researchers to assess agronomic techniques where small plot trials have limited value.

**Key words:** forage kale; PGR

# Thoughts about a new nitrogen regulation system in Denmark

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**Abstract**

The current Danish nitrogen (N) regulation system is based on a general N consumption model. This N regulation system will likely be replaced by one of two regulation systems in the near future. The purpose of the new regulation system is to increase yield and quality of the harvested crops while further minimizing environmental impact. In practice this means that more N can be applied on environmentally robust areas, where a robust area is defined as an area with a low N leaching potential and/or is not close to a water catchment area. This new regulation system could be defined as a 'differentiated N regulation system' based on a 'top down' management or 'bottom up' leaching model.

**Key words:** perennial ryegrass; red fescue; seed production

# The potential impact and opportunities from nutrient management regulation on the New Zealand herbage seed industry

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**Abstract**

The New Zealand herbage seed growers will need to ensure their farm management practices do not result in nutrient losses that exceed catchment based limits. Unlike many countries New Zealand has adopted limits for nutrient loss based on outputs, with no limit on the application (input) of nutrients. As it is not currently possible to measure outputs, management within the limits will rely on the use of models which accurately estimate losses for a farm system averaged over a reasonable time frame. Herbage seed crops offer both opportunities and problems to growers when minimising nutrient losses. Excellent research has provided growers with good information on the N requirements of grass seed crops, thus minimising the risk of over application of nitrogen. However, it will be important to understand potential nitrogen losses when establishing the next crop after grass seed crops and more importantly after legume seed crops. The Overseer® model is likely to be used to estimate nitrogen losses from the farm system and its applicability to herbage seed cropping systems is discussed.

# *Dactylis glomerata* seed yield response to Plant Growth Regulators's

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**Abstract**

The seed yield response of orchard grass (cocksfoot *Dactylis glomerata* L.) to stem shortening plant growth regulators (PGRs) chlormequat chloride (CCC) and trinexapac-ethyl (TE) were evaluated in eight field trials; five irrigated and three non-irrigated. Across all trials and treatments the average seed yield increase was 53% resulting in an extra 290 kg/ha. In irrigated trials the average response to PGRs was 68%, resulting in an extra 380 kg/ha of seed, compared with an average 27% response in non-irrigated trials. A mixture of CCC+TE (1500+200 g/ha) applied at Zadoks growth stage (GS) 32 or split between GS 32 and GS 37—39 provided the largest and most consistent seed yield response. Reduced lodging was a factor in one trial only. In all trials, reduced stem length was associated with increased seed yield even in the absence of lodging. Seed yields increased at 12.5 kg/ha for every cm reduction in stem length from 130 cm (untreated) to 76 cm.

**Key words**: cocksfoot; orchard grass; trinexapac-ethyl

# Effects of Tillage and Establishment Systems on Annual Ryegrass Seed Crops

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**Abstract**

Annual ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] seed crops have been produced on some Oregon farms continuously for decades without rotation of crops or farming practices. Our objective was to determine the effects of tillage and establishment systems (systems) on Gulf annual ryegrass seed crops over a 9-year period. Six systems were employed in the study: (i) continuous conventional tillage (CT), (ii) continuous no-till (NT), (iii) NT/CT cycle alternate year tillage (NT/CT), (iv) volunteer/CT cycle alternate year tillage (Vol/CT), (v) burn and NT/CT cycle alternate year tillage (Burn + NT/CT), (vi) volunteer/NT/CT cycle with tillage every 3rd year (Vol/NT/CT). Environment x system interaction effects governed seed production characteristics. Three types of yield environments were observed during the 9 years: high, intermediate, and low. High yield environments had higher temperatures (+1.2°C) and lower precipitation (-48 mm) in April-June than in low or intermediate yield environments. Across environments, yields were greatest with Burn + NT/CT, CT, and Vol/NT/CT and lowest with NT. Stability analysis revealed that Burn + NT/CT, CT, and Vol/NT/CT systems produced up to 40% greater yields than the mean of all systems in low yield environments. Yield variation among systems was lower in high yield than in low yield environments. Increasing tillage frequency from zero in NT to once every other year in NT/CT boosted yields so that they were equivalent to CT. Yield differences among systems were primarily attributable to seed number. Moderate tillage frequency with disturbance of crop residues and occasional residue removal are required to produce the best long-term seed yields in annual ryegrass.

**Key words**: seed yield; stability analysis; yield components

# Planting Density and Nitrogen Application Affects *Cleistogenes songorica* Seed Yield

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**Abstract**

Native grasses play an important role in the sustainable development of grassland animal husbandry, land protection and the lawn industry. However, with seed yields often low and variable, it is necessary to develop techniques for increasing seed production in these species. From 2007 to 2012, we evaluated nine planting densities (5, 10, 15, 20, 25, 30, 35, 40 and 50 plants $m^{-2}$) on seed yield for a drought tolerant grass species, *Cleistogenes songorica*. From 2013 to 2014, we evaluated three N timing with three N rates: only spring (50, 100, and 150 kg $ha^{-1}$), only autumn (50, 100, and 150 kg $ha^{-1}$), spring and autumn (50+100, 75+75, and 100+50 kg $ha^{-1}$) on seed yield for *C. songorica*. The optimum plant density was 30 plants $m^{-2}$, with a mean seed yield of 500 kg $ha^{-1}$ over the 6 yr. In 2013, the highest seed yield was obtained at 100 kg $ha^{-1}$ of N only applied in spring, with more than twice the seed yield than no N application. In 2014, the highest seed yield was obtained at 75+75 kg $ha^{-1}$ of N applied in spring and autumn, with more than 13 times the seed yield than no N application. Applying more N in autumn resulted in more fertile tillers. These results suggest that 30 plants $m^{-2}$, 75 kg $ha^{-1}$of N applied in autumn and 75 kg $ha^{-1}$applied the following spring are suitable for seed production of *C. songorica*. Optimizing seed harvest method for *C. songorica* seed production is also discussed base on the preliminary investigation conducted in 2014.

## Boron Effects on Red Clover Seed Production and Quality

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**Abstract**

Boron (B) is a critical micronutrient for many legume crop species, including clovers grown for seed. B is required for reproduction and a deficiency can adversely affect the formation of terminal growing points and flowering. Soils in western Oregon, USA are often B deficient, less than 1 ppm, where red clover (Trifolium pretense L.) seed crops are commonly grown. This two year study was conducted on a Woodburn silt loam with a pre-plant soil test level of 0.4 ppm. Five rate and timing combinations were used to determine the effect of B fertilizer on red clover seed yield and quality. Treatments consisted of 1.1 kg B ha-1 soil applied in fall, 2.2 kg B ha-1 a soil applied in fall, 1.1 kg B ha-1 foliar applied in summer at bud emergence (BBCH 50), 2.2 kg B ha-1 split among the two timings, and an untreated control. Treatments applied in 2012 were repeated on the same plots in 2013. In both years, seed yield, seed weight and total above ground biomass were measured and B tissue concentration was measured prior to B application, at peak bloom (BBCH 65), and one to three days prior to swathing. In 2013, seed quality analyses were conducted on clean seed samples to determine seed viability and vigor. Boron fertilizer increased plant tissue B concentrations by 66% in 2012 and 33% in 2013 when applied to foliage at BBCH 50. There were no differences in seed yield, seed weight or percent cleanout between B applications rates or application timings. There were no differences among treatments in any of the seed quality analyses. The results of this study suggest that red clover seed crops are not adversely affected by B soil test levels of 0.4 ppm as previously thought.

## Grass seed crops soil moisture utilisation

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**Abstract**

Denmark annually produces grass seed from an expansive area. However, environmental restrictions results in limited availability to irrigation. Understanding soil moisture utilisation in perennial ryegrass (*Lolium perenne* L.) and red fescue (*Festuca rubra* L.) will be a valuable tool for managing agronomic inputs more effectively. Soil moisture was measured using 'Time Domain Reflectometry' (TDR) like devices at 6 soil depths to cover 100cm of soil profile. For the year 2014, soil moisture limitations were observed for both crops during the growing season. Soil moisture measurements aided agronomic management inputs, as well as providing a better understanding of these.

**Key words:** Denmark, area, environmental restrictions, irrigation

# Pre-harvest foliar application of calcium influences seed yield and yield components of alfalfa

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**Abstract**

In order to investigate the influence of pre-harvest foliar application of calcium on seed yield and yield components of alfalfa (*Medicago sativa* L.), a field experiment was carried out with a randomized complete block design during 2009 to 2012. Calcium chloride in five levels (0, 0. 2‰, 0.4‰, 0.6‰ and 0.8‰) were applied as foliar sprays at the start of flowering and full flowering, respectively and data were recorded for seed yield and yield components including racemes per square meter, flowers per raceme, pods per raceme, and seeds per pod. Results indicated that the year, calcium application and the interaction of year and calcium application affected significantly on seed yield and all yield components except the number of seeds per pod. The highest seed yield was obtained at 0.4‰ concentration (585.41 kg $ha^{-1}$) in the 2012 experiment year (746.82 kg $ha^{-1}$). Additionally, the number of flowers per raceme (r=0.557**) and the number of pods per raceme (r=0.613**) was significantly and positively correlated with alfalfa seed yield, respectively. Path analysis depicted that pods per raceme had positive and highest-direct effect (pc=0.596**) on the seed yield while racemes per square meter had negative direct effect on yield (pc=-0.223).

**Key words:** alfalfa, calcium, climatic conditions, seed yield components

## Preliminary evaluation of *Stipa bungeana* for the turf use purpose

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**Abstract**

*Stipa bungeana*, perennial grass, is widely distributed on the Losses Plateau. It is very drought tolerance with fine leaf, and thus was proposed as a potential turf grass in the semi-arid and arid environment. This study evaluated turf quality of *S. bungeana* established at different densities with *Festuca arundinacea* as control. The study was conducted in Yuzhong experimental station (35º57′N, 104º10′E, a.s.l. 1720 m) of Lanzhou University from 2013 to 2014. Sowing densities of *S. bungeana* were 10000, 15000, and 20000 plants/m$^2$, and *Festuca arundinacea* 15000 plants/m$^2$. Index of plant density, uniformity, colour, texture and elasticity were measured every year. The results showed that 1) In the established year, *S. bungeana* had a significant higher density ($P<0.05$) than *F. arundinacea*, and the texture of *S. bungeana* was better than that of *F. arundinacea* ($P<0.05$). However, the uniformity of *F. arundinacea* was better than *S. bungeana*. Colour of *F. arundinacea* was greener or darker than *S. bungeana* ($P<0.05$). Elasticity of these four treatments had no significant ($P<0.05$). 2) Sowing density of 20000 plants/m$^2$ of *S. bungeana* performed the best in the established year. 3) In the second year, *F. arundinacea* was basically dead after winter, indicating that the cold resistance capability of *S. bungeana* were stronger than *F. arundinacea*. In the second year, sowing density of 10000 plants/m$^2$ of *S. bungeana* performed better than the other two sowing density. Meanwhile, study on the effect of plant density on the seed production of *Stipa bungeana* was also conducted. The results indicated that between-row spacing (30 cm, 40 cm and 50 cm) had no significant effect on actual seed yield of *S. bungeana* in the first year, ranking as 50 cm (10.20 g/m$^2$) ＞30 cm (10.04 g/m$^2$) ＞40 cm (9.56 g/m$^2$). Because the influence of density on seed yield and yield components may vary with years, continual observe on the performance of seed production in the following years to confirm our results is necessary.

# Siberian wildrye seed weight and seed number between different source-sink ratios under variable nitrogen availability

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**Abstract**

Seed weight and seed number play a great role in field crop seed production. However seed weight and seed number showed a competitive relationship under limited resource in grass seed production. Siberian wildrye (Elymus sibiricus L.), a tall-growing, perennial bunchgrass, is usually utilized to build rangeland and recover degenerated grassland for its drought and cold tolerance. In order to investigate the Siberian wildrye difference in average seed weight and seed number between different source-sink ratio under variable nitrogen availability, a spilt plot experiment with four replications was carried out from 2013 to 2014 at the grassland research station of China Agricultural University located in Yuershan, Hebei province, northern China. The main plots were nitrogen applications (0, 90,180 kg N $ha^{-1}$) and sub plots were source-sink ratio (half spike by hand trimming from the upper half of the spikes after anthesis and unaltered spike). Average seed weight and seed setting rate of spikelets were significantly increased by trimming spike and nitrogen application, and the florets per spikelet was also significantly enhanced with nitrogen application rate increased, but no difference was found between the control and trimmed spike. The result indicated that improving the source of Siberian wildrye could increase the average seed weight and seed number. Our insights provided in this report are helpful for the comprehension of grass seed production and grass seed breeding.

# Modelling Analysis to Improve Seed Yield of Western Wheatgrass (*Elytrigia smithii* (Rydb) Nevski)

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**Abstract**

This study examined the key yield component for increase seed yield through field trials designed for increasing the seed yield and the efficiency in breeding on Western Wheatgrass.

**Methods:** Using 5 groups of multi-factor orthogonal field experimental designed blocks in Jiuquan (39° 37′ N, 98° 30′ E), Gansu province, northwest of China, from anthesis to seed harvest, each one block of the yield components: fertile tillers/m$^2$ ($Y_1$), spikelets/fertile tillers ($Y_2$), florets/spikelet ($Y_3$), seed numbers/spikelet ($Y_4$), seed weight ($Y_5$) and seed yield (Z) were determined by hand with big sample size in field in succesive three years, and statistically investigated the correlations of Z and $Y_1$ to $Y_5$ through path coefficient analysis and ridge regression analysis.

**Results**: $Y_1$, $Y_4$, $Y_3$ and $Y_2$ were significantly ($P<0.001$) correlated with the seed yield whereas $Y_2$ were not significant correlated with $Y_3$, $Y_4$ and $Y_5$ by Peason correlation analyses. The effect of yield components on seed yield was in order of contribution as following: $Y_1 > Y_4 > Y_3 > Y_2 > Y_5$ ; Increasing $Y_1$ is the most productive on the seed yield in the components, then is the $Y_4$ and $Y_3$. The model of seed yield with its 5 components was founded, whereas the total effects (directs plus indirects) of the components were positively contributed to the seed yield by path analyses..

**Conclusion**: $Y_1$ and $Y_4$ is most important yield components for improving seed yield, and $Y_5$ the least. All of ridge regression coefficients are > 0, that theoretically means increasing any one of yield component ($Y_1$ to $Y_5$) will increase seed yield. $Y_1$ was the major component

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presenting the most important and effective effect in the 5 components in the plant seed production. Therefore, selection for high seed yield through direct selection for large $Y_1$, $Y_4$ and $Y_3$ would be effective for breeding in the grass.

# Effect of some agronomic factors on seeding emergence of *Artemisia sphaerocephala*

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**Abstract**

Seedling emergence is an important stage in the establishment of a plant species, and is the stage in the plant's life cycle that is most sensitive to environmental conditions. In order to determine the best agronomic management practices for optimal seeding emergence for *Artemisia sphaerocephala*, two field experiments were conducted under two sand burial depth treatments (0.5cm and 1.5cm) and two irrigation intervals (7 days and 14 days). In addition, the effect of three different sowing dates (13, 20, and 27 July, 2014) and three seeding rates (2, 4, and 6 kg/ha) were also determined in one of the experiments. Results showed that emergence percentage decreased significantly with increasing sand burial depth and irrigation intervals, whereas emergence percentage showed a decline with increasing seeding rates, regardless of sowing date.

**Key words:** *Artemisia sphaerocephala*; irrigation regime; sand burial; seeding rate; sowing date

# Effects of Growth Regulator on Seed Production of Italian Ryegrass

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**Abstract**

Italian ryegrass（*Lolium multiflorum* Lam）usage in China is increasing and the need for seed is increasing. But lodging of ryegrass during seed fill is a serious issue in its principal production area of coastal area of Jiangsu Province, resulting in reduced seed yield and also hindering mechanical harvest. Three growth regulators, trinexapac-ethyl (TE), paclobutrazol (PB) and maize roborant (MR) were used at the jointing stage of Italian grass (cv 'Changjiang 2') to observe the effects on growth and seed yield. The experiment was carried out in Linhai farm of Yancheng city in Jiangsu Province of China during October, 2010 to May 2011. The concentrations of TE were 141, 282, 423 and 564 g/ha, PB 50, 100, 200, 400 g/ha, and MR 0.855, 1.71, 2.56, 3.42L/ha, respectively.

Plant height was reduced 15% and there was no plant lodging with 564g TE/ha application. TE shortend spike length, increased kernel number per spike and spike number per plant significantly. With the application rate of TE increasing, seed yield was increased and lodging rate was reduced, but the seed yield began to decline at the concentration 564g/ha. Treatment of 423g/ha effectively prevent plant lodging, increased kernel number and seed yield.

Paclobutrazol treatment promoted plant tillering and increase effective panicles 42% at 400g/ha treatment compared with control. However, the duration of PB effect was short. With application times increasing, culm length of Italian ryegrass became shorter and stem diameter of the second section increased. The effect was enhanced with an increased rate of PB.

Low concentration treatment of MR increased the effective panicle number and prevented lodging. MR maize roborant at 1.71 L/ha increased seed yield by 41.4% compared with control, but at caused seed abortion and reduce seed yield.

The results indicated that growth regulators could suppress culm length, and increase the number of vascular bundles in stem, which might be the reason why the plant growth regulator could effectively prevent plant lodging. After TE treatment of the lodging rate decreased and seed yield was higher than those of PB and MR treatments, and the highest theoretical average seed yield was up to 7400 kg/ha, increased by 42% compared with control.

**Key words:** Italian ryegrass; lodging; growth regulator; seed production

## Organic red clover seed production

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**Abstract**

Red clover (*Trifolium pratense* L.) was domesticated in Spain around 1000 AD and, from there, it was introduced for use as forage 600 years later in Northern European. Red clover was grown for its beneficial properties and in many regions red clover represented 30 to 50% of the crop rotation. In Denmark grain production doubled during this period; however with the introduction of artificial fertilizer the production of red clover rapidly declined.

Currently, there is increasing interest for red clover for forage production due to its high productivity, high quality and drought tolerance. Further there is a growing interest to use red clover as a bioenergy crop.

Red clover is an outcrossing, insect-pollinated species. It has a long corolla tube, and requires long-tongued bumblebees for effective pollination. In agricultural areas with intensive cropping systems, the number of bumblebees and other native pollinators are declining due to a lack of nesting sites. Furthermore, in Denmark a large proportion of the long-tongued bumblebees are threatened species.

In Denmark the area of red clover seed production is steadily declining; while the average seed yield has not improved. Conventional seed producers are losing interest in red clover; however, organic seed producers see red clover as very important seed crop.

Organic seed producers will cut their crop in spring in order to control weeds and obtain high protein forage, which many sell to organic dairy farmers. However, the timing of cutting risks negatively affecting seed yields, depending primarily on the soil moisture at the time of cutting.

Aarhus University has initiated a new research project with the aim of increasing seed yield of red clover in both conventional and organic seed producing systems. An important part of the project is determining the pollination of red clover. Following, advanced knowledge dissemination involving beekeepers, seed industry personal and academics will take place.

# Studies on harvesting methods for Seed Production of *Cleistogenes songorica*

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**Abstract**

*Cleistogenes songorica* is an important native grass of northwest China, which has very strong drought resistance. However seed harvesting of *C. songorica* is difficult because its small size seeds are tightly enclosed in leaf sheaths. Therefore the aim of present study was to find out the appropriate method for *C. songorica* seed harvest on which time spent and seed lost during processing are both acceptable. The main methods included rubbing with hand、beating with stick and grinding with roller. The roller method was further divided into 3 different roller weights (50, 75, and 100kg) and 3 grinding time treatments (10, 20, 30 minutes). The different weight of roller was obtained by adding different amount of water into the roller. There were total 11 treatments used and each of them was applied to the same weight of plant (1kg). The hand rubbing was used as control in which the time spent for complete harvesting 1 kg plant was 155 min. The 50 min needed by using stick beating and 15% of seed lost in this treatment. Among all roller treatments, as the roller weight increased the seed yield loss increased, maximized yield lose reached to 50% in the highest weight (100kg) roller treatment. While 50kg roller grinding 20min got the minimum loss of seed yield (6.3% lost), so this method is recommended as an appropriate method of harvesting seed of *C. songorica* based on present study.

# The influence of moisture on lentil seeds germination and growth

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**Abstract**

Lentil (*Lens culinaris*) is an important dietary source of some developing countries. Moisture is an important factor to plant growth. The treatments in the study were 0.15, 0.20 and 0.25 ml per seed and enough water as control. Four days later, all of seeds could absorb enough water after adding enough water to plots. The results were analysed by Duncan analysis. The results showed that radicle growth was affected by moisture deficit ($p<0.05$) as well as numbers of lateral root, time of germ emergence and germ length. When the water was added to sufficient, the influence had a little improvement. Compared 0.2ml and 0.25ml, at last, they has no significant difference ($p>0.05$), although there had difference ($p<0.05$) before addition enough water. So in some moisture scope, addition enough water affect could be improvement. Moisture deficit can have a small impact to lentil seedling growth and may extend the time of growth, but whether it could influence reproductive growth needs to study.

# Pasture seed production in Tasmania, Australia, current issues and future opportunities

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**Abstract**

Tasmania has a small but specialised pasture seed industry that relies heavily on species and cultivars that have been developed to meet the forage production needs of local livestock industries and to fill important agro-ecological gaps, primarily in the cool temperate, low to medium (<700mm) rainfall zones.

By using an approach based on seed collection, introduction and evaluation, and working closely with industry, a number of new species and cultivars have been developed and registered for commercial use. Species of particular significance to Tasmania and other similar climatic regions would include a persistent winter active cocksfoot, *Dactylis glomerata* spp *hispanica* L., the world's first commercial cultivar of a deep tap-rooted perennial Talish clover, *Trifolium tumens* Steve. Ex M.B, a stoloniferous, long-lived red clover, *Trifolium pratense* L., a browsing perennial legume shrub, *Dorycnium hirsutum* L., and the only known commercially available coloured brome, *Bromus coloratus* Steud.

Considerable opportunity exists for expansion of pasture seed production in Tasmania based on its geographic location and the availability of cultivars of existing sown temperate and new alternate species. Current investment in irrigation by State and Federal Governments will further increase development of this opportunity.

**Key words:** forage; grass; legume; pasture; seed industry

# Effect of fertilizer application on seed production of *Brachiaria decumbens* basilisk'

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**Abstract**

In order to find out the optimal fertilization application of *Brachiaria decumbens* 'basilisk' for seed production in Yunnan province, the application of nitrogen，phosphate and potassium was chosen as the control variables with 15 treatments of N，P and K designed by using three factors quadratic general rotary combination, expecting to learn the effect on seed yield, 1000 seeds weight and seed viability under different fertilization application. The results showed that reasonable application of nitrogen，phosphate and potassium was the key technology in the 'basilisk' seed production. N application could improve the seed production performance of 'basilisk' at the lower P or lower K levels while which it could not increase seed production at the higher levels of P and K. K application could improve the 1000 seeds weight of 'basilisk' at the higher levels of N. Reasonable P application could improve the seed yield of 'basilisk' at the lower levels of K. However, at the higher K levels, excessive P application was no good for 'basilisk' seed production. Lower N application was observed to have a negative effect on seed viability of 'basilisk'. It was concluded that fertilizer rate of 250 kg urea, 350kg calcium superphosphate and 270kg potassium sulphate per hectare was the optimal fertilization application for'basilisk' seed production in Yunnan province.

