

# Proceedings of the 7th International Herbage Seed Conference



Dallas, Texas, USA



11-13 April 2010

**Proceedings of the  
Seventh International Herbage Seed Conference  
Dallas, Texas USA  
11 – 13 April 2010**

G. R. Smith, G. W. Evers & L. R. Nelson (eds)

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# International Herbage Seed Conference 2010

## PROGRAM

### Sunday, April 11

19:00 Welcome Reception

### Monday, April 12

CHAIR: Birte Boelt

08:15 – 08:25 **Opening**

Birte Boelt, President, International Herbage Seed Group, Denmark

08:25 – 08:30 **Welcome**

David Baltensperger, Department Head, Soil and Crop Sciences , Texas A&M University

08:30 – 08:45 Introduction to Texas

Lloyd Nelson, USA Texas

08:45 – 09:00 Texas Foundation Seed Service – a production and commercialization unit for Texas

AgriLife Research

Steve Brown, Director, TFSS

09:00 – 09:30 **Invited Speaker:**

Changes in seed crop management – the Oregon experience, 1990-2009

William C. Young, USA Oregon

09:30 – 09:50 **Invited Speaker:**

Texas herbage seed production in contrasting climates

Gerald W. Evers, USA Texas

09:50 – 10:15 **Break**

### Session:

CHAIR: Don Loch

10:15 – 10:30 The influence of planting density on seed yield and seed yield components of

*Cleistogenes songorica*

X. Wei, China

10:30 – 10:45 Variation in seed shattering in a germplasm collection of *Panicum coloratum* L. var.

*makarikariensis* Goossens

Andrea Tomas, Argentina

10:45 – 11:00 Harvest methods and seed yield potential in *Brachiaria* hybrids

Esteban A. Pizarro, Uruguay

- 11:00 – 11:15 Alfalfa seed production in semi-humid climate of southeast Europe  
D. Karagic, Serbia
- 11:15- 11:30 On-farm conversion of straw to bioenergy – A value added solution to grass seed residue  
G. W. Mueller-Warrant, USA Oregon
- 11:30 – 12:00 Posters and Discussions
- 12:00 – 13:00 **Lunch**

**Session:**

CHAIR: Lars Havstad

- 13:00 – 13:15 Perennial ryegrass (*Lolium perenne* L.) seed crop response to spring nitrogen: a comparison of New Zealand and Oregon results  
Phil Rolston, New Zealand
- 13:15 – 13:30 In-season assessment of plant nitrogen status for perennial ryegrass seed production using remote sensing  
Mike Flowers, USA Oregon
- 13:30 – 13:45 Modeling critical NDVI curves in perennial ryegrass  
René Gislum, Denmark
- 13:45 – 14:00 Harvest loss in ryegrass seed crops  
M. P. Rolston and R. J. Chynoweth, New Zealand
- 14:00 – 14:15 Predicting spring nitrogen for perennial ryegrass seed crops from NDVI  
Richard Chynoweth, New Zealand
- 14:15 – 14:30 Nitrogen fertilization management for seed production of tall fescue  
Marie-Laure Casals, France
- 14:30 – 14:45 Questions and Discussion of above papers
- 14:45 – 15:00 Posters and Discussion of Poster Papers
- 15:00 – 15:30 **Break**

CHAIR: Jorge Castano

- 15:30 – 16:00 **Invited Speaker:**  
Stresses associated with germination and establishment of overseeded turfgrasses  
M. D. Richardson, USA Arkansas
- 16:00 – 16:15 Evaluation of vigour tests for determination of seed storage potential in red clover and timothy  
Lars Havstad, Norway

- 16:15 – 16:30 Reliability of salinity screening *Lolium* genotypes using field grown versus greenhouse techniques  
Lloyd Nelson, USA Texas
- 16:30 – 16:45 Pericarp imposed seed dormancy in *Zygochloa xanthoxylum* (Bunge) Maxim. favours its adaptation to desert environments  
X. Hu, China
- 16:45 – 17:00 Posters and Discussions
- 17:00 Announcements
- 19:00 **Dinner at Hotel**

## Tuesday, April 13

CHAIR: Bill Young

- 08:00 – 08:15 Light, lodging and flag leaves – what drives seed yield in ryegrass?  
Jason Trethewey, New Zealand
- 08:15 – 08:30 Seed yield components and their potential interaction in grasses  
Birte Boelt, Denmark
- 08:30 – 08:45 Stem rust in perennial ryegrass seed crops: epidemiological and genetic research at USDA-ARS  
William Pfender, USA Oregon
- 08:45 – 09:00 Seed yield variation in a red clover breeding population  
Dave Monks, New Zealand
- 09:00 – 09:15 History of crimson clover in the USA  
Gerald Smith, USA Texas
- 09:15 – 09:30 Discussion and Posters
- 09:30 – 10:00 **Break**

CHAIR: Phil Rolston

- 10:00 – 10:30 **Invited Speaker**  
Chemical diversity of bioprotective alkaloids of endophytic fungi in cool season grasses .  
Carolyn Young, USA Oklahoma
- 10:30 – 10:45 Control of *Vulpia myuros* in red fescue  
S. Mathiassen, Denmark
- 10:45 – 11:00 Annual grasses in crop rotations with grass seed production  
P. K. Jensen, Denmark
- 11:00 – 11:45 Business Meeting

- 11:45 – 12:00 Discussion and Closing
- 12:30 Depart for tour of Noble Foundation in Ardmore, Oklahoma
- 22:00 Return from tour to hotel

### **Wednesday, April 14**

Post-Conference Tour (Must be registered for post-conference tour)

- 08:00 **Depart from hotel**
- 08:45 Arrive at Texas AgriLife Research Urban Solutions Center at Dallas at 8:45 and hear presentation by staff on turf-grass research.
- 11:30 **Lunch at Dallas Center**
- 12:00 Depart Dallas Center for 3 hr trip to College Station
- 15:30 Arrive at George Bush Presidential Library on Campus of Texas A&M University
- 18:00 Depart Library to check in at Hampton Inn in College Station
- 19:00 Depart hotel to eat dinner
- 20:00 Return to hotel

### **Thursday, April 15**

- 08:15 **Depart hotel and travel to sports field complex at Texas A&M University**
- 08:30 View soccer, softball and Kyle Field turf
- 10:00 Travel to Borlaug Biotech Center and take break
- 10:30 Presentation by staff at Borlaug Biotech Center
- 11:30 Depart for lunch at G Rollie White Conference Center
- 12:00 **Lunch**
- 13:00 Depart to Agronomy Research Station for several presentations by plant breeders showing and discussing their programs.
- 15:00 **Break**
- 15:30 Depart for CERES Research Station for presentation on high-yielding energy crops
- 17:00 Depart for return trip to hotel
- 19:00 Depart hotel for dinner

### **Friday, April 16**

- 08:15 **Depart hotel for 3 hr. trip to Fredericksburg**
- 10:45 Arrive at Wildseed Farms to tour largest wild flower seed producer in the USA
- 12:00 **Lunch at Wildseed Farms**
- 13:00 Depart to visit town of Fredericksburg
- 13:30 Arrive in Fredericksburg, visit many tourist shops, and sites.
- 19:00 Depart for San Antonio
- 20:30 Arrive at La Quinta Convention Center Hotel in San Antonio, near River Walk

### **Saturday, April 17**

- 08:15 **Depart hotel for 2 hr trip to Pogue Agri Partners Seed Company**
- 10:15 Tour seed company and hear presentation on seed production on native species.
- 12:00 **Lunch at Pogue Seed Farm**
- 13:00 Depart for San Antonio
- 15:30 Arrive back at hotel
- Remainder of day is free time for touring River Walk, Alamo, and other sites

Hotel expense is covered for night of April 17<sup>th</sup> with registration

**Sunday, April 18**

Delegates are on their own to depart as scheduled

# Introduction to Texas

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## Introduction

This presentation will be about the history and people of Texas, Texas A&M University, and the role Texas AgriLife Research encompasses for agriculture in the State of Texas.

Texas is known as the Lone Star State, due to the fact Texas was once a country or nation before it joined the United States. If a Texan is traveling internationally, and you ask him where he is from, he will say Texas; however, if he is from any other state, he will say USA. The reason for this is Texans want you to know exactly where they are from and they are proud of it (McDonald, 2007). Texas has been under the control of six different nations, namely Spain, France, Mexico, Texas, the Confederacy of Southern States, and of course the USA. The first inhabitants were the Native Americans or American Indians. There were several tribes of Indians which included Forest Indians, Plains Indians and Inter Mountain Seed Gatherers. They had to adapt to the climate to survive in the wet Gulf Coast Region, or extremely dry West Texas Mountains. When the Spanish introduced horses, the Comanche adopted the horse culture and became the greatest light cavalry in the world. The demise of the Indian culture was brought on by the interaction with Americans moving west and European emigrants who moved in to claim much of their land. Even more importantly, many common diseases were highly contagious and often fatal to Indians who had little resistance.

**Six flags over Texas:** The first Spaniard to visit Texas was Alonzo Alvarez de Pineda in 1519, when he sailed along the Gulf Coast (McDonald, 2007). The Spanish were interested in Texas for two reasons, they had settled in Mexico, where they had found gold and brought much gold back to Spain. Secondly, French and English activities east of Texas were viewed as a threat, so Texas was a buffer between Mexico and the other foreign powers. In 1682, the French explorer La Salle sailed down the Mississippi and claimed all territory drained by the river including Texas (only a small part of Texas is drained by the Mississippi River). The first Spanish Mission was erected by De Leon in 1690 and several other missions were opened over the next 100 or so years. In 1821 the Mexican revolution resulted in the establishment of the independent Mexican government. By this time, American settlers were moving into East Texas and were at first welcomed to help develop the region. Farmers were given 177 acres of land. By 1833, so many Americans were moving to the region that was to become Texas that the Mexican government passed laws and raised taxes which began to cause resentment among the settlers. This would soon result in the Texas Revolution. The battle of the Alamo was on March 6, 1836, although the mission had been under siege for several days. The Mexican General Santa Anna was defeated at San Jacinto on April 21, 1836, and shortly thereafter the Republic of Texas was established. Sam Houston was elected President. The Republic of Texas had a population of 40,000, almost none who lived in a town. There was no system of local, county or even state government. Texas joined the United States in 1845 with more than 100,000 citizens, of which

40,000 were slaves (McDonald, 2007). In 1861, Texas withdrew from the United States and joined the Confederate States of America; however, Texas was back in the USA by 1865. Many of the early settlers were from the Southern US as they moved westward. Significant numbers of French, Polish, Czechs, and Germans also emigrated directly from Europe and numerous towns kept native customs, and even spoke their native language for a generation or more. The present day population of Texas is about 25,378,000 of which 45% are Anglo, 38% Hispanic, 12% Black and 5% other (Texas Dept. of State Health Services).

After the Civil War, there was a great amount of lawlessness in Texas. This resulted in the old image of Texans often seen in western movies. In fact much of it was true as there were often feuds, gunfights and eventually the Texas Rangers were recruited to keep the peace. There were Cattle Kingdoms formed in South Texas and later on in North Texas. Cattle drives in the 1850s moved cattle north to Missouri, Nebraska and Kansas. One of the main cattle trails came through Fort Worth, close to where this meeting is taking place.

**Modern Day Texas.** The oil industry has made Texas an important and rich state in the twentieth century. The Spindletop oil field near Beaumont was extremely important in World War I, and the East Texas Oil Field fueled the war effort in World War II. These and many other oil fields are still active today and new oil and gas wells are being drilled every day in Texas. Many wind generators are present in West Texas, where strong winds are normal. The estimated numbers by the end of 2008 were 5877 wind turbines located on 56 wind farms, producing 8,876 MW of electrical power (Nelson et al. 2008). The number of wind generators is likely to be near 10,000 today, and provides a significant amount of electrical energy for Texas.

**Agriculture.** Almost all crop species can be grown for seed production somewhere in Texas, due to the wide environmental conditions which exist here. Due to climatic conditions, profitable seed production is not possible on most species. Many summer annuals adapted to northern climates can be grown in Texas in the winter, when only light frosts occur. Examples of this would be spring oats (*Avena sativa*) and spring wheat (*Triticum aestivum*) and many annual clovers (*Trifolium* spp). There is great potential to test plant species collected in northern Russia and China as winter annuals in Texas. Many of the important grass species used in the eastern half of Texas were introduced. Examples of cool season species would include *Lolium* spp., *Secale cereale*, *Avena sativa*, and *Triticum aestivum*, *Trifolium* spp, *Lespedeza stipulaceae* and many more. Some warm-season forage crops would include *Cydonon* spp., *Paspalum* spp., *Trifolium* spp., *Vigna unguiculata*, and *Pisum* spp., etc. West from Central Texas, or Fort Worth, less crop production occurs (unless under irrigation), and more rangeland is present. Rangelands grow the tall and short prairie grasses and a multitude of forbs and shrubs species. Native bluestems, mostly *Andropogons*, and introduced bluestems *Bothriochloa* spp., buffalograss, some native from South Texas and also introduced from South Africa, and switchgrass (*Panicum virgatum*) especially in South Texas are commonly found (Smith and Anciso). Seed production and seed yields on most of the range grasses are quite low and difficult to harvest in most cases. Seeds often shatter prior to harvest, but they tend to reseed themselves, if not over grazed. Rainfall in most of the rangelands is limiting and very unpredictable, thus careful management of livestock and grazing levels is required to maintain desired forage species in the range ecosystem. In West Texas large agriculture cropping enterprises are present due to modern irrigation systems. Vast aquifers of underground water have allowed relatively inexpensive

water to be used to grow cotton (*Gossypium hirsutum*), corn (*Zea mays*), wheat (*Triticum aestivum*), peanuts (*Arachis hypogaeae*) and grain sorghum (*Sorghum vulgare*) crops. Water levels in the aquifers have dropped significantly in recent years, and crops can no longer be grown in some regions.

**Texas AgriLife Research** was formerly called the Texas Agricultural Experiment Station and is part of the Texas A&M University System. Texas A&M University at College Station was founded in 1876, and in 2010 has 38,000 plus undergraduates and 9,000 graduate students. It is located on a 5,000 acre campus in College Station. The College of Agriculture has 40 undergraduate degrees and 50 graduate degrees. There are 400 nationally recognized faculty members, including one Nobel laureate. Texas AgriLife Research is the agricultural research agency of the Texas A&M University System. Many of the scientists with this agency do not teach and are located off-campus at 13 Research and Extension Centers. These centers were strategically located to conduct research across the wide array of environments of Texas. They are located on the high plains in Northwest Texas, the southern tip in semi-tropical Weslaco, to the desert conditions of El Paso, to the very wet conditions near the Gulf Coast at Beaumont. The Texas AgriLife Research and Extension Centers and their main emphasis in research are as follows. Figure 1 provides the location of each of the centers and the main campus or headquarters of Texas A&M University at College Station.

Amarillo	Cattle feedlot nutrition, wheat breeding, precision agriculture, etc.
Beaumont	Rice breeding and production systems, developing bio-energy crops, etc.
Corpus Christi	Improved cropping systems, animal reproduction, shrimp production
Dallas	Urban management of turf, horticulture and water resources
El Paso	Water-use efficiency, water quality including pathogen detection, etc.
Lubbock	Abiotic & biotic stress of crops including cotton, corn, grain sorghum, etc.
Overton	Ryegrass & clover breeding, pasture management & horticulture
San Angelo	Sustainable rangelands with cattle, sheep, goats & wildlife
Stephenville	Sustaining healthy ecosystems, biofuel feedstocks & peanut breeding
Temple	Modeling with soil & water natural resources and cropping systems
Uvalde	Wildlife and beef cattle systems, vegetable quality research and water
Vernon	Rangeland restoration, livestock & forage production systems & wheat
Weslaco	Citrus & sugarcane production, molecular, biotechnology & genomics

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# Research and Extension Locations

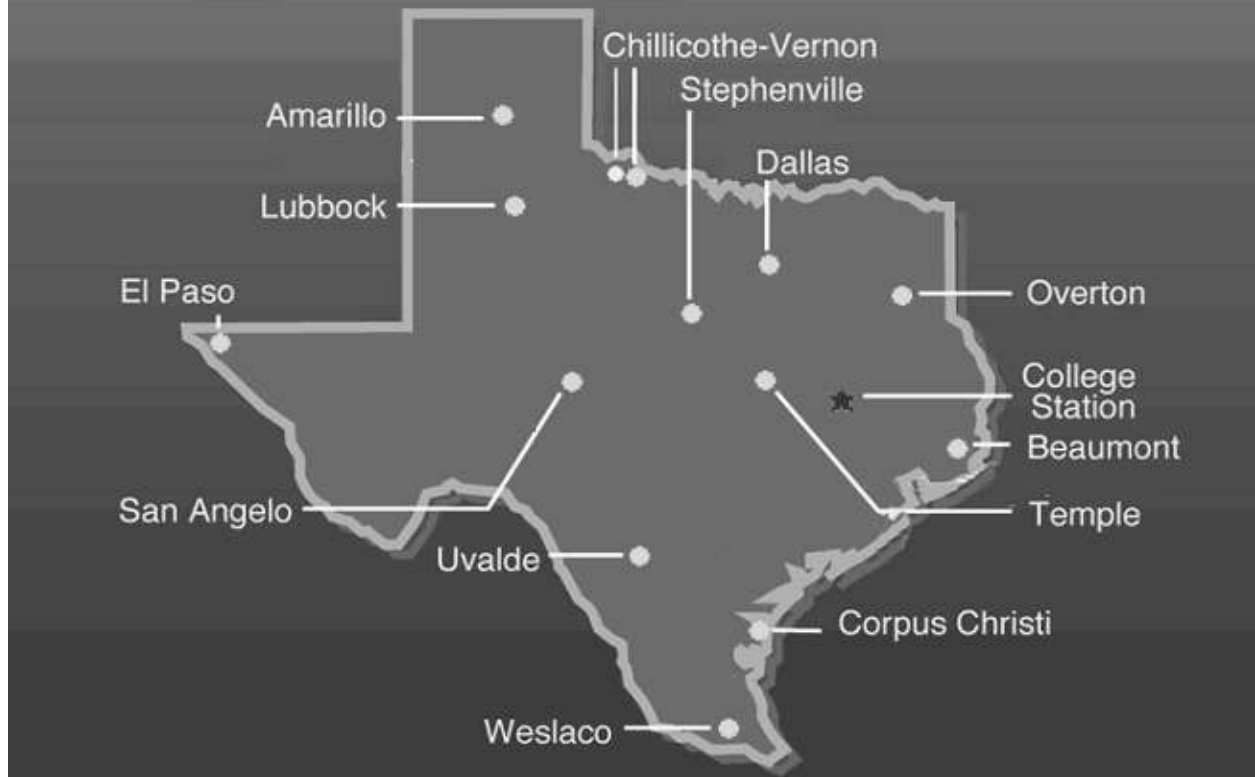


Fig. 1. Texas AgriLife Research and Extension Centers and Research Stations in Texas.

## **Texas Foundation Seed Service – A production and commercialization unit for Texas AgriLife Research**

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### **Abstract**

The Texas Foundation Seed Service (TFSS) is a non-profit, stand-alone unit of Texas AgriLife Research that services most of the plant material improvement efforts of AgriLife plant breeders. Traditional duties include production and marketing of genetically pure foundation class seed of new cultivars for distribution to licensees of the products. TFSS is a liaison between plant improvement programs and the private sector seed companies that both sponsor research and license products. As a part of our duties, we handle business aspects of research commercialization in an effort to allow scientists to concentrate on their respective research projects.

The Texas Foundation Seed Service (TFSS) located in Vernon, Texas, is a non-profit, stand-alone unit of Texas AgriLife Research that services most of the plant material improvement efforts of AgriLife statewide. The service exceptions include citrus and sugarcane (Weslaco Center), rice (Beaumont Center), and vegetables (Vegetable and Fruit Improvement Center in College Station). Operations of TFSS are intended to be self-supporting by generating revenue through sales and services similar to a commercial business.

The original mission of TFSS was to produce and market genetically pure seed of new cultivars developed by the scientists of AgriLife. Although the name implies that TFSS works only with seed, it is involved with production and distribution of vegetatively propagated plant materials too. TFSS works as a liaison between the various plant breeding programs of AgriLife Research and those companies that have interest in licensing and marketing these plant material improvements.

As a part of our work with plant material improvement programs, we either contract production of seed, or work with licensees regarding seed production on most of the following crops:

Forages – Annual ryegrass, clovers, medics, bundleflowers, native and introduced warm-season grasses, cowpeas, beans, and summer dormant cool-season grasses.

Forages include both native (157 million acres) and introduced (111 million acres) pastures in Texas that provides about 70% of the nutrients consumed by livestock. Forage crops are the foundation on which the Texas livestock industry is built. Currently, cash receipts are more than

\$7 billion for cattle, \$3 billion for wildlife, and more than \$700 million in hay to Texas ranchers. Forages also enhance water quality, serve as sinks for the disposal of agricultural and municipal wastes, are renewable sources of energy, provide food and habitat for wildlife, and are used to revegetate disturbed lands and public right-of-ways. TFSS is the sales and marketing vehicle for the three USDA Plant Materials Centers (PMC) located in Texas and has a seat on the USDA PMC Advisory Committee (Texas).

**Turfgrasses** – Seeded and vegetatively propagated types include – Turf type annual and intermediate ryegrass, Texas x Kentucky bluegrass hybrids, creeping bentgrass, zoysiagrass, buffalograss, and St. Augustine grass.

The turfgrass industry contributes an estimated \$7 billion annually to the economy of Texas. With over 50,000 acres of dedicated turfgrass production and over 3.5 million acres of turfgrass in lawns, sports fields, golf courses, parks, and other turf commercial and industrial sites, turfgrass ranks as one of the highest valued agricultural crops in the state. The scope of the turfgrass industry provides substantial and continuing employment opportunities for the citizens of Texas.

**Peanuts** (*Arachis hypogaea*)– runner types and Spanish types

Texas production (2008) was 257,000 acres with a farm gate value of \$185.8 million. (NASS website)

**Small Grains** – Wheat (*Triticum aestivum*), oat (*Avena sativa*), and barley (*Hordeum vulgare*) – all used for grain only, dual-purpose, and forage only

Planted acres in Texas (2008) were about 6.4 million acres with about 3.4 million of these acres used for forage and hay for livestock. Harvested acreage yielded a farm gate price for producers of \$756.8 million. (NASS Website)

**Sorghum** (*Sorghum vulgare*) – parental lines for use in grain hybrids, forage hybrids, and biofuel hybrids (sweet sorghums and high biomass cellulosic hybrids)

Texas is the largest producer of hybrid sorghum seed in the world. Hybrid seed produced in Texas is shipped throughout the sorghum production areas of the world. Planted acreage of all types of sorghums in Texas was about 3.6 million acres with grain sorghum harvest generating producer values of \$ 648 million and yielding in excess of 1.9 million tons of silage. (NASS website)

**Corn** (*zea mays*)– inbred lines

Corn production in Texas (2008) for grain was planted on 2.3 million acres yielding a producer value of \$1.2 billion. In addition to grain production, silage production was planted on 180,000 acres producing 3.7 million tons of livestock feed. (NASS website)

**Cotton** (*Gossypium hirsutum*) – germplasm enhancement and cultivar development

Texas is the largest cotton producing state in the U.S. Planted acreage in 2008 was 5 million acres. Harvested acreage was 3.25 million acres yielding 4.45 million bales with a farm gate value of \$1.14 billion. Cottonseed values, for oil and livestock feed, from this crop increased value an additional \$363.6 million. (NASS website)

In addition to the previously mentioned plant improvement programs, TFSS is also involved with specialty type commercial crops types listed below. These crops include both greenhouse and field production and are another source of economic impact for the producers of Texas.

**Ornamentals** – Crape myrtle, roses, *Brugmansia*, hardy hibiscus, and other flowering trees and shrubs

Texas is considered to be the third largest output state in nursery and greenhouse production based on wholesale sales values. The nursery and greenhouse production industry in Texas employs 22,700 people and encompasses 21 million square feet of covered greenhouse and shade production area plus an additional 22,162 acres of outdoor production. Wholesale value of nursery and greenhouse sales for Texas in 2007 was \$2.064 billion. Of total sales, 72% was sold in Texas and 26% was sold in other regions of the U.S. Export sales represented 2% of sales. (The Economic Impact of the Green Industry in Texas)

Texas AgriLife Research may be somewhat unique among public research organizations in the fact that the Legislature of the State of Texas has mandated that we protect intellectual property and license the use of inventions to companies that have the ability to broadly distribute products to the producers of Texas and beyond. One purpose for these mandates was that the legislature was anticipating a reduction in traditional funding of public research programs. As a result of our efforts to meet these challenges, we have been successful in broadly licensing products and currently have sales of plant materials developed by AgriLife in about 35 states in the U.S. and approximately 20 countries throughout the world.

Since 2002, the role of TFSS has been greatly expanded to meet these challenges. In addition to the more traditional functions of ‘foundation seed’ organizations, we work closely, not only with the various plant improvement programs, but also with various internal business units and external corporate entities that have interest in various research projects. These efforts were developed to allow scientists to work on their specific research projects without the worry of business issues that are related to commercialization of their research outcomes.

TFSS works with the AgriLife Contracts and Grants office to develop sponsored research agreements, Non-Disclosure Agreements (NDAs), Material Transfer Agreements (MTAs), and production contracts. With the AgriLife Corporate Relations Office, TFSS works with corporate entities to develop public/private partnerships that lead to sponsored research agreements and ultimate licensing of research outcomes. This method is more of a pull-through economic

principle and encourages both AgriLife scientists and corporate research leaders to collaborate to provide research solutions for specific problems.

TFSS interaction with the Texas A&M University Systems (TAMUS) Office of Technology Commercialization (OTC) is on a very frequent basis. TFSS markets many of the plant material improvements to prospective licensees, assists OTC with licensing terms and drafting both non-commercial (evaluation) license agreements and commercial license agreements, manages executed license agreements and monitors agreements for compliance and infringement issues, and collects royalties for OTC on licensed plant materials. OTC then distributes the licensing revenues to the various stakeholders under guidelines set out under TAMUS Intellectual Property (IP) policy.

TFSS also provides royalty collection/license management services for several other land grant institutions that market plant materials into the Texas and SW US markets. Another service provided by TFSS is production and/or seed conditioning services to public and private breeding programs. With some of the revenues generated by TFSS, we are able to provide some longer term investment (funding) in AgriLife plant breeding programs. In most cases, this funding is for assistance in the production of small purification or breeder seed blocks in programs that have commercial potential but lack adequate and timely funding for these small and very expensive production blocks.

TFSS, as a representative for AgriLife, is very active in state, regional, and national seed and agricultural organizations. We also work very closely with the seed certification agencies located in every state where AgriLife licensed products are produced for commercial sales. TFSS acts as a resource for many of our commodity board stakeholders as well as AgriLife internal committees such as the Intellectual Property Management and Commercialization Team, Small Grains Advisory Committee, and Plant Review Committee.

Change is a given for agribusiness and the only constant is that we will continue to see change in the way agribusiness is conducted and in the speed of technological improvements moving to the marketplace. TFSS is fortunate to be a part of the AgriLife team that has led some of the changes and in many cases AgriLife is looked to as an operating model for other universities and public institutions as they move from more traditional funding mechanisms to the development of public/private partnerships and public institution licensing of most or all plant material improvements.

NASS website – National Agricultural Statistics Service

[http://www.nass.usda.gov/Statistics\\_by\\_State/Texas/index.asp](http://www.nass.usda.gov/Statistics_by_State/Texas/index.asp)

Information current as of January 26, 2010

The Economic Impact of the Green Industry in Texas

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## **Changes in seed crop management – the Oregon experience, 1990-2009**

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### **Abstract**

Oregon has continued as a major producer of cool-season forage and turf grass seed over the past 20 years. However, seed production technologies have not remained static. Indeed, several significant challenges have confronted Oregon seed growers, as well as new opportunities. Clearly, the most daunting affront to growers' traditional production system was legislation to reduce reliance on open-field burning of post-harvest crop residues. In the absence of burning, straw marketing opportunities emerged, but not without concern for anti-quality alkaloids present in endophyte-infected turfgrasses. In addition, an appreciation for the nutrient value of grass straw left on the fields challenged other producers to manage the full straw load in their cropping system. In the late 1990s, a new generation of plant growth regulators (PGRs) became available. These foliar-applied chemicals reduce growth through a reduction in plant level of gibberellins, and have become widely used on several species for providing lodging control and increased seed yield. Other production inputs, such as nitrogen, weed, disease and insect management have been "fine-tuned" to avoid environmental risks and to maintain profitability for growers. Although the Willamette Valley is by far the most important area of grass seed production in the state, the Umatilla / Morrow county area (in the lower Columbia Basin of north central OR) has significantly expanded production of cool-season grasses in the last 20 years, and currently has more acreage than Union county (northeast OR) and Jefferson county (central OR) areas combined. Collectively, Oregon's Willamette Valley produces almost two-thirds of the total production of cool-season grasses in the United States of America (USA).

### **Introduction**

Oregon is a major producer of cool-season forage and turf grass seed and has long been recognized as a center of expertise in seed production. Most of the state's acreage is located in the Willamette Valley, an area known as the "grass seed capital of the world." Mild and moist winters with dry summers favoring seed development and harvest make the Valley an ideal place to produce high quality seed. Grass seed is produced on family-owned farms, with more than 60% of the total labor requirements provided by family members. Although an exact tally of seed farms and the acreage of each is unknown, it is assumed there are slightly fewer, but larger farms today than 20 years ago. These trends have been shown in recent USDA Census of Agriculture data.

### **Production trends**

Farm gate value of Oregon's grass seed industry has increased approximately 125% over the last 20 years. In an attempt to "soften" the somewhat cyclical nature of production and prices

received by growers, the above percentage increase in value was computed by comparing the average value from 1990, 1991 and 1992 to the average value from 2007, 2008 and 2009. The dollar amount of those two means is US\$184,480,000 and US\$412,750,000, respectively. The range in value of Oregon's grass seed crop production over the last 20 years was US\$169,030,000 (1991) US\$480,060,000 (2007).

Comparing the current year's production with 1990's data shows an increase of 12% (approximately 20,000 hectares) over the last 20 years (Table 1). However, not all species have shown similar changes. For example, tall fescue (+72%), rough bluegrass (+59%) and hard fescue (+29%) have all increased significantly more than the average. Conversely, colonial bentgrass (-71%), Chewings fescue (-43%), creeping bentgrass (-38%) and Kentucky bluegrass (-30%) have all decreased significantly over the last two decades.

Seed yields for most grass species grown have seen significant increases in the last 20 years (Table 1). Only colonial bentgrass and orchardgrass are shown to yield less today than in 1990. Annual ryegrass has made a modest gain of 5%, but all other species show a double-digit percentage increase in kilograms per hectare with a range from +29% (perennial ryegrass) to +64% (Chewings fescue).

Table 1. Hectares of grasses grown for seed and seed yield in Oregon, 1990 and 2009, and percent change over 20 years.

	Hectares of production			Seed yield (kg/ha)		
	1990	2009	Percent change	1990	2009	Percent change
Annual ryegrass ( <i>Lolium multiflorum</i> Lam.)	44,218	48,001	+9	2,117	2,215	+5
Perennial ryegrass ( <i>Lolium perenne</i> L.)	43,878	43,505	-1	1,333	1,721	+29
Tall fescue ( <i>Festuca arundinacea</i> Schreb.)	37,062	63,816	+72	1,355	1,906	+41
Kentucky bluegrass ( <i>Poa pratensis</i> L.)	10,376	7,278	-30	885	1,182	+34
Rough bluegrass ( <i>P. trivialis</i> L.)	488	774	+59	924	1,310	+42
Orchardgrass ( <i>Dactylis glomerata</i> L.)	8,080	6,905	-15	952	874	-8
Chewings fescue ( <i>F. rubra</i> L. var. <i>commutate</i> Gaudin)	7,173	4,113	-43	818	1,340	+64
Red fescue ( <i>F. rubra</i> L.)	3,592	3,443	-4	784	1,166	+49
Hard fescue ( <i>F. brevipila</i> Tracey)	871	1,122	+29	672	927	+38
Colonial bentgrass ( <i>Agrostis capillaris</i> L.)	3,151	911	-71	437	388	-11
Creeping bentgrass [ <i>A. stolonifera</i> L. var. <i>palustris</i> (Huds.) Farw.]	2,900	1,794	-38	504	784	+56
Total Grass	161,787	181,661	(+12)	--	--	--

Oregon State University plays an important role assuring seed quality. Certification activities began in Oregon in 1916, and in 1937 it was legislatively directed that the Oregon Seed Certification Service would be coordinated through the OSU College of Agriculture Science. The certification program helps assure buyers that Oregon seed is quality seed. To meet certification standards, a grower's field must pass a seedling inspection and a crop inspection prior to harvest, and cleaned seed must meet germination and purity requirements. Over 390

seed conditioning plants are located in Oregon to prepare the seed for market once the harvest operation is complete.

In 2009, 44% of Oregon's grass seed production was certified; however, the percentage of each species that was certified ranged from 99% (rough bluegrass) to 4% (annual ryegrass) (Table 2). Data used in the calculations for Table 2 are from Oregon Seed Certification Service's annual summary reports for 1990 and 2009, and the total Oregon production data collected by the OSU Extension Economic Information Office for the same years. Over the last 20 years a greater proportion of red fescue (+60%), colonial bent (+21%) and Kentucky bluegrass (+16%) is being certified. However, overall, there has been a decrease (-9%) in the percentage of grass seed crops certified (Table 2). This decline has been led by tall fescue and perennial ryegrass (both -17%), and orchardgrass (-13%).

Table 2. Change in percentage of Oregon certified seed crops relative to total acreage produced, 1990 and 2009.

Species	Percent certified		Percent change
	1990	2009	
Hard fescue	100	94	-7
Rough bluegrass	100	99	<1
Creeping bentgrass	92	85	-7
Chewings fescue	89	82	-8
Colonial bentgrass	68	82	+21
Red fescue	49	79	+60
Tall fescue	80	66	-17
Kentucky bluegrass	50	58	+16
Orchardgrass	55	48	-13
Perennial ryegrass	52	43	-17
Annual ryegrass	4	4	0
Total	49	44	-9

### Field burning

Certainly the most dramatic change in Oregon's seed crop management in the last 20 years has been the limitation of fire (open-field burning) as the dominant means of handling post-harvest straw residue. The practice of open-field burning of grass seed fields began in the late 1940s, and was widely used into the 1980s. Between 1980 and 1985 growers registered to burn essentially every hectare in production, and 75-80% of those hectares were permitted to be burned. Between 1985 and 1990, the percentage of total hectares in production that were allowed to be burned fell to 40%. However, in this same time period hectares grown in the Willamette Valley increased from 119,920 to 160,400 (+34%). Thus, growing frustration over smoke emissions continued to be voiced by an increasingly urban, non-agricultural population, and in 1991 the

Oregon Legislature imposed a phase down in the practice of open-field burning. A final limit to only 26,325 hectares of allowed burning by 1998 was established in this law.

Prior to 1992, hectares were identified by species when registered for burning; however, once a permit was issued it was not restricted to which field (i.e., species) was burned. Thus, in 1990 one can see that only 64,538 of the 108,402 hectares (60%) registered were actually burned (Table 3). This level of burning accounted for 40% of the Willamette Valley grass seed acreage that year. Twenty years later only 11,868 hectares were burned – just 7% of production.

Table 3. Hectares of Willamette Valley (WV) grass seed crops registered for open-field burning (1990) and actual acreage burned (1990 and 2009) in Oregon.

Species	1990		2009	
	WV hectares registered	WV hectares burned	WV hectares burned	Percent of hectares grown
Annual ryegrass	34,807	--	5,700	11.96
Perennial ryegrass	22,291	--	622	1.50
Tall fescue	25,477	--	26	0.04
Fine fescue	17,250	--	5,371	81.86
Orchardgrass	4,321	--	45	0.65
Bentgrass	3,878	--	104	0.62
Kentucky bluegrass	378	--	--	--
Totals	108,402	64,538	11,868	7.08

Between 1992 and 1998 growers rapidly began adopting non-thermal post-harvest residue management cropping systems (Table 4). The industry actually achieved the legislatively allowed limit of 26,325 hectares two years prior to the 1998 deadline. Today, only the fine-leaf fescue species utilize open-field burning as a significant portion of the acreage grown.

Table 4. Hectares of open-field burn post-harvest residue management used by Willamette Valley grass seed growers for each species, 1992-98.

Species	Open burn hectares <sup>1</sup>						
	1992	1993	1994	1995	1996	1997	1998
Bluegrass	62	98	70	68	37	19	54
Bentgrass	2,248	1,297	1,264	1,026	1,204	1,000	761
Fine fescue	9,260	8,901	8,829	7,301	6,773	5,522	5,736
Orchardgrass	495	263	241	394	388	172	206
Per. ryegrass	2,776	3,670	3,745	4,208	4,097	2,642	1,979
Tall fescue	5,905	4,142	3,378	3,784	3,127	2,513	1,845
Ann. ryegrass	12,864	11,268	14,655	16,221	14,887	10,863	7,550
<b>TOTAL</b>	<b>33,610</b>	<b>29,639</b>	<b>32,182</b>	<b>33,002</b>	<b>30,513</b>	<b>22,731</b>	<b>18,131</b>

<sup>1</sup>Oregon Department of Agriculture annual summaries of field burning

Nevertheless, foes of open-field burning have continued to push for greater regulation of this practice. Hence, the 2009 Oregon Legislature imposed further restrictions that banned open-field burning in Linn, Lane and Benton counties (in the southern Willamette Valley) and limits the practice to only 6,075 hectares of “Identified Species” and seed fields on “Steep Terrain.” (“Identified Species” are colonial bentgrass, and Chewings and creeping red fescue, and “Steep Terrain” are fields with highly erodible slopes.)

### Straw markets

Concomitant with reduced open-field burning was the availability of approximately 888,850 to 1,029,325 Mg (tonne) of grass seed straw from Willamette Valley fields. Prior to 1990, a small export market for grass straw had been developing through the efforts of the Agricultural Fiber Association (a non-profit association of straw merchants). Volume had grown from <3,000 Mg in 1980 to approximately 200,000 Mg in 1990. The export of grass straw was almost exclusive to Japan where straw was being used as a source of roughage in the dairy industry. While grass seed straw is generally considered a low-quality forage source, the ruminant animal and its microbial population can utilize it with proper nutritional management.

Through the 1990s the straw export market grew rapidly as increased availability of unburned crop residues were removed from seed fields by baling. During the phase-down period for field burning, straw was offered to contract balers and straw merchants as no cost; thus, the price of straw offered to end-users relative to other livestock feeds was competitive. Seed growers constructed straw storage barns to hold product until straw merchants needed to move bales to compressors used to process bales for efficient containerized shipping via deep-water freighters.

The Oregon Department of Agriculture (ODA) first began record-keeping on tonnage of grass straw exports in the 1997-98 market year; that year, 300,000 Mg was exported. Grass straw species exported were perennial ryegrass (58%), tall fescue (37%), orchardgrass (3%) and bentgrass (2%). Destination of these exports was Japan (89.5%), Korea (6.7%) and Taiwan (3.8%). The most recent grass straw statistics from ODA (2008-09) report 565,400 Mg of straw exported to Japan (45%), Korea (54%) and Taiwan (1%), and the percentages by grass species were tall fescue (49%), perennial ryegrass (39%), annual ryegrass (8%), orchardgrass (3%) and bentgrass (1%). Thus, in the last 20 years grass straw exports have increased approximately 183%, or to the point that over one half (52%) of the estimated total production is shipped off-shore.

Tall fescue and perennial ryegrass comprise most of the Oregon's straw exports. Most of the grass seed varieties grown today are turf-type, which in recent years have been developed with high levels of fungal endophyte infection. Concern for the safe use of these grass straw residues has led to a routine analysis by the OSU College of Agricultural Science's Endophyte Service Laboratory for toxin content prior to shipment to consuming markets. This testing service is now used by Oregon straw exporters to insure that ergovaline and lolitrem B levels are below the thresholds known to produce clinical signs in livestock. These chemical analyses provide assurances against deleterious effects to livestock fed Oregon grass straw.

### **Post-harvest residue management**

As previously noted, growers currently burn only 7% of the total seed crop acreage in the Willamette Valley. Thus, the majority of post-harvest residues are managed without fire. Management of the post-harvest residues is an important practice in grass seed production as crop residues impact certain diseases, weeds and insects, and can adversely affect seed yield in some grass species. A broad range of grasses are grown for seed production in Oregon – these species can differ markedly in their seed yield response to post-harvest residue management. Currently, perennial grass seed fields not burned are either managed using a clean, non-thermal cropping system, or managed with the full straw load.

*Clean, non-thermal.* This method is based on straw removal by baling, and removal from the field. Stubble reduction following straw removal with a flail mower may or may not be employed. The goal in this system is to remove large volumes of straw and stubble that might interfere with subsequent crop development and other management operations. This option is widely used in perennial ryegrass, tall fescue, and to a lesser extent Chewings fescue.

*Full straw load.* This method involves no straw removal as the straw is allowed to decompose in the field. Straw length may be reduced by using a straw chopper on the combine and/or by a flail mower after harvest. The straw composts in place in the seed field thereby allowing nutrients in grass straw to recycle and improve several beneficial characteristics of the soil. This option is widely used in orchardgrass, tall fescue, and to a lesser extent perennial ryegrass.

Cropping systems for annual ryegrass seed production have changed significantly from the time when open-field burning and no-till planting was a common and important practice. Prior to the 1990, over 50% of the acreage was burned. Currently, a majority of the annual ryegrass seed crop acreage is successfully managed with conventional tillage and planting systems. However, alternative no-till and volunteer systems are being used to a limited extent. In the volunteer

system, a seed crop is produced from seed shattered from the previous crop and is essentially a no-till and no-plant system of production for un-certified seed production. When no-till planting is used, a sprout of annual ryegrass volunteers and other weed seedlings are first sprayed with glyphosate herbicide before seeding. These systems offer a way to reduce tillage and fuel expenses, and reduce concerns about dust and air quality.

### **Plant growth regulators**

In the late 1990s, a new generation of plant growth regulators (PGRs) became available. These foliar-applied chemicals reduce growth through a reduction in plant level of gibberellins, and are applied to fields to reduce crop lodging, facilitate swathing, and to increase seed yields. Two PGRs are currently registered for use on grass seed crops in Oregon: Palisade<sup>®</sup> (trinexapac-ethyl) and Apogee<sup>®</sup> (prohexadione-calcium), both foliar applied products with similar modes of action in plant species.

The use of these two PGRs has become an accepted crop production program for many Oregon seed producers. OSU and private researchers have conducted many experimental trials with PGRs on perennial grass seed species and have shown consistent results from applications to perennial ryegrass, tall fescue, and fine-leaf fescue species with seed yield increases ranging from 15 to 40%.

Current industry estimates from Syngenta Crop Protection, Inc. (Palisade<sup>®</sup>) and BASF Corporation (Apogee<sup>®</sup>) suggest that between 60 to 70% of grass seed acres were treated with PGRs in 2009. These estimates, based on recent product sales, indicate that three-fourths of the fine-leaf fescue acreage is treated; two-thirds of the perennial ryegrass acreage is treated; one-half of the tall fescue acreage is treated; and one-third of the orchardgrass acreage is treated.

### **Conclusion**

Grass seed production was introduced in the Willamette Valley in the 1920s as an alternative crop for the south valley. Ryegrasses were especially well adapted to the wet soils and soon became an important crop. Grass seed also established itself as an excellent alternative crop for the highly erodible foothill soils found on the valley's eastern flank. Since the 1940s the industry has made steady growth, with many national and international seed companies located in the Willamette Valley. Today, grass seed crops are grown on more than one half of all cropland in Willamette Valley counties. Consistent production at current levels is anticipated in the future assuming demand for forage and turf grass seed products does not significantly change in the global economy.

## Texas herbage seed production in contrasting climates

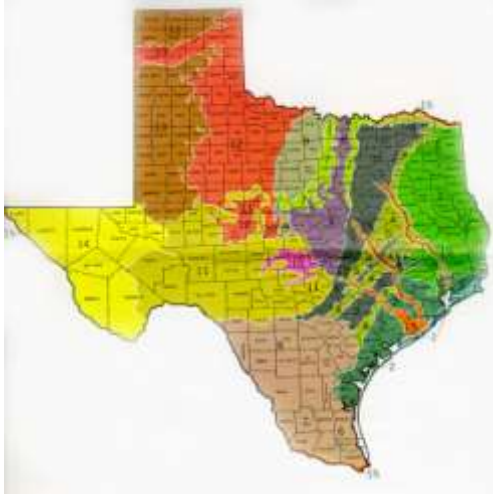
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### Abstract

Texas is a large state with diverse soils, rainfall, and growing seasons. Rainfall ranges from 200 to 1400 mm and the frost free growing season from 185 to 320 days. There are 15 distinct land resources areas in Texas. Lack of a consistent dry period for seed maturation and harvest is the main factor for limited grass and legume herbage seed production in Texas. The small amount of herbage seed production in Texas is primarily introduced and native warm-season perennial grasses grown in South and West Texas where annual rainfall is low and irrigation is available. The Natural Resources Conservation Service, a federal agency, collects, evaluates, and increases seed of native plants at 27 plant material centers. Three are located in Texas. Registered and certified planting material of Tifton 85 bermudagrass and turf grasses are also produced in Texas.

Texas is a large and diverse state. It is 1288 km from the northern tip to the southern tip and 1244 km from the eastern edge to the western edge. Soils range from poorly drained clay soils in southeast Texas where rice (*Oryza sativa*) is grown to deep sandy soils in East Texas. The southern tip of Texas has a tropical climate and is in the USDA Plant Hardiness zone 9b (average minimum temperature of -1.2 to -3.8<sup>0</sup>C) with an average growing season of 320 days. In contrast the northwest tip of Texas is in the USDA Plant Hardiness Zone 6a (average minimum temperature of -20.6 to -23.3<sup>0</sup>C) with an average growing season of 185 days. Average annual rainfall ranges from 1400 mm on the eastern edge and decreases moving west to 200 mm on the western edge. Rainfall generally peaks in spring and is low in mid to late summer. However rainfall is very erratic from year to year. Any part of Texas can be in a drought or have a flood any month of the year. Forage producers in South Texas describe the rainfall pattern as prolonged drought interrupted by periodic floods. Many cool- and warm-season agriculture crops and forages are grown in Texas due to the wide combination of soils, rainfall, and temperature.

*Land Resource Areas in Texas*



**Fig. 1. Land resource areas of Texas.**

Grasslands are important in all 15 land resource areas of Texas because livestock production is a major agriculture enterprise (Fig. 1). Among the states, Texas ranks first in number of cattle operations, beef cows, sheep, goats, and horses. A general description of the soils and list of major forage species for each land resource area follows:

1. Coastal Marsh – Saline clay and loam soils in marsh areas on Upper Texas Gulf Coast that is grazed by beef cattle in late-winter and spring.
2. Coastal Prairie – Poorly drained loam and clay soils predominate. Rice is the main crop in the eastern part and corn (*Zea mays* L.), grain sorghum (*Sorghum vulgare* Pers.), and cotton (*Gossypium hirsutum* L.) in the western part. Primary introduced grasses are dallisgrass (*Paspalum dilatatum* Poir.), common bermudagrass (*Cynodon dactylon* (L.) Pers.), and annual ryegrass (*Lolium multiflorum* Lam.). White clover (*Trifolium repens* L.) is grown in wet areas and annual medics (*Medicago* spp.) in the dry areas.
3. East Texas Timberland – Deep sandy acid soils predominate. Native vegetation was soft- and hardwood forest with small open areas of native grasses. Major introduced forages are bermudagrass, bahiagrass (*Paspalum notatum* Flugge), annual ryegrass, rye (*Secale cereale* L.) and annual clovers (*Trifolium* spp.).
4. Claypan (Post Oak) – Thin layer of sandy loam topsoil over claypan. Oak (*Quercus* spp.) trees are common. Bermudagrass, bahiagrass, annual ryegrass, small grains, and annual clovers are the major introduced forages.
5. Blackland Prairie – Characterized by black, calcareous clay soils. Originally it was a native grass prairie that has been converted to cropland for cotton, corn, and grain sorghum production. Major grass genera are *Panicum* spp., *Bothriochloa* spp., *Andropogon* spp., *Bouteloua* spp., and *Eragrostis* spp. Oat (*Avena sativa* L.) is the primary cool-season annual grass. Adapted cool-

season annual legumes are sweetclover (*Melilotus alba* Medik.), rose clover (*Trifolium hirtum* All.), and berseem clover (*Trifolium alexandrinum* L.)

6. Rio Grande Plain – Sandy loam soils with moderate fertility. The area is primarily rangeland consisting of native grasses, forbs, and legumes. Overgrazing has led to invasion of brush species like mesquite (*Prosopis* spp.). Most of the area is managed for wildlife and beef cattle.

7. Cross Timbers and 8. Grand Prairies – Soils are neutral to slightly acid and sandy. Topography is rolling to hilly. Oak tree species predominate with mid- to short native grasses. Main introduced grasses are hybrid bermudagrasses and wheat (*Triticum aestivum* L. subsp. *aestivum*) that is grown for forage and grain.

9. North Central Prairies – Moderately deep to deep soils with loamy surface soils and clay subsoils. Native warm-season perennial grasses predominate and wheat is grown for grain and forage.

10. Central Basin and 11. Edwards Plateau – Shallow stony to gravelly clayey soils. Topography is rolling to hilly. Native grasses predominate. Cedar (*Juniperus* spp.) and Shinner oak (*Quercus mohriana* Buckl.) are common. This area is a major sheep and goat production area.

12. Rolling Plains – Shallow to moderately deep soils with loamy surface layers over clay, loam, or limestone. Mostly rangeland with native grasses and forbs. Wheat and cotton are grown.

13. High Plains - Sandy and loamy soils predominant. Major crop production area, especially cotton. Rangeland is composed of native grass and forbs.

14. Trans-Pecos – Mostly nearly level sand, loam, or clay soils. Rangeland is native grasses, forbs and shrubs. Some irrigated cotton production. Salinity is a problem.

15. Bottomlands – Loam to clay loam fertile soils that are moderately to poorly drained. Moderately drains are soils used for vegetable and crop production and poorly drained soils used for pasture. Major forage species are dallisgrass, bermudagrass, and white clover.

### *Texas Herbage Seed Production*

There is a substantial acreage of small grains grown in Texas. They are planted in autumn and harvested in late spring to early summer. Foundation, registered, and certified seed of oat, wheat, and triticale (X *Triticosecale* Wittmack) are produced in Texas. Lack of a consistent dry period for seed maturation and harvest is the main factor for limited grass and legume herbage seed production. The small amount of herbage seed production in Texas is primarily introduced and native warm-season perennial grasses grown in South and West Texas where annual rainfall is low and irrigation is available. The only certified herbage seed production at this time is buffelgrass [*Pennisetum ciliare* (L) Link.], Burr medic (*Medicago polymorpha*), and Little burr medic (*Medicago minima*). Seed from planted and natural stands of native grasses is harvested

by land owners. Seed yields are low because of indeterminate growth resulting in seedheads at various stages of maturity. Because of poor quality and low germination they are sold on a pure live seed basis.

The Natural Resources Conservation Service is a federal government agency that works hand-in-hand with producers to improve and protect their soil, water, and other natural resources. One component of the NRCS are 27 plant materials centers located throughout the United States that cooperate with state and Federal agencies, commercial businesses, and seed and nursery associations. The purpose of the program is to provide native plants that can help solve natural resource problems. Beneficial uses for which plant material may be developed include biomass production, carbon sequestration, erosion reduction, wetland restoration, water quality improvement, streambank and riparian area protection, coastal dune stabilization, and other special conservation treatment needs. Staff at the plant material centers look for plants that show promise for meeting an identified conservation need and test their performance. They maintain Foundation Seed of cultivar releases that are distributed by the Texas Foundation Seed Service to commercial seed growers.

There are three plant materials centers located in Texas (Fig. 2). The Nacogdoches location serves East Texas, western Louisiana, southwest Arkansas, and southwestern Oklahoma. The Kingsville location serves South Texas, and the Knox City location serves the Southern Great Plains area in Texas and Oklahoma.



Fig. 2. Natural Resource Conservation Service plant material centers in Texas.

The most prominent grass species in eastern Texas and the southeastern US are hybrid bermudagrass [*Cynodon dactylon* (L.) Pers.] cultivars. All hybrid bermudagrass cultivars must be established vegetatively because only 3 to 5 % of the seed is viable. Specialized equipment has been developed for the digging and planting of sprigs (parts of the lower stem, stolons, rhizomes, and roots). Sprigs are sold by the bushel (2.84 hectoliter), with a recommended planting rate of 30 to 40 bushels/acre (34 to 46 hl ha<sup>-1</sup>). Registered and certified sprigs of 'Tifton 85' bermudagrass are produced and sold in Texas. Production of turf sod for urban areas is also a major industry in southeast Texas. Foundation, registered, and certified sod of bermudagrass, buffalograss [*Buchloe dactyloides* (Nutt) Engelm.], centipede grass [*Eremochloa ophiuroides* (Munro) Hack.], St. Augustine grass [*Stenotaphrum secundatum* (Walt.) O. Ktze], and zoysia (*Zoysia* spp.) are produced in Texas.

### **Summary**

Seed yields of forage grasses and legumes grown in Texas are generally low because there is not a predictable wet and dry season. Indeterminant growth of perennial grasses and seed shattering of legumes are additional problems. Seed yields of native perennial grasses are low but can be profitable for landowners some years because of no cost inputs except harvesting and cleaning the seed.

# The influence of planting density on seed yield and seed yield components of *Cleistogenes songorica*

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## Abstract

The present study explored the optimum plant density through testing seven plant density treatments with 5, 10, 15, 20, 25, 30 and 50 plants/m<sup>2</sup> under irrigated conditions in the northwest of China (Gansu province) during three consecutive growing seasons from April 2007 to August 2009. Results showed that planting density was significantly positive correlated with aboveground biomass and seed yield of *C. songorica* ( $P < 0.05$ ). For three successive seasons, a significant higher aboveground biomass (3023 kg/hm<sup>2</sup>, 3127 kg/hm<sup>2</sup>, 2860 kg/hm<sup>2</sup>, respectively) was observed under 50 plants/m<sup>2</sup> treatments than the other treatments, except for 30 plants/m<sup>2</sup>. However, the highest seed yield were obtained in three growing seasons (632kg/hm<sup>2</sup>, 636kg/hm<sup>2</sup>, 388kg/hm<sup>2</sup>, respectively) under 30 plants/m<sup>2</sup> treatment. In addition, correlation analysis revealed that the seed yield were significantly positive correlated with the number of fertile shoots/m<sup>2</sup> for each treatment ( $r^2 = 0.924^{**}$ ,  $0.741^{**}$  and  $0.780^{**}$  in 2007, 2008 and 2009, respectively), which implied that the number of fertile shoots/m<sup>2</sup> was the most important component in determining seed yield and the optimal plant density for seed production was 30 plants/m<sup>2</sup>.

## Introduction

*Cleistogenes songorica* is one of the most important native perennial grasses in the northwest desert grassland of China because of its high economic and ecological value in arid region (Chen et al., 2002; Huang, 2008). Previous study had investigated the optimal soil condition for *C. songorica* seedling establishment in arid and semiarid areas (Tai, 2008). To better utilize this grass for economic and ecological purpose, understanding the effect of cultivation techniques on its forage and seed yield is very important, however, to our knowledge this has not yet been. The main objective of the present study was to evaluate the effects of plant density on seed yield and its components, aboveground biomass and harvest index of *C. songorica* under arid field conditions in the northwest of China, and further to determine the optimum plant density for seed production of test species.

## Material and Methods

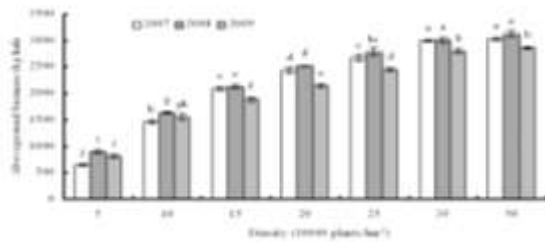
The field study was conducted from April 2007 to August 2009 at Hexi Corridor, Gansu province China (latitude 100°29'E, longitude 38°24'N, altitude 1450 m a.s.l.). The experiment was a completely randomized block design with seven plant density treatments (5, 10, 15, 20, 25, 30 and 50 plants/m<sup>2</sup>) and four replications. Ten plants from each plot were selected randomly at ripening stage (September in each year) of *C. songorica* for seed yield components

measurements. Plants were collected within 1m×1m from each plot to determine aboveground biomass, and plant materials was dried at 60°C for 48 h, then weighed. Plants were harvested within another 1m×1m from each plot to obtain the seed, air-dried and weighed.

## Results

### *Aboveground biomass*

Aboveground biomass increased with increasing plant density, and it was significantly higher ( $P<0.05$ ) under 50 plants/m<sup>2</sup> treatment than that under any other plant density except for 30 plants/m<sup>2</sup> in the three experimental years (Figure 1). In addition, the aboveground biomass of all treatments was higher in 2008 than that in 2007 and 2009.



**Figure 1.** Effects of plant density on aboveground biomass of *C. songorica* in three years

### *Effects of density on seed yield and harvest index*

Plant density significantly affected seed yield and harvest index of *C. songorica*. Seed yield and harvest index were increased when the plant density increased from 5 plants/m<sup>2</sup> to 30 plants/m<sup>2</sup>, then decreased when the plant density rose to 50 plants/m<sup>2</sup> during three experimental years. In addition, seed yield of all treatments performed better in 2007 and 2008 than that in 2009 when the number of plants/m<sup>2</sup> was more than 10, but differences were not significant ( $P>0.05$ ) differences between 2007 and 2008 (Table 1). Harvest index of *C. songorica* were the highest (21.1% in 2007, 21.3% in 2008 and 15.9% in 2009, respectively) under 30 plants/m<sup>2</sup> treatment while they were the lowest (6.1% in 2007, 12.2% in 2008 and 8.5% in 2009, respectively) under 5 plants/m<sup>2</sup> treatment.