**Key words**: *Brachiaria decumbens* 'basilisk'; Fertilization application; Seed yield

## Reproductive biology of Stylosanthes

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**Abstract**

Stylosanthes spp. is a dominant tropical pasture legumes and commercially used in a range of agricultural systems in many tropical and subtropical regions. Studying the basic mechanics of reproductive biology is very important in understanding how to maximize the yield and quality of the seed in stylo. In this study, the florescence, fertilization, embryo development and seed formation of stylo were studied by means of anatomic methods. The results show that almost all the flowers have accomplished self-pollinating at 6.00-7.00 Am while the petals are still closed. The flowers begin to bloom between 8.00- 9.00 Am with the keels are still closed, and meanwhile the pollen grains begin to germinate on the stigma. 3 to 4 hours after blooming, the stamen and pistils extent out of the keels and the pollen tubes grow down through the stylar canal. 5 to 6 hours after blooming, the fusion of sperm and egg cell occurs, and that of the sperm and polar nuclei occurs at the same time. After 22-23 h of anthesis the zygote started its first division to form a two-celled pro-embryo and experienced a series of development stages such as single-row celled, bat-like, globular, heart, torpedo, juvenile and spreading-growth stages. The development of the embryo was of the solanad type. After the enlarging stage, the embryo began the accumulation of starch and protein sequentially. The primary endosperm nucleus began to carry on mitosis 8h after anthesis .The development of endosperm was designated as nuclear type. The endosperm started to degenerate as the embryo grew up to fill almost the whole embryo sac and disintegrated completely but with a thin layer of remains left when the embryo matured. Seed mature 25-30d after blooming.

# Seed morphological characteristics and production technology of *Stylosanthes* species

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**Abstract**

The seed characteristics have a significant effect on seed yield and production technology. The seed and pod morphological characteristics of different *Stylosanthes* species were observed under stereo microscope. The results show that the morphology of seed and pods is different among different stylo species. The pods of both *S. hamata* and *S. subsericea* have the longest coiled beak, and *S. guianensis* and *S. grandifolia* have shortest beak. In some stylo species, pods have two articles, for example, *S. seabrana*, *S. macrocephala* and *S. fruticosa*. Seed testa have variable color, including back, light yellow, yellow, dark yellow, dark brown and red brown, and variable shape, including reniform (kidney), ovoid, spherical and irregular, among the main stylo species. *S. fruticosa* has the biggest seed with 3.85 g 1000-seed weight among the stylo species investigated.

Commercial stylo seed production is based mainly on four state farms in Hainan province. Stylo seed production is managed as an annual crop. Seedlings are produced in raised beds at a sowing rate of 37 - 50 kg/ha with high fertilization. At transplanting fertilizer is applied at a rate of 150 - 225 kg of superphosphate, and 4500-7000 kg of organic fertilizer/ha. When the stand is more than 85% ripe, the plants are cut. About 30% of the seeds are harvested from the cut plant, while the remaining 70% of the seeds are found on the ground and recovered by sweeping them plus the soil for processing. After drying and threshing, the material is sieved and the seeds are separated from soil and other trash. Finally, the seeds are placed in 25 kg bags and stored under low temperature. Average seed yield is about 375 kg/ha.

# Research Advances in Leguminous forage germplasm resource of *Tephrosia candida* in South China

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**Abstract**

*Tephrosia candida* DC. is a perennial legume forage shrub, native to SE Asia and India, tolerant to acid soils. It is also grown as a temporary shade crop, soil-improvement, reclamation, living fences, and as cover crop and for green manure. It fixes large amounts of atmospheric nitrogen and is used in agroforestry systems. As a pasture plant cattle sometimes avoid it, perhaps because at least part of the plant is poisonous. Our research was on its subtoxicity to mice and rabbits and proved it is safe forage for rabbits. The feeding experiment results showed, the crude protein content of *T. candida* in initial bloom stage is 18.3%. 0%, 25%, 50% and 75% of *T. candida* companied with *P. purpureum*; and 10%, 20%, 30%, 40% *T. candida* powder were fed to 60 rabbits as fodder for 90 days to study their production performance. As to fresh forage, rabbits feed with 100% *T. candida* had the highest of daily weight gain of 17.6± 1.8 g/day. The best performing group had 50% *T. candida* with 50% of *P. purpureum*, had not only the best palatability but also the best economic benefit that was 1.32 times than that of the control group fed *P. purpureum* only. The trials demonstrated that *T. candida* has potential to be used as both fresh and powder forage materials

**Key words**: *Tephrosia candida*; toxicity test; pathological change; daily weight gain; root nodule bacteria

# Productivity and karyotype analysis of hexaploid oat cv. Qingyan No.1

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## Abstract

Qingyan No.1 oat (*Avena sativa* L.) has been bred from progenies of cross-breeding pedigree between and Qingyongjiu No.146 (female parent) and Bayan No.3 (male parent) for several years, and its productivity were determined compared to Qingyin No.2 (Control 1), Jiayan No.2 (Control 2) and Qinghai No.444 (Control 3), which are widely planted native cultivars in Qinghai province.

At the agriculture region of Minhe county (1745 m a.s.l.), the grain yield of Qingyan No.1 (3068 kg/ha) was 7.8% and 10.9% higher, and the fresh hay yield at flowering (41 072 kg/ha) was 7.0% and 12.2% higher than that of the Control 1 and Control 2, respectively. At the semi-pastoral region of Huzhu, Ping'an, Datong and Huangzhong county with averaged altitude is 2585 m a.s.l., the mean grain yield of Qingyan No.1 (5075 kg/ha) was 14.1% and 14.4% higher, and the fresh hay yield at flowering (43877 kg/ha) was 13.7% and 14.1% higher than that of the Control 1 and Control 2, respectively. At the pastoral region of Haibei prefecture (3203 m a.s.l.), the grain yield of Qingyan No.1 (4 446.6 kg/ha) was 22.6% and 24% higher, and its dry hay yield at flowering (9325.5 kg/ha) was 7.9% and 10.7% higher than that of the Control 2 and Control 3, respectively. The nutrition results showed that, the crude protein and Calcium content in the seed of Qingyan No.1 were 160 mg/g and 1.44 mg/g, which were greatly improved compared to Control 2. Qingyan No.1 was proved to be a Hexaploid oat and its chromosome karyotype have been analyzed, including the number and the length ratio of the chromosome, the length of the short arm and the long arm, the arm ratio and the karyotype formula. All the results certified that Qingyan No.1 is a Hexaploid oat with early maturity, good quality and high-yielding characters, with highest grain yield occurred in the semi-pastoral region.

## Seed yield and hardness of *Sophora alopecuroides* L. in different habitats

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**Abstract**

*Sophora alopecuroides* L. is a perennial legume and medicinal plant. This study focused on the seed yield and hard seed rate of *S. alopecuroides* L. in different habitats. The results show that:

(1) Seed yields per unit area in arid habitats were higher than in moist habitats. The maximum seed yield was 1623 kg/ha recorded in the arid habitat of Left Banner and the least one was 92 kg/ha recorded in the moist habitat of the Yanchi County;

(2) *S. alopecuroides* had high rates of hard seeds. The hard seed rates of the populations from arid habitats were higher than of those from moist habitats.

**Key words:** hard seed rate; seed yield; *Sophora alopecuroides* L.

# Influence of irrigation regime on alfalfa seed production in the Yellow River irrigated region of Gansu, China

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**Abstract**

Soil moisture is an important factor affecting alfalfa seed production. In the Yellow River irrigated region, as precipitation is scarce, soil moisture is mainly from irrigation. A study was conducted to find a better irrigation regime in this region. Different irrigation regimes (3, 4, and 5 water applications) were assessed. Results showed that maximum seed yield was achieved when plants were irrigated 3 times with yields of 686 kg/ha and 890 kg/ha for 2013 and 2014, respectively. Thus, an irrigation regime consisting of 3 water applications was suggested for the local production of alfalfa seed.

**Key words**： Alfalfa; irrigation regime; seed yield

# Study on the seed yield components and seed yield of three *Melilotoides ruthenica* strains

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**Introduction** *M. ruthenica* is very important in degraded pastures improvement, artificial grassland establishment and ecological restoration, but the low seed production has limited its use.

**Methods** Three *M. ruthenica* strains (A, B, C) were bred by Institute of Grassland Research, CAAS. All seed yield components were measured in the field with 10 replicates in the 3rd year after planting. Actual seed yield (SY) measured at seed mature stage, potential and expressional seed yield (ESY) were estimated by seed yield components. ESY=effective branches/ ha*inflorescences/ effective branch*pods/inflorescence * seeds per pod * TKW/1000,

**Results** There were significant differences among *M. ruthenica* strains for SY components (Table 1). Strain C had the highest number of branches/m$^2$ and effective branches/m$^2$. Strain A had the highest number of inflorescences/effective branch and TKW. Strains B had the highest number of flowers/inflorescence, pods/inflorescence, seeds/ pod and lowest number of ovules/pod.

| Seed yield (kg/ha) | Strains | | |
|---|---|---|---|
| | A | B | C |
| Potential | 13822 | 8090 | 11908 |
| Expressional | 3277 | 4868 | 3813 |
| Actual | 154 | 168 | 173 |
| Ratio Actual potential seed Yield | 0.011 | 0.021 | 0.015 |

**Conclusions**

The result of my research show the dropping of flowers is one of the primary causes of the large drop between potential and actual SY. Another important cause is that part of the ovules could not develop into seeds. The actual seed production had significant positive correlation with potential seed production, expressional seed production and TKW. The actual seed production of the three *M. ruthenica* strains have large possibility to be improved by use of efficient cultivation technique.

**Keywords:** actual seed yield; potential seed yield; expressional seed yield

# Session 3. Endophyte utilization and pest control

# Vertically transmitted endophytes as biocontrol agents

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**Abstract**

Vertically (plant–to–seed) transmitted endophytes with biocontrol properties could circumvent the need for the application of exogenous pest and/or disease control methods in crop protection. The role of vertically transmitted endophytes as potential biocontrol agents has long been considered promising. However, with the exception of *Epichloë* spp. in forage grasses this promise has not been achieved. Endophyte associations in herbage species are reviewed, and seed production practices which may limit successful vertical endophyte transmission are highlighted.

**Key words:** bacterial endophytes; fungal endophytes; pest and disease control; seed crop management; seed transmitted

## Introduction

Endophytes are ubiquitous in vascular plants (Zabalgogeazcoa, 2008), with the evolutionary and ecological significance of these symbiotic relationships becoming increasingly apparent (Rodriguez and Rodman, 2008). Endophytes comprise a diverse range of microorganisms, primarily fungi (Rodriguez et al., 2009) and bacteria (Truyens et al., 2015). These organisms, for either all or part of their life cycles, colonise plant tissues internally, causing unapparent and asymptomatic infections (Wilson, 1995). The infections can be localised or systemic, through several relationships including latent saprobism, parasitism, commensalism or mutualism (Schultz and Boyle, 2005).

Historically, fungal endophyte symbioses have been mainly recognized for the benefits they confer on host plants, by protecting them from, or enhancing resistance to, insect herbivores (Schardl et al., 2004). It is now known, however, that endophytic fungi can enhance plant growth and yield, increase tolerance to environmental stresses including drought and heavy metal contamination, improve nutrient acquisition and recycling, and increase resistance to pathogens (Zabalgogeazcoa, 2008; Rodriguez et al., 2009; Truyens et al., 2015).

A large number of bacteria capable of endophytic colonization have been identified, and many are known to be endophytic in different hosts including crop plants (Truyens et al., 2015). Some are capable of enhancing plant growth, for example by fixing atmospheric nitrogen and solubilizing phosphate (Lopez et al., 2011), while others increase plant resistance to pathogens (Kloepper and Ryu, 2006).

Endophyte reproduction occurs through three different strategies, involving either horizontal (contagious) or vertical (plant-to-seed) transmission, or a combination of both (White, 1998).

Vertical transmission, in which the fitness of both partners is linked, is predicted to select for an increasingly mutualistic interaction (Tintjer et al., 2008). The advantage of vertical transmission from a biological control perspective is the establishment and persistence of the desired endophyte in the host plant and progeny (Wulff et al., 2002). By being vertically transmitted, endophytes can more readily assure their presence in new plants. Such bio-active endophytes could enable development of innate biological systems for host protection against plant pests and pathogens, circumventing the need for exogenous applications of agrichemicals in crop protection. This review summarises the literature on vertical transmission of fungal and bacterial endophytes in herbage species and examines their potential as biocontrol agents.

**Vertically transmitted fungal endophytes**

Sanchez Marquez et al. (2007), using morphological and molecular methods, identified 109 different fungal endophyte species from Spanish *Dactylis glomerata* plants. This assemblage consisted of grass-specific as well as generalist species, and the number of species found was similar to that reported for other grasses (Zabalgogeazcoa, 2008). Whether any of these fungi are vertically transmitted or have biocontrol properties was not reported, however. Apart from the *Epichloë* endophytes of grasses (*E*-endophytes), studies of vertically transmitted endophytes are scarce (Gallery et al., 2007).

Endophytic fungi have been characterized as Clavicipitaceous and Non-clavicipitaceous (Rodriguez et al., 2009, Table 1). The former (Class I endophytes, Table 1) include *Balansia* spp., *Epichloë* spp. and *Claviceps* spp. (Bacon and White, 2000) and are limited to cool-and warm-season grasses in the Poaceae. Those that are vertically transmitted colonise the scutellum and embryonic axis of host seeds.

**Table 1.** Symbiotic criteria used to characterize classes of fungal endophytes (Rodriguez et al., 2009).

| | Clavicipitaceous | Non clavicipitaceous | | |
|---|---|---|---|---|
| **Criteria** | **Class 1** | **Class 2** | **Class 3** | **Class 4** |
| Host range | Narrow | Broad | Broad | Broad |
| Tissue(s) colonized | Shoot and rhizome | Shoot, root, rhizome | Shoot | Root |
| *In planta* colonization | Extensive | Extensive | Limited | Extensive |
| *In planta* biodiversity | Low | Low | High | Unknown |
| Transmission | Vertical and horizontal | Vertical and horizontal | Horizontal | Horizontal |
| Fitness benefits[1] | NHA | NHA and HA | NHA | NHA |

[1]NHA = non-habitat adapted (e.g. drought tolerance/growth enhancement); HA = habitat adapted (e.g. after selection pressures such as pH, temperature and salinity).

## 1. Vertically transmitted Clavicipitaceous endophytes

### *1.1 Invertebrate pest resistance*

The list of invertebrate pests deterred by *E*-endophyte infected grasses includes over 45 insect pests and 12 nematode species (Kuldau and Bacon, 2008). Secondary metabolites produced by the *E*-endophytes are strong insect feeding deterrents (Rodriguez et al., 2009). These include the alkaloids peramine, ergovaline and lolitrems, lolines, and epoxy-janthitrems (see Table 2). The advantages of wild-type *E*-endophytes in pasture grasses for insect deterrence were in many situations initially outweighed by the disadvantages of *E*-endophyte toxicity to livestock (Bacon et al., 1977). The only option for preventing livestock toxicoses was to eliminate the intake of the toxins lolitrem B and ergovaline. In the original European habitat of *Lolium* spp., plant with diverse strains of endophytes with different alkaloid profiles were collected and brought to New Zealand. From this material *E*-endophytes which produced the insect feeding deterrent alkaloids but not the mammalian toxins were identified, isolated and inoculated into *L. perenne.* In New Zealand these are known as "novel *E*-endophytes" (Easton et al., 2001), because there are artificial associations between the endophytes and hosts (see Table 2). The impact of these novel *E*-endophytes on New Zealand agriculture has been to allow more reliable pasture production as a result of reduced pest populations with minimal effects on livestock health (Easton and Fletcher, 2007). Approximately 80% of the seed for the New Zealand ryegrass endophyte market now contains novel *E*-endophytes (Milne, 2007). The development of these non-mammalian-toxic endophytes in pasture grasses has allowed a sustainable and environmentally acceptable improvement in animal health and production, their contribution being approximately $200 million per annum to the New Zealand economy (Johnson et al., 2013). As well as bioactivity and animal performance studies, the molecular biology of the *E*-endophytes has now been extensively studied (Fleetwood et al., 2008). The predicted genes for the synthesis of indole diterpenes, ergot alkaloids, lolines and peramine has been determined, so that the research focus can now shift to manipulating the known biosynthetic pathways and analysing secondary metabolite genes with as yet unknown products, some of which may confer additional protection to host plants against pests (Fleetwood et al., 2008).

### *1.2 Bird pests*

*E*-endophytes which produce high levels of alkaloids (especially ergovaline) also deter forage eating bird pests such as *Branta canadensis* (Canada geese). A *Festuca arundinacea* turf cultivar developed with an *E*-endophyte association (Avanex®) has been recently released in New Zealand for use at airports (to reduce the possibility of bird strikes), and for recreational turf areas (Pennell et al., 2010).

### *1.3 Disease resistance*

The presence of *E*-endophytes has been reported to increase host resistance to infection by pathogens (Christensen 1996; Tian *et al*., 2008; Panka et al., 2013a). For example Panka et al. (2013b) reported that *E*-endophyte infected *Festuca arundinacea* plants were more resistant to infection by *Rhizoctonia zeae* (see also Table 3). Plant defence responses to pathogen infection involve physiological and biochemical processes. Chemicals including several indole compounds, phenolic compounds and volatile organic compounds (VOCs) such as (Z)-3-hexen-1-yl acetate, (Z)-3-hoxenal, linalool and methyl salicylate have been associated with reductions in diseases on *Phleum pratense*, *L. perenne* and *F. arundinacea* (Panka et al., 2013a). The mechanism of enhanced disease resistance is not clear, but is most probably not based on direct inhibition of the pathogen through antibiosis or competition (Panka et al., 2013b). Rather, the presence of *E.* endophytes appears to allow the host to activate a defence reaction faster than in non-*E.* endophyte infected plants in response to a pathogen attack (Panka et al., 2013a; Wiewióra ete al., 2015).

**Table 2.** Alkaloid profiles of *E*-endophytes currently available in New Zealand

| ***E*-endophyte** | **Peramine** | **Ergovaline** | **Lolitrem B** | **Lolines** | **Epoxy-Janthitrem** |
|---|---|---|---|---|---|
| *L. perenne* | | | | | |
| Wild type[1] | √√√[3] | √√√ | √√√ | x[4] | x |
| AR1[2] | √√√ | X | x | X | x |
| AR37[2] | X | X | x | X | √√√√ |
| NEA22[2] | √√ | √ | √ | X | x |
| AR5[2] | √√√ | √√ | x | X | x |
| *F. arundinacea* | | | | | |
| Wild type[1] | √√ | √√√√ | x | √√√ | x |
| AR542/AR584[2] | √√ | X | x | √√√ | x |

[1]Also known as "common-toxic" (Johnson et al., 2013); [2]Novel endophytes; [3]production intensity; [4]not produced

**Table 3**. Positive effect of *E*-endophyte presence in host on plant resistance to fungal pathogens (adapted from Wiewióra et al., 2015)[1]

| **Host** | **Pathogen** |
|---|---|
| *Lolium perenne* | *Fusarium* spp., *Drechslera* spp., *Puccinia coronate* |
| *Festuca arundinacea* | *Puccinia coronata, Rhizoctonia* spp. |
| *Festuca pratensis* | *Drechslera* spp., *Puccinia coronata*, *Rhizoctonia* spp., *Sclerotinia* spp. |
| *Phleum pratense* | *Cladosporium phlei, Puccinia graminis* |

[1]These authors also report nil responses to other pathogens for these hosts, and negative responses for *Claviceps purpurea* and *Pythium* spp.

### *2. Non-clavicipitaceous endophytes*

Only Class 2 endophytes (Table 1) can be transmitted vertically; they are often passed from maternal plants via seed coats (Redman et al., 2002), which may be more by chance than a transmission strategy. This group of endophytes generally colonize the roots, stems and leaves of host plants, are rarely found in the rhizosphere, and typically have high infection frequencies (90-100%) in plants grown in high-stress habitats. Examples include *Phoma* spp., *Fusarium* spp. and *Cladosporium* spp. (Rodriguez et al., 2009).

Many Class 2 endophytes partially protect hosts against fungal pathogens, reflecting the possible production of secondary metabolites, fungal parasitism, or induction of systemic resistance exhibited by these species (Rodriguez et al., 2009). Similar to the response in Class 1-endophyte-colonized plants, upon exposure to virulent pathogens Class 2-endophyte-colonized plants activated their host defences more rapidly than those not containing endophyte (Redman et al., 1999). In the absence of pathogen exposure these host defences were not activated.

Class 2 endophytes generally allow host plants to better cope with abiotic stresses including heat, drought and salt stress. To date, none have been reported from herbage species, although *Fusarium culmorum* colonises all non-embryonic tissue of *Leymus mollis* (coastal dune grass), conferring salt tolerance (Rodriguez et al., 2009).

**Vertically transmitted bacterial endophytes**

Endophytic bacteria have been found in virtually every plant species studied to date (Roseblueth and Martinez-Romero, 2006). Most appear to originate from plant rhizospheres or phyllospheres, but many can be found in seed tissues. Truyens et al. (2015) reported that 131 bacterial genera from 25 different plant species have been confirmed as naturally occurring seed endophytes. These include *Bacillus, Pseudomonas, Paenibacillus, Micrococcus, Staphylococcus, Pantoea* and *Acinetobacter* spp. Bacterial endophytes can be transmitted from the vegetative parts of plant hosts to the seed endosperms via vascular connections, through gametes directly into embryos and endosperms, and as colonisers of reproductive plant meristems (Truyens et al., 2015).

Bacterial endophytes have been reported from seeds of *L. multiflorum* and *P. pratense* (Ikeda et al., 2006), *Panicum virgatum* (Gagne-Bourge et al., 2012) and *Agrostis capillaris* (Truyens et al., 2014). Ikeda et al. (2006) identified *Pseudomonas fluorescens* and *Curtobacterium plantarum* in seeds of both of the grass species they examined. *Pseudomonas fluorescens* has been described as a beneficial organism that contributes to plant protection (Haas and Keel, 2003). Gagne-Bourgue et al. (2012) confirmed vertical transmission in *P. virgatum* for *Bacillus subtilis*, *Microbacterium testaceum* and *Curtobacterium flaccumfaciens*. They also demonstrated the presence of toxins including lipopeptides and surfactins in culture filtrates of *B. subtilis* and *M. testaceum*. The production of toxins by these bacterial endophytes and their antimicrobial activities is well documented (Romero et al., 2011). Surfactins have strong activities against several plant pathogens (e.g. Vitullo et al., 2012). Other mechanisms include the production of siderophores which leads to competition with pathogens for iron, the production of VOCs, and the activities of quorum-sensing molecules which may participate in the interaction of plants with pathogens and symbionts by induction of plant gene expression (Truyens et al., 2015).

While many endophytic bacterial species have been isolated from seeds, for many demonstration of vertical transmission is lacking (Lopez-Velasco et al., 2013). Information on vertically transmitted bacterial endophytes in herbage grasses is as yet limited and there are no reports for herbage legumes, although bacterial endophytes have been reported from the roots of *Medicago* and *Trifolium* spp. (Dudeja et al., 2012).

**Factors affecting vertical transmission of *Epichloë* endophytes**

Imperfect transmission, where an endophyte is not vertically transmitted to all plant offspring, is common in natural ecosystems (Lewis et al., 1997). Endophyte loss can occur during all stages of host plant life cycles (e.g. from seeds to seedlings or from mature host plants to seeds), with the degree and type of loss varying between host and endophyte genera (Afkhami and Rudgers, 2008). This may be associated with mortality of endophytes in stored seeds (Ravel et al., 1997) or at other stages of the host lifecycles (Afkhami and Rudgers, 2008). With the exception of the *E*-endophytes, literature on the factors affecting vertical transmission of endophytes is sparse. The following sections therefore refer only to *E*-endophytes.

***(i) Genetic factors***

*E*-endophytes are host-specific, leading to host-adapted fungal races compatible with only certain host genotypes (Saikkonen et al., 2004). In agronomic systems, the limited genetic diversity of a small number of grass cultivars with the *E*-endophyte partners, combined with adequate resources (water, nutrients) tend to ensure persistence, as opposed to greater variability in transmission in wild-grass populations (Saikkonen et al., 2004). The complexity of the interactions between *E*-endophytes and their hosts (Saikkonen et al., 2010) is further illustrated by endophyte alkaloid levels which can vary 5 to 8-fold in concentration among genotypes of the same cultivar (Pennell et al., 2010). *E*-endophyte levels can differ among individual plants (Ball et al., 1995). Even within an individual plant, tillers can contain or be free from *E*-endophyte (Ju et al., 2006).

***(ii) Environmental factors***

For *E*-endophytes, hyphal mass and endophyte frequency vary in relation to environmental conditions (Mack and Rudgers, 2008), as indicated by fluctuations with the seasons (Rasmussen et al., 2007). *E*-endophyte presence in tillers tends to be greatest over the warm summer months (during seed maturation) and least in late winter/spring (during the onset of reproductive growth). The temperature required for growth of some *E*-endophytes may be more than 5$^{o}$C greater than that for plant growth (Ju et al., 2006).

It is possible that iron availability may also affect *E*-endophyte transmission. In grasses, iron acquisition is regulated by the secretion of grass siderophores into the soil. In *E*-endophyte infected grasses, the secretion of endophyte-derived siderophores into plant apoplasts is additionally required for iron uptake (Johnson et al., 2007). Fungal siderophores are necessary for maintaining iron homeostasis of the entire grass-endophyte association. Any loss in endophyte siderophore production results in infected plants that are no longer symbiotic, are stunted, and display symptoms of iron deficiency (Johnson et al., 2007).

***(iii) Seed crop management***

Nitrogen, while not affecting endophyte transmission, can reduce *E*-endophyte concentrations in seed (Stewart, 1986). Why this occurs is not known, but may be associated with reduced hyphal density in tillers (Norriss et al., 2009) because the application of nitrogen increases numbers of tillers per plant.

Certain fungicides used to control foliar diseases in seed crops may negatively affect *E*-endophytes. While Rolston et al. (2002) reported that the systemic fungicides then in use did not adversely affect *E*-endophyte survival, more recent research has demonstrated that triazole fungicides can result in poor *E*-endophyte transmission (M.P. Rolston, unpublished data).

***(iv) Seed harvesting***

Hill et al. (2005) demonstrated that *E*-endophytes enter developing seeds well before the seeds reach physiological maturity. These authors considered that harvesting methods which maintain seed viability also maintained *E*-endophyte viability. If seed harvest is delayed by poor, weather after crop swathing, however, *E*-endophyte viability loss can be 0.4% per day (Abel, 2010).

(***v) Seed drying and storage***

*E*-endophytes lose viability at a greater rate than seeds (Gundel et al., 2009). Seeds are sensitive to drying temperature, but *E*-endophytes are more so (Rolston et al., 1993). Rolston et al. (1986) showed that as long as seed drying temperature did not exceed 30°C, *E*-endophytes remained viable. Best storage recommendations to maintain *E*-endophyte viability are a seed moisture content of <11%, low temperature (<5°C) and low relative humidity (<50%) (Easton, 1999). Storage in these conditions can maintain *E*-endophyte viability for up to 15 years, but storage conditions which do not control temperature and humidity can kill *E*-endophytes in a matter of months (Rolston et al., 1986). Rolston and Agee (2007) described the quality control requirements to deliver *E*-endophyte associations to market specifications.

**Conclusions**

Hodgson et al. (2014) recently demonstrated that vertical transmission of fungal endophytes was widespread in forbs (including *Plantago lanceolata*), particularly for two endophyte species *Alternaria alternata* and *Cladosporium sphaerospermum*. The 109 fungal endophytic species reported by Sanchez Marquez et al. (2007) were from non-farmland *D. glomerata* populations, not sown pastures. Card et al. (2013) reported *E.* endophyte presence in non-domesticated species of *Hordeum*, yet their presence in modern barley cultivars has not been reported. Has this endophytic abundance in seeds of wild-type plant populations been adversely affected in modern herbage cultivars by plant breeding and seed production factors, particularly the use of agricultural chemicals and seed drying and storage? This requires further investigation.

It is likely that no plant species are devoid of naturally-occurring endophytes (Rosenblueth and Martinez-Romero, 2006), and endophytes with biocontrol properties are increasingly being reported. Is it therefore possible that vertically transmitted endophytes can provide innate biological control for herbage species? For the *E*-endophytes this is already occurring.

With this one exception, however, the potential for vertically transmitted endophytes to provide effective biocontrol cannot be realistically determined until the following questions have been thoroughly investigated:

- which non-pathogenic microorganisms occur on or within seeds of herbage species and are endophytic?
- if endophytic, do these organisms possess biocontrol activity against one or more important pests/pathogens of respective crops or do they have properties that may be detrimental to host plants?
- although they may be detected in seed, are the endophytes vertically transmitted and what are the the transmission rates?
- can a desirable vertically transmitted endophyte be successfully introduced into another host plant?
- will seed crop production practices allow endophyte survival and successful vertical transmission?
- can vertically transmitted endophytes present in non-cultivated ancestor plants be re-introduced into modern cultivars?

Future research in this emerging field must seek to answer these questions, and this new knowledge could be turned into practice for effective biocontrol in herbage species. We conclude that considerable advances in the benefits conferred to herbage crops by vertically transmitted endophytes will eventually occur.

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# The relationships between *Epichloë* endophyte and *Festuca* seeds germination and morphology

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**Abstract**

In the present study, endophyte and seed viability and seed morphology of 12 native *Festuca* spp. from west of China were evaluated. Six ecotypes of *F. sinensis* -endophyte association from Gansu and Sichuan were confirmed which had 100% endophyte viability in seeds indicating that endophytes were successfully transmitted by maternal host. One novel *F. nitidula*-endophyte association was firstly identified in China which had 83% endophyte viability in seeds. Endophytes exhibited different levels of viability which varied from 0-100% in seeds and 0-92% in seedlings. Compared with endophyte viability in seeds, the noticeable declines in seedlings were observed for all associations after seeds were stored at room temperature for 7 months which suggests importance of maintaining endophyte viability of these studied novel associations in optimal conditions for further study. These varied endophyte viability in seeds and seedling for different ecotypes revealed that endophyte viability was affected by host species or host ecotypes. For the indices of seed germination rates, seed length and width and 1000 seeds weight, there were significant difference ($P<0.05$) between different species or different ecotypes within the same species which exhibited the strong effects of host genetic background. There was no significant correlation between endophyte viability and these tested indices which indicated the presence of endophyte did not affect seed viability and morphology in natural or optimal conditions. However, endophyte significantly promoted host seeds germination under NaCl stress in the case of one ecotype of *F. sinensis*. These results suggest that the improvement effect from endophyte under stress conditions performed better than that under suitable conditions and the effects on seeds quality and viability depend on the complicated interactions between host genotypes, endophyte genotypes and environmental conditions. The more comprehensive evaluation of these novel *F. sinensis* -endophyte associations should be conducted to improve the understanding of endophyte effects in local environment.

**Key words**: Endophyte; *Festuca* spp.; germination; morphology; NaCl stress

## Introduction

*Epichloë* endophytes are thought to interact mutualistically with their host plant, mainly by providing the grass host with major fitness enhancements (Bush*, et al.*, 1997, Saikkonen*, et al.*, 2004, Siegel and Bush, 1997). The asexual stage of *Epichloë* life-cycle involves growth out of the embryo into the intercellular spaces within the emerging leaf tissue, predominantly in the basal stem regions, followed by colonisation of reproductive tillers and vertical seed-borne transmission to the succeeding generation(Schmidt and Christensen, 1999). The benefits conferred by an endophyte on the host grass include increased survival of the seed until germination, which in turn promotes endophyte survival(Clay, 1987, Novas*, et al.*, 2003). Clay (1987) demonstrated that seeds from infected perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*) plants exhibited higher levels of germination than comparable seeds from uninfected plants. However, such endophyte effects on seed germination have not been consistently observed in other studies, including those in perennial ryegrass (Rolston*, et al.*, 1986), Arizona fescue (*F. arizonica*) (Faeth*, et al.*, 2004, Neil*, et al.*, 2003), Italian ryegrass (*L. multiflorum*) (Gundel*, et al.*, 2006a), and drunken horse grass (*Achnatherum inebrians*) (Zhang and Nan, 2010). The inconsistent outcomes from these studies provide evidence for complicated interactions between the endophyte and the host under a range of environmental conditions(Cheplick, 1997, Cheplick, 1998, Clay, 1993, Marks*, et al.*, 1991), suggesting a requirement to assess viability of both seed and endophyte for each novel association.

*Festuca sinensis* is native to highly chilled and semi-arid regions of China(Nan and Li, 2000) and is an important perennial bunchgrass cool season. It has been reported that some *F. sinensis* ecotypes from Gansu Province infected with *Epichloë* endophyte which improved the host fitness (Peng*, et al.*, 2013). In the present study, the broad range of ecotypes from west of China were collected for novel endophyte discovery program as the excellent performance of *F. sinensis* endophyte associations. The viability of both seed and endophyte for each novel association need to be assessed because the endophyte effects vary with host and environment.

**Methods**

Seeds of 4 native *Festuca* spp. including 12 ecotypes (Table 1) were obtained from Institute of Grassland, Qinghai Academy of Animal Husbandry and Veterinary Sciences. The seeds were collected in summer of 2013 and despatched to Lanzhou university in February, 2014. The seeds were stored in -20℃ at College of Pastoral Agriculture Science and Technology, Lanzhou University until required.

The seeds were assessed for endophyte infections by aniline blue staining and microscopic examination(Nan, 1996a). Afterwards, the well filled, healthy-looking seeds were planting for further endophyte detection in seedlings. Plastic trays (30 cm×25 cm×8 cm) were filled with soil (commercial fine sandy soil) which had been sterilized in an oven at 130°C for 30 min. Trays were placed in a temperature controlled greenhouse (18°C-24°C) with natural illumination. They were watered as required. Two months after sowing, the endophyte infection status of seedlings was determined by microscopic examination of host leaf sheath pieces stained with aniline blue(Nan, 1996b).

One hundred seeds from each seed lot were counted manually and placed in a Petri dish (150 ×15 mm) containing two pieces of filter paper (Whatman D = 150mm). The seeds were distributed evenly and distilled water was added to keep the paper moist. Each seed lot was replicated four times, and each dish was incubated at a temperature-

controlled laboratory (18℃-24℃) under natural day lengths. Moisture was maintained through daily check, and after 14 days germinated seedlings were counted and recorded. The length and width of seeds were measured by a Vernier caliper (Mitutoyo Japan). Each seed lot was replicated 20 times. One hundred seeds from each seed lot were counted manually and weighed on an electronic balance (Sartorius, 2007 MP, Germany). This was repeated 8 times for each line and the weight of 1000 seeds was calculated using the following equation(Ellis*,* *et al.*, 1985):

1000 seeds weight = the mean of 100 seeds weight × 10

The endophyte negative and endophyte positive seeds from one ecotype of *F. sinensis* (Xiahe, Gansu) were employed to evaluated endophyte effects on seeds germination under NaCl stress. The method was same as above excepted with that the 6ml solution NaCl (0, 50, 150, 200 mmol/L) was added to set up the control and NaCl stress treatment. The dishes were incubated at 30℃ for 8 hours and 20℃ for 16 hours in total dark.

All of the data were analyzed and mean differences determined using one way ANOVA in SPSS (version 13.0, SPSS, Inc., Chicago, USA).

**Results and Discussion**

The endophyte viability in seeds and seedling of total 12 ecotypes of *Festuca* spp. were shown in Table 1. The results showed that all of the 6 ecotypes of *F. sinensis* had 100% endophyte infection rate in seeds which indicated that *F. sinensis* formed natural stable associations with *Epichloë* endophyte and endophytes were successfully transmitted by maternal host. Only 1 ecotype of *F. kirilowii* had low very endophyte infection of 4% and the other ecotype of *F. kirilowii* was endophyte free. The 3 ecotypes of *F. kryloviana* had different endophyte infection rates in seeds which varied from 0% to 46%. *F. nitidula* had 83% endophyte infection in seeds which was firstly identified in China. Endophyte viability in *F. sinensis* seedling varied from 27% to 97% which was highest in the ecotype from Sichuan (92%) and lowest in the ecotype from Guchengzheng, Qinghai (27%). The endophytes in 1 ecotype of *F. kryloviana* and *F. nitidula* also had viability. The varied endophyte viability in seeds and seedling revealed that the it was affected by host species or host ecotypes. The novel associations of *F. sinensis* and endophyte in Gansu Province has been reported in 1996 (Nan, 1996a) and the more ecotypes of *F. sinensis* associations from Gansu Province were confirmed in the following study(Yang*,* *et al.*, 2011). In the present study, 6 more ecotypes of *F. sinensis* associations from Qinghai and Sichuan were identified which were consistent with the previous studies and also extended the range of host ecotypes. Endophyte viability in seedlings of all tested 10 *Festuca* associations were always lower than the corresponding viability in seeds which confirmed the previous conclusion that endophyte viability decreases more rapidly than that of seed (Rattray, 2003, Rolston*,* *et al.*, 1986) and endophyte in stored seed may become non-viable when conditions of storage are not carefully controlled (Rolston*,* *et al.*, 1986) although the seed itself retains its ability to germinate. Endophyte stability *in planta* (in seed and in vegetative tissue) is very important as loss of tiller infection and a failure of the endophyte to be transmitted via seed from incompatible associations has been observed (Christensen, 1995). However, in the present study, viability of each endophyte associations decreased to less than 60% excepted with ecotype from Sichuan. This highlights the importance of improving endophyte viability of these studied novel associations for future breeding program.

**Table 1.** Endophyte presence in seeds and seedlings of 12 native *Festuca* spp.

| Species | Origin | Sample ID | Viability in seeds | Viability in seedling |
|---|---|---|---|---|
| *Festuca sinensis* | Bazanggou, Qinghai, | 09-41 | 100% | 56% |
| *F. sinensis* | Bazanggou, Qinghai, | 09-57 | 100% | 57% |
| *F. sinensis* | Shagou, Qinghai | 09-84 | 100% | 51% |
| *F. sinensis* | Guchengzheng, Qinghai | 09-99 | 100% | 27 % |
| *F. sinensis* | Shihuiyao,Qinghai | 09-141 | 100% | 37% |
| *F. sinensis* | Hongyuan, Sichuang | 111 | 100% | 92% |
| *F. kirilowii* | Bazanggou, Qinghai, | 09-42 | 4% | 0% |
| *F. kirilowii* | Shagou, Qinghai | 09-67 | 0% | 0 % |
| *F. kryloviana* | Heimahe, Qinghai | 08-066B | 0% | 0% |
| *F. kryloviana* | Heimahe,Qinghai | 08-071 | 8% | 0% |
| *F. kryloviana* | Batan, Qinghai | 08-120 | 46% | 12% |
| *F. nitidula* | Jiaxing, Tibet | 09-291 | 83% | 77% |

The seed germination rates of the *Festuca* spp. ranged from 15.5% to 94.5% which indicated different viability of seeds following harvest (Figure 1). There were significant difference ($P<0.05$) between different species or different ecotypes within the same species. One ecotype of *F. sinensis* (09-57), *F. kirilowii* (09-42) and *F. nitidula* (09-291) had significantly higher ($P<0.05$) germination rates than the other ecotypes. One ecotype of *F. sinensis* from Sichuan had significantly lower ($P<0.05$) germination rates than all the other ecotypes although it had highest endophyte viability in seedlings. The 4 tested ecotypes (09-42, 09-67, 08-066B and 08-071) which had medium to high germination rates although there was no viable endophyte in seedling which suggested that endophyte may not improve the host germination. Although endophyte positive and negative seeds from the same ecotype were not compared in the present study, there was no significant correlations between endophyte viability in seedlings and seeds germination rates for the other 8 ecotypes ($y=0.663x+2.316$, $R^2=0.503$) which suggested the endophyte presence may not affect seed germination ability. Conflicting results have been obtained regarding the capacity of endophyte presence to increase the survivability of the seed up to germination, which in turn promotes *Epichloë* survival. This is due to the complicated nature of interactions between the endophyte and host under specific environmental conditions (Clay, 1987, Faeth*, et al.*, 2004, Gundel*, et al.*, 2006a, Neil*, et al.*, 2003, Rolston*, et al.*, 1986, Zhang*, et al.*, 2010). A number of studies have indicated that the endophyte effects on seed viability depended on both the genetic background of populations and the form of the evaluation process(Gundel*, et al.*, 2009, Gundel*, et al.*, 2007, Hill and Roach, 2009). The present results suggested that likelihood of the different germination ability of studied *Festuca* spp. was more possible due to the different genetic background of populations.

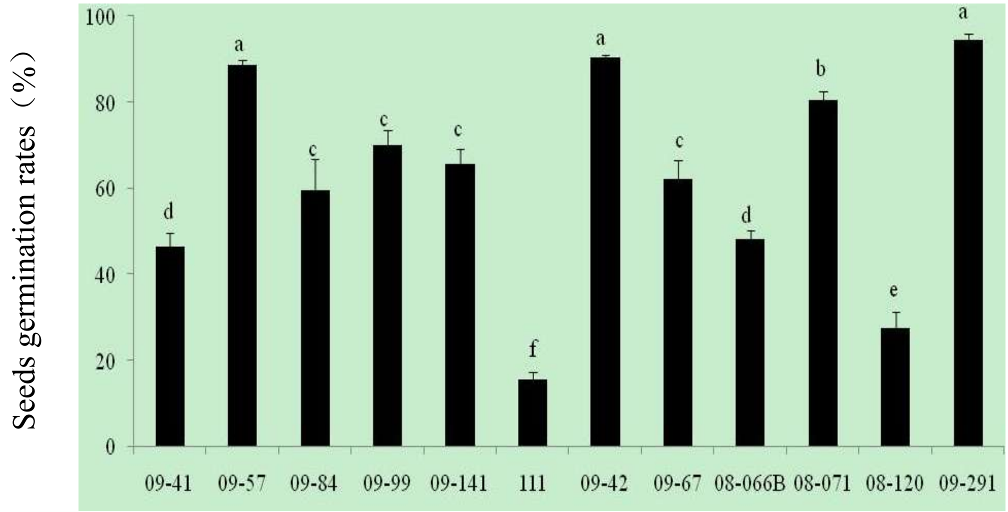


**Figure 1** The seeds germination rate of 12 native *Festuca* spp. seeds.

Note: The number on X-axis stands for the sample ID of different ecotypes shown in Table 1. Letters above bars indicate significant differences between seed lots($P<0.05$). Y error bars denote standard errors of the mean.

Table 2 **Seed length, seed width and weight of one thousand seeds for 12 *Festuca* spp.from west of China**

| Species | Sample ID | seed width(mm) | Seed length(mm) | Weight of 1000 seeds (g) |
|---|---|---|---|---|
| *Festuca sinensis* | 09-41 | 1.08±0.03b | 6.73±0.15ab | 0.96±0.06d |
| *F.sinensis* | 09-57 | 1.05±0.02bc | 6.03±0.13bc | 0.89±0.02d |
| *F.sinensis* | 09-84 | 1.07±0.02bc | 6.57±0.15abc | 1.21±0.00b |
| *F.sinensis* | 09-99 | 1.03±0.02bc | 6.16±0.20bcd | 0.94±0.03d |
| *F.sinensis* | 09-141 | 1.09±0.02b | 6.60±0.12abc | 0.75±0.02e |
| *F.sinensis* | 111 | 1.01±0.02c | 5.83±0.15d | 0.91±0.01d |
| *F.kirilowii* | 09-42 | 1.04±0.03bc | 5.72±0.28d | 1.06±0.03c |
| *F.kirilowii* | 09-67 | 1.18±0.04a | 6.96±0.13a | 1.28±0.02a |
| *F. kryloviana* | 08-066B | 0.77±0.02d | 5.67±0.19d | 0.44±0.01g |
| *F.kryloviana* | 08-071 | 0.83±0.02d | 5.68±0.17d | 0.54±0.02f |
| *F.kryloviana* | 08-120 | 0.78±0.02d | 6.17±0.29bcd | 0.35±0.02h |
| *F.nitidula* | 09-291 | 1.01±0.02c | 5.84±0.21d | 0.72±0.01e |

Note: letters indicate significant differences between seed lots ($P<0.05$)

The length and width of seeds and weight of 1000 seeds were measured for 12 *Festuca* spp. (Table 2). Significant differences were observed between different species or different ecotypes within the same species. Seeds of all of the 3 ecotypes of *F. kryloviana* had lowest seed width and length ($P<0.05$). Seeds of *F. nitidula* had lower seed width and length ($P<0.05$) compared with the other 2 species of *F. sinensis* and *F. kirilowii*. The weight of 1000 seeds had similar performance. Within the same specie, there was still significant difference between ecotypes. These difference suggested the host genetic background play major role for seed morphology difference. There was no significant correlations between endophyte viability in seedlings and seed morphology for the 6 ecotypes of *F. sinensis* (length: $y = -30.48x + 245.9$, $R^2 = 0.243$; width: $y = -372.4x + 445.9$, $R^2 = 0.287$; weight: $y = 10.96x + 42.86$, $R^2 = 0.005$) which suggested the endophyte presence may not affect seed morphology of *F. sinensis* under this studied conditions. This may preclude the hypothesis that the presence of the endophyte has changed germination by an indirect effect through extension of maternal plant growth during seed development and ripening (Gundel*, et al.*, 2006b).