**Table 1 .** Effect of plant density (plants/m<sup>2</sup>) on seed yield (kg/hm<sup>2</sup>) of *C. songorica* in three years

Density	2007	Significance		2008	Significance		2009	Significance	
	yield	<i>Y</i>	<i>T</i>	yield	<i>Y</i>	<i>T</i>	yield	<i>Y</i>	<i>T</i>
5	39.7	c	e	188.5	a	d	129.0	b	f
10	149.8	b	d	316.6	a	bc	166.5	b	ef
15	273.6	a	c	333.1	a	bc	189.2	b	de
20	340.9	a	c	410.4	a	b	214.7	b	cd
25	458.5	a	b	488.2	a	ab	293.2	b	b
30	631.5	a	a	636.2	a	ab	387.6	b	a
50	447.0	a	b	410.2	a	b	242.5	b	cd

*Y* and *T* indicate significant level at 5% among years and density treatments, respectively.

*Simple correlation and regression analysis between seed yield and its components*

The results showed that the most important yield component of *C. songorica* was the number of fertile shoots/m<sup>2</sup> that was significantly ( $P<0.05$ ) positive correlated with seed yield over three years ( $r=0.924^{**}$ ,  $0.741^{**}$  and  $0.780^{**}$  in 2007, 2008 and 2009, respectively) (Table 2). In addition, seed yield also significantly positive correlated with thousand seed weights ( $r=0.475^*$  in 2007,  $r=0.755^{**}$  in 2008) and number of seeds/spikelet ( $r=0.454^*$  in 2008).

**Table 2.** Correlations between seed yield and its components of *C. songorica* in three years

Year	Components	Fertile shoots/m <sup>2</sup>	Spikelet /shoot	Seeds /spikelet	1000-seed weight	Seed yield
2007	Fertile shoots/m <sup>2</sup>		0.032	0.371	0.288	0.924 **
	Spikelet/shoot			0.307	0.260	0.180
	Seeds/spikelet				0.242	0.381
	1000-seed weight					0.475 *
2008	Fertile shoots/m <sup>2</sup>		0.532 *	0.577 **	0.712 **	0.741**
	Spikelet/shoot			0.069	0.366	0.270
	Seeds/spikelet				0.231	0.454*
	1000-seed weight					0.755 **
2009	Fertile shoots/m <sup>2</sup>		0.218	0.444 *	-0.235	0.780 **
	Spikelet/shoot			0.010	0.068	0.288
	Seeds/spikelet				0.095	0.400
	1000-seed weight					0.017

**Discussion**

This field experiment showed that seed yield, forage yield and harvest index were markedly increased with the plant density increasing up to a certain level, then decreased as the density further increased. Similar results were also found in many previous studies (Moore et al., 1993, Brahim et al., 1998, Defoor et al., 2001, Cusicanqui and Lauer, 1999). Kebe et al. (1998) reported that low seed yield occurred at extremely low and high densities, as a result of the number of branches/unit area reduced. The fertile shoots number/m<sup>2</sup> was the most important component in determining seed yield of *C. songorica* observed in present study further supporting the above mentioned study. Seed yield and forage yield in 2008 were higher than that in the other two years, this may be due to the annual accumulated temperature ( $\geq 10^{\circ}\text{C}$ ) in 2008 (3555.5 $^{\circ}\text{C}$ ) is higher than that in 2007 (3214.3 $^{\circ}\text{C}$ ) and 2009 (3489.7 $^{\circ}\text{C}$ ). This study suggests that the optimal plant density for seed production of *C. songorica* is 30 plants/m<sup>2</sup>.

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# Variation in seed shattering in a germplasm collection of *Panicum coloratum* L. var. *makarikariensis* Goossens

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## Abstract

*Panicum coloratum* L. var. *makarikariensis* Goossens (makarikari grass) is a warm-season perennial forage, to be potentially used in Argentine in sites characterized by heavy soils with highly variable climate, with successive alternate periods of drought and flooding. Before its use is generalized in the country, some issues related to difficulties in seed production of adequate amount and quality should be addressed. Phenotypic variability in seed retention reported in *P. coloratum* var. *coloratum* has raised the possibility of genetic improvement in the species. The aim of this work was to evaluate seed shattering variability in a makarikari grass collection located at INTA Rafaela Experiment Station, consisting of 5 populations collected at different sites, that serves as base of a breeding program started in 2006.

Seed shattering was evaluated using a seed trap in 10 plants per population. Panicles were set into the trap at the peak of anthesis. Seeds were collected weekly in a paper bag, taken to the lab and counted. Seed retention was calculated at each harvest date, as the ratio of the number seeds retained per panicle over the total number of seeds produced. Trap setting was performed in the summer of 3 consecutive years (2007-2008 and 2009). Data of wind intensity and rainfall were gathered for those years. Results showed that populations differed markedly in the dynamics of seed production and shattering. In 2007, peak of anthesis occurred sequentially among populations. Seed shattering took place in a similar fashion. In the following years, anthesis and shattering were synchronized among populations. The variability in SR among years found in this study suggested a large environmental component in this character in makarikari grass indicating that genetic improvement may be slow. Further studies are needed to help elucidate specific mechanisms for resistance to shattering in makarikari grass.

## Introduction

*Panicum coloratum* L. var. *makarikariensis* Goossens (makarikari grass) is a warm-season perennial grass, to be potentially used as forage in rangelands of Central Northern Santa Fe (Argentina). The site is characterized by heavy soils with highly variable climate, resulting in a succession of drought and flooding periods. The makarikari grass is naturally adapted to black, cracking clay soils in sub-humid environments (Lloyd 1981). Inflorescence development is indeterminate and seed maturation is nonuniform within the same panicle. Seed shatters readily

at maturity or earlier if weather conditions are severe (Tischler & Ocumpaugh 2004). Seed shattering and non-uniformity of seed ripening have been considered major limitations to consistent seed production because yield can be dramatically reduced if harvests are delayed for any reason, but seed quality can be compromised if harvested before physiological maturity (Roe 1972). Issues related to seed production should be addressed before promoting its utilization in the region. Fortunately, phenotypic variability in seed retention in some accession of *P. coloratum* var. *coloratum* has been previously reported, suggesting the possibility of genetic improvement in the species (Young 1986; Young 1991).

The present study was initiated to assess seed shattering variability in a germplasm collection in Argentina to evaluate the possibility of undertaking an efficient program of selection and breeding to develop a shattering resistant material.

### **Materials and Methods**

Seed shattering dynamics were evaluated in a germplasm collection established at INTA (National Institute of Agricultural Technology) Rafaela Experiment Station (31°11'41'' S; 61°29'55'' W) in October 2006. The collection included 5 populations coming from a wide range of soils, precipitation and different management regimes in north- central Argentina. Three populations were collected in Córdoba (Typic Haplustol, 600 mm annual precipitation): DF was under grazing of cattle and goats; UCB and MR were not grazed. The other two populations (ER and BR) were from Corrientes (Vertic Argiudol, 1500 mm annual precipitation, grazed by beef cattle). Populations consisting in 32 individual plants planted spaced at 0.6 m distance, were widely separated from each other to prevent cross pollination.

A seed trap especially designed was used to collect all the seeds produced per panicle. The trap consisted of a cylindrical steel structure over a pole and covered by a nylon stocking. Panicles were set into the trap at the peak of anthesis, when at least 2/3 of all florets had gone through anthesis. Ten plants per populations were evaluated. Trap setting was performed in 3 consecutive summers (2006-07, 2007-08 and 2008-09, hereafter 2007-2008 and 2009, respectively). In 2007, traps' setting up was performed on 03/6 in DF, 03/15 in UCB, 03/20 in ER and BR. A strong rainfall event the last week of March 2007 delayed trap setting in MR until 04/04 (Figure 1). Date of anthesis was uniform among populations in 2008 and 2009; therefore all traps were set on 02/04/08 and on 02/27/09. The seeds trapped in the nylon-stocking as they ripened, were collected weekly in a paper bag, taken to the lab and counted for seed shattering evaluation. In 2007, seed collection started on 03/15 in DF, on 03/21 in UCB, on 04/04 in BR and ER and on 04/11 in MR. Seed collection commenced 02/19 for all populations in 2008 and on 03/10 in 2009. Consequently, seeds were collected at 8 successive dates in 2007, 5 dates in 2008 and 9 in 2009. The samples were hand-cleaned, seeds were separated from remaining glumes and only mature seeds were counted. At the last harvest, panicles were cut, taken to the lab and the threshed seeds were counted and added up to get the total number of seeds per panicle.

Seed retention (SR) was calculated at each harvest date, as the ratio of the number seeds retained per panicle over the total number of seeds produced by each panicle. In addition, data of wind intensity and rainfall were gathered for those years. Statistical analysis of SR data was performed using Mixed Model procedure of SAS® using the compound symmetry covariance structure. For the purpose of comparisons only 5 harvesting dates per year were considered. Main effects and the interactions of year, population and date of harvest were made by using analysis of variance. Mean comparisons were made using LSD. The significance level for all comparisons was  $P < 0.05$ .

## Results

Total precipitation during the growing season (November to April) for the 2006-07, 2007-08 and 2008-09 were 1224.9 mm, 495.9 mm and 579.5 mm, respectively. The 70-yr average precipitation for the site over the same period is 707 mm. Storms with copious rainfall events and wind velocities over  $10 \text{ km.h}^{-1}$  were frequent in the summer of 2007. Lower precipitation and less intense winds characterized the summer of 2008 and 2009, compared to 2007 (Figure 1).

Results of ANOVA are shown in table 1. Interactions were all significant, and then factors were analyzed separately. Populations differed markedly in the dynamics of seed production and shattering (Figure 1). In 2007, the first cycle after plants were transplanted from their original site, peak of anthesis occurred earlier in DF, one week later in UCB, then in BR and ER and finally in MR. Populations DF was also the first to start shattering seeds in 2007. Populations UCB and BR were intermediate while ER and MR retained the seeds until April. A massive seed falling (peak shattering) in DF and UCB was associated to a big storm by the end of March. On the contrary, anthesis and shattering were synchronized among populations in 2008 and 2009. Strong surface winds and rainfall could be associated to a notorious seed falling event (peak shattering) in all 5 populations in 2008. Winds were constant though calmer than before during the summer of 2009, nonetheless seed shattering was concentrated the first week of April.

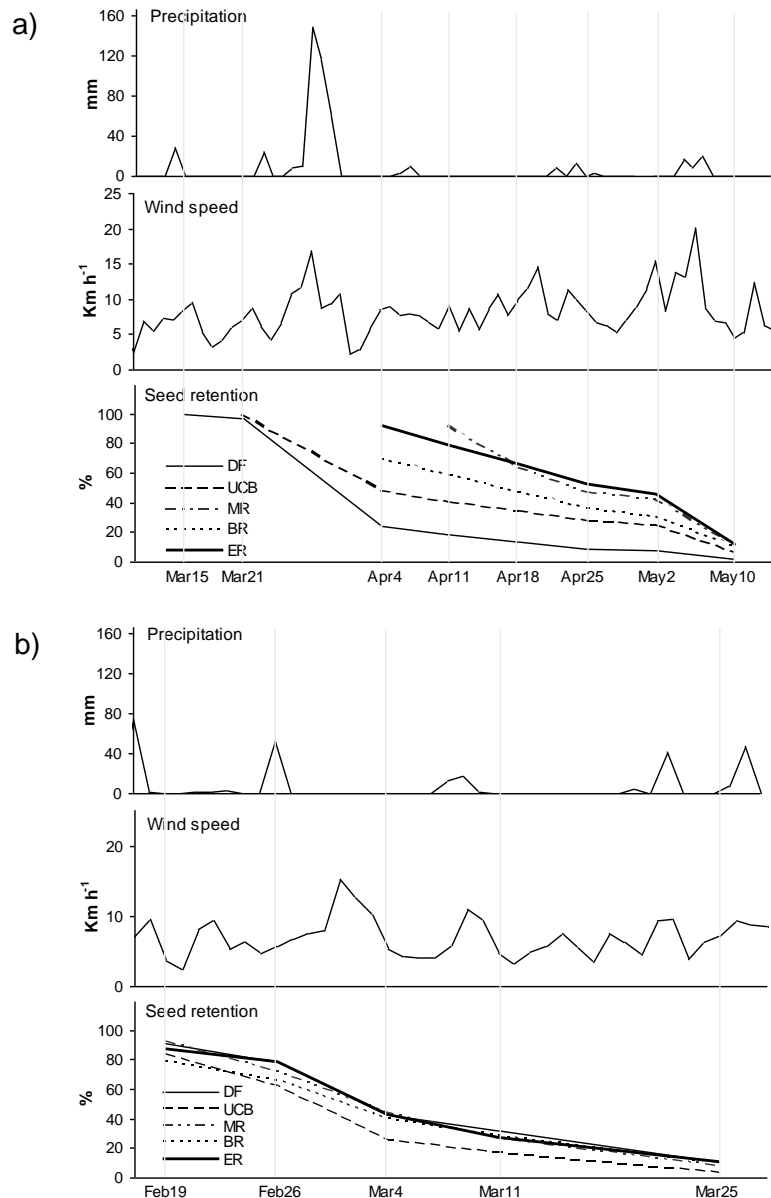
**Table 1.** Analysis of variance for seed retention percentage for 5 populations of makarikari grass evaluated 3 successive years at 5 consecutive harvesting dates in Rafaela, Santa Fe, Argentina.

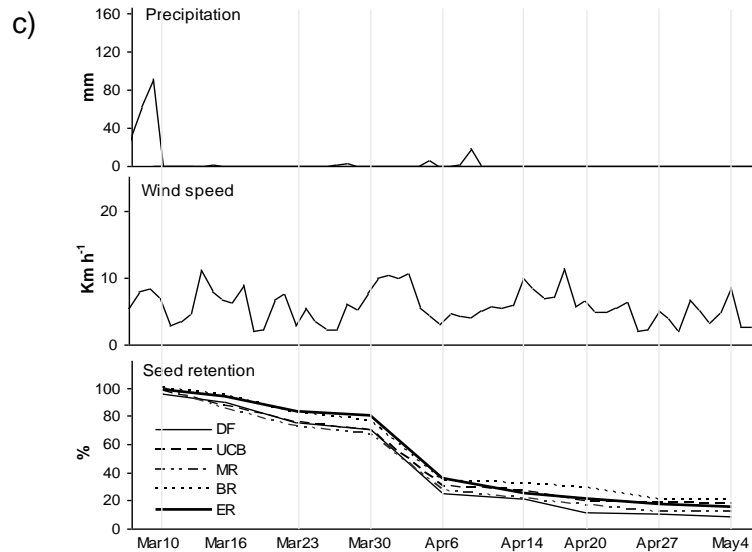
Source	df	F- value
Population (P)	4	7.3***
Year (Y)	2	65.5***
Harvest date (D)	4	726.8***
P x Y	8	31.6***
P x D	16	4.1***
Y x D	8	24.5***
P x Y x D	32	3.2***

\*\*\* Significant at the 0.001 level.

Mean seed retention percentages (SR) per population per year are shown in table 2. Within populations, mean SR percentage was significantly different among years ( $p < 0.001$ ). Populations ER and MR showed the best SR performance in 2007 while DF and UCB highest retention was in 2009. Seed retention was comparable in 2007 and 2009 for BR population. The worst retention performance for BR, ER, MR and UCB were in 2008 while DF retained the least in 2007. Within years, differences in mean SR among populations were highly significant in 2007 ( $p < 0.001$ ); the highest SR were observed in MR and ER, BR and UCB were intermediate and DF was the one with the least retention. Differences in SR were less pronounced in 2008 ( $p < 0.05$ ) and populations showed comparable performance in 2009 ( $p = 0.28$ ).

**Figure 1.** Seed retention (%) in populations of *Panicum coloratum* L. var. *makarikariensis*, rainfall (mm) and wind velocity at 2 m on the ground level ( $\text{km h}^{-1}$ ), registered in the summer of a) 2007, b) 2008 and c) 2009. Seed collection dates are shown in the X axis.





**Table 2.** Mean seed retention percentage and standard errors for 5 populations of makarikari grass evaluated 3 successive years at 5 consecutive harvesting dates in Rafaela, Santa Fe, Argentina.

Population	2007	2008	2009‡
MR	79.71 <sup>aA</sup>	47.87 <sup>cA</sup>	53.72 <sup>b</sup>
ER	77.79 <sup>aA</sup>	49.16 <sup>cA</sup>	63.54 <sup>b</sup>
BR	60.13 <sup>aB</sup>	44.72 <sup>bAB</sup>	61.23 <sup>a</sup>
UCB	51.57 <sup>bB</sup>	37.70 <sup>cB</sup>	59.12 <sup>a</sup>
DF	34.04 <sup>cC</sup>	48.33 <sup>bA</sup>	58.37 <sup>a</sup>

Within rows, different small letters indicate significant differences at 0.05 level.

Within columns, different cap letters indicate significant differences at 0.05 level.

‡ Population means were not significantly different in 2009 at 0.05 level.

## Discussion

Results from this study showed that patterns of seed retention changes among populations and among years in a germplasm collection of makarikari grass growing in central-Argentina. Population ER from Corrientes exhibited the least shattering percentage in 2007 but, no differences in SR among populations were detected in 2009, even though a tendency was apparent. A delayed seed shattering in ER in 2007 could be related to a deferred anthesis associated to a differential origin. Floral transition may be affected by signals that feed into both environmental and endogenous (or autonomous) pathways (Colasanti & Coneva 2009) and it was reported that weather conditions during the previous season affected flowering capacity and seed yield in reed canarygrass (*Phalaris arundinacea* L.) (Sahramaa *et al.* 2004).

In this study, time elapsed since the peak anthesis to peak shattering was variable among years and populations. In 2007, time elapsed since the peak anthesis to peak shattering was shorter in

DF and longer in ER and MR. However, peak anthesis and seed shattering were synchronized among populations in 2008 and 2009. A substantial seed fall occurred 2 weeks after anthesis in 2008, and 4 weeks after anthesis in 2009. Optimal harvest time has been conveniently correlated to growing degree units after peak anthesis in several forage grasses (Berdahl & Frank 1998) but it was found to be variable between years in eastern gamagrass (*Tripsacum dactyloides* L.) (Lemke *et al.* 2003). Further studies need to be performed to find an indicator to help managers decide optimum harvest timing in makarikari grass.

## Perspectives

The variability in SR among years found in this study suggested a large environmental component in this character in makarikari grass indicating that genetic improvement may be slow. However, results could be successful if selection for SR was performed on specific characters not related to environmental interactions, such as inflorescence structure and abscission of the glumes (Falcinelli *et al.* 1984; Whalley *et al.* 1990). Therefore, morphological and histological studies on inflorescence structure and seed abscission may be needed to help elucidate specific mechanisms for resistance to shattering in makarikari grass.

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## Harvest methods and seed yield potential in *Brachiaria* hybrids

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### Abstract

Until 2000, *Brachiaria* spp. cultivars were derived without genetic modification directly from natural germplasm collected in Africa. A breeding program was initiated at CIAT in 1988. Trials demonstrated the superiority of Mulato II, a vigorous, semi-erect grass with very deep and branched roots giving it excellent drought resistance. Mulato II has excellent nutritional value: CP is in the range of 14 to 22% and IVDMD from 55 to 65%. These values, similar to temperate grasses, are unusual in warm-season grasses. Seeds yields are encouraging and are related with management, harvest methods, and geographical locations.

### Introduction

Until 2000, *Brachiaria* spp. cultivars were derived without genetic modification directly from natural germplasm collected in Africa.

A breeding program, based on sexual germplasm kindly provided by C.B. do Valle (EMBRAPA-CNPGC), was initiated at CIAT in 1988, to combine desirable attributes found in accessions of *B. brizantha* and *B. decumbens*. (Miles *et al.*, 2004).

Since the release of cv. Mulato II, a series of agronomic experiments have been conducted. Trials, demonstrated the superiority of Mulato II, a vigorous, semi-erect grass with very deep and branched roots giving it excellent drought resistance, confirmed by results in the Brazilian Cerrado, Central America, Mexico, Asia, as well as in the Argentine Chaco (Pizarro *et al.*, 2008). Mulato II is the most innovative alternative to improve ruminant livestock productivity in the tropics (Pizarro *et al.*, 2008).

Mulato II has the potential to produce in excess of 500 kg ha<sup>-1</sup> of pure seed, as evidenced by experiments where an effort is made to recover all pure seed produced, either by bagging inflorescences or by opportune recovery of fallen seed (Hare *et al.*, 2007c; Chávez Chena *et al.*, Personal communication). How much of this potential is realized in practice will depend on many factors, including harvest method.

Choosing an appropriate method for seed harvest depends firstly on the particular species, especially its growth habit and seed structure, the synchrony of crop development and relative amounts of standing and fallen seed. Secondly, it depends on the availability of machinery or hand labor and finally, on previous experience.

Several methods are commonly used for harvesting forage grass seed. For the genus *Brachiaria* both manual and mechanized harvest has been employed. Mechanized harvest is further divided into direct heading and recovery of fallen seed.

### **Seed harvesting procedures used in *Brachiaria* hybrids: Manual harvesting**

**Thailand:** (Field trials were conducted in Ubon Ratchathani province, Thailand (15° N Lat., 130 masl, AAR 1538 mm).

### **Effect of time of planting on seed production of Mulato II** (Hare *et. al.*, 2007a)

Seven tiller planting dates were compared in a five replicate, randomized complete block field trial. Tillers with roots were divided from 1-yr-old Mulato II plants.

**Table 1.** Effect of time of planting Mulato II on seed yields and seed yield components

<b>Time of planting</b>	<b>Inflorescences m<sup>2</sup></b>	<b>Racemes inflorescence</b>	<b>Spikelets raceme</b>	<b>Seed yield kg ha<sup>-1</sup></b>
May 16	163	4.7	35.0	138
June 1	138	5.2	34.6	109
June 16	122	5.3	34.0	80
July 1	104	5.0	32.7	54
July 15	59	2.8	25.5	20
August 1	23	2.7	21.9	6
August 16	-	-	-	-
LSD(P<0.05)	28	0.7	6.4	28

Planting earlier in the season produced higher seed yield as well as a greater numbers of inflorescences and seeds than subsequent plantings. Planting in August produced either very low seed yield (5 kg ha<sup>-1</sup>) with low thousand seed weight, or no seed at all. Raceme and spikelet numbers were reduced when tillers were planted from mid-July onwards.

### **Effect of harvesting method on seed yield and seed quality of Mulato II** (Hare *et al.*, 2007c)

Four harvest methods were compared in a four-replicate, field experiment. In the first three methods, inflorescences were tied up into "living sheaves" and seed knocked into large cloth bags daily, twice daily, or on alternate days. For the fourth method, inflorescences were enclosed in mesh bags. In the fifth treatment, fallen seed was swept up from the ground.

**Table 2.** Effect of harvesting method on Mulato II seed yields and seed viability

Harvest method	Seed yield kg ha <sup>-1</sup>	TSW g <sup>-1</sup>	Seed viability %
Knocking daily	230	8.79	92
Knocking twice daily	271	8.68	92
Knocking 2 days	255	8.94	89
Nylon net bag	509	9.03	91
Ground sweeping	87	8.20	84
LSD P<0.05	73.2	0.38	5.8

The method of tying nylon net bags over the seed heads to collect seed produced the highest Mulato II seed yield, which was twice the yield than from the 3 methods of knocking seed heads. Sweeping Mulato II seed from the ground produced a much lower seed yield, lighter seed and seed with lower viability than other harvesting methods possibly owing to ant predation or seed rotting on the ground.

#### **Yapakani – Bolivia** (J. H. Antezana Rojas)

In Yapakani, (17° 4' S – 53° 83' W, 500 masl, AAR 1800 mm), Santa Cruz Province, Bolivia, farmers with limited capital have hand-harvested seed of cv. Mulato II, using sickles to sever seed heads above the leaf canopy. These are bound in sheaves and stacked in the field to "sweat". About two weeks later, the stokes are collected and threshed, normally by beating the seed out on a sheet with sticks.

Seed quality can be low (up to 80% dead seed with sweating and hand threshing, compared with only 30% with mechanized harvest). The dead seed is mainly due to overheating during sweating.

#### **Recovery of fallen seed: Chiapas – Mexico:** A. Chávez Chena, R. R. Ramón, A. Balbuena and I. G. Miranda

Field trials were conducted in Chiapas (15°27' N - 92°16' W, 2800 masl, AAR 2000 mm) for two consecutive years with four replications.

The recovery of fallen seeds can potentially collect a greater proportion of total seed yield than methods previously described which target the standing crop.

Moreover, the viability of seed recovered from the ground is normally high, because seeds are mostly mature when shattering occurs.

**Table 3. Effect of the length of harvesting period on seed yield**

Length of harvesting period days	Harvested pure seed kg ha <sup>-1</sup>	
	Paraíso*	Jacaranda II*
0	533a**	619a**
7	508a	713a
14	242b	581b
21	239b	542b
28	173b	593b
35	188b	422b
42	100c	344c
49	92c	281c
56	119c	212c
63	88c	110d

\*Paraíso= 3 years crop; Jacaranda II=2 years crop. \*\* Values with different letter differ significantly (P < 0.05)

Seed fields are invariably rolled immediately after sowing to facilitate harvesting by the ground-sweeping methods. In some years, late crops are attacked by “honey dew” (an ergot caused by *Claviceps* spp.) thereby reducing yields and seed quality.

Although there are good seed yields in both areas, the field seed losses are significantly very high in a 60 days harvesting cycle. Mechanical sweeping need to be carried out within two to three weeks in order to recover at least 50% of the seed (Table 3).

Ground sweeping was not successful in Thailand in research trials. However, farmers in one village only use ground sweeping. Yields of over 500 kg ha<sup>-1</sup> are common. In Brazil and Mexico, this method has been the predominant seed harvesting method of *Brachiaria* species for the past two decades, producing up to 700 kg/ha from either manual or machine sweeping.

We think that due to the length of the harvesting period, a lot of the seed is eaten by ants and perhaps a smaller amount rots on the ground. Brachiariagrass hybrid seeds are relatively soft when they shed and can be easily eaten by ants.

Moist conditions during harvest, from either rain or heavy dews, could contribute to brachiariagrass hybrid seed rotting on the ground.

### **Combine harvesting** (J. H. Antezana Rojas)

Due to the fact that mechanical seed sweeping equipment is costly and the harvest season is very long and a significant amount of seed inevitably is left in the field, or carried away by ants, and perhaps a smaller amount rots on the ground, there is a renewed interest in combine harvesting brachiaria seed.

Direct headed seed is especially at risk because of its high initial moisture content (up to 50 - 60%), and rapid deterioration can occur if freshly harvested seed is left in moist unventilated bulk for more than a few hours, especially with small dense seeds that pack tightly.

In Bolivia, the trials carried out in Montero (17° 20'S - 63° 10'W, 300masl, AAR 1300 mm), the final yield was 100 kilogram pure seed per hectare. Further improvement can be achieved, since seed swept samples collected by hand from the ground after mechanical harvesting reached more than 350 kg pure seed ha<sup>-1</sup>. New experiments are in the pipeline.

### **Discussion and perspectives**

When the components of seed yield are examined it appeared that the number of inflorescences and seeds m<sup>2</sup> were the most critical components of yield. Planting early in the wet season, May or June (Northern Hemisphere), produced the most brachiariagrass hybrid seed in the current study.

Planting early enables farmers to cut forage from their seed crops before closing. The forage of the brachiariagrass hybrids cut before closing was of a very high quality in all trials, with a high proportion of leaf (55-60%) and a high leaf crude protein concentration that averaged over 15%.

The time of final closing date defoliation was found to be extremely important for seed production of brachiariagrass hybrids in Thailand (Hare *et al.*, 2007b) and Bolivia (J. H. Antezana Rojas, personal communication) as well as the length of the harvesting period as was evaluated in Mexico.

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# Alfalfa seed production in semi-humid climate of the southeast Europe

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## Abstract

The main characteristic of the seed production of alfalfa (*Medicago sativa* L.) in South East Europe is an extremely wide variation of yield, depending on weather conditions in a specific year, from 50 kg ha<sup>-1</sup> to 1000 kg ha<sup>-1</sup>. In the most important agricultural region of Serbia, during thirteen years (1997-2009) and on four locations, there were measured alfalfa seed yields and monitored basic meteorological data, such as maximum, minimum and average daily air temperatures, insolation, rainfall and number of days with rainfall. The effect of ecological factors on seed yield was pronounced. The lowest average yield was obtained in 2005 (49 kg ha<sup>-1</sup>), the highest in the year 2008 (530 kg ha<sup>-1</sup>), while the yield in 1998 was somewhat lower than the long term-average for the Serbia (283 kg ha<sup>-1</sup>). Highly significant positive correlations were obtained between seed yield on one side and mean and maximal air temperatures and solar radiation on the other: 0.633<sup>\*\*</sup>, 0.676<sup>\*\*</sup> and 0.355<sup>\*\*</sup>, respectively. Highly significant negative correlations existed between seed yield and precipitation data: for growing season (May-August),  $r = -0.667^{**}$ ; for June-August,  $r = -0.660^{**}$ , and for July-August,  $r = -0.761^{**}$ , respectively. The main limiting factor of alfalfa seed production in semi-arid climatic conditions of southeast Europe is a high rainfall during the growing season of alfalfa seed stand (May-August), especially in the stages of flowering and maturity (July-August).

Key words: *Medicago sativa* L., seed, yield, lodging, environment, correlation.

## Introduction

Alfalfa is the most important forage legume due to its high forage yields of excellent quality. The area under alfalfa in the South East Europe takes between 3.5 % and 5 % of the total arable land. In Croatia, it is grown on 42.000 ha (Stjepanović *et al.*, 2009), in Hungary 150.000 ha and in Romania on 500.000 ha (Valentina, pers. comm.). In Serbia, with its central position in the South East Europe, alfalfa is cultivated on about 200,000 ha ([www.fao.org](http://www.fao.org)). According to the data of the Department of Statistics, the production of the certified alfalfa seed in 2009 was carried out 2.900 ha, while it is estimated that the non-certified alfalfa seed is produced on more than 1.000 ha (Karagić *et al.*, 2007).

Alfalfa has a genetically determined potential render extremely high forage yields, which are often in a negative correlation with seed yields (Bolanos-Aguilar *et al.*, 2002). Good alfalfa

cultivars are characterized by slender, soft and easily digestible stems. Such plant morphology and mechanical features of the stem make them prone to lodging.

Because of these characteristics, alfalfa seed yield is strongly affected by ecological factors and it varies significantly in dependence of weather conditions, more than the other crops. Average long-term variations in seed yield are 14,5% in corn, 17,6% in winter wheat and up to 57% in alfalfa (Zarinov & Kljuj, 1990).

In regions with semi humid climate, weather conditions in the year of growing are the main source of variation in alfalfa seed yield. In years with high rainfall, alfalfa plants are lush and they lodge easily. Lodged plants are not suitable for pollination and low seed yields are consequently produced (Huyghe *et al.*, 2001). In conditions of high soil moisture, there are no effective cultural practices to avoid the lodging of seed crop and seed losses. The most efficient measure is the selection of suitable production region. Moderate soil moisture content in combination with high air temperatures is required for seed production (Goloborodko & Bodnarcuk, 1998).

The objectives of this paper were to analyze the alfalfa seed yields obtained in Serbia and to establish correlations between the seed yields and the ecological factors.

### Materials and Methods

Impact of weather condition on alfalfa seed yield was studied in thirteen experimental years (1997-2009) on four locations. The experimental sites were located in northern Serbia, at 45°83' N, 20°46' E (Kikinda), 45°77' N, 19°11' E (Sombor), 45°20' N, 19°51' E (Novi Sad), and 44°58' N, 19°36' E (Mitrovica). This area has a continental semi-arid to semi-humid climate, a mean monthly air temperature of 11.0°C, a total annual precipitation of around 600 mm, and a highly uneven distribution of precipitation. Table 1 shows the monthly mean air temperatures and monthly sums of precipitation for the period May-August.

**Table 1.** Basic meteorological parameters of four locations in Serbia (1997-2009)

Climatic factor	Locality	Month				Average/ Total
		May	June	July	August	
Mean air temperature (°C)	Kikinda	17,6	21,0	22,6	22,3	20,9
	Sombor	17,6	20,7	22,1	22,0	20,6
	Novi Sad	17,7	20,8	22,3	22,2	20,7
	Mitrovica	17,7	20,7	22,1	22,0	20,6
	Average	<b>17,7</b>	<b>20,8</b>	<b>22,3</b>	<b>22,1</b>	<b>20,7</b>
Precipitation (mm)	Kikinda	48,5	70,9	58,6	54,2	232,2
	Sombor	49,1	81,2	78,8	55,0	264,1
	Novi Sad	62,2	92,8	76,2	58,4	289,6
	Mitrovica	56,4	80,1	66,5	55,2	258,2
	Average	<b>54,1</b>	<b>81,3</b>	<b>70,0</b>	<b>55,7</b>	<b>261,0</b>

Rather near the meteorological stations there has been established a long-term trial with two alfalfa cultivars (NS Banat ZMS II and NS Mediana ZMS V) in the spring of 1996. Both cultivars were sown with a row spacing of 12.5 cm and with a seeding rate of 15 kg ha<sup>-1</sup>. The trial was set up according to a randomized block design with four replicates. The plot size was 10 m<sup>2</sup> (2 x 5 m) with 16 rows per plot. The alfalfa seed is produced in the period starting with the second and ending with the fifth year of growth. On each location the sowing is repeated every fourth year. The first cut is done in the stage of full flowering (late May), when the cut biomass is removed from the field, while the seed is produced in the second growth.

Alfalfa seed was harvested in a single passage of a Hege harvester, after desiccation with Diquat performed when about 70% of pods on normally developed plants were in the stage of physiological maturity (mid-August). Seed yield was calculated on the basis of measurements of processed seed per plot. The obtained results were statistically processed by the analysis of variance. The differences among mean values were compared using the least significant difference test (LSD). Because the analyses of variance did not show significant differences in seed yield between the tested cultivars, the values are reported as means of the two varieties. The simple correlation coefficients were calculated between the seed yields of the tested cultivars and the monitored meteorological parameters, with 104 pairs, and for three periods: seed stand period (from May till August), the period of budding to harvest (June-August), and for the period of flowering to harvest (July-August).

## **Results and Discussion**

The maximum variation in seed yield was caused by weather conditions in the growing year. In 2008, which had favorable ecological conditions, the seed yield was 10.8 times higher than in 2005, which had extremely unfavorable conditions (Table 2). The highest average seed yield was on the location of Kikinda (348 kg ha<sup>-1</sup>), while the differences in seed yields between the other locations were not significant.

**Table 2.** Alfalfa seed yield depending on location in the period 1997-2009

Year (Y)	Location (L)				Average
	Kikinda	Sombor	Novi Sad	Mitrovica	
1997	217	220	50	360	<b>212</b>
1998	300	270	250	310	<b>283</b>
1999	44	120	50	180	<b>99</b>
2000	450	480	510	380	<b>455</b>
2001	290	244	220	246	<b>250</b>
2002	330	230	300	270	<b>283</b>
2003	310	480	300	230	<b>330</b>
2004	340	210	250	200	<b>250</b>
2005	70	15	50	60	<b>49</b>
2006	320	160	220	100	<b>200</b>
2007	530	400	480	390	<b>450</b>
2008	880	300	580	360	<b>530</b>
2009	440	500	500	560	<b>500</b>
Average	<b>348</b>	<b>279</b>	<b>289</b>	<b>280</b>	<b>299</b>

LSD	Y	L	Y x L
0.05	22.06	17.11	41.15
0.01	31.18	23.82	64.63

**Table 3.** Correlation between alfalfa seed yield and some climatic factors (1997-2009)

Climatic factor	Analyzed period		
	May-August	June- August	July - August
Mean air temperature	0,575	0,611	0,633
Max air temperature	0,676	0,649	0,632
Min air temperature	-0,156	-0,118	-0,161
Insolation	0,154	0,355	0,307
Precipitation	-0,667	-0,660	-0,761
No. of rainy days	-0,628	-0,636	-0,692

Highly significant positive correlations were obtained between seed yield on one side and mean and maximal air temperatures and solar radiation on the other: 0.633\*\* (July-August), 0.676\*\* (May-August) and 0.355\*\* (June-August), respectively (Table 3). Highly significant negative correlations existed between seed yield and precipitation data: for growing season (May-August),  $r = -0.667^{**}$ ; for June-August,  $r = -0.660^{**}$ , and for July-August,  $r = -0.761^{**}$ , respectively. Numerous authors (Žarinov & Ključ, 1990; Huyghe *et al.*, 2001; Bolanos-Aguilar *et al.*, 2002; Karagić *et al.*, 2007; Stjepanović *et al.*, 2009) agree that variation in alfalfa seed yield is primarily due to weather conditions in the year of growing. Among them, the total amount and distribution of rainfall were most important.

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## **On-farm conversion of straw to bioenergy – A value added solution to grass seed residue**

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### **Abstract**

Analysis of the geospatial distribution of straw from grass seed and cereal crops across the PNW indicates that optimally-sited bioenergy conversion plants of 1 million kg y<sup>-1</sup> capacity should be able to obtain needed straw from within a radius of a very few km, opening up the possibility of using farm-scale equipment such as forage choppers, wagons, silage blowers, and bunkers to handle the straw from field to syn-gas generator. The economic advantages of not needing to bale and truck the straw long distances will at least partially offset efficiencies of scale likely present in larger plants operating at many times the capacity of farm-scale units.

Perhaps the most contentious aspect of intensive grass seed production systems has been the management of post-harvest residues. Conflicts over possible adverse effects of smoke from field burning on human health and economic impacts of regulating burning on the grass seed industry have raged in courtrooms, legislatures, elections, and the mass media for decades. Because use of burning to dispose of grass seed and cereal straw throughout the Pacific Northwest (PNW) is now banned or restricted in most areas, agricultural producers have actively sought cost-effective alternatives. In higher rainfall regimes such as Oregon's Willamette Valley, thorough chopping of the full straw load in the dry, late summer facilitates its decomposition in the wet fall and winter while remaining compatible with high yields of quality seed. Growers using this method view retention of nutrients and building of soil OM as adequate trade-offs for the nuisance of chopping straw and increased problems with slugs and weeds. Other growers bale their residues for domestic use and overseas export as livestock feed, often receiving little more than the cost of baling. In collaboration with partners including the electrical power industry, researchers at the National Forage Seed Production Research Center have built a pilot plant in Spokane for conversion of straw to syn-gas, which can then generate electricity fed back into the regional power grid. The nominal size of the plant is 1 million kg y<sup>-1</sup>, comparable to straw produced on medium-sized PNW grass seed or cereal farms. Testing of the syn-gas generator is focusing on the impact of operating conditions on CO and H<sub>2</sub> content of the syn-gas and on impurities in it that could damage the diesel engine powering the electrical generator.

Knowledge of the geospatial distribution of straw from grass seed and cereal production in the PNW is vital to the accuracy and reliability of feasibility studies comparing scales of operation of proposed bioenergy conversion plants. Because existing data on straw availability were limited to county-wide summaries, our first step in identifying optimum locations for straw-

based bioenergy conversion plants was to map the location of all grass seed and cereal production in the PNW using remote sensing methods. For satellite imagery necessary for remote sensing classification, we used MODIS 16-day composite NDVI, 250 m by 250 m pixels, covering the periods from April 23 through August 29 in 2005, 2006, and 2007. Crop areas and yields per ha within counties were obtained from yearly USDA-NASS summary statistics for winter, spring, and durum wheat, barley, and oats. Areas and yields per ha for grass seed crops were primarily obtained from OSU Extension Service estimates within Oregon and USDA-NASS summaries in Idaho and Washington. Ground-truth data for the remote sensing classifications of cereals were derived from USDA-NASS National Crop Land Data layers (NLCD) covering southern Idaho in 2005, Washington in 2006, and the entire PNW in 2007. Ground-truth data for grass seed crops were a mixture of our in-house, western Oregon GIS and the NLCD. Maps of crop locations were converted into straw yields by use of county-wide average crop yields and harvest indices, and then subtracting crop-specific estimates of residue requirements to protect soils from erosion. Larger quantities of straw were “left behind in the field” for annual crops such as winter wheat or Italian ryegrass than for perennial grasses whose crowns and roots help protect the undisturbed soil from erosion.

Our estimates of total available cereal and grass seed straw in the PNW were 7.01, 6.27, and 5.63 million metric tons in 2005, 2006, and 2007. We used the individual year estimates and multi-year averages of available straw in procedures that identified the optimal locations for each new bioenergy plant, based on local density of straw and location of all previously sited plants. Each new plant was sited at the position of the maximum straw density over a neighborhood adequate to supply all the straw needed for plants with capacities of 1, 10, and 100 million kg y<sup>-1</sup>. Straw assigned to each new plant was then removed from the raster and the location of the maximum density of remaining straw recalculated.

Approximately 6,200 farm-scale plants (1 million kg straw y<sup>-1</sup> capacity) distributed across landscape would be required to convert all the available straw in the PNW into bioenergy. Approximately 620 medium-sized plants (10 times greater capacity than the farm-scale units) would be needed to process all the available straw (Fig. 1). The first 10 million kg y<sup>-1</sup> plant built could obtain all its straw from within a distance of only 2 km, while the 124<sup>th</sup> plant (20% of 620) would only need a range of 4 km to meet its needs. Relative to the average distance required to supply straw to the first 10% of plants, a range of twice that distance was sufficient for 70% of the smallest sized plants, and 60% of the medium- and largest-sized ones. The final 10-20% of straw is extremely hard to justify going after for all plant sizes. Locations of the most easily supplied plants clearly show the regions across the PNW where a straw-based bioenergy industry is likely to initially develop. Maps of the 6,200 smallest-sized plants tend to show a more egalitarian distribution of optimal locations across all production areas in the PNW. In contrast, the strongest regional differences in how far straw would have to be transported occurred for the largest-sized plants. Distribution of the 62 largest (100 million kg y<sup>-1</sup>) capacity plants (Fig. 2) differed somewhat from that of the medium capacity plants (Fig. 1), with the best 20% of sites

for the largest plants all occurring in the Willamette Valley, except for a single one in the eastern Snake River Valley of southern Idaho. The next best locations (stars) occur over a broader set of regions, including the Palouse Hills and the Columbia Basin in eastern Washington.

One obvious concern with the methods we used to identify optimal plant locations is that they are based on a single estimate of production. Because the specific crops grown within individual fields often change from year to year, a logical question is what impact this yearly variation has on the efficiency of plant siting. In other words, if plant locations are optimized for crop (and straw) distribution patterns of one year (e.g., 2005), how well would those locations function as centralized collection points for another year (e.g., 2006)? Since the bioenergy conversion plants are unlikely to be mobile, a relatively simple way to evaluate the impact of yearly variation in cropping patterns was to measure how much straw was available around plants whose locations and collection distances were optimized for one year when a second year's straw distribution was assumed. Practical limitations in programming methods used to optimize plant locations caused some variability to exist in amount of straw present within the defined ranges around each plant even when the same year was used to define locations (and collection ranges) and measure straw availability. Using the CV of the straw availability at each plant for the "same year" analysis as the standard, a ratio of the CVs can be calculated showing how much less stable the straw supply would be in some other year compared to the one used to locate the plants. The worst combination we found was when medium-sized plants were located based on 2007 straw distribution and tested using 2005 straw distribution, with a CV ratio of 11.6-fold (Table 2). The smallest CV ratios occurred when the 3-year average straw distribution was used to define plant locations, with ratios for 2005, 2006, and 2007 ranging from 1.7 to 2.2 X for the smallest plants, 2.6 to 3.9 X for the medium sized plants, and 1.5 to 2.2 X for the largest plants. The individual CVs generally followed a pattern of slowly decreasing with increasing plant size, with mean CVs for all combinations of years-defining and year-testing straw availability averaging 50.0, 32.3, and 27.1% for the smallest-, medium-, and largest-sized plants.

In a "young" straw as bioenergy industry, yearly variation in cropping practices and straw yields around individual plants will merely generate small changes in the distance required to supply sufficient straw for plants. In a "mature" bioenergy industry, the yearly variations will likely also impact how close to full capacity the plants can operate and the prices paid for straw. The largest scale straw-to-bioenergy plants currently under development in the Willamette Valley are designed to utilize 150 million kg y<sup>-1</sup>. Even a plant that large would only need 2.3% of the total available straw in the PNW. As a consequence, there is ample opportunity for market forces to determine how much straw will continue to be exported as livestock feed, how much will be converted into electricity and other energy products, and what mix of small-scale, on-farm and large-scale, industrial park bioenergy projects will operate to convert the straw into bioenergy.

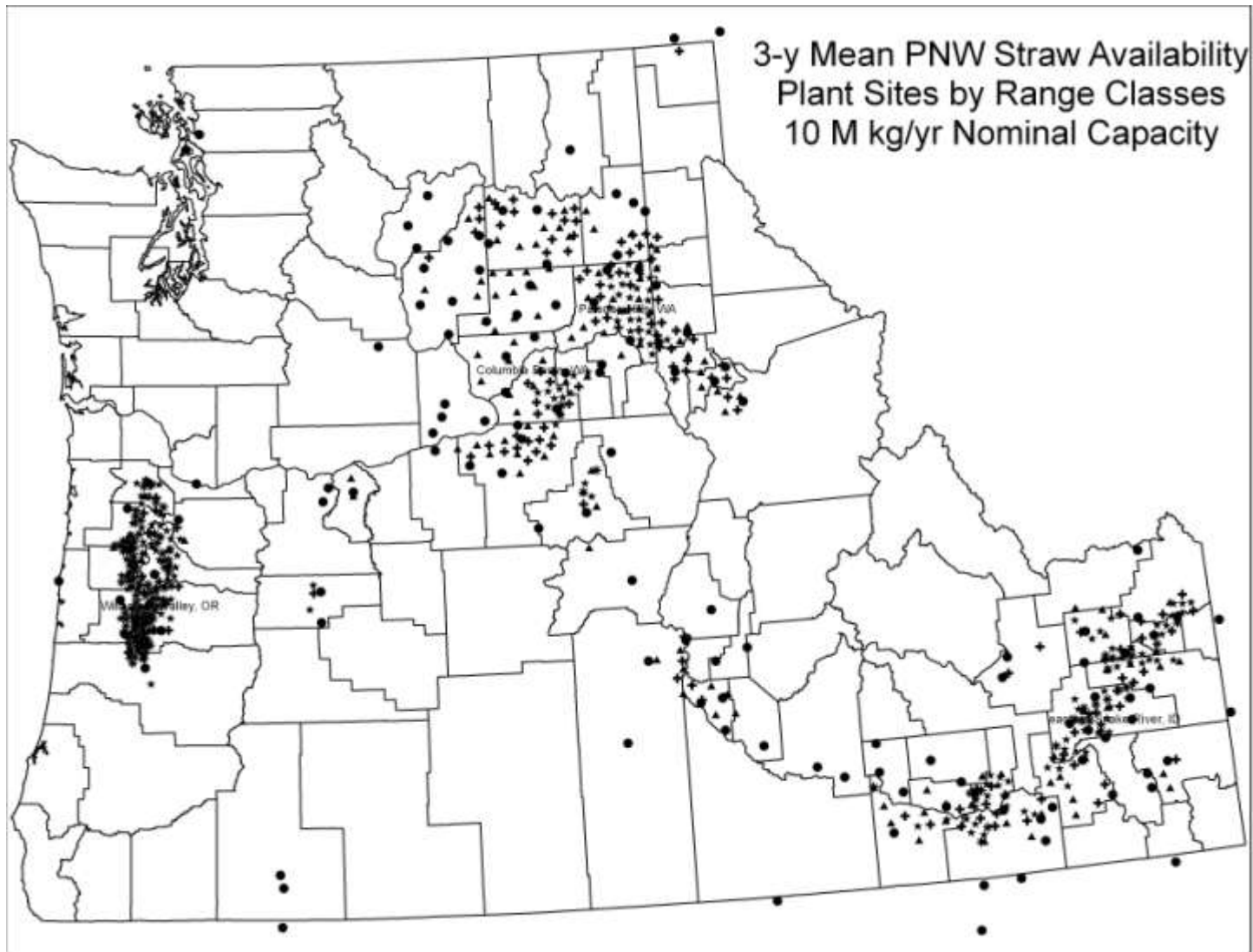
**Table 1.** Average distances required to provide sufficient straw to supply bioenergy conversion plants for each 10 percentile increment in total straw assigned using 3-year average density.

Incremental Percentiles of Total Available Straw Assigned to Optimal Plant Site Locations										
State	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
<b>10<sup>6</sup> kg y<sup>-1</sup> capacity</b>	<b>Average Range Required for Adequate Straw to Meet Plant Capacity (km)</b>									
Idaho	1.2	1.5	1.7	1.8	2.0	2.1	2.3	2.9	4.9	31.2
Oregon	0.9	1.2	1.4	1.5	1.7	1.9	2.1	2.4	3.4	19.2
Washington	1.5	1.7	1.8	2.0	2.1	2.4	2.8	4.0	5.9	16.6
entire PNW	1.2	1.4	1.6	1.8	1.9	2.1	2.4	3.0	4.6	23.0
<b>10<sup>7</sup> kg y<sup>-1</sup> capacity</b>										
Idaho	3.7	4.3	4.8	5.5	6.1	6.9	8.2	11.5	17.6	88.9
Oregon	2.3	2.7	3.1	3.5	4.0	4.9	5.8	7.4	11.1	39.6
Washington	4.6	5.1	5.5	6.0	6.9	8.2	9.9	12.3	18.0	66.5
entire PNW	3.5	4.0	4.4	4.9	5.5	6.6	7.7	10.2	15.4	64.9
<b>10<sup>8</sup> kg y<sup>-1</sup> capacity</b>										
Idaho	14.0	16.4	17.5	18.1	25.3	27.5	29.0	46.3	98.2	276.5
Oregon	7.9	9.2	10.7	11.7	13.2	14.8	21.8	28.4	47.0	186.4
Washington	14.8	16.2	19.6	20.9	24.7	26.4	32.2	39.5	44.7	296.2
entire PNW	11.7	14.3	15.2	16.2	21.1	23.6	26.0	39.2	68.3	243.5

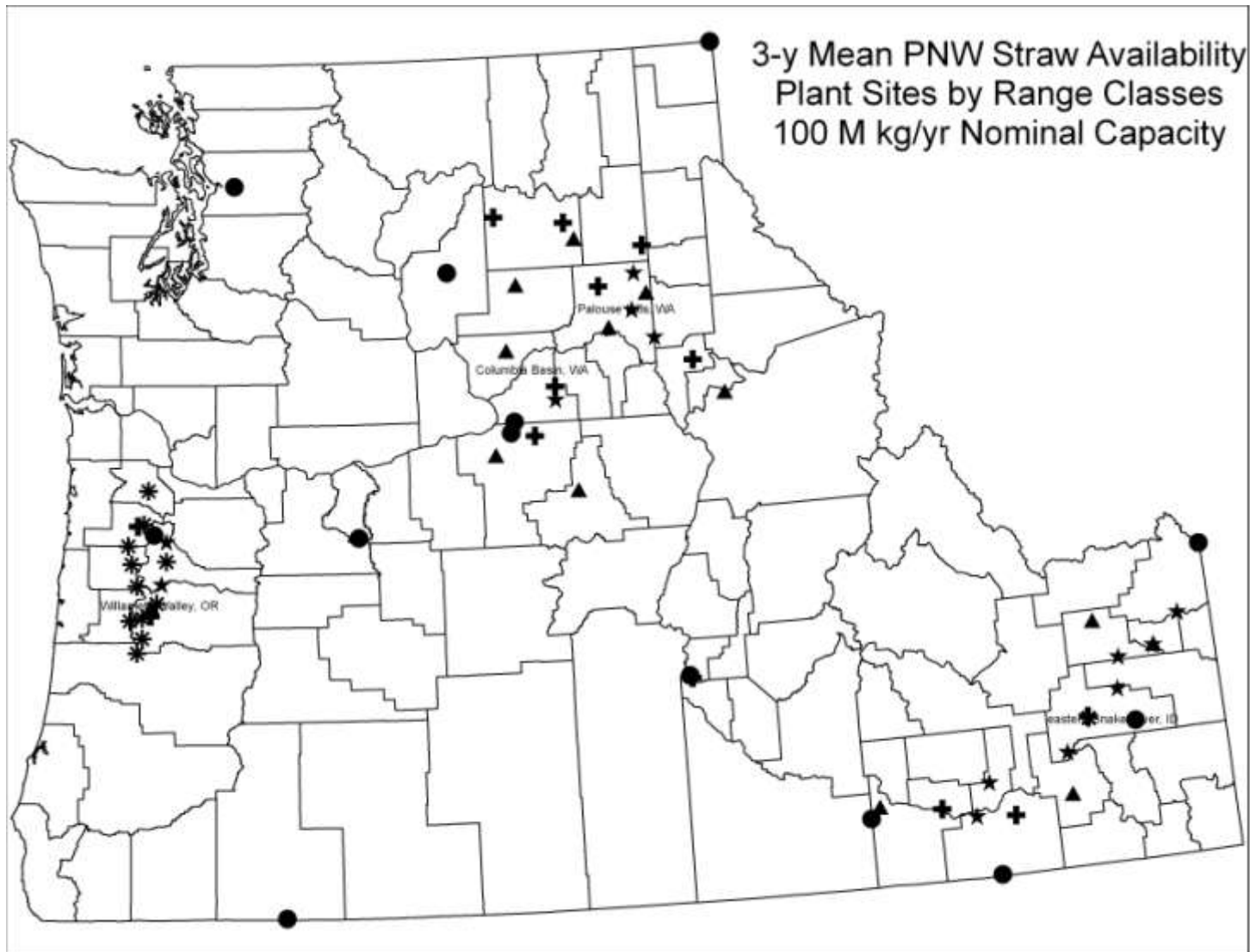
**Table 2.** Straw availability at plant site locations optimized for straw source year and nominal plant capacity and evaluated against 2005, 2006, 2007, and 3-year average straw density rasters.

† Same data source used in defining plant site location series and measuring straw availability.

Data source used to define plant site location series		Data source used in measuring straw availability	Straw availability at defined plant site locations			
Year	Nominal plant capacity		Mean	Standard deviation	CV	Ratio of standard deviation to site location data source
	( $10^6 \text{ kg y}^{-1}$ )	(Raster year)	( $10^6 \text{ kg y}^{-1}$ )		(%)	
2005†	1	2005†	1.13	0.29	26.13	1.00
2005	1	2006	1.01	0.77	76.94	2.63
2006	1	2005	1.26	0.87	69.33	3.04
2006†	1	2006†	1.13	0.29	25.51	1.00
2007	1	2005	1.38	1.60	115.87	5.65
2007	1	2006	1.23	1.17	94.55	4.12
2007†	1	2007†	1.11	0.28	25.53	1.00
2007	1	3-y avg.†	1.24	0.88	71.12	3.12
3-y avg.	1	2005	1.21	0.54	44.66	2.09
3-y avg.	1	2006	1.08	0.44	40.61	1.70
3-y avg.	1	2007	0.97	0.58	59.67	2.24
3-y avg.†	1	3-y avg.†	1.09	0.26	23.77	1.00
Mean	1		1.16	0.59	50.00	2.11
2005†	10	2005†	10.81	2.64	24.41	1.00
2005	10	2006	9.60	4.45	46.34	1.69
2006	10	2005	10.97	4.58	41.80	2.71
2006†	10	2006†	9.82	1.69	17.22	1.00
2007	10	2005	11.75	9.03	76.85	11.61
2007	10	2006	10.51	6.37	60.56	8.19
2007†	10	2007†	9.45	0.78	8.23	1.00
2007	10	3-y avg.	10.57	4.96	46.97	6.39
3-y avg.	10	2005	10.60	3.18	29.97	3.64
3-y avg.	10	2006	9.49	2.30	24.20	2.63
3-y avg.	10	2007	8.52	3.44	40.33	3.94
3-y avg.†	10	3-y avg.†	9.54	0.87	9.14	1.00
Mean	10		10.09	3.33	32.31	3.04
2005†	100	2005†	117.75	30.53	25.93	1.00
2005	100	2006	104.63	34.02	32.51	1.11
2006	100	2005	126.67	48.30	38.13	1.53
2006†	100	2006†	113.29	31.65	27.94	1.00
2007	100	2005	122.90	55.86	45.45	5.78
2007	100	2006	109.96	36.74	33.41	3.80
2007†	100	2007†	98.81	9.66	9.78	1.00
2007	100	3-y avg.	110.55	31.03	28.07	3.21
3-y avg.	100	2005	108.91	23.19	21.30	2.23
3-y avg.	100	2006	97.59	16.01	16.41	1.54
3-y avg.	100	2007	87.65	22.50	25.67	2.17
3-y avg.†	100	3-y avg.†	98.04	10.38	10.59	1.00
Mean	100		110.33	30.48	27.08	1.81



**Figure 1.** Optimized locations for 10 million kg y<sup>-1</sup> capacity bioenergy plants based on 3-yr average straw availability. Symbols indicate quantiles of range required to supply straw, with asterisks, stars, crosses, triangles, and circles denoting 2 to 4, 4 to 6, 6 to 7, 7 to 12, and 12 to 600 km miles. County boundaries are outlined.



**Figure 2.** Optimized locations for 100 million kg y<sup>-1</sup> capacity bioenergy plants based on 3-yr average straw availability. Symbols indicate quantiles of range required to supply straw, with asterisks, stars, crosses, triangles, and circles denoting 8 to 15, 15 to 19, 19 to 28, 28 to 46, and 46 to 488 km. County boundaries are outlined.