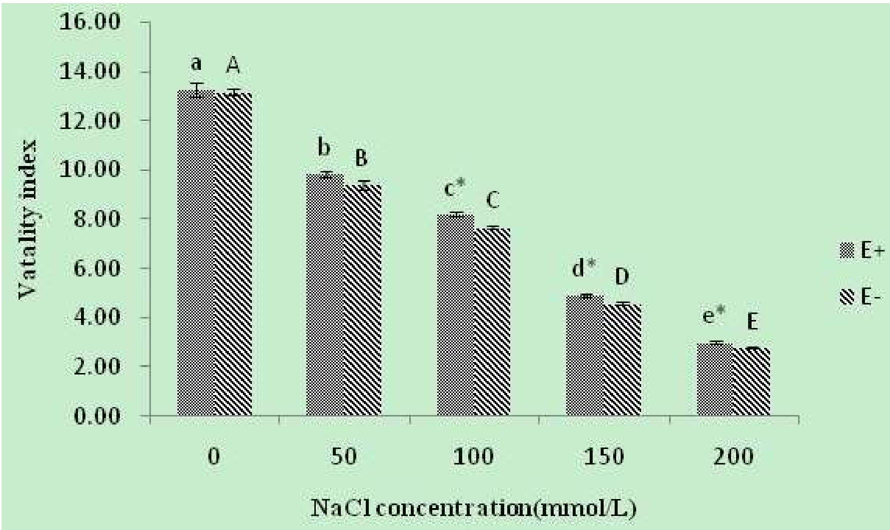


**Figure 2** The seed vigor index of E+ and E- seed from *F. sinensis* (Xiahe, Gansu) Note: The lower case letters above bars indicate significant differences between E+ seed lots under different NaCl treatments ($P<0.05$), the upper case letters above bars indicate significant differences between E- seed lots under different NaCl treatments ($P<0.05$). Y error bars denote standard errors of the mean, * indicate significant difference between E+ and E- under the same NaCl treatment.

The vigor index of *F. sinensis* seeds significantly decreased with increasing NaCl concentration ($P<0.05$) which suggested that NaCl had strong inhibition on seeds germination. There was no significant difference between E+ and E- seeds under control condition and lower NaCl concentration (50 mmol/L) which was consistent with the above germination tests and indicated that endophyte did not affect seed germination without stress. However, the vigor index of E+ seeds were significantly higher ($P<0.05$) than those of E- seeds under higher NaCl concentration(100,150, 200 mmol/L) which showed the positive effects of endophyte on seeds vigor under stress. These results showed that the improvement effect from endophyte under stress conditions performed better than that under suitable conditions which were consistent with the previous studies about effects of endophyte on seeds germination of *Festuca sinensis* under water stress (Peng, *et al.*, 2013) and *Achnatherum inebrians* under cadmium (Zhang *et al.*, 2012) or NaCl stress (Gou, 2007).

**Conclusion**

Seed and endophyte viability and seed morphology in native *Festuca*-endophyte associations were evaluated and the broad range of endophyte associations allowed significant improved understanding of their performance in west of China. One more novel *F. nitidula*-endophyte association was identified. Seed and endophyte viability were affected by host species or host ecotypes. The decrease of viability in seedlings after 7 months storage indicated the loss of viable endophyte after storage and highlighted the importance of improving endophyte viability of these studied novel associations. Seed viability and morphology were strongly affected by host genetic background. The presence of endophyte did not affect seed viability and morphology in natural or optimal conditions, however, endophyte significantly promoted host seeds germination under NaCl stress. These results suggest that the effects of endophyte presence on seeds quality and viability depend on the complicated interactions between host genotypes, endophyte genotypes and environmental conditions.

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# Endophyte-grass technology – obstacles in the production and delivery of a quality seed product

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**Abstract**

The viability of fungal endophyte (*Epichloë* species) within seed generally declines faster than seed viability. This can result in forage grass populations harbouring a reduced endophyte infection frequency that is less than anticipated. As the inclusion of endophyte in seed provides for pest control and improved agronomic performance of plants; reduced infection levels can subsequently lead to efficacy issues. Seed is a perishable product, with endophyte-infected seed requiring extra care than endophyte-free seed, particularly with respect to crop management, harvest conditions, post-harvest management storage and the seed delivery environment, that can all impact on endophyte viability. Endophyte viability tests are time specific, and like seed germination, a time limit between tests needs to be considered.

**Key words:** tall fescue; ryegrass; *Epichloë*; MaxQ; AR1; AR37; Avanex; seed production.

# Fungicides for the control of stem rust in susceptible perennial ryegrass seed crops

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**Abstract**

In turf type perennial ryegrass seed crops, stem rust reduces seed yields through reducing green stem area and the number of saleable seeds/m$^2$. In New Zealand fungicides are the main control method used for limiting stem rust infection in susceptible cultivars. To investigate the control of stem rust, 15 fungicide treatments were applied consisting of prothioconazole Proline® a DMI fungicide, alone or in mixture with Strobilurins (part of the QoI family), where application frequency ranged from one to four times. All treatments increased seed yield above the untreated with the best treatments providing a 105% increase in seed yield. Single application timings increased seed yield compared with the untreated control but multiple applications of fungicide were required to maintain green stem area. Seed yields were consistently greater when products were applied in combination. Combinations of Proline® mixed with pyraclostrobin (Comet®) or azoxystrobin (Amistar®) consistently produced greater seed yield and better disease control compared with individual products applied alone. To control stem rust growers should apply combinations of Proline® mixed with Comet® or Amistar® in a preventative programme at approximately 20 day intervals following head emergence.

**Key words:** *Lolium perenne*; *Puccinia graminis*; stem rust; seed yield

# Effect of insecticides used for pest management in red clover seed crops on pollination activity and bumblebee colony development

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**Abstract**

The repellent effect of three insecticides on bumblebee and honeybee pollination activity was evaluated in a large scale on-farm trial with red clover seed crop in SE Norway in 2013. On average for 1, 2, 3, 4, 5 and 7 days after spraying, 17 and 40 per cent less honeybees and 26 and 20 per cent less bumblebees were observed on plots sprayed with the pyrethroids lambda-cyhalothrin and Alfa-cypermetrin, respectively, compared to non-sprayed control plots. No similar negative repellent effect on pollination activity was found on plots sprayed with the neonicotinoid thiacloprid. All insecticides reduced the predator density and had a positive influence on seed yield. Of the three insecticides, thiacloprid, which showed no repellency against pollinating insects and had the strongest positive effect on seed yield (22% over non-sprayed control plots), was regarded as the most promising for red clover pest management. In a follow-up study in 2014, the long-term effect of thiacloprid on bumblebee (*Bombus terrestris*) colony development in nests set out in sprayed and non-sprayed red clover seed fields at various locations in SE-Norway, was carried out. Spraying was performed when 23-44 % of flowers were in full bloom. Analyses showed that thiacloprid was taken up during pollination, as residues were found in bumblebees from three out of five sprayed seed fields. The bumblebees with the highest residue level (0.009 mg thiacloprid/kg fresh weight) only managed to develop (weak) colonies in one out of five possible nests. Also bumblebees from the other thiacloprid sprayed fields normally developed smaller and weaker colonies than from comparable non-sprayed fields. Although further experiments are required, this indicates that spraying should be restrained to the period before flowering to reduce the uptake of thiacloprid from nectar and pollen during pollination.

**Key words**: Bumblebees; pollination; pyrethroids; neonicotinoids; weevils

**Ditylenchus dipsaci nematodes on alfalfa seed crops (Medicago sativa L.): how to mange for healthy seeds**

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**Abstract**

Alfalfa stem nematode, *Ditylenchus dipsaci*, also known as the bulb and stem nematode, is a migrating endoparasitic nematode. This nematode can infect more than 1200 species and is widely present all over the world. The race (strain) of stem nematode that infests alfalfa can survive on several other hosts but reproduces only on alfalfa (*Medicago sativa*), bean (*Phaseolus vulgaris*), pea (*Pisum sativum*) and sainfoin (*Onobrychis viciifolia*). Stem nematode symptoms are mostly observed at green-up stage in the early spring or just after the first cutting. Patches of stunted plants having swollen nodes and shortened internodes are the most recognizable symptom. *D. dipsaci* is recognized as the most serious nematode problem for this crop leading to lower forage and seed production. This nematode is transmitted by seeds, seed dust, weeds and soil. Moreover it has been listed as a quarantine pest on alfalfa seeds by EPPO in Europe. Fumigation with methyl bromide was widely used in the past on alfalfa seeds to control *D. dipsaci*. Since the use of methyl bromide was forbidden in Europe in 2010, it has been crucial to find another ways to control this nematode in alfalfa seed crops. Thus, this study focuses on alternative treatment methods to guarantee seed health but also on epidemiology in order to reduce or prevent soil contamination. The main alternative treatment to methyle bromide fumigation was thermotherapy based on a hot steam treatment (Thermoseed, Incotec). The efficiency of different conditions was tested on contaminated seed lots and the results show that thermotherapy represents good perspectives for controling nematodes. Mechanical methods (ventilation, sieving…) appear also to be efficient to eliminate *D. dipsaci* from seeds. The epidemiology studies were carried out in contaminated fields by following many plants from the green-up stage to harvest. Our results show that the epidemiology of *D. dipsaci* is complex and that it is not possible to predict the link between the observed symptoms, the contamination of the plant and the contamination of seeds. More research on this topic needs to be undertaken.

# Phenoxy herbicides responses in white clover seed production

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**Abstract**

Field hygiene is important to obtain high white clover (*Trifolium repens* L.) seed yields. Weed pressures need to be controlled to minimise competition and seed cleaning loses. This trial was established to evaluate different rates and forms of phenoxy 2,4-D herbicides on target weeds: *Crepis carpillaris* L. and *Sonchus arvenisi* L. and *Senecio quadridentatus* Labill. White clover seed yield responses were increased with all treatments when compared with that of the control. The seed yield of the best treatment was 1080 kg/ha. Yields increased as rates of phenoxy GAI (grams of active ingredient) increased. The higher rates of GAI gave a larger plant growth regulator effect, which had a positive response on seed yields.

**Key words**: field hygiene; phenoxy herbicide; 2, 4-D; growth regulator; seed yield

# Effects of endophyte *Epichloë festucae* var. *lolii* response to fungal pathogens on variations of physiological characteristics and peramine in *perennial ryegrass*

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**Abstract**

Interactions of perennial ryegrass (*Lolium perenne*), endophytes (*Epichloë festucae var. lolii*) and plant fungal pathogens is a research area with great impacts in both theory and production. However, the area is less studied than other areas of endophyte research worldwide. In order to compare the responses of endophyte infected plants (E+) and endophyte free plants (E-) of perennial ryegrass varieties to fungal pathogens isolated from turf type ryegrass in Lanzhou, the intact plants of cultivar 'Pinnacle' were inoculated with *Alternaria alternata* and *Bipolaris sorokiniana* and the activities of polyphenol oxidase (PPO), phenylalanine ammonialyase (PAL), alkaloids content and the effects of leaves extraction on spore germination were recorded with the development of disease. Compared with E- plants, the infection rates of E+ plants decreased by 31.6% and 35.3%, respectively, and the lesion length of E+ plants decreased by 37.7% and 23.8%, respectively. The enzyme activities of PPO and PAL for both E+ and E- plants firstly increased with disease development and then decreased. Compared with E- plants, enzyme activities of E+ plants increased by 75% to 140%. However, the alkaloids contents did not change with disease development. Compared with E- plants, the spore germination rate of *Alternaria alternata* and *Bipolaris sorokiniana* treated with the ethanol extraction of E+ leaves decreased by 2%-16% and 10%-19%, respectively. Hopefully, the present research will provide a solid base for further study the molecular mechanism of interaction between *Epichloë festucae* var. *Lolii* and perennial ryegrass under disease stress.

**Key words:** perennial ryegrass; endophyte; fungal pathogen; plant physiology; alkaloids; protective enzyme activity

# Does endophyte symbiosis resist the allelopathic effect of invasive plant in degraded grassland?

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**Abstract**

Allelopathic effects and plant associated systemic endophytic fungal are often thought to play role in the invasion of exotic plant species. Here, we tested the inhibitory effects of aqueous inflorescence, stem and root extracts of hemiparasitic weed *Pedicularis kansuensis* on seed germination and seedling growth of endophyte-free (E-) and -infected (E+) grass species, *Stipa purpurea* and *Elymus tangtorum*, which are native to the alpine steppe and meadows of the Qinghai-Tibetan Plateau. The weed extracts significantly inhibited both seed germination and seedling growth of target grass species. However, the negative effects were related to the concentration and type of the extract, and the susceptibility of target species depended on the endophyte infection. Inflorescence extract had stronger inhibition effect on target species than stem and root extracts, and the inhibition effect was directly proportional to the extract concentration. E+ target plants were less susceptible to the extracts than their E- counterparts. Our results demonstrate allelopathic potential of *P. kansuensis* and suggest that its invasion should select for E+ plants leading to increased frequencies of endophyte infected plants in *E. tangutorum* and *S.purpurea* populations. The dilemma between allelopathy facilitated reproduction and high dependency on neighboring host plants may destabilize invasion success of the species, thus partially explaining why the species show patchy distribution in the Qinghai-Tibetan Plateau.

# Detection of the seed-borne asexual *Epichloë* endophyte from *Elymus* hosts in China

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**Abstract**

Asexual *Epichloë* endophytes from Chinese *Elymus* species were surveyed and detected. Approximately 160 *Elymus* spp. samples (plant and/or seeds) were collected from four provinces in China. The direct microscopic examination showed that there were 10 species infected with endophytes, including *Elymus antiquus*, *E. cylindricus*, *E. dahuricus*, *E. dahuricus var. cylindricus*, *E. dolichatherus*, *E. excelsus*, *E. nutans*, *E. sibiricus*, *E. tangutorum* and *E. tibeticus*, and the endophyte infection rates appeared as follows: 75.0% in *E. cylindricus*, 32.9% in *E. dahuricus*, 28.9% in *E. excelsus*, 26.6% in *E. dahuricus var. cylindricus*, 23.7% in *E. dahuricus*, 15.9% in *E. nutans*, and 6.3% in *E. tibeticus*. In addition, we found that *E. antiquus*, *E. dolichatherus* and *E. sibiricus* were not infected by endophytic fungi. Further, endophyte infection varied among populations of same species. Only one plant was infected in all the 16 *E. tibeticus* collected from same population, so further endophyte infected plants or seed of *E. tibeticus* need to be collected to provide a wider population for further study. The 11 populations of *E. nutans* collected from Gansu Province of China did not have detectible endophytic fungi but the infection rate from Maqu population was 49%. The altitude of Maqu population is about 3503m, but others range from 1714m to 2911m. The infection rate is positive correlation with altitude, and the correlations between infection rates and environmental factors need further research.

**Key words:** *Epichloë*, seed-borne endophyte; *Elymus* spp.; infection rate; population

# The relationship between endophyte infection and perennial ryegrass (*Lolium perenne*) seed morphological characteristics

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**Abstract**

Perennial ryegrass (*Lolium perenne*), a highly competitive and productive pasture species, is the most commonly sown pasture species worldwide. It establishes quickly and is highly tolerant to treading damage and hard grazing. Endophyte of *Epichloë festucae* var. l*olii* formed mutualistic associations with perennial ryegrass which has been widely studied. In the present study, the endophyte infection rates in seeds of seven commercial perennial ryegrass cultivars (Pon., Gen., Eme., Lar., Pav., Exq., Pin) which were popular in local seeds market were evaluated. Furthermore, the relationship between endophyte infection and seed morphological characteristic were studied. Endophyte infection rates were detected by microscope with aniline blue staining. The results showed that the seeds from four perennial ryegrass cultivars were infected by endophyte ranged from 52% to 70%, however, the seeds from the other cultivars were not infected. Among the four infected cultivars, Eme. seeds had the highest endophyte infection rate with 70%. Thousand seed weight of the seven cultivars were significantly different ($F_{6,14}$=80.8, $P$<0.05). Seeds of Pon. had the highest thousand seed weight, whereas seeds of Pav. had the lowest thousand seed weight. There was a negative relationship between thousand seed weight and endophyte infection ($F_{1,5}$=40.0, $P$<0.01, $R^2$=0.88). There were also significant difference between seed length (long axis) of seven cultivars ($F_{6,63}$=19.4, $P$<0.05). However, there was neither positive nor negative correlation between seed length and endophyte infection. Therefore, the endophyte survey for local perennial ryegrass seeds market will provide suggestion for local farmer to select the appropriate cultivars. Our work tries to reveal the relationship between endophytic fungi and perennial ryegrass seed quality, which provide theoretical basis for use of endophytic fungi for grass breeding.

**Key words:** *Lolium perenne*; seed morphological; endophyte; infection rate

# Integrated pest management in Danish grass seed production

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**Abstract**

Knowledge Center for Agriculture participates in a project financed by the European Community. The task of the project is to minimize the use of pesticides, without minimizing the yield or the quality of the different crops. In Europe we are already very limited in the use of pesticides. These limitations have given us a few problems. We have problems with the amount of weeds in the different crops, but also a beginning problem with resistance to the active substances we are allowed to use. These problems apply not only to grass seed production but are a general problem not only in Denmark but all over Europe. A number of farms are participating in the project. One of them is a grass seed producer from Sealand. He has five different grass seed crops on the farm, 38 % of his 480 ha are with grass seed. That means very big challenges concerning the purity of the crop and to fight the weeds. Crop rotation and all other actions that concern no-pesticide weed control are included in his approach to growing his grass seed crops. Last summer we had an “Open Farm” where 800 farmers visited the farm; which was a great success I think. In this Poster I will present how we are dealing with Integrated pest management (IPM) in Denmark on a grass seed farm, how we got started and the current status of the project.

# Alternative Methods To Control *Tychius Aureolus* In Alfalfa Seed Crops (*Medicago sativa* L.)

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**Abstract**

*Tychius aureolus* Kiews (alfalfa seed weevil) belongs to the Curculionidae family. This weevil is a pest of alfalfa seed crops. Females lay eggs on alfalfa pods and then the larvae develop and eat the seeds. Damages can lead to yield losses up to 30%. For many years, *T. aureolus* has been more and more present in France and at the same time, the chemical protection has been reduced according to the new insecticides regulations. Furthermore, the development of new methods of control, complementary or alternative to chemicals, is crucial to control this pest. Since 2008, our studies have been conducted to understand the biology of the pest better in order to develop alternative methods of control.

This study focuses on two control alternative methods which were tested in the two main regions of alfalfa seed production in 2013 and 2014. The first control was made mechanically. It consisted of a shallow tillage following the first cutting. For the second control, we used semiochemicals. For example, we used different volatile organic compounds (VOC) from flowers or pods that we tested. The results show that i) the shallow tillage leads to lower insect emergences and ii) the attraction of the VOC from flowers and pods seems different. Overall, our results show that both methods represent good perspectives to use "integrated pest management" against *T. aureolus* in alfalfa seed crops. However, more research on this topic needs to be undertaken.

## The distribution of locoweed endophyte in *Oxytropis ochrocephala* population, plants and seeds

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**Abstract**

*Oxytropis ochrocephala* is one of important locoweed species in northwest China since it is poisonous to livestock and causes locosim. The toxicity of locoweed comes from swainsonine which was mainly produced by endophyte (*Undifilum* spp.). The object of this study is to determine the distribution situation of endophyte in *O. ochrocephala* population, plant tissues and seeds in Haiyuan County, Ningxia Province. Among the plants collected at this site in October 2013, 100% plants contained endophyte, however, endophyte resided in 88% shoots and other were free from endophyte. For individual shoots that contained endophyte, endophyte was distributed in 20-100% stem segments (averaged was 71%) but not in each segments. For seeds collected from individual shoots, 0-70% seeds contained endophyte. Germination percentage of seeds was positively correlated to isolation percentage of endophyte from seeds ($P<0.05$, $R^2=0.88$). No significant correlation was found between isolation percentage of endophyte from shoots and isolation percentage of endophyte from seeds ($P>0.05$, $R^2=0.406$). Conidia of the endophyte were commonly found on the surface of leaves, which likely contribute uneven distribution of the endophyte within plant tissues.

**Key words:** locoweed endophyte; isolate rate; distribution

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# Evaluating the response of slug populations and activity to tillage practices in annual ryegrass grown for seed

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**Abstract**

Slugs remain one of the most damaging economic pests of the grass seed industry in Western Oregon, USA. No-till production systems create ideal slug habitat and can result in higher slug pressure, which is a factor that keeps growers from adopting no-till. While in theory tillage should reduce slug populations, slugs can also reach economic-infestations levels in conventionally tilled fields. More information is needed on the use of tillage as a slug management tool. The objective of this study was to determine the impact of four tillage systems on slug emergence and total numbers during the fall of one season. The study was conducted on a research farm near Corvallis, Oregon in the fall of 2014. The study plots were part of an existing long-term annual ryegrass seed production trial established in 2005, and four tillage treatments were studied in 2014: 1) continuous conventional tillage (CT); 2) continuous no-till (NT); 3) alternate-year tillage (NT/CT); and 4) third-year tillage (volunteer/NT/CT). Treatments 1 and 3 were conventionally tilled in October 2014, while treatments 2 and 4 were left to volunteer. Slug blankets were used to track slugs numbers in a series of ten counts from October 12 to November 28. Over the sampling period slug counts were naturally affected by weather, and slug populations tended to increase with moisture. Alternate-year tillage resulted in the lowest (6.3 slugs/blanket) and most consistent population of slugs over the sampling period. Continuous NT resulted in the earliest slug emergence and greatest number of slugs (26.3 slugs/blanket). Tillage did delay slug emergence; however, there was no significant difference between continuous CT and tillage every third year (18.5 and 21.1 slugs/blanket, respectively). This study suggests that alternate-year tillage provided the best options for control with bait because of the low and consistent slug activity.

# Detection and identification of *Fusarium* species isolated from imported grass by DNA barcoding

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**Abstract**

To determine a suitable DNA barcode for the genus *Fusarium*, the internal transcribed spacer rDNA(ITS), elongation factor 1α（EF-l α, mitochondrial small subunit rDNA(mtSSU), and beta-tubulin（*β*-tubulin）genes were selected as candidate markers. A total of 91 sequences were analyzed with 22 strains of 8 *Fusarium* species isolated from five different herbage seed lots from different countries. The ease of nucleotide sequence acquisition, intra- and inter-specific divergences, and the frequency distribution of the genetic distance were treated as criteria to evaluate the feasibility of a DNA barcode. The results showed that the four genes for *F. graminearum*, *F. culmorum*, *F. incarnatum*, and *F. equiseti* couldn't identify accurately, including *F. acuminatum*, *F. avenaceum*, and *F. tricinctum* complex species. But intra- and inter-specific divergences of EF-l α gene was less in overlap than the other, and inter-specific difference was significantly greater than the intra-specific. According to the EF-l α tree, all of the species were separated very well. Therefore, EF-l α gene was better than the other genes for the genus identification.

**Key words:** genetic distance; intra- and inter-specific divergences; PCR and sequencing success; sequence analysis

# Soil nematodes community characteristics analysis of *Festuca arundinacea* in Shunyi, Beijing

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**Abstract**

From August 2014 to May 2015, the nutrition group and its quantity of the soil nematodes in yellow grass and normal lawn were studied, to identify the cause of withered and yellow in *Festuca arundinacea*. After one year's study, the results show that *F. arundinacea* related nematodes nutritional groups including plant parasitic nematodes, insect nematodes, bacterivorous nematode and omnivorous/predator nematodes. Through morphological identification, the nematodes in *F. arundinacea* soils in the region involved 5 orders 8 families 15 genera, in which including plant parasitic nematodes 2 orders 4 families 7 genera and non-plant nematodes 3 orders 4 families 8 genera. Soil nematodes number is 200-380 Per 100 g soil. The dominant plant parasitic nematodes is about 72.6%, the number of the genera of *Pratylenchus*, *Helicotylenchus* and *Aphelenchoides* in yellow grass is 3 times of the normal lawn. The results in March 2015 show, the number of *Pratylenchus* is 54.3% of all nematodes. All the data shows the *Pratylenchus* winter survival rate is high, the resistance is strong, it is difficult to control. This paper shows the cause of withered and yellow in *F. arundinacea*, provides the basis of lawn nematode control in shunyi district.

**Key words:** lawn; nematode trophic group; soil health; turf

# Session 4. Seed quality: from field to storage

# An overview of seed dormancy, with particular reference to the most common kinds in forage species: nondeep physiological dormancy and physical dormancy

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**Abstract**

Seed dormancy may be physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY), and combinational (PY+PD). Each of the five classes is described, and particular attention is given to dormancy in herbage seeds with PD and PY and how it is broken. PD is the most common class of dormancy in all the major vegetation zones of the world followed in order by PY, MPD, MD, and PY+PD. PD is subdivided into three levels: non-deep, intermediate, and deep, and seeds of most species with this class of dormancy, including grasses, have non-deep PD. There can be much variation in dormancy between seed lots, which may make it difficult to obtain high and uniform germination percentages and rates. The primary sources of this variation include genetics and the parental environment. With regard to parental environmental effects (transgenerational plasticity) higher germination percentages (low dormancy) in seeds with non-deep PD generally are associated with high temperatures, short days, light with a high red/far-red ratio, drought (low soil moisture), and high nutrient availability during seed development on the mother plant. In the case of PY, exposure of the mother plant to drought stress or to a long daily photoperiod may decrease germination, i.e. increase dormancy.

**Key words:** parental environment; physical seed dormancy; physiological seed dormancy; seed dormancy classes

## Introduction

When one grows herbage species from seeds what he/she would like to be able to do is sow seeds and a few days later find that each one of them has produced a healthy seedling. However, even with the aid of incubators and greenhouses seed germination percentages of many species often are low, which leaves the scientist trying to figure out how to promote germination. If seeds are viable but do not germinate when sown under conditions generally favorable for germination of nondormant seeds, it can be concluded that they are dormant. Now, what should one do? People who work with seeds know about various dormancy-breaking treatments such as cold stratification, warm stratification, afterripening, scarification, and treatment with gibberellin that can be used to promote germination. Consequently, dormant seeds are subjected to these treatments, but all too often the results are disappointing. That is, although the seeds were viable, only a low percentage of them germinated.

Obviously, then, the correct dormancy-breaking treatment or sequence of treatments has not been used.

There are five major *classes* of dormancy, and for some of them *levels* (i.e. subdivisions of the class) have been distinguished. Further, specific environmental conditions are required to overcome each class and level of dormancy. Obviously, if the seeds are in the incorrect environmental conditions, they are not going to come out of dormancy and will not germinate when sown in the greenhouse, nursery, or field. Thus, for the seed industry it is important to be able to identify the classes and levels of dormancy and know how to break each of them. The objectives of this paper are to (1) distinguish between dormant, conditionally dormant, and nondormant seeds, (2) describe the five classes of seed dormancy, (3) discuss the environmental conditions required to break physiological dormancy and physical dormancy, and (4) provide some information on the primary sources of variation in seed lots of species with nondeep physiological dormancy, the most common kind of dormancy on earth.

**Dormancy terminology**

If freshly matured, viable seeds fail to germinate after 4 weeks at any of several combinations of environmental conditions such as temperature and light, they are **dormant** (see Baskin and Baskin, 2014). In germination tests that extend beyond 4 weeks, there is a possibility that dormancy loss will have occurred during the incubation period, thus making it impossible to determine if fresh seeds were dormant. On the other hand, if fresh seeds germinate to high percentages (c. 75-80) over a range of test conditions in 4 weeks or less, and this range and germinate rate (speed) do not increase after seeds have been given a dormancy-breaking treatment, they are **nondormant,** or perhaps morphologically dormant (see below). If fresh seeds germinate to high percentages over a narrow range of conditions after 4 weeks, they may be in a *state* of **conditional dormancy**, which is common in seeds with nondeep physiological dormancy (see below). If fresh seeds are in a state of conditional dormancy, the percentage and rate of germination, as well as the range of conditions over which they germinate, increase following a complete dormancy-breaking treatment. It should be noted that dormancy and nondormancy are the extreme ends of a continuum that occurs in seeds with PD. All degrees of dormancy between dormancy and nondormancy are designated as conditional dormancy, even though the strength of dormancy between these two states varies considerably, i.e. decreasing from dormancy → nondormancy.

We have compiled seed dormancy and germination data for 13634 species from the major vegetation zones of the world, and seeds of 75.9% of the species are dormant at maturity (Baskin and Baskin, 2014). There are five classes of seed dormancy: physiological, morphological, morphophysiological, physical, and combinational (Baskin and Baskin, 2004). A closer look at these five classes will help explain why fresh seeds do not germinate and provide insight into how dormancy is broken in nature.

**Physiological dormancy** (PD) means there is a "physiological inhibiting mechanism" (*sensu* Nikolaeva, 1969, 1977) in the embryo that prevents it from generating enough growth potential to overcome the mechanical restraint of the seed coat and/or other covering layers such as endosperm, perisperm, and in the case of grasses also the palea and lemma (e.g. Duclos et al., 2013). Dormancy break occurs at cool (c. 0-10ºC) wet, warm (>15ºC) wet, or warm dry conditions (afterripening), depending on the species. After the embryo becomes fully nondormant, it has enough growth potential to push through the layers surrounding it. PD is the most common class of dormancy in all vegetation zones on earth.

At the biochemical/molecular level, the primary regulators of dormancy and germination in seeds with PD are abscisic acid (ABA) and gibberellin (GA), and endogenous levels of these two plant growth regulators (hormones) determine whether a seed will remain dormant or germinate. ABA induces and maintains dormancy, and GA stimulates germination. Further, sensitivity of the embryo to ABA decreases and that of GA increases during dormancy break. Ethylene and jasmonates also play a role in regulating dormancy and germination in seeds with PD (Graebner et al., 2012; Linkies and Leubner-Metzger, 2012; Bewley et al., 2013).

In seeds of some species with **morphological dormancy** (MD), the embryo is undifferentiated (organless), or it may be differentiated but underdeveloped [low embryo length : seed length (E:S) ratio]. Thus, the embryo needs to complete growth and development (inside the seed) before the seed can germinate, as shown in the following diagram.

**Embryo undifferentiated → underdeveloped → fully developed**

**Seed maturity**

**Embryo underdeveloped → fully developed**

Although seeds with MD germinate in ≤ 4 weeks, they differ from nondormant seeds in that time is required for completion of growth of the embryo prior to germination (the dormancy period). If seeds with an underdeveloped embryo have only MD, they do not require any pretreatment *per se* for the embryo to grow, just the appropriate conditions for growth of the embryo. In seeds of some species, the underdeveloped embryo may be physiologically dormant, and thus the seeds will not germinate when they are incubated on a moist substrate at otherwise appropriate temperature and light conditions for germination in ≤ 4 weeks (Baskin and Baskin, 2014). These seeds have **morphophysiological dormancy** (MPD). In seeds with MPD, PD has to be broken, either prior to, after or in some cases before and after (e.g. Phartyal et al., 2009) the embryo elongates, depending on the species, and the embryo must complete growth and development before the seed can germinate.

In seeds with **physical dormancy** (PY), which is hypothesized to have evolved from PD (Willis et al., 2014), germination is prevented because the seed coat (sometimes the fruit coat) is impermeable to water. Impermeability is due to the presence of one or more palisade layers of lignified cells in the seed or fruit coat, which becomes water impermeable during the final stages of seed maturation, when the drying seed reaches a moisture content of < 15% for seeds of most species with PY. During development of water impermeability, the seed coat away from the hilum becomes impermeable before the hilum closes, and it is the hilum through which the final few percentages of water loss occur. Thus, if seeds are collected before they dry enough to become water impermeable, they may germinate without requiring any pretreatments. In which case, the fresh seeds may be (incorrectly) determined to be nondormant.

PY is known to occur in 18 flowering plant families, including the Fabaceae (Leguminosae). In seeds with PY, a specialized morphoanatomical structure ("water plug" or "water gap") occurs in the impermeable seed or fruit coat. Several kinds of water gaps have been identified, and in legumes it is called the lens. In the breaking of PY, water gap structures are dislodged or disrupted in response to environmental cues such as heat from fire, high temperatures, or fluctuating temperatures, thereby creating an entry point for water into the seed. Even in the breaking of PY by such methods as dipping the seeds in boiling water or soaking them in concentrated sulfuric acid, the seed coat may become permeable only via opening of the water gap. From a world perspective, PY is the second (after PD) most common class of seed dormancy (Baskin and Baskin, 2014).

In addition to a water-impermeable seed coat, seeds of a relatively few species in seven of the 18 families with PY also have a(nondeep) physiologically dormant embryo, i.e. seeds have **combinational dormancy** (PY+PD). In the Cucurbitaceae, no species are known to produce seeds with only PY, but those in the genus *Sicyos* have PY+PD. In some species, such as winter annual legumes, PD is broken *via* afterripening before PY is broken; however, in other species PY must be broken before PD can be broken *via* cold stratification, i.e., seeds must be imbibed to be cold stratified (Baskin and Baskin, 2014). Seeds of three of four forage species of *Vicia* (Fabaceae) from the Tibetan Plateau studied by Hu el al. (2013) had PY+PD.

A group of seeds not included in the five classes of dormancy, as described here, is dust seeds. Dust seeds are very small (most <1.0 mm in length), the embryo is undifferentiated (organless) and the amount of endosperm ranges from absent to "copious" (many cells). The undifferentiated embryo does not developed into an embryo *per se* before a plant is produced. For example, in orchids the undifferentiated mass of cells that is the embryo grows directly into a "protocorm," from which a plant is produced. Dust seeds occur in mycoheterotrophic (e.g. orchids) and holoparasitic (e.g. *Orobanche* spp., some of which are bad weeds in field crops) and have specialized kinds of MD or MPD (Baskin and Baskin, 2014).

**Breaking Physiological dormancy**

In subtropical/tropical regions, PD is broken while seeds are exposed to warm wet (warm stratification) or warm dry (afterripening) conditions. However, in other climatic regions, depending on the species, warm wet, warm dry, or cold wet (cold stratification) conditions may be required for dormancy loss to occur.

Three levels of the class PD have been distinguished: deep, intermediate, and nondeep (Nikolaeva, 1969, 1977). In general, seeds with deep PD require 10-16 weeks of cold stratification to become nondormant, while those with intermediate PD require 8-14 weeks of cold stratification to become nondormant. However, a pretreatment period of afterripening or of warm stratification may reduce the length of the cold stratification period required to break intermediate PD. A distinguishing feature of fresh (nontreated) seeds with deep PD is that growth of the seedling produced by the isolated (from the seed) embryo of nontreated seeds is abnormal, and in some cases the embryo does not grow at all. In nontreated seeds with nondeep and intermediate PD, isolated embryos give rise to normal seedlings, although compared to embryos from treated (nondormant) seeds, there may be a bit of a lag in beginning of growth of the embryo into a seedling. Nondeep PD is broken in seeds of many species by 2-8 weeks of warm stratification (or sometimes by 8-12 weeks of afterripening), but it is broken in seeds of other species by 2-10 weeks of cold stratification. Seeds of all species with PD in subtropical/tropical climatic regions, and most of those in other parts of the world, have nondeep PD.

Seeds with nondeep PD exhibit a continuum of changes as they go from dormancy (D) to conditional dormancy (CD), and finally to nondormancy (ND), i.e.,

D → CD → ND. Seeds coming out of dormancy exhibit various patterns at the temperatures at which they can germinate while in conditional dormancy. Six patterns or types of nondeep PD are known (Nur et al., 2015), but most species have either Type 1 or Type 2. In the early phases of conditional dormancy, seeds with Type 1 germinate to high percentages only at low (e.g. 15/6°C) temperatures (Vegis, 1964; Baskin and Baskin, 2014). As dormancy break progresses through the dormancy continuum (CD), seeds can germinate at higher and higher temperatures until they become nondormant, at which time they can germinate over the full range of temperatures possible for the species, ecotype, or cultivar, e.g. 15/6 to 30/15 °C, if light, moisture and oxygen are not limiting. The temperature response pattern in seeds with Type 2 nondeep PD is the reverse of that of species with Type 1. Thus, as dormancy break progresses through the dormancy continuum seeds with Type 2 first germinate at high (e.g. 30/15°C) temperatures and then at lower and lower temperatures until they become nondormant. Further, seeds with types 1 and 2 nondeep PD respond to various other environmental factors, such as amount and quality (e.g. R/FR ratio, green) of light, soil water potential, soil nitrate, and exogenously-applied plant hormones (i.e. ABA, GA) in the same way as they do to temperature. For example, the sensitivity of some seeds to light increases through the dormancy continuum, and thus they require less light to germinate (or may even germinate in darkness) in the late than in the early stages of conditional dormancy and when nondormant. Further, whereas green light might inhibit germination in the early stages of conditional dormancy, it might not do so in the late stages or when the seeds become nondormant. Consequently, a green “safe” light may not be safe to use in monitoring germination of seeds incubated in darkness!

Although seeds with deep or intermediate PD may germinate at the same low temperatures (e.g., 5°C) at which dormancy is broken, the temperature regime required to break dormancy in seeds with nondeep PD frequently is not the optimal temperature for germination, especially while seeds are in conditional dormancy. For example, the optimal temperature for dormancy break in seeds of winter annuals, many of which have a Type 1 response pattern during dormancy break, is summer temperatures (e.g., 30/15°C), but the only temperatures at which seeds will germinate when they are in the early phases of conditional dormancy are low autumn (e.g., 15/6°C) temperatures. Later, after several months of exposure to high summer temperatures, seeds eventually become nondormant and thus gain the ability to germinate over a wide range of temperatures, e.g. 15/6 to 30/15°C, depending on the species. Thus, if one is eager to obtain seedlings before seed dormancy is fully broken, he/she can expose the seeds to high summer temperatures for 1-2 months, and then incubate the conditionally dormant seeds at a low or moderate (20/15ºC) temperature. In summer annuals, many of which have a Type 2 response pattern during dormancy break, responses to temperature are just the reverse of what they are for winter annuals. Thus, cold stratification breaks PD, but in the early phases of conditional dormancy high temperatures (e.g., 30/15ºC) are optimal for germination (Baskin and Baskin, 2014).

Seeds of many species with nondeep PD in the temperate zone have the ability to reenter dormancy (secondary dormancy) after they become nondormant, if environmental conditions prevent them from germinating during their normal germination season. As they reenter D, they go from ND to CD and then to D, i.e. ND ➔ CD ➔ D. Depending on the species, seeds may go all the way through CD into D, or they may stop somewhere along the CD continuum. In the first case, seeds cycle between D and ND, and in the second case between CD and ND, until they either germinate or die. Further, seeds may be dormant at maturity, become nondormant and cycle between CD and ND, i.e. D → CD ↔ ND; be conditionally dormant at maturity and cycle between CD and ND, i.e. CD ↔ ND; and be conditionally dormant at maturity, become nondormant and remain nondormant, i.e. CD →ND → ND. We are not aware of a well-documented case in which a seed that is fully ND at maturity can be induced into CD or D. In the soil environment, darkness is an important environmental factor that prevents many buried seeds from germinating at the time of year when they are nondormant. Low winter temperatures induce seeds of many winter annuals into secondary dormancy (or into CD), and D or CD is broken the following summer. High summer temperatures induce seeds of many summer annuals into secondary dormancy (or into CD), and dormancy or conditional dormancy is broken the following winter (Baskin and Baskin, 2014).The biochemistry/molecular biology of dormancy cycling in seeds of the annual species *Arabidopsis thaliana* (Brassicaceae), which have nondeep PD, has been described by Footitt et al. (2011, 2012).

Seeds with nondeep PD collected from the same species at different locations in the same year; at the same location in different years; from different plants in the same population; from the same plants at different times during the growing season; or from different locations on the same plant can differ in their dormancy-breaking and germination requirements. This variation can be due to differences in parental environment, genetics, or genotype x environment interaction (Baskin and Baskin, 2014). Many factors of the parental plant environment, including $CO_2$ level, competition, photoperiod, length of growing season, light quality, mineral nutrition, soil moisture, solar irradiance, temperature, salinity, and herbicides, can have transgenerational effects on dormancy/germination and on other features of growth and functioning of the $F_1$ progeny and in some cases of the $F_2$ progeny and beyond (Baskin and Baskin, 2014). Parental environmental effects or transgenerational phenotypic plasticity (nongenetic inheritance) may be mediated by changes in (1) maternal provisioning to the seed of storage reserves (seed size) and seed coat structure and thickness, which can play a role in the regulation of dormancy/germination; and (2) epigenetics, in which gene expression is modified by abiotic and biotic environmental stress through molecular mechanisms (other than DNA sequence change) such as DNA methylation.

Transgenerational effects can be adaptive, i.e. increase in the product of maternal fecundity and offspring fitness when the offspring grow in (not dispersed away from) their mother's environment and environment is predictable between generations. Further, these transgenerational effects can evolve if there is within-population genetic variation in the response of the phenotype to the maternal environment, i.e. a statistically significant maternal genotype x maternal environment effect on progeny trait of interest, e.g. seed germination (Roach and Wulff, 1987; Donohue and Schmitt, 1998; Kalisz and Purugganan, 2004; Galloway, 2005; Galloway and Etterson, 2007; Bossdorf et al., 2008; Herman and Sultan, 2011; Holeski et al., 2012; Latzel et al., 2014).

**Breaking physical dormancy**

Under laboratory conditions, mechanical scarification is a safe and effective way to make the seed or fruit coat permeable to water, i.e. break dormancy. Other methods used to make seeds permeable include scarification with concentrated sulfuric acid and wet or dry heat treatments, e.g. 70-110°C dry heat for 1-15 min or 100°C (boiling water) for 1-20 sec (Baskin and Baskin 1997). However, the response of impermeable seeds to boiling water and dry heat varies with the species, and neither treatment works for some species. In general, wet heat is more effective than dry heat in breaking PY. Scarification with concentrated sulfuric acid, dipping in boiling water or exposure to dry heat in an oven can make seeds permeable by opening the water gap (Baskin and Baskin 2014).

Only a few studies have described in detail the mechanism of water-gap opening. Perhaps the most detailed such study was done by Gama-Arachchige et al. (2012, 2013a), who found that opening of the water gap in seeds of the weedy winter annuals species *Geranium carolinianum* is a two-step process. In step I, high summer temperatures cause the insensitive seeds to become sensitive by weakening the bond between the water gap palisade and subpalisade cells. The $Q_{10}$ of step 1 ranged from 2.0 and 3.5, indicating chemical processes. In step II, exposure of sensitive seeds to autumn temperatures caused differential shrinking of the palisade and subpalisade cells and thus formation of a gap between them. This was followed by several subsequent changes in the water gap region, leading to formation of a "hinged-valve" water gap. The $Q_{10}$ of step II was between 0.02 and 0.10, indicating purely physical processes (Gama-Arachchige et al., 2013a). This study clearly shows that physical dormancy-break in *G. carolinianum* is controlled by temperature, i.e. high temperatures make insensitive seeds sensitive, whereupon on their exposure to lower temperatures the water gap opens and the seeds imbibe water and germinate. However, the hinged-valve is only one of 24 kinds of water gaps regions described by Gama-Arachchige et al. (2013b) for seeds and fruits in 16 of the 18 families of flowering plants known to have PY, and thus we would expect at least the details of the mechanism of opening to differ among them (e.g. see Jayasuriya et al., 2009).

Timing of germination of seeds with PY in nature is controlled by responses of the seeds (and specifically opening of the water gap) to environmental cues, and fluctuating and high temperatures sometimes in connection with high soil moisture are the most important factor controlling the breaking of dormancy (Baskin and Baskin, 2014). In contrast to popular opinion of ecologists and plant biologists, who think that microbial activity and soil abrasion are the main factors that break PY in nature, there is little or no evidence to support these ideas, which in fact are not ecologically or evolutionarily rational (Baskin and Baskin, 2000). Neither has freezing and thawing, except via ultra-low temperature (e.g. liquid nitrogen at -196°C), been shown to be effective in breaking PY (Baskin and Baskin, 2014).

The temperature regimes required to break PY vary with the species. Seeds of Townsville stylo (*Stylosanthes humilis*) and Caribbean stylo (*S. hamata*) (Fabaceae) became permeable during the dry hot season in Northern Australia and thus germinated when the wet season began (McKeon and Mott, 1982). Van Assche et al. (2003) found that if water-impermeable seeds of the spring-germinating legumes *Melilotus albus, Lotus corniculatus, Medicago lupulina,* and *Trifolium repens* were incubated on wet filter paper at 5ºC for 2 months, high percentages of them became permeable and germinated when they were moved from 5 to 15/6°C or from 5 to 20/10ºC. However, seeds moved from 5 to a constant temperature of 10 or 23ºC did not become permeable. Thus, germination in spring occurs only if seeds are subjected to low winter temperatures followed by low alternating temperature regimes of early spring. Any seeds buried in soil in spring would not be subjected to the daily alternating temperature regimes required to make them permeable; consequently, they would not germinate and thus remain in the soil seed bank.

In seeds of subterranean clover (*Trifolium subterraneum,* Fabaceae), Taylor (1981, 2005) discovered that dormancy break occurs in two steps. The first step, or conditioning phase, occurred if nonconditioned (insensitive) seeds were incubated dry at constant (15 to 80°C) temperatures, and the rate at which this step was completed, i.e. seeds become conditioned (sensitive), increased with an increase in temperature. In the second step, seeds that have been conditioned (made sensitive) became water-permeable (i.e. PY broken) if they were subjected to an alternating temperature regime of 60/15°C in a drying oven, but constant temperatures of ≥50°C were somewhat effective. Subsequently, it has been found that seeds with PY in some species, e.g. *Ipomoea lacunosa* (Convolvulaceae), can cycle between being sensitive to dormancy-breaking conditions and insensitive (Jayasuriya et al., 2008). Further, seeds with PY can cycle many times between the sensitive and insensitive states. However, whereas seeds with nondeep PD can cycle between dormancy and nondormancy numerous times if they remain viable, those with PY cannot reenter dormancy once the water gap opens as diagramed below.