# Perennial ryegrass (*Lolium perenne* L) seed crop response to spring nitrogen: a comparison of New Zealand and Oregon results

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## Abstract

Nitrogen (N) uptake and seed yield from eight site years in Oregon (OR) and nine locations in New Zealand (NZ) are compared. The production systems between the two regions are very different. Despite these differences, a striking similarity exists between the OR and NZ data sets. Using a Cate-Nelson technique for data separation, the N content above which yield was not limited by N was 143 kg/ha.

## Introduction

Characterizing N uptake by ryegrass seed crops is necessary to develop prediction models for spring N application. The ability for a prediction model to be useful in differing production regions is dependent on factors including similarities or differences in plant N uptake. Production systems and environments between the main ryegrass seed production regions of New Zealand (NZ) and Oregon (OR) are very different.

In the Canterbury area, NZ, the 600 to 800 mm of rainfall is received uniformly throughout the year. In contrast, the 1000 to 1200 mm of rain falling in the Willamette Valley of OR is primarily received from mid-autumn to mid-spring. To supplement rainfall in Canterbury, NZ, approximately two-thirds of the area producing perennial ryegrass seed is irrigated compared to less than five percent of the area in the Willamette Valley, OR. The first year seed crops in Canterbury, NZ reach spikelet initiation at approximately 520 GDD (Growing Degree Days base 0°C) from July 1 where spikelet initiation in first or second year crops in the Willamette Valley, OR occurs at approximately 370 GDD from January 1. The forage seed varieties grown in Canterbury, NZ are routinely grazed and the predominantly turf varieties grown in the Willamette Valley, OR are rarely grazed.

This paper compares N uptake data and seed yield from N rate trials in NZ and Oregon forage and turf perennial ryegrass and discusses utility of the data for prediction of spring N rate in perennial ryegrass seed production.

## Methods

**New Zealand.** Nine N rate, seed yield response trials in grower fields were undertaken from 2006/07 to 2008/09 in Canterbury and mostly in two districts; Ashburton, coastal (30 m asl); and

Methven (300 m asl). The sites were irrigated and managed by the grower for all inputs except for nitrogen (N). The plant growth regulator trinexapac-ethyl was used at all trial sites usually at 400 g ai/ha. Trials were grazed with sheep during winter and spring and closed for grazing at the beginning of stem elongation (usually late September). Trial plots were 32 m<sup>2</sup> and with four replicates in a randomized block design.

Applied N as urea was evaluated with rates increasing in 50 kg/ha increments usually covering the range of 0 to 250 kg N/ha. Spring N was applied as a split application with equal amounts at each application, the first application at closing and the second application about two weeks later. At late seed fill (about 7 to 10 days before harvest) a 0.25m<sup>2</sup> quadrant was sampled from each plot for an assessment of crop mass and components of yield. Straw from this sample and seed after harvest were analyzed for N content at a commercial laboratory. N uptake by the crop (seed and straw) was calculated from this data.

At harvest a 17 m<sup>2</sup> swath was cut from the centre of all plots with a modified plot windrower, and then harvested with a plot combine. Seed samples were machine dressed on a small-scale air-screen separator to achieve a 1<sup>st</sup> Generation seed purity standard. Cleaned plot samples were weighed and converted to a yield per ha.

**Oregon.** Between 1998 and 2009, eight replicated nitrogen rate experiments in perennial ryegrass fields were evaluated in the Willamette Valley or low hills on the valley margin and none were grazed. Plant growth regulator to decrease lodging was used at the discretion of growers, but used at most sites after 2000. All sites had a nil treatment and increments of approximately 50 kg N/ha to a maximum of 200 to 350 kg N/ha. In 2007 and 2009, the plots were located on Oregon State University's Hyslop Crop Science Farm, Corvallis; and the other sites were in grower fields, and except for addition of N, managed by growers.

Either a single or split application of urea was made in grower fields to plots between 400 and 800 GDD with a Gandy Orbit-air spreader. Plots were 6.2 m wide and 75 to 120 m long, allowing for harvest with grower equipment. Growers cut a single swath through the middle of each plot that dried and was then harvested with their combines on the same schedule held for the field. Seed from each plot was transferred from the combine to a Brent Yield cart for weighing. A sub sample of seed from each plot was cleaned and the yield from the combine adjusted with this data.

Plots at Hyslop were 50 m<sup>2</sup>. Treatments were swathed and after drying, a plot combine was used to separate straw from seed. After the seed was weighed, a bulk sample was taken for cleaning using a two-screen cleaner. Clean seed yield for each treatment was determined using the cleanout percentage from the bulk sample.

Biomass, aboveground N accumulation, and yield components were determined from 30 cm sections of row by clipping all plant tissue above the soil surface after anthesis. Two or more samples were collected for each treatment from random locations and combined to create a

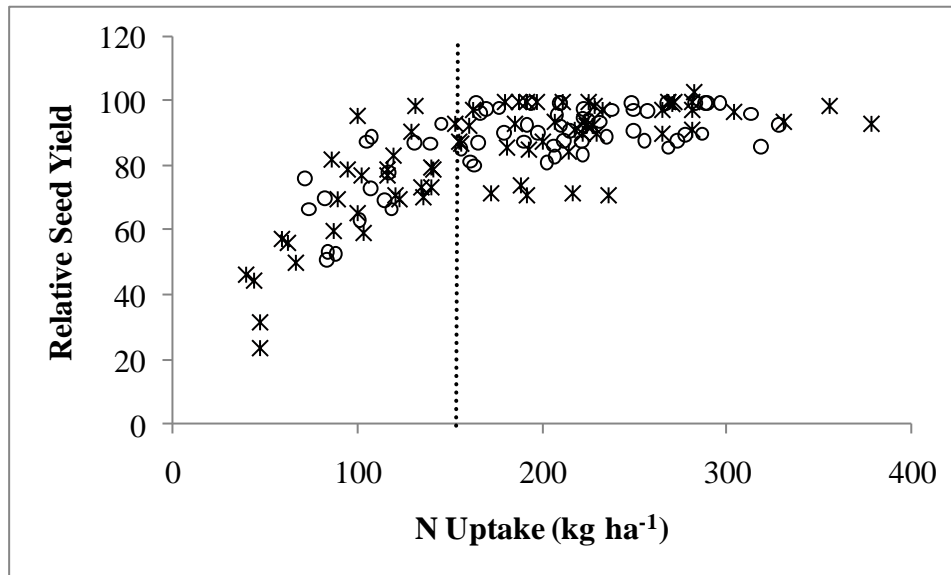
single composite sample. Composite samples were analyzed for dry biomass and whole-plant N concentration. Relative seed yield was calculated with the highest treatment value for a trial set at 100%.

## Results

Perennial ryegrass seed yield increased with N uptake until the aboveground plant content was 143 kg/ha (Figure 1). The average seed yield response in the linear response phase was 3.7 and 5.6 kg seed/kg N for NZ and ORs respectively, reflecting the higher mineral N level of NZ sites. N uptake beyond need for seed yield is stored in straw. Straw N concentration commonly doubles from 10 to 20 mg/kg while seed N remains constant within range 20 to 24 mg/kg.

The range of mean seed yield for a location was 1700 to 3000 kg/ha in Canterbury, NZ and 1400 to 2300 kg/ha in the Willamette Valley, OR. In the Canterbury area, the average perennial ryegrass seed yield with nil N was approximately 1600 kg/ha compared to only 1200 kg/ha in the Willamette Valley, OR. These differences likely reflect soil N contribution of approximately 100 kg/ha in New Zealand compared to only 60 kg/ha in Oregon (Hart et al 2007).

Crops in the Willamette Valley, OR produced more aboveground harvest biomass, 11,000 to 16,000 kg/ha compared to 9,000 to 15,000 kg/ha in Canterbury Area, NZ. The aboveground biomass mean of all nil N treatments was similar in both areas, between 8 and 9,000 kg/ha.



**Figure 1.** Relative seed yield and ryegrass N uptake from nine New Zealand (o) and eight Oregon trials (\*). Critical N uptake (--) calculated at 143 kg N ha<sup>-1</sup> (Cate-Nelson 1971).

## **Discussion**

Seed yield change from N rate application is site specific and commonly does not translate to another year or site. For many years, N rate trials have been conducted in both production areas with the same result. No combination of soil N measurements adequately predicted spring N rate.

Rather than attempting to use soil parameters to predict spring N requirement, an alternative approach is to measure N concentration to determine a critical level (Rowarth et al. 1998). N concentration was measured in early reproductive growth with available time to add N and influence seed yield. Sampling perennial ryegrass seed production fields to assess N concentration has limitations: (i) is the time and effort needed to collect samples; (ii) is the time from sampling and tissue analysis to fertilizer application, and (iii) is the rapid dilution of N with stem elongation as N concentration is sensitive to the rapid change in plant growth and can change quickly after N application. A dilution curve approach (Gislum et al. 2009) overcomes dilution effects but not delays in laboratory analysis of N.

To adequately capture changes in biomass and tissue N concentration, N uptake estimation using remote sensing is currently pursued (Flowers et al, 2009). Data used in this paper shows that perennial ryegrass grown for seed assimilates N in a similar amount in two production region despite differences in the environment and crop management. This fact allows data combination into a single model for remote sensing and other predictive research.

The N uptake required for maximum relative seed yield is not an absolute number as it will change with data provided. Even so, it is a reasonable estimate that can initially be used for comparison of early season N uptake estimates from remote sensing.

## **Conclusion**

Perennial ryegrass grown for either turf or forage seed, requires the same amount of total N, approximately 150 kg/ha before N limits seed yield in both Canterbury, NZ and the Willamette Valley, OR. The consistent amount of N in the crop provides opportunity for a universal model of N use by the crop.

## **Acknowledgement**

Dr Chikako van Koten, statistician at AgResearch for data analysis; funding for the New Zealand trials was from a seed growers levy administered by the Foundation for Arable Research. Funding for the Oregon trials was provided by the Oregon Seed Council and Oregon Ryegrass Growers Seed Commission.

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# **In-season assessment of plant nitrogen status for perennial ryegrass seed production using remote sensing**

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## **Abstract:**

The high nitrogen (N) fertilizer rates applied to perennial ryegrass combined with the winter rainfall pattern in western Oregon may result in reduced profitability for producers and N loss to the environment. Plant tissue testing to determine the in-season plant N status may improve N management. However, tissue testing can be costly, time consuming, and difficult for growers to adopt. A possible solution to these problems is remote sensing. The objective of this study was to determine if remote sensing in the form of aerial images could be used to assess the in-season plant N status of perennial ryegrass. Research was conducted at four sites in 2007, 2008, and 2009. Strong relationships were found between spectral measurements and both whole-plant N concentration ( $r^2 = 0.46$ ) and N uptake ( $r^2 = 0.61$ ) across site-years. Additionally, critical values obtained from these relationships were similar to those found through tissue testing, indicating that spectral measurements may be used to replace tissue tests to assess in-season plant N status of perennial ryegrass.

## **Introduction:**

Growers in the Willamette valley region of Oregon rely on yield goal estimates and experience to formulate spring nitrogen (N) rates. However using this approach, N rates may be insufficient or excessive in any given year. Thus, improved methods that optimize spring N rates are required.

Soil based approaches have been unsuccessful in perennial ryegrass (Hart et al., 2006). A plant based approach, in-season tissue testing, has shown some promise. However, tissue tests have several limitations. They are relatively expensive, difficult, and time consuming to obtain when considering the number of samples required to accurately describe the within field spatial variability found in most Willamette valley fields.

Remote sensing in the form of aerial photographs or an on-the-go sensor might offer a solution to these limitations. Reflectance in the visible and near infrared (NIR) spectrum can be related to chlorophyll, N concentration, biomass, and vigor of plants (Gates et al., 1965; Knippling, 1970).

Thus, similar to tissue tests, remote sensing may be related to whole-plant N concentration or N uptake.

In 2006, a research project was initiated to examine the use of remote sensing to assess the in-season N status of perennial ryegrass for seed production. Specifically, we wanted to determine if spectral measurements from aerial images could assess the plant N status of perennial ryegrass.

## **Materials and Methods:**

### *Site and Agronomic Description*

Research was conducted at the Hyslop Crop Science Farm near Corvallis, Oregon in 2007 through 2009. In 2008, research was also conducted at an additional on-farm location (Macpherson Farm) located near Peoria, Oregon. In 2007 and 2008 at Hyslop, a randomized complete block design with 21 N treatments and four replications was used. Nitrogen treatments were arranged in a factorial design with three fall N rates (0, 45, and 90 kg N ha<sup>-1</sup>) and seven spring N rates (0, 45, 90, 135, 180, 225, and 270 kg N ha<sup>-1</sup>). In 2009 at Hyslop a randomized complete block design with seven spring N treatments (0, 45, 90, 135, 180, 225, and 270 kg N ha<sup>-1</sup>) and 12 replications was used. At the Macpherson site, a randomized block design with three N treatments and three replications was used. Nitrogen treatments consisted of a control (0 kg N ha<sup>-1</sup>), a fall application of 0 kg N ha<sup>-1</sup> followed by 202 kg N ha<sup>-1</sup> in the spring, or a fall application of 45 kg N ha<sup>-1</sup> followed by 157 kg N ha<sup>-1</sup> in the spring.

Urea (46-0-0) was used as the N source for all applications. Treatments at each site were sampled in April to determine in-season plant N status. Samples were collected from one-foot sections of adjacent rows by clipping all plant tissue above the soil surface. Samples were analyzed for dry biomass and whole-plant N concentration. Nitrogen uptake for each treatment was calculated by multiplying dry biomass by whole-plant N concentration.

At maturity, treatments were swathed, allowed to dry, and combined. Seed was weighed and a bulk sample was taken for cleaning. Clean seed yield for each treatment was determined using the cleanout percentage from the bulk sample.

### *Aerial Images*

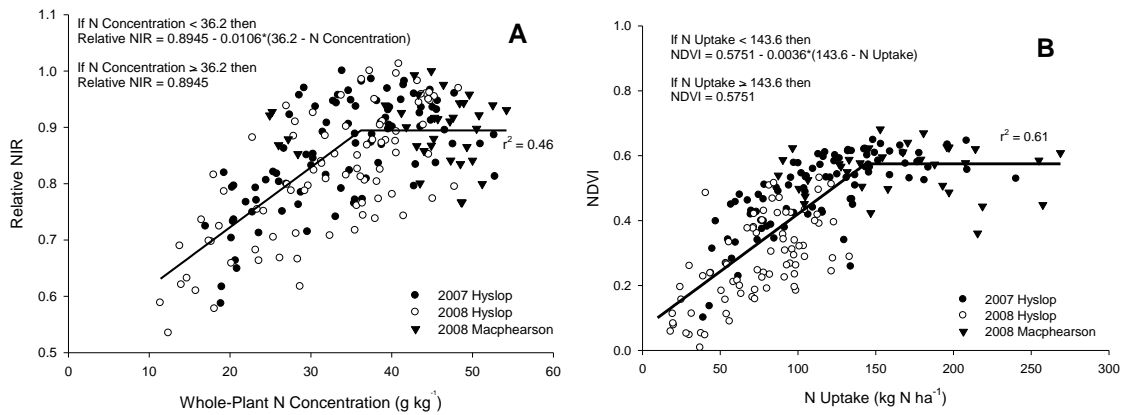
Aerial images of each site were obtained in conjunction with the April tissue samples. Aerial images consisted of four spectral bands; blue (400-500 nm), green (500-600 nm), red (600-700 nm) and NIR (700-900 nm). The procedure described by Flowers et al. (2001) was used to derive spectral reflectance values for each band using ERDAS Imagine software (ERDAS, 2006). In addition to examining the reflectance from individual bands, the normalized difference vegetation index (NDVI; Yang and Anderson, 1999) was calculated and analyzed.

Reflectance values from the individual bands and the spectral index NDVI were compared to the whole-plant N concentration and N uptake values determined from the plant tissue samples.

The relationship between whole-plant N concentration and N uptake with the NIR band and NDVI was examined across sites using a non-linear procedure in SAS (SAS, 2002). A high N reference approach (Blackmer and Schepers, 1995) was also examined by determining the relative NIR or NDVI values for each treatment using the mean NIR or NDVI values from the highest N rate treatment.

## Results and Discussion:

To examine the spectral measurements across site-years the Hyslop 2009 dataset was removed due to its unusually high whole-plant N concentrations and small N uptake values that resulted from poor plant growth in the spring. At the remaining three sites, differences in whole-plant N concentration across site-years could be accounted for using a high N fertilizer reference approach (Fig. 1a). In addition, the linear plateau model determined a critical whole-plant N concentration value of  $36 \text{ g kg}^{-1}$ . This critical value is higher than that determined by the tissue testing analysis (data not shown) and previous studies (Young et al., 1998 and Rowarth et al., 1998). However, the difference among critical values is not likely to be the largest problem with implementation of this approach. The need to use a high N fertilizer reference strip is likely to be a much greater obstacle to grower adoption.



**Figure 1.** Relationship between nitrogen (N) uptake and relative near infrared (NIR) values (A) or the normalized difference vegetation index (NDVI; B) at three sites in 2007 and 2008.

Unlike the results for whole-plant N concentration, NDVI was able to account for differences in N uptake across site-years without the use of a high N fertilizer reference (Fig 1b). This enhances the appeal of using spectral measurements or sensor readings and may increase grower adoption of such techniques. In addition, the critical value determined by the linear plateau model is  $144 \text{ kg N ha}^{-1}$ . This is similar to the critical N uptake value of  $158 \text{ kg N ha}^{-1}$  determined by the analysis of the tissue tests (data not shown). Thus, both the plant tissue samples and spectral measurements are providing the same information in regards to N uptake.

## **Conclusion:**

Perhaps our most important finding was a strong relationship between NDVI and N uptake across site-years (Fig. 1b). This relationship does not depend on the use of a high N reference and also has a critical value very close to that determined by the analysis of the tissue tests (data not shown).

Our results are very promising and indicate that spectral measurements from aerial images can directly replace sampling for in-season assessment of N status of perennial ryegrass. This could have a major impact in improving the management of N fertilizer in perennial ryegrass grown for seed. Future research should focus on developing and validating a robust model for predicting spring N fertilizer rate as well as examining the many commercial sensor platforms available for producers.

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# Modelling critical NDVI curves in perennial ryegrass

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## Abstract

The use of optical sensors to measure canopy reflectance and calculate crop index as e.g. normalized difference vegetation index (NDVI) is widely used in agricultural crops, but has so far not been implemented in herbage seed production. The present study has the purpose to develop a critical NDVI curve where the critical NDVI, defined as the minimum NDVI obtained to achieve a high seed yield, will be modelled during the growing season. NDVI measurements were made at different growing degree days (GDD) in a three year field experiment where different N application rates were applied. There was a clear maximum in the correlation coefficient between seed yield and NDVI in the period from approximately 700 to 900 GDD. At this time there was an exponential relationship between NDVI and seed yield where highest seed yield were at NDVI ~0.9. Theoretically the farmers should aim for an NDVI of 0.9 and intervene in case of a lower NDVI, which means to apply more nitrogen (N). However, this might be impossible in some years. From a practical point of view aims are therefore to obtain the highest NDVI as late as possible in the growing season and if NDVI has to increase this is done by an additional N application at a time where the crop is able to take up and utilise the applied N.

## Introduction

Critical nitrogen (N) dilution curves (CNC) are defined as the critical plant N concentration at different shoot biomasses levels (Lemaire & Gastal, 1997). If CNC can be linked to seed yield by defining the minimum plant N concentration at different biomasses to give maximum yield suggests that N is the limiting factor to obtain a high seed yield. It is very appealing to introduce CNC in practical seed production as discussed by Gislum and Boelt (2009a). Besides a situation where N is not the only limiting factor to obtain a high seed yield the use of CNC is not for practical agriculture in its present form. However, the concept of monitoring the crop during the growing season and intervene according to the result of the monitoring to achieve a high seed yield is great.

The use of optical sensors measuring canopy reflectance and influx of light to calculate a crop index e.g. normalized difference vegetation index (NDVI), to predict final yield or for variable-rate N fertilization is well known within agricultural. Raun et al. (2005) showed the possibility to use an optical sensor-based algorithm for N fertilization in winter wheat and discussed both the effects on yield and the possibilities to decrease environmental contamination due to excessive N fertilization. The environmental effects of using optical sensors to adjust N fertilization was

further discussed by Roberts et al. (2010), who supported the environmental benefits from using optical sensors in corn to adjust N fertilization, but on the precondition that the sensor information can be processed by a decision-rule algorithm into an N rate that approximates the optimal N rate. Not only the possibility to increase yield by a higher utilization of N applied but also the environmental benefits from using optical sensors was a great inspiration for us when we developed a partial least square regression model to predict seed yield in perennial ryegrass (Gislum & Boelt, 2009b). Even though the number of publications on optical sensors, N and yield in agriculture is enormous the concept has not been implemented in practical seed production around the world.

If optical sensors are going to be part of practical seed production it is critical that the method has to be cheap to buy and run, and easy and robust to use and most important be economically profitable for the seed grower. The technical part of this we will leave for the engineers to solve, but the agronomic part is left for us to solve. Based on our agronomic knowledge from several field experiments with different N application rates and N application strategies we are aware that things change during the growing season and N is seldom the only limiting factor for a high seed yield. Based on this it would be obvious to use the concept of CNC and monitor the crop during the growing season. The obvious solution to make CNC more applicable in agriculture would of course be to use optical sensors in combination with the concept behind CNC.

The purpose of this experiment was therefore to develop a critical NDVI curve where the critical NDVI, defined as the minimum NDVI obtained to achieve a high seed yield, was modelled during the growing season.

## **Materials and Methods**

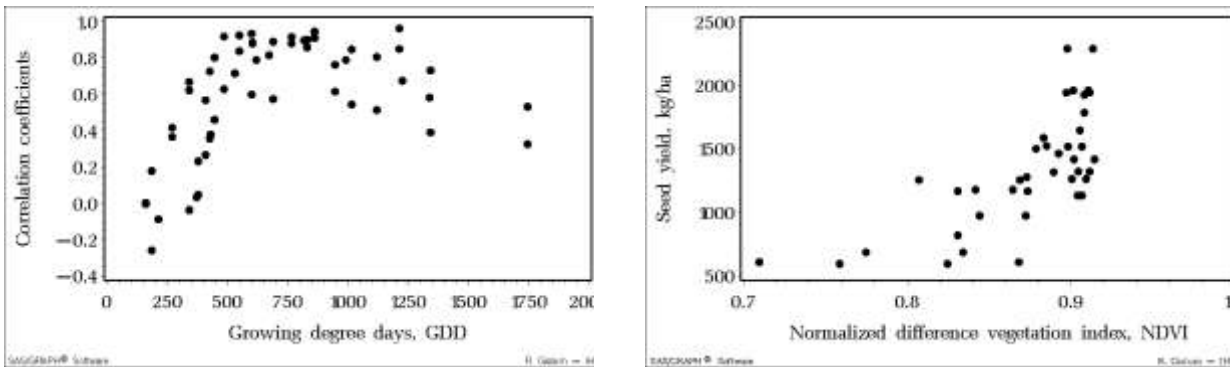
Field experiments with perennial ryegrass were established at Roskilde (1996 and 1998) and at Flakkebjerg (1999) in Denmark. The cultivars used in 1996 were Borvi (diploid) and Grasslands Nui (diploid) but in 1998 and 1999 Grasslands Nui was replaced by Tivoli (tetraploid). For all years and for both locations the purpose of the experiments was to evaluate the effect of different N application rates and application strategies on seed yield. The N application rates were: (spring) 0, 50, 100, autumn/spring 30/120 and 60/140 kg N ha<sup>-1</sup>. The experimental design was a factorial design with four replicates where N application rate was the main factor.

Canopy reflectance was measured at different times during the growing seasons with a hand-held spectroradiometer (Skye SKR 1800, Skye Instruments Ltd., UK). Solar radiation and canopy reflectance were measured at 640-660 nm (red) and at 790-810 nm (infrared). Five separate measurements were made each covering 1.5 m<sup>2</sup> within each plot. Accumulated growing degree-days (GDD) at base temperature 0°C were calculated from January 1 in each year.

The five spectral reflectance measurements in each plot at each sampling date were averaged after outlier detection and the mean values were used in the analysis. The total number of canopy reflectance measurements was 1140. From the canopy reflectance data normalized difference vegetation index (NDVI) were calculated using  $NDVI = (R_{infrared} - R_{red}) / (R_{infrared} + R_{red})$  where  $R_{infrared}$  represent canopy reflectance in the infrared and  $R_{red}$  represent canopy reflectance in the red regions, respectively. At each measurement of canopy reflectance, the linear correlation coefficient ( $R^2$ ) between NDVI and seed yield was calculated. All analyses were performed using the procedures PROC GLM and PROC NLIN module within the Statistical Analysis System version 8, software package (SAS, 1999).

## Results

Analysis of the correlation coefficients between NDVI and seed yield at the different GDD revealed a large variation ranging from negative correlation coefficients, correlation coefficients close to zero and very high correlation coefficients (figure 1 left). The maximum correlation coefficient was from approximately 700 to 900 GDD.



**Figure 1.** Left: accumulated growing degree days (GDD) plotted against correlation coefficients ( $R^2$ ). The total number of data points is 55. Right: Normalized difference vegetation index (NDVI) plotted against seed yield (kg/ha) in the period from 700 to 900 GDD.

The relationship between NDVI measurements from 700 to 900 GDD and the seed yield showed an exponential relationship where it was not possible to define maximum NDVI to obtain highest seed yield (figure 1 right).

## Discussion

The purpose of modelling a critical NDVI curve during the growing season was determined to be too optimistic due to no significance difference in NDVI at the first period of growth in the spring. During this period there was also a very low or even no correlation between NDVI and seed yield, which will make a critical NDVI curve of no value. Focus was therefore moved to the narrow time period (700 to 900 GDD) where the correlation coefficient is at a maximum. This period is almost identical to the period shown by Gislum and Boelt (2009b). During the 700 to

900 GDD period it is possible to replace a critical NDVI curve by a NDVI value to obtain a high seed yield. During this period the goal should be to obtain an NDVI value as high as possible, which is ~0.9. Results from a similar study showed that the most important spectral information used to describe seed yield is information from (rainfall or soil moisture) and N (Gislum and Boelt, 2009b). Unfortunately the period from 700 to 900 GDD is during the period before and at heading and during this time N application is not recommended in all years. The decision should be made each year.

With the purpose to make the present results applicable in herbage seed production the conclusion is that aims should be to reach an NDVI value at approximately 0.9 at 700 to 900 GDD. The best possible way to increase NDVI is to apply more N and the time for measuring NDVI would therefore have to be adjusted according to the time where N application will still have an effect on the seed yield.

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# Harvest loss in ryegrass seed crops

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## Abstract

Harvest loss in farmers' perennial ryegrass crops was assessed using collection trays over 3 harvest seasons. In two years losses were portioned between cutting losses, losses in the windrow, pick-up losses and losses during threshing. In the 3<sup>rd</sup> year 9 farmer fields were monitored and total harvest loss assessed. Harvest losses averaged 570 kg/ha, representing 24% of the saleable seed at harvest. Losses associated with cutting were large, especially from disc mowing. Losses in the windrow during drying varied, but in a crop that had rain on the swath the loss was >400 kg/ha. Despite the harvest losses the average crop yield was 1920 kg/ha. Reducing harvest losses is a key management objective to achieving high seed yields.

## Introduction

There is a large gap between the number of developing seeds two weeks before harvest and the number of saleable seeds produced (Rolston *et al.* 2007). Two components of this gap are the occurrence of undersized seed and seed losses at harvest. There have been few studies made on harvest losses in perennial ryegrass (*Lolium perenne*) seed crops, and they mainly compare direct heading with windrow swathing (Nellist & Rees, 1967; Hampton & Hebblethwaite, 1985). Reviews on harvest approaches for herbage seed include Hopkinson & Clifford (1993) and Simon *et al.* (1997). Previous studies have often looked at harvest loss indirectly by measuring seed yield responses to different harvest approaches. In Oregon the effect of cutting with dew (early morning) or without dew (afternoon) was assessed in annual ryegrass (*L. multiflorum*), and seed yields were 250 kg/ha higher with cutting with dew (Silberstein *et al.* 2005). In perennial ryegrass seed loss under swathing at different seed moistures (36 to 29%) was associated with a reduced seed yield of 65 kg/ha (4%), while seed numbers equivalent to 9 kg/ha (46 seeds/0.11 m<sup>2</sup>) were counted on the ground (Silberstein *et al.* 2005).

In New Zealand many seed crops are cut at ca. 38 to 42% seed moisture content (SMC) using multi-disc mowers, while some are swathed using self-propelled windrowers with either belt or auger feeds. Threshing of the crop usually occurs 5 to 9 days after windrowing when the SMC is between 12 and 14%. Seed loss stages in harvesting are described by Hopkinson & Clifford (1993). Trials to determine the magnitude of seed loss and to determine what stage in the harvest operation contributes most loss were started in 2006/07 harvest season. The four stages in the harvest process were defined as (i) cutting (disc mowing or windrowing); (ii) drying in the

swath; (iii) combine pick-up - the loss as the swath is lifted onto the combine draper front; and (iv) offal trail - seed not separated from the straw and lost over the back riddles.

Seed maturity assessed by measuring seed moisture content (SMC) has also been shown to affect seed yield, presumed in part to be associated with reduced seed loss (Simon *et al.* 1997). Harvest loss in ryegrass seed crops in New Zealand has not been previously studied. In this paper we report on harvest loss assessments made with collection trays.

## Method

The seed losses were studied in 13 fields managed by seed growers in the Canterbury region of New Zealand and covers a 3 years (Table 1). Harvest loss was assessed with oblong aluminum foil trays either 16x20 cm for crops sown in 30 cm rows; or 10x18 cm in crops sown in 15 cm rows. In sites 1-4 on the day of cutting 6 trays were placed with the upper lip at the soil surface in a line at 3m intervals in the direction of machinery travel. An 8 cm staple was used to anchor the tray and a hole to allow rain water to drain. At sites 5 to 13, 15 trays were used, and on average 13 trays were recovered. Where components of loss were assessed (4 sites), these trays were replaced after each step carefully removing trays to avoid shaking extra seed into them. The collected trays were placed into paper bags, dried and the seed cleaned using screens and air to produce a saleable seed sample that was weighed and the loss per ha calculated. Loss figures were adjusted down to account for the “drawing-in” of the crop during cutting by 20% and 35% respectively for disc mowing and windrowing respectively. The yield of the field was based on the machine dressed yield provided by the seed company contracting the seed.

## Results

Harvest losses were large, averaging 570 kg/ha with a range from 190 to 1300 kg/ha (Table 1). The largest loss was in a field that the grower was late in cutting; while the lowest loss was in a windrowed crop, swathed in damp conditions. When the loss is expressed as a percentage of the saleable seed available for harvest, an average loss of 24% (range 6 to 43%) was recorded (Table 1). If the worst loss (43%) is excluded because of known late cutting, the average loss was 22%. Despite these losses the average seed yield was 1920 kg/ha (Table 1).

When cutting method was compared the average harvest loss was 29% versus 16% for disc mowing (n=8) and windrowing (n=5) respectively and significantly different (P=0.02 for a one-way ANOVA).

A breakdown of loss by harvest stages undertaken with four trial sites identified that losses at cutting and losses during drying in the swath accounted for 49 and 43% of the losses respectively (Table 2). The large loss during swath drying in Trial 3 was associated with a 25 mm rain event the day before the crop was due to be combined (Table 2).

## Discussion

Larger than expected harvest losses were recorded in these assessments. Growers have control over some of these losses, especially losses associated with cutting. Weather-related losses, e.g. from rain after cutting, cannot be controlled. The data strongly suggests that seed losses are less when crops are windrowed compared to mowing, and when the 2009/10 data is available the number of data sets that can be compared will double.

**Table 1.** Site number, year of assessment, method of cutting, harvest loss, field seed yield and loss calculated as percentage of total saleable seed available for harvest.

Site	year	Method	Loss	SY	% loss
1	2005/06	mow	660	2400	22
2	2007/08	mow	1300	2500	34
3	2007/08	windrow	560	2500	18
4	2007/08	windrow	190	3100	6
5	2008/09	mow	350	950	27
6	2008/09	mow	430	2000	18
7	2008/09	windrow	370	1660	18
8	2008/09	mow	600	1700	26
9	2008/09	windrow	510	2670	16
10	2008/09	mow	640	1290	33
11	2008/09	windrow	560	2200	20
12	2008/09	mow	940	1230	43
13	2008/09	mow	270	790	25
Average			570	1920	24

**Table 2.** Percent of harvest loss attributed to each step of harvest process.

Site	1	2	3	4	Average
Step	% loss				
cutting	32	60	32	74	49
drying	50	32	75	15	43
combine	12	7	12	12	11
offal	6	2	6	x <sup>1</sup>	5

<sup>1</sup>not measured

The lowest loss recorded, 6%, is probably an unrealistic goal for all growers to reach. However if growers could reduce average harvest losses by 50% of the levels reported, average seed yields would increase by 265 kg/ha and improve crop profitability significantly. A greater understanding of how to manage losses is needed, including cutting methods (especially windrowing versus mowing), speed of cutting, effect of crop dividers, seed moisture and environment (temperature, humidity and dew) interactions, degree of lodging. A combination of harvest timing, machine options and agri-chemicals may be required to reduce harvest loss. A preliminary evaluation of a harvest aid “Pod-Lock” in 2008/09 did not improve seed yields.

In NZ disc mowers became popular as a replacement for reciprocating knife mowers, coinciding with larger crop fields, increasing crop bulk from high N rates (250 to 300 kg N/ha) and before the introduction of straw shortening plant growth regulators (PGR). These factors combined to result in crops with considerable new vegetative growth through the lodged crop. The combination of these factors made traditional mowing slow and the adoption of disc mowing was rapid. In recent years with the adoption of lower N rates (150 kg N/ha) and the use of trinexapac-ethyl (TE) as a PGR, lodging and regrowth can be prevented and the crop architecture at cutting changed. Further trials were initiated during the 2009/10 harvest season but data are not yet available. The trials include a comparison of harvest losses associated with changed crop architecture using TE. Seed yields are increased with delayed lodging achieved with TE (Rolston *et al.* 2007). However some growers argue that while more erect crops are easier to cut, they are more vulnerable to seed shattering from high wind events. Understanding the effect of wind during seed maturity will be an important component to developing methods to reduce harvest loss.

Reducing the time between cutting and combine harvesting is associated with higher yields (and presumably lower losses), but requires access to seed drying facilities (Hampton & Hebblethwaite, 1985). Future harvest loss trials should look if an interaction occurs between cutting method and days from cutting to threshing. Windrows have more bulk and are generally slower to dry.

## **Conclusion**

The average harvest losses were large, 24% of saleable seed available for harvest. The size of loss was also very variable between fields; suggesting management options could reduce losses.

## **Acknowledgements**

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# Predicting spring nitrogen for perennial ryegrass seed crops from NDVI

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## Abstract

Nitrogen (N) fertiliser makes up approximately 15 % of the total cost of growing a perennial ryegrass seed crop. Therefore accurate estimates of applied N fertiliser are important both financially and environmentally. Optical sensors may have the ability to determine the nitrogen requirements and the output expressed as normalized difference vegetation index (NDVI).

In two experiments carried out in the 2008/09 season a treatment based on estimating nitrogen requirements using NDVI calculations was implemented. Results were promising with NDVI reading increasing as N rate increased until a saturation level. The NDVI readings were then converted in to a nitrogen nutrition index (NNI) where resulting treatments ranked in the top statistical seed yield group in both experiments. In each experiment this technique applied a lower or equal amount of N fertiliser compared to standard best practice while achieving similar seed yields.

If NDVI and NNI can be used to predict N requirement of perennial ryegrass this method could provide a tool for variable rate N applications based on crop variability within a field.

## Introduction

Perennial ryegrass (*Lolium perenne* L.) seed production is responsive to the application of nitrogen (N) fertiliser, averaging 36% response above control treatments in 17 trials throughout Canterbury (Rolston *et al.* 2008). The availability of nitrogen to the ryegrass seed crop is a major determinant of seed yields. At various points throughout the growing season N content varies between 5 (possible in early spring) and 1.5% (typical at harvest) of herbage dry matter. Nitrogen influences dry matter production, tillering and therefore number of inflorescences which develop to maturity. Currently best practice in New Zealand is to record soil mineral N in early spring to determine the application rate of nitrogen for each field. Recent work by the authors has identified that testing soil mineral N is a reliable indicator to the amount of applied N required to achieve optimum seed yields in New Zealand. Currently application rates can be estimated by;  $185 - \text{measured soil mineral N (kg ha}^{-1}\text{)}$  (Rolston *et al.* 2008). However this approach relies on an average target application rate and may be excessive or insufficient in any given year. Therefore if a method exists of using 'real time' data for crop N status (and also biomass production, growth rate, chlorophyll content etc) then profitability could be improved.

Optical sensors have the potential to determine stress in arable crops and therefore may have the ability to determine the nitrogen requirements. The “Greenseeker” is an active type optical sensor that measures canopy reflectance in the red (~650nm) and near infrared (770nm) wave bands. The output is then converted to normalized difference vegetation index (NDVI) as calculated by. Normalized difference vegetation index has been used previously to provide an estimate of crop nitrogen (N) concentration in wheat (Flowers *et al.* 2003).

With a lack of New Zealand data, results published by Flowers *et al.* 2007 were used to estimate N% and unpublished New Zealand data used for estimating dry matter per hectare based on NDVI values (FAR unpublished data 2008). From these data sets a nitrogen nutrition index (NNI) (Lemaire & Gastal 1997) was estimated with critical levels as described by Gislum and Boelt (2009). It was assumed that 40 kg N would raise plant N tissue by 0.5 % based on data presented by Cookson (1999).

Typically spring nitrogen is applied in two or three applications at 14 to 21 day intervals, to limit the potential for leaching and/or volatilisation losses. The aim of this study was to investigate tools for determining the amount of additional nitrogen required after the first application.

## Methods

Two trials, each with 22 treatments including applied N from 0 to 240 kg N ha<sup>-1</sup> in 40 kg increments, were undertaken within irrigated commercial seed crops at Greendale and Methven (Canterbury, New Zealand) with cv Grasslands Samson and cv Alto respectively. Plots were 10 m long and 3.2 m wide with four replicates in a randomized block design. The crops were managed by the grower for all inputs except N. The soil N levels (Table 1) are based on analysis by Hills Laboratories<sup>1</sup> for samples collected in September before closing. The mineral N (MnN) is the combined NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> levels. A “Greenseeker” NDVI assessment was made 21 days after the initial spring N application. Two treatments used NDVI values for making a decision on total spring N.

At approximately 40% seed moisture content a 1.7 m swath was cut from the centre of all plots with a modified plot windrower. Seed was then harvested at 12% seed moisture content with a plot combine. Seed samples were machine dressed on a small-scale air-screen separator to achieve a 1<sup>st</sup> Generation seed purity standard and converted to a yield/ha.

## Results and Discussion

### *Soil Mineral N*

Soil mineral N levels were slightly below the long term average (41 kg ha<sup>-1</sup>, range 10 – 122 kg ha<sup>-1</sup>) (Rolston *et al.* 2008) at both sites (Table 1) indicating that winter leaching had removed much of the available N below the rooting zone.

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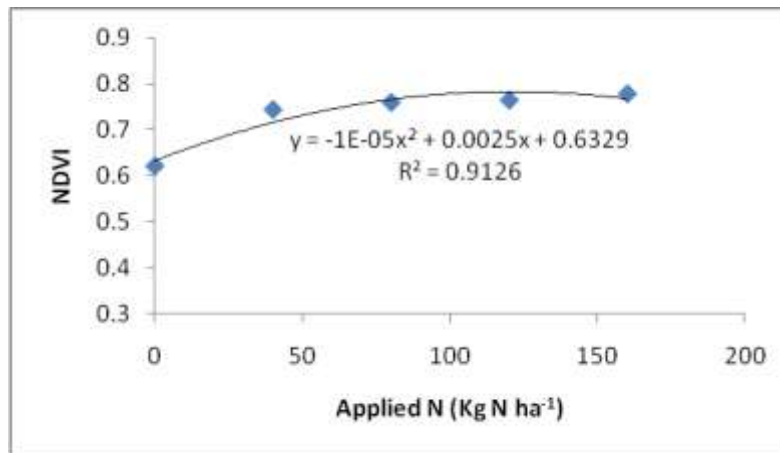
<sup>1</sup> Hills Laboratories see [www.hill-labs.co.nz](http://www.hill-labs.co.nz)

**Table 1.** Recorded soil Mineral N (MnN, combined  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) levels in early spring at two sites, Canterbury, New Zealand.

Depth	Greendale	Methven
	Soil N (kg/ha)	
0-30cm	35	25
30-60cm	12	6

### NDVI

The application of N at closing significantly increased ( $P < 0.05$ ) the NDVI for all treatments when assessed three weeks later (prior to the second N application) (Figure 1). Variation in NDVI for individual plots with the same base N application existed. One possible explanation for this is variation in the number of urine patches produced by grazing animals prior to closing, which could lead to variability in results. Saturation in NDVI occurred from approximately 40  $\text{kg ha}^{-1}$  of applied N.



**Figure 1.** Normalized difference vegetation index response to applied N at Greendale, cv Grasslands Samson  $\text{LSD}_{0.05} = 0.047$ .

### Nitrogen Response

Both experiments showed increased seed yield to applied N up to approximately 140  $\text{kg N ha}^{-1}$  and had relatively flat optimum response zones, approximately 130 – 170  $\text{kg applied N ha}^{-1}$  (Figure 2). Some of the variability within Nitrogen rates can be explained by differences in application timings.

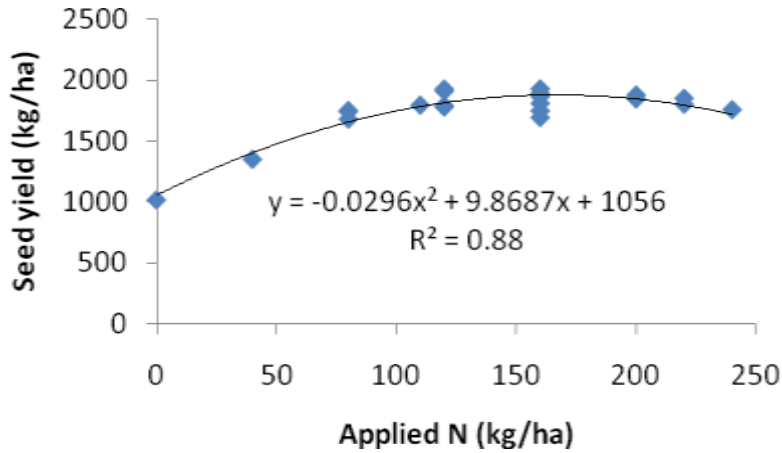


Figure 2. Seed yield response to applied N rates with quadratic model for Greendale ( $LSD_{0.05} = 160$ ).

#### *Greenseeker treatments*

In both experiments the Greenseeker treatments were associated with the highest seed yielding groups ( $P < 0.05$ ) and tended to recommend N application rates close to the beginning of the plateau at both experimental sites (e.g.  $130 \text{ kg ha}^{-1}$  at Greendale). The commercial standard for application at the sites would be approximately  $160\text{-}175 \text{ kg N ha}^{-1}$ . At the Methven site the Greenseeker treatment gave the highest yielding plot at  $2340 \text{ kg/ha}$  (Table 2). This result requires further investigation to confirm the calibration data which was obtained from published literature where turf cultivars were grown. These results require caution as they may not apply to those cultivars which reflect/absorb radiation differently e.g. turf verses forage and/or diploid verses tetraploid.

**Table 2.** Nitrogen application rates and associated seed yields at two sites (not all data presented), Canterbury New Zealand.

Nitrogen application timing and rate (N kg ha <sup>-1</sup> )			Applied N	Greendale	Methven
Closing	3 weeks later	Mid ear emergence	(N kg ha <sup>-1</sup> )	Seed yield (kg/ha)	
0	0		0	1010	1240
0	120		120	1780	2080
0	160		160	1690	2010
40	80		120	1790	2290
40	120		160	1740	1970
80	40		120	1910	2160
80	80		160	1890	2060
80	30	20/40*	130/150	1790	2340
120	0		120	1920	2140
120	40		160	1800	2050
160	0		160	1929	2060
160	80		240	1750	2020
LSD 5%				160	220

\* Greenseeker-Greendale 20 and Methven 40 kg N ha<sup>-1</sup> at mid ear emergence

## Conclusion

- Preliminary trials suggest that an optical sensor has the potential to estimate N requirements, however further calibration is required.
- NDVI has the potential for identifying early season variation, however saturation at higher biomass levels suggests limitations later in the season

## Acknowledgment

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# Nitrogen fertilization management for seed production of tall fescue

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## Abstract:

In France, rules relative to nitrates use (instruction n°91/676/CEE) prescribe 3 different compulsory instructions to farms located in sensitive areas: firstly calculation of N supply by method of nitrogen balance per crop, secondarily edition of a forecasting fertilization scheme, thirdly registration of quantities used and spraying dates in respect to forbidden periods.

For grass seed production, and especially tall fescue, number of analyses of many experiments carried out by FNAMS, in different areas and years have led to define for the first time the exact N needs for the crop (first trials serial between 1986 and 1994). Then, a good ratio was found between N level uptake and seed yield: 160 kg N uptake /ha (at full ear emergence, from aerial part and root system) is necessary to obtain the maximum seed yield. A N balance method has been described for the N fertilization management of this grass specie.

Following then, different experiments confirmed the validity of this N balance method. In 2007 and 2008, trials have been carried out in different areas of France: south west, centre and north east. In those areas several fields had been selected and three treatments have been compared: T1 without nitrogen (to estimate N soil contribution), T2 calculated with N balance method and T3 based upon the usual farmer practice. For the majority of experiments, the T2 modalities led to reduce the N supply of approximately 10 kg N/ha without any significant difference on seed yield, but varied from 40 kg N/ha to -15 kg N/ha. On the contrary, reduction of N supply (some T3 modalities, compared to T2 - N balance method), decreased significantly seed yield.

These experiments also focussed similar N needs between two different fescue types: turf type (variety TOMAHAWK) and forage type (variety DULCIA), and also between first or second year of production. These results demonstrate that a more extensive use of N balance method could improve N fertilization management in these crops, and reduce luxurious N intake.

**Key words:** grass seed production, tall fescue (*Festuca arundinacea*), N fertilization

## Introduction:

In France, tall fescue is cultivated on small areas, on various latitudes like North-east, West and South-west which are submitted to various conditions of soils and climates. Before 2003, N fertilization was essentially empiric, based upon visual observations or based on other crops like cereal.

In 2010, France has launched the fourth procedure referring the nitrate policies (CEE n°91/676 of the 12 December 1991). The program covering 2009-2012, restricts the use of N in view to respect environment by elimination of the most dangerous practices.

Considering the new context, seed growers located in vulnerable areas are constrained to adjust N fertilization with possibility to refer to three methods. In seed production, only balance method can be used to determine the quantity of N needed for seed crop. FNAMS researches have established the N needs for tall fescue to reach the maximum yield. This quantity can be used in the balance method to calculate the spring N supply.

### **Material and methods:**

Field experiments were carried out on tall fescue from 1986 to 2003 in four locations in France: Angers (north-west), Troyes (north-east), Lavaur and Condom (south-west), Bourges (middle). On these 4 locations and for each year, four rates of spring nitrogen application were compared: 0, 60, 120 and 180 kg N ha<sup>-1</sup>. Different cultivars were used: Clarine, Barcel, Bariane (forage type) and Apache, Sinfonia, Villageoise (turf type). Each trial is conducted on autumn sowing (august), with 4 replications per object and harvested with an experimental combiner to obtain seed production data after drying and cleaning. In each experiment, a plants sample is clipped at the end of ear emergence, dried (80°C during 24 h), weighted (t ha<sup>-1</sup>) and analysed for N content (Dumas method by flash combustion with automatic N analyser). At least N uptake is calculated (kg ha<sup>-1</sup>). A total of 158 dataset has been compiled to define the optimum N uptake to achieve the highest seed yield.

In 2007 and 2008, 17 experiments were carried out on different areas of France. The N fertilization levels varied in each trial. Three treatments are compared (table 1): T1 - a control plot without nitrogen (N=0), T2 – N supply calculated with the N balance method and T3 - based upon the usual farmer practice. In all experiments, at ear emergence, plants samples were cut. Methods to carry out trials and to calculate N uptake are the same than described before.

### **Results and Discussion**

- Relationship between N uptake and seed yield

The maximum seed yields obtained on different trials are very variable (from 800 to 2350 kg ha<sup>-1</sup>), so results are expressed in percentage of the maximum seed yield obtained in each trial. The figure 1 shows the relationship between N uptake at the end of ear emergence and the seed yield for all trials. The response curve can be divided in three parts: (1) until 110 kg ha<sup>-1</sup> of uptaken nitrogen, yields increase rapidly in all trials, (2) between 110 and 130 kg ha<sup>-1</sup>, most the maximum seed yields are obtained, (3) above 130 kg ha<sup>-1</sup>, the increased of uptaken N has no effect on yields.

From the logistic curve  $Y = a / [1 + b \times \exp(-c \times X)]$  (with Y = yield in % and X = nitrogen uptake in kg ha<sup>-1</sup>), we can consider that the optimum amount of N uptake in aerial part at ear

emergence is, on average, 120 kg ha<sup>-1</sup> for the tall fescue. The total N uptake by the plant is 30% more, if we take into account uptake by the roots and the lowest parts of the crop which are not cut.

So, we consider the total and optimum nitrogen uptake at ear emergence is 160 kg ha<sup>-1</sup> (120 kg N ha<sup>-1</sup> aerial part + 40 kg N ha<sup>-1</sup> root part).

- Nitrogen balance method

In the second part of study, the N mineral supply is calculated for 17 tall fescue seed crops (table 1) with the next equation:

$$\text{N supply} = (\text{Plant N} + \text{N}_0) - (\text{RSH} + \text{Mr} + \text{Mh} + \text{Mo})$$

With: Plant N: plant N uptake at ear emergence, N<sub>0</sub>: N unavailable by the plant, RSH: soil nitrogen content at the end of winter; Mr: mineralization of vegetal residue; Mh: mineralization of humus; Mo: mineralization of organic matter

For these 17 situations, N supply N uptake, at ear emergence, and seed yield are presented in table 2. The results show that for the majority of trials in first harvest (n° 1 to 9), the modality T2 (N calculated with balance method) led to reduce the N mineral supply of about 10 kg ha<sup>-1</sup> without any significant different in seed yield. For these situations, the maximum seed yield is generally reached in T2.

For situations in second harvest (n° 10 to 16), the N supply by the N balance method (T2) is generally higher than the usual farmer practice. The modality T2 increases the N supply of about 3 kg ha<sup>-1</sup> (with an important variation from +40 kg N/ha to -40 kg N/ha), and increase the seed yield with a significant difference, in more than 75% of situations. Use the nitrogen balance method, for tall fescue in secondary harvest, is very pertinent. This method induces most of the time to reach the maximum of yield compared to an empirical method.

In situations 8, 9, 17 and 16, nitrogen balance method is used with the same total nitrogen uptake (160 kg N/ha) for two types of fescue: turf type (variety TOMAHAWK) and forage type (variety DULCIA). The results show a similar nitrogen uptake at ear emergence (140 kg N/ha) but yields mark the difference between variety TOMAHAWK (2137 kg ha<sup>-1</sup>) and DULCIA (935 kg ha<sup>-1</sup>). The experiments pointed out similar needs between two different fescue types: turf type and forage type even if the yield level is too different.

In situation 2, farmer practice is more important than nitrogen balance method, the difference between T2 and T3 is equal to - 28 kg N/ha. The nitrogen mineral supply by the farmer induces the beating down of crop by overfeeding. Nitrogen supply, by calculated method, is more right.

In all cases, yield obtained by nitrogen balance method is higher than seed yield T3 (figure 2), and the nitrogen uptake at ear emergence is not too far from 120 kg N/ha (the mean is 145 kg N ha<sup>-1</sup>).

## Conclusion

The results of these experiments show that the maximum seed yield of tall fescue was reached at about 160 kg ha<sup>-1</sup> of total nitrogen uptake at the end of ear emergence. The different experiments confirmed the validity of the nitrogen balance method. In few cases, the soil contribution to the total requirement of the crop can be high.

The total N uptake by tall fescue is equal in the different types (turf and forage) as well as in different year of harvest (1<sup>st</sup> or 2<sup>nd</sup> harvest).

Figure 1: Yield response to nitrogen uptake in aerial part, at ear emergence, for tall fescue (total of 158 datasets)

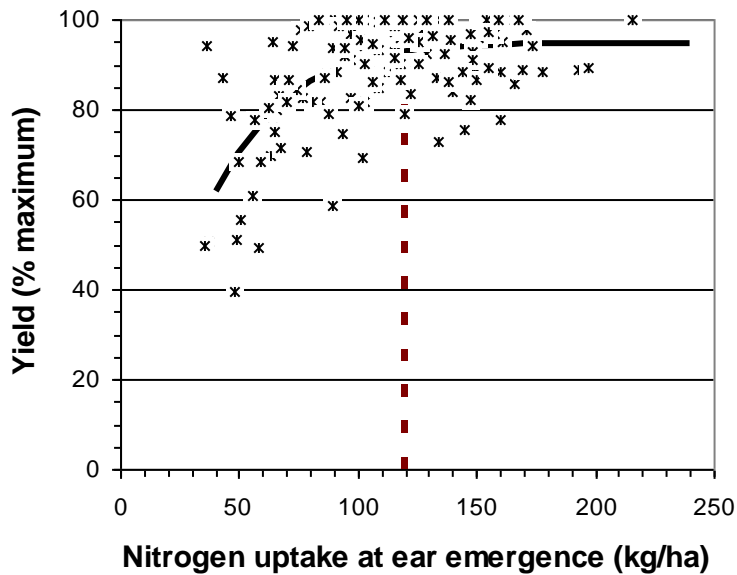


Figure 2: Effect of N fertilization (balance method) on seed yield compared to usual farmer practice (2007 and 2008) – From 1 to 9 (left) crops in first year of production, from 10 to 16 (right) crops in second year of production

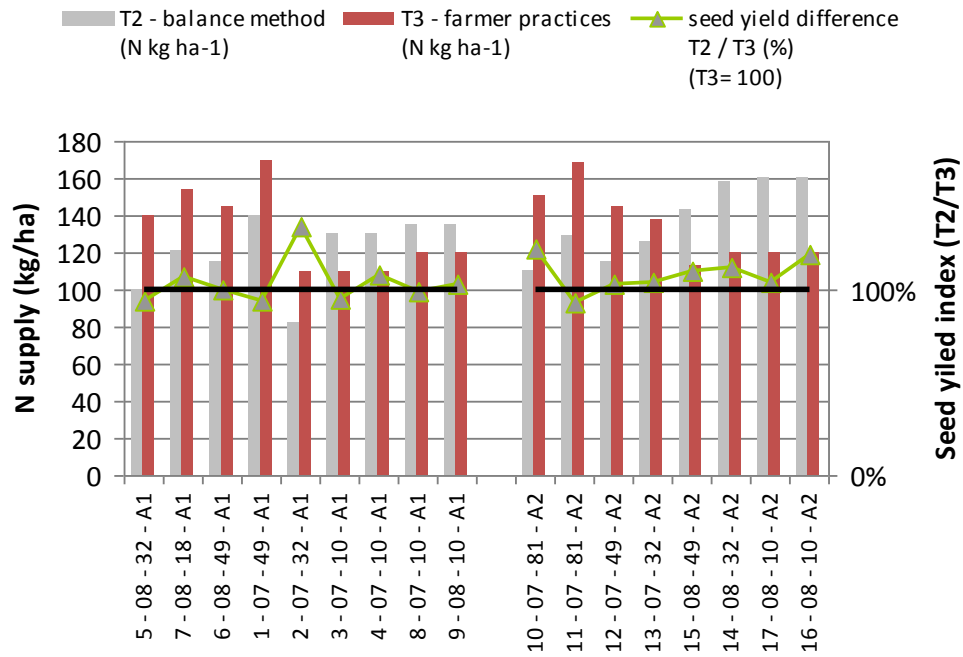


Table 1: Seed crops of tall fescue studied in 2007 and 2008 with N fertilization comparison.