**insensitive seed (PY) ↔ sensitive seed (PY) → nondormant seed (cannot reenter PY)**

## Concluding remarks

Nondeep PD and PY are the two most common kinds of seed dormancy on earth, and they are the ones likely to be encountered in seeds of wild and domesticate forage species. These are the kinds of dormancy found in seeds of the two great groups of forage plants: grasses (nondeep PD) and legumes (PY). Knowledge of the plant life cycle (phenology), and in particular when seeds mature and when they germinate in the field, will provide a clue to the conditions required for breaking seed dormancy. For example, if seeds mature in spring or early summer and seedlings emerge in autumn, the high temperatures of summer may be required to release seeds with nondeep PD from dormancy, i.e. *via* afterripening or warm stratification. With regard to PY, summer temperatures may break dormancy by causing the water gap to open or make the seeds sensitive, thereby allowing them to become nondormant and germinate at low temperatures in autumn (Gama-Arachchige et al., 2012). For seeds that mature in the growing season of one year and do not germinate until the growing season of the following year, cold stratification during winter may break dormancy in seeds with nondeep PD. Although low winter temperatures are not known to break PY, they can make the seeds sensitive, and then the seeds can become nondormant and germinate in response to fluctuating temperatures in spring (Van Assche et al., 2003). In sum, then, knowledge of the phenology of the seed maturity and seedling emergence stages of the life cycle of the herbage species of interest can provide the researcher insight into the treatments required to break dormancy and the grower with information about the best time to sow seeds in order to get a good stand.

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# Seed set and seed development in perennial ryegrass and the effects on seed quality

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**Abstract**

Outcrossing species generally have a low seed/ovule ratio, which may be a result of embryo abortion or pre-fertilization ovule sterility. It might also be due to poor pollination for example in a heavily lodged grass seed crop. Increasing ovule and floral fertility seems to have obvious potential for improving seed yield without compromising the vegetative development and to optimise the economic return from the agronomic inputs.

The major importance of seed set for grass seed production has led to the definition of floret site utilization (FSU). Elgersma (1991) distinguished between the biological FSU, which is the percentage of florets present at anthesis resulting in a viable seed and the economic FSU, which is the percentage of florets present at anthesis resulting in a cleaned pure seed. Different studies reported large variations in biological FSU, but on average 20-50% of the florets seem biologically unproductive with losses occurring during pollination, fertilization and seed development.

In the literature we find contrasting statements:

- Fertilization does not decline from the basal to the distal floret within a spikelet in spaced plants, and unproductive florets are found in all floret positions.
- The capacity of florets to set seed declines from the basal to the distal florets within the spike, and even more pronounced within the spikelet under field conditions.

In a three year field experiment we found that the number of florets per spikelet was 8.2 from which 4.3 seeds were produced. The FSU was in average 55.4% with the interval being 36.8 – 66.1% in the three experimental years. Seed set was further analysed in a four year pot experiment. Here FSU was in average 61.6%. Seed set in the basal floret was 78.3% whereas the floret in position eight from the base has a seed set of 49.7%. Our results show that unproductive florets are found in all floret positions.

We analysed caryopsis length of seeds from one experimental year using the spectral imaging system VideometerLab instrument (Videometer A/S). Our results indicate that the length of the caryopsis relative to the length of the palea is consistent throughout seeds in position one to eight from the base of the spikelet.

# Developing viability testing methods for native seeds to promote their use in re-vegetation of desertification lands - a summary of recent works

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**Abstract**

To promoting the use of native seeds in re-vegetation of desertification land, the tetrazolium testing procedures for viability have been developed for three psammophytes growing in north China, *Nitraria tangutorum*, *N. sibirica* and *Agriophyllum squarrosum*. Now, the viability percentages of the three species can be obtained within two days.

**Key words**: *Agriophyllum squarrosum*; *Nitraria tangutorum*; germination; tetrazolium; viability

# Physiological and biochemical responses to ultrasonic treatments on aged grass seeds, germination and seedling growth

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**Objective:** The study tried to enhence aged seeds germination, vigour and seedling growth using ultrasonic treatments on three grasses, smooth bromegrass (*Bromus inermis* L.), tall fescue (*Festuca arundinacea*) and Russian wildrye (*Psathyrostaehys juncea* Nevski).

**Methods:** The three grasses seeds are respectively storaged 3 to 12 years in room temperature. To evaluated the sonication effect on the three aged grasses seeds germination and seedling growth using an orthogonal matrix design, three ultrasonic factors were selected, including sonication time (factor A), sonication temperature (factor B) and ultrasound output power (factor C). The activity of SOD, POD, CAT and MDA content of the treatments were determined. Also, each treatment were evaluated for germination percentage (GP); The selected seedlings of the treatments were evaluated for plumular and radicular lengthes (PL and RL). The binary quadratic regressions were performed to investigate the coupling effects of pair-wise factors and to assess optimum ultrasonic conditions.

**Results**: The variance analysis showed that factors A, B, and C were significant in GP, PL and RL ($p < 0.01$), and there were significant coupling effects both pair-wise and among the three factors. The multivariate analysis of variance was significantly different. The range analyses showed that effects of factor B had the highest averaged R value, then orderly came factor A and B. The activity of SOD, POD, CAT and MDA content were changed.

**Conclusion**: The ultrasonic has positive effects on the aged seeds germination and seedling growth. It provides a basic evidence for applying ultrasonic to pretreat the aged grass seeds. The conditions of the three factors were optimized. In addition, as a simple, cheap and time saving method, ultrasonic treatment has the potential to be used in improving the seedling growth.

# Effect of light on seed germination and its practical implication

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**Abstract**

Light is one of the most important environmental factors control seed germination and thus seedling establishment. Understanding the effect of light on seed germination may pose great potential in improving seedling emergence as well as weed control. The role of light in controlling seed germination varied with species, genotypes, germination condition and their interaction. The objective of this study is to review and summary current advances in light regulated seed germination, and tries to provide some hints for agricultural practice.

**Key words:**light; seed germination; weed control

# Effect of increasing temperatures on germination of two varieties of *Panicum coloratum*

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**Abstract**

*Panicum coloratum* is a subtropical forage grass, native to South Africa, with great potential to improve pasture production in marginal areas in Argentina. It is composed by several varieties; the two most known are var. *makarikariense* and var. *coloratum*. Varieties differ in their range site adaptability making them suitable for different environmental conditions. In this study, the germination response along a range of temperatures from 5 to 45 ºC was compared between the two varieties of *P. coloratum*. Fifteen seeds of each variety were set in a Petri dish, imbibed in distilled water, and put in a germination chamber compartmentalized to maintain temperatures of 5, 10, 15, 20, 25, 30, 35, 40 and 45ºC under an alternated cycle of 12/12 hours light/darkness. Each temperature treatment consisted of five replicates. Seeds were considered germinated when either the radicle or the plumule were visible. Beside germination percentage (GP), indices as mean germination time in days (MGT) and index of germination velocity (IG) were calculated as: $MGT= \Sigma(t_i*n_i)/\Sigma n_i$ where $t_i$ is the number of days and $n_i$ is the number of seeds germinated per day; and $IG=\Sigma G/t$, where G is the accumulated germination percentage per day and t is the duration of the trial in days. GP, MGT and IG were analyzed by ANOVA with temperature and varieties as main factors. Interaction temperature*variety was only significant for GP. A steady increase in GP with increasing temperature was observed in the two varieties up to the highest temperature (45ºC). No seed germination was registered at temperatures of 5 and 10ºC. Varieties did not differ in MGT and IG (mean values were 5.57 days and 49.65, respectively). Shortest MGT was 2.38±0.62 days at 45ºC and maximum was 8.62±0.62 days at 15ºC. Lowest IG was registered at 15ºC to a top at 45ºC (8.23±4.49 and 75.6±4.49, respectively). Results showed that most favorable germination conditions are set to higher temperatures and are not differentiated between varieties. Temperatures over 45ºC need to be evaluated in order to determine optimum germination temperature of the species.

**Key words**: germination; temperature; varieties; warm-season grass

# Development of a PCR protocols to rapidly distinguish between similar herbage seeds

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**Abstract**

Most forage seeds are significant for livestock, supporting a high economical global seed export market. Adulterations in pasture and forage seeds can radically lower the forage value and create weed components in the grassland. Distinguishing adulterating seeds from one another is difficult because of higher similarities in colour, shape, size and weight. Existing methods to distinguish these seeds are usually time-consuming and inaccurate. So our lab developed two novel molecular methods to distinguish similar species: the first method is based on variable sites in homologous genes, two pairs of primers were designed and used with simple PCR and PAGE techniques to identify whether the specific band is existing or not, such as sorghum-sudangrass hybrids (*Sorghum bicolor* (L.) Moench x *S. sudanense* (Piper) Stapf) mixed with different proportions of sorghum (*S. bicolor* (L.) Moench). The results showed that this method can clearly distinguish sorghum-sudangrass hybrids mixed with 1% sorghum seeds. The second method is based on the large inserted fragment of homologs between species, designed one same pair of primers to examine different amplicon sizes such as alfalfa (*Medicago sativa*) and sweet cover (*Melilotus* spp L.), and *Lolium perenne* L and *L. multifolorum.* Our results showed the pair of primers was clearly different between the two species, supporting the reliability, sensitivity and simplicity of this method. The two methods have the potential to be extensively applied in the production of herbage seeds.

# Physiological and ultrastructural changes of embryo cell related to storage of Siberian wildrye (*Elymus sibiricus* L) seeds with different moisture content

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**Abstract**

This study was conducted to examine the changes in antioxidant enzyme activity, superoxide anion radical content, malondialdehyde (MDA) content and the ultrastructure of the embryo cells in Siberian wildrye (*Elymus sibiricus* L) seeds with different moisture levels (4%, 10%, 16%, 22%, 28%, 34%, and 40%) and stored for 3, 6 and 12 months at 4 °C, and to determine the tendency of physiological changes occurred during seed deterioration. The results showed that the seed germination percentage decreased as moisture content increased from 4% to 40%, decreasing significantly in the 16-40% moisture range as the storage duration increased. The activity of antioxidant enzymes, superoxide anion production rate and MDA content of seeds were all maintained higher levels at 4-16% moisture content. While superoxide dismutase (SOD) activity and MDA content both continued to decrease at 22-40% moisture content, ascorbate peroxidase (APX) and catalase (CAT) activity and superoxide anion production rate all increased. Storage duration had a different effect on the antioxidant enzyme activities, superoxide anion production rate and MDA content. Under condition of 4-16% moisture content, the sensitivities of SOD, APX and CAT were different for the oxygen free radical scavenging with seed stored from 3 months to 12 months. Ultrastructures of cell membrane and mitochondria were normal at 4-10% moisture but began to transform at 16%, then observed by the formation of cavities, breakdown of organelles at 28-40%. During the low temperature storage of Siberian wildrye seeds, the integrity of membrane system and activities of antioxidant enzymes were the key factors for maintaining seed vigor at 4-10% moisture content. The ultrastructural destroy was the reason for the loss of seed germinability as moisture content higher than 22%, despite of activities of antioxidant enzymes.

**Key words:** moisture content; seed deterioration; Siberian wildrye; storage duration; ultrastructure

## Single counts of radicle emergence to predict seed vigor of *Elymus nutans* and *Avena sativa*

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**Abstract**

Predicting seedling establishment is very important for grass production. The simplest way to measure germination and quality of seed lots is to control radicle emergence (RE) in a set of specific environment conditions, usually in optimal conditions. Radicle emergence can be defined as the radicle just breaking through the seed coat (pericarp/testa), while that breaks the endosperm but does not appear excluded. *Elymus nutans* is a perennial grass and *Avena sativa* is an annual grass, both of them are important grasses with high adaptively and forage quality. Due to the poor establishment of these two grasses, vigor test appears to be very important. Single counts of radicle emergence are considered to be more rapid and alternative than the existing methods. This work was conducted to test the field emergence (FE) and mean field emergence time (MET) in different sowing times with 20 seed lots of Elymus nutans and 15 seed lots of *Avena sativa* in Xiahe county of Gansu Province from April to June in 2014. Single counts of radicle emergence were tested in the laboratory in every 4 hours. The results showed that (1) in every sowing time, FE was negatively correlated with MET for both species; (2) single counts of RE were significantly positively correlated with FE, while negatively correlated with MET (44 h at 25℃ for *Elymus nutans* and 56h at 20℃ for *Avena sativa*). Therefore, single counts of radicle emergence in certain times of germination can be used to measure tested forage grass species.

# Seed Moisture Content Determination of Several Forage Species

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**Abstract**

Seed moisture content of 43 seed batches of 27 forage species were determined with two methods which including low and high constant temperature. The results showed that the high constant temperature oven method 1 hour is suitable for seed moisture determination of *Elymus sibiricus*, *Haloxylon ammodendron*, *Myosotis silvatica* and *Sanvitalia procumbens*, while high constant temperature oven method 2 hour is suitable for seed moisture determination of *Agriophyllum squarrosum*, *Agropyron cristatum*, *Bromus inermis*, *Elymus sibiricus*, *E. dahuricus*, *E. nutans*, *Festuca elata*, *Haloxylon ammodendron*, *Lotus cornioulatus*, *Onobrychis viciaefolia*, *Poa annua*, *Rumex patientia*, *Sanvitalia procumbens*, *Trifolium repens*, *T. hybridum* and *Uraria crinite*.

**Key words**：high constant temperature oven method；low constant temperature oven method；seed moisture content

# Impacts of exogenous ascorbic acid on germination, membrane permeability and ultrastructure of embryo cells of aged *Elymus sibiricus* L. seeds

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**Abstract**

Our objective was to determine the effects of exogenous ascorbic acid (AsA) treatment on germination, seedling vigour, membrane lipid peroxidation and ultrastructure of embryo cells of Siberian wildrye (*Elymus sibiricus* L.) seeds during artificial accelerated ageing. This experiment was designed that seeds were aged at 45°C and 100% relative humidity for 0, 24, 48 and 72 h to prepare for samples. The results indicated that germination percentage, shoot length, root length, shoot weight, root weight and vigour index declined gradually, leachates electrical conductivity value and malondialdehyde (MDA) content of seeds increased progressively as artificial accelerated ageing time prolonged from 0 h~ 72 h. In addition, ultrastructure observation of embryo cells showed that serious damage occurred in seeds with 72 h artificial accelerated ageing, including the broken cellular and nuclear membrane, pyknotic nucleolus and swollen mitochondria. However, exogenous AsA treatment had obviously protective effects on alleviating the damage of ageing to seeds, especially for seeds artificially aged for 24 h ~ 72 h. AsA treatment significantly ($P < 0.05$) enhanced seed germination percentage and vigour index during 24 h ~ 72 h ageing time, and correspondingly electrical conductivity and MDA content significantly ($P < 0.05$) decreased. Meanwhile, seedling growth parameters shoot length, root length, shoot weight and root weight of AsA-treatment seeds were significantly ($P < 0.05$) improved at 72 h ageing time. In addition, AsA treatment also had effective function on protecting integrality of membrane system and structure of mitochondria, especially when seeds were damaged seriously at 72 h ageing time. Therefore, the application of exogenous AsA could alleviate the activities of lipid peroxidation and the damages of membrane integrity to maintain seeds vigour.

# Optimization of hydro-priming condition for alfalfa seed

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**Abstract**

This study determined the optimal conditions for hydropriming of *Medicago sativa* seed by manipulating priming temperature, duration and water addition. The optimal response to hydropriming was achieved at 10℃ for 36h with 80% water of original seed weight.

**Key words**: Germination speed; seed priming; seed vigor

## The effect of sterilisation on seedling root fluorescence of *Lolium* species

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**Summary**

The seedling root fluorescence test is widely used as an ISTA approved test in commerce as an indicator *of Lolium multiflorum* contamination of *Lolium perenne* seed lots. Despite its widespread use some unexplained variation has been observed in results.

Tests were undertaken of sterilised seeds of *Lolium multiflorum* and *L. perenne* using heat treatment, or with the antibiotics streptomycin and neomycin. All treatments showed a significantly lower % of seedlings fluorescing than unsterilized seeds. Further research is required to determine the reason for this.

**Table.** Seedling fluorescence of three *Lolium multiflorum* and *L. perenne* cultivars and breeding lines after various sterilisation treatments

| | Asset Total UV%* | Asset Bright UV% | KLp511 Total UV% | KLp511 Bright UV% | Base Total UV% | Base Bright UV% | One50 Total UV% | One50 Bright UV% |
|---|---|---|---|---|---|---|---|---|
| Control | 98.2 | 98.2 | 45.6 | 45.1 | 32.8 | 28.9 | 12.1 | 12.1 |
| Neomycin | 88.0 | 73.0 | 31.3 | 28.8 | 25.0 | 21.3 | 3.8 | 3.8 |
| Streptomycin | 89.5 | 84.1 | 28.4 | 24.7 | 29.7 | 29.1 | 2.4 | 2.4 |
| Hot water 60$^o$ >15 min | 88.8 | 88.8 | 27.1 | 24.1 | 25.1 | 21.3 | 5.1 | 5.1 |
| LSD 5% = 8.3 | | | | | | | | |

*Total UV% refers to seedlings with any fluorescence (bright or dull);

**Bright UV% refers to only those which fluoresce brightly

# Effects of Endophytic Fungi Under Artificial Ageing Treatment on Seed Physiology of *Hordeum brevisubulatum*

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**Abstract**

An experiment was conducted to study the change of vigor of *Hordeum brevisubulatum* seed under the conditions of 58±1℃ and 100% relative humidity, and the artificial ageing time was set as 0, 5, 10, 15, 20, 25, 30 and 35min. The results indicate that in the process of artificial ageing seed germination percentage, germination energy and vigor index of *Hordeum brevisubulatum* seed as well as plumule length and root length decreased. The conductance and the soluble sugar correlated with the seed extract increased with artificial ageing, and they had a significant negative correlation with germination percentage, germination energy, plumule length, root length and vigor index ($P<0.01$). This study suggested that compared with endophyte-free seeds, the endophyte enhanced seed germination of *H. brevisubulatum* with longer plumule and root lengths, reduced the damage of plasmalemma and decreased the soluble sugar content and conductance of seed extract. This resulted in reduced seed deterioration and extended the storage time.

# A method to maintain the genetic purity of alfalfa (*Medicago sativa* L.) varieties in Italy

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**Abstract**

In Italy for all varieties listed in the National Register there is an obligation of preservation in genetic purity. When they apply for registration, the breeder, or the holder of the variety, should indicate: i) the name of the head of the variety conservation; ii) where the conservation is carried out; iii) the method used for the maintenance.

Alfalfa (*M. sativa*) is a perennial and outcrossing species and is the most widely planted forage legumes for hay, pasture and silage in the world because of its highly nutritious forage and broad adaptability. Alfalfa also has significant benefits for sustainable cropping systems because its deep root system and perennial nature limit soil erosion and improve the soil. Alfalfa breeding programs are based on recurrent phenotypic selection, with or without progeny testing, to accumulate desirable alleles at high frequency into a population.

In alfalfa an appropriate maintenance of varietal purity is done by following two strategies. The first strategy consists of conservation of seed foundation in the freezer; the amount of this seed must be adequate to establish fields for the production of certified seed. The second strategy is represented by conservative selection. In this paper the second strategy will be considered.

In spring 2012 about 1,000 plants of four alfalfa varieties, three from the North and one from the Center Italy, were transplanted in experimental field located where the varieties were developed. The experimental fields were spatially isolated from other potential contaminations of alfalfa pollen sources. In both experiments, plants were established in a spaced plant design with 90 cm between plants in both directions. During the 2012-2013 and 2013-2014 seasons a negative selection was made by removing the plants out type. Specifically diseased plants, that had yellow and variegated flowers, and all those that were different from the ideotype for traits like: vegetative regrowth, leafiness, plant habit and flowering time, have been eliminated. In July 2014 seed was harvested on the remaining plants. Part of the harvested seed were stored in the freezer and the remaining seed will be used to establish fields for the production of certified seed.

# Appropriate Temperature and Time for a Controlled Deterioration Vigor Test in Seeds of Switchgrass (*Panicum virgatum* L.)

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**Abstract**

Switchgrass (*Panicum virgatum* L.) is a highly versatile grass, used for soil and water conservation, livestock production, and biomass production for conversion to energy, for which a proper method of testing seed vigour has not been successfully developed. Controlled deterioration is one of the widely used vigor tests to determine the quality of small-sized seeds. This study was conducted to identify the appropriate combination of temperature and time for controlled deterioration vigor test to switchgrass seed lots according to their physiological potential. Four seed lots of the cultivars 'Alamo' and 'Cave-in-rock' were used. They were grown at the Northwest A&F University forage research center, and harvested in 2012 and 2013, respectively. The controlled deterioration test was carried out at three different temperatures: 40, 45 and 50℃ with 10 duration periods of 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 hours, and with a seed moisture content of 20%. We measured seed germination percentage, germination index, vigor index and seedling growth indexes in the laboratory. The results showed that controlled deterioration under conditions of moisture content adjusted to 20% at 45℃for 96 h were appropriate procedure to assess the seed vigor of switchgrass. With seed lots harvested in 2013, seed germination percentage increased significantly compared to the control for temperatures under 50℃ aged for 12 h up to 72 h; and then decreased with longer aging time. This is because more than 90% of these two cultivars new harvested seeds could be dormant, and appropriate high temperature was a conducive method to break dormancy. This research suggested that the controlled deterioration test could be successfully utilized to evaluate seed vigor of switchgrass, and we recommend its use in seed quality control programs.

# Forage pea seed quality as affected by seed moisture at harvest and genotype

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**Abstract**

Forage pea (*Pisum sativum* L.) is an annual legume crop rich in protein, traditionally grown in the regions of South East Europe for high quality fresh forage, haylage or hay. However, cultivating this crop on a more significant acreage is hindered by high seed rates needed (150-330 kg $ha^{-1}$) and low seed quality. One of the most important factors that affect the physical seed quality is the seed moisture content at harvest. The primary objective of this study was to identify the optimum seed moisture content at harvest time in order to maximize physical seed quality of forage pea. A three-year experiment was conducted at the Institute of Field and Vegetable Crops in northern Serbia during 2010-2012.

The seed moisture content, interim germination ($4^{th}$ day = germination energy), final germination ($8^{th}$ day), proportion of abnormal seedlings and 1000-seed weight were analysed at the eight harvest stages (H1-H8) and in 10 forage pea genotypes (G1-G10). At the first harvest stage (H1), the seed moisture content was 20.4%, while at the eight one (H8) it was 10.3%. The highest seed quality, with an interim germination of 82% and a final germination of 90%, was at H6 when the seed moisture content was 12.9%. The lowest seed quality was at H1, with an interim germination of 68% and final germination of 78%.

The highest interim germination ($4^{th}$ day) was 82% with a final germination of 92% and the lowest proportion of abnormal seedlings (2%) had been observed in the earliest-maturing genotype (G9), which is characterized by determinant stem growth and very uniform seed maturation. The lowest interim germination (70%), final germination (76%) and the highest proportion of abnormal seedlings (4%) had the genotype G1 with the longest growing season, indeterminant stem growth and non-uniform maturation.