Nitrogen mineral supply (T2) is calculated with N balance method (FNAMS abacus)

N° Trials : (Year, Location and French department ) N-W: north-west S-W: south-west M: middle N-E: north-east	Cultivars (T: turf type F: forage type)	N Plant Needs (kg ha-1)		N Soil Furniture (kg ha-1)				Compared objects		
		Plant N uptake	N unavailable	Soil nitrogen content at the end of winter	Mineralization of vegetal residues	Mineralization humus	Mineralization organic matter	T2 - N calculated by balance method (and real N supply) (kg ha-1)	T3 - Farmer practice N mineral supply (kg/ha)	Différence T2 - T3 (kg /ha)
<b>Crops in first year of production (A1)</b>										
1 (07 / N-W / 49)	TOMAHAWK (T)	160	20	11	0	32	0	140 (140)	170	- 30
2 (07 / S-W / 32)	VILLAGEOISE (T)		20	82	0	25	0	73 (81)	110	- 28
3 (07 / N-E / 10)	TOMAHAWK (T)		20	7	20	22	0	130 (130)	110	+ 20
4 (07 / N-E / 49)	DULCIA (F)		20	7	20	22	0	130 (130)	110	+ 20
5 (08 / S-W / 32 )	VILLAGEOISE (T)		30	88	-20	22	0	100 (100)	140	- 40
6 (08 / N-W / 49 )	VILLAGEOISE (T)		20	13	20	28	0	119 (115)	145	- 30
7 (08 / M / 18 )	VILLAGEOISE (T)		15	9	20	33	0	113 (121)	154	- 33
8 (08 / N-E / 10 )	TOMAHAWK (T)		30	12	20	23	0	135 (135)	120	+ 15
9 (08 / N-E / 10 )	DULCIA (F)		30	12	20	23	0	135 (135)	120	+ 15
<b>Crops in second year of production (A2)</b>										
10 (07 / S-W / 81)	DULCIA (F)	160	30	42	0	27	0	120 (110)	151	- 41
11 (07 / S-W / 81)	VILLAGEOISE (T)		20	21	0	20	0	139 (129)	169	- 40
12 (07 / N-W / 49)	VILLAGEOISE (T)		20	34	0	32	0	115 (115)	145	-30
13 (07 / S-W / 32)	BELFINE (F)		20	28	0	25	0	126 (130)	138	- 12
14 (S-W / 32 )	VILLAGEOISE (T)		30	10	0	22	0	158 (158)	120	+ 38
15 (N-W / 49 )	TOMAHAWK (T)		20	13	0	21	0	146 (143)	113	+ 30
16 (N-E / 10 )	TOMAHAWK (T)		30	10	0	23	0	157 (160)	120	+ 40
17 (N-E / 10 )	DULCIA (F)		30	8	0	23	0	159 (160)	120	+ 40

Table 2: Effect of N fertilization on seed yield, N uptake and dry matter of tall fescue (2008)

		T2 - N Balance Method				T3 - usual farmer practice			
N° Trials : (Location / French Department ) N-W: north-west S-W: south-west M: middle N-E: north-east	Cultivars (T: turf type F: forage type)	N mineral supply	Seed yield	N uptake (aerial part)	Dry matter	N mineral supply	Seed yield	N uptake (aerial part)	Dry matter
		(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(T ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(T ha <sup>-1</sup> )
<b>Crops in first year of production</b>									
1 (07 / N-W / 49)	TOMAHAWK (T)	140	<b>784</b>	193	11.5	145	<b>837</b>	210	11.3
2 (07 / S-W / 32)	VILLAGEOISE (T)	82	<b>1055</b>	115	5.7	110	<b>789</b>	154	6.9
3 (07 / N-E / 10)	TOMAHAWK (T)	130	<b>1563</b>	132	10.2	110	<b>1640</b>	115	9.7
4 (07 / N-E / 49)	DULCIA (F)	130	<b>863</b>	124	10.5	110	<b>801</b>	117	11.0
5 (08 / S-W / 32 )	VILLAGEOISE (T)	100	<b>1440</b>	121	5.0	140	<b>1540</b>	118	7.9
6 (08 / N-W / 49 )	VILLAGEOISE (T)	115	<b>2150</b>	187	12.9	145	<b>2150</b>	201	12.0
7 (08 / M / 18 )	VILLAGEOISE (T)	121	<b>1520</b>	119	11.5	154	<b>1420</b>	149	12.3
8 (08 / N-E / 10 )	TOMAHAWK (T)	135	<b>2550</b>	149	14.2	120	<b>2570</b>	172	14.7
9 (08 / N-E / 10 )	DULCIA (F)	135	<b>1090</b>	148	12.8	120	<b>1060</b>	160	14.2
<b>Crops in second year of production</b>									
10 (07 / S-W / 81)	DULCIA (F)	110	<b>509</b>	164	10.7	151	<b>417</b>	148	10.4
11 (07 / S-W / 81)	VILLAGEOISE (T)	129	<b>784</b>	138.5	7.6	169	<b>841</b>	172	7.8
12 (07 / N-W / 49)	VILLAGEOISE (T)	115	<b>999</b>	131	9.4	145	<b>973</b>	141	9.0
13 (07 / S-W / 32)	BELFINE	126	<b>850</b>	166	10.5	138	<b>820</b>	141	11.7
14 (S-W / 32 )	VILLAGEOISE (T)	158	<b>1550</b>	176	10.0	120	<b>1380</b>	109	8.4
15 (N-W / 49 )	TOMAHAWK (T)	143	<b>1380</b>	138	9.3	113	<b>1250</b>	125	8.6
16 (N-E / 10 )	TOMAHAWK (T)	160	<b>2120</b>	131	10.83	120	<b>1780</b>	124	10.8
17 (N-E / 10 )	DULCIA (F)	160	<b>780</b>	143	11.6	120	<b>750</b>	129	11.9

## Stresses associated with germination and establishment of overseeded turfgrasses

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### Abstract

Overseeding is a practice that is commonly applied to dormant turfgrasses to provide an actively-growing turf surface during winter and early spring. This practice has been widely used across the south and transition zone on golf courses, athletic fields, and other recreational turf surfaces. Grasses used for overseeding are often more sensitive to biotic and abiotic stresses compared to the dormant warm-season grass and there is a need to identify new genetic resources and cultural practices that allow better utilization of this practice. This paper summarizes results from trials that investigated the effects of salinity and traffic on several commonly-used turfgrass species. Overall, the results from these trials indicate the ryegrass species, including diploid perennial ryegrass, tetraploid perennial ryegrass, and intermediate ryegrass, remain the most tolerant overseeding species of abiotic stresses such as salinity. In addition, these species are also more tolerant of traffic than grasses such as meadow fescue and annual ryegrass. Continued research is needed to identify grasses and management strategies that allow successful overseeding in harsh environments.

### Introduction

Warm-season grasses such as bermudagrass (*Cynodon* spp.) continue to be the predominate turfgrass species used for golf courses, sports fields, and home lawns in tropical and transition zone areas of the world. Although bermudagrass has many positive attributes, such as good wear tolerance and recuperative potential, excellent heat and drought stress tolerance, and broad pest resistance, the species experiences a long winter dormancy period in many use areas. Because of this extended dormancy period, bermudagrass is often over-seeded with a cool-season turfgrass to provide an actively growing green surface for winter and early-spring sporting activities (Dudeck and Peacock, 1980; Schmidt and Shoulders, 1977).

Numerous grass species have been successfully used for overseeding, including annual (Italian) ryegrass (*Lolium multiflorum* Lam.), perennial ryegrass (*L. perenne* L.), intermediate ryegrass (*L. multiflorum* Lam. x *L. perenne* L.) creeping bentgrass (*Agrostis stolonifera* L.), rough bluegrass (*Poa trivialis* L.) and fine fescue (*Festuca* spp.) (Kneebone and Major, 1969; Schmidt and Shoulders, 1977; Richardson, 2004). Although all of these cool-season grasses have been successfully used in various overseeding situations, there is a continued need to develop new species and cultivars with applications to overseeding. In recent years, efforts to improve

turfgrass characteristics of both meadow fescue (*Festuca pratensis* Huds.) and tetraploid ryegrass (*L. perenne* L.) have led to new species with applications to overseeding dormant bermudagrass turf (Richardson et al., 2007).

Overseeding grasses, like all turfgrasses, often experience a range of environmental, biological, and cultural stresses such as diseases, limited water, poor-quality water, salinity stress and traffic from equipment or players. However, overseeding grasses are rarely provided with an extended establishment window in which the turf can be hardened and prepared for some of these stresses. As such, developing overseeding grasses with improved tolerance of biotic and abiotic stresses and developing management practices to minimize damage to seedling overseeding grasses are important research objectives.

Increased demand for potable water in southern locations where overseeding is practiced has increased the use of lower quality irrigation sources at many golf courses and sports turf facilities. Across the U.S., 12% of all golf facilities use recycled water, while 37% of golf facilities in the southwestern U.S. use some form of recycled water (Throssell et al., 2009). Although data is not available for other turf facilities, it is assumed that recycled water use is increasing around the country, especially in areas where potable sources are restricted. Although certain overseeding grasses such as perennial ryegrass can germinate in the presence of moderate salinity (Johnson et al., 2007), it is unclear how other overseeding species might respond to moderate or higher levels of salinity. Regardless, it is obvious that alternative grasses or cultivars with improved tolerance of poor-quality water would be of great benefit to turfgrass managers.

A major stress observed in all overseeding applications is the effects of traffic and play on the overseeding grasses, especially in the weeks following seeding, where seedlings are vulnerable to injury from equipment or humans. Although overseeding grasses have been shown to respond differently to traffic at maturity (Summerford et al., 2008; Minner and Valverde, 2005), there have been limited studies that have investigated the effects of traffic on overseeded turf during the seedling stage.

This paper will highlight studies conducted by our research program over the past 6 years that have investigated the effects of species, pre-plant cultivation, and salinity on the germination and establishment of overseeding grasses in a transition-zone environment.

## **Materials and Methods**

### *Salinity effects on germination*

A greenhouse study was conducted in which four overseeding species, including diploid perennial ryegrass, tetraploid perennial ryegrass, intermediate ryegrass, and meadow fescue. The four species were germinated in hydroponic solutions adjusted to four irrigation water salinity levels. The salinity levels included 7500, 10000, 12500, and 15000 mg L<sup>-1</sup> NaCl solutions, which

correspond to electrical conductivity values of ~12, 16, 20, and 24 dS / m. These levels were chosen based on results from a preliminary study where there was no inhibition of germination of any of these species up to 5000 mg L<sup>-1</sup> NaCl. The hydroponic system consisted of tubs (28 by 35 by 14 cm) in which a foam insulation board (thickness = 1.5 cm) was cut to fit inside the perimeter of the tub and floated on the hydroponic solution. Twelve holes (3.8 cm diam.) were cut into each board and a nylon screen (18 x 16 mm) was affixed with silicon glue to the bottom side of the insulation board, which placed the screen in contact with the solution. The base solution for each salinity treatment consisted of a complete, nutrient solution that delivered 50 ppm N using a fertilizer formulation (5-11-26, HYDRO-SOL, Peters Professional) specifically designed for hydroponic culture. Air was continuously supplied to each solution via an air stone (Aqua Mist, Penn Plax, Inc.) connected to an aquarium pump (Silent Air, Penn Plax, Inc.). Each solution concentration was replicated four times.

Twenty-five seeds of each species were placed in each of 3 subsample cells on top of the screen that was in contact with each solution. Germination was monitored frequently (every 1-2 days over the next 14 days) and a seed was considered to have germinated when both the radicle and coleoptile had emerged. Once a seed had germinated, it was counted and removed from the solution.

#### *Pre-plant cultivation and traffic response of overseeded grasses*

In Sep. 2007 and 2008, five cool-season turfgrasses, including annual ryegrass, intermediate ryegrass, meadow fescue, diploid perennial ryegrass, and tetraploid perennial ryegrass were overseeded into a mature (>4 yr) stand of bermudagrass (cv. Riviera) turf at the University of Arkansas Agricultural Research and Extension Center at Fayetteville. Species were seeded at similar seeding densities (Table 1). Plots were assigned one of three pre-plant cultivation treatments of core-aerification, verticutting, or an untreated control. Core-aerification treatments included a single pass with a greens aerifier (Toro Greens Aerator, Toro Co., Bloomington, MN) set up with 1.25 cm hollow-tines on a 5 x 5 cm spacing. Verticutting treatments were applied with a tractor-mounted verticutter (Jacobsen Turf, Charlotte NC) containing blades on 2.5 cm centers and set to a depth that reached the soil surface. Traffic was applied using a Cady traffic simulator (Henderson et al., 2005) making four passes at 1, 2 or 4 weeks after planting (WAP) or an untreated control.

The experimental design was a strip-strip plot with species considered the whole plot and pre-plant cultivation and traffic treatments applied randomly as strips across species treatments. The plot size for each species replicate was 3.6 x 3.6 m, while both the pre-plant cultivation and traffic treatments were applied in 1.2 m strips across the species plots. All treatments were replicated four times. Digital image analysis was used to determine turfgrass coverage of the

overseeded species when the bermudagrass turf was dormant (Richardson et al., 2001). For brevity, only the 2008 data will be presented in the present paper.

## **Results and Discussion**

### *Salinity effects on germination*

Germination was first observed with intermediate ryegrass in the lowest salt solution at 4 days after seeding (DAS) (Fig. 1). All of the ryegrasses began germinating in the 7000 and 10000 mg l<sup>-1</sup> solutions at 5 DAS. In the 12500 and 15000 mg l<sup>-1</sup> solutions, ryegrass germination was first observed at 7 DAS. In all solutions except the 15000 mg l<sup>-1</sup>, germination of meadow fescue was first observed at 8 DAS, which is consistent with earlier reports comparing these species (Richardson et al., 2007). Minimal germination of meadow fescue was observed at the highest salt concentration (Fig. 1).

All of the ryegrasses obtained similar germination levels in each solution by 14 DAS and exceeded 80% germination in the lowest two salt concentrations (Fig. 1). There was a reduction in final germination of the ryegrasses observed at the 12500 and 15000 mg l<sup>-1</sup>, with maximum germination of approximately 60% and 45%, respectively (Fig. 1). Meadow fescue followed a similar trend, except the final germination of this species was reduced to approximately 75% in the 10000 mg l<sup>-1</sup> solution, 20% in the 12500 mg l<sup>-1</sup> solution, and less than 5% in the highest salt concentration.

The overall conclusion from this study is that tetraploid ryegrass has similar salt tolerance, relative to seed germination, as other commonly-used ryegrass species. As these grasses are being applied to many overseeding situations, further research on its salt tolerance as a mature plant would be worthwhile. Germination of meadow fescue appears to be more sensitive to increasing salt levels compared to the ryegrasses and would not be currently recommended in areas where low-quality water is being used. However, it should be noted that the salt concentrations used in this study were quite high. The 10000 mg l<sup>-1</sup> solution would approximate a 1:1 mixture of fresh water and sea water and there was minimal germination inhibition observed in any species at that salinity level.

### *Pre-plant cultivation and traffic response of overseeded grasses*

Aerification and verticutting both provided better establishment for over-seeding grasses compared to the untreated control although differences were not dramatic (Fig. 2). A similar trial was conducted in the 2009 season on a site with higher soil compaction and the pre-plant cultivation treatments had a bigger impact on initial establishment (data not shown).

Traffic applied 4 WAP was consistently more detrimental than traffic applied at 1 and 2 WAP in all overseeding species (Fig. 2). The reason for this is currently unclear, but apparently traffic

applied later in the fall was more detrimental, as the turf had less time to recover before growth slowed due to low temperatures. In one year of our studies, the pre-plant aerification also helped reduce damage from traffic on seedlings (data not shown). This may be the result of reduced compaction from the aerification. Regardless, these results suggest that cultivation can enhance establishment of overseeding grasses and this could be very beneficial in transition-zone environments where there is a limited establishment period in the fall.

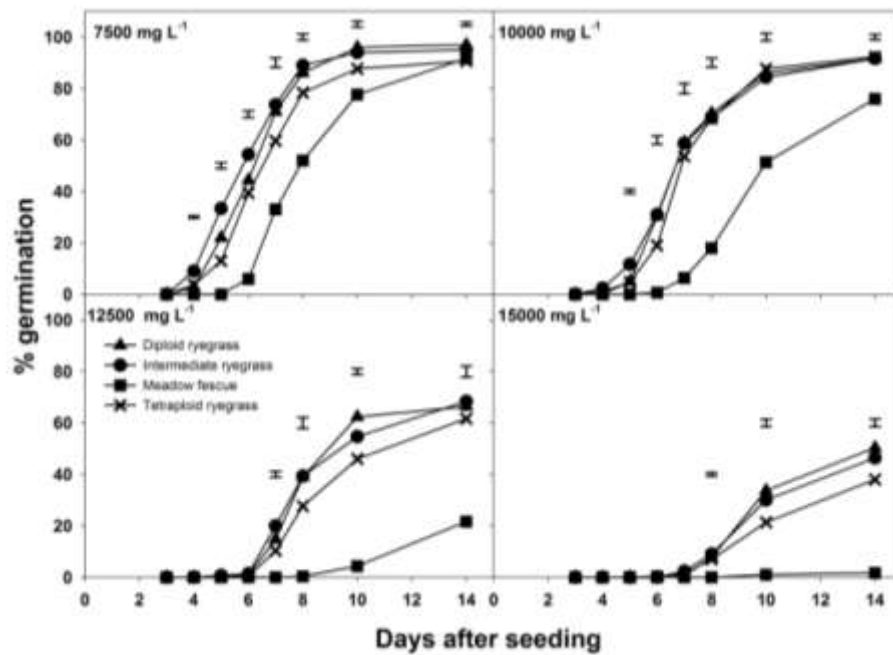
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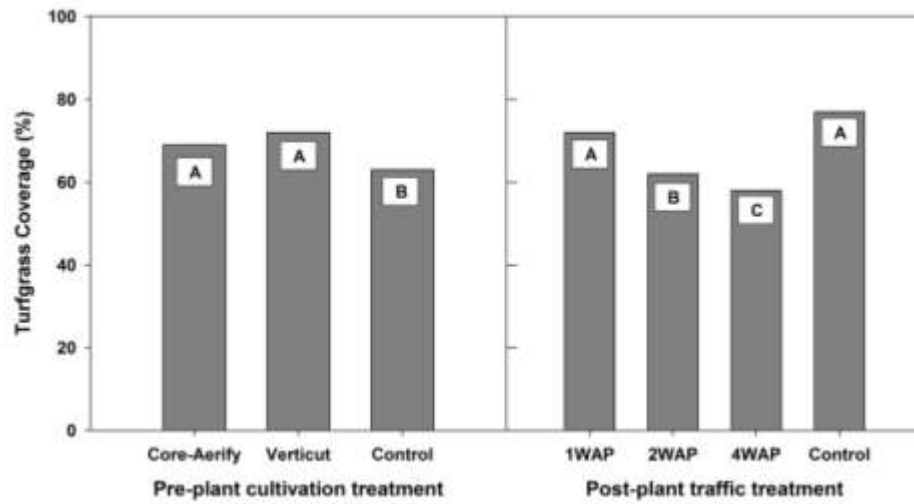
**Table 1. Overseeding species used in field study and their corresponding seeding rates.**

Species:	Cultivar	Seeding Rate	
		(g m <sup>-2</sup> )	Seeds / cm <sup>2</sup>
Annual ryegrass	Gulf	68	3.4
Intermediate ryegrass	50% Transist / 50% Tranzeze	63	3.4
Meadow fescue	AMF29	63	3.4
Diploid perennial ryegrass	Integra	59	3.4
Tetraploid perennial ryegrass	T3	93	3.4

**Figure 1.** Germination of four, cool-season turfgrass species in solutions with various NaCl concentration. Error bars represent the least significant difference (P=0.05) for comparing species within a salinity level.



**Figure 2.** Establishment of five overseeding turfgrass species as affected by cultivation and traffic. Different letters represent significant difference between either cultivation treatments or traffic treatments, as determined by a least significant difference test (P=0.05).



# **Evaluation of vigour tests for determination of seed storage potential in red clover (*Trifolium pratensis* L.) and timothy (*Phleum pratense* L.)**

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## **Abstract**

Three different seed vigour tests, Accelerated ageing (AA), Germination index (GI) and Polyetyleneglycol (PEG), were evaluated with regard to ranking six reference seed lots of timothy cv. Grindstad and red clover cv. Lea according to their physiological potential for storage. The seed reference lots, which all had germination percentages between 70-100%, had sustained different degrees of ageing before examination. In timothy, both the AA-test and the GI-test managed well to separate the low-vigour seed lots, having a poor storage potential, from the high-vigour seed lots with better storage longevity. The clearest separation was obtained using the GI-test, either as complete test (six out of six seed lots was significantly separated) or as a simplified test (five out of six seed lots was significantly separated). The germination index of the two tests was based on counting of germinating seeds either on a daily basis for ten days or after counting on day 3 and day 5, respectively. Also the AA-test managed to separate five out of six seed lots of timothy after optimal treatment for 56 h at 45°C. In red clover, the AA-test gave the clearest separation between seed lots. At optimal conditions for 24 h at 43-45°C, as many as five of the six red clover seed lots could significantly be separated from each other. Also the PEG-test discriminated well between reference seed lots of both species, but was considered as more labour-intensive than the two other vigour tests. The recommended seed vigour test for timothy (simplified GI-test) and red clover (AA-test for 24 h at 43-45°C) has later been adopted for use in practical seed testing routines at the Norwegian National seed laboratory.

## **Introduction**

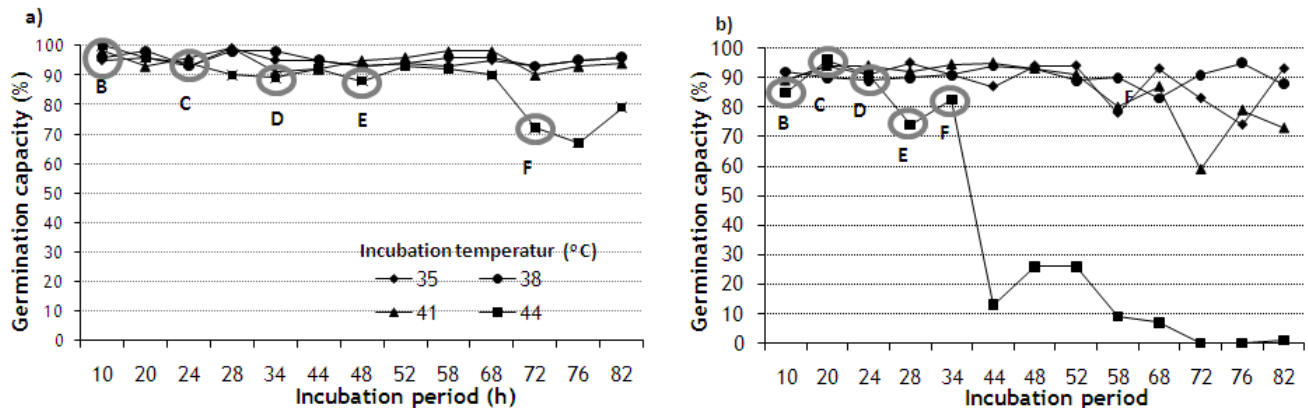
In the early 2000s Norwegian seed companies had to discard large amount of forage seed, especially of timothy, due to germination losses on storage. In order to minimize losses, methods for ranking seed lots with regard to storage longevity were highly requested by the seed companies. Seed vigour tests have earlier been found as good predictors for seed longevity in storage (Wang & Hampton 1991). The maximum seed vigour occurs at physiological maturity, after which vigour decline during ageing on plant and during storage.

The aim of the present project was to evaluate three different seed vigour tests, Accelerated ageing (AA), Germination index (GI) and Polyetyleneglycol (PEG), with regard to ranking seed lots of timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.) according to their physiological potential for storage.

## **Materials and methods**

### *Seed reference set*

The seed vigour testing was performed at the seed laboratory at Bioforsk Øst Landvik in 2007. The original seed material used for vigour testing was supplied from commercial production of timothy cv. Grindstad and red clover cv. Lea. From the original seed lot, seed samples were artificially aged under humid conditions (about 100% RH in airtight plastic containers) at four different temperatures (35, 38, 41 and 44 °C) for various incubation periods. After ageing, germination capacity was determined according to ISTA (2008) but with one replicate of 100 seed per treatment. In both species, only seed aged at the highest temperature (44 °C) obtained a severe loss of germination capacity during the 82 h incubation period (Figure 1a and 1b). Thus, five samples of both species with different physiological age, incubated for various lengths at this temperature, was selected as a seed reference set for later use in the seed vigour testing programme. The initial germination capacity of the selected reference seed lots (B-F) was between 70-100% as shown in Figure 1 and 2. In addition, seed from the original seed lot (A: untreated seed, 97 % germination in both species) was selected as a reference (i.e. six reference lots in total for each species). According to the different degrees of physiological ageing, reference seed lot F was ‘oldest’ (low storage potential) and seed lot A ‘youngest’ (high storage potential).



**Figure 1.** Germination capacity (%) of the selected reference seed lots (marked with grey circles) of a) timothy cv. Grindstad and b) red clover cv. Lea after being aged at different temperature and incubation length.

#### Germination index test

The germination index test was carried out as a standard germination test (ISTA 2008) where four replicates of 100 seeds of the six reference seed lots of each species were laid out on the Jacobsen germination apparatus, but with a more frequent counting regime. The day/night temperature was regulated to 25 / 20 °C and 20 / 20 °C for timothy and red clover, respectively. The first registration of emerging seedlings was performed after 2 and 3 days in red clover and timothy, respectively. New seedlings were then daily counted for 7 days before a last counting after 10 days completed the registration program. Seed was registered as germinated when the length of the emerging seedling (timothy) or the total length of radicle and seedling (red clover) was 2 mm or longer. The germination index (GI) was calculated based on the following formula:  $GI = (\text{number of new seedlings after day } n_1 / n_1) + (\text{number of new seedlings after day } n_2 / n_2) + \dots + (\text{number of new seedlings after day } n_i / n_i)$ .

#### PEG-test

The six different reference groups of each species was osmotic stressed by imbibing the seed at five concentrations (0.00, -0.15, -0.30, -0.45 and -0.60 MPa) of Polyethylene glycol (PEG 6000) based on the equation:  $\psi_o = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T$  where C is the concentration of PEG 6000 in grams per kilogram H<sub>2</sub>O and T is the temperature in degrees Celsius (Michel and Kaufmann, 1973). Filter papers were placed in Petri dishes and moistened with 3 ml distilled water (control) or the respective concentrations of PEG solutions before seeding samples of 50 seeds per dish (total: 8 x 50 seed = 400 seed per treatment). The Petri dishes were sealed with parafilm to avoid evaporation and placed on the Jacobsen germination apparatus at the same conditions as in the germination index test. The number of germinating seeds was counted and removed after 7 and 10 d (timothy) or after 5 and 10 d (red clover).

#### *Accelerated aging test*

A preliminary AA-screening test indicated that seed from the six different reference groups was best discriminated at around 44 °C (data not shown). Thus, a more detailed accelerated ageing test was performed in both species at 43 and 45 °C for 32, 48 and 72 h (timothy) or 24, 32 and 48 h (red clover). For each combination of temperature and ageing period, seeds from the six reference seed lots were lightly dusted with the fungicide thiram and placed on a wire mesh in a plastic box 30 mm above water surface (around 100% RH). Following the incubating period, seed was dried at room temperature for 24 h and germination capacity (4 replicates x 100 seed per treatment) evaluated using standard methods (ISTA 2008).

In order to discriminate differences between the artificially aged reference seed lots, analyses of variance were performed separately for each species (timothy and red clover), always regarding each replicate (100 seeds) as a random variable for each of the three vigour tests. In the tables, significant differences are indicated by different letters according to Duncan multiple comparison test at P<0.05.

## **Results and discussion**

In timothy and red clover, six and three of the total six reference set lots of each species was significantly separated by the GI-test, respectively. In addition, a less labour-intensive model, based on the number of germinating seeds on day 3 and day 5 in the GI-test, was calculated for timothy. This simplified GI-test managed to discriminate five out of six seed reference lots (Table 1).

**Table 1.** Germination index (GI) for the six reference seed lots of timothy and red clover.

Reference seed lot	Timothy		Red clover
	Germination index	Simplified GI, (3 + 5 days)	Germination index
A	24.5a	20.5a	38.0a
B	23.0b	18.6b	38.4a
C	21.8c	15.5c	38.8a
D	20.0d	12.1d	38.5a
E	18.0e	12.1d	34.3b
F	11.4f	0.9e	28.3c
Sign	<0.01	<0.01	<0.01

The clearest separation by the PEG-test was found at an osmotic pressure of -0.6 MPa and -0.15 MPa for timothy and red clover, respectively. Under these conditions, significant differences in germination capacity between five of the six reference seed lots was detected in both species. However, the test was considered as more labour-intensive for practical use in seed laboratories than the two other seed vigour tests.

**Table 2.** Effect of osmotic pressure (PEG-concentration) on germination capacity of six reference seed lots of timothy and red clover.

Reference seed lot	Timothy					Red clover				
	0,00 Mpa	-0,15 MPa	-0,30 Mpa	-0,45 MPa	-0,60 MPa	0,00 Mpa	-0,15 MPa	-0,30 Mpa	-0,45 MPa	-0,60 MPa
A	98a	99a	96a	82a	68a	97a	96ab	83a	39bc	20a
B	95a	98ab	96a	78a	55b	98a	97a	83a	68a	13b
C	98a	98ab	87b	55b	30c	97a	91b	84a	53ab	2c
D	95a	92ab	76c	60b	12de	88b	84c	79a	30c	2c
E	93a	87b	84b	51b	15d	91b	76d	54b	23c	3c
F	63b	76c	65d	32c	4e	68c	61e	43c	21c	1c
Sign, %	<0.01	<1	<0.01	<0.01	<0.01	<0.01	<1	<0.01	<1	<0.01

The AA-test managed to separate five out of six seed lots of timothy, with regard to germination capacity, after optimal treatment for 56 h at 45°C. In red clover, incubation for 24 and 48 h at 43°C or for 24 h at 45°C gave similar separation (five out of six) (Table 3). The results indicate that red clover has a relatively tolerant optimal temperature regime (43-45 °C) when incubated for 24 h.

**Table 3.** Effects of accelerated aging at various temperatures and incubation periods on germination capacity of the six reference seed lots of timothy and red clover.

Ref. seed lot	Timothy						Red clover					
	43°C			45°C			43°C			45°C		
	32t	48t	56t	32t	48t	56t	24t	32t	48t	24t	32t	48t
A	93a	90a	90a	90a	85a	79a	95a	79a	73a	89a	79a	6a
B	89ab	88a	90a	88a	84a	77a	91a	79a	58b	80b	73b	10a
C	90ab	88a	85b	89a	71b	64b	80b	71a	30c	48c	14c	1b
D	88bc	81b	81bc	79b	64c	53c	63c	57b	19d	46c	16c	0b
E	85c	85ab	78c	81b	62c	34d	48d	35c	0e	14d	0d	1b
F	47d	22c	20d	19c	3d	1e	14e	4d	0e	5e	1d	0b
Sign,%	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2

### Conclusions

Based on the seed vigour test results (Table 1-3) and for practical usability in seed laboratories, the simplified GI-test and the AA-test for 24 h at 43-45°C was recommended for timothy and red clover, respectively. In order to rank commercial seed lots of the two species with regard to their storage potential, these two seed vigour tests have later been adopted for use in practical seed testing routines at the Norwegian National seed laboratory.

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# Reliability of salinity screening *Lolium* genotypes using field grown versus greenhouse techniques

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## Abstract

*Lolium multiflorum* and *perenne* as well as some other turf species were evaluated during the 2008-2009 growing season in the greenhouse at Overton, as well as in the field at Pecos, Texas. The immersion technique conducted in the greenhouse was repeatable and we believe effective in screening genotypes of salt tolerance. Screening genotypes in the field at Pecos, may be effective for the type of salinity present in that environment; however, the “salt” from NaCl added to water in the greenhouse versus high salt in soils of west Texas apparently is different. Therefore results from these two environments were not compatible.

## Introduction

High salinity in water from aquifers and soils in many regions of Texas is problematic to the turf industry. Marcum (2004) rated most cool-season grasses as very susceptible to high salinity with perennial ryegrass having more tolerance than *Poa trivialis* and annual ryegrass. Rose-Fricker and Wipff (2001) screened Kentucky bluegrass cultivars at 10,000 mg salt L<sup>-1</sup> and perennial ryegrass at 17,000 mg L<sup>-1</sup> when grown in sand in salt tanks. Brilman (2006) screened perennial ryegrass for germination in 10,000 mg salt L<sup>-1</sup> and seedlings at concentrations of 12,000 mg salt L<sup>-1</sup> and reported differences among genotypes for rate of germination and for seedling growth. Nelson (2009) described a technique where ryegrass seedlings were grown in sand in flats periodically immersed in high salinity water and differences between genotypes were observed. This experiment was conducted to compare field screening of ryegrass with the immersion technique and if results were compatible.

## Materials and Methods

Twenty-seven genotypes were tested in a field trial at Pecos, and in two greenhouse experiments at Overton, Texas. A detailed salinity test (Saturated Past Extract) of a soil sample collected at Pecos indicated sodium levels at 609 mg kg<sup>-1</sup> (26.501 meq/L) and calcium at 675 mg kg<sup>-1</sup> (33.683 meq/L). Soil pH was 8.2. Water analysis from the Pecos well water was 477 mg kg<sup>-1</sup> sodium, 1125 mg kg<sup>-1</sup> chloride with total dissolved salts at 3853 mg kg<sup>-1</sup>. At Pecos, one g of seed of each entry was planted in 1 m rows on Oct. 28 (3 replications). No significant rainfall occurred; therefore all water was applied by flood irrigation. Salinity damage ratings were taken on a 1 to 9 scale where 9 was most severe. During the spring of 2008 a glasshouse study was established at the Texas AgriLife Research and Extension Center at Overton, Texas. The glasshouse salinity screening technique involved planting seed in Quikrete Play Sand and 3% peat moss by volume, which was placed in plastic inserts in trays. Each tray was 27 cm wide by 42 cm in length. The plastic insert had 4 x 4 cm squares 4 cm deep. Oceanic Natural Sea Salt Mix was added to the well water and was placed in a plastic tank filled to a depth of 15 cm.

Treys were immersed in high salinity water for 2 min. every 3 to 4 days. Salt concentrations began at 5000 mg kg<sup>-1</sup> (8 d S/m) and were gradually increased over time until near the end of the screening period, when salt concentrations were 9,800 mg kg<sup>-1</sup> (15.8 d S/m). Five replications were treated with the above salt treatments. A control, immersed in well water, consisting on one replication was used to make comparisons with the salt treated plants. Ratings were also made on entries from this treatment. The corrected ratings are the actual rating, minus the rating from the untreated (salt) plants in “rep 6”. This trial was repeated (Exp. B) to test reliability of procedure. The rating for each entry should increase from one date to the next date. Late in the rating period of both experiments many ratings were near 8 on a 1 to 9 scale, where 9 = dead plants, indicating many plants were dead.

## Results

At Pecos, ratings were made at three dates (Table 1). By comparing salt damage ratings at all three dates, it was apparent some entries demonstrated tolerance to salt damage. PSB (Pecos BLK) and Pecos BLK 07-08 were selected from space plants for 2 or more generations at Pecos. They appear to be somewhat salt tolerant; however, it may be that they are becoming adapted to that soil type and environment and not just high salinity. Other genotypes which had low salinity damage ratings were K-31 tall fescue, TMI-Puccinella distant, an alkali grass, and TXR2008-TF-PR-85, an intermediate ryegrass.

In greenhouse trials at Overton (Tables 2), data are shown for 2 dates on each experiment. Most entries had ratings higher than 7 indicating severe salt damage. Generally speaking the tall fescue entries had lower ratings than annual or perennial ryegrass. TMI-Puccinella distans was the most tolerant entry in both experiments A & B, just as it was in the field trial. Soprano perennial ryegrass was more tolerant to salt than other ryegrass entries. The PSB and Pecos BLK 07-08 entries did not show tolerance to high salt in the immersion treatments in the greenhouse. It was obvious from the data that we had a significant amount of senescence in the untreated (salt) plants. This could have been due to poor light quality, poor water quality, high temperature in the greenhouse, especially in the Exp. B, or some other factor. It was useful to compare the actual rating and the corrected rating for each entry for each date. If an entry had very high actual ratings such as 9 indicating all plants were dead, and also its corrected rating was 6, (indicating the control had a rating of 3), then this entry was very susceptible. SS3 had a high salt damage rating of 8 on 9 Feb; however, the corrected rating was 3 for that date. This indicates that it may be relatively tolerant to salt damage. Utilization of a correction factor as stated above did not seem to improve the reliability of rating the entries for salt damage. The C.V.'s for the corrected means were higher than the actual ratings which may be a result of the mathematical process in that the corrected means were small, or we analyzing smaller numbers. We ran a simple correlation analysis of the means from the field trial against the means shown in Table 2 for experiments A & B. The field means were not significantly correlated with means from either greenhouse experiment. Means from Exp. A were highly correlated with means from B ( $r = 0.51$ ,  $p = 0.006$ ). Salinity means in Exp. A were highly correlated to corrected means from Exp. A, as would be expected. In Exp. B, salinity means were not correlated with the corrected means. This may have been due to environmental conditions, as Exp. A was conducted during the middle of the cool season, and B was conducted in the late spring, when mid-day greenhouse temperatures were quite warm. The high temperatures resulted in the senescence (high rating) on the plants which were not treated with high salinity.

## **Acknowledgment**

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**Table 1.** Salinity damage ratings in field at Pecos, TX in 2009.

Entry		Date of Rating		
		4 February	21 March	8 May
Phenom	PR <sup>‡</sup>	4.3 <sup>†</sup>	3.0 <sup>†</sup>	3.7 <sup>†</sup>
Sidewinder	TF	4.7	4.0	4.3
Cochise IV	TF	5.3	5.3	5.0
TF – 152	TF	6.0	4.3	5.3
K 31	TF	2.3	2.7	2.3
Gulf	AR	2.3	3.0	4.0
TXR2004-TF-EM	IR	1.7	2.3	4.0
06 B Lp	PR	5.3	4.0	3.7
PSG-4MSH	PR	6.0	2.7	4.0
08-8 Lh	IR	4.7	4.3	3.3
IS-PR 385 BLK	PR	4.7	4.3	5.0
TMI-Puccinella dis	AIG	6.3	1.7	1.0
TXR2008-TF-PR-85	IR	4.0	2.0	1.3
TXR2008-PR-1	PR	4.3	3.3	4.7
RKS	PR	5.7	4.7	5.0
Soprano	PR	5.3	3.7	4.0
Soprano Myco	PR	5.7	3.7	5.7
TXR2006-TF-AxP	IR	4.7	2.7	3.7
TXR2008-TF-AxP F4	IR	5.0	3.3	3.3
TXR2006-TF-PR-LS	IR	2.7	3.0	3.7
Panterra	AR	1.3	3.0	4.7
SS3	AR	4.0	2.0	2.7
TAMTBO	AR	4.0	3.3	3.3
TFCT-1	AR	4.0	2.7	2.7
TF-F-LM	AR	3.7	2.7	2.7
PSB (Pecos BLK)	AR	4.3	2.7	2.7
Pecos BLK 07-08	AR	2.0	2.0	2.7
Mean		4.2	3.2	3.6
C.V.		33.8	35.3	36.8
LSD (0.05)		1.9	1.5	1.8

Planted Oct 28, 2008.

<sup>†</sup>Ratings were taken on a 1-9 scale where 1 = best and 9 = most stunted.<sup>‡</sup>Indicates turf species where PR = perennial ryegrass; AR = annual ryegrass; TF = tall fescue; IR = intermediate ryegrass; and AIG = alkali grass.

**Table 2.** Greenhouse salinity screening experiment A and B, Overton, TX 2009.

		Date plants were rated for salt damage							
Entry		Experiment A				Experiment B			
		26 Jan		9 Feb		22 Apr		22 May	
		Act <sup>†</sup>	Cor <sup>‡</sup>	Act <sup>†</sup>	Cor <sup>‡</sup>	Act <sup>†</sup>	Cor <sup>‡</sup>	Act <sup>†</sup>	Cor <sup>‡</sup>
Phenom	PR <sup>§</sup>	4.2	3.2	7.6	4.6	5.8	1.8	7.4	2.2
Sidewinder	TF	4.8	3.8	6.6	4.6	3.6	0.6	5.8	1.8
Cochise IV	TF	3.6	1.6	6.2	4.2	3.8	0.8	5.2	1.2
TF – 152	TF	3.6	1.6	6.2	2.2	4.6	2.6	5.6	3.6
K 31	TF	4.6	2.6	7.2	4.2	4.0	3.0	5.6	3.6
Gulf	AR	4.2	3.2	8.0	5.0	4.6	1.6	8.0	4.4
TXR2004-TF-EM	IR	5.0	3.0	7.6	3.6	5.6	1.6	8.0	4.0
06 B Lp	PR	3.6	2.6	7.0	6.0	4.6	2.6	7.2	3.6
PSG-4MSH	PR	4.0	3.0	6.6	3.6	6.6	1.6	8.2	3.2
08-8 Lh	IR	6.0	5.0	9.0	6.0	5.6	2.4	7.2	2.4
IS-PR 385 BLK	PR	4.4	3.4	7.6	5.6	4.8	0.8	8.2	3.2
TMI-Puccinella dis	AIG	4.4	3.4	5.4	1.4	3.6	0.8	6.2	1.2
TXR2008-TF-PR-85	IR	5.0	4.0	8.2	5.2	5.2	1.2	8.4	4.4
TXR2008-PR-1	PR	3.6	0.8	6.8	3.2	5.2	2.2	8.4	3.4
RKS	PR	3.8	2.8	7.2	5.2	5.0	1.0	6.8	2.8
Soprano	PR	3.4	2.4	5.4	3.6	5.0	1.0	7.6	3.6
Soprano Myco	PR	3.2	2.2	6.8	4.2	4.6	0.6	8.0	4.0
TXR2006-TF-AxP	IR	4.4	3.4	7.8	4.8	5.8	1.8	8.0	3.0
TXR2008-TF-AxP F4	IR	4.6	2.6	8.0	4.0	5.8	1.8	7.8	2.8
TXR2006-TF-PR-LS	IR	4.6	3.4	7.8	4.8	6.0	2.0	7.6	3.6
Panterra	AR	4.8	3.8	8.2	5.2	6.4	2.4	7.8	1.8
SS3	AR	4.6	1.6	8.0	3.0	7.0	1.2	8.4	2.4
TAMTBO	AR	4.8	3.8	8.0	5.0	4.4	2.4	8.0	3.4
TFCT-1	AR	4.8	4.0	8.2	5.2	5.8	1.8	8.0	3.0
TF-F-LM	AR	4.2	3.2	7.8	4.8	5.0	1.0	7.8	3.8
PSB (Pecos BLK)	AR	5.2	4.2	8.2	6.2	6.2	1.2	8.0	2.0
Pecos BLK 07-08	AR	5.0	4.0	8.4	5.4	5.0	1.0	7.6	3.6
Mean		4.4	3.1	7.4	4.5	5.2	1.6	7.4	3.0
C.V.		29.8	42.8	13.6	22.8	21.8	61.5	15.4	29.5
LSD (0.05)		NS	1.4	1.1	1.1	1.2	1.0	1.2	0.9

<sup>†</sup> Actual salt damage rating on a 1 to 9 scale, where 1 = best and 9 = dead.

<sup>‡</sup> Corrected rating were actual ratings minus rating for untreated plants of that genotype.

<sup>§</sup> PR = perennial ryegrass, AR = annual, IR = intermediate, TF = tall fescue, and AIG = alkali grass.

# **Pericarp imposed seed dormancy in *Zygophyllum xanthoxylum* (Bunge) Maxim. favours its adaptation to desert environments**

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## **Abstract**

This study investigated the role of the seed pericarp in regulating germination of *Zygophyllum xanthoxylum* (Bunge) and its influence on the long term persistence of buried seeds under field conditions. Laboratory studies showed that the presence of the pericarp almost completely prevented seed germination; removal of the pericarp or flushing seeds with tap water significantly increased the level of germination. We suggest that the pericarp is responsible for imposing seed dormancy in *Z. xanthoxylum*, acting through the combined effects of mechanical resistance and chemical inhibitors. When seeds were buried in the field, pericarp, burial time and burial depth all showed significant effects on field germination and the number of decayed seeds. Seeds without a pericarp germinated across the entire burial period, but seeds with pericarp mainly germinated during the extended wet season. The presence of a pericarp significantly reduced the extent of seed decay. This implies that the pericarp has an important role in determining the time and place of seed germination and favours seedling establishment and seed persistence (longevity) under field conditions. These conclusions may have practical relevance for the use of this species in grassland rehabilitation and plant conservation programs.

## **Introduction**

The germination traits of the naturally dispersed seed unit govern the population dynamics in both natural and agricultural ecosystems (Baskin & Baskin, 1998) and are of particular importance when considering the rehabilitation of degraded and desertified lands. For *Z. xanthoxylum*, an economically and ecologically important fodder shrub in the arid environment, its dispersed seeds are enclosed by a three winged perianth which originated from extending exo- and endocarps (Beier *et al.*, 2003). Previous research related to the seed germination characteristics of *Z. xanthoxylum* has used naked seeds (Tobe *et al.*, 2001; Zeng *et al.*, 2002), and these results may lead to some false expectations about field performance.

For the present study, we investigated the effect of the pericarp of *Z. xanthoxylum* on seed germination characteristics and its implications for adaptation of the species to local environments.

## **Materials and methods**

### *Materials*

The seeds were collected from the Alxa desert in northern China (105°34'E, 39°05'N, and 1360 m.a.s.l.) in 2007. After drying at room temperature, seeds were enclosed in a plastic bag and stored at 4°C.

### *Laboratory experiments*

Four types seeds were used to test the germination as presented by Zeng et al.(2002): 1) seeds with pericarp (WP), 2) seeds without pericarp (WOP), 3) seeds with the pericarp near the radical end (RRP) removed, 4) seeds with the pericarp near the cotyledon end (opposite to radical, RCP ) removed. Germination was recorded every day in the first fourteen days and a final germination percentage was recorded at 28 days. Also, WP, WOP, RRP or RCP were flushed with tap water for 24 hours (WP+F, WOP+F, RRP+F, RCP+F, respectively), and then test germination as described above.

### *Field experiment*

Seeds with and without a pericarp were placed into permeable nylon bags (20 cm × 15 cm) and buried at two depths (surface and 5 cm) on 25 September 2007, with five recovery times (two monthly intervals beginning on 8 December 2007).

Recovered samples were pooled into a 11-cm diameter Petri dish and tested for germination as described in Section 2.2. The numbers of germinated (field germination), decayed (dead) and dormant seeds (viable, non-germinated seeds) were assessed after 28 days. Enforced dormancy was defined as seeds that subsequently germinated in the laboratory.

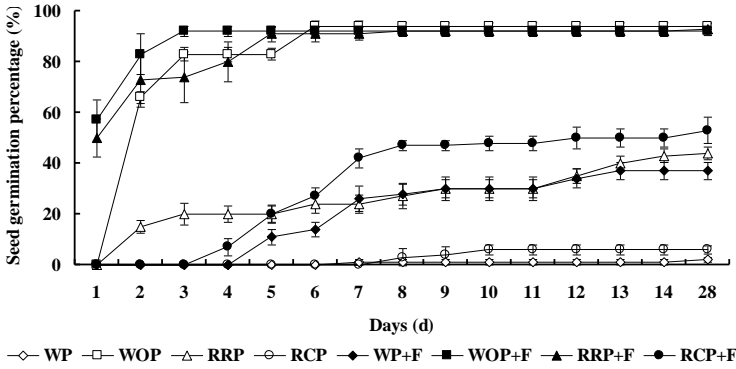
### *Statistical analysis*

Germination data were arc-sine transformed in order to meet the assumption of normality of variance. All data are presented as means  $\pm$  SE.

## **Results**

### *Effects of pericarp on seed germination in the laboratory*

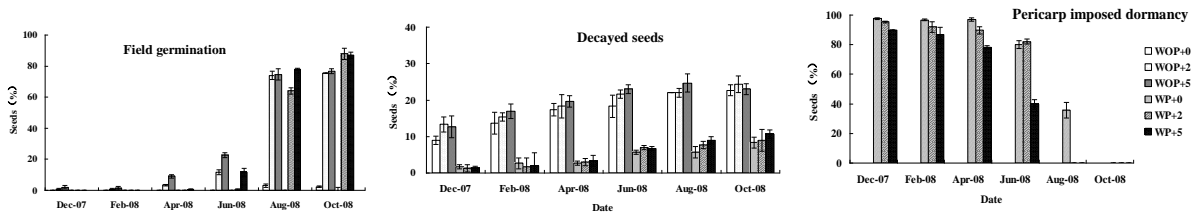
Seeds germinated significantly higher than that in the control (WP) when the pericarp was removed or partly removed or flushed with tap water except seeds with the pericarp removed near cotyledon end only (RCP). Seeds with pericarp or RCP germinated to less than 6%, seeds with pericarp near radical end removed (RRP) significantly increased germination to 44%, and completely removal of the seed pericarp germinated to 94%. Also, flush treatment increased seed germinate but response depends on the seedlot treatment, e.g. it increased RRP germination from 44% to 93%, and increased RCP or WP from 6% and 2% to 53% and 37%, respectively(Figure 1.).



**Figure 1.** Cumulative germination of *Zygophyllum xanthoxylum* seeds subjected to different physical treatments (WP, seeds with pericarp; WOP, seeds without pericarp; RRP, seeds of pericarp near radical removed; RCP, seeds of pericarp near cotyledon removed; F, flush with tap water for 24hours)

*Effect of pericarp and seed burial on seed persistence in the field*

Field germination was significantly influenced by pericarp, burial depth and burial time (Figure 2.). Seeds without pericarp germinated earlier compared to seeds with pericarp, and as burial depth increased, seed germination increased early in the season (from February to June ) but no difference was observed between 2 and 5cm burial depths late in the season (from August to October). Most buried seeds (at 2 and 5 cm) with or without a pericarp germinated during the August to October period. However, nearly all seeds exposed on the soil surface failed to germinate at any time of the year. A significantly higher proportion of decayed seed was observed in treatments without a pericarp compared to seeds with a pericarp, regardless of burial depth, and increased over time.



**Figure 2.** Status of seeds recovered over time in the burial experiment, expressed as a proportion of field germination (%), decayed seeds (%) and pericarp imposed dormancy (%). WOP+0, 2 or 5 are seeds without pericarp buried at 0, 2 or 5 cm depth; WP+0, 2 or 5 are seeds with pericarp buried at 0, 2 or 5 cm depth.

**Discussion**

This study clearly shows that pericarp is responsible for seed dormancy in *Z. xanthoxylum* because the pericarp almost completely inhibits seed germination and removal of the pericarp

increases germination to its potential percentage (Figure.1). Also the pericarp seems to not affect gas exchange because removal of the pericarp near cotyledon (RCP) did not increase seed germination significantly, but interesting, removal of the pericarp near radical (RRP) largely improves seed germination, this implies that a mechanical resistance on radical protrusion is imposed by the pericarp. Further, the pericarp mechanical resistance just partly explains pericarp imposed seed dormancy because RRP germinated to 44% only which indicate other factors are involved in the dormancy of the remaining seeds. In the present study, water flushing significantly increased germination percentage of seeds with pericarp or with a part of pericarp, and this result is consistent with previous research (Clrak, et al., 2007) and implies a germination inhibitor existed or generated by the pericarp. Based on this, we suggest that pericarp imposed dormancy in *Z. xanthoxylum* seed is a combination of mechanical resistance and chemical inhibition generated by pericarp.

Seed dormancy has an important role in determining the time and place of seed germination in the field to spread the risk of germination failure, and thus may help ensure long-term seed survival, especially for wild species growing in harsh environments (Baskin & Baskin, 1998). It is clear from the current field experiment that pericarp imposed dormancy is responsible for the lack of germination from December 2007 to June 2008. During this period seeds with pericarp do not germinate even when returned to optimum conditions for germination (Figure. 2), and this is consistent with previous research in other species (Hu *et al.*, 2009b). Seeds without a pericarp can germinate readily at any time of the season depending on environmental conditions. Hu *et al.* (2009a) also reported that seeds with a pericarp exhibited a lower germination but higher seedling establishment compared to seeds without a pericarp because most seedlings from naked seeds had a greater spread of germination time and many died from subsequent drought conditions.

Seed decay is another factor affecting seed persistence in addition to changes in dormancy. Present experiment clearly showed that the presence of a pericarp significantly reduced the number of decayed seeds regardless of burial depth and burial time. Low levels of seed decay early in the season may be a consequence of low soil moisture at the experimental site (similarly slowing the process of seed aging). When soil moisture rapidly increases late in the season, environmental conditions become optimum for seed germination and most seeds germinate rather than lose viability and decay. In addition, more than 90% of the variance in terms of seed decay could be explained by the pericarp, suggesting that the presence of the pericarp plays a deterrent role in limiting seed decay across a range of conditions.

### **Acknowledgements**

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# Light, lodging and flag leaves-what drives seed yield in ryegrass?

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## Abstract

The effect on seed yield components of reduced photosynthetic capacity of perennial ryegrass (*Lolium perenne* L.) was investigated. Firstly, seed yield responses to plant growth regulators were examined. Lodging during flowering and seed fill had a negative effect on seed yield. Days to 50% lodging is a good predictor of seed yield. Plant growth regulator responses from trinexapac-ethyl rates and timings can be explained by delays in the onset of lodging and improved photosynthetically active radiation (PAR) interception at the mid canopy level. Secondly, following flowering, photosynthetic capacity was reduced by defoliation or shading the flag leaf, stem, or head of individual ryegrass tillers. Reduced PAR to the flag leaf and stem did not affect thousand seed weight or seed yield when compared with control plants whereas reducing PAR to the head had a significant effect. The seed head itself may be a more important source of carbohydrate than the flag leaf during seed fill.

## Introduction

Early lodging in perennial ryegrass reduces seed yields (Young et al., 1996). Early lodged crops often trigger secondary vegetative tillering, which may result in nutrient competition with seed fill (Rolston et al. 2007). The plant growth regulator trinexapac-ethyl (TE) inhibits gibberellic acid biosynthesis (Rademacher 2000) and reduces lodging by irreversibly shortening internodes. Seed yield increases in perennial ryegrass (*Lolium perenne* L) from TE rates of 300 to 400 g ha<sup>-1</sup> are typically 30 to 50% and the rate response to TE is often linear to rates of 800 g ha<sup>-1</sup> (Rolston et al. 2004).

Reducing lodging may also increase the amount of photosynthetic radiation within the crop (Rolston et al. 2007). Chemical and mechanical prevention of lodging of perennial ryegrass increased the mobilisation of carbon from the flag leaf to both the reproductive head and the vegetative tillers (Hampton *et al.* 1987). Factors that reduce the amount of photosynthetically active material present on the plant such as lodging or defoliation are likely to influence the amount of carbohydrate that reaches the seed. Competition for photosynthate between plant organs of economic importance and the remaining vegetative structures, that may also support seed fill, is therefore especially important. The aim of this study was firstly to determine seed yield response to TE rate and lodging. Secondly, the importance of the flag leaf, stem, and reproductive head in contributing to seed quality was investigated by reducing photosynthetic active capacity from flowering through to harvest.

## Methods

### *Seed yield response to TE rate*

Trials were undertaken in farmers' ryegrass seed production fields in the Canterbury region of New Zealand. There were 6 trials that included four cultivars (Bealey, Commando, Extreme, Hillary, Nui) and four locations (Dunsandel, Lincoln, Methven, Wakanui). All inputs except TE were managed by the grower. Trials had four replicates and plots were 3.2m x 9 m. Each trial consisted of between three and five rates of TE (250 g L<sup>-1</sup>), with different closing dates or comparing single versus split rates of TE. Lodging was recorded weekly on a 0 (no lodging) to 100% (fully lodged) linear scale from full head emergence Zadoks GS 59 through anthesis and seed fill, and days to 50% lodging was calculated. Components of seed yield were assessed from 0.25m<sup>2</sup> quadrats cut at late seed fill.

Photosynthetically active radiation (PAR) was measured at the flag leaf level using an AccuPAR LP-80 linear PAR Ceptometer (Decagon, Washington) and the percentage of intercepted PAR relative to the upper canopy was calculated.

At harvest a 1.7 m swath was cut from all plots with a modified plot windrower, and then harvested with a plot combine. The crop was windrowed at ca. 40% seed moisture content (SMC) and combined at <14% SMC. Seed samples were machine dressed to achieve a 1<sup>st</sup> Generation seed purity standard (MAF 2008). Cleaned plot samples were weighed and converted to a yield per ha.

### *Reduced photosynthetic capacity*

Perennial ryegrass (cv. Grasslands Commando) was sown in April 2007 at 7.5 kg ha<sup>-1</sup> at the AgResearch Lincoln farm, Canterbury. Plots (9 x 4m) were replicated four times. Spring N was applied in October as urea with two equal applications giving a total of 150 kg ha<sup>-1</sup> applied N. The plots were irrigated as required to ensure plants were not under moisture stress.

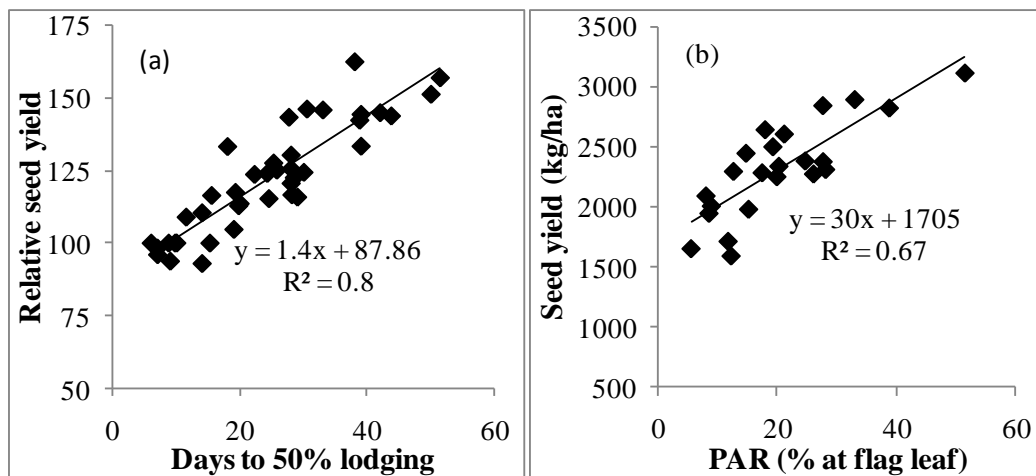
TE at 1.5 L/ha and Opus fungicide (epoxiconazole 125 g/L) at 0.2 L/ha were mixed and applied as one application on October 24. A tank mix of Amistar (azoxystrobin 250 g/L) at 0.5 L/ha and Proline (prothioconazole 480g/L) (400 mL/ha) was applied to prevent stem rust. Post anthesis, 30 tillers per replicate had the blade of the flag leaf removed at the collar (treatment 1). A control treatment containing 30 tillers with flag leaf blade attached was also tagged. Stems (20 per replicate) were enclosed in foil below the head (treatment 2) as well as the seed head of additional tillers (20 per replicate) (treatment 3). Five tillers per replicate were also prevented from lodging (treatment 4). Tillers were left to develop through to harvest.

All treatments were hand harvested at approximately 38% SMC. Tillers were air dried, threshed and cleaned to a 1<sup>st</sup> Generation Seed Certification standard. Seed yield of treatment plots was assessed from 1m<sup>2</sup> quadrats, and separately for marked tillers (g/tiller). GenStat (v 10) was used

for statistical analysis using a general ANOVA model. Samples were designated as treatments and replicates designated as blocks.

## Results

The seed yield response to 400 g ha<sup>-1</sup> TE averaged 720 kg ha<sup>-1</sup> (42%) from an average of 1720 kg ha<sup>-1</sup> in the untreated control. The seed yield response is in the range reported by Rolston et al. (2004). With no TE, seed fill occurs when the plant is in a lodged state compared to the TE 400 rate where plants can complete seed fill in either an erect or semi-erect state. Seed yield increased 22 kg ha<sup>-1</sup> (1.4%) with each days delay in days to 50% lodging, and the response was linear with an R<sup>2</sup>= 0.80 (Fig. 1a).



**Figure 1.** Relative seed yield versus days after flowering to 50% lodging (a) and Seed yield versus percentage of photosynthetically active radiation (PAR) at the flag leaf (b).

There was a strong correlation between light interception at the flag leaf and seed yield. Seed yield increased 30 kg ha<sup>-1</sup> for every 1% increase in PAR (Fig 1b). Seed yield response in these trials was the result of a higher conversion of fertile florets to saleable seed, resulting in more seeds per unit area. The TE treatment had no effect on seed head density, spikelet numbers per head or florets per spikelet.

Blocking all available PAR to the stem and flag leaf had no effect on TSW when compared to the control treatment (Table 1). No statistical difference was observed between lodged and unlodged tillers. In contrast, reducing PAR to the head during seed fill had a significant effect ( $P < 0.01$ ) on TSW (Table 1). This TSW result was reflected in seed yield per tiller, which was also decreased when heads were devoid of PAR during seed fill. A one-tailed t-test confirmed that the treatment with heads shaded was depressed compared with all others ( $P < 0.01$ ), equating to an average difference of 470 kg ha<sup>-1</sup> of saleable seed. No difference in seed yield was observed when the flag leaf was removed compared to the control treatment.

**Table 1.** Thousand seed weight (TSW) for all treatments

Treatment	TSW (g)
Stems wrapped	2.95a
Heads wrapped	2.55b
F-leaf removed	3.06a
F-leaf attached	3.10a
Unlodged tiller	3.10a
LSD (5%)	0.28

## Discussion

Results of the present study showed a strong correlation between seed yield, lodging and PAR at the mid canopy level. Increased seed yields are often correlated with an increased number of seeds per spikelet (Chynoweth *et al.* 2008). The TE treatment had no effect on seed head density, spikelet numbers per head or florets per spikelet. Early lodged crops often trigger secondary vegetative tillering, which is postulated as either resulting in nutrient competition with seed fill or reducing the level of photosynthetic radiation to the crop (Rolston *et al.* 2007). Early lodging may also reduce the amount of photosynthetic radiation within the crop.

In the present study, reducing all available PAR to the seed head had a significant effect and decreased average TSW by 16%. Similarly, in a glasshouse experiment, Warringa *et al.* (1998) reported a 10% difference in seed weight when whole tillers of perennial ryegrass were shaded by 75%. These results suggest that shading induced by lodging may have a negative effect on perennial ryegrass seed yields through reduced light interception of the seed head itself.

## Conclusion

The plant growth regulator TE increases ryegrass seed yields by delaying the onset of lodging and increasing light interception at the mid canopy. The number of days from full head emergence to when the crop is 50% lodged is highly correlated with seed yield. Seed growers are recommended to use rates of TE to achieve a crop that is  $\leq 50\%$  lodged at harvest. Furthermore, head photosynthesis contributes explicitly to seed fill. The reproductive head itself may be more important than the flag leaf in contributing to seed weight and determining tiller seed yield.

## Acknowledgement

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# Seed yield components and their potential interaction in grasses - to what extent does seed weight influence yield?

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## Abstract

In a first-year seed crop of red fescue (*Festuca rubra* L.) the degree of lodging was controlled by the use of Moddus (Trinexapac-ethyl). Seed weight was found to increase by the decreasing degree of lodging prior to harvest. The higher seed weights were accompanied by higher yields even though the number of reproductive tillers and floret site utilization (FSU) were unaffected by the treatments.

Seed yield is affected by several yield components and reflects the interaction between the seed yield potential (e.g. number of reproductive tillers, number of spikelets and florets/spikelet per reproductive tiller), the utilization of the potential (e.g. seed set, seed weight) and the realization of the seed yield potential, defined as the number of florets forming a saleable seed. The realization of the seed yield potential is affected by seed retention, seed weight and other traits associated with yield loss during the harvest and post-harvest processes.

## Introduction

The number of reproductive tillers per unit area is an important component in establishing the seed yield potential; however, its importance varies among grass species. In slow-establishing species or species with a high vernalization requirement tiller number and tiller size in autumn are important factors in maintaining a high yield potential in first-year seed crops. Tiller development influences transition rate from vegetative to reproductive growth with large tillers being more successful than smaller tillers (Boelt 1999; Meijer 1987).

The total number of spikelets and florets per inflorescence depends on the number of primary branches and on the number of florets produced per primary branch. It is believed that the number of primary branches increases with apex size (Colvill and Marshall 1984). Therefore autumn-produced tillers usually have more spikelets per tiller and in addition, they are also found to have more florets per spikelet (Ryle 1964). This is in agreement with a more recent study of Yamada et al. (2004), who found a positive correlation between plant height, tiller size, spike length and the number of spikelets per spike.

The seed set is of major importance for grass seed production. Elgersma (1991) distinguished between the *biological* floret site utilization (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 are reporting large variations in biological FSU, but on average 20-50% of the florets are biologically unproductive with losses occurring during pollination, fertilization and seed development.

Seed development depends on the position of the seed within the inflorescence. Seed weight decreased from the basal to the distal spikelets and with an even steeper gradient within the spikelet (Anslow 1964). From a glasshouse experiment with spaced plants of perennial ryegrass, Warringa et al. (1998) concluded that the amount of carbon assimilates in flowering tillers does not limit seed growth. Reducing light intensity by 75% had only minor effects on seed dry weight.

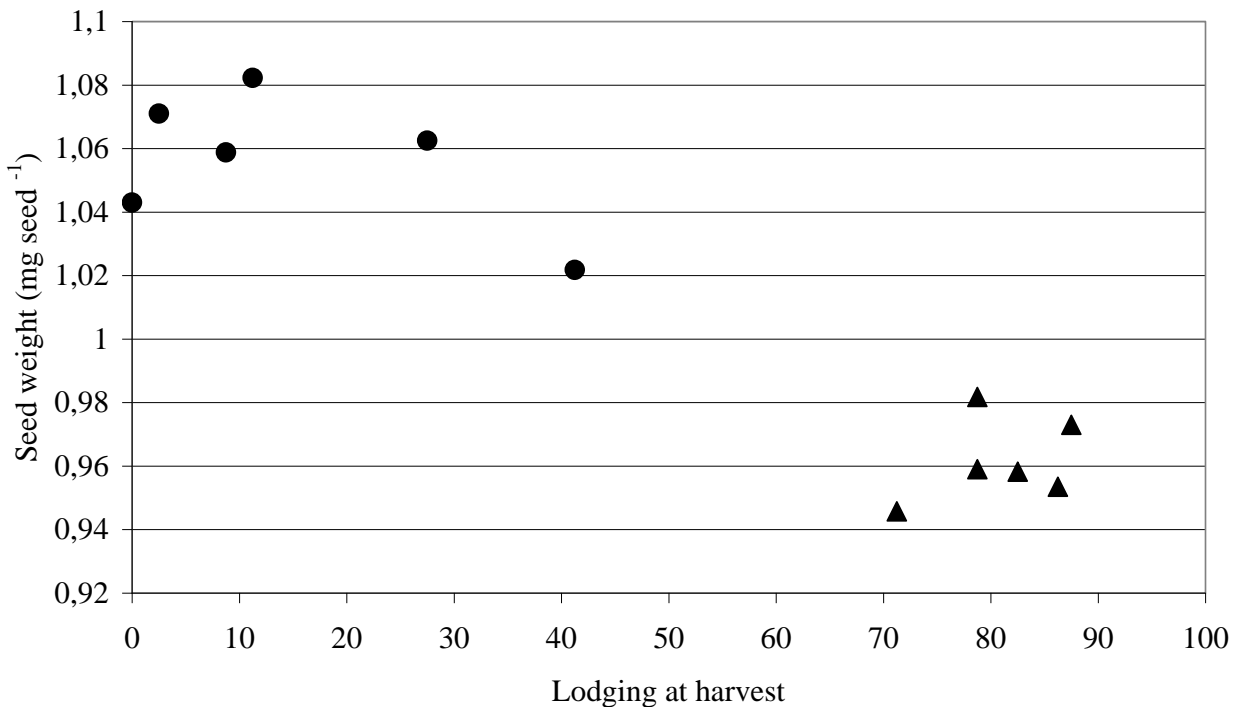
Information on how seed weight is influenced by seed crop management is very limited, and often it is confounded with variation in other seed yield components such as reproductive tiller number.

## **Materials and methods**

A field experiment was conducted in 2007 and 2008 at Aarhus University, Research Centre Flakkebjerg where the growth regulator Moddus (Trinexapac-ethyl) was evaluated in different doses in a first-year seed crop of red fescue (*Festuca rubra* L.) cv. Maxima. In the seed production year one plant sample per plot was cut within an area of 0.25 m<sup>2</sup> prior to seed harvest and the number of reproductive tillers was registered. The number of florets per spikelet at flowering and the number of seeds per spikelet prior to harvest were registered in 30 reproductive tillers in selected treatments in each of four replicates. Lodging was assessed at flowering and prior to harvest using a scale where 0 equals 'all reproductive tillers erect' and 100 equals 'all reproductive tillers completely lodged'. Before shedding, 8 m x 2.5 m area per plot was harvested directly with a trial combine harvester, and seeds were air-dried to 12% moisture before determining seed yield. After seed drying and cleaning, one sample from each replicate was analyzed for purity, and seed yield expressed as 100% clean seed was calculated. Thousand seed weight was calculated on basis of eight individual counts of 100 seeds in each plot.

## **Results**

Variation in the degree of lodging at harvest was recorded in response to growing season and the applied dose of Trinexapac-ethyl. In the harvest year 2007 lodging ranged from 71-88; whereas the responses in 2008 were 0-41. Seed weight ranged from 0,94 to 1,08 mg seed<sup>-1</sup>, the lowest seed weight obtained when lodging at harvest was registered at 71 and highest when lodging was registered at 11 (figure 1).



**Figure 1.** The effect of lodging at harvest on seed weight in red fescue 2007 (▲) and 2008 (●).

The number of reproductive tillers and FSU were not influenced by treatments and varied only slightly between years. The average number of reproductive tillers was 3830 and 3644 m<sup>-2</sup> in 2007 and 2008 respectively. FSU was high in both years with 79,3% and 78,2% in 2007 and 2008 respectively.

Seed yield was 1743 kg ha<sup>-1</sup> in 2007 in average of all treatments whereas yield was significantly higher in 2008 with 2642 kg ha<sup>-1</sup>. In both years application of Trinexapac-ethyl influenced seed yield positively.

### Discussion

It is generally recognized that variance in seed yield are best explained by the number of seed per unit area (Hampton & Hebblethwaite, 1983). Often yield components are negatively correlated and a high number of reproductive tillers leads to less and/or smaller seeds on each individual tiller. This assumption is however often based on limited registrations of weight and number of seeds per reproductive tiller or based on glasshouse experiments. Our experiment shows that seed weight is increased by 14,9% when lodging at harvest is reduced from 71 to 11 in red fescue without having any concomitant effect on other yield components such as reproductive tiller number density and FSU. The increase in seed weight was followed by a higher seed yield.

In grass species with poor seed retention, major losses may occur if the crop does not lodge at maturity to an extent where it prevents seed shattering from wind or rain. Further seed loss in the harvesting process may be substantial and in some cases, the harvested seed may even fail to meet certification standards in terms of physical purity and germination ability. The variation of seed size and maturity level both within and among the inflorescences is considered a key factor determining seed loss before and during harvest as well as in the post-harvest management processes of drying and cleaning. During seed cleaning, the saleable seed is separated from impurities, weed seeds, empty seeds etc. on the basis of seed size, shape and gravity. Variation in seed size leads to losses in the separation process where physical impurities and weed seeds are separated from the saleable seed. Therefore a seed crop more uniform in seed maturity level and seed weight will realize a higher proportion of the potential seed yield and in addition it often has a higher seed quality.

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## **Stem rust in perennial ryegrass seed crops: epidemiological and genetic research at USDA-ARS**

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### **Abstract**

In production of perennial ryegrass seed in the Northwest USA, stem rust is the primary disease constraint in terms of management cost and potential yield loss. The USDA-ARS research program on this problem is focused on improved decision making for fungicide use, and tools for genetic improvement. Data from 10 years of field and greenhouse research was used to construct an epidemiological model. This model is based on the effects of weather (temperature, leaf wetness) on plant growth and pathogen activity, and includes the effects of two major fungicide classes (triazoles and strobilurins) on disease development, particularly on the spread of disease within a plant. The model is implemented as a decision aid on a publicly-accessible internet site. Users enter scouting data and select a location for weather data to be loaded into the model. The website displays the model results, including the relationship of modeled disease development to an action threshold. The decision aid has performed well in large-scale field demonstration plots across 10 location-years, providing an average economic advantage of \$39 per acre (range: \$0 to \$140) compared to standard fungicide programs. In genetic research, a perennial ryegrass mapping population was constructed from a cross between resistant and susceptible parents. The progeny (193 individuals) were tested by inoculating with two different pathotypes of the stem rust fungus, in separate experiments. Using a genetic map of these parents constructed with SSR and RAD markers, QTL analyses were conducted. The goal of this genetic research is to produce genetic markers for marker-assisted selection of stem rust resistance, as well as to provide information about genomic organization of disease resistance in perennial ryegrass.

### **Stem rust on perennial ryegrass and tall fescue in the Pacific Northwest USA**

In perennial ryegrass (*Lolium perenne*) and tall fescue grown for seed, the economically most important disease in major seed production areas of the United States is stem rust caused by *Puccinia graminis* subsp. *graminicola* (Welty & Azevedo, 1984). Perennial ryegrass seed yield losses up to 98% due to stem rust damage have been recorded (Pfender, 2009a). The disease is also a significant production constraint in New Zealand, where yield losses of 35% were documented (Hampton, 1986). Since the 1980s, there has been a research program at the USDA-ARS Forage Seed Research Laboratory in Corvallis, OR, USA. Starting in the late 1990s the research focused on developing an epidemiological model that could be used in a decision

support system to optimize fungicide use in disease management. Within the last few years another component, stem rust resistance genetics in *Lolium*, has been added to the effort.

### **Epidemiological research and fungicide decision support system**

The goal of the epidemiological work at USDA-ARS has been to quantify the effects of environmental factors on disease development, and to summarize that knowledge in the form of a mathematical model. The model is intended to depict the linked disease cycle components, and is driven by weather data that can be collected with automated weather stations in the field.

#### *Stem rust epidemic model*

We determined that the probability of infection, given the presence of stem rust inoculum, can be calculated from temperature and leaf wetness occurring during nighttime hours and in the few hours after sunrise. If the leaves are wet and temperature is greater than 2C, the fungus spores can germinate and grow. At cold temperatures, a long period of wetness is required for adequate growth to cause infection, but the required wetness duration is shorter at warmer temperatures. The temperature X wetness duration must be adequate during both the dark period (for spore germination) and the early morning period (for penetration into the plant). We derived an equation for calculating infection probability from overnight and morning weather data recorded in the field (Pfender, 2003). After the fungus has successfully penetrated the plant, some time is required for the infection to develop sufficiently to produce the next generation of spores. The duration of this time period between infection and production of new spores (the latent period) is determined primarily by temperature. The latent period duration can be calculated by accumulation of heat units (e.g. degree days) (Pfender, 2001). The processes of infection and the latent period form the core of the stem rust epidemic model.

An additional important aspect of stem rust development is the ability of the fungus to spread from infections on the sheath to the stem or flower that is extending from within the infected sheath (Pfender, 2004). This disease spread occurs because the pustule on the sheath is producing spores on the inner sheath surface as well as the outer surface. The result is that a single infection on the flag sheath, for example, can produce hundreds of infections on the inflorescence head and stem. Our observations indicate that up to 80% of the infections that appear on ryegrass stem sections at the height of an epidemic are the result of this process. The amount of disease due to this within-plant component is dependent not only on the frequency of lesions on sheaths, but also on the rate of extension of the internodes. Therefore the epidemic model includes a component for stem elongation (Pfender, 2006), which is driven by heat units. The full epidemic model includes infection probability, latent period and within-plant spread. The model works on a daily time step, and the amount of inoculum at the beginning of each day is proportional to the amount of disease (sporulating pustules) at the end of the previous day.

Although within-plant spread seems to be a general feature of stem rust in perennial ryegrass, we have noted that varieties differ in the prominence of the sheath lesions that act as sources for the

process. In the turf varieties we have worked with the sheath pustules are usually prominent, and it is easy to see the relationship between the sheath pustule and the resultant stem lesions. However in some forage varieties such as Linn, pustules may be relatively inconspicuous on the outside surface of the sheath although they are sporulating on the inner surface. In these cases it is only with some difficulty that one can trace the origin of extensive stem lesions (including those underneath a sheath) to the source lesion on the sheath. This difference among varieties has important implications for disease scouting.

The effectiveness of fungicides is an important factor to include for the epidemic model to be used as a decision support for disease management. We measured protective and post-infection ("kick-back") activity of azoxystrobin and propiconazole fungicides acting against infections on treated plant surfaces, and derived equations for efficacy as a function of time between infection and fungicide application (Pfender, 2006). We also noted that the strobilurin suppressed the within-plant spread process of stem rust by preventing sporulation from the inner surface of sheath lesions, whereas the propiconazole had very little such activity. Therefore, if a fungicide is applied when sheath lesions are beginning to shed spores onto the enclosed stem internodes, very different fungicide efficacies will be apparent for the two fungicide types starting about a latent period later. The stem rust model accounts for the differing activities of fungicides as affected by the timing of tiller extension (Pfender, 2009c).

#### *Stem rust decision aid*

For use as a decision aid, an action threshold is required. The threshold should be based on the relationship between disease severity and yield loss. We determined that the best predictor of yield loss due to stem rust is the severity of disease during the 2 to 3 weeks centered on anthesis. A damage function was derived that estimates yield as a function of healthy plant area duration (total plant area minus diseased plant area) (Waggoner, & Berger, 1987) during this time window (Pfender, 2009a), and the action threshold for the decision aid is set such that significant yield loss is avoided.

The decision aid is currently implemented on a publicly available internet web site. Users of the site choose a geographical source for weather data input, and enter information for plant growth stage, their most recent disease scouting results and any fungicide applications. A simulation is run with this information and results are displayed as a graph of outputs running from March 1 to the current day. The graph shows daily infection probabilities and two categories of simulated disease severities. One line is plotted for visible disease, and another for the total number of infections, including latent infections that have not yet become visible. The threshold criterion is compared with the total infection level (visible plus latent). The graphical output also shows the timing and estimated effects of fungicide applications.

An important aspect of information delivery for the decision support system is the use of representative weather data for the user's location. Currently we are using a limited number of

weather stations in grass seed fields; the stations have communications gear and automatically transmit newly-acquired data to our laboratory computer every morning for incorporation into the season-long weather database for that location. To obtain better geographical coverage, there is an ongoing multidisciplinary, multi-state effort to produce relatively high-resolution (~1 km grid) weather estimation for parts of the western U.S. The weather estimates are produced from the 60-km gridded US National Weather Forecast, processed through climatological and physical process models. Our goal is to use this 1-km weather data, including 7-day forecast, as the weather data source for the stem rust decision support system.

Rigorous validation of the simulation model is in progress, but has not yet been completed. However, the model has been tested in large-scale replicated plots in grower fields over the last 5 years, 2 locations per year (Pfender et al., 2009). In these 10 location-years use of the decision support produced economic results (revenue from seed yield minus fungicide costs) at least as good as current standard practice. In three location-years there was no significant difference between results of standard practice and decision-supported practice, and in five location-years there was a saving on fungicide costs with no yield penalty. In two cases there were yield increases, one of which was achieved with fewer fungicide applications (but different timing) than the standard treatment.

### **Genetics of stem rust resistance in *Lolium***

In field and greenhouse trials we determined that the cultivar 'Kingston' (PGG Wrightson Seeds, New Zealand) typically has a lower level of stem rust than other varieties we tested. To gain some insight into genetics of stem rust resistance, we created a mapping population by crossing two plants (resistant and susceptible) that we selected from 'Kingston' after repeated stem rust testing under controlled conditions. A population of 193 F1s from this cross was mapped.

#### *Mapping*

Linkage maps for the male and female parents of the mapping population were constructed with SSR (simple sequence repeat) and RAD (restriction-site associated DNA) (Baird et al., 2008) markers. Tall fescue SSR markers, previously developed (Saha et al., 2006) by researchers at the Samuel Roberts Noble Foundation (Ardmore, OK, USA) were screened against parental DNA and genotyped on the progeny by M. Saha. Additional SSR markers, also run at the Noble Foundation, were originally developed for *Lolium* by other research groups (Gill et al., 2006). Population-specific RAD markers were developed from parental DNA and genotyped on the progeny by Floragenex (Eugene, OR, USA). For RAD library preparation, *Lolium* genomic DNA was digested with SbfI and fragments were ligated to P1, a modified Solexa adapter (Illumina, Inc.). After PCR amplification, libraries were run on an Illumina Genome Analyzer II located at the University of Oregon. Raw Solexa data were processed using Floragenex custom programs. In the RAD marker development phase, sequence data from each parent were grouped into highly similar sequences with no more than a two-bp mismatch allowed. Sequence

groupings from the parents were then compared to identify alleles from SbfI-tagged loci, expected to segregate in three configurations in an F1 population: testcross (heterozygous SNP in one parent, homozygous in the other), cut-site testcross (heterozygous SNP in one parent, absence of sequence in the other presumably caused by a nucleotide polymorphism in the SbfI site), and intercross (heterozygous SNP in both parents). F1 genotypes were scored based on comparison of F1 sequences with the parental marker panel.

Maps were assembled for each parent from the F1 segregation of co-dominant and male- and female-specific SSR and RAD alleles, using JoinMap 4 software and CP population type codes (Kyazma, Wageningen, Netherlands). We used the test for independence LOD score, which is not affected by segregation distortion, to group markers into seven linkage groups for each map (significance level of 6.0 LOD).

### *Phenotyping*

Disease phenotypes were determined in inoculation assays conducted in a greenhouse, with bulk inoculum (field-collected, genetically mixed) or single-pustule isolates (genetically uniform). We had previously demonstrated pathotype specificity in stem rust of *Lolium perenne*, by purifying and increasing two different, single-pustule isolates of the pathogen (Pfender, 2009b). Isolate 101 is avirulent on one of the mapping population parents, and resistance is inherited as a single dominant gene that is heterozygous in the resistant parent. Isolate 106 is virulent to some degree on both parents. Phenotypes were scored as number of pustules per plant. There were three replicate (cloned) plants per F1 individual in each experiment, and each experiment was conducted at two different times. QTL analysis was conducted in MapQTL5 for the male and female parent maps. Kruskal-Wallis analysis and automatic cofactor selection were used to choose cofactors for use in MQM analysis.

### *QTL Results*

There is a distinct location for resistance to pathotype 101, on linkage group 7. The peak, with LOD scores of 10 to 28, is located at about 40cM on both male and female maps. The same peak can be seen in the QTL analysis of the bulk-inoculum experiments. QTL analyses of data from phenotypes produced by pathotype 106 lack the peak at 40cM on LG 7, but have a significant peak on LG 1 on the male and female maps. A peak at the same LG1 location is seen in all QTL results for the bulk-inoculum phenotypes as well, although the peak appears at a slightly displaced position on the male map for one of these experiments. Whereas the resistance to isolate 101 (on LG7) has a pathotype-specific qualitative resistance phenotype, the resistance to isolate 106 is of a quantitative nature. Other possible QTL locations are: LG2 where there are distinct peaks (but less than genome-wide significance level for LOD score) for several experiments on the female map, and LG6 where consistent, but similarly non-significant, peaks occur in the male and female maps for several experiments.

These findings are the first report of linkage-group locations for qualitative and quantitative stem

rust resistance loci in *Lolium perenne*. The use of RAD markers, which have defined sequences, will make it possible to make probes for the markers that are associated with these QTL peaks.

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# Seed yield variation in a red clover breeding population

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## Abstract

Variation in flower number and seed yield of 330 individuals from a breeding pool were assessed for seed yield. The average per plant seed yield was 32 g plant<sup>-1</sup>. Per plant seed yield ranged from <10 g plant<sup>-1</sup> (7% of plants) to >60 g plant<sup>-1</sup> (4% of plants). Seed yield was independent of time of peak flowering. There was a positive correlation between seed yield and flower number; but flower number only explained 18% of the variation in seed yield. A sub-sample measured for corolla tube length showed no correlation between length and seed yield.

## Introduction

New Zealand's herbage seed producers are discouraged from growing red clover because of low returns (associated with low seed yield), limiting local market supply and increasing price kg<sup>-1</sup> to end users. End users are therefore discouraged from sowing red clover because of the increase in seed costs and decrease demand. This has stalemated the industry and New Zealand has produced about 150 T seed year<sup>-1</sup> for the last 5 years (Anonymous, 2009). New cultivars, like 'Sensation', have only replaced older technology rather than increased the market. A significant increase in red clover seed yield ha<sup>-1</sup> would increase supply and bring cost to the end user down while still ensuring a suitable return to the producer. Recent work confirming the heritability of seed yield components, including inflorescences per plant, has increased the interest in seed yield specific screening (Herrmann *et al.*, 2006).

This paper reports the results from a red clover breeding programme that, for the first time, looked at both seed yield and agronomic qualities as selection criteria.

## Materials and Methods

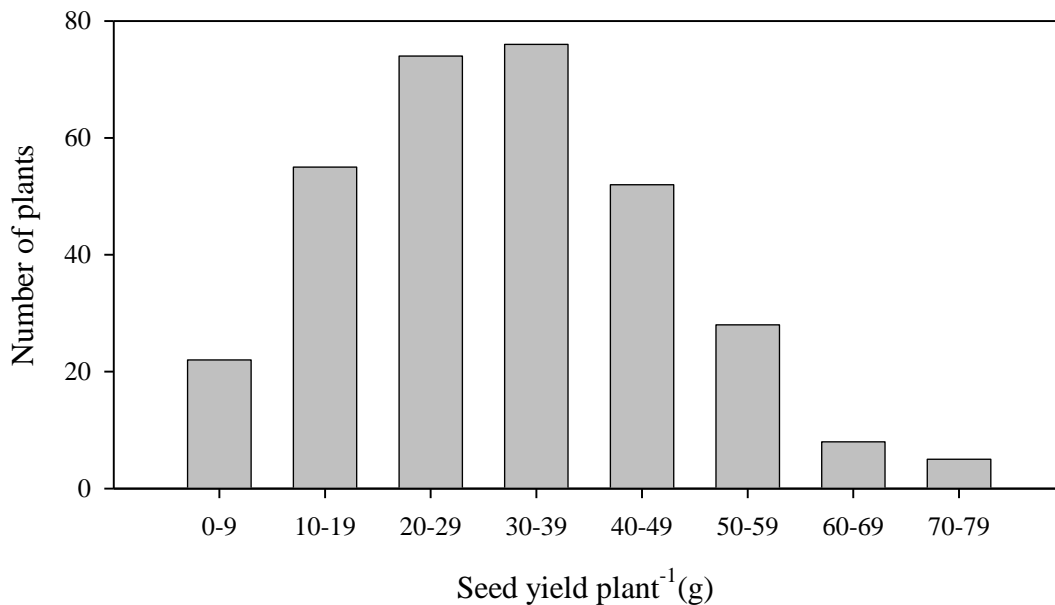
The trial was undertaken at Lincoln, Canterbury, New Zealand (43° 63'S, 172° 47'E, 11 m a.m.s.l.). On 15 April 2008 a diploid red clover breeding pool was sown in root trainers and in a glasshouse on 1 July 2008 seedlings were transferred in trays to a shade house to harden off. On 2 October 2008, 330 seedlings were transplanted in to the field (a deep Wakanui silt loam with good moisture holding capacity) at the AgResearch Lincoln Farm as single, spaced plants on 850 mm squares.

Natural populations of long-tongued bumblebees (*Bombus hortorum*) and introduced short-tongued bumblebees (*B. terrestris*) were present during flowering (late November – late

February). On 20 January 2009 a subsample of 29 plants were chosen at random and three newly opened inflorescences per plant were removed. Five florets from the bottom-most 2 rows were then measured from the tip of the standard petal to the base of the sepals. On 18 March 2009 plants were harvested by hand following an application of diquat dibromide desiccant. Harvested material was dried in a glasshouse and seed was extracted using a belt thresher and screen dresser.

## Results

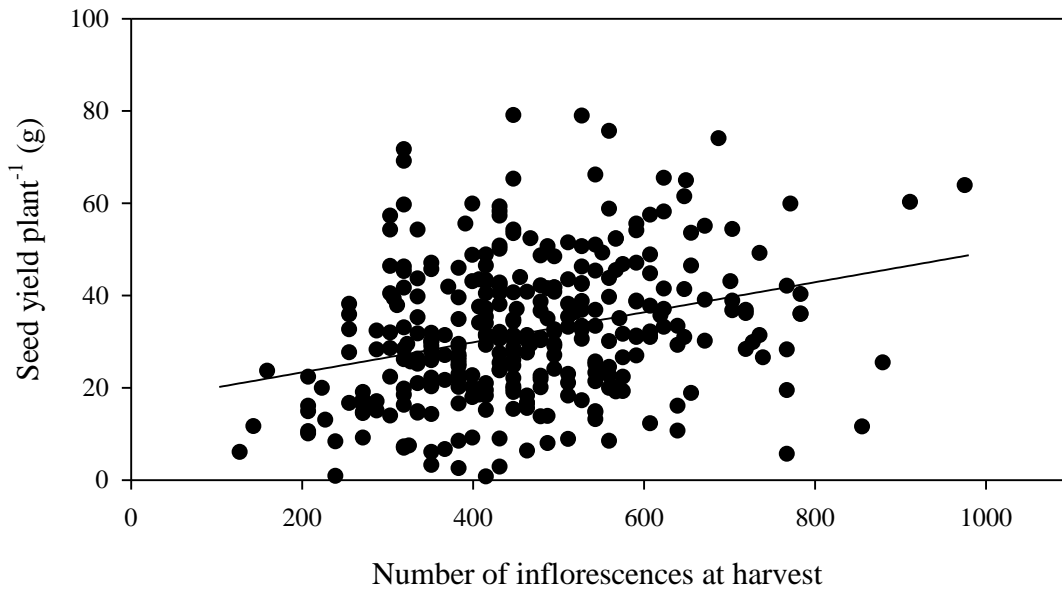
The average per plant seed yield was 32 g. Seed yield ranged from 1 g to 79 g with 22 plants having <9 g (7%) and 5 plants >70g (4%) and 68% of plants within 1 standard deviation (15.3 g) of the mean (Figure 1).



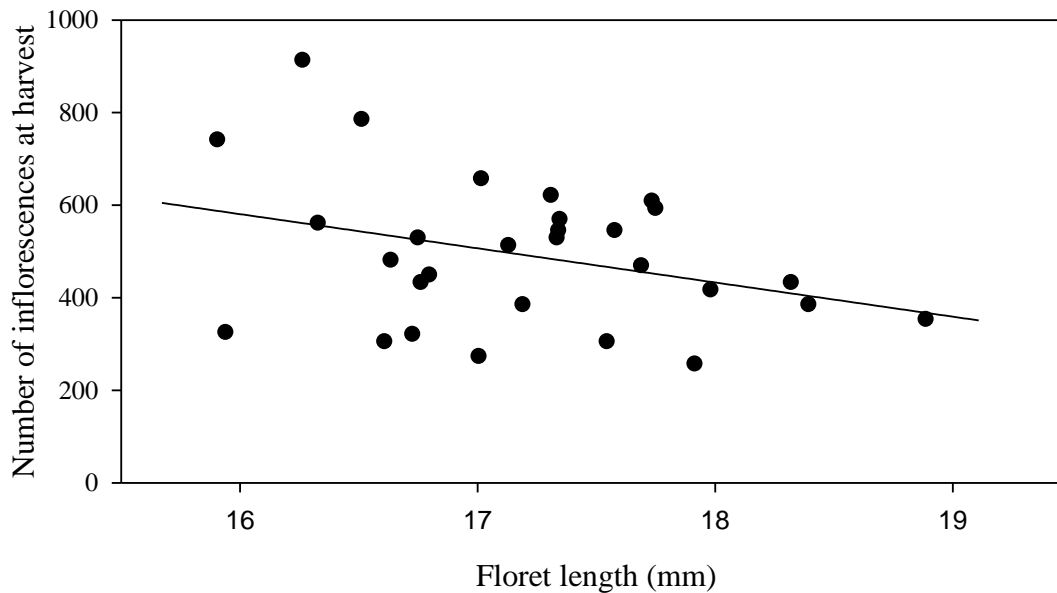
**Figure 1** The number of plants within different seed yield bands

The average number of inflorescences at harvest was 465 per plant (Figure 2). Average number of inflorescences at harvest ranged from 128 to 976 with 67% within 1 standard deviation (215) of the mean. The seed yield per plant had a minor positive correlation ( $R^2 = 0.09$ ) with number of inflorescences at harvest. There was a range of 32 days in the date of peak flowering. Seed yield was independent ( $R^2 = 0.002$ ) of the time of peak flowering (maximum number of inflorescences with >50% of florets available for pollination) (Figure 3). All plants had reached peak flowering by 3 February 2009.

The average floret length on 20 January 2009 had a negative correlation to the number of inflorescences at harvest ( $R^2 = 0.11$ ) (Figure 3). There was no correlation between average floret length and yield plant<sup>-1</sup> ( $R^2 = 0.01$ ).



**Figure 2** The seed yield per plant against the number of inflorescences at harvest



**Figure 3** The number of inflorescences plant<sup>-1</sup> at harvest against the average length of 15 newly opened florets on 20 January 2009 for a subsample of 29 plants.

### Discussion

A large difference in genetic potential for seed yield was identified (Figure 1 and Figure 2). Both seed yield and total inflorescence number varied in a breeding line nearing cultivar release status.

There may be potential to improve seed yield through selection of material from the positively skewed tail of the production curve. However, only 13 plants produced >60 g seed plant<sup>-1</sup> and screening for seed yield earlier in the breeding programme or screening larger numbers in a family structured population may result in greater advancement.

The best predictor of seed yield was the number of inflorescences (Figure 2), in agreement with past literature (Oliva *et al.*, 1994; Herrmann *et al.*, 2006). However, the length of florets and the date of peak flowering were not related to seed yield. The interaction between inflorescence number (Figure 3), pollinator type, pollination method (proper vs. side cut) and seed yield is not fully understood (Clifford and Scott, 1989). In both open and caged situations, the number of flowers successfully pollinated is variable (Rao *et al.*, 2007). Variation in pollinator visitation in red clover may result for multiple reasons including different levels of nectar, or colour, size and shape of the inflorescence and environmental and geographical conditions (Shuel, 1952; Brian, 1954; Marden, 1984). Further work with plant and pollinator interactions will lead to better management and increased red clover seed production.

Another consideration in selecting red clover plants for reproductive and agronomic performance from the spaced plant system is plant density and subsequent growth habit compared with production systems. In New Zealand, red clover seed producers typically sow diploid cultivars at 5-6 kg ha<sup>-1</sup> in 30 cm rows and plants stand erect, reaching 50 cm height with ease. Sown on 850 mm squares, New Zealand's grazing-type material typically lie prostrate or only semi-erect. The influence of this on successfully selecting material for pastoral applications is currently under investigation.

## Conclusions

The experiment identified large differences in seed yield within a breeding pool of red clover and suggests that breeders could improve the seed yield potential of new cultivars by including seed yield as a breeding objective. There is also a need to better understand the interaction between plant spacing, selection and performance in a pastoral situation.

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# History of crimson clover in the USA

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## Abstract

Crimson clover (*Trifolium incarnatum* L.) is widely planted in the US southern region as a winter pasture legume and is an important seed crop in Oregon. The history of use and improvement of crimson clover in the US stretches back to the mid 1800's with several changes in management and breeding objectives. Crimson clover was first used in the US as a non-reseeding pasture legume and as a green manure crop. The development of reseeding strains and more recently, improved cultivars with high hard seed levels was critical to modern crimson clover breeding programs and lead to increased use of this annual clover in US agriculture.

## Introduction

Crimson clover is the most important annual clover to US agriculture, with primary use as a winter annual forage legume overseeded on warm-season perennial grass pastures in the southeast US. Crimson clover is an important component of forage production systems from Virginia to east Texas and the beautiful crimson flowers enhance the landscapes of both pastures and roadsides in this region. Crimson clover USA seed production (all in Oregon) averaged 1769 Mt/year for 2006 through 2008 (Young, 2010) with a conservative estimated seed sales value of \$4.0 million per year. Crimson clover is native to southern Europe and has been grown in the USA for more than 150 years but with increasing use in the last 60 years (Piper, 1935). As introduced from Europe, this forage legume did not have high hard seed levels and was not reliable in producing volunteer (reseeding) stands in the humid subtropical climate of the eastern and southeast USA (Hollowell, 1947).

## Early use

Duggar (1898) noted the potential of crimson clover as a green manure crop for use in cotton cropping systems in Alabama. Interest in the use of crimson clover as a grazing crop in the southeast increased in the 1940's as reseeding strains became available (Donnelly and Cope, 1961). Crimson clover was grown as a seed crop and as a combination grazing and seed crop in the southeast US for 40 years beginning in the 1930's. Crimson clover seed yields in Alabama ranged from 110 to 900 kg ha<sup>-1</sup>, depending on soil type, plant nutrition and insecticide treatments (Donnelly and Cope, 1961). Insecticides were necessary to control clover head weevil (*Hypera meles* F.).

Crimson clover seed production in the southeast US declined rapidly in the 1960's and early 1970's (personal communication, Dr. Jim Bostick, Alabama Crop Improvement Association). Some possible reasons for this decline are: loss of seed harvest and processing infrastructure; shift from clover and grass pastures to nitrogen fertilized grass pastures; inconsistent seed yields because of unpredictable rainfall during seed harvest; and clover head weevil damage to the crimson clover seed crop.

### **Crimson clover in Texas**

The exact beginning of crimson clover use in Texas is difficult to discern but for Rusk County, Texas the first crimson clover planting is well documented. Reseeding crimson clover was introduced to east Texas in 1949 from Alabama (Anon., 1951) and interest in this new clover increased rapidly as results from the first plantings were noted. The attributes that made crimson clover a success at this time in Texas were: three months of late winter and spring grazing; reseeding stands; and cash income from seed crops. Texas Governor Allan Shivers proclaimed the week of April 30, 1951 as Crimson Clover Week. Dr. Bruce McMillan, a prominent Rusk County agricultural leader, was instrumental in the introduction of this new clover from Alabama.

### **Crimson clover improvement**

“Dixie” crimson clover was developed in Georgia in the early 1950's in response to the need for a cultivar with improved reseeding traits that could also be produced as certified seed. Dixie is a composite of three crimson clover farm strains that exhibited excellent field reseeding, high forage yields and high hard seed test results in laboratory evaluations (Hollowell, 1953). As recent as 1959, common crimson clover had less than 5% hard seed at harvest (Bennett, 1959), but improvement in the hard seed level through recurrent selection could be demonstrated. “Chief” crimson clover was developed in Mississippi (Hollowell, 1960) through nine cycles of recurrent selection for hard seed with the final generation stabilized at 65% hard seed (as measured at harvest with hand-cleaned seed).

The hard seed trait in Dixie crimson clover was shown to be very stable over years and environments in a nine year study conducted in Alabama, Mississippi and Georgia in the 1950's (Knight et al., 1963). The hard seed level of Dixie was consistently 60 to 80% at harvest with little effect from seed production location or year. In a three year experiment in Texas beginning in 1994, Dixie crimson clover averaged 33% hard seed at harvest (Evers and Smith, 2006) but did produce acceptable reseeding stands ( $>100$  seedlings  $m^{-2}$ ) in each year if grazing was terminated by mid-April. This indicates a reduction in hard seed level for this cultivar (reduced from levels reported in 1963) and may explain the variability in crimson clover reseeding in years with sporadic fall rains.

Both “Flame” and “AU Robin” are crimson clover cultivars selected for early maturity out of Dixie (Baltensperger, et al., 1989; van Santen, et al., 1992). Parental lines of AU Robin were

selected based on bloom date, dry matter yield and nitrogen yield. Flame was selected from a population of Dixie that had reseeded for seven years in warm-season perennial grass sod under winter grazing and summer hay management. Additional crimson clover cultivars are described in Table 1.

Table 1. US cultivars of crimson clover.

Cultivar	Year of Release	Organization	Eligible for Certification in Oregon
Dixie	1953	Georgia AES	X
Chief	1960	Mississippi AES; ARS	X
Auburn	1961	Alabama AES	
Frontier	1963	Mississippi AES; ARS	X
Tibbee	1972	Mississippi AES; ARS	X
Flame	1989	Florida AES	X
AU Robin	1992	Alabama AES	X
AU Sunrise	2000	Alabama AES	X

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# Chemical diversity of bioprotective alkaloids of endophytic fungi in cool season grasses

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## Abstract

Epichloë endophytes (Epichloë and Neotyphodium species) are important fungal symbionts that form mutualistic symbioses with cool season grasses. The asexual *Neotyphodium* species have been utilized in agriculture to enhance the persistence of grasses such as tall fescue and perennial ryegrass. These fungi are known to produce a range of bioprotective alkaloids, peramine, lolines, ergot alkaloids and lolitrems (indole-diterpenes) that provide anti-insect and anti-mammalian properties that protect their host. Of most interest in the agricultural setting are endophyte/grass associations that produce only the beneficial anti-insect alkaloids. Methodologies are well established to detect and quantify the presence of these alkaloids. More recently the genes required for the biosynthesis of the four bioprotective alkaloids have been cloned and characterized from a number of endophytes present in agriculturally important grasses. As found with other fungal secondary metabolite biosynthesis genes, the genes for three of the four alkaloids (ergot alkaloids, indole-diterpenes and lolines) are present in co-regulated gene clusters. This genomic organization enabled the identification of sets of genes that are likely to be involved in the biosynthesis of each alkaloid. The cloning and characterization of these alkaloid biosynthesis genes now provides an opportunity to understand the biochemical pathways by means of targeted mutagenesis and determine metabolite diversity across naturally occurring endophytes based on the presence and absence of genes.

## Introduction

Endophytes (microorganisms that colonize plants) are found everywhere in nature, but many of these associations are not fully understood (Rodriguez & Redman, 2008). However, clavicipitaceous fungi are known to inhabit cool season grasses and these fungal endophytes have been well studied for a number of decades in agriculturally important forages (Bacon et al., 1977; Sampson, 1933). The Epichloae broadly consists of the sexual *Epichloë* and asexual *Neotyphodium* species that systemically infect the aerial parts of the grass plant. They are known to aid with the general persistence of the plant and have been deemed akin to a “supergene” because of the growth advantage they provide their host.

In the 1930s a tall fescue cultivar “Kentucky 31” had shown great promise with persistence especially during times of drought (reviewed in Bacon, 1995). What was not understood at the time was that a systemic infection of the fungus *Neotyphodium coenophialum* provided the grass with persistence (Bacon et al., 1977). Unfortunately, this same fungus also gave Kentucky 31 a bad name due to the poor animal performance and reproductive problems that it caused grazing livestock (reviewed in Bacon, 1995). Kentucky 31 had an even greater effect on horses in Kentucky – a state that prides itself on an extensive thoroughbred industry – triggering a great

deal of investigation into the causes of the disease symptoms. Scientists eventually established that not only was tall fescue infected with an endophyte but that the fungus could produce an ergot alkaloid now known as ergovaline and this alkaloid was responsible for the effects on cattle such as fescue foot and fescue toxicosis (Bacon et al., 1977; Porter et al., 1981). A similar endophyte, *Neotyphodium lolii*, was found in perennial ryegrass (Fletcher & Harvey, 1981) and this fungus is known to cause ryegrass staggers due to the production of the indole-diterpene lolitrem B (Gallagher et al., 1984).

Phylogenetic analysis using the genomic sequences of *tefA* and *tubB* from *N. coenophialum* indicated it was an interspecific hybrid that consisted of the progenitor species *Epichloë festucae*, *E. typhina* and the *Lolium*-associated endophyte closely related to *E. baconii* (Tsai et al., 1994; Moon et al., 2004). The endophyte from perennial ryegrass is less complex and appears to be an asexual *E. festucae* (Schardl et al., 1994). Subsequently it has been shown that many asexual epichloë endophytes are interspecific hybrids and many of these have an *E. festucae* progenitor (Craven et al., 2001a; Craven et al., 2001b; Iannone et al., 2009; Moon et al., 2004; Moon et al., 2007).

### **Alkaloid Production by Endophytes**

One well documented aspect of the cool season grass endophytes is the ability to produce secondary metabolites (also known as natural products) that provide anti-insect and anti-mammalian properties (Popay & Bonos, 2005; Siegel et al., 1990). The epichloae are able to produce four classes of alkaloids, ergot alkaloids, lolines (pyrrolizidines), peramine (pyrrolopyrazine) and lolitrems (indole-diterpenes) with known biological activities. The production of lolines and peramine are considered positive traits because of their anti-insect properties while the ergot alkaloids and lolitrems are best known for anti-mammalian activities that have negative effects on livestock. Prior to the cloning and characterization of the genes required for the biosynthesis of these compounds much work was done on establishing methodologies for detecting and quantifying the alkaloids using techniques such as thin layer chromatography (TLC) (Fannin et al., 1990), high performance liquid chromatography (HPLC) (Lodge-Ivey et al., 2006; Rottinghaus et al., 1991; Spiering et al., 2002b) and enzyme-linked immunosorbent assay (ELISA) (Hill & Agee, 1994). These techniques were also useful for identifying isolates that only provided beneficial alkaloids to the association (Latch et al., 2000a; Latch et al., 2000b). Identification of proposed intermediates identified within the plant extracts of endophyte infected material also provided insight into the predicted biosynthetic pathways for some of the alkaloids (Gatenby et al., 1999; Munday-Finch et al., 1996; Munday-Finch et al., 1997).

The asexual life cycle of the *Neotyphodium* species contain the endophyte within the plant. During the inflorescence development the fungus is able to colonize the developing seed and embryo (Sampson, 1933). Fungal/grass associations can be manipulated in order to replace a native, common toxic endophyte with a strain that might cause less mammalian toxicity while still retaining the positive benefits. The lifestyle of the asexual endophytes that are utilized in forage grasses allow for replacing these endophytes from the “hot” mammalian toxic variety to one known to be mammalian friendly such as MaxQ (Bouton et al., 2002) and AR1 (Fletcher, 1999). The naturally infecting fungus can be selectively killed off using heat and humidity to

produce a seed that will germinate and become an endophyte free seedling. This seedling can then be artificially inoculated with a different, more mammalian friendly, strain (Latch & Christensen, 1985).

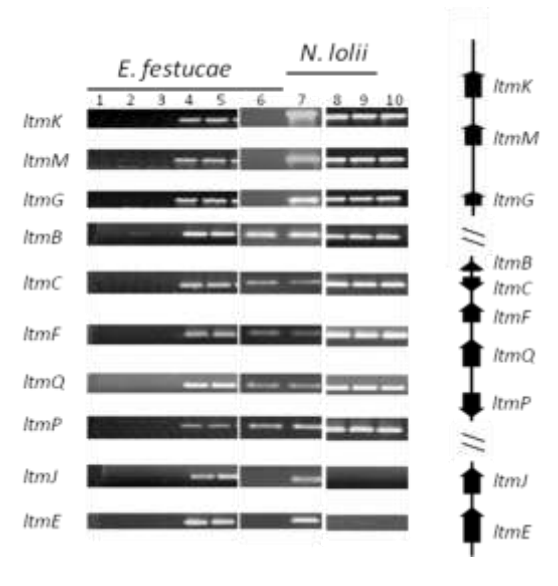
In more recent years, with the advancement of molecular biology methodologies, researchers also focused on the identification of DNA sequences encoding gene products required for the biosynthesis of the alkaloids. As found with other fungal secondary metabolite biosynthesis genes (Keller & Hohn, 1997), the genes for three of the four alkaloids (ergot alkaloids, indole-diterpenes and lolines) are present in co-regulated gene clusters (Fleetwood et al., 2007; Spiering et al., 2005; Young et al., 2006). The genomic organization of a co-regulated gene cluster helps define the boundaries of the cluster and subsequent identification of potential gene products that would be essential for alkaloid biosynthesis. Targeted gene deletions, gene silencing and heterologous gene expression have allowed for the characterization of many genes and provided evidence of specific pathway steps for all four of the known alkaloids (Panaccione et al., 2001; Spiering et al., 2005; Tanaka et al., 2005; Wang et al., 2004; Young et al., 2005; Young et al., 2006).

Using PCR with primers specific to each alkaloid biosynthetic pathway gene, we can now rapidly profile isolates for their ability to produce specific alkaloids. Therefore, the presence of biosynthetic genes helps define the potential biochemical pathways of the symbiotum (association of the endophyte and plant). In other words, if the gene cluster for a specific alkaloid is missing from an isolate, the compound cannot be synthesized as the gene products (the enzymes required for alkaloid biosynthesis) are not produced. This also means that if key pathway genes are missing (or non-functional) then critical pathway steps and resulting metabolites will not be produced. Alternatively, later pathway steps which are missing or non-functional can still result in the biosynthesis of pathway intermediates that may have biological activity.

The most extensive analysis of metabolite genes has been performed for the lolitrem B biosynthesis genes (*ltm* genes) across a number of sexual and asexual epichloae and these data helped define the pathway as a complex biosynthetic grid (Young et al., 2009). These data concluded that many sexual species lack all 10 *ltm* genes and presents the reason they are unable to produce lolitrems. Spiering et al (2002) also showed a correlation with the presence of two genes, *lolC* and *lolP*, with *Epichloë* and *Neotyphodium* isolates that are able to produce lolines (Spiering et al., 2002a).

The *E. festucae* and asexual isolates with an *E. festucae* progenitor showed the greatest variation with respect to the presence of *ltm*

**Figure 1.** PCR profiling for the lolitrem biosynthesis genes (*ltm*). The *ltm* gene cluster is shown to the right of the PCR analysis

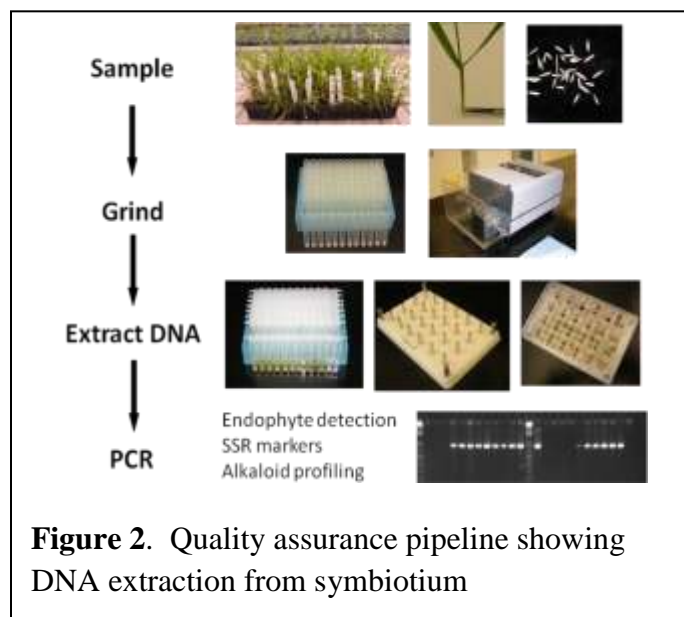


genes and subsequent ability to produce lolitrem B or indole-diterpene intermediates (Young et al., 2009) (Fig. 1). The variation across these isolates ranged from the presence of no genes to the presence of five, eight, nine or all 10 *ltm* genes (Fig. 1). Isolates with only five *ltm* genes were still unable to synthesis indole-diterpenes as two of the early essential pathway genes, *ltmG* and *ltmM*, were missing. It was perhaps surprising to find that the mammalian friendly *N. lolii* isolate AR1 contains eight of the 10 *ltm* genes and is able to produce lolitrem intermediates such as the terpendoles (Young et al., 2009). As evidenced by animal performance trials and feeding studies, the toxicity of the terpendoles produced by AR1 is much lower than that of lolitrem B and does not cause problems to grazing animals (Bluett et al., 2005a; Bluett et al., 2005b; Gatenby et al., 1999).

The lolitrem biosynthesis pathway also presents an opportunity to generate metabolite diversity. It appears that some biosynthesis enzymes such as P450 monooxygenases and aromatic prenyl transferases are more promiscuous and are capable of accepting more than one substrate. This in turn provides a complex biosynthetic grid resulting in the production of more metabolites than can be produced by a linear pathway (Young et al., 2009).

*Epichloë festucae* is known to generate the greatest range of alkaloids as this species can produce ergot alkaloids, lolitremes (indole-diterpenes), peramine and lolines (reviewed in Clay & Schardl, 2002). However, at present, there has been no single isolate that has produced all four classes of alkaloids. It is therefore, no surprise that asexual endophytes with an *E. festucae* progenitor are also likely to produce these compounds and will represent the alkaloid variation as well.

### Quality assurance for grass/endophyte associations



We have developed a quality assurance pipeline that allows us to rapidly screen grass tillers and seeds for the presence of an epichloë endophyte from a range of cool season grasses. The pipeline involves the isolation of genomic DNA from the symbiotium followed by PCR with primers specific to the endophyte for detection of the fungus, identification of isolates using SSR markers and profiling of the alkaloid genes (Mittal, Hopkins and Young unpublished) (Fig. 2). However, it is equally useful for marker assisted selection for host traits (Young, Mittal, Saha and Hopkins, pers. com). We have successfully used this method to identify

contamination of “hot” (mammalian toxic) endophytes within seed batches and plot trials, profiling endophyte alkaloid potential of material from collection trips, and for endophyte infected grasses such as Canada wildrye (Saha et al., 2009), western wheatgrass and orchard grass. The development and utilization of novel (mammalian friendly) endophyte/grass

associations requires additional testing throughout the cultivar development process, providing quality assurance of low level (or no) contamination.

The focus of this paper has been on the alkaloids we are knowledgeable of, but genome sequencing can provide a snapshot of an endophyte's capability with regards to areas that we know little about. The sequenced *E. festucae* genome ([www.endophyte.uky.edu](http://www.endophyte.uky.edu)) is known to contain other potential alkaloid biosynthesis genes of which we are unsure of the resulting products (Schardl et al, unpublished) and research has begun characterizing a number of these genes. As the cost of genome sequencing comes down, we will soon be able to rapidly screen endophytes to determine their full potential and dissect other important traits that these fungi supply to the symbiotum using comparative genomics approaches.

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# Control of *Vulpia myuros* in red fescue

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## Abstract

The efficacy on *Vulpia myuros* of more than 20 herbicides was examined in pot experiments. *V. myuros* was tolerant to most herbicides and the selectivity in red fescue of effective herbicides was marginal. In field experiments iodosulfuron + mesosulfuron (Atlantis WG) improved the efficacy compared to pendimethalin (Stomp) alone but the effect was temporary and did not provide a satisfactory control lasting until seed harvest.

## Introduction

*Vulpia myuros* is a winter annual grass weed which has been reported as a weed problem in Australia, the USA, the Netherlands and Denmark. In Denmark it was first reported as a weed problem in red fescue in the late 1990s. Since then, the infested area has expanded rapidly, and today it can be found in all winter annual crops. *V. myuros* is not competitive in a dense crop, but it establishes in bare spots of the field from where it infests the area. It can cause considerable yield losses due to crop competition, but in red fescue the impact on seed quality is more important.

## Materials and methods

### *Pot experiments*

Seeds of *V. myuros* and red fescue were sown in 2-L pots filled with a potting mixture of soil, sand and peat. The pots were placed in a glasshouse and were sub-irrigated with deionised water. After emergence the number of plants per pot was reduced to a pre-set number.

Herbicide application was carried out at different growth stages from pre-emergence to the 3- to 4-leaf stage. Effective herbicides were subsequently tested for selectivity in red fescue. All spray solutions were prepared in deionised water and applied using a laboratory pot sprayer. The plants were harvested 3 weeks after spraying, and foliage fresh and dry weights were recorded.

### *Field experiments*

Field experiments were carried out at Research Centre Flakkebjerg. *V. myuros* and red fescue were established in separate strips in monoculture and in a mixture. The species were undersown in winter wheat or spring barley. Herbicide applications were carried out across the strips at 5

timings. Timing A and B were applied in the cover crop with timing A on grass seedlings at BBCH 10-11 (Meier *et al.*, 2001) , and timing B 2 to 3 weeks later. Timing C was shortly after harvest of the cover crop and D was 10 to 14 days later. Timing E was in the winter before the first seed harvest.

The herbicide treatments are shown in table 1. The application rate of Stomp (400 g L<sup>-1</sup> pendimethalin) was 1.5 L ha<sup>-1</sup> at timing A and 2 L ha<sup>-1</sup> at the timings C and D. Atlantis WG (30 g kg<sup>-1</sup> mesosulfuron + 6 g kg<sup>-1</sup> iodosulfuron) was applied at a dose of 0.25 kg ha<sup>-1</sup> in mixture with 0.625 L ha<sup>-1</sup> Biopower, Reglone (374 g L<sup>-1</sup> diquat) was applied at a dose of 1 L ha<sup>-1</sup> and Kerb (500 g L<sup>-1</sup> propyzamid) was applied at 0.25 L ha<sup>-1</sup>.