# Determining optimal germination condition and setting seedling evaluation guidelines for fifteen herbage and herbal medicinal seeds

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**Abstract**

Seed germination is one of the most important tests for assessing the potential planting value of seed. Standardized method in the test is a prerequisite for achieving reliable and comparable results among different seed testing laboratories. In this research, seeds of fifteen wild plants (*Achnatherum sibilicum, Artemisia ordosica, Artemisia sphaerocephala, Artemisia wudanica, Astralagus adsurgens, A. adsurgens, Caragana intermedia, Ceratoides lateens, Elymus dahuricus, Hedysalum leave, Hedysalum scoparium, Lactuca indica, Lespedeza davurica, Polygonum divarcatum* and *Stipa krylovii*), which were not yet listed in the official testing books, were subjected to different germinating conditions in order to find the most suitable conditions for seed germination testing. The conditions include 6 different temperature regimes (constant and alternative temperatures), with illumination or in dark, and on 3 different germinating beds. Germination percentage and germination speed were measured and used as indicators to evaluate seed germination performance. Times of first and final counting were determined based on seed germination progress. Seed germinating behavior and seedling morphogenesis features were observed in order to classify the seedlings into different evaluating categories. The results showed that most of the tested seeds are orthodox seeds with optimum germinating temperature of 20-25$^{0}$C, no special preference for light or dark. All of the three germinating beds were satisfactory for germinating testing, but for economical consideration and convenience of preparation, top of paper bed was recommended in routine testing practice. With referencing the similar species listed in the international or national seed testing rules, “standardized” methods and procedures were recommended for the 15 tested species in the testing laboratories. It was also hoped that the methods and procedures to be adopted by the concerned organizations in the future revised versions of the seed testing books.

**Key words**: germination testing; seedling evaluation; herbage seed; herbal medicinal plants; wild plants; standardization

## Study on the Storage of Ultradried *Haloxylon persicum* seeds

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**Abstract**

Moisture content of *Haloxylon persicum* seeds was dried to 10.57%, 5.23%, 4.24%, and 3.25% in a desiccating container with silica gel, and stored at 35°C, 25°C and 4°C for 12 months. This study was to determine whether the vigor of the *H. persicum* seeds can be maintained at a higher level and the characteristics of their storage can be improved by ultradry storage. The data from 12 months showed that the optimum moisture content for storage varies with temperature. After ultradrying the seeds were accelerated aged (45°C, 2 days), some physiological and biochemical indices were tested. The results indicated that POD, SOD and CAT activities of the ultradried seeds were higher than those of the control seeds, while malondialdehyde were lower than the control group. The results showed ultradrying treatment did not induce any significant changes in seed germination and moisture content of seed was a key index for storage at ambient temperature (15°C) and 3.25% seem to be the best moisture content for ultradried seeds in our research. The storability of ultradried seeds was improved significantly. Based on these results it has been concluded that ultradrying was effective and safe to the seeds of *H.persicum*.

## Dormancy type and dormancy release of *Anemone rivularis* seed

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**Abstract**

*Anemone rivulars* is a perennial herbaceous plant whose rhizomes and leaves are used for Chinese herbal medicine. In addition, *A. rivulars* is an important plant of alpine meadow on the Tibetan plateau, playing an important role on grassland ecosystem, the stability of the biological diversity and the health of livestock production. We studied the dormancy type and dormancy release of *A. rivulars* seeds, revealing its life cycle characteristics to provide a basis for managing alpine meadow steppe, recovering degraded grassland and cultivating *A. rivulars.*

The results showed that there is no significant allelopathic effect of aqueous extract liquid of *A. rivularis* seedcase on the seed germination of *A. rivularis.* Removing the seedcase can break the seed dormancy of *A. rivularis* effectively. The dormancy type of *A. rivularis* seed (achene) is combinational dormancy (physical dormancy + physiological dormancy, PY+PD). The differences of *A. rivularis* seed germination percentage between storage period of 40, 80, 150 and 180 days at room was not significant ($P$>0.05), but all were significantly higher than that stored for 0 d treatment ($P$<0.05). The germination percentage of the *A. rivularis* seed of the stratification with trample under alternative temperatures of 25/15 ºC and 20/10 ºC was 38.0％and 35.3％, and was significantly higher than that of the stratification treatment without trampling (was 22.7％and 14.0％), respectively ($P$<0.05); while seed with un-removed seedcase stored at dry and room temperature in the same period (November to April) did not germinate. After stratification at the grazing land of Tianzhu alpine meadow from October to August of the following year, 40.2％ *A. rivularis* seed germinated. Un-germinated seeds can germinate under the temperature ranged from 30/20 ºC to 20/5 ºC, the germination percentage (15.3％) at 30/20 ºC was the highest among that, so the majority of seeds still remain dormant.

# Analysis on imported forage seeds in Beijing port

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**Abstract**

With the improvement of forage structure in China, especially the unprecedented development of urban green areas, the demand for excellent grass species is increasing and we have the introduction of a large number of forage seeds from abroad. But there are risks when we import large amount of forage seeds, especially that quarantine pests will invade our country.

In recent years, the number of forage grass seed lots arriving via Beijing port has been the first in the country. According to the statistics between 2009 to 2013 years, the quantity of imported seeds is nearly 75,000 tons . How to control the potential for disease invasion is a severe situation for us by now. Through risk analysis we have determined the key quarantine objects and targeted detection is our main focus. Now we have done a lot of work, and achieved certain results. We hope to continue to improve the work to control the invasion of disease through the above ways, in order to protect pasture production safety in China.

**Key word:** Beijing port, Forage seed, risk analysis

Foundation project: Scientific and technological project from Beijing Entry-Exit Inspection and Quarantine Burea—Risk assessment on Imported grass seeds（2015BK024）

# Session 5. Seed development in developing countries, and extension, tech transfer and training

# The difficulties of smallholder village farmer forage seed production in Thailand and Laos

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**Abstract**

Forage seeds in northeast Thailand and northern Laos are produced by over 1,000 smallholder village farmers. The seeds are all harvested by hand. This paper examines the difficulties of managing production and maintaining seed quality control.

1. Logistics. Villages are scattered over large distances, and in northern Laos the terrain is mountainous and the roads to villages predominantly unsealed. This makes regular extension trips difficult and many fields can not be inspected and training of the farmers can not always be done as well as should be.

2. Low seed yields. Farmers in Thailand apply fertilizer but often not enough to substantially increase seed yields. Farmers in Laos apply no fertilizer at all, with yields less than half that of Thai farmers.

3. Seed quality. Many farmers do not dry to a low enough moisture content and this can create problems later on with seed germination and seed vigour during seed transportation, storage and seed processing. Some farmers mix low quality seed of other species with similar looking seed into the commercial seed to be purchased.

4. Outside buyers. Agricultural traders often try to “steal” seed by offering higher seed prices for unclean seed before we get to buy clean dry seed from the farmers. This can result in shortfalls in certain cultivars, with potential buyers losing confidence in our delivery system.

5. Government bureaucracy. In Laos, the forage seed programme must be approved by local government organizations in each district and province.

6. Land use competition. Many farmers do not own their own land for forage seed production and must rent land. Other crops compete for rented land and drive the costs of rent up.

**Key words:** Brachiaria, guinea grass, village farmers, seed harvesting, seed quality

## Introduction

Tropical Seeds LLC, a subsidiary of a Mexican seed company, Grupo Papalotla, employs Ubon Forage Seeds group at Ubon Ratchathani University, Thailand, to manage seed production, seed sales and export, and to conduct research on existing and new forage species (Hare, 2014). In the 2014-15 seed harvesting season, 130,000 kg of seed comprising of eight forage grasses [Mulato II hybrid brachiaria (*B. ruziziensis* x *B. decumbens* x *B. brizantha*), Cayman hybrid brachiaria (*B. ruziziensis* x *B. decumbens* x *B. brizantha*), Cobra hybrid brachiaria (*B. ruziziensis* x *B. decumbens* x *B. brizantha*), Mombasa guinea (*Panicum maximum*), Tanzania guinea (*P. maximum*), Ubon paspalum (*Paspalum atratum*)], two forage legumes [Ubon stylo (*Stylosanthes guianensis*), Greenleaf desmodium (*Desmodium intortum*)] and one green manure legume [Sunn hemp (Crotalaria juncea)], were harvested for seed by over 1,000 village farmers in northeast Thailand and northern Laos. The smallholder seed-production programme has produced positive social and economic outcomes for the village growers and enabled smallholder farmers in other tropical countries to receive high quality seed (Hare, 2014).

All the seeds are hand-harvested, with methods ranging from knocking ripe seeds out of the seedheads for many of the grasses (Kowithayakorn and Phaikaew, 1993), sweeping seeds from the ground for Ubon stylo and some Mulato II seed crops (Hare, 2014) and picking ripe seed pods of Sunn hemp and Greenleaf desmodium. In some villages, small electric sieve seed cleaners are used, but for the majority of the seed crops, the seeds are hand-sieved through mesh and nylon screens and winnowed using cane trays and small electric fans. Seeds are dried on nylon sheets inside houses and out in the sun.

The management of the smallholder seed production programme is intensive and requires constant supervision to maintain seed quality and produce larger quantities of seed. There are many difficulties in farmer supervision, trying to increase seed yields, maintaining seed quality and purity, village and government bureaucracy and the influence of outside traders. This paper will examine these difficulties and how they are overcome or avoided.

**Logistics**

In northeast Thailand all the seed production villages can be driven to on good tar-sealed roads. Forage seed production fields are 500 m to 3 km in distance from the villages and range in size from less than 500m$^2$ up to 1 hectare. The majority of these seed fields can be reached by pickup along tar-seal, concrete, gravel or dirt roads. However, in a few villages, the fields are in hilly land, and access to the fields is either by motorbike or by walking. Critical field inspections for off-types and weeds often can not be done in these fields in the wet season, when small tracks become muddy or blocked with water from overflowing streams.

The access situation in northern Laos is more difficult. Access into many villages is only by motorbike along dirt roads which can become impassable at times in the wet season. The fields are usually some distance from the villages, 1-3 km, up on steep hill slopes and can only be reached by walking. Fields range in size from 200m$^2$ up to 1000 m$^2$ and are scattered over wide areas. Only a small number of the fields can be inspected by the Mulato II seed company in Laos. Instead we reply on appointed farmer representatives to monitor the seed crops and village self-regulation to ensure seed crop sanitation but often this is unsatisfactory, as they too can not inspect all the fields.

The time of seed purchase involves organization and management. The seed of each crop is purchased in the villages. In Thailand, bags are distributed to each village one to two weeks before seed purchase. The farmers bag their clean seed and all the seed from each village is brought to a central place, such as the grounds around a temple, the village meeting hall or the frontage of a house. The seed in each bag is inspected for impurities and tested for moisture, weighed and loaded on to trucks. Farmers are paid in cash that day. Trucks have to be hired to transport the seed back to the sheds at Ubon Ratchathani University.

In Laos, seed purchasing takes several weeks. Bags are not distributed to the farmers prior to seed purchase and farmers are not paid in cash on the day of seed purchase. Instead each farmer receives a promissory note for their seed. On the day of seed purchase, the farmers bring their seed to the village meeting hall where each bag of seed is poured into the company's bags, inspected for impurities and moisture, and loaded on to pickups. In some remote villages with narrow dirt tracks, the seed must be brought out on the backs of motorbikes. The seed from each village is brought together at a central point in the district town and loaded on to larger trucks and taken to the provincial city. There the documentation for transporting the seed out of the province is done and the seed trucked over 1200 km to the capital Vientiane. Export documents are obtained and the seed is then trucked on one large truck and trailer into Thailand and 700 km down to Ubon Forage Seeds in Ubon Ratchathani.

Ubon Forage Seeds weighs every bag of seed before storing the seed in its warehouse. Money for the seed is then transferred back to the company in Laos, who then must return to each village to pay the farmers.

There are often problems with getting all the correct documentation in Laos and frequently there are delays. Trucks can also break down. In Thailand, there are numerous police check points where inspections for overloading and other regulations are enforced.

**Low Seed Yields**

Yields from many temperate grass and forage legume seed crops may exceed 1,000 kg/ha, and more than 2,000 kg/ha for ryegrass (*Lolium*) and *Bromu*s species. With tropical forage seed crops, the average seed yields under good management are between 600 and 1,000 kg/ha. Seed yields above 1,000 kg/ha are rare for tropical forage grasses but sometimes are reached with tropical forage legumes. These low yields can be atributed to a number of factors which are described by Loch and Ferguson (1999). Most of the tropical species are relatively new to agriculture and were only developed in the last 30-40 years. They flower over a long period which makes it difficult to decide the optimum harvest time. Only a handful of cultivars have undergone hybridization and rigorous selection and those that have, such as the hybrid brachiaria cultivars and hybrid buffel grass cultivars, have very poor seed-set (less than 2%) (Miles and Hare, 2007). Furthermore, they have not undergone selection for seed production.

Therefore to get reasonable seed yields from tropical forage seed crops is always a challenge for village farmers in Thailand and Laos. Nearly all the seed crops in Thailand are grown on very poor acid soils which are deficient in nitrogen, phosphorous, potassium and sulphur. Farmers in Thailand do apply some fertilizers, particularly compound NPK fertilizers, but usually not in sufficient amounts to encourage maximum inflorescence density. A rate of 100 kg/ha/year of N is sufficient to increase seed yields (Loch et al., 1999). Generally, farmers in Thailand only apply about 20-30 kg/ha/year of N. In 2015, clean seed yields of *Panicum* *maximum* and *Paspalum atratum* grasses, hand harvested by village farmers tying seed heads together and knocking the seed out, were between 400-500 kg/ha.

Farmers usually buy fertilizer on credit from local cooperatives and fertilizer companies. They would like to apply more fertilizer but it is expensive. In the past we have supplied fertilizer on credit and deducted the fertilizer costs at the time of seed purchase. However, there has never been any noticeable increase in seed yields when farmers purchased their own fertilizer and than when we supplied it. Some farmers spread the fertilizer on their rice and other crops. This has lead to insufficient quantities of grass seed to sell for us to deduct the fertilizer costs. It also was a huge job trucking in tonnes of fertilizer to each village and so we discontinued this practice.

In Laos, farmers do not apply fertilizer to grass seed crops. Seed yields are usually half of those harvested in Thailand (Hare, 2014). Not applying fertilizer is due to a number of factors. Soils are for the most part fairly fertile and traditional vegetable and leaf crops grow reasonably well without fertilizer. Our grasses also grow well vegetatively, but then fail to set a lot of seed. It is difficult for the farmers in remote villages to buy fertilizer if they wanted to, because the retail agricultural shops are far away. The range of different types of fertilizers is limited and the quality dubious. Another factor is government policy, with many districts designated “organic areas” with chemicals and fertilizers prohibited. Using animal manure and compost is encouraged, but these are not applied in large enough amounts to substantially increase seed yields.

To encourage maximum reproductive tiller development, many grass seed crops must be given a closing cut about three months before anthesis (Hare et al., 1999; Phaikaew et al., 2002; Hare et al., 2007a). A lot of farmers are reluctant to do this for fear of harming the emerging seed heads. They have been used to growing rice, maize and other annual crops which are never given a closing cut after planting. Those farmers that would like to implement a closing cut find it burdensome. They must cut the whole crop at one time and there is too much vegetative material to feed their livestock. Without closing cuts, many crops lodge or become very rank and form a low number of seed heads.

Row spacing has been shown to be an important factor in contributing to seed yields in tropical grass seed crops grown at low soil nitrogen levels (Hacker and Jones, 1971; Boonman, 1972; Phaikaew et al, 2002). Wide row spacings of 80-100 cm are encouraged. This also facilitates weeding. In Laos, often the spacings are excessive between plants, ranging from 150 – 200 cm and the resulting low plant density is another contributing factor to low seed yields.

Farmers in harvesting *Panicum maximum* cultivars in Thailand and Laos and hybrid brachiaria cultivars in Laos, tie the seed heads before anthesis and knock the ripe seed out (Kowithayakorn and Phaikaew, 1993; Hare 2014). This method produces good seed yields if the farmers give top priority to grass seed production and knock the seed heads daily or even twice daily (Hare et al., 2007b). Many farmers, however, do not give harvesting close attention, and only knock the seed heads every 2-3 days, resulting in a lot of seed being shed and consequently seed yields are low.

**Seed quality**

Hand harvesting, drying and cleaning, if done correctly, will produce high quality seed of a high purity and a high germination. Farmers must receive thorough training and supervision in seed processing from harvest to cleaning the final product. Seeds that are knocked from the seed heads must be knocked out gently to get mature seed. If the seeds are pulled vigorously off the seed heads, a high proportion of these seeds can be immature leading to low germinations. Farmers over time, can become casual and want to harvest the crops quickly. They don't want to return every day to harvest the seed. Some farmers therefore pull the seeds off the seed heads to save time and also to avoid seed shedding of ripe seed.

Hand-knocked grass seeds have a high moisture content (over 30%) and must be dried promptly to avoid germination lose and fungi built-up. The drying process must be done in the shade as rapid drying outside under the hot sun will lead to shriveled seed of low germination. Some farmers do not dry promptly. They just leave the fresh seed in open bags in their houses and think the seed will dry slowly this way. Other farmers want the seed to dry quickly and so immediately spread the seed out in the hot sun. Some farmers do not want to dry the seed to below our standard of 10%, as they think they will lose money with the weight loss. We can not inspect all the villages during seed harvesting and processing. We therefore inspect every bag of seed at the time of purchase and check for moisture, purity and seed weight.

Another problem which developed two years ago was that some farmers mixed their seed with cheaper seed of a different cultivar but of the same species. Both cultivars have almost identical seed which are very hard to differentiate between in open bags. We were not looking for this seed cultivar contamination at the time of seed purchase, as the village involved had been harvesting high quality seed for a number of years. It was not until complains started coming in from customers that we were alerted to the problem. In the laboratory the seeds can be differentiated, though it is not easy. Currently we have stopped seed production in this village.

With seed harvested by ground sweeping in Thailand (Mulato II and Ubon stylo), it is important to have thorough weed control in the growing season. In some countries, weed seeds found in our ground-harvested seed crops are prohibited weeds. For example, *Sida* spp., *Crotalaria* spp. and *Richardia braziliensis* are common in Thailand but prohibited in many other countries. There must be regular field inspections throughout the growing season and farmers instructed to hand weed their fields. Not all farmers are conscientious about rouging weeds. No herbicides are used on forage seed crops in Thailand and Laos for weed control.

**Outside buyers**

From time to time we have had problems with outside traders coming into the villages and trying to buy our seed from our contract growers. The problem is mainly in one species, *Panicum maximum* cv. Tanzania. Traders offer the farmers a higher price for freshly harvested seed, not dried or cleaned, than the price we buy clean, dry seed for. Some farmers are very tempted by these offers as it saves them the expense and time in drying and cleaning. We are then faced with the problem of having insufficient amounts of seed for our customers.

The traders may dry the seed if it is very moist, but usually not low enough (<10%) for medium-term safe storage. The seed is also not cleaned. They store the seed in ordinary warehouses at ambient temperatures, resulting in low germination seed reaching the market place in Thailand and other countries in Southeast Asia. The traders sell this seed at prices cheaper than our good seed.

This practice is very difficult to control as we are over 100 km away from these villages. We have to rely on the honesty of the farmers not to sell our seed to other traders. Slowly this situation is coming under control, with the farmers regulating themselves and the high reputation our good seed has in contrast to the low reputation of trading companies' seed.

**Government bureaucracy**

In Thailand the seed programme proceeds smoothly with no interference from government agencies. We are able to go directly into villages, sign contracts with the farmers, buy the seed and transport the seed to the university and export overseas. In Laos the situation is more difficult. There the programme must be approved by the local government in each district. If meetings are held in the villages, the local government agricultural officer must be invited. The grass seed programme becomes part of the government plan for agriculture. When the seed is purchased, permission must be requested to truck the seed out through various provinces to Vientiane the capital. Local taxes must be paid. If the proper procedures are followed, the programme runs smoothly.

**Land use competition**

In Thailand cassava is a major competitor with forage seeds for land, particularly with seed crops of Mombasa, Tanzania and Mulato II. Hare (2014) discussed the costs and incomes received by farmers for various crops and forage seeds. Increases in the price of cassava can seriously impact on how much land is available to plant forage seeds. We currently have villages that a few years ago were producing up to 10,000 kg of grass seed per year. Now they produce no grass seed at all and the land has been planted into cassava and rubber.

In other villages, many farmers do not own the upland on which they grow forage seeds. They must rent this land. Outside business people who want to grow cassava are coming in and pushing the land rentals up. These rentals have doubled in two years from US$30/ha per year up to US$60/ha per year, forcing the village forage seed growers to either reduce the amount of land they will rent or to seek poorer, cheaper land some distance from their villages.

**Changing species and cultivars**

Village farmers over time become very proficient at producing and harvesting seed of certain species. The Mulato II seed farmers in Thailand sweep the seed from the ground because harvesting fits after the rice harvest and suits their crop management patterns. They dislike having to tie seedheads of any crop and therefore refuse to produce panicum seeds because of hand knocking from tied seed heads at harvest. They refuse to produce Ubon stylo from hand seeping because they say the seeds are too small to clean thoroughly. The Ubon stylo seed growers in Thailand refuse to sweep seed of other species from the ground saying the ants eat the seed before they can harvest. The panicum seed growers in Thailand refuse to sweep from the ground because they say the ground preparation is too difficult. They also refuse to harvest any legume that has pods. Laos farmers only like hand knocking from seed heads and refuse to sweep fallen seed from the ground.

It is therefore difficult for us to get the farmers to change from one species to another if market demands for one species declines and another increases. For example, in 2015 market demands for our Mulato II and panicum seeds has fallen and we have to cease seed production in many villages. We have offered the farmers crotalaria seed to produce because we have a demand for this seed. However, many farmers have refused to plant crotalaria because pods must be hand-picked and they say this is too time-consuming.

**Conclusion**

Village farmer seed production, which involves hundreds of farmers producing seed on small areas of land, is a difficult management operation. At times it becomes a social orientated enterprise that has to deal with many factors outside a normal business operation. There are difficulties with organizing the production over large areas, maintaining high seed quality, dealing with bureaucracy, farmers not honoring contracts, low seed yields, other competing cash crops and continual farmer training as new species are introduced.

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# Extension - delivering opportunities to the New Zealand herbage seed industry

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**Abstract**

Information flow to the New Zealand herbage seed producer can be very effective as it is a small cohesive industry. Some key factors that help ensure good uptake of new information are, firstly the farmers recognize that to remain competitive they need to be aware of, evaluate and implement new practices rapidly into their farming system. This desire for new knowledge is then supported by many of the researchers and consultants working collaboratively to ensure they deliver the same messages. A third key factor is that the production area in New Zealand is geographically condensed and the same extension personnel are working with farmers across the country. Farmers are introduced to new ideas and management practices early in the research through an effort to undertake much of the research on commercial farms. Integral to the successful delivery and uptake of new information are the industry good supported Grass Seed Production discussion groups. These groups have been operating for over 23 years and the key messages related to optimizing productivity are discussed with farmers in their local fields on two or three occasions each year. These face to face discussions are supported by regular email updates, written reports and presentations. In future new methods are being explored to ensure New Zealand farmers have the best information.

**Key words**: extension tools, extension approaches

## Introduction

It has been said that there is sufficient scientific knowledge in the world - the problem is getting it to the users. Estimates of time for technology transfer to the user are depressing - surveys indicate that it takes seven years to make a change in 1% of growers, another seven to achieve 10% (the critical threshold for adoption) and another seven for the rest. In the herbage seed industry in New Zealand we are trying to reduce that 21 year challenge. The type of technology and the cost to implement the technology has a marked impact on the rate of uptake. For example, it took 55 years from 1920 for 90% of US farms to have tractors (high cost, high risk, benefit not clear) whereas it took 25 years from 1935 to achieve 90% use of hybrid maize (low risk, medium cost, high benefit) and eight years from 1997 to achieve 80% uptake of use of GE maize hybrids in the US (low risk, low cost, medium benefit) (Pardey et al., 2010). Although the risk, cost, benefit equation may well influence the time frame for uptake of these technologies, it is generally accepted that new technologies are implemented more rapidly in agriculture today than a few decades ago.

In 2011 New Zealand produced approximately 38,000 tonnes of herbage seed and, of this $70 million dollars of herbage seed was exported (Hampton et al., 2012). Seed is produced from over 14 species and exports went to more than 30 countries with the major markets being Australia, America and the EU (Pyke et al., 2004; Rowarth et al., 1995). The number of species and end user requirements means there is a complex jigsaw of information required by growers to sustainably produce economically viable yields of high quality seed.

Over the last three decades changes in funding (e.g., government subsidies on fertilizer) and regulation (nitrogen and pesticide restrictions) have meant changes in emphasis from maximum production to maximum efficiency of use of inputs (Rowarth, 1997). This, coupled with a dramatic increase in numbers of herbage cultivars entered into certification, increased demands for quality, traceability and sustainable production systems, and the removal of the government supported extension service, means that the New Zealand herbage seed growers have had to be extremely adaptable in order to stay viable.

Extension is a compromise between effectiveness and cost: one-to-one programs are effective but costly (Rowarth et al., 1993); press articles are relatively cheap, but untargeted and, as they are unlikely to contain detail, can, at best, be used to generate interest. Seminars and field days, particularly if focused on a particular topic, and linked with a technical bulletin, are believed to have both reach and impact (Pyke et al., 1997). While awareness can be created from agrimedia reports, good uptake generally requires greater interaction through field events or similar (Lissaman et al., 2013).

Encouraging the uptake of technology by growers is essential if the value of investment in research is to be achieved. Since it was established in 1995, the Foundation for Arable Research (FAR) has been responsible for identifying the applied research needs, commissioning research and delivering the outcomes to provide benefits to farmers. The herbage seed industry in New Zealand has been assisted by targeted extension programs involving scientists working directly with groups of growers (Rowarth et al., 1993; Rolston, 1995). These programs resulted not only in increased yields but also in communication of research needs from growers to scientists (Rowarth et al., 1993).

Extension is recognized internationally as essential for the herbage seed industry, with groups in many countries devoting significant effort to delivering outcomes to growers. However, the successes of extension are not well documented, and in fact, over the seven IHSG conferences to date only three papers have addressed extension.

**Extension**

Within the herbage seed industry there are some key points that are essential to the uptake of technology:

- Involve the growers and target the correct group of growers.
- Keep the message concise.

- Provide a number ways for growers to access the information – push vs pull.
- Remove the risk through delivery of high quality information
- Identify the benefits - not the results or the outcomes.

**Involve the growers**

Involving growers wherever possible in applied research activities will help them to understand the research. It also helps to ensure that the research is practical, focused, relevant, will provide benefits and takes into consideration the issues that growers will need to face when they implement the outcomes of the research on their properties. Keeping growers informed of the research as it progresses helps to encourage uptake by growers when the research is completed, and undertaking it on growers' properties provides confidence that the practice will work and will be applicable to them on their property.

Different growers have different interests, different abilities to understand new technology and different abilities to take risk. Thus the information should be targeted to cater for a reasonably broad group of growers. While the general concept of different ability to take up new technology, as expressed in the diffusion of innovation curve, with groups such as the innovators, followers and laggards (Rogers 1995), applies to herbage seed producers the fit of people to a group may change depending on how readily they understand and accept the technology and what the technology or information will deliver to them.

Fortunately herbage seed growers in New Zealand embrace new ideas and technologies readily, suggesting that the shape of the uptake curve will differ with more innovators and early adopters. This is clearly evident in how rapidly the uptake of the PGR trinexapac ethyl occurred in New Zealand, with estimates of over 95% uptake in three years from the launch of the product (Rolston et al., 2004). However, with FAR research data indicating at least a 20% yield increase and a margin over the cost of the chemical of over $1100/ha then rapid uptake was expected. The benefit to the New Zealand industry is over $8 million per annum. This is not the only example of rapid uptake. Nitrogen use and closing dates are other examples where growers have rapidly adopted new practices.

**Keep the message concise**

The message to growers should be concise. Growers are busy people and need to get the important information quickly. This may mean presenting only the data from trials that is of value in aiding the farmer to make a decision. However, if they want more detail they should be able to easily source the extra information they need. FAR format written and most verbal messages to ensure the key points are clearly articulated but further information is available for those requiring further detail. With the volume of information available, or being pushed to farmers, it is essential the message is concise, particularly if we wish to maximize the value of smart phones, apps and texts. 55% of NZ dairy farmers preferring to receive information by text (Dodd 2015).

**Provide a number of options in ways that growers can access the information**

Different people want to access information in different ways, so it is important that information is made available to growers in more than one way. Most growers prefer to have relevant information pushed to them. If they are searching for new information they may go to a website (pull) but generally they are too busy. Thus it is important the right information is transferred in a way the grower can understand (non-scientific), at the right time of year, in the right quantity, at the right price, and that it clearly identifies the benefits (often dollars) from implementing outcomes of the research (push). Increasingly an extension exercise is considered to be complete when information has been posted on a website, a large electronic filing cabinet often with a filing system that may make little sense to the farmer user.

There is a clear expectation from many research organizations and from government that growers should view the web as a primary source of information. While it has increased in usage in recent years it is not widely used, with only 32% of New Zealand farmers (Lissaman et al*.,* 2013) regularly sourcing information from the web. This is lower than the 65% of arable farmers who indicated they used the web in a survey in 2002 (FAR unpublished data) and lower than the 96% of New Zealand dairy farmers reported to be online (Dodd, 2015).

In New Zealand, the use of the web is limited in some areas by the ability for farmers to operate with good connectivity, and the use of hand held portable devices is also limited by rural connectivity.

**Remove the risk, deliver high quality information**

Key to removing perceived risk is ensuring that the research delivers high quality, relevant and believable information. High quality research can be confidently delivered to growers and the level of risk is minimized. If a field trial is not well managed and the practices used are not relevant to grower practices then it will be extremely difficult for growers to have confidence in the information. If the trial has good significance levels this will help growers accept the data, but greater reliance is placed on the research visually appearing good and the person discussing the results being confident in what is presented.

The research should be designed so that the farmer question "What will happen on my property?" can be answered. This may involve good use of appropriate models, doing the research at a range of sites, with a range of cultivars or environments, or by involving key growers in the research. Farmers can then decide if it will work for them, under their growing conditions and with their expertise.

FAR has tried to ensure that applied research trials address this for growers. For example, research on nitrogen (Rolston et al., 2010) includes results from 17 trials over four years with eight cultivars. This has now expanded to be 28 trials over nine years with 13 cultivars (R. Chynoweth, FAR pers. comm.)

**Identify the benefits**

The benefits to the grower are not the results of the science or the outcomes of the research but what it means to them and their life. Many of the benefits will be judged by the influence on the $$ returns in the short term. However, farmers will also implement research if the dollar benefits can be clearly seen in the future, if the job will be made easier or can be undertaken more safely, if more leisure time will result, or if it will improve the sustainability or environmental acceptability of farming practices in the short or long term.

The financial benefits were clearly demonstrated with trinexapac-ethyl, but the cost benefit can be applied to other management changes. For example, research on fungicides for disease control in Italian ryegrass showed an average $282 margin over fungicide cost per hectare over 5 trials over four years (FAR 2007), an increased profitability per hectare which can be achieved with minimal risk.

Most arable farmers realize the importance and value of arable research and information transfer. FAR consults with farmers to make sure the research is addressing the issues of concern to farmers and that information transfer methods are appropriate. A 2002 random survey of arable farmers showed over 90% support for the ways in which they receive information and 88% of farmers said FAR had significantly improved the information available to them as growers (FAR unpublished data).

It is imperative that farmers are involved in all parts of the research and information transfer process if the research is to add value and be implemented within their business.

FAR use all of the following methods to transfer information:

- Arable Updates: written summaries (2-4 sides of A4) of a research project or information, with a concise key points;
- Newsletters: progress reports on research, information on what research has been funded;
- Discussion groups, Seminars and Field days: researchers discuss the key points of the research;
- Media articles: where growers comment on what research means to them;
- Email: to deliver immediate results of research or information that is timely is available;
- Training courses: presenters go into detail on a specific topic to upskill farmers;
- Strategy documents and booklets: information from a number of trials on a single topic compiled for farmers;
- Website: much of the written information is in a repository on the website;
- Demonstration: research and involvement of farmers in the research.

**Successful extension in herbage seed – Ryegrass 2000 and beyond**

The most successful and enduring extension method used in the New Zealand herbage seed industry are the discussion groups. These groups were started by Dr Phil Rolston in 1993 (Rolston, 1995) and are still extremely popular. For a number of years NZ Arable (a private research and advisory company) facilitated three regional grower discussion groups and FAR became the facilitator from early 2000's. In 2014 nine Ryegrass Discussion Group field meetings were held on growers' properties, predominantly in the Canterbury region. These discussion groups are spread both geographically and chronologically to try to ensure most grass seed growers can attend a meeting on farm in their area at key times in the growing season when they are making major decisions. The number of growers attending each meeting is usually a maximum of 30, so those attending feel comfortable to ask questions relevant to their farms.

These discussion groups are probably unique in that the facilitators are represented across four different organisations; AgResearch (Crown Research Institute), FAR (grower levy R&D organisation), NZ Arable and PGG Wrightson Seeds (a commercial seed company) and that the facilitators are some of the most experienced research and extension personnel in New Zealand. Uptake of new production methods from the information presented at these discussion groups is likely to be high. Payne *et al.* (2009) reported 4.6 changes were made by farmers attending these sort of meetings compared to two changes made by farmers not attending.

These discussion groups have made a very significant impact to the herbage seed industry in New Zealand and this is clearly identified by five points:

- They are probably the longest running discussion groups in New Zealand agriculture and have now celebrated their 22nd birthday.
- An aspirational production goal of 2000kg/ha was part of the group name (Ryegrass 2000) in 1995 (Rolston 1995), but due to the opportunity provided by these groups for growers to see first-hand the trials showing the effects of the new PGR's and strobilurin fungicides, the improvement in production by New Zealand growers this aspirational yield was often surpassed by 2000 and a new target of 3000kg/ha was developed.
- When yields of 2000kg were achieved, a plan to wind down the discussion groups was strongly opposed by growers and there is demand for these discussion groups outside the Canterbury area.
- In 2014 each discussion group meeting was attended by approximately 30 growers. As each region had two separate meetings at least 150 different growers attended the discussion groups. There are approximately 800 growers of herbage seed in New Zealand which means approximately 18% of growers attended the discussion groups.
- 20% of people attending discussion group meetings are seed, agri-chemical and fertilizer company field reps who in turn are in contact with 20+ other seed growers giving a significant multiplier effect

Clearly other extension systems such as seminars, written articles and especially seed company representatives have also impacted on uptake of new knowledge, but no other single method has had such a long term and lasting effect as the discussion groups.

**Looking ahead**

Sustainable production systems are increasingly required by the consumer and much of FAR's work will be in synthesizing information from several research programs to create management protocols that meet these requirements (decreased costs, decreased risk to production, increased yield and protection of resources). At the same time, the importance of producing and maintaining a quality (weeds and vigor) product are recognized (Rowarth *et al*., 1998), and research and extension in these areas is also underway.

The web, apps and email are becoming increasingly important tools in delivering information efficiently to farmers. Relationships with other countries and research groups are also extremely important, and with modern communication systems are getting increasingly easier.

The New Zealand Herbage Seed Industry is successful in achieving rapid uptake of information mainly because we have a number of very good growers who are constantly searching for and implementing new practices on their farms. This, combined with the delivery of information by a small group of trusted researchers and consultants providing high quality information in a familiar on-farm discussion type format, will continue to make a difference to and ensure ongoing viability of their businesses.

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# Research progresses in alfalfa seed production technology in China: an overview

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**Abstract**

This paper reviews the research progresses in alfalfa seed production technology in China since 2000, when the real commercialization of seed production was initiated, with the coming of the first heat wave of alfalfa production. Plant density regulation, irrigation and fertilization constitute the technological foundation of alfalfa seed production.

Insect control, pollination management and harvesting machine adjustment (small-farm-type invention) are the “bottleneck” technologies which we shall break in the near future. Alfalfa seed chalcid and lygus bugs are major insects which infect alfalfa seed in China currently. Some insects such as thrips and cutworms damage flowers (even pods).

In addition, the popularization of the established technologies will be emphasized in the future.

Seed supply of the recognized varieties is limited due to the low seed yields (for example, averaging less than 314 kg/ha in 2013 at national level according to the statistics released by National Animal Husbandry Station), which can largely explain why the quantity of the imported alfalfa seed in China surged from 368 tons in 2010 to 2,500 tons in 2014.

**Key words:** alfalfa; research progress; seed production technology

## Grass seed production research in the Hexi Corridor

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**Abstract**

It has long been recognized that optimal grass seed yield can be attained under appropriate climate conditions. The Hexi Corridor, located in Gansu Province, is one of the major seed production centers for maize, vegetable, and flower in China. The climate in the Hexi Corridor is characterized by dry, sunny, and warm conditions. In recent years, and in part due to insufficient local supplies of high-quality grass seed in China, many agronomical studies have been conducted in the Hexi Corridor to assess its potential as a main grass seed production area. Different species have been tested to identify the best management and agronomical practices, including planting strategies, fertilization, harvest, and residue management. Three-year average seed yields for *Elymus kamoji* (ohwi) S.L. Chen, *Lolium arundinaceum* Schreb. (syn *Festuca*), *Bromus inermis* Leyss, *Elymus trachycaulus* (Link) Gould ex Shinners ssp. *trachycaulus*, *Elymus sibiricus* L., and *Psathyrostachys juncea* (Fisch.) Nevski were 1350, 1120, 1140, 900, 1160, and 2760 kg/ha, respectively. Chinese sheepgrass [*Leymus chinensis* (Trin) Tzvel], introduced from northeastern China, had a seed yield of 430 kg/ha, which is significantly higher than that for natural grassland in northeastern China. However, seed yield for Orchardgrass (*Dactylis glomerata* L.) is very low in the Hexi Corridor due to the limited number of fertile tillers per square meter under local conditions. Timothy (*Phleum pratense* L.) seed yields varied greatly during the studied period, thus suggesting a limited adaptation to the Hexi Corridor. The results of this study appear to indicate that the Hexi Corridor has potential to become one of the main grass seed production centres' in China.

**Key words**: *Elymus*; *Leymus*; native grasses; seed yield

# The challenge of perennial grass seed production in China

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**Abstract**

China currently imports large quantities of seed of perennial grasses yet many indigenous local species, such as *Leymus chinensis,* are required for revegetation of the vast northern grasslands but lack any historical production or mechanization processes. Producing seed of these local perennial species will require large areas of suitable land with available irrigation, government support, and introduction of larger scale windrowing and harvest technologies. Grass seed production has potential to be integrated with the alfalfa seed production systems which already exist on a limited scale in China. The development of a large scale local seed industry will require a concerted and coordinated effort in research, implementation, staff training, appropriate machinery and training, herbicide management, seed testing and investment to develop a profitable long term industry capable of delivering consistent quantities and qualities at economic prices. The paper looks at each of the following issues in detail:

1. Region of Production.
2. Species, cultivars and breeding
3. Suitable seed certification and quality control systems
4. Production infra-structure
5. Seed cleaning facilities
6. Suitable and reliable supply chains to market
7. Seed production research and extension to seed growers
8. Investment and government support to develop an industry
9. Investment and government support to develop an industry
10. Commercial structure of industry (i) Village based seed production system; (ii) State run seed production; (iii) Commercial companies producing seed under Government quality assurance

# Herbage seed production and extension in the south region of Xinjiang, China

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**Abstract**

Alfalfa seed production and extension in HeTian region of Xinjiang, China. The XinJiang daye alfalfa is a famous alfalfa breed in China and is unique variety in order to keep pure pedigree in HeTian region. Our work is how to help farmers to improve alfalfa seed yield. The seed yield have been increased from 150 kg/ha (10 kg /mu) to 345 kg/ha (23 kg/mu) by controlling the seeding rate and with irrigation. The highest seed yield was 680 kg/ha (45.4 kg/mu). In our experimental area the field tours were often organized for farmers, and we gave training about seed production for farmers in Uygur language and Chinese. Meantime the indigenous insects that were pollinating alfalfa were researched. The most common bee is in the leaf-cutter bee family. The seed yield was improved by bee pollination.

Sweet clover seed production and extension in BaiCheng county, Aksu, Xinjiang China. Winter wheat is suited for growing in BaiCheng county nearby Tianshan Mountain because of heavy snow and short growing season. But cotton is not suitable. Thus winter wheat interplanted with sweet clover have been extended about 9,000 ha in BaiCheng county and sweet clover seed production has become more important. There is a 1 ha model district for seed production. The seed yield were improved from 450 to 600 kg/ha (30-40 kg /mu) to 1275 kg/ha (85 kg/mu), and the highest yield was 1,600 kg/ha (107 kg /mu) using fertilizer at N90P90 kg/ha in our experimental area. We trained farmers to produce seed using fertilization technology and harvest technology. The technologies were welcomed by farmers.

# Seed production of *Brachiaria* hybrid cv. Mulato II in two counties of Mexico

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**Introduction.** *Brachiaria* hybrid cv Mulato II, released in 2005, is a forage of high production and nutritive quality adapted to marginal soils. These qualities encouraged its seeding in tropical America and Southeast Asia. The aim of this study was to analyze the production of *Brachiaria* hybrid cv Mulato II during 2003-2010 in two counties of Mexico.

**Methods.** The data corresponds to 32 farms, divided into 12 in the county of Jiquipilas and 20 in the county of Ocozocoautla. The data were organized by type of soil, year in which the seedbed was installed, annual seed production and average area per farm devoted to the seed production. The soils of both counties were grouped into three categories: sandy soils, loamy soils and clay soils.

**Results and discussion.** *County seed production of Mulato II.* The seed production of Mulato II was 9% higher in Ocozocoautla than in Jiquipilas, although this difference was not significant ($p>0.05$).

*Seed production by soil type.* In the loamy soil were obtained higher seed yields than in sandy or clay soils. Sandy soils in contrast, show a negative asymmetry whereas clay soil has a more symmetrical distribution, but its seed yield is lower.

*Amount of harvests, crop age and soil type.* Relating crop age and the amount of harvests, it was obtained a positive relationship between both parameters for both Jiquipilas ($R^2=0.90$) to Ocozocoautla ($R^2=0.79$). These results would imply that in Jiquipilas the contracts are for three years, while in Ocozocoaultla the contracts last for five years. This hypothesis would be the most acceptable because it was not obtained relationship between soil type and quantity of harvests. According to the coefficient of association, the type of soil would not limit the time of crop for seed production only.

*Paddock size, seed yield, crop age and amount of harvests.* The paddock size was related to seed yield and was obtained a moderate correlation between both parameters for Jiquipilas. These results would imply that the smaller the paddock of Jiquipilas, better is care farming activities (land preparation, fertilization, weed control).

**Conclusions**

- The best seed yields were achieved in loamy soils>sandy soils>clay soils;
- However, soil type did not explain the differences in productivity, possibly because the incidence of other variables not included in the database.
- In Jiquipilas, the production yield is higher in smaller plots, with more focused attention in the care of the crop.

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# Soil seed bank of *Lespedeza daurica* under different grazing intensity in a steppe grassland of northwest China

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The extensive grasslands in northwest China are degenerating with reduced plant species diversity. *Lespedeza daurica*, a dominant species in the grassland, is a very important forage for native livestock. However, over the past years the abundance of *L. daurica* has been greatly reduced. One of the causes of degeneration is overgrazing. The ability to regenerate from seed in the soil is likely to be very important for *L. daurica*. In this current study, the relationship between intensity (i.e. control, light, medium and heavy intensity, representing 0, 4, 8, 13 sheep per ha, respectively) of sheep grazing on changes in the size of the soil seed bank and composition of aboveground vegetation was investigated. Because *L. daurica* seed is highly physically dormant (seed germination is less than 5%), it is difficult to accurately evaluate its content in the soil seed bank using a glasshouse tray germination test. In this experiment, soil samples firstly were sieved through 5 mm mesh. Secondly, all *L. daurica* seed of each sample was picked out carefully by hand. Totally, ca.$1\times10^4$ seed were recovered from all soil samples. The results showed that there is a significant correlation between soil seed density and aboveground plant density ($F_{1,30}$=43.6, $P$<0.01,$R^2$=0.58). Also, there are significant differences of soil seed density of *L. daurica* in the four grazing intensity paddocks ($P$<0.05). Seed of *L. daurica* was most abundant in soil of the lightly-grazed paddock, while its abundance was greatly reduced in the heavily-grazed paddock. In addition, seed mass and seed size differed significantly between the heavily-grazed paddock and the other three grazing frequency paddocks. Seed mass was highest in the mediate paddock, while seed size (long axis and short axis) was highest in the non-grazed paddock.

# Seed Production Extension programming in Western Oregon – addressing challenges and opportunities

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**Abstract**

The top production challenges identified by herbage seed producers in Western Oregon, USA, are slug and vole pests, tough-to-control grass weeds, the lack of suitable rotational crops, and social pressures demanding stricter management practices. The Oregon State University Extension Service has a long history of serving seed producers in the Willamette Valley through needs assessment and Extension program development. The Field Crops Extension Program conducts applied research to address production challenges, and provides growers with the most up-to-date information on pest management and optimal production practices. On-farm research in grass and legume seed production is coordinated with interested growers across the Valley, and collaborated with local and regional researchers. Trial results and agronomic updates are communicated to producers in various formats: individual field visits; bi-annual workshops; grower meetings; industry meetings; field tours; annual seed production reports; monthly newsletters; emails lists; and social media. Current research has led to improved yields through new pesticide registrations, optimized plant growth regulator (PGR) applications, and improved fertilizer recommendations. Future research interests in the southern Willamette Valley include: row spraying and grazing to manage white clover seed crops; alternative PGR applications to improve grass seed yields; added options for transitioning into certified annual ryegrass seed production; plant breeding to improve disease resistance; and the use of new technologies to determine cutting times. Extension will continue to support Oregon growers as they pursue new opportunities within changing markets and growing conditions.