The herbicide efficacy was visually assessed in the monoculture strips of *V. myuros* and crop damage in the red fescue strips. The plots with the mixed plant populations showed the combined effect of herbicide efficacy and crop competition.

## Results and discussion

### *Pot experiments*

The efficacy of more than twenty different herbicides was tested in pot trials during the last 10 years. The early results showed that *V. myuros* was susceptible to Stomp (pendimethalin) and Boxer (prosulfoarb). The highest efficacy was obtained when applied pre-emergence. A later experiment showed that Fusilade Max (fluazifop), Primera Super (fenoxaprop-ethyl), Grasp (tralkoxydim), Topik (clodinafop), Select (cycloxydim) and Aramo (tepraloxymid) had low effects indicating that the ACC-ase inhibitors which control several annual grasses cannot be used for control of *V. myuros*. Among the sulfonylurea herbicides, Hussar (iodosulfuron), Monitor (sulfosulfuron) and Lexus (flupyr-sulfuron) had some but not sufficient efficacy. In contrast, MaisTer (iodosulfuron + foramsulfuron) and Atlantis WG (iodosulfuron + mesosulfuron) had a high efficacy, but a subsequent experiment showed that MaisTer was not selective in red fescue. Iodosulfuron + mesosulfuron had a moderate effect on red fescue and can be used in low doses. Other herbicides with high efficacy were Fenix (aclonifen), Kerb (propyzamid), Tiara (flufenacet) and Command (clomazone). Unfortunately, these herbicides also had a high effect on red fescue. Calaris (mesotrion + terbuthylazin) and Sumimax (flumioxzin) had a low effect. Roundup Bio (glyphosate 360 g L<sup>-1</sup>) controlled *V. myuros* in 1 L ha<sup>-1</sup> – a dose that is not tolerated in red fescue.

Herbicide application was carried out at different growth stages in some of the experiments. The results showed clearly the importance of starting the spraying at early growth stages. None of the herbicides were able to control *V. myuros* when the plants had 3-4 leaves.

### *Field experiments*

The strip design provides an opportunity to assess the efficacy on *V. myuros* and the selectivity in red fescue in the monoculture strips and the combined effect of herbicide and crop competition in the strips with mixed populations. The results in winter wheat showed that the efficacy of 1.5 L ha<sup>-1</sup> Stomp applied in the autumn was insufficient and only reached 32% (Table 1). The efficacy was significantly improved when Atlantis WG was applied in combination with Stomp (treatments 1 and 2). While an early tank mix application had 82.5% effect on *V. myuros*, a split application with Stomp in October followed by Atlantis 3 weeks later improved the efficacy to 96%. The assessments in October 2008 showed that two applications of Stomp in red fescue with the first application shortly after winter wheat harvest and the second application 2 weeks later had 66% efficacy. While the benefit of starting the control with Stomp in the winter wheat crop was marginal, there was still a high response on *V. myuros* to treatments including Atlantis WG. The treatment with Reglone (1 L ha<sup>-1</sup>) during the winter had a low effect on *V. myuros* the following summer. The visual assessments in May in the seed crop still showed 20% higher efficacy for treatments including Atlantis WG in the winter wheat crop compared to Stomp alone, but the overall efficacy level had decreased to 55-58% and was not satisfactory. All the treatments were also conducted in combination with 0.25 L ha<sup>-1</sup> Kerb applied during the winter. Kerb had a very high effect on *V. myuros*, but unfortunately damaged red fescue. Treatments including Atlantis WG caused some damage on red fescue but the symptoms were reduced by time and no differences between treatments 1 to 5 were noticed in the spring in the seed harvest year. In contrast, all treatments including Kerb were seriously damaged and crop coverage ranked from 25 and 36% and seed stem coverage was less than 10% in June.

**Table xx.** Herbicide treatments and efficacy on *V. myuros* in a winter wheat field experiment. Means followed by the same letter do not significantly differ.

Date	In winter wheat		After harvest of winter wheat				Red fescue 2009	
	2007		2008				Winter	Spring
	08.10	31.10	19.08	14.08	02.09	27.10	02.01	20.05
Timing	A	B	C		D		E	
Treat- ment			% control		% control		% control	
1	Stomp + Atlantis WG	-	83 a	Stomp	Stomp	91 ab	Reglone	58 a
2	Stomp	Atlantis WG	96 a	Stomp	Stomp	92 ab	Reglone	55 a
3	Stomp	-	32 b	Stomp	Stomp	73 bc	Reglone	34 b
4	-	-	-	Stomp	Stomp	66 c	Reglone	38 b
5							Reglone	24 c

In spring cereals the efficacy of Stomp was only 5%. Combinations of Stomp and Atlantis WG gave 73% effect when applied in a tank mix at timing A, and similar to the winter wheat experiment the efficacy was improved (to 87%) when Stomp was applied at timing A and Atlantis WG at timing B. The visual assessments in October reflecting the efficacy of sprayings in the spring barley as well as after harvest of the cereal crop (A, B, C and D), showed a high efficacy (94-96%) of treatments 1 and 2 and a low efficacy (40-44%) of treatments with only one or no Stomp application in the cereal crop (treatments 3 and 4). Reglone applied in January had a low effect on *V. myuros* while Kerb had a high effect. The ground cover of *V. myuros* in May was 1-2% for all treatments including Kerb but 3-11% for all treatments including Reglone.

Atlantis WG is not registered for use in spring barley and caused severe crop damage by foliage yellowing and plant stunting. Growth reduction in red fescue after the C and D applications of Stomp was 3% for treatments 3 and 4 and 7-8% for treatments 1 to 4. Red fescue coverage in May in the seed harvest year was 84-90% for all treatments that had Reglone and 31 to 43% for all treatments with Kerb in the winter.

## Perspectives

The pot experiments did not revealed any new effective herbicides for control of *V. myuros*. The field experiments showed that *V. myuros* control is improved when Atlantis WG is included in the weed control strategy; however, the effect was temporary, and none of the treatments were able to provide a satisfactory control lasting until seed harvest. In conclusion, control of *V. myuros* requires more effort than herbicides and future experiments will include integrated weed management such as timing of crop establishment, sowing depth and crop density.

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## **Acknowledgement**

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# Annual grasses in crop rotations with grass seed production

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## Abstract

In the article an overview of occurrence of important annual grasses as weeds in grass seed production is given. The article also gives the main conclusions concerning factors of importance in IPM strategies. These involve particular attention to the handling of volunteer seeds. In general seed survival is strongly reduced at the soil surface compared to incorporated seeds. Crop rotations and cropping systems that allow the volunteer seeds to be left as long as possible at the soil surface or in stubble favour a fast decrease of the soil seedbank of the investigated annual grasses.

## Introduction

In grass seed production purity of the product is of main importance. Many annual grasses are difficult or impossible to control chemically in grass seed crops and at the same time seed characteristics make it difficult to separate the seeds from some of the cultivated grasses. A main issue in an integrated strategy is to reduce or eliminate the seedbank of a species that possesses the above mentioned characteristics. Such strategies have been investigated and developed for some important annual grasses in Denmark such as *Apera spica-venti* (Jensen; 2009) and *Poa trivialis* and *Vulpia Myuros* (Jensen; 2010). However if volunteer seeds from a formerly grown species establishes in another grass seed crop a corresponding undesired contamination of the crop arises. Therefore correct handling of volunteer seeds of cultivated grass species is also important and has been investigated (Jensen; 2010). This paper and the presentation gives an overview of occurrence of important annual grasses in grass seed production in Denmark and it summarises the experiences obtained concerning non-chemical strategies to reduce pressure from annual grasses.

## Materials and methods

The conclusions on IPM strategies are a review of results obtained from different investigations during the last 15 years in different projects on non-chemical methods. The investigations have been published and the references are given.

The Danish area with production of grasses, clovers and other pasture legumes for seed production totals approximately 80.000 ha in average a year (Anon, 2009). During the growing season all fields are inspected and important weeds are assessed on a scale from 1-5,

Character	Weed abundance
1	A few plants in the field
2	Less than 1 per. 10 m <sup>2</sup>
3	1 – 2 per 10 m <sup>2</sup>
4	3 – 5 per 10 m <sup>2</sup>
5	> 5 per 10 m <sup>2</sup>

DLF-TRIFOLIUM has a market share of 80-90% and DLF-TRIFOLIUM has since 2004 stored all data from their field inspections in a database. This database has been at the disposal of a new project at the department of Integrated Pest Management. The database covers the period 2004-2009, and extracts from the database gives a survey of the most important weeds and especially annual grasses in Denmark.

## Results and discussion

### *Weed survey*

The field inspections of grass, clover and pasture legumes for seed production is carried out at a time where the weeds remaining in the field is a result of all preceding field operations influencing the weed in question. The field inspections therefore give a good impression of the actual weed problems related to the seed production of a certain species in Denmark at the time.

The most frequent grass weeds found in seed production was *Elytrigia repens*, *Poa annua*, *Poa trivialis*, and *Bromus hordeaceus*. *Vulpia* species is a special problem in fescue production and especially red fescue production. Among the cultivated grasses, *Lolium perenne* and *Dactylis glomerata* are most frequently found as weed infestations in fields with seed production of other grasses.

### *IPM methods*

IPM methods are important as chemical control options are limited or unavailable concerning many combinations of crops and weeds. The most important tools concerning annual grass weeds are:

1. Effective chemical control in the crop rotation where possible
2. Leave volunteer seeds at the soil surface as long as possible, this reduces longevity of both weedy and cultivated grasses and can be a very effective way to reduce the seedbank

3. The stale seedbed technique can be a very effective method to deplete the seedbank and reduce contamination of a subsequent grass seed crop
4. Spring establishment of seed crops reduces contamination with winter annual grasses.

An example of the influence of straw disposal technique and soil cultivation is shown in Figure 1.

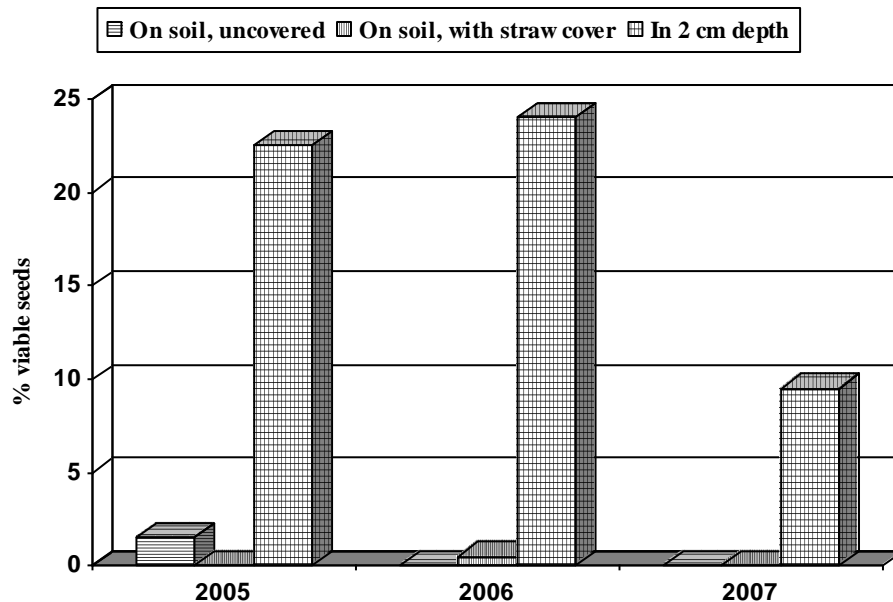


Figure 1. Longevity of seeds of *Poa trivialis* kept at the soil surface with or without straw cover or at a depth of 2 cm from maturity in July to the end of September the same year.

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## **APOSTART-derived SCAR markers discriminate between apomictic and sexual *Poa pratensis* L. genotypes**

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Apomixis is a challenging trait and offers unique opportunities for developing superior varieties since it enables us to develop hybrids or genotypes that breed true regardless of heterozygosity. Apomixis would make it possible to fix the genotype of a superior plant variety bred for a particular environment or market niche so that clonal seeds could be continuously and cheaply produced independent of pollination. If apomixis were well understood and harnessed, it could be exploited to indefinitely propagate superior hybrids or specific genotypes bearing complex gene sets. Until the gene(s) that promote and control apomixis are molecularly understood, this trait can only be introgressed into agricultural crops through traditional breeding methods, most of which are slow and laborious and require progeny tests for the selection of apomictic genotypes after each round of backcrossing

In our previous work by applying the cDNA-AFLP transcriptional profiling technique we isolated messengers from developmental staged inflorescences of *Poa pratensis* L. In particular, more than two thousands transcript-derived fragments were visualized 179 of which were differentially expressed between apomictic and sexual genotypes. A major finding is that most of the genes expressed in florets displayed similar patterns during sexual and apomictic pathways. Of the about 8% of mRNAs differentially expressed between apomictic and sexual genotypes, the vast majority were attributable to genes with a differentiated temporal expression during flowering. As few as 1.6% mRNAs specific to sexual or apomictic genotypes were found, providing that a highly conserved developmental program exist in embryos during zygotic embryogenesis and apomeiotic parthenogenesis. In particular one of these genes (APOSTART) was found to be flower-specific and showed a differentiated expression in reproductive tissues between apomictic and sexual genotypes of *P. pratensis*. We developed a SCAR marker from an APOSTART apomictic-specific allele and then tested for its potential use in MAS programs. APO-SCAR primer pair was first tested on an F<sub>1</sub> population of 68 individuals segregating for the mode of reproduction and on its parental genotypes. The APO-SCAR pair of primers produced a single amplification product of 225 bp, present in the apomictic paternal genotype but absent in the maternal sexual plant.

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# Effects of intraspecific competition on growth and seed yield of contrasting sulla genotypes

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## Abstract

*Sulla* (*Hedysarum coronarium* L.) is a short-lived perennial legume native to the Mediterranean basin, where it is grown extensively as a 2-year forage crop. Seed is often obtained as a secondary product from second-year crops established to produce forage. Very little information is available on seed yield capacity in the first year of the crop cycle and on the influence of agronomical techniques on the reproductive process. The present study aimed to evaluate the effect of intraspecific competition on dry matter accumulation (both in epigeic organs and in taproots) and seed production in the first year of a crop cycle for two contrasting genotypes. Two field trials were carried out in a Mediterranean environment during the 2003/2004 and 2004/2005 growing seasons. The experiment was set up as a split-plot design with four replications. The treatments were as follows: 1) genotype: Gangi or Resuttano (both originated from inside Sicily but differed in terms of growth rate during the 2 years of the cycle); and 2) intraspecific competition realized by means of two plant densities: 100 and 600 plants m<sup>-2</sup>. The results showed that in the first year of the cycle, the two landraces had very different behavior: Resuttano tended to accumulate C reserves in taproots, whereas Gangi used the photoassimilates mainly for epigeic growth and the gametic reproduction process. As a result, Gangi produced about 550 kg ha<sup>-1</sup> seed, whereas Resuttano had a negligible seed yield. The increase in intraspecific competition increased above- and belowground biomass at the beginning of flowering irrespective of genotype but had no effect on crop growth or seed yield at maturity.

## Introduction

*Sulla* (*Hedysarum coronarium* L., syn. *Sulla coronaria* [L.] Medik.) is a short-lived perennial legume native to the Mediterranean basin (Talamucci, 1998), where it is grown extensively as a 2-year forage crop for grazing and/or hay or silage production. The species plays a key role in cereal-based systems of semi-arid regions, particularly in organic and low-input agriculture. It is commonly used to enhance the productivity and sustainability of farming systems (e.g., to supply nitrogen and to maintain soil organic matter). Recently there has been a newfound interest in *sulla* both in traditional and in nontraditional areas because of its excellent adaptability to marginal and drought-prone environments (Borreani *et al.*, 2003; Annichiarico *et al.*, 2008). Moreover, it has several non-agricultural uses; for example, it is planted for soil protection (Watson, 1982) and revegetation of disturbed lands (Flores *et al.*, 1997) as well as for honey

production and landscape architecture (Talamucci, 1998). Seed is often obtained as a secondary product from stands established to produce forage. Usually seed is harvested from second-year crops and from selected areas where more vigorous plants and fewer weeds are found (Stringi & Amato, 1998). Very little information is available on seed yield capacity in the first year of the crop cycle and on the influence of agronomical techniques on the reproductive process. The present study aimed to evaluate the effect of intraspecific competition on dry matter accumulation (both in epigeic organs and in taproots) and seed production in the first year of the crop cycle for two contrasting genotypes.

## Materials and Methods

Two field trials were carried out during the 2003/2004 and 2004/2005 growing seasons in a Mediterranean environment (37°30'N – 13°31'E; 178 m a.s.l., Sicily, Italy) on a deep, well-structured soil classified as a Vertic Haploxerert. The treatments were as follows: 1) genotype: Gangi and Resuttano; and 2) intraspecific competition realized by means of two plant densities: 100 and 600 plants/m<sup>2</sup>. The two landraces, both originating from inside Sicily, differed in terms of productivity (aboveground biomass and seed) in the first year of the cycle and regrowth capacity after the summer stasis (Amato *et al.*, 2007). The experiment was set up as a split-plot design with four replications, with genotype as the main plots and intraspecific competition as sub-plots. Sub-plots were 7.0 × 4.5 m (18 rows, 0.25 m apart and 7 m long). Before sowing, 69 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was applied. Plots were hand-sown on 7 January 2004 and 10 January 2005. Sulla was seeded at double rate (200 and 1200 seeds/m<sup>2</sup>, respectively for 100 and 600 plants/m<sup>2</sup>) and hand-thinned 20 days after emergence to obtain the desired plant densities. All plots were hand-weeded. Within each sub-plot three 1.25 × 0.75 m areas were randomly chosen for destructive plant samplings at the beginning of flowering (which was similar for the two landraces; i.e., in the first days of May), after 30 days (which coincided with the end of flowering for Gangi, the last to stop flowering), and at maturity. At each stage, number of plants, plant height, total aboveground biomass, botanical fractions (leaves, stems, and racemes), and taproot biomass (layer 0–20 cm) were recorded. At the beginning of flowering, leaf area was measured with a LICOR 3100C leaf area meter on a 50-g subsample of leaves per plot. In the remaining plot area, seed yield and seed yield components were recorded at maturity. Analysis of variance on the combined 2-year data set was performed according to the experimental design. Data analysis was performed using SAS version 9.1 (SAS Institute, 2004). Year was treated as a random factor, whereas all other factors were treated as fixed.

## Results and Discussion

In both growing seasons, at the beginning of flowering low plant density was ~90 plants m<sup>-2</sup>, whereas for high plant density the number of plants per unit area was half of that fixed (~300 plants/m<sup>2</sup>). Plant density did not further vary by treatment until maturity. At the beginning of

flowering, Gangi accumulated a higher average aboveground biomass than Resuttano (+25%; Table 1). However, Resuttano produced a leafier forage (leaf:stem ratio, 8.1 vs. 1.5) and had a similar leaf area index. The two landraces also differed considerably in terms of taproot dry matter accumulation, which was more than double in Resuttano than in Gangi. The increase in plant density led to an increase in both leaf area index and biomass yield (both above and below ground) irrespective of genotype. During the flowering period, both landraces showed a dramatic increase in total biomass, but in Gangi the increase was mainly due to an increase in aboveground biomass (from 6.50 to 10.26 t DM ha<sup>-1</sup>), whereas in Resuttano most of the increase occurred in taproots (from 1.35 to 7.11 t DM ha<sup>-1</sup>). At the end of the flowering stage, the average concentration of taproot water soluble carbohydrates (WSC) was slightly greater for Gangi than for Resuttano (149 vs. 138 g kg<sup>-1</sup> DM, respectively), but because of the much greater taproot dry matter production, Resuttano had about a 5-fold greater amount of WSC than Gangi.

**Table 1.** Sulla seed crop parameters (LSR = leaf:stem ratio; LAI = leaf area index; ADM = aboveground dry matter; TDM = taproot dry matter; WSC = water soluble carbohydrates) at various growth stages as affected by genotype and plant density.

			Beginning of flowering				End of flowering				Maturity
Year	Geno- type <sup>†</sup>	Plant density <sup>‡</sup>	LSR	LAI	ADM	TDM	ADM	TDM	Taproot	WSC	Seed
					t/ha	t/ha	t/ha	t/ha	g/kg	g/m <sup>2</sup>	kg/ha
2004	Gan.	100	0.89	4.01	7.46	0.61	11.85	1.58	150	24	590
		600	0.77	3.76	9.94	0.78	10.38	1.37	151	21	515
	Res.	100	3.70	4.29	5.65	1.61	6.83	7.02	138	97	73
		600	4.63	4.20	6.72	1.98	5.94	6.80	127	86	10
2005	Gan.	100	2.72	2.86	3.25	0.38	8.74	1.25	145	18	535
		600	1.59	4.36	5.35	0.55	10.05	1.44	148	21	547
	Res.	100	10.92	2.78	3.21	0.80	3.91	6.78	142	96	7
		600	13.15	4.31	5.25	1.01	4.67	7.85	144	113	1
Year (Yr)			**** <sup>§</sup>	***	***	***	*	ns	ns	ns	ns
Genotype (G)			***	ns	**	***	***	***	*	***	***
Plant density (D)			ns	***	***	**	ns	ns	ns	ns	ns
Yr × G			**	ns	***	**	ns	ns	*	*	ns
Yr × D			ns	***	ns	ns	**	ns	ns	ns	ns
G × D			ns	ns	ns	ns	ns	ns	ns	ns	ns
Yr × G × D			ns	ns	ns	ns	ns	ns	ns	ns	ns

<sup>†</sup> Gan. = Gangi; Res. = Resuttano. <sup>‡</sup> 100 = 100 plants/m<sup>2</sup>; 600 = 600 plants/m<sup>2</sup>.

<sup>§</sup> \*, \*\*, \*\*\*,  $P \leq 0.05, 0.01, 0.001$ , respectively; ns,  $P > 0.05$ .

Intraspecific competition did not affect any trait either at the end of flowering or at seed maturity. In the interval between the end of flowering and maturity a further increase in taproot dry matter (~15% on average) was observed for both genotypes (data not shown).

The seed yield of Gangi was, on average, 547 kg ha<sup>-1</sup> (with no significant difference between the two growing seasons), whereas Resuttano produced a negligible seed yield in both years.

This study reveals the very different behavior of two sulla genotypes during the first year of a 2-year crop cycle; these differences could be related to different population survival strategies. Our results show that the Resuttano landrace tends to accumulate C reserves in taproots, whereas the Gangi landrace uses the photoassimilates mainly for epigeic growth and the gametic reproduction process. Greater C and N reserves in the taproots of alfalfa allow for greater plant survival and faster regrowth (Dhont *et al.*, 2002); it is likely that this is also the case for sulla. The present study shows that the possibility of producing sulla seed in the first year of a crop cycle depends strongly on genotype. Further research is needed to investigate the behavior of these contrasting genotypes during the second year of the crop cycle, particularly with regard to seed yield and regrowth rates after the summer stasis.

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## Seed yield components and yield per plant in populations of *Panicum coloratum* L. var. *makarikariensis* Goossens

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### Abstract

*Panicum coloratum* L. var. *makarikariensis* Goossens (makarikari grass) is a warm-season perennial bunchgrass native to South Africa, adapted to subtropical, subhumid environments with heavy soils, that tolerates seasonal flooding and relatively long periods of drought. The objective of this study was to evaluate the extent and nature of variation in seed yield components in a germplasm collection with a limited genetic base. Research material included 5 populations coming from a wide range of soils and different management regimes in north-central Argentina. Number of seeds per panicle was determined using a seed trap in each of 10 individual plants per population. Mature and empty seeds were determined. Number of panicles per plant was counted at the end of the harvest period. Weight of 1000 mature seeds was estimated by averaging the weight of 3 samples of 100 seeds. Number of mature seeds per plant and yield (g) of seeds per plant were obtained by multiplication of the factors involved. Data was analyzed using ANOVA and population means were compared by LSD at the 0.05 level of significance. Correlations and path coefficient analysis were performed on the seed data. Populations differed markedly in seed yield per plant and the number of mature seeds per panicle. Minor differences were detected in seed weight and no difference was evident in number of panicles per plant. Therefore, the component most associated to yield per plant was the number of mature seeds per panicle. Further studies need to be performed to evaluate genetic variation and heritability of this trait to be included in a breeding program.

### Introduction

*Panicum coloratum* L. is a warm-season cross-pollinated perennial bunchgrass native to South Africa that has been used around the world to improve pastures and in range revegetation (Lloyd 1981), given its wide adaptability and its ability to tolerate seasonal flooding in addition to long periods of drought (Tischler & Ocumpaugh 2004). It present there are two varieties: var. *coloratum* (kleingrass), extensively grown in Texas, was developed primarily for utilization as gamebirds, and var. *makarikariensis* Goossens (makarikari grass), utilized in Queensland, Australia, on heavy clay soils subjected to heavy grazing (Lloyd 1981; Tischler & Ocumpaugh 2004). In Argentina, *P. coloratum* has been introduced as a forage grass several times (Petruzzini *et al.* 2003) from different sources but its use has not been used extensively in the country. To increase the sowed area, production of good quality seed is crucial and therefore, a breeding program has been recently initiated at the National Institute of Agricultural Technology (INTA)

in Argentina focused on several aspects of seed production and seedling establishment. The program started with a small germplasm collection, consisting of accessions adapted to different areas and management regimes.

Seed yield is one of the characters commonly evaluated in breeding programs at the plant level and analyzed in terms of its components (Hearn & Holt 1969; Elgersma 1990; Diz *et al.* 1994): panicles per plant (number pan  $\text{pl}^{-1}$ ) (number panicles  $\text{plant}^{-1}$  or no. panicles  $\text{plant}^{-1}$ ), mature seeds per panicle (number seeds  $\text{pan}^{-1}$ ) and weight of mature seed ( $\text{g seed}^{-1}$ ). This study evaluated the extent and nature of variation in seed yield per plant and the associated components in the germplasm collection at INTA to be used in the breeding program. Specific objectives were i) assess variability in seed yield and seed yield components and ii) determine correlations among seed yield components and partition the correlation through path coefficient analysis to assess relative importance of direct and indirect effects.

## Materials and Methods

Seed yield components were evaluated in a germplasm collection established at INTA Rafaela Experiment Station ( $31^{\circ}11'41''$  S;  $61^{\circ}29'55''$  W) in October 2006. The collection includes 5 populations coming from a wide range of soils and precipitation in north-central Argentina: DF, UCB and MR from Córdoba, ER (and?) BR from Corrientes (Typic Haplustol, 600 mm annual precipitation in Córdoba, and Vertic Argiudol, 1500 mm of annual precipitation in Corrientes, respectively). Each population consists in 32 individual plants, at 0.60 m distance in a 8 x 4 matrix. Populations were planted apart to avoid cross pollination.

Estimates of seed yield components were obtained from 10 individual plants per population in the summer 2008-09. Number of panicles per plant (change  $\text{n}^{\circ}\text{pan.pl}^{-1}$ ) was counted at the end of the harvest period. In order to prevent seed losses through shattering, a seed trap especially designed was used to collect all the seeds produced per panicle. The trap consisted in a cylindrical steel structure over a pole that was covered by a nylon stocking. Panicles were set into the trap when at least 2/3 of all florets had gone through anthesis to ensure out-crossing. Seeds were collected from the trap weekly, from 03/21/09 to 04/17/09, taken to the lab, counted, added up and weighted. The number of mature and empty seeds was determined in each panicle. Weight of 1000 seeds (change  $\text{g.1000 seed}^{-1}$ ) was estimated by averaging the weight of 3 subsamples of 100 mature seeds per plant. Number of mature seeds per plant (change  $\text{n}^{\circ}\text{seeds.pl}^{-1}$ ) and yield per plant (change  $\text{g.pl}^{-1}$ ) of seeds per plant were obtained by multiplication of the factors involved. Separated ANOVAs were conducted to test for population differences for each trait measured. Comparisons of population means were conducted by LSD test at 0.05 level of significance? for traits that had significant variations among the populations. Correlation and path coefficient analyses were done by standard methods (Dewey & Lu 1958). The causal relationships for the path coefficient analysis involved the four seed yield components primarily measured as predictor (cause) variables and seed yield as the response (effect).

## Results

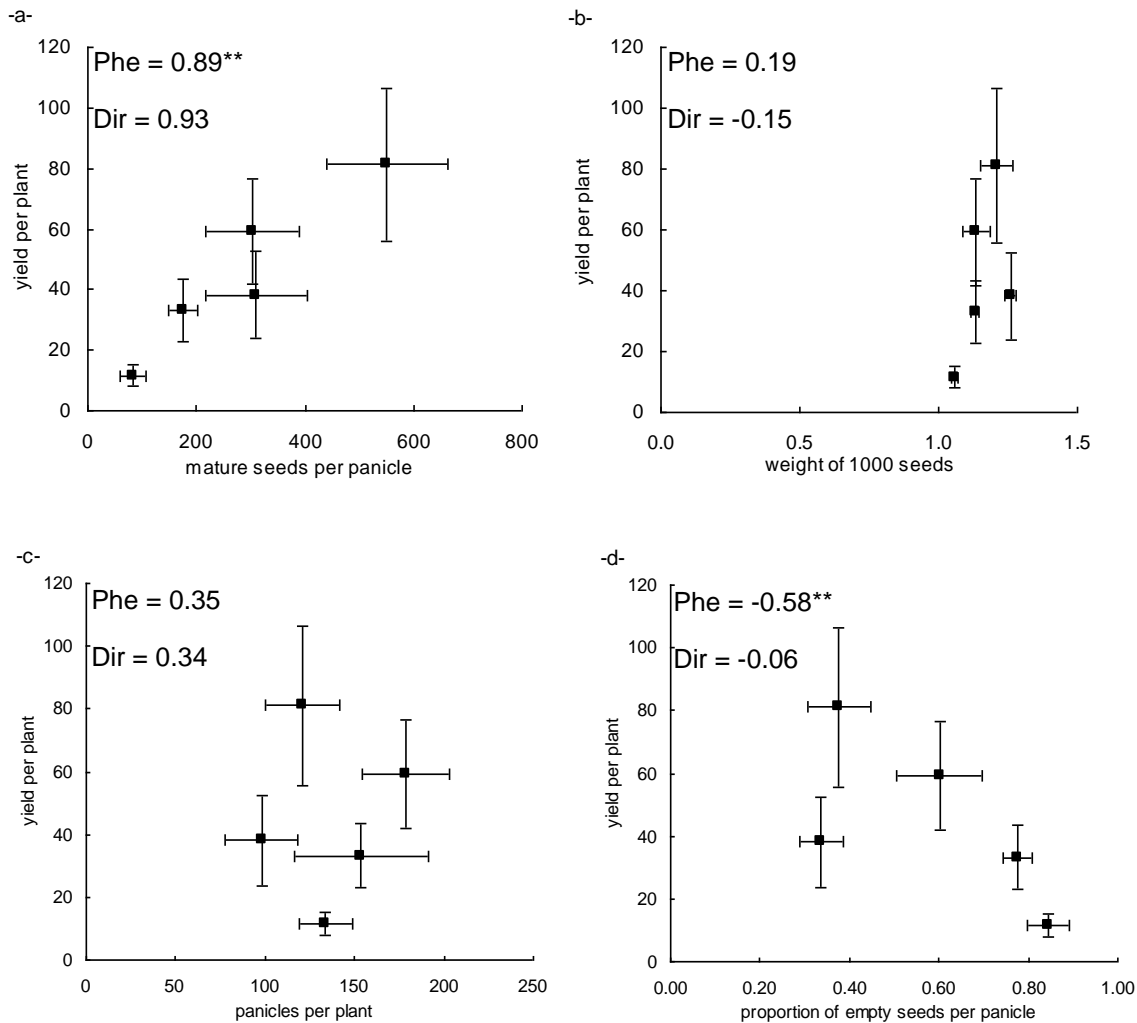
Variation among populations was detected in seed yield per plant, with ER the highest, BR and MR the lowest and DF and UCB intermediate (Table 1). Interestingly, in terms of the components of yield, while a similar ranking was detected for number of mature seed per panicle, variation in the weight of the seed was only slightly significant and no differences among populations were detected in the number of panicles per plant (Table 1). These findings suggest that variation of yield per plant was mainly associated with variation in number of mature seed per inflorescence.

**Table 1.** Means of yield per plant (change g.pl<sup>-1</sup>), mature seeds per inflorescence (change seeds.pan<sup>-1</sup>), 1000 seeds weight (change g.1000 seed<sup>-1</sup>) and number of inflorescences per plant (change pan.pl<sup>-1</sup>), in 5 populations of *Panicum coloratum* var. *makarikariensis* at Rafaela, Argentina in 2009.

Population	Yield per plant	Mature seeds per panicle	1000 seeds weight	Panicles per plant
DF	50 <sup>b</sup>	398 <sup>b</sup>	1.278 <sup>a</sup>	98 <sup>a</sup>
ER	86 <sup>a</sup>	572 <sup>a</sup>	1.226 <sup>ab</sup>	123 <sup>a</sup>
BR	22 <sup>c</sup>	161 <sup>c</sup>	1.058 <sup>d</sup>	129 <sup>a</sup>
UCB	54 <sup>b</sup>	321 <sup>b</sup>	1.162 <sup>bc</sup>	144 <sup>a</sup>
MR	27 <sup>c</sup>	161 <sup>c</sup>	1.131 <sup>c</sup>	152 <sup>a</sup>

Values followed by a common letter within a column are not significantly different as indicated by LSD test at  $p = 0.05$

Figure 1. Relationships between yield per plant (change  $\text{g.pl}^{-1}$ ) and (a) mature seeds per panicle (change  $\text{seeds.pan}^{-1}$ ), (b) weight of 1000 seeds (change  $\text{g.1000 seed}^{-1}$ ), (c) number of panicles per plant (change  $\text{pan.pl}^{-1}$ ), and (d) proportion of empty seeds, in 5 populations of *Panicum coloratum* var. *makarikariensis* at Rafaela, Argentina in 2009. Phe = total phenotypic correlation coefficient; Dir = direct effect.



Correlations were partitioned into direct and indirect effects through path coefficient analysis. Number of mature seeds per panicle had the greatest positive effect on seed yield per plant (Fig. 1a) while weight of 1000 seeds and number of inflorescences per plant were negligible (Fig 1b, 1c), and the proportion of empty seeds per panicle was negative but not significant. The indirect effects were not significant ( $P > 0.10$ ) for all variables analysed. The ranking of populations in the total number of seeds produced per panicle (Table 2) (the original text is fine) was different than the ranking of population for the number of mature seeds produced per panicle (Table 1), which means that populations showed differences in the proportion of empty (immature) seeds produced (Table 2, Fig. 1d).

**Table 2.** Means of total seeds per panicle (change seeds.pan<sup>-1</sup>) and proportion of empty seeds, in 5 populations of *Panicum coloratum* var. *makarikariensis* at Rafaela, Argentina in 2009.

Population	Total seeds per panicle	Proportion of empty seeds
DF	568 <sup>c</sup>	0.32 <sup>c</sup>
ER	981 <sup>a</sup>	0.38 <sup>c</sup>
BR	784 <sup>abc</sup>	0.80 <sup>a</sup>
UCB	718 <sup>bc</sup>	0.56 <sup>b</sup>
MR	765 <sup>abc</sup>	0.78 <sup>a</sup>

Values followed by a common letter within a column are not significantly different as indicated by LSD test at  $p = 0.05$

## Discussion

Results from this study showed that there were marked differences in quantitative seed production traits among populations of makarikari grass. Variation in seed yield among cultivars have been reported in many forage grasses (Voltas *et al.* 1999; Lemke *et al.* 2003; Herrera-C *et al.* 2008). In this study, differences were not apparently related to the site where the populations were collected as populations from the same origin showed differential seed production (i.e. ER and BR were both collected in Corrientes).

Yield per plant was strongly and positively associated to the number of mature seeds per panicle (Fig. 1a), in agreement with previous reports for this and other species (Lloyd 1981; Diz *et al.* 1994; Wu *et al.* 2008). The lack of relationships between the seed weight and yield per plant found in makarikari grass (Fig. 1b), agree with the notion that seed size (weight) is a more conservative, highly-heritable character than seed number (Sadras & Denison 2009). However a significant positive genotypic correlation of both components, seed weight and seed number, was observed for *Pennisetum* sp. (Diz *et al.* 1994).

Our results showed that for populations of *P. coloratum* var. *makarikariensis*, the number of panicles per plant was not correlated to the seed yield per plant (Fig. 1c), contrasting with previous work in bermudagrass (Wu *et al.* 2008), that showed a large effect of panicle number on the seed yield per plant. Interestingly, this work showed that the number of total seeds per panicle was quite similar between populations (Table 2), implying that genesis of reproductive structures (flowers) was similar between populations. However, the number of mature seeds per panicle was different populations (Table 1), which cause a negative relationship between the proportion of empty seeds and the seed yield (Fig. 1d). The relatively high proportion of immature seeds in some populations could be due to unsuccessful fertilization and/or seed abortion. In addition, a source (photosynthesis) limitation due to nutritional and water deficits, during the period of fertilization of flowers may not be expected because plants were grown with high availability of water and nutrients, and all populations flowered and filled their seeds in a same time frame (January to April 2009). Hence, proportion of empty seeds seemed to be quite variable and characteristic of each population. In perennial grasses, unlike cereals, the vegetative

sinks (e.g. daughter tillers) are an active system of nutrient demand during the reproductive stage, being stem a specially important sink (Ryle 1970; Ryle & Powell 1972). In this study, populations BR and MR had more empty seeds, also were more leafiness and stoloniferous than the other populations (personal observation, A. Tomás) suggesting they might provide a greater proportion of assimilates to the vegetative parts than the other populations studied. Otherwise, the high proportion of empty seeds in MR and BR might be associated to a high number of sterile florets. In addition, the number of sterile florets was shown to be variable within and between species in several forage grasses (Elgersma 1990; Makela & Kousa 2009).

In conclusion, the variability observed among populations in seed production per plant associated to variability in the mature seeds per panicle and the proportion of empty seeds per panicle, suggests that these traits might be suitable to be utilized in a breeding program to improve the seed production in *P. coloratum* var. *makarikariensis*. However, further studies are needed to calculate estimates of the heritable genetic variation

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## Clover seed production – in organic and conventional cropping systems

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### Abstract

Seed yields in organic farming systems are lower than in conventional systems, by 7-13% in grasses and 76% in white clover (*Trifolium repens*) compared to yields in conventional systems (Lund-Kristensen et al., 2000).

The major yield reduction was found to be caused by the white clover seed weevil (*Apion fulvipes*) and the lesser clover leaf weevil (*Hypera nigrirostris*) and no region in Denmark was found to be free from this pest. White clover seed crops free of pests have the potential of a 40% higher seed yield, and the yield reduction in organic white clover seed production can largely be explained by insufficient pest control methods (Boelt, 2005).

A series of experiments have been initiated to reduce the yield reduction from pests in organic crops, however, none of these have been able to produce seed yields in white clover comparable to yields in conventional cropping systems. Results from these experiments will be presented, information from a video illustrating the life cycle of the clover seed weevil and the present status of white clover seed production in organic and conventional cropping systems in Denmark.

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## Control of *Apion trifolii* in red clover seed production

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### Abstract:

Clover seed weevil (*Apion trifolii* L.) is the main pest on red clover seed production (*Trifolium pratense* L.). Larvae consume clover seeds and can cause severe yield losses. Seed growers are usually advised to administer two or three applications of insecticide to control pests before the female lays their eggs in the flower bud.

Some efficient active ingredients have been banned these last years and only one (*bifenthrine*) is still able to control *apion trifolii* attacks. But the amount of permitted *bifenthrine* has been reduced during flowering time since 2005 and recently *bifenthrine* has been also withdrawn in the EU. So no more efficient registered pesticide will be available from the 30<sup>th</sup> May 2010. With the means currently available, it is not possible to avoid attacks without chemical solutions. Growing organic red clover is difficult especially because of *apion trifolii*. One possibility to avoid attacks of clover seed weevil could be to delay the spring mowing so eggs and larvae could be destroyed and the next flowering would be less sensitive to the weevil damage. But our experimental results indicate that the egg-laying period is so long that it requires a very late cutting which leads to an inappropriate date of seed harvest.

Recently, new insecticides have been tested in FNAMS trials to control this insect successfully. *Spinosad* which could be used for organic production and *acetamépride*, and *thiaclopride* have already shown interesting results. They are promising alternative and need to be registered for red clover seed production. The specific action of *spinosad* (ovi-larvicidal activity) should allow a new threshold of treatment to be defined.

**Key words:** Clover seed weevil (*A. trifolii* L.), *acetamipride*, *thiaclopride*, *spinosad*, alternative control.

### Introduction

Clover seed weevil (*Apion trifolii* L.) is the main pest on red clover seed production (*Trifolium pratense* L.). Larvae consume clover seeds and can cause severe yield losses. Two or three insecticide applications of *bifenthrine* are usually advised to the seed growers to control pest before the female lays their eggs in the flower bud. To avoid problem of resistance, research has been carried out since 2000 to find new chemical solutions or alternative methods. Recently *bifenthrine* has been banned also and the problem becomes greater.

## Materials and Methods:

Field experiments were carried out from 2004 to 2009 in three locations in France: near Angers (northwest of France), Chateauroux and mainly near Bourges (central France). The threshold to apply insecticide before flowering time is one hundred clover seed weevils caught in 25 semicircles of butterfly net. At ripeness, 4 × 50 inflorescences are collected before harvest to establish the number of good seed /inflorescence, which is one of the best ways to appreciate the pesticide's efficiency.

Also, the clover crops are harvested directly with an experimental combiner. Seed samples are cleaned and seed yield is calculated for each sample.

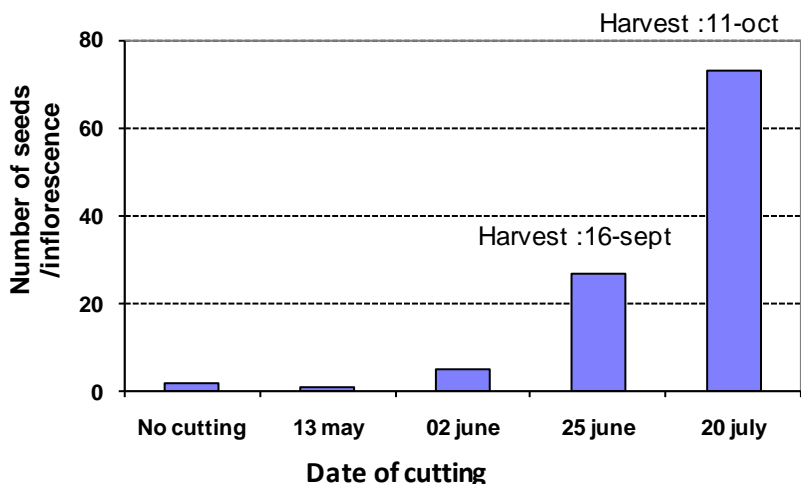
## Results and discussion:

### - Alternative method:

Some trials have been carried out for organic farming. *Roténone*, *natural pyrethrinoid*, *azadirachtine*, plant manure have been tested unsuccessfully to control *apion trifolii* attacks. (Bouet 2003). Another method has been tested without chemical: One possibility to avoid attacks of clover seed weevil could be to delay the spring mowing; so eggs and larvae could be destroyed and the next flowering would be less sensitive to the weevil damage.

Four dates of cutting from May (normal date) to July are compared to one modality without cutting. Our experimental results indicate the egg-laying period is so large that it requires a very late cutting which leads to an inappropriate date of seed harvest.

Figure 1: Effect of the date of cutting on the number of seeds /inflorescence in organic red clover seed production – (FNAMS/ARVALIS Chateauroux, 2004)

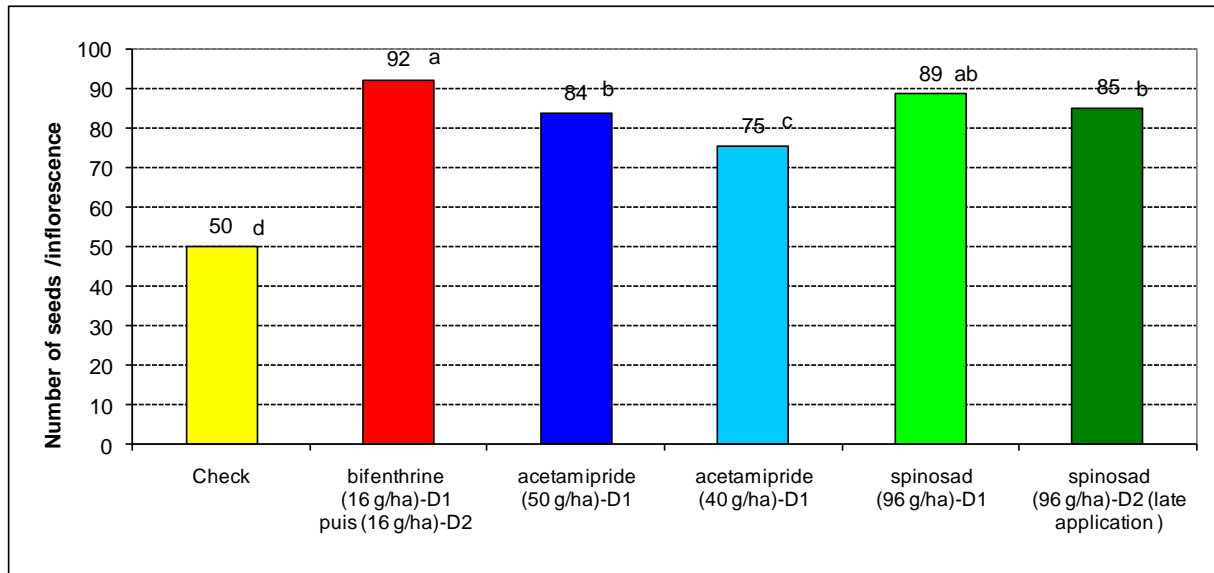


### - Acetamipride trials:

*Acetamipride* (50 g. a. i. /ha) has been studied for five years and has shown very good results to control *apion trifolli* damage. *Acetamipride*'s efficiency on *apion trifolli* adult is always prompt and more persistent than the reference (*bifenthrine*). The number of seed per inflorescence is

similar to the reference but with less application (ex: 2008) which is showing a high efficacy level. At a lower rate (40g a. i./ha.), *acetamipride*'s efficiency seems to decrease regarding to the results on number of seed/inflorescence (Bourges 2008).

Figure 2: Insecticide effects on the number of seeds /inflorescence in red clover seed production (FNAMS Bourges, 2008) (Date of spraying : D1= 25/06/08 D2= 01/07/08)



- **Thiaclopride trials:**

Only one year trials (2009) has been carried out recently with *thiaclopride* (62.5g a.i./ha + *deltaméthrine* 6.25g . a. i./ha). This association has shown the same results as *acetamipride* in a single application with a prompt and persistent efficiency on adult. Number of seed /inflorescence and seed yield obtained are better than with one application of *bifenthrine*.

Figure 3: Insecticide effects on apion trifolii population in red Clover seed production (FNAMS Bourges, 2009)

D1: Date of spraying: 12/06/09

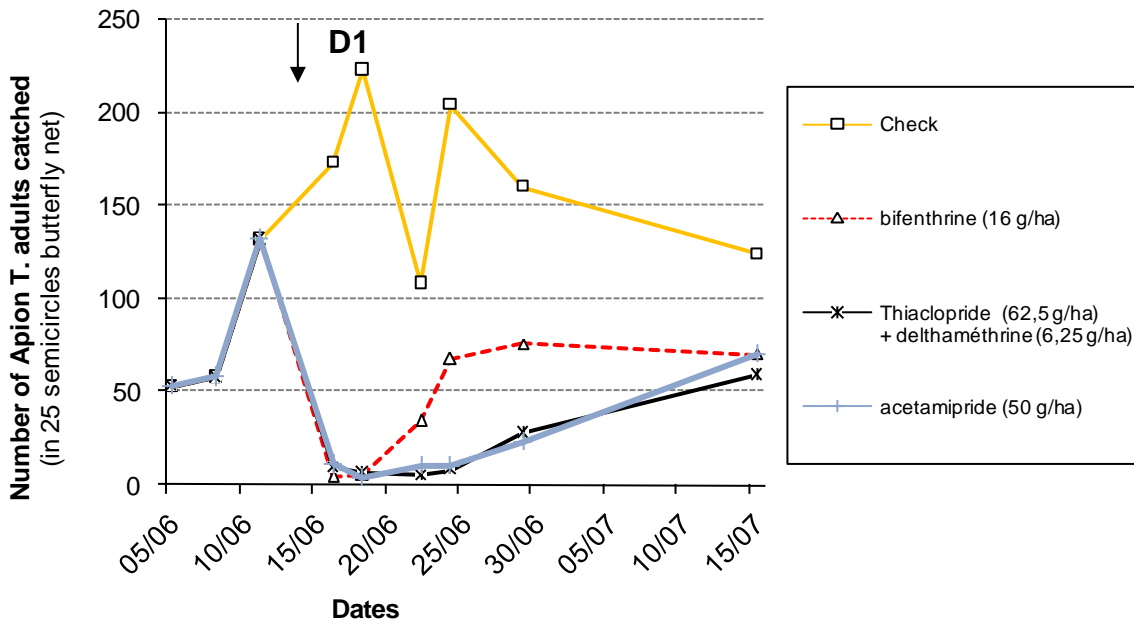
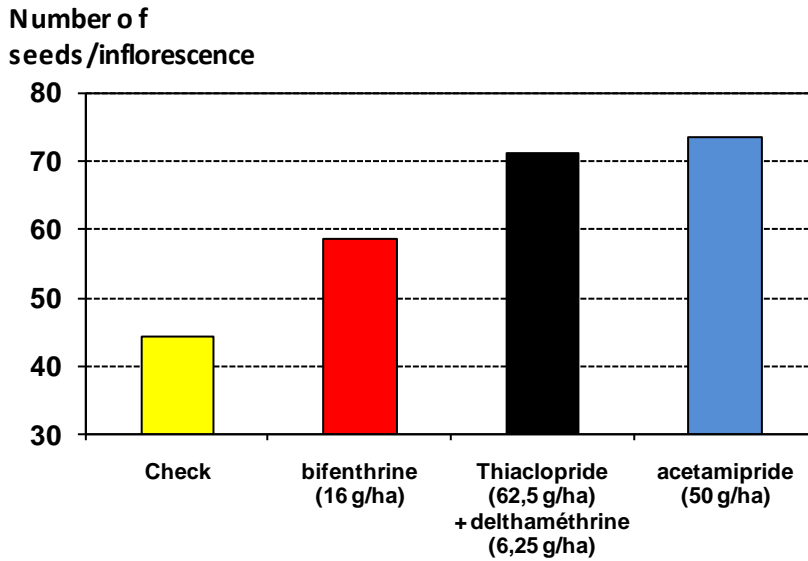


Figure 4: Insecticide effects on the number of seeds /inflorescence in red clover seed production (FNAMS – Bourges, 2009) (Date of spraying : D1= 12/06/09)



- *Spinosad* trials:

*Spinosad* has been tested during four years at the rate of 96g. a.i. /ha (figure 2) (this active ingredient could be used also for organic crop). It shows an incomplete control of seed weevils

population. However, in spite of this lower efficacy level on adult, it gives interesting results on seed yield with a number of seed/inflorescence similar to the reference *bifenthrine*. Looking at these effects, there is one possible explanation: *spinosad* has ovi-larvicidal activity which has been proved in 2008 with a late application. Seed yield with a normal date of application of *spinosad* are the same as those obtained with a late application. That means the threshold to apply *spinosad* could be larger than with a standard pesticide. But without bee label, *spinosad* could be used only before flowering time.

**Conclusion:**

These results provide new chemicals solutions to avoid *Apion Trifolii* damage which need to be registered for red clover seed production. For organic seed production, no alternative method has been found and only *spinosad* could be used before flowering time.

# **Evaluation of Palisade (trinexapac-ethyl) on fifteen Kentucky bluegrass varieties grown for seed in central Oregon**

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## **Abstract**

The growth regulator, Palisade (trinexapac-ethyl), was evaluated on 15 Kentucky bluegrass (*Poa pratensis* L.) varieties grown for seed at the Central Oregon Agricultural Research Center. One set of plots is a first-year stand and the other is a second-year stand. The influence of Palisade on seed yield, plant height, and lodging were documented. Treatments were applied at the boot stage and varieties were harvested based on maturity. Seed yields were significantly increased for 12 of 15 varieties in the first year field, and 4 of 15 in the second year field. Application of Palisade uniformly reduced plant height and decreased lodging, with the exception of one data point.

## **Introduction**

Research to evaluate Palisade on Kentucky bluegrass was conducted in commercial seed fields of 'Merit' or 'Geronimo' from 1999 to 2003. Yields were increased by 31 to 36 percent 4 of the 5 years when Palisade was applied at 22 oz/acre from the second node (Feekes 7) to heads just becoming visible (Feekes 10.1). Late application when the heads extended just above the flag leaf (Feekes 10.4) produced the greatest reduction in plant size, while plants tended to outgrow the effect of earlier Palisade applications. No differences between treatments in weight per 1,000 seeds were observed, and percent germination was not adversely affected.

## **Methods and Materials**

This research initiated in 2007 was conducted at the Central Oregon Agricultural Research Center (COARC) near Madras. A split-plot design was used, with 10-ft by 60-ft main plots and 3 10-ft by 20-ft subplots. Subplots were randomized and included Palisade, Beacon (primisulfuron), and an untreated check in the first-year field planted to 2008, and Palisade and an untreated check in the second-year field planted during 2007. Main plots were replicated four times in a randomized complete block design. Palisade was applied at 24 oz/acre on May 8, 2009 when most varieties were in the boot stage.

Application was made with a CO<sub>2</sub>-pressurized, hand-held boom sprayer at 40 psi and 20 gal/acre water using TeeJet 8002 nozzles. Plant height was measured in the first-year field on May 22 and June 3 and in the second-year field on May 27 and June 3. Percent lodging was estimated on July 1. A section 6-ft x 17 ft of each plot was swathed as varieties matured from July 2 to 9. This was followed by combining of the plots at an appropriate timing. Equipment used was a plot-sized swather and Wintersteiger plot combine. Seed samples were transported to the Hyslop Farm near

Corvallis where they were debearded, run through a small scale Clipper cleaner, and clean seed weight was determined.

## **Results and Discussion**

### *1<sup>st</sup> Year Field*

Seed yield on the first year field was significantly higher for 12 of the 15 varieties treated with Palisade (Table 1). Yield across all varieties averaged 35 percent higher than the untreated check. Average yield for untreated plots across varieties was 1025 lb/acre compared with 1340 lb/acre for those treated with Palisade, with varieties varying from a 12 to 92 percent increase. The trend was a reduction in plant height of 2.0 inches on May 22 and 2.6 inches on June 3, with a 37 percent reduction in lodging across varieties.

### *2<sup>nd</sup> Year Field*

Seed yield on the second year field was significantly higher for 4 of the 15 varieties, with the trend for increased yields (Table 2). The exception was the variety 'Crest'. The average yield increase with Palisade across varieties was 22 percent. However, seed yields on the second year field were down significantly from last year, with an average of 702 lb/acre for the untreated plots compared to 822 lb/acre for those treated with Palisade. It is believed this overall reduction in yield was due to a lack of control for winter grain mite and ergot. The trend was a reduction in plant height of 3.4 inches on May 27 and 4.5 inches on June 3, with a 34 percent reduction in lodging across varieties.

As a first year field last year, this field produced an average yield across varieties of 1266 lb/acre for the untreated plots and 1383 lb/acre for plots treated with Palisade. The average yield increase across varieties was 11 percent for Palisade treated plots. The trend was a reduction in plant height of 1.3 inches, and a 44 percent reduction in lodging across varieties.

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**Table 1.** Effect of Palisade growth regulator on seed yield, lodging, and plant height on a first year field of 15 Kentucky bluegrass varieties planted August, 2008 at the COARC, Madras, Oregon.

Variety	Clean seed yield (lb/acre)				Evaluation Dates					
					7/1/09		5/27/09		6/3/09	
	Check	Palisade	% Check	Signif.	Lodging (%)		Plant ht (in)		Plant ht (in)	
Atlantis	1206	1516	126	***	69	43	15.3	13.3	27.8	25.3
Merit	1305	1602	123	**	66	11	13.0	10.5	23.0	19.0
Rhapsody	888	1101	124	*	26	3	14.0	10.8	24.3	19.3
Valor	776	942	122	ns	63	8	11.3	9.5	19.0	14.0
Bariris	614	1179	192	***	100	93	17.0	16.0	26.5	25.3
Crest	1261	1467	116	*	48	8	12.3	10.0	21.8	19.3
Monte Carlo	743	911	123	ns	50	16	11.3	9.8	19.0	15.8
Shamrock	1682	1918	114	**	83	28	18.0	14.3	27.8	25.5
A00-891	1311	1712	131	***	58	16	14.3	14.0	21.3	21.3
A00-1400	663	1012	153	***	59	31	12.0	10.3	23.0	20.0
Bandera	1060	1190	112	ns	15	3	11.5	9.0	21.3	17.3
Bordeaux	890	1302	146	***	81	13	16.0	11.5	25.0	21.3
Volt	1211	1514	125	***	85	48	19.3	16.5	28.3	26.0
Zinfandel	783	1216	155	***	66	15	14.0	12.5	21.8	19.5
A01-299	981	1529	156	***	46	19	15.5	15.8	22.8	24.3

<sup>1</sup> Comparison with paired t-test: ns=non-significant, \* for P=0.10, \*\* for P=0.05, \*\*\* for P= 0.01

**Table 2.** Effect of Palisade growth regulator on seed yield, lodging, and plant height on a second year field of 15 Kentucky bluegrass varieties planted August, 2007 at COARC, Madras, Oregon.

Variety	Clean seed yield (lb/acre)				Evaluation Dates					
					7/1/09		5/27/09		6/3/09	
	Check	Palisade	% Check	Signif.	Lodging (%)		Plant ht (in)		Plant ht (in)	
Atlantis	1002	1049	105	ns	23	3	17.0	14.0	25.8	20.5
Merit	860	884	103	ns	13	1	13.3	9.8	21.3	15.8
Rhapsody	412	661	160	**	15	0	12.0	10.3	18.8	14.8
Valor	487	584	120	ns	31	0	11.3	7.5	17.0	11.3
Bariris	203	344	169	ns	85	60	20.0	14.3	27.0	24.5
Crest	838	708	84	ns	25	0	13.8	10.0	20.8	15.3
Monte Carlo	561	636	113	ns	71	6	11.3	9.5	17.0	13.0
Shamrock	818	928	113	ns	69	14	19.0	14.0	27.0	22.3
A00-891	951	1187	125	*	30	3	12.5	9.8	21.0	17.3
A00-1400	480	657	137	ns	56	10	12.3	11.3	17.8	15.5
Bandera	655	748	114	ns	24	5	13.8	10.3	20.3	15.8
Bordeaux	751	757	101	ns	53	10	15.0	10.0	24.5	16.5
Volt	950	1133	119	ns	81	10	20.3	16.0	28.0	23.8
Zinfandel	732	980	134	**	41	16	12.0	9.3	16.8	12.0
A01-299	825	1070	130	**	33	1	15.0	11.5	19.3	17.3

<sup>1</sup> Comparison with paired t-test: ns=non-significant, \* for P=0.10, \*\* for P=0.05, \*\*\* for P= 0.01

## Variations on potential and harvested seed yield of *Lotus tenuis* sown at different densities during spring and fall.

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*Lotus tenuis* is a forage legume broadly adopted by beef cattle breeders in the Flooding Pampas of Argentina, because of its positive attributes like high nutritive value, non bloating effect, natural re-seeding, and tolerance to grazing, flooding and alkalinity conditions. In Argentina, national production of certified seed of *L. tenuis* evolved from 10 t in 1999 to 84 t in 2007. Although seed yields under commercial production are low (< 200 kg ha<sup>-1</sup>), but yields of 1200 Kg seed/ha are possible when grown under non stress conditions. Forage seed crops are currently planted in the fall at ca. 6 Kg Ha<sup>-1</sup> in this region, and there is no information on how planting date and plant density affect seed yield for this species. The main goal of this work was to analyze the influence of plant density on potential and harvested seed yield of *Lotus tenuis*, in spring and fall crops. A field experiment was carried out in the Estación Experimental Agropecuaria Balcarce (INTA, 37° 45' S, 58° 18' W; 130 m.s.n.m ). Nine 2.1 m x 6 m plots (17.5 cm wide rows) were planted in the spring of 2006 (September 21<sup>th</sup>) and the autumn of 2007 (April 12<sup>th</sup>) in a Typic Argiudoll Soil. The plots were maintained free of weeds, harmful insects and irrigated to keep soil moisture. After emergence, the seedling stands were hand-thinned attaining three plant densities: 26, 43 and 87 pl m<sup>-2</sup> (low, medium and high, respectively). Thus, a 3 densities x 3 blocks (related to topography) design was established and analyzed with statistical software R at each planting date. A 56 cm diameter wired circle was used to delimit a 0.25 m<sup>2</sup> harvest area. Two sub samples per plot were harvested at maturity (>50 % of mature brownish pods in a 0.33 m<sup>2</sup> area), clipping with hand-scissors and leaving a ca. 3 cm high remanent herbage. The effect of three plant densities on potential and harvested seed yield was analyzed at each planting date. Potential seed yield was estimated by means of a linear relationship between pods weight and seed number. Seed yields increased with plant density, for both spring and fall crops (**Table 1**). The highest potential seed yield was recorded in spring crops which almost doubled fall crops yields for all the densities. This finding also suggests the presence of a wider gap between harvested and potential seed yield when *Lotus* is sown in the spring. According to these results the gap between harvested and potential seed yield is smaller in fall crops of *Lotus tenuis* sown at high densities. Spring crops with low percentage of pod dehiscence and timely harvest are needed to reduce this gap.

**Table 1.** Potential and actual seed yield of *Lotus tenuis* (kg ha<sup>-1</sup>) for three plant densities and two planting dates. Means with equal letter indicates no significant differences between densities within sowing date (Tukey HSD at 5 %).

Planting Date	Density	Seed Yield (kg ha <sup>-1</sup> )	
		Potential	Harvested
Spring	low	655.2 <b>b</b>	222.5 <b>b</b>
	medium	1174.2 <b>a</b>	508.7 <b>a</b>
	high	1413.9 <b>a</b>	523.8 <b>a</b>
Fall	low	342.6 <b>b</b>	295.6 <b>b</b>
	medium	485.4 <b>b</b>	427.8 <b>b</b>
	high	766.9 <b>a</b>	706.4 <b>a</b>

# Determination of optimum desiccation timing in white clover seed crops

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## Abstract

Time of harvest experiments, defined as date of desiccation, were carried out at three sites on the Canterbury plains, in Lincoln, Leeston and Chertsey. Crop management was carried out by the host farmer up to desiccation. Plots were desiccated at weekly intervals beginning three weeks after peak flowering and extending to eight weeks post peak flowering. Harvest consisted of two strips cut by a rotary lawn mower, dried and threshed and yields calculated as kg ha<sup>-1</sup>. There was a linear yield increase of 40% from weeks 3 to 7. Based on thermal time optimum harvest date was approximately 725 C° days after peak flowering.

## Introduction

White clover (*Trifolium repens* L.) is an important crop on arable farms in the Canterbury region of New Zealand where it provides an ideal break crop in the wheat, grass seed and vegetable seed crops rotation. However white clover seed crops can be highly variable in their harvested seed yield which can have large effects on profitability. Common yields range from 200 to over 1200 kg ha<sup>-1</sup> depending on grower management and weather conditions experienced during the summer period. Commonly white clover is desiccated prior to harvest using diquat (600g active ingredient ha<sup>-1</sup>) and combine harvesting follows 3-5 day later. The timing of desiccation is often a difficult decision due to the indeterminate nature of white clover where flowering often occurs over several weeks. General guidelines to the harvesting of herbage seeds were published by Simon et al. (1997) which include suggestions on harvest timing including days after flowering, seed color and seed moisture content etc.

The use of thermal time has been successfully used in computer simulation to estimate the time from anthesis until maturity e.g. Sirius wheat model (Jamieson et al. 1998). Within the Sirius wheat model the time from anthesis until maturity is split into three sections, i) anthesis to the beginning of grain fill, this is based on one phyllochron. ii) Grain filling, typically based on 550 C° days (base temperature 0 °C) with thermal time based on air temperature. iii) An interval of 200 C° days is allowed for the grain to dry down after the end of grain filling. Data supporting the use of thermal time on cereals in relation to seed development has also been presented by Loss et al. (1989) and reviewed by Hay and Kirby (1991). Moot (2000) suggested thermal time alone gave the best correlation for the prediction on the seed filling period in 32 different brassica cultivars when trialed under Canterbury growing conditions.

The aim of the current study was to investigate if the timing of desiccation (quantified using days and thermal time) and harvest timing have any effect on the harvested seed yield in white clover.

## Methods

Trial sites were selected in two farmer fields and the Chertsey FAR demonstration site. The sites and cultivars were:

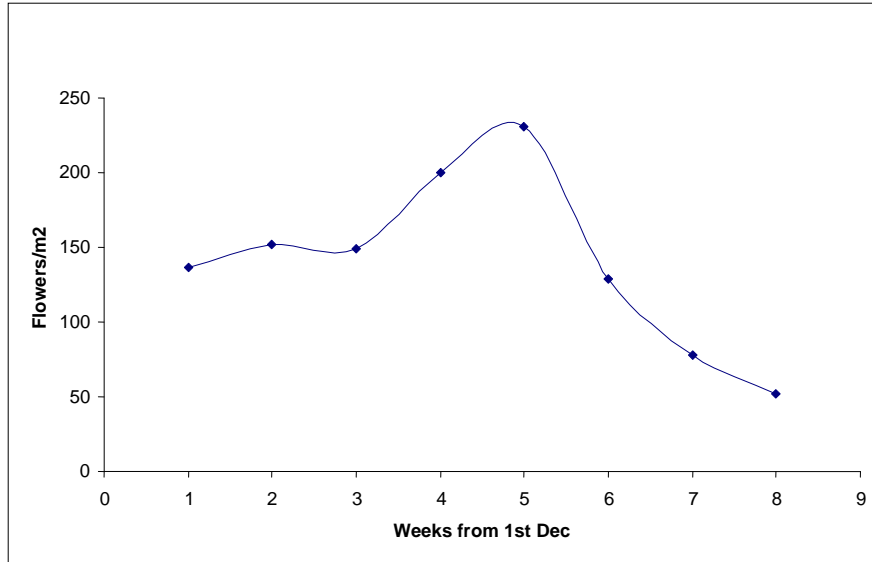
- i. Leeston on a good soil with cv Riesling, a mid-large leaf-size cultivar
- ii. Lincoln on a heavy soil prone to winter water logging and more exposed to cool easterly winds in summer with cv Tribute, a medium leaf size cultivar,
- iii. Chertsey on a shallow Templeton soil with cv Tribute.

Flower numbers were counted weekly with a 0.25m<sup>2</sup> quadrat, with two counts per plot, one at each end of the plot. Flowers were counted if they had 3+ open florets and had less than half the florets turned down from pollination. Temperatures were recorded at the nearest available weather station, for Lincoln this was located approximately 5 km from the trial site, for Leeston temperature data was sourced from Pendarves, approximately 16 km away and at Chertsey a weather station was located approximately 150 m from the trial area.

Prior to harvest all management was undertaken by the host farmer. Plots were desiccated at weekly intervals and harvested with two strips cut by a rotary lawn mower, dried and threshed. Yields from these plots were adjusted relative to the mean yield of an adjacent machine harvested topping trial. Statistical analysis of data used GenStat v9.

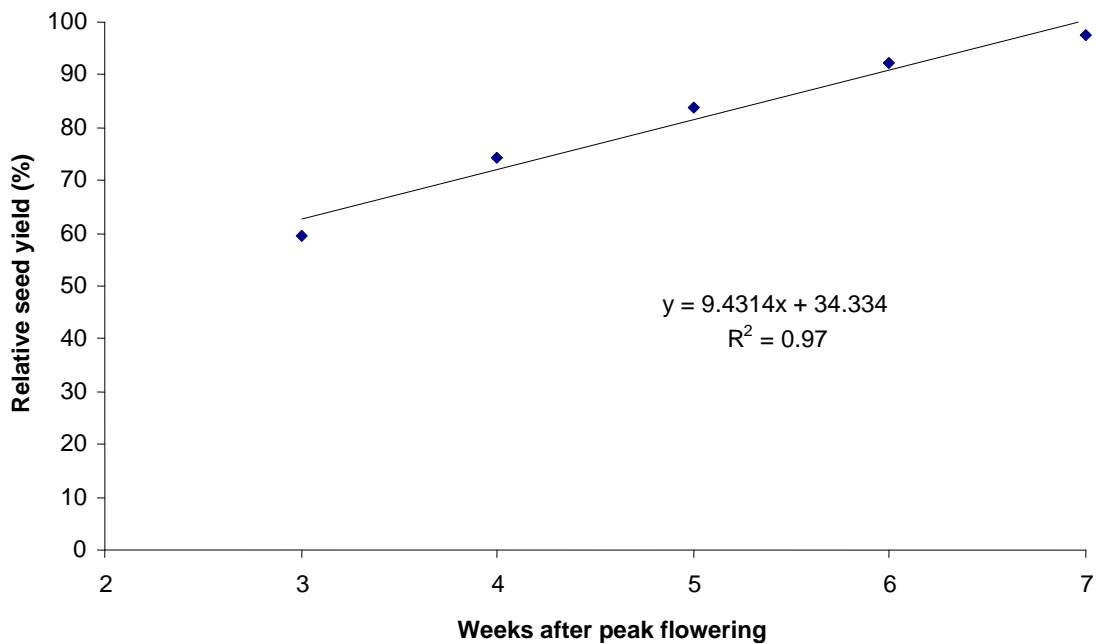
## Results

Flower head production increased per week at all sites and peaked approximately 5 weeks after December 1<sup>st</sup>, for example at the Leeston site flowering peaked at 250 flower heads/m<sup>2</sup> on the 5<sup>th</sup> January (Figure 1.).



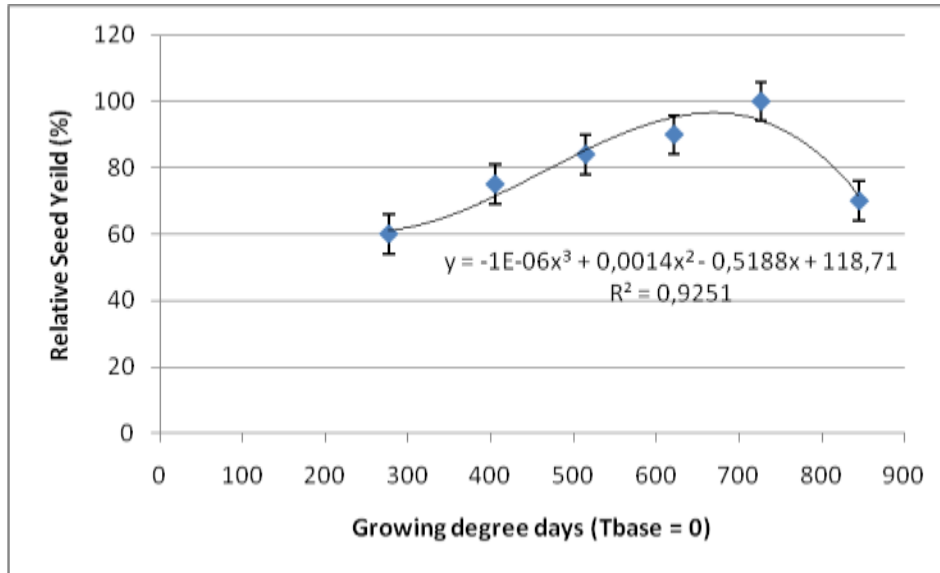
**Figure 1.** Weekly flower counts for time of harvest trial at Leeston cv Riesling (peak 5 Jan).

Combined data using weeks three to seven from peak flowering showed a linear increase ( $R^2=0.97$ ) in seed yield, with relative yield increasing at 9% per week between three and seven weeks after peak flowering (Figure 2).



**Figure 2.** Combined data for seed yield (relative to an adjacent trial) and five harvest dates for weeks 3 to 7 past peak flowering.

When the data for week 8 is added (data from two sites available) the response is a quadratic response with an optimum harvest time of six weeks after peak flowering. The decline in seed yield eight weeks after harvest is large and significant. Based on thermal time, optimum harvest date is approximately 725 C° days after peak flowering (Figure 3). There was some variation between sites in weekly temperature accumulation however, at week 7 only 40 C° days separated all sites.



**Figure 3.** Relative seed yield (three sites combined) (optimum = 100%) and five harvest dates expressed against thermal time from peak flowering (2006/07) (harvest date = desiccation date).

## Discussion

Flower numbers gradually increase during late November, followed by a rapid increase in late December / early January. Harvest generally occurs in February, the exact timing depending on soil moisture and other environmental conditions e.g. temperature. Based on thermal time, optimum harvest date is approximately 725 C° days after peak flowering. In 2006/07 this was approximately seven weeks after peak flowering. Seed development is generally well represented by thermal time. This result was consistent across three sites with two different cultivars and should be transferable from season to season and site to site. It is assumed that thousand seed weight (TSW) and number of seeds harvested account for the increases in seed yield. Loss of seeds through decomposition and sprouting are likely to contribute to the lower yields after seven weeks.

The timing of desiccation should occur when the maximum number of seed heads are ripe and likely to be harvested. Premature desiccation will lead to lower seed yields and lower TSW. Generally the majority of seed should be changing color at the time of desiccation. If desiccation occurs too late lower yields will result. Our results indicate that 725 C° days predicts this optimum time.

## Conclusions

- Harvesting too soon after peak flowering reduced seed yield. The optimum time was six to seven weeks after peak flowering, with seven being best. Waiting 8 weeks resulted in yield loss.
- Seven weeks was equivalent to 725 C° days (base temperature 0°C) at all sites

### **Acknowledgement**

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## Seed yield responses to climate

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Field data from more than 2000 farmers in the period from 2002 to 2006 has been used to analyse how different climate conditions affect seed yield. The field data consists of different cultivars of perennial ryegrass (*Lolium perenne* L.) grown at different soil types, in different climatic regions and using different management systems. The climate data consists of temperature, radiation and precipitation and soil available water content was calculated. Climate data used in the experiment was taken from 40 \* 40 km grids in Denmark.

The results showed that especially radiation up until harvest was a very important factor to describe seed yield. There was a positive effect of higher radiation 1 to 5 weeks before harvest and the highest effect was the week before harvest. The results furthermore showed a negative effect of a high radiation during the winter, but a positive effect of high temperature in the same period. The positive effect of high radiation shortly before harvest was not surprising. The negative effect of high radiation during the winter while there was a positive effect of high temperature seems contradictory but might be explained by the effect of high temperature on soil temperature.

These results are interesting for the farmers as it shows the importance of taking the optimum harvest day. From a more scientific point of view, the results are important for the breeders as it shows that utilization of radiation in the ears should be the focus.

# Annual ryegrass seed production in acidic soil

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## Abstract

A three-year study of lime application for annual ryegrass seed production in Oregon, USA was completed in 2008. Lime application significantly increased the three-year average seed yield ( $p=0.05$ ) on a field with an initial pH of 4.2. The average seed yield with no lime application was  $2815 \text{ kg ha}^{-1}$ , compared to  $3070 \text{ kg ha}^{-1}$  for annual banded lime application of  $165 \text{ kg ha}^{-1}$ ,  $3050 \text{ kg ha}^{-1}$  when  $5,600 \text{ kg ha}^{-1}$  lime was applied preplant, and  $3175 \text{ kg ha}^{-1}$  when  $11,200 \text{ kg ha}^{-1}$  lime was applied preplant. KCl extractable aluminum (Al) increased exponentially as soil pH decreased and seed yield increased linearly as KCl extractable Al decreased.

## Introduction

Annual or Italian ryegrass (*Lolium multiflorum* Lam.) is grown on approximately 50,000 ha in the southern Willamette Valley of Oregon, primarily on moderately and poorly drained acidic soils. The soil pH in fields typically is below 5.5 (2:1 soil water), the value at which lime is recommended. Annual ryegrass forage production studies in Texas, Louisiana, and Florida have shown an increase in production when lime was applied to fields with a soil pH below 5.0, especially on sandy loam soils (Haby 1995). Despite acidic soil in western Oregon, seed yields comparable to or greater than the industry average ( $2,240 \text{ kg ha}^{-1}$ ) are commonly obtained at strongly acidic pH levels ( $<4.5$ ) that would limit seed production of perennial ryegrass and other perennial seed crops in the area.

Seed producers are cautious about purchasing lime since it is expensive. An alternative strategy is to band granular lime at planting. In theory, granular lime will neutralize acidity in the zone of germination, improve seedling growth and establishment, and ultimately maintain seed yields on low pH soils; however this benefit has never been documented in Western Oregon seed fields.

The purpose of this trial was to: (1) evaluate the changes in soil chemical properties and annual ryegrass seed yield from lime application on acidic soil and (2) compare seed yield when granular lime is banded at a low rate to traditional broadcast lime application.

## Material and Methods

A field was selected for this study with an initial pH of 4.2 (2:1 soil water), yet had a history of  $2,500 \text{ kg ha}^{-1}$  or greater annual ryegrass seed yield. This yield is above the industry average for

Oregon. The field had been in production of annual ryegrass (cv. Gulf) for over 30 years, managed mostly under a conventional tillage system where the full straw load was flail chopped and worked back into the soil each year. The field had never been limed. Gulf annual ryegrass was the variety grown historically in this field, and was the most commonly grown diploid annual ryegrass cultivar in Oregon. We continued with this variety during the trial period.

In August of 2005, 5,600 and 11,200 kg ha<sup>-1</sup> of paper mill by-product lime were applied and preplant incorporated to a depth of 12 cm. At planting, treatments receiving no lime and 165 kg ha<sup>-1</sup> granular lime (trade name CalPril) annually applied in a band were established. All lime treatments are expressed as 100 score material. Soil pH, 1 N ammonium acetate extractable Ca, and KCl extractable aluminum were measured during the experimental period. The trial was arranged in a randomized complete block design with three replications. Individual plots were 18 m wide by 125 m long. The annual ryegrass cv. Gulf was planted in September. Seed yield was measured for three years. Grower equipment was used for harvest by first making a 5 m swath the length of center of each plot, allowing the grass to dry, and threshing with a combine. A weigh wagon was used to measure plot yields. Sub-samples of the harvested seed were collected to determine 1000 seed weight, percent cleanout, and calculate total clean seed weight.

## Results and Discussion

Lime application produced a small but significant increase, 230-360 kg ha<sup>-1</sup>, in annual ryegrass seed yield, Table 1. In spite of a 4.2 soil pH in the treatment receiving no lime, seed production was 2820 kg ha<sup>-1</sup>, which is 25% above the regional average. The application of granular lime and 5,600 kg ha<sup>-1</sup> by-product lime produced the same yield statistically. The greatest seed yield, 3180 kg ha<sup>-1</sup>, was obtained from the incorporation of 11,200 kg ha<sup>-1</sup> of lime.

**Table 1.** The changes in three year average seed yield, soil pH and extractable Ca from lime applications on annual ryegrass seed yields on a strongly acid soil in Western Oregon, USA

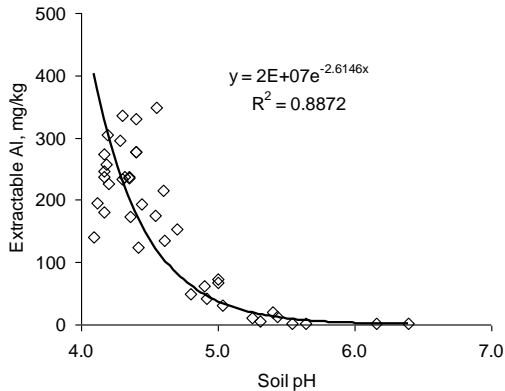
Lime Rate	pH		Ca		Seed Yield
	10/05	06/08	10/05	06/08	
kg ha <sup>-1</sup>			--- cmol/kg ---		kg ha <sup>-1</sup>
0	4.2	4.4	2.1	2.2	2815
165 <sup>2</sup>	4.2	4.3	1.8	2.1	3070
5,600 <sup>1</sup>	5.4	4.7	6.1	4.4	3050
11,200 <sup>1</sup>	6.0	5.1	11.3	6.5	3175
P Value	0.0007	0.0122	0.0067	0.0035	0.0066
LSD (0.05)	0.24	0.16	1.86	0.77	155

<sup>1</sup> By-product, lime score of 72, applied 20 August, 2005 to provide an equivalent amount of 100 score lime.

<sup>2</sup> Granular lime \$250 USD tonne and byproduct lime \$65 USD tonne

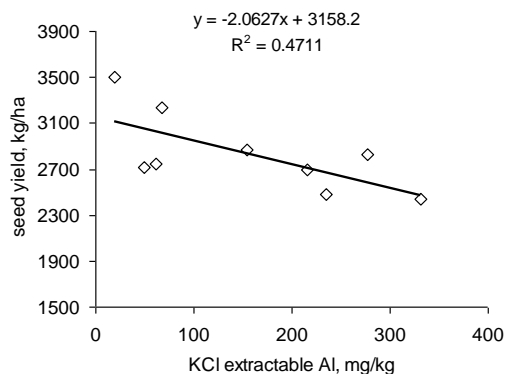
Soil pH and extractable Ca were increased by the conventionally applied lime treatments. The 11,600 kg ha<sup>-1</sup> lime rate raised the soil pH from 4.2 to 6.0 in the first season following application. The 11,200 kg ha<sup>-1</sup> preplant lime treatment increased soil pH and Ca to levels

considered adequate in the Oregon State University nutrient guide for annual ryegrass seed production (Hart *et al*, 2003). The conventional lime treatments maintained soil pH and Ca values above those from the untreated plots for the three-year period of this study. Soil pH and Ca levels from the conventional lime treatments decreased with time due to annual plowing and mixing of lime plus acidification associated with ammonium-N application. The band application of granular lime did not change soil pH or Ca. This outcome was expected as the rate of application was low.



**Figure 1.** KCl extractable Al change with 2:1 soil:water pH

Aluminum (Al) toxicity is considered a primary plant growth limiting factor for strongly acidic soils. As soil pH decreased, extractable Al increased exponentially, Figure 1, and grass seed yield decreased linearly, Figure 2.



**Figure 2.** Annual ryegrass seed yield change with KCl extractable Al. Data from treatments receiving no lime and both rates of broadcast lime in 2008.

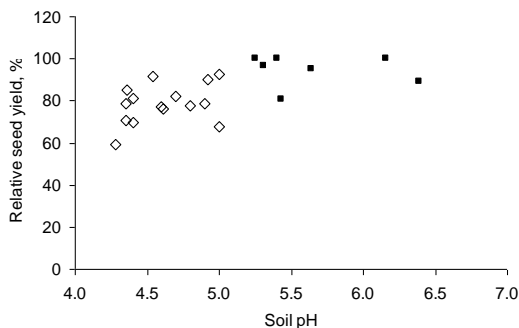
The soil pH at which Al becomes toxic to plants is dependent on the soil, plant species, and variety grown. In Willamette Valley soils, a pH of 4.7 has been considered a threshold level where Al concentration begins to increase exponentially and affect the growth of grass roots in forage and seed production systems. The increase in extractable Al measured in this trial also

showed a sharp increase at approximately pH 4.7, Fig. 1. At soil pH 4.7, the extractable Al was approximately 100 mg/kg. Maximum annual ryegrass seed yield was measured when the extractable Al was below 100 mg/kg, supporting the choice of a soil pH 4.7 as threshold value for sufficient Al to limit root growth in this area.

Even when KCl extractable Al was three times the amount where toxicity was thought to affect root growth, 300 mg/kg, seed yields were above the industry average of 2240 kg ha<sup>-1</sup>. One possible reason that seed yield was maintained under these conditions was an Al-complex by organic acids, thus ameliorating the effect of Al toxicity on root growth. This explanation is possible since total soil C at the site was 2.5 %. Another possibility is that the Gulf annual ryegrass cultivar grown in Oregon has developed tolerance to lower pH conditions. The seed stock of Gulf annual ryegrass used in this trial came from fields with similar low soil pH. These are plausible reasons for the annual ryegrass to grow well in acidic conditions.

Even though a reasonable relationship exists between KCl extractable Al and annual ryegrass seed yield, use of extractable Al to predict lime need is not recommended. The test is not universally available and critical Al levels are expected to vary with soil and crop. The strong relationship between KCl extractable Al and soil pH shown in Figure 1 shows that soil pH is an adequate indicator of the amount of Al in the soil and therefore, need for lime.

Data from the last two years of this project can be used to show that soil pH adequately indicates lime need. The relative seed yield and soil pH data was sorted into two groups, above 5.3 and below 5.3. The two groups of data plotted in Figure 3 support the OSU recommendation that lime is needed when the soil pH is below 5.5. Yield decreases as soil pH decreases when the pH is below 5.5 and yield does not change as the soil pH increases when the pH is above 5.5.



**Figure 3.** Annual ryegrass relative seed yield as changed by soil pH below 5.3, open diamonds, and above 5.3, solid squares.

Annual ryegrass seed yield increases with lime application on strongly acid soils. A low rate of granular lime is an economical option for maintaining seed yield when the soil pH is below 5.5. Conventionally incorporated lime applications, provide greater assurance of increasing soil pH, reducing extractable Al, and increasing seed yields on acidic soils of the Willamette Valley.

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## **A primary study on seed dormancy mechanism and breaking technique of *Leymus chinensis***

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### **Abstract**

*Leymus chinensis*, commonly known as alkali-grass, is an ecologically and economically important fodder grass due to its high nutritional value, palatability, high-yield and high tolerance of drought, salinity and low fertility (Huang *et al.*, 2002). It mainly was found in the eastern region of the Eurasian steppes, the northern and eastern parts of the People's Republic of Mongolia. In China, it is mainly distributed in the Northeast China Plain, the Northern China Plain, and the Inner Mongolia Plateau of China (Zhang *et al.*, 2007). Seed dormancy of *L.chinensis* is one of the most important factors limiting its culture and utilization. The present study investigated the seed dormancy mechanism and dormancy breaking technique of *L.chinensis* and the results showed that: Piercing naked seed compared with intact seed the germination rate, water absorption rate, viability by contrast, 6%, 63%, 0% significantly increased to 60%, 86%, 94%; Water soaking one day, 30% NaOH soaking 80min, soaking in 30% NaOH for 60 min after soaking with water for one day could increase germination rate of *L.chinensis* intact seeds significantly from 6% to 36%, 60%, 84%, while the different concentration of gibberellin treatment to intact seeds had no significant changes compared with the control; Seeds soaked with 30% NaOH by 60 min after soaked with water one day, and then imposed 200  $\mu\text{g/g}$  GA<sub>3</sub>, could germinate to 91%, close to the seed viability 94%. This study showed that glume and seed coat did not affect seed imbibition, but affected absorption of macromolecular substances such as GA<sub>3</sub>, and also some germination inhibitors leakage which led to seed dormancy. These results implied that palea, seed coat, and germination inhibitors contained in the seeds may play an important role in controlling seed dormancy of *L. chinensis*.

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# Kentucky bluegrass (*Poa pratensis* L.) germplasm for non-burn seed production

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## Abstract

This long-term study consists of 10 Kentucky bluegrass (*Poa pratensis* L.) entries; eight are USDA/ARS Plant Introduction (PI) accessions and two are commercial cultivars ('Kenblue' and 'Midnight'). The selected PI accessions had expressed high seed yield without burning of post-harvest residue and good turfgrass quality. Several agronomic yield parameters were then evaluated over a 2-yr period and individual plants were selected within each accession, or check, with the highest seed weight, highest seeds panicle<sup>-1</sup>, highest panicles area<sup>-1</sup>, and highest seed yield. Remnant seed of the original USDA/ARS population were also included. Turfgrass plots were established in 2006 and seed production plots (irrigated and non-irrigated) in 2007 at Pullman, WA. The turfgrass trial was evaluated monthly (2007 to 2009) according to NTEP (National Turfgrass Evaluation Program) protocol to determine turfgrass quality. In 2008 and 2009, seed production plots were harvested and seed yield was determined. Only 2009 results will be presented. The prior selection for seed yield components had a variable response and seed yield appeared to be dependent primarily on accession. Accession PI 368241 showed the best promise of being able to provide good turfgrass quality and good seed yield under non-burn management in both non-irrigated and irrigated production. Selection within Kenblue for seed head<sup>-1</sup> had good turfgrass quality and seed yield under non-irrigated production. These studies will be followed for several additional harvests to determine if a non-burn Kentucky bluegrass can be developed for sustainable grass seed production in the Pacific Northwest, USA.

## Introduction

Burning of Kentucky bluegrass seed production fields in the fall normally maximizes seed yield the following year. Open-field burning of post-harvest residue also allows seed fields to remain in production for many years (Canode, C.L. & Law, A.G., 1979). With the regulation of field burning in Washington, burning has become highly restricted (< 20 ha burned in 2009) and growers have been forced to utilize shorter rotations. Our work and others has shown that genetic variation in Kentucky bluegrass to improve grass seed production without burning (baling of post-harvest residue) does exist (Lamb, P.F. & Murray, G.A., 1999; Johnson *et al.*, 2003). To sustain grass seed production at economically viable levels, new bluegrass germplasm that maximizes yield potential for several years in non-burn management systems needs to be identified, selections made, germplasm enhancement carried out, and ultimately high seed yielding bluegrass cultivars capable of multiple harvests with good turfgrass quality be made available to growers.

## Materials and Methods

The Kentucky bluegrass accessions used came from the previous work of Nelson (1996), Johnston *et al.* (1997), Johnston, W.J. & Johnson, R.C. (2000), and Johnson *et al.* (2003). Eight PI accessions possessing good turfgrass quality and seed yield under mechanical residue removal were further evaluated in a space-plant nursery at Pullman, WA for two years (Johnson *et al.*, 2010) to identify, within each accession, the plant with the most panicles area<sup>-1</sup>, the plant with the highest number of seeds panicle<sup>-1</sup>, the plant with the highest seed weight, and the plant with the highest seed yield. A fifth category was seed from the original USDA/ARS population for each accession. This resulted in 50 entries (10 accessions x five selection criteria) used in this study. Turfgrass and seed production trials were established at the Washington State University (WSU) Turfgrass and Agronomy Research Center (TARC) at Pullman, WA. The soil was a Palouse silt loam (Pachic Ultic Haploxerolls, fine silty, mixed mesic).

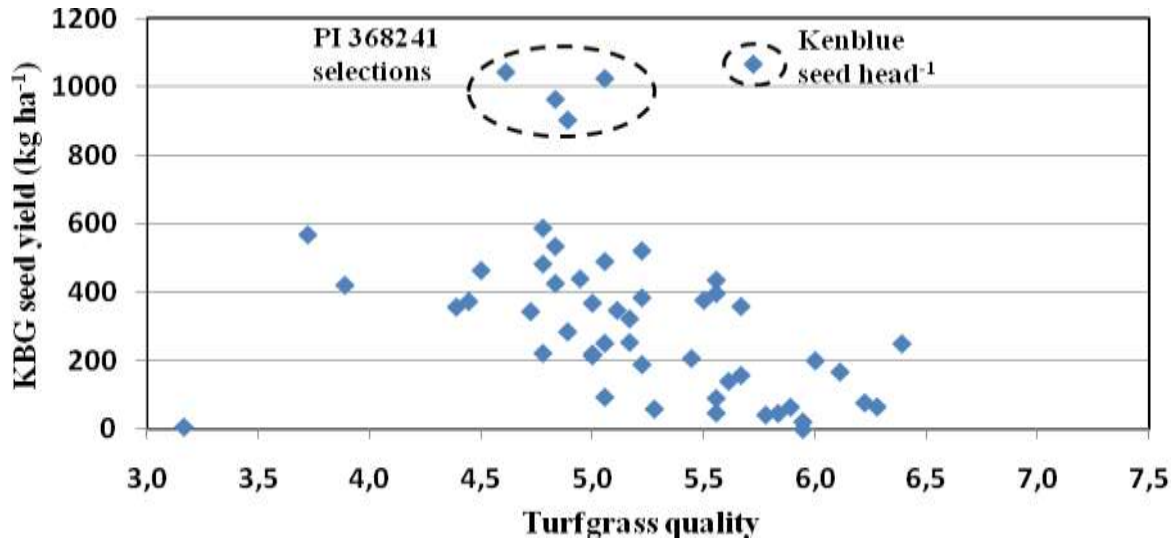
The turfgrass evaluation trial was planted September 2006. Each experimental unit was 2.25 m<sup>2</sup> and was seeded at 11 g m<sup>-2</sup>. The experiment was a randomized complete-block (RCB) design with three replications. Turfgrass quality ratings were based on National Turfgrass Evaluation Program (NTEP) protocol with a 1 to 9 scale, where 1 represented brown or dead turf and 9 represented ideal turfgrass quality. Two seed production plots were established in the summer of 2007 at the TARC. Whole-plot treatments consisted of two irrigation environments, irrigated and non-irrigated, and subplots consisted of the 50 entries with three replications. Each experimental unit was 2.1 x 1.8 m and consisted of seven rows spaced 30.5 cm apart (irrigated) and five rows planted on 35.5-cm-row spacing (non-irrigated). Seed production plots were harvested using a sickle-bar mower, residue put into cloth bags, and air dried. Panicles were threshed using a small-plot combine, hammermilled, and cleaned. Clean seed weight for each experimental unit was recorded. Analysis of variance was completed using SAS and means were separated with least significant differences (LSD) for all parameter with Fisher's Protected LSD ( $P = 0.05$ ), or using LS Means comparisons ( $P = 0.05$ ) (SAS Institute, 1990).

## Results and Discussion

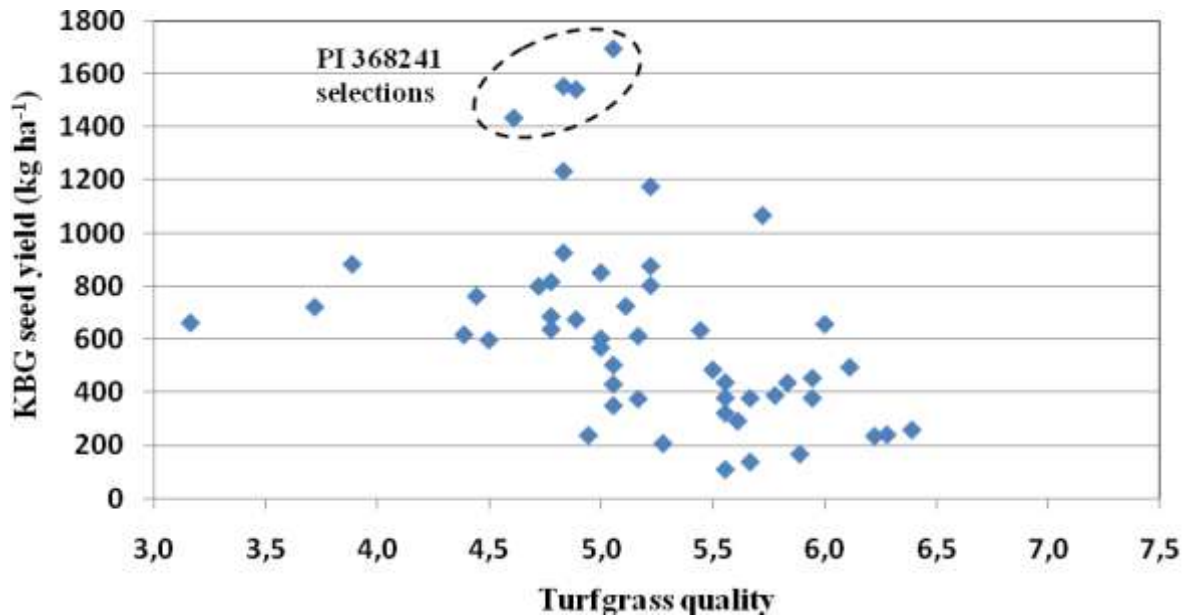
Since Kentucky bluegrass is a facultative apomictic species (Huff, D.R. & Bara, J.M., 1993), with the apomictic aspect dominating reproduction, uniformity is promoted from one generation to the next within a given genotype. However, the facultative component of Kentucky bluegrass reproduction allows for some genetic recombination (Huff, D.R. & Bara, J.M., 1993) and the introgression of new genes, albeit at a relatively low frequency. We have observed variation in plant type and seed yield within accessions (Johnson *et al.*, 2003), suggesting there is a potential for seed yield improvement by selecting within accessions (Johnson *et al.*, 2010).

From 2007 to 2009, the prior selection methodology (Johnson *et al.*, 2010) was evaluated to determine how to improve seed yield and turfgrass quality in Kentucky bluegrass. In 2009, non-irrigated and irrigated seed production plots planted in 2007 were harvested for the second year (2009 data presented). Turfgrass trials were evaluated for the third year (2009 data presented). In 2009, as in 2008, the selection for yield components had a variable response and appeared to be dependent on accession. Overall, there was an increase in seed yield due to irrigation; however, regardless of non-irrigated or irrigated seed production, accession PI 368241 continued to show promise of being able to provide good turfgrass quality and good seed yield under non-

burn residue management (Fig. 1 and 2). Under non-irrigated seed production, selection within Kenblue for seed head<sup>-1</sup> had good seed yield and turfgrass quality (Fig. 1). It is critical to follow these studies for several additional harvests to determine if a non-burn Kentucky bluegrass can be developed for sustainable grass seed production in the Pacific Northwest.



**Figure 4.** Kentucky bluegrass non-irrigated seed yield vs. turfgrass quality (rated 1-9; 9 = excellent quality) at Pullman, WA, 2009.



**Figure 2.** Kentucky bluegrass irrigated seed yield vs. turfgrass quality (rated 1-9; 9 = excellent quality) at Pullman, WA, 2009.

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## Comparing herbicide selectivity in field- and pot-grown grass seed crops

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Effective chemical weed control measures are a prerequisite for producing high quality grass seeds. Very few herbicides are registered for use in grass seed crops and weed control relies to a large extent on so-called off-label approvals that are not supported by the pesticide manufactures, i.e. the use is on the risk of the user. New weed problems are emerging continuously and particularly grass weeds are becoming an increasing problem in the grass seed production. Selectivity is the main issue when looking for new chemical solutions for weed control in grass seed crops. The documentation required for off-label approvals originates from field experiments paid for by the growers. The objective of the present study was to examine whether regular field experiments where yields are taken could be fully or partly replaced by a less costly and less time-consuming testing procedure based on pot experimentation and field experiments with logarithmic sprayers.

Experiments were conducted in the field with a range of broadleaved and grass weed herbicides using a logarithmic sprayer delivering an exponential decrease in the dose in response to time or applying 6 herbicide doses using a plot sprayer. Concurrently with the field trials we conducted pot experiments out-doors and in glasshouses examining the selectivity of the same herbicides under contrasting conditions by varying climatic conditions, soil type, growth stage of application etc. The hypothesis we would like to test was the following: by using a test protocol taking into account the parameters known to affect herbicide selectivity it is possible to predict field performance based on results from pot experiments or alternatively from field experiments with logarithmic sprayers. If confirmed this would allow a faster and less costly test procedure because non-selective herbicides could be omitted from the tests at an early stage and field testing of potential candidate herbicides could be reduced.

Two of the herbicides tested were Atlantis WG (30g/kg mesosulfuron-methyl+6 g/kg iodosulfuron-methyl-Na+90 g/kg mefenpyr-diethyl) and Agil 100 EC (100 g/L propaquizafop). Application of 10 g/ha Atlantis WG resulted in more than 80% crop damage on *Lolium perenne* L., *Festuca arundinacea* Shreb. and *Poa pratensis* L. while *Festuca rubra* L. and *Dactylis glomerata* L. were more tolerant (20-40% crop damage). The ranking of the 5 grass seed crops was similar in the field trials with the logarithmic sprayer but, in general, crop tolerance was higher in the field compared to the pots. In the regular field experiments application of 200 g/ha Atlantis WG caused yield reductions of 10 and 22% in *D. glomerata* and *F. rubra* whereas yield losses in the 3 other grass species ranged from 50 to 100%.

*In the pot experiments Agil 100 EC caused no damage to F. rubra, L. perenne was tolerant to low doses while D. glomerata, F. arundinacea and P. pratensis were severely damaged. A very good correlation was found between field experiments with a logarithmic sprayer and pot experiments regarding the ranking of the species as well as the dose rates tolerated by the crop. In the regular field experiments Agil 100 EC did not significantly reduce the yield of F. rubra, F. arundinacea, P. pratensis and L. perenne while D. glomerata yields were reduced to 0.*

It is concluded that pot experiments can be used to establish the ranking of susceptibility of the grass species to the herbicides but not the actual doses that can be tolerated by the crop under field conditions.

# Development of new tetraploid *Chloris gayana* cultivars with improved salt tolerance from ‘Callide’ and ‘Samford’

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## Abstract

*Chloris gayana* (Rhodes grass) is an outbreeding species with both diploid and tetraploid genotypes. Repeated mass selection for improved salt tolerance during germination and during growth and for forage quality was applied to 3 breeding populations derived from the tetraploid cultivars ‘Callide’ and ‘Samford’. Three new synthetic tetraploid cultivars, each based on elite single-plant clones selected from the final generation, were produced. ‘Sabre’ (10 clones, 5 generations) and ‘Toro’ (13 clones, 4 generations) were derived from ‘Callide’, while ‘Mariner’ (12 clones, 4 generations) was derived from ‘Samford’.

## Introduction

Rhodes grass (*Chloris gayana*) is among the more salt-tolerant subtropical/tropical pasture grasses, and is a major forage species, both in Australia and overseas (Loch *et al.*, 2004). It is well adapted to a wide range of medium-textured soils in the 600-1500 mm rainfall belt in the subtropics and tropics, and has been widely sown for the past 100 years in grazed pastures or short-term pasture leys, for hay or silage production, and for soil conservation and remediation. Rhodes grass is an outbreeding species with a great deal of between-plant variation in salt tolerance and other agronomic attributes, which could be exploited by breeding to tap market opportunities for salt-tolerant grass cultivars with good agronomic characteristics. The commercial success of ‘Finecut’ (bred in the early 1990s) demonstrates the gains that can come through targeted selection for improved agronomic attributes.

Over the past 30 years, the tetraploid cultivar ‘Callide’ has become the premier grazing type among the current Australian Rhodes grass cultivars, and seed is also sold into the Middle East where it is grown under irrigation for fresh forage and hay. Despite this, there has been no formal breeding work on ‘Callide’, which is quite variable in terms of flowering time, growth habit, leafiness, coarseness of stem, and many other agronomic attributes. Similarly, ‘Samford’ (which also produces high quality forage, but has not achieved the same market penetration as ‘Callide’) is derived from East African germplasm represented internationally by ‘Boma’ and ‘Elmba’. This paper reports on a breeding program to improve salt tolerance while at the same time selecting for improved agronomic attributes in ‘Callide’ and ‘Samford’.

## **Materials and Methods**

### *Breeding Strategy*

Mass selection was applied to seedling populations derived from ‘Callide’ and ‘Samford’ Rhodes grass (2001-06) with the aim of improving both the level of salt tolerance and the agronomic attributes. Based on first generation performance, seedlings selected from ‘Callide’ were divided into two breeding populations: one comprising leafy, early flowering (less daylength-sensitive) plants with a preference for fine-stemmed types; and the other based on leafy, late flowering plants, rejecting only the very coarse-stemmed types. Selection within the single ‘Samford’ population focused on leafy, late-flowering, finer-stemmed genotypes.

In the first ‘Callide’ generation, selection for salt tolerance related only to plant growth and survival under high salinity, and was followed by selection for improved agronomic characters. Selection for germination under saline conditions was then added, giving a three-stage selection process for generations 2-4(-5) in ‘Callide’ and for generations 1-4 in ‘Samford’: (i) germination under saline conditions; (ii) growth and survival under saline conditions; and (iii) agronomic performance under non-saline field conditions.

### *Breeding Procedures*

2000 ‘Callide’ seedlings were grown individually in a peat-vermiculite mix in 50 mm square tubes. At early tillering, the lower one-third of the tube was immersed in a NaCl solution, and the concentration progressively raised (0.2 M every 2-3 days) to 0.7 M NaCl, which was maintained for c. 2 months during which approximately 85-90% of the seedlings died. The surviving plants were then established on a spaced-plant grid for field agronomic evaluation.

Because salt tolerance for germination and salt tolerance for growth tend to be unrelated, screening for germination under saline conditions was applied to generations 2-4(5) in ‘Callide’ and generations 1-4 in ‘Samford’. Preliminary trials showed that germination was progressively delayed (by osmotic effects) and then reduced as the level of salt increased, reaching virtually nil germination by the 0.3 M NaCl level. At the start of each new generation, seed of the 3 breeding populations from the preceding generation was sown and lightly covered by sand in a “flood-and-drain” hydroponic system with 0.2 M NaCl plus complete plant nutrients. Despite heavy seeding rates, low numbers of germinated seedlings were obtained due to the heavy selection pressure exerted for salt tolerance. Following germination, the trays of young seedlings (up to c. 1-2 cm tall) growing in sand were placed in salt solutions to c. 3 cm depth. Salt levels were progressively increased to 0.7 M NaCl, and the most visibly tolerant seedlings later transferred to the field as spaced plants.

## **Results and Discussion**

Following 4 (or 5) cycles of selection, 3 new synthetic Rhodes grass cultivars (each based on 10-13 elite plants selected from the final generation) were constituted as shown in Table 1. In each case, the selected single-plant clones were divided vegetatively and established as balanced polycross breeder's blocks (each 500-1000 m<sup>2</sup>, with the component clones each contributing equally to the overall cultivar) for seed multiplication in isolation from all other Rhodes grass plants in north Queensland. The aim was to reduce the risk of genetic drift by taking only 2 of generations of seed increase to produce commercial seed.

**Table 1.** New synthetic Rhodes grass cultivars constituted following repeated mass selection.

<b>Cultivar</b>	<b>No. of clones</b>	<b>Origin</b>	<b>No. of generations</b>	<b>Agronomic selection criteria</b>
Sabre	10	Callide	5	Early flowering, dense, leafy growth habit; fine stems
Toro	13	Callide	4	Late flowering, dense, leafy growth habit
Mariner	12	Samford	4	Late flowering with a dense, leafy growth habit; fine stems

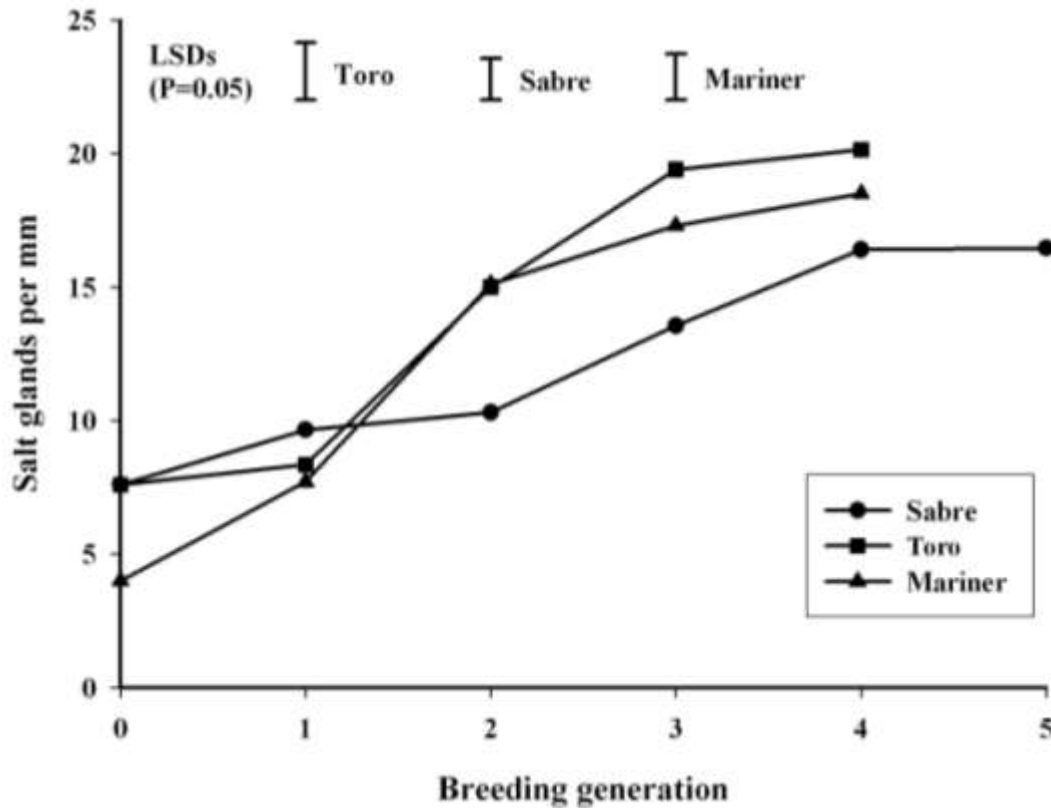
A growing trial to describe the new cultivars for Plant Breeder's Rights registration (Loch and Zorin, 2009) showed that 'Toro' and 'Mariner' are later-flowering and 'Sabre' earlier-flowering than 'Callide' and 'Samford'. Probably because of selection against extremely prostrate genotypes, 'Sabre' and 'Toro' spread laterally a little slower than 'Callide' and on average are more erect in habit. They produce stolons with shorter, thinner internodes and more branches at the nodes, and thinner culms than 'Callide'. 'Sabre' produces slightly shorter culms with fewer nodes and longer leaves than 'Callide' and 'Toro', and does not exert its inflorescences as far above the leaf canopy as the latter two cultivars. Stems of 'Mariner' are thicker than for 'Samford' (reflecting the removal of the finer-stemmed early-flowering genotypes through selection) and comparable to 'Sabre' and 'Toro'. The last 2 cultivars produce smaller inflorescences with fewer raceme branches and ('Toro' only) shorter racemes than 'Callide'. 'Mariner' produces larger inflorescences with longer racemes than 'Samford', comparable in overall size to 'Sabre', but with more (though shorter) racemes.

Additional work is on-going to document the levels of salt tolerance achieved through selection in the 3 new cultivars, both under controlled glasshouse and laboratory conditions and in the field. In this respect, the gains in salt tolerance reported by Malkin and Waisel (1986) from 5 generations of mass selection from 'Pioneer' Rhodes grass are encouraging. Plants vary in salinity tolerance at different developmental stages. However, available evidence from other species (e.g. Mano and Takeda, 1997) suggests that plants tolerant of salt during germination may not necessarily show a comparable level of tolerance during growth (and vice versa), hence the two-stage selection process for salt tolerance applied from the second 'Callide'

generation onwards. Other breeders have adopted similar approaches when selecting for salt tolerance (e.g. Rose-Fricker and Wipff, 2001; Rose-Fricker *et al.*, 2003).

In conclusion, it is interesting to speculate as to the possible mechanism(s) contributing to higher salt tolerance of Rhodes grass. While a number of possible factors have been identified (Loch *et al.*, 2004), the density of salt glands may be important as suggested by de Luca *et al.* (2001).

This is supported by counts of salt gland density made on the selected plants retained from each of the 5 breeding generations, showing that the density of salt glands in each case had been increased 2- to 3-fold by selection for salt tolerance (Figure 1). Nevertheless, more detailed studies of this and other salt tolerance mechanisms in Rhodes grass will obviously remain a fruitful avenue for other researchers in the future.



**Figure 1.** Changes in salt gland density in 3 Rhodes grass breeding populations over 4 or 5 generations of selection for salt tolerance.

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# Possibilities for use of new herbicides in selected grass species grown for seed in Czech Republic

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## Abstract

In small plot field trials conducted for three years the selectivity of some herbicides in selected grass species was studied. In the trials basic and double doses of herbicides were tested. Evaluations were made of potential phytotoxicity of herbicides in the grass species and their influence on number of fertile tillers, total seed yield, thousand seed weight, germination, germination energy and number of seed per fertile tiller. Based on trials (tritosulfuron 250 g kg<sup>-1</sup> + dicamba 500 g ha<sup>-1</sup>) applied at 0.2 kg ha<sup>-1</sup> and Aurora super SG (carfentrazone-ethyl 1.5 % + MCP-P 60 %) at 1 kg ha<sup>-1</sup> have been allowed for minor use in seed crops of perennial ryegrass, red fescue, cocksfoot, timothy, Kentucky bluegrass (KGB) and loloid as well as festucoid type of the festulolium. Arrat has been allowed for minor use also in meadow fescue. Husar (iodosulfuron 50 g kg<sup>-1</sup> + mefenpyr-diethyl 150 g kg<sup>-1</sup>) applied at 200 g ha<sup>-1</sup> shown good selectivity in KGB and cocksfoot but when it was applied in double dose the seed yield declined. In both types of the festulolium and timothy it is possible use the Callisto 480 SC (mesotrione 480 g ha<sup>-1</sup>). However, Callisto caused small chlorosis after application, mainly in loloid type of festulolium. Arkem (metsulfuron 200 g ha<sup>-1</sup>) at 30 g ha<sup>-1</sup> applied in TM with CZ-600 (MCP-P 600 g ha<sup>-1</sup>, 1.5 l ha<sup>-1</sup>) shown good selectivity in KGB, cocksfoot and timothy. Nevertheless, Arkem applied in double dose evoked the symptoms of phytotoxicity and decreased the seed yield of cocksfoot.

Key words: grasses, herbicide, selectivity, seed yield

## Introduction

Grass seed crops are minor but important part of Czech agribusiness. High ratio of grass seed is exported and improves the balance of agro trade. However, grass seed yield falls behind the average yields of European Union. One of the causes is non-recognition of seed crops as a result of high weed infestation or non-recognition of harvested seeds due to contamination by uncleanable weed seed. The present assortment of herbicides registered in the Czech Republic for use in grass seed crops is poor and do not include the herbicides for control of changing weed spectrum. Czech grass seed producers need new and more effective herbicides.

## Materials and Methods

Tests of herbicide selectivity were conducted in small plot trials at Grassland Research Station at Zubří (North-east Moravia, 360 m a.s.l., cambisol soil, average air temperature 7.5 °C, precipitation 864 mm). The trials were conducted with perennial ryegrass (*Lolium perenne* L.) cv. Olaf, meadow fescue (*Festuca pratensis* Huds.) cv. Roznovska, red fescue (*F. rubra* L.) cv.

Tagera, timothy (*Phleum pratense* L.) cv. Sobol, loloid type of *Festulolium* cv. Lofa, festucoid type of *Festulolium* cv. Hykor, cocksfoot (*Dactylis glomerata* L.) cv. Dana and Kentucky bluegrass (*Poa pratensis* L.) cv. Slezanka. The plot size was 10 m<sup>2</sup>. Test plots were situated to randomized complete block design. For each herbicide the base dose (2 times) and double dose (2 times) were tested. The herbicides and doses applied are given in Table 1.

All treatments were performed with wheelbarrow sprayer driven by compressed air (Lurmark 01F80 nozzles, a pressure 0.25 MPa, spraying volume 300 l ha<sup>-1</sup>) in GS 25-29 (mid to end of tillering). The standard treatment (MCPA + clopyralid + fluroxypyr) was used for comparison (control). Fertilizers application: autumn 45 kg N, 20 kg P<sub>2</sub>O<sub>5</sub> and 60 kg K<sub>2</sub>O per ha, spring – only nitrogen at dose depending on grass species 70-100 kg ha<sup>-1</sup>. The field trials were established in 2005 and for 3 years (2006-8) the selectivity of selected herbicides were evaluated (visual crop damage assessment). The trials were combined directly with plot combine Wintersteiger Elite. Harvested seed was dried and subsequently cleaned by laboratory cleaner Westrup for seed yield determination. Seed quality (TSW, germination) and number of seed per fertile tiller were analyzed in the lab of GRS Zubri. The results were analyzed by ANOVA and Tukey's post hoc test on significance level 95 % (Statistica 8.0).

**Table 1** Herbicides, active substances, doses and tested grass species

Herbicide	active ingredient	basic dose g a.i. per ha	KBG	cocksfoot	Hykor	Lofa	timothy	meadow fescue	red fescue	perennial ryegrass
Arrat	tritosulfuron	50								
	+ dicamba	100	x	x	x	x	x	x	x	x
Aurora super	carfentrazone-ethyl	15	x	x	x	x	x	x	x	x
	+MCPA-P	600								
Arkem + CZ 600	metsulfuron-methyl	6	x	x	x	x	x	x	-	-
	+ mecoprop-P	900								
Callisto 480 SC	mesotrione	144	-	-	x	x	x	x	-	-
Husar	iodosulfuron	3								
	+ mefenpyr-diethyl	30	x	x	-	-	-	-	-	-

x herbicides was applied in the grass species

## Results and discussion

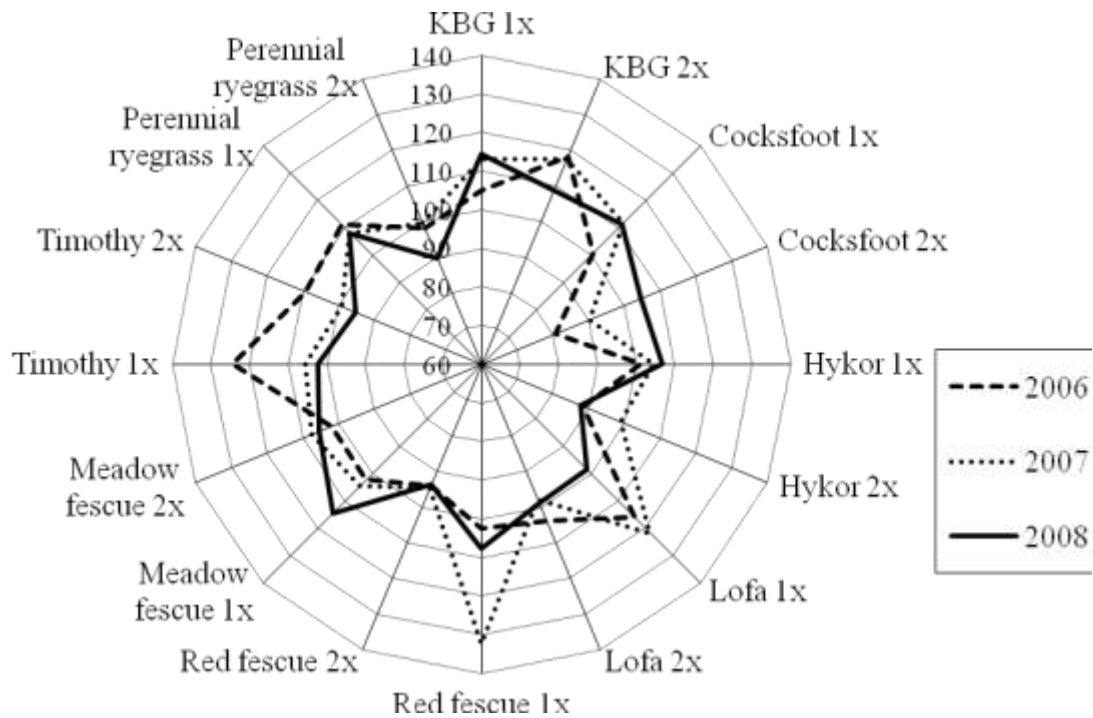
### Arrat

The field trials shown a good selectivity of Arrat (tritosulfuron + dicamba) in both doses (base and double) on all tested grass species. However, slight symptoms of damage crops were recorded on both types of *Festulolium* (growth retardation, chlorosis) and perennial ryegrass (chlorosis). If the base dose was tested the seed yields of all grasses were comparable or higher to standard treatment. Nevertheless, if the double doses was used the seed yields of some species (cocksfoot, *Festulolium*, red fescue) were significantly lower compared to control. Relative seed yield tested grass species treated by base dose (1x) and double dose (2x) of herbicide Arrat is shown in figure 1 (control = 100 %). Based on trials the Arrat in dose 0.2 kg ha<sup>-1</sup> has been allowed for minor use in seed crops of each grass species under study.

### *Aurora super SG*

The good selectivity of *Aurora super SG* (carfentrazone-ethyl + MCP-P) in both doses was recorded on all tested grass species. However, slight growth retardations were observed on cocksfoot and KBG treated by double dose of herbicide. Small chlorosis, mainly in first harvest year, was recorded on *Festulolium* cv. Hykor. If the base dose was tested the seed yields of all grasses without meadow fescue were comparable or higher to standard treatment.

**Figure 1** Relative seed yield (%) selected grass species treated by Arrat

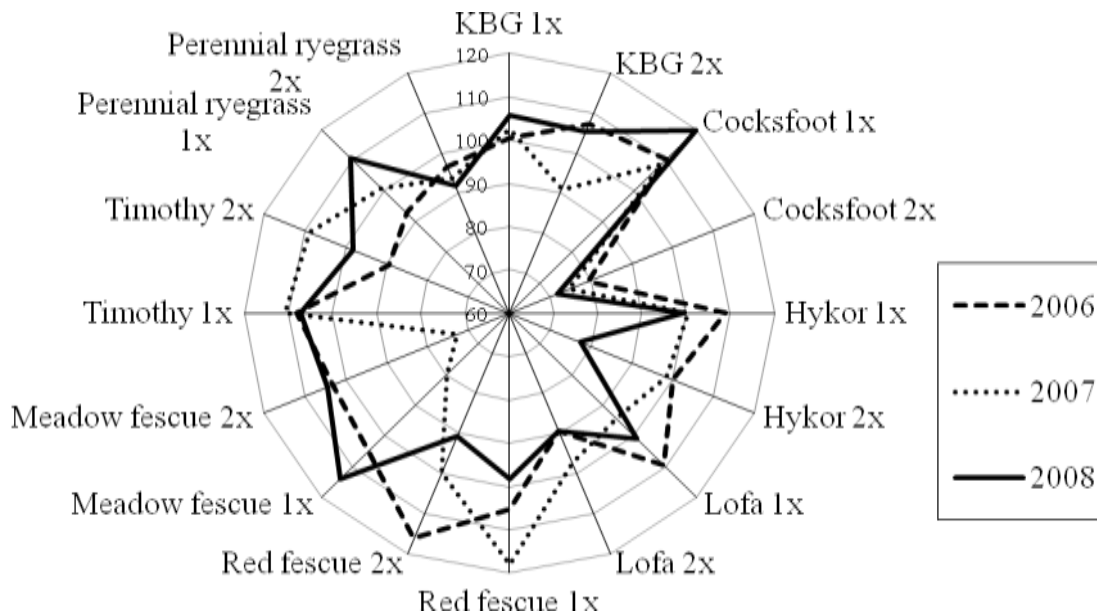


Nevertheless, if the double doses was used the seed yields all the species were significantly lower compared to standard in one harvest year at the least. Based on trial results the *Aurora super SG* in dose  $1.0 \text{ kg ha}^{-1}$  has been allowed for minor use in seed crops of perennial ryegrass, red fescue, *Festulolium*, timothy, cocksfoot and KBG.

### *Arkem*

Crops treated by *Arkem* (metsulfuron-methyl) showed relatively strong symptoms of injury, mainly growth retardation. Damage of meadow fescue and *Festulolium* in the first harvest year was very strong and so the *Arkem* was rejected from trials in the given species for subsequent years. This is corresponding with results obtained by Mathiassen *et al.* (2007). Inferior symptoms were observed only on KBG, cocksfoot and timothy. The seed yields were compared to the standard but if double dose was used the seed yield of cocksfoot became lower. Based on results of trials the *Arkem* in dose  $30 \text{ g ha}^{-1}$  has been allowed for minor use in seed crops of KBG, cocksfoot and timothy.

**Figure 2** Relative seed yield (%) selected grass species treated by Aurora super SG



#### *Callisto 480 SC*

The herbicide Callisto 480 SC (mesotrione) caused strong chlorosis after application, mainly on Festulolium and timothy if a double dose was used. However, later the symptoms of chlorosis fade away. If the base dose was tested the seed yields of all grasses without meadow fescue were comparable to standard. Nevertheless, if the double dose was applied the seed yields of Festulolium and timothy in the third harvest year were lower compared to control. Based on trials the Callisto 480 SC in dose  $0.3 \text{ l ha}^{-1}$  has been allowed for minor use in seed crops of Festulolium and timothy.

#### *Husar*

The herbicide Husar (iodosulfuron + mefenpyr-diethyl) was applied only in KBG and cocksfoot. The slight symptoms of growth retardation were recorded on both species, mainly if double dose of Husar was applied. The seed yield was satisfied but when it was applied in double dose the seed yield of cocksfoot was significantly decreased. Nevertheless, due to good control of *Poa annua*, one from most problematic weeds in grass seed crop (Cagaš *et al.* 2006), this herbicide is allowed for minor use in seed crops of KGB and cocksfoot at  $200 \text{ g ha}^{-1}$ .

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# Effects of trinexapac-ethyl (Moddus) in seed crops of eleven temperate grass species in Central European conditions

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## Abstract

In small plot field trials conducted for two years the effects of plant growth regulator (PGR) trinexapac-ethyl (TE) in selected grass species were studied. Eleven temperate grass species were used in trials: Perennial ryegrass, annual ryegrass, meadow fescue, red fescue, Kentucky bluegrass, timothy, cocksfoot, loloid and festucoid type of festulolium, yellow oat grass and tall oat grass. Evaluations were made of lodging, plant height, number of fertile tillers, total seed yield, thousand seed weight, germination, germination energy and number of seed per fertile tiller. In each grass species TE reduced lodging and reduced plant height. Grasses treated by PGR achieved a higher seed yield associated with a higher number of seeds per panicle. Based on trials, Moddus (trinexapac-ethyl 250 g l<sup>-1</sup>) in rate 0.8 l ha<sup>-1</sup> preferably applied at growth stage GS 31-32 or 0.4 l ha<sup>-1</sup> applied 2 times (GS 29 and GS 32) has been allowed for minor use in seed crops of each grass species under study in the Czech Republic.

Key words: grasses, PGR, trinexapac-ethyl, seed yield

## Introduction

Czech grass seed production has a significant position in European Union, however unfortunately only due to overall area of grass seed crops. The production and seed yields fall under average of EU. The main cause of low yields is poor basic agronomical practices and low inputs. To achieve higher seed yields sufficient supply of nitrogen is necessary which has a significantly effect on the photosynthesis and thereby total productivity of plant. However, increasing of nitrogen supply increases growth and prolongation of stems that are more inclinable to lodging. Lodging has been identified as one of the most important factors reducing grass seed yield. Losses due to lodging have been estimated to be as great as 60 % (Rolston *et al.*, 1997). Lodged stems are exposed to higher competitive for light and nutrients. Developing seeds may abort or fall due to less effective photosynthesis and decreasing of assimilate supply. Lodged stands are also more predisposed to diseases. Last but not least is the complication of harvest and increased seed losses due to no-cutting of lodged stems and increased moisture of thrashed material etc.

In the Czech Republic only chlormequat-chloride (CCC) is registered for stem length shortening and reduction of lodging. CCC works on the beginning of gibberellin biosynthesis by inhibiting of kaurene synthesis (precursor of gibberellins). The plant growth regulator trinexapac-ethyl is widely used on grass seed crops abroad (Chastain *et al.*, 2003, Haldrup 2007, Rijckaert 2007, etc.). Rademacher (2000) claims that trinexapac-ethyl (TE) is inhibiting the activity of enzyme 3- $\beta$  hydroxylase that the transforms inactive gibberellins form GA<sub>20</sub> on highly active forms GA<sub>1</sub>

and GA<sub>4</sub>. In consequence this inactivation the stem growth is reduced and stalk wall is stronger. Finally, the plant height is lower and the crop is less susceptible to lodging. The main topic of our research was to verify the influence of TE on seed yield of selected temperate grass species and its quality in Central Europe conditions.

## Materials and methods

Tests of herbicide selectivity were conducted in small plot trials at Grassland Research Station at Zubri (North-east Moravia, 360 m a.s.l., cambisol, average air temperature 7.5 °C, precipitation 864 mm). The trials were conducted with perennial ryegrass (*Lolium perenne* L.) cv. Olaf, annual ryegrass (*Lolium multiflorum* Lam. var. *westerwoldicum* Wittm.) cv. Jivet, meadow fescue (*Festuca pratensis* Huds.) cv. Roznovska, red fescue (*F. rubra* L.) cv. Tagera, timothy (*Phleum pratense* L.) cv. Sobol, loloid type of *Festulolium* cv. Lofa, festucoid type of *Festulolium* cv. Hykor, cocksfoot (*Dactylis glomerata* L.) cv. Dana, Kentucky bluegrass (*Poa pratensis* L.) cv. Slezanka, tall oat grass (*Arrhenatherum elatius* (L.) Beauv. ex J. S. et K. B. Presl) cv. Roznovsky and yellow oat grass (*Trisetum flavescens* (L.) P. Beauv.) cv. Roznovsky. The plot size was 10 m<sup>2</sup>. Each trial was arranged in a randomized complete block design (with other pesticide treatments) with four replications. Two tested treatments with trinexapac-ethyl (TE) were applied: single application of dose 0.2 l TE ha<sup>-1</sup> at GS 31-32 and split application 2 times 0.1 l TE ha<sup>-1</sup>, 1<sup>st</sup> application at GS 29 and 2<sup>nd</sup> application at GS 32.

All treatments were performed with wheelbarrow sprayer driven by compressed air (Lurmark 01F80 nozzles, a pressure 0.25 MPa, spraying volume 300 l ha<sup>-1</sup>) in GS 25-29 (mid to end of tillering). The standard treatment (MCPA + clopyralid + fluroxypyr) was used for weed control. Fertilizers application: autumn 45 kg N, 20 kg P<sub>2</sub>O<sub>5</sub> and 60 kg K<sub>2</sub>O per ha, spring – only nitrogen at dose depending on grass species 80-110 kg N ha<sup>-1</sup>. The field trials were conducted for 2 years (2007-8). The trials plots were combined directly with plot combine Wintersteiger Elite. Harvested seed was dried and subsequently cleaned by laboratory cleaner Westrup for seed yield determination. Seed quality (TSW, germination) and number of seed per fertile tiller were analyzed in the lab of GRS Zubri. The results were analyzed by ANOVA and Tukey's post hoc test on significance level 95 % (Statistica 8.0). Due to insertion to the randomized blocks with pesticide treatments the number of degree of freedom (27) was satisfactory for statistical analyses.

## Results and discussion

The positive effect of trinexapac-ethyl on seed yield was recorded in larger or smaller rate on all of selected grass species. However, there was insignificant decreasing of seed yield of some species in 2007. With regard to dry weather in growing seasons (especially in 2007) the grasses only very few lodged. It is possible to suppose, that if grasses would more lodged the difference in seed yield between untreated plots and plots treated by TE were to be higher. The effect on TE application on seed yield of selected grass species is shown in the Table 1. Thousand seed weight (TSW) was at most grass species insignificant lower on treatments when the TE was applied in comparison with untreated plots. Minimal and also insignificant differences between treatments were recorded in germination and germination energy (data not shown).

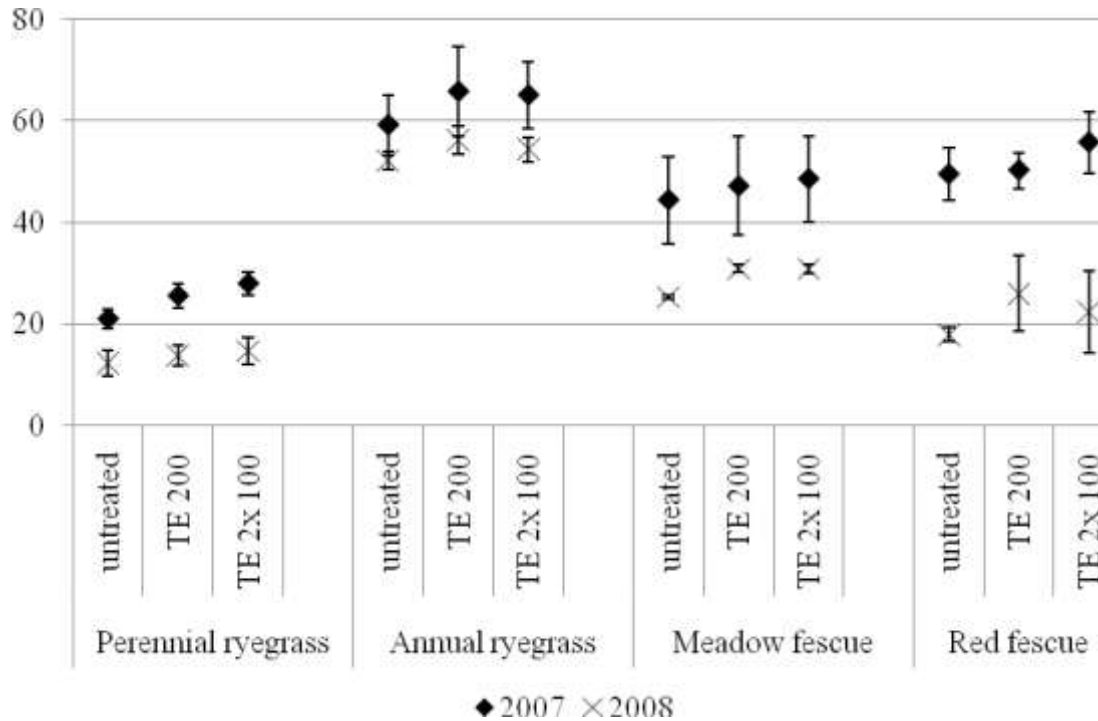
Significant differences were recorded in the number of seed in inflorescence (see. Figure 1). At the most grass species the number of the seed was higher at treated grasses; however, at the some species then number of the seed was lower in consequence with higher number of fertile stems. Positive influence of trinexapac-ethyl application on increase of number of perennial ryegrass seed was observed by Silberstein *et al.* (2002), who found out that TE increases number of created ripe seed, while potential number of seed isn't impressed with. Application TE so has positively effect on Floret site utilization (FSU). According to Young *et al.* (2007) the nitrogen rate increasing has significant effect on increasing of both, actual and potential seed number, but insignificant effect on FSU in perennial ryegrass.

Reduction in plant height and lodging was recorded on all the treated plots. Application of trinexapac-ethyl on cool season grass seed crops can significant increases seed yields mainly due to number of seed increasing. There is slight variation in the increasing of seed yield between grass species, but average increasing of seed yield is about 12-15 %. Based on trials the PGR Moddus (trinexapac-ethyl 250 g l<sup>-1</sup>) at 0.8 l ha<sup>-1</sup> preferably applied at growth stage GS 31-32 or 0.4 l ha<sup>-1</sup> applied 2 times (GS 29 and GS 32) has been allowed for minor use in seed crops of each grass species under study in the Czech Republic.

Table 1 The effect of trinexapac-ethyl on seed yield selected grass species

species	treatment	Seed yield					
		2007		2008		average	
		kg ha <sup>-1</sup>	Tukey	kg ha <sup>-1</sup>	Tukey	kg ha <sup>-1</sup>	rel.
Perennial ryegrass	untreated	933.6	d	554.6	e	744.1	100
	TE 200	1230.1	ab	708.0	a	969.1	130
	TE 2x 100	1238.2	ab	696.0	abc	967.1	130
Annual ryegrass	untreated	1748.4	a	1655.0	c	1701.7	100
	TE 200	1792.8	a	1842.6	ab	1817.7	107
	TE 2x 100	1860.2	a	1757.5	a	1808.8	106
Meadow fescue	untreated	718.8	de	356.2	ab	537.5	100
	TE 200	765.1	cd	378.2	ab	571.7	106
	TE 2x 100	800.8	bcd	521.4	a	661.1	123
Red fescue	untreated	902.0	ab	1194.5	bc	1048.3	100
	TE 200	995.4	a	1348.8	a	1172.1	112
	TE 2x 100	982.7	a	1227.0	ab	1104.9	105
Kentucky blue grass	untreated	346.3	bcd	517.0	b	431.7	100
	TE 200	471.3	a	676.0	a	573.6	133
	TE 2x 100	413.7	ab	628.7	ab	521.2	121
Timothy	untreated	834.7	a	640.0	ab	737.3	100
	TE 200	859.1	a	706.6	a	782.8	106
	TE 2x 100	903.0	a	729.2	a	816.1	111
Cocksfoot	untreated	838.3	abcd	625.5	ab	731.9	100
	TE 200	799.8	abcd	762.8	a	781.3	107
	TE 2x 100	862.0	abc	694.7	ab	778.4	106
Festulolium Lofa	untreated	893.8	bcdef	734.9	bc	814.4	100
	TE 200	1055.4	abc	767.0	ab	911.2	111
	TE 2x 100	998.1	abcde	877.2	a	937.6	115
Festulolium Hykor	untreated	938.4	a	952.4	abc	945.4	100
	TE 200	937.2	a	1099.1	a	1018.1	107
	TE 2x 100	950.6	a	1147.9	a	1049.2	111
Tall oat grass	untreated	516.6	bc	367.4	c	442.0	100
	TE 200	671.5	a	506.1	ab	588.8	133
	TE 2x 100	549.3	b	513.7	a	531.5	120
Yellow oat grass	untreated	266.8	c	207.6	a	237.2	100
	TE 200	285.1	a	231.3	a	258.2	109
	TE 2x 100	302.9	b	239.2	ab	271.0	114

**Figure 1** Effect of trinexapac-ethyl on number of seed per inflorescence in selected grass species including confidence intervals ( $p=0.05$ )



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# Effect of row spacing and plant growth regulators on the alfalfa seed yield

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## Abstract

The trials were conducted for comparing the seed yield components and seed yield to utilize row spacing and plant growth regulators in the fields of alfalfa cv Zhongmu 2. The results showed that alfalfa seed yield increased with the row spacing from 30cm to 90 cm, but were similar between 90cm and 120cm. As the concentration increased, ethephon and paclobutrazol significantly improved the inflorescences per square metre, and increased seed yield, especially at the highest treatment concentration. For NAA, there were no significantly differences for alfalfa seed yield among different concentration levels.

## Introduction

Alfalfa (*Medicago sativa* L.) is a important forage crop in China, especially in the semiarid cropping region of north-western China. In this region the climate is suitable for alfalfa seed production with low humidity and moderate air temperature. Many studies have been conducted on the effects of thinning and between-row spacing on alfalfa seed yield at multiple locations. Recommended between-row spacings in these studies were quite different and varied from 20 to 91 cm (Askarian *et al.*, 1995; Zhang *et al.*, 2008). Plant growth regulators have opened new prospects for increased seed production in grasses and legumes (Lorenzetti, 1993).

## Materials and Methods

Alfalfa (*M. sativa* cv Zhongmu 2) was planted in western Inner Mongolia in April 2008. Different treatments were designed with row spacing (30cm, 60cm, 90cm, 120cm) and plant growth regulators (ethephon, paclobutrazol and naphthichetic acid) in the fields. Ethephon concentration was 0(CK), 0.5, 1.0, 1.5, 2.0 kg/ha; paclobutrazol concentration was 0(CK), 0.2, 0.4, 0.6, 0.8 kg/ha; and naphthichetic acid (NAA) concentration was 0(CK), 0.02, 0.04, 0.06, 0.08 kg/ha. Different plant growth regulators were sprayed on the leaves of alfalfa growing in 60cm row space during the early flowering and peak flowering. Yield components and seed yield were measured during seed development.

## Results and Discussion

With the row spacing enlarged, the inflorescences per square metre increased, and there weren't significant difference between 90 and 120 cm row spacing. Ovule number per floret at 90cm spacing was highest, and seed yield increased with the row spacing from 30 to 120 cm, but was similar between 90cm and 120cm. With increased concentration of ethephon and paclobutrazol, the

inflorescence number and seed yield were improved, but there weren't significantly different for floret and ovule number. For NAA, 0.02 and 0.04 kg/ha treatments achieved the higher inflorescence number and seed yield, but there weren't significantly different with CK. It could be suggested that there are different roles on the yield components for ethephon, paclobutrazol and NAA, and understanding the key factors will be helpful for alfalfa seed production in the western China.

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## Seed yield potential of wild vetch (*Vicia* spp.) species

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The flora of Serbia is rather rich in vetch (*Vicia* spp.) species. Many of them bring an essential contribution to the quality of pasture and meadow communities and soil fertility. Among such are narrow-leaved vetch (*V. sativa* subsp. *nigra* (L.) Ehrh.), large-flowered vetch (*V. grandiflora* Scop.), hairy vetch (*V. villosa* Roth) and Hungarian vetch (*V. pannonica* Crantz). With the potential for 50 t ha<sup>-1</sup> of green forage and 12 t ha<sup>-1</sup> of forage dry matter in hairy vetch and more than 40 t ha<sup>-1</sup> of green forage and nearly 14 t ha<sup>-1</sup> of forage dry matter in large-flowered vetch, as well as with a forage dry matter crude protein content of more than 200 g kg<sup>-1</sup>, these vetch species are richer in nitrogen content than other forage legumes such as lucerne or pea and are able to answer the demands for a quality source of both forage and green manure.

One of the crucial tasks of all breeding programs of annual forage legumes is an improvement of seed production, making a newly developed variety able to be successfully commercialized. Vetches are perhaps the most difficult of all annual forage legumes for this job, being notorious for its excessive and indeterminate growth, non-uniform maturity and large seed losses due to a prominent pod dehiscence. The best example of a wide discordance between forage and seed yields is hairy vetch, in which the seed yields in rainy seasons are less than 500 kg ha<sup>-1</sup>.

The potential solutions for the enhanced seed production of diverse annual vetch species may be achieved by certain modifications of plant architecture, like in pea, with the identification and the introgression of the genes responsible for determinate stem growth and a more uniform maturity. At the same time, an increased seed yield may be achieved by the selection and the development of multi-pod genotypes, such as reported in large-flowered vetch, where only two or three fertile nodes with three or four pods each, maturing at the same time, may provide reliable seed yields without having a negative impact on forage yields. In the end, it is Hungarian vetch that often has higher seed yields than common and many other vetches due to a less prominent pod shattering.

# Germination of *Lolium multiflorum* genotypes in high salt conditions

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## Abstract

Results from this study indicated best levels to select ryegrass genotypes for germination under high salt levels would be from 13,000 to 16,000 mg L<sup>-1</sup>. At 19,000 mg L<sup>-1</sup>, little differentiation was apparent, although some seed did germinate. Second, high seed quality is important in germination studies, and low seed quality of the experimental lines may have masked possible salinity tolerance at germination. Lastly, if these experimental lines have salt tolerance during the seedling stage, this resistance was not effective during the germination process.

## Materials and Methods

Five annual ryegrass genotypes ('Gulf', 'Panterra', TXR2009-SS, Pecos Bulk, and SS<sub>3</sub>) were subjected to five concentrations of salinity during germination. The latter three germplasm lines were selected for salt tolerance under either field conditions at Pecos, Texas or under greenhouse growing conditions. Therefore seed was extremely light, and of low quality compared to seed of the first 2 germplasms, which was produced in Oregon. The germination protocol for ryegrass from the Proceedings of the Association of Official Seed Analysis, Vol. 60, No. 2 was followed as closely as possible. One hundred seed of each entry were placed on germination paper in 1.5 x 14 cm Petri dishes. There were 4 replications, which resulted in 100 Petri dishes for the entire study. Salt concentrations of NaCl in distilled water were 0 (check), 10,000 (16.0 dS/m<sup>-1</sup>), 13,000 (20.8 dS/m<sup>-1</sup>), 16,000 (25.6 dS/m<sup>-1</sup>), and 19,000 mg L<sup>-1</sup> (30.4 dS/m<sup>-1</sup>), respectfully. Data are presented on percent germination after 7 and 14 days after seed were placed in a germination chamber at 15 C nights (12 hr) and 25 C days (with light). Data was analyzed as a randomized complete block as a factorial with 4 replications.

## Results

Significant differences were observed for genotypes (G), salt concentrations (SC) and for G x SC levels. The mean % germination of genotypes averaged over all salt concentrations indicated Gulf had the highest level at 14 days of 70% followed by Panterra (66%), TXR2009-SSBk (54%), SS<sub>3</sub> (41%) and Pecos Blk (38%). Percent germination for salt levels averaged over genotypes were 92% at 0, 81% at 10,000, 61% at 13,000, 28% at 16,000, and 8% at 19,000 mg L<sup>-1</sup>, respectively. Germination percentages at 7 days were from 10 to 20 % lower; however, followed the same trend as at 14 days. Since there was a significant interaction between G x SC, the performance of each genotype must be looked at under different salt concentrations. Gulf had more tolerance to high salt concentrations than other genotypes until a concentration of 19,000 mg L<sup>-1</sup>, where little germination occurred for any line. Panterra was the next best genotype and followed a similar trend. The three experimental lines selected for salt tolerance after germination all had less tolerance to germination under high salt concentrations in this study. After 14 days, Gulf and Panterra had some seed germinate even at 19,000 mg L<sup>-1</sup> (13 and

20%, respectively). The experimental lines had less than 4% germinate at 19,000 mg L<sup>-1</sup> salt concentration.

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## Effect of sowing density on seed yield and quality of Westerwold ryegrass (*Lolium multiflorum ssp. westerwoldicum*) in Finland

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### Abstract

Effect of sowing density on seed yield and quality of Westerwold ryegrass (*Lolium multiflorum ssp. westerwoldicum*) was investigated at MTT Agrifood Research Finland at Ylistaro research station (latitude N 62°57') in 2005-2008. Completely randomized block experiment with four replicates had five sowing density treatments: 200, 300, 500, 700 and 900 germinating seeds /m<sup>2</sup>. Sowing rate ranged from 8.4 to 37.8 kg ha<sup>-1</sup> depending on germination and seed size of the used seed lot. Cultivar 'Pollanum' was used in the experiment. The experiments were on loam soil. Nitrogen was applied at 80 kg ha<sup>-1</sup> prior to sowing. The trial was sown by Oyjord plot experiment sowing machine. The whole experiment was direct combine harvested at same date by Wintersteiger Plot Harvester. Plot size was 13,75 m<sup>2</sup>. Objective of the study was to clarify how sowing density would affect evenness of maturity and seed yield. Previous studies have indicated that uneven maturity is the biggest problem in seed production of annual ryegrass in Finnish conditions. Late tillering in sparse stands has been one factor causing uneven maturity. Green tillers at harvest make harvesting difficult and can risk the quality of harvested seed. The average seed yield in this trial was 1264, 1247, 499 and 739 kg ha<sup>-1</sup> in 2005, 2006, 2007 and 2008, respectively. Increasing sowing density increased number of fertile tillers and seed yield, and reduced seed moisture content at harvest. Effect of sowing density on thousand seed weight and on germination was positive, although weak. On average the seed yield was 23 % lower at sowing densities 200 and 300 seed /m<sup>2</sup> in 2005-2008 than at the higher sowing densities. Seed yield at sowing density treatments (500, 700, 900 seed/m<sup>2</sup>) did not differ of each other. On average the number of fertile tillers decreased from 1120 to 790 ears /m<sup>2</sup> when sowing density decreased from 900 to 200 seeds /m<sup>2</sup>. Average seed moisture content at harvest decreased from 48,2 percent to 42,1 percent at sowing densities 200 and 900 seeds/m<sup>2</sup>, respectively, indicating more even maturation at the higher sowing density treatments.

**Key words:** westerwold ryegrass, seed yield, sowing density, seed quality

# Effects of plant growth regulation in seed crops of Italian ryegrass (*Lolium multiflorum* L.)

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## Abstract

Despite the official registration of Moddus (trinexapac-ethyl) for growth regulation in C1-seed crops of all ryegrass species in Belgium since 2002, seed growers of Italian ryegrass have not adopted this new management practice. The main reasons for this non-adoption were the limited number of trials (2), the less pesticide-minded cattle farmers and the critical profitability due to very low growers' seed prices during 2002-2006.

A new series of trials was conducted for Italian ryegrass seed crops in three consecutive years, i.e., 2007-09. In all trials, eight PGR treatments and an untreated control were evaluated on a tetraploid variety 'Meroa'. The respective rates of Moddus (250 g a.i. l<sup>-1</sup>) were 0.4 – 0.4+0.4 – 0.8 – 1.2 l ha<sup>-1</sup>, either with or without methylated seed oil surfactant (Actirob B). Moddus was applied at the 2-3 node growth stage and again 10-20 days later for the split treatment.

Across years, the optimal seed yield response ranged from 7.3 to 32.6% over the untreated checks, and also the optimal application rates were different between years, i.e., 1.2 – 0.4 – 0.4 l ha<sup>-1</sup> for 2007-08-09, respectively. This could be attributed to a wet or dry season. The mean optimal seed yield over the years increased by about 17% (347 kg ha<sup>-1</sup>).

The addition of the surfactant was not always profitable, except for 2007 (very wet). The split treatment (0.4+0.4) rarely resulted in a higher seed yield when compared with the full rate (0.8). Seed yield responses are discussed in relation to seed yield components, thousand seed weight, straw yield, lodging, stem shortening and seasonal meteorological conditions.

## Introduction

Italian ryegrass (*L. multiflorum*) is the most commonly grown grass species for seed production in Belgium (51°N); i.e., on average 3,232 ha for the period 2006-09 in comparison with 1,257 ha in the period 1982-86. Currently, it represents 57% of the total grass seed area. Belgium is one of the better agro-climatic production sites for seed production of Italian ryegrass. The growing interest in Italian ryegrass seed crops over the years fits very well in the favourable region of Flanders with its moderate, humid climate, mild winters and long growing season. This crop is fully integrated on cattle farms (dairy) and mixed farms as a combined forage-seed production system. The very intensive, but sustainable production system consists of subsequent productions: i.e., a forage cut (prewilted) in May, a seed cut + grass seed straw in July-August, possibly followed by a forage cut or grazing during autumn, and finally a forage cut in the spring of next year. The latter is directly followed by silage maize. In other words, the

by-products of this grass seed crop are fully exploited in dairy and mixed farms. The combined forage-seed production system is a profitable speculation for Belgian seed growers. On the other hand, the more globalised market for grass seeds and very fluctuating market prices necessitate higher and more stable seed yields for Italian ryegrass over the years.

While the use of plant growth regulators (PGR) has become common practice in seed production of most grasses, preliminary seed production research has been somewhat neglected for Italian ryegrass worldwide. Some research is known on annual ryegrass-Westerwold type. Oregon studies (Melbye *et al.*, 2007) with trinexapac-ethyl (Palisade, Moddus) and prohexadione-calcium (Apogee, Regalis) reported an average seed yield increase of about 10% ( $250 \text{ kg ha}^{-1}$ ) in annual ryegrass over seven site-years (1999-2006). In New Zealand, seed yield increases from the use of Moddus in annual ryegrass were even higher; over 75% (Pyke, 2007). In Belgium, the first PGR research (2 trials) on Italian ryegrass (biannual type) was conducted in 2000 and 2001, and this finally led to the Belgian registration of Moddus for use in all ryegrass species. The mean optimal seed yield increased by 17% ( $192 \text{ kg ha}^{-1}$ ) for a diploid cultivar and the optimal rate of Moddus ranged from 100 to  $200 \text{ g a.i. ha}^{-1}$  depending on growing conditions (Rijckaert, 2007).

However, seed growers of Italian ryegrass did not pick up this new management practice. Therefore, a new series of trials was started in order to ameliorate the economical return of Italian ryegrass seed crops over the years. In this article, we summarise the main effects of Moddus applied alone or in combination with a surfactant, on seed yield and related characters.

## Materials and Methods

Three growth regulation experiments (2007-08-09) were conducted at ILVO, Plant Sciences Unit, Merelbeke, on a sandy-loam soil in first year seed crops of Italian ryegrass cv. Meroa (tetraploid). All trials were sown using a precision drill (Øyord system) against 800 germinable seeds per  $\text{m}^2$ , during late September to early October.

Eight PGR treatments and an untreated control were compared in a randomized complete block design with four replications. Namely Moddus ( $250 \text{ g l}^{-1}$  trinexapac-ethyl) was applied at 4 rates: i.e.,  $0.4 - 0.4 + 0.4 - 0.8 - 1.2 \text{ l ha}^{-1}$ , respectively without and with addition of methylated canola oil (Actirob B) at 0.25% by spray volume. The first application stage (T1) was GS 32-33 (Table 1) and this was followed by a second spray for the split application, 10-20 days later (T2). Treatments were applied using a 2.5 m wide boom sprayer (compressed air) with 5 flat nozzles; spray volume;  $250 \text{ l ha}^{-1}$  and pressure: 181 kPa.

Gross plot size was  $2.5 \times 11 \text{ m}$  where only the central part was harvested i.e.,  $1.5 \times 10 \text{ m} = 15 \text{ m}^2$  in order to avoid border effects and possible drift from the applied chemicals.

The total mineral nitrogen fertilisation was  $200 \text{ kg ha}^{-1}$  which was divided in 120 units for the forage cut and 80 units for the seed cut. After the preceding forage cut in May (Table 1), plots were subjected to the experimental treatments.

**Table 1.** Some details of three PGR experiments on Italian ryegrass trials 2007-08-09

	Trial n° 1 harvest 2007		Trial n° 2 harvest 2008		Trial n° 3 harvest 2009	
<b>Time of defoliation</b>	03/05/07		15/05/08		20/05/09	
Harvest date	25/07/07		31/07/08		29/07/09	
Moisture content	30.7%		40.7%		27.4%	
Treatments	T1	T2	T1	T2	T1	T2
- planned GS	32-33	+ 10-20D	32-33	+ 10-20D	32-33	+ 10-20D
- application date	25/05/07	14/06/07	04/06/08	17/06/08	02/06/09	12/06/09
- average n° of nodes	1.8	3.6	2.8	4.0	2.0	3.5
Rainfall <sup>1</sup> - (temp. <sup>2</sup> ) <sup>3</sup>						
- May – 61,0 (13.8)	96.7 (14.1)		30.8 (16.0)		66.4 (14.2)	
- June – 77,9 (16.1)	91.6 (17.3)		79.1 (15.8)		69.5 (16.0)	
- July – 82,5 (18.1)	154.5 (17.1)		51.7 (17.8)		67.7 (18.2)	

<sup>1</sup> rainfall in mm<sup>2</sup> mean temperature in °C<sup>3</sup> average figures for period 1992-2009 in first column

When appropriate (except in 2007), the following parameters were evaluated: lodging, plant height, culm and ear length, fertile tiller density and secondary regrowth at harvest time. Finally, seed was harvested by cutting with a forage plot harvester designed for efficient bagging. The harvested swath was air dried in jute bags to a moisture content of 12% before threshing and seed cleaning. Cleaned seed, thousand seed weight (TSW), germination, straw yield, cleaning efficiency, harvest index and number of seed per unit area were determined.

## Results and discussion

Very high clean seed yields of Italian ryegrass were obtained in both dry seasons of 2008 and 2009; the untreated controls yielded 2,358 and 2,150 kg ha<sup>-1</sup>, respectively. In both years, the seed crop was preventively protected by one fungicide application. In 2007 on the other hand, the control only achieved 1,441 kg ha<sup>-1</sup>. This could be attributed to the very wet season and a late crown rust attack without any fungicide protection. Seed yield responses to PGR treatments were distinctly different between years and results are presented per year.

Across years, the optimal seed yield response ranged from 7.3 to 32.6% over the untreated checks, and also the optimal application rate of Moddus was different between years i.e., 1.2 – 0.4 – 0.4 l ha<sup>-1</sup> for 2007-08-09, respectively. Only in 2008, seed yield increases were not significantly different from the control, but the optimal rate tended to 0.4 l ha<sup>-1</sup>. In 2007, PGR treatments with surfactant Actirob B tended to enhance the effect on seed yield when compared to the respective doses without surfactant, but it was only significant at the rate of 0.8 l ha<sup>-1</sup>. In 2008 and 2009, this positive effect from the surfactant could not be observed.

**Table 2.** Effect of PGR on seed yield and related parameters in Italian ryegrass – 2007

Treatments <sup>1</sup>	Seed yield (rel. %)	Straw yield (rel. %)	Harvest index (%)	Seeds m <sup>-2</sup> calculated (x 1000)	TSW (dg)
	(=1,441 kg ha <sup>-1</sup> )	(=5.15 ton ha <sup>-1</sup> )			
1. 0 = control	100.0 b	100.0 ab	21.9 c	38.1 b	<b>378 c</b>
2. 100	105.5 b	101.2 a	22.5 bc	40.5 ab	<b>375 c</b>
3. 100 + 100 <sup>2</sup>	108.2 b	97.1 abcd	23.8 bc	40.8 ab	<b>382 c</b>
4. 200	109.6 b	96.7 abcd	24.1 bc	41.1 ab	<b>384 c</b>
5. 300	132.4 a	94.1 cd	28.2 a	46.1 a	<b>414 b</b>
6. 100 S <sup>3</sup>	110.5 b	98.3 abc	23.9 bc	42.5 ab	374 c
7. 100 + 100 <sup>2</sup> S	112.3 b	94.6 bcd	24.9 b	42.3 ab	384 c
8. 200 S	126.6 a	92.1 de	27.8 a	44.5 ab	410 b
9. 300 S	132.8 a	89.4 e	29.4 a	43.8 ab	438 a
P-value	<0.001	<0.001	<0.001	<0.05	<0.001
LSD (P<0.05)	12.6	3.9	1.8	4.3	21

<sup>1</sup> rate in g a.i./ha of Moddus (trinexapac-ethyl)<sup>2</sup> split application<sup>3</sup> S = surfactant methylated canola oil (Actirob B)

The split application of Moddus (treatment n°3 - n°7) was mainly introduced in order to be able to apply in practice the optimal dose, varying in accordance to soil moisture content and expected weather conditions. In this way the second spray should be carried out or not. A possible detrimental effect on seed yield from the split application could only be confirmed in one out of six cases in above trials (Table 2). Therefore, this approach of split application could be recommended when economical return will coming up; e.g. low grass seed prices, high product costs.

Seed yield increases in the wet season of 2007 were associated with lower straw yields, especially for treatments n° 5, 8 and 9 and higher thousand seed weights for the same treatments, both on a significant level. Harvest index and seed number per unit area responded accordingly. This indicated that these PGR treatments resulted in a less vegetative seed crop or a more favourable micro-environment for good pollination, seed setting, seed filling, ripening and harvesting during the extremely wet season of 2007.

The seasons 2008 and 2009 on the other hand, were quite dry when compared to the normal weather conditions in Belgium (Table 1). Because the optimal conditions for flowering and seed ripening were also present in the untreated controls – i.e., an upright seed crop, PGR treatments were less responsive to seed yield increases. So during dry growing seasons, plant growth regulation could not really offer an advantage against the controls.

**Table 3.** Effect of PGR on seed yield and related parameters in Italian ryegrass – 2008

Treatments	Seed yield	Straw yield	Seeds m <sup>-2</sup>	Lodging <sup>1</sup> (flowering)	Culm length	Lodging (near harvest)
	(rel. %)	(rel. %)	(x 1000)	04/07/08 (0 – 10)	24/07/08 (cm)	22/07/08 (0 – 10)
	= 2,358 kg ha <sup>-1</sup>	= 7.15 ton ha <sup>-1</sup>				
1. 0 = control	100.0	100.0 a	48.9	<b>4.3 a</b>	<b>80.4 a</b>	<b>7.3 a</b>
2. 100	106.5	96.1 ab	52.8	<b>2.1 b</b>	76.7 ab	5.9 b
3. 100 + 100 <sup>2</sup>	99.8	89.2 b	48.8	<b>1.1 bc</b>	72.9 ab	5.6 b
4. 200	105.3	94.8 ab	52.3	<b>1.6 b</b>	75.8 ab	4.8 c
5. 300	99.5	94.4 ab	49.4	<b>1.0 bc</b>	76.1 ab	4.1 c
6. 100 S <sup>3</sup>	108.1	92.1 ab	54.3	1.6 b	75.0 ab	5.9 b
7. 100 + 100 S	104.7	93.7 ab	51.8	1.9 b	73.8 ab	4.5 c
8. 200 S	103.3	89.7 b	51.4	1.0 bc	71.4 b	3.5 d
9. 300 S	104.0	92.6 ab	52.2	0.4 c	70.6 b	2.8 e
P-value	0.39	<0.05	0.17	<0.001	<0.05	<0.001
LSD (P<0.05)	-	5.9	-	0.7	5.1	0.6

<sup>1</sup> lodging score: 0 = erect; 10 = completely lodged<sup>3</sup> S = surfactant methylated canola oil (Actirob B)**Table 4.** Effect of PGR on seed yield and related parameters in Italian ryegrass – 2009

Treatments	Seed yield	Straw yield	Seeds m <sup>-2</sup>	Lodging (flower.)	Culm length	Lodging (near harvest)
	(rel. %)	(rel. %)	(x 1000)	06/07/09 (0 – 10)	22/07/09 (cm)	24/07/09 (0 – 10)
	= 2,150 kg ha <sup>-1</sup>	= 7,07 ton ha <sup>-1</sup>				
1. 0 = control	100.0 b	100.0 a	45,9 b	<b>3.8 a</b>	<b>83,2 a</b>	<b>8,0 a</b>
2. 100	117,9 a	95,2 b	55,2 a	<b>2.6 b</b>	<b>78,7 ab</b>	<b>8,1 a</b>
3. 100 + 100 <sup>1</sup>	118,1 a	93,9 bc	53,2 a	<b>1.0 cd</b>	<b>68,1 cd</b>	<b>5,6 cd</b>
4. 200	121,4 a	92,1 bcd	57,7 a	<b>1.1 cd</b>	<b>78,1 ab</b>	<b>6,4 bc</b>
5. 300	118,7 a	89,0 cd	55,6 a	<b>0.3 cd</b>	<b>66,5 cd</b>	<b>4,4 de</b>
6. 100 S <sup>2</sup>	119,1 a	93,2 bcd	55,4 a	<b>1.6 bc</b>	<b>76,3 b</b>	<b>7,3 ab</b>
7. 100 + 100 S	117,1 a	92,1 bcd	53,2 a	0.6 cd	<b>69,6 cd</b>	4,4 de
8. 200 S	114,1 a	89,1 cd	52,8 a	0.0 d	72,4 bc	4,3 de
9. 300 S	117,1 a	86,0 d	54,9 a	0.0 d	<b>64,2 d</b>	3,5 e
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (P<0.05)	7.6	3.5	4.2	1.0	5.1	1.1

<sup>1</sup> split application<sup>2</sup> S = surfactant methylated canola oil (Actirob B)

In both years, lodging differences at flowering were rather small between the PGR treatments, while the controls only lodged 4.3 and 3.8 respectively; this is caused by drought (Tables 3-4). The lodging scores before harvest showed a nearly upright seed crop following the higher rates of Moddus; namely 0.8 and 1.2 t ha<sup>-1</sup>. The reduced lodging effect was more pronounced when the surfactant was used, especially in 2008, but also in 2009.

Further culm length measurements gave a good evaluation of the effectiveness of the straw shortening. In 2008, only the higher rates of Moddus + surfactant (treatments n°8-9) provoked a significant reduction in culm length against the control. Other treatments only showed a tendency to shortening. In 2009, the reduction in culm length was more pronounced and the split application resulted in a significant reduction when compared to the same rate at one time (0.8). This phenomenon was partly translated to the lodging score before harvest.

Seed yield differences between PGR treatments were negligible in 2008 and did not need further explanation. The lack of seed yield response when compared to the untreated control, was probably due to the drought stress just after PGR applications.

In 2009, the similar seed yield increases from all PGR treatments were associated with lower straw yields and resulted in higher seed numbers per unit area. However, no further seed yield increase occurred with the increasing rate of Moddus. This could also be attributed to the very dry conditions from 15 th of June until harvest, where higher growth inhibition could not ameliorate any further the micro-environment for optimal seed production.

In both years, the thousand seed weight and the number of fertile tillers were not affected by treatments (data not shown).

### **Conclusion**

Based upon the extremely different testing years with different optimal rates of Moddus i.e., 1.2 – 0.4 – 0.4 t ha<sup>-1</sup> in 2007-08-09 respectively, we believe a split application of Moddus will more easily approach the optimal rate over several years. The addition of a surfactant like Actirob B is highly recommendable.

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# **Effect of date and rate of nitrogen fertilization on state of nutrition, photosynthesis rate and yielding of lawn cultivar of red fescue (*Festuca rubra* ssp. *commutata*) grown for seeds**

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## **Abstract**

Nitrogen concentration in leaves at the beginning of growth responds to nitrogen rates applied in autumn, which gives a possibility of using this indicator for predicting needs for spring fertilization. Diversification of the chlorophyll index at the beginning of shooting according to fertilization level in early spring can be useful for determination of the supplementary rate. In respect of seed yield, in the first production year applying of 20 kg N ha<sup>-1</sup> in autumn and 40 kg ha<sup>-1</sup> in spring during starting of growth is sufficient. For maintaining a similar productivity in the second year, at the autumn application of 20 or 40 kg N ha<sup>-1</sup> it is necessary to apply not less than 60 kg ha<sup>-1</sup> in early spring.

## **Introduction**

Availability of nutrients, mainly nitrogen, is the basis for plant productivity. Monitoring of soil nitrogen availability (Hart *et al.*, 2007), the chlorophyll index (Fotyma & Bezdusznik, 2000) or nitrogen content in plants (Gislum & Boelt, 1999) is useful for the optimization of nitrogen fertilization. The aim of this study was to assess the state of nutrition, photosynthesis rate and yield of red fescue grown for seeds under conditions of varied rates and times of mineral nitrogen fertilization.

## **Materials and methods**

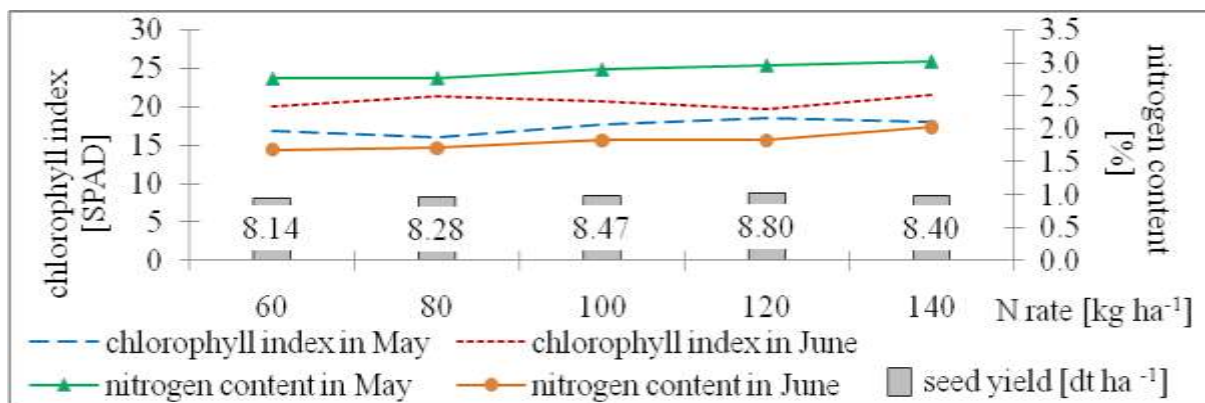
The study was based on a strict field experiment located in the Kujawy and Pomerania region (53°09' N; 17°35' E). It was established on a podzolic soil of a content of 10.2 NO<sub>3</sub><sup>-</sup> and 3.57 NH<sub>4</sub><sup>+</sup> mg kg<sup>-1</sup> D.M. of soil. A lawn cultivar of chewing red fescue was sown in 2004 and 2005 and then utilized for two successive years (respectively, 2005-2006 and 2006-2007). After harvesting the cover crop (spring barley) in the establishment year or seeds in the production years mineral nitrogen was applied at rates of: 20, 40 or 60 kg ha<sup>-1</sup> (autumn fertilization). At the end of March/beginning of April, directly before the start of growth, 40, 60 and 80 kg N ha<sup>-1</sup> was applied in a single rate or divided into half with application of 40 kg ha<sup>-1</sup> at the beginning of shooting. State of plant nitrogen nutrition was determined based on total nitrogen content in leaves (the Kjeldahl method) and the chlorophyll index (leaf greenness) using a

chlorophyllometer. Measurements were made before starting of growth (April), at the beginning of shooting (May) and on the flag leaf at seed milk maturity (June). Photosynthesis rate was measured with a portable gas analyser (Li-Cor 6400). Significance of differences were determined by Tukey's test, at the level  $\alpha=0.05$ .

## Results and discussion

Highest seed yields of red fescue were obtained after the application of 120 kg N ha<sup>-1</sup> per year (Fig. 1). After the application of a rate reduced by half the decrease in yield was only 7.5 %. On the basis of measurements in May and June it was indicated that an increase in the rate from 60 to 140 kg N ha<sup>-1</sup> resulted in proportional growth of nitrogen concentration in leaves (coefficients of determination ( $R^2$ ) 0.95 and 0.88, respectively).

**Figure 1.** Chlorophyll index, N content and seed yield of red fescue depending on total rate of mineral nitrogen, mean from first and second production year



Nitrogen content in leaves at the beginning of growth and shooting was similar and decreased at seed milk maturity (Table 1). Decrease in nitrogen concentration in ontogenic development makes it difficult to use this measure to estimate plant nutrition state (Fotyma & Bezdusznik, 2000). In production years, at the beginning of growth nitrogen concentration in leaves was the largest if 60 kg N ha<sup>-1</sup> was applied in autumn. A similar response to this level of autumn nitrogen fertilization is also reported by Gislum and Boelt (1999). In the present study, at the beginning of shooting (in May) an increase in the spring rate of nitrogen from 40 to 60 kg ha<sup>-1</sup> and also from 60 to 80 kg ha<sup>-1</sup> caused an increase in nitrogen concentration in leaves, but the differences were significant only in the first production year.

Chlorophyll index increased from April to June (Table 1). No response of this index to nitrogen rates applied in autumn was observed. In production years, at the beginning of shooting the chlorophyll index was significantly higher after a single application of 80 kg N ha<sup>-1</sup> as compared

with 40 kg. A similar reaction was indicated on the flag leaf (in June), but only in the first harvesting year.

Nitrogen fertilization level in autumn and early spring did not have an effect on the photosynthesis rate of the flag leaf in seed milk maturity in the first or the second production years (Table 2). In the second year of seed harvesting the application of half of 80 kg N ha<sup>-1</sup> at the beginning of shooting caused an increase in photosynthesis rate of the flag leaf in relation to the single application of 40, 60 or 80 kg N ha<sup>-1</sup> in early spring.

**Table1.** Nitrogen content and chlorophyll index of red fescue prior to starting of growing in spring (April), at the beginning of shooting (May) and of flag leaf at milk stage (June)

Nitrogen rate [kg ha <sup>-1</sup> ]	Nitrogen content [%]						Chlorophyll index [SPAD]					
	April		May		June		Time of measuring		May		June	
							April	May				
	I		II		I		Year of production		I		II	
I							II					
autumn	20	3,06	3,33	3,24	2,50	1,84	7,70	5,84	20,1	15,7	20,8	23,1
	40	2,93	3,41	3,20	2,55	1,76	7,46	7,01	19,0	16,7	20,4	22,6
	60	3,32	3,76	3,31	2,60	1,88	7,25	6,89	19,7	16,4	19,7	21,2
LSD		0,22	0,32	ns	ns	ns	ns	ns	ns	ns	ns	ns
spring	40	-	-	3,10	2,40	1,69	-	-	17,1	15,2	18,4	23,7
	60	-	-	3,25	2,58	1,72	-	-	19,5	16,0	20,6	20,6
	80	-	-	3,40	2,67	1,85	-	-	22,3	17,6	21,7	21,1
	40+40	-	-	-	-	2,05	-	-	-	-	20,4	23,7
LSD		-	-	0,14	ns	ns	-	-	3,21	2,05	3,00	ns

ns - not significant

**Table 2.** Photosynthesis rate of flag leaf and seed yield of red fescue

Nitrogen rate [kg ha <sup>-1</sup> ]	Photosynthesis rate [ $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ ]		Seed yield [dt ha <sup>-1</sup> ]		
	Year of production				
	I	II	I	II	
autumn	20	8,83	6,09	8,72	8,42
	40	11,03	6,90	8,82	8,32
	60	10,64	6,54	8,66	7,94
LSD	ns	ns	ns	0,35	
spring	40	9,62	2,08	8,48	7,39
	60	11,65	3,45	8,81	8,45
	80	8,31	3,80	8,77	8,60
	40+40	11,09	16,70	8,87	8,46
LSD	ns	2,09	ns	0,81	

ns - not significant

In the first year, no significant effect of autumn and spring fertilization levels on seed yield was indicated (Table 2). In the second year, following the autumn application of 20 or 40 kg N ha<sup>-1</sup>, application of at least 60 kg was necessary in spring. After the application of 40 kg ha<sup>-1</sup> during starting of growth, seed yield was the smallest, but an additional application of 40 kg at shooting stage, at a high photosynthesis rate of the flag leaf during seed formation, significantly increased the yield. The study conducted indicates that the total level of nitrogen fertilization should not be more than 60 kg ha<sup>-1</sup> in the first production year and 80 kg in the second. Similar rates for red fescue (55-80 kg ha<sup>-1</sup>) are recommended by Fairey (2006).

## Conclusions

1. Red fescue at the beginning of growth shows the response of nitrogen content in leaves to the level of autumn fertilization with nitrogen, and at the beginning of the shooting stage of the chlorophyll index to the rates applied in early spring. Analysis of plant nutrition state by means of those indexes may be useful for determination needs for spring fertilization with nitrogen in production years.
2. Increasing rates of autumn fertilization with nitrogen above 20 kg ha<sup>-1</sup> do not result in a significant increase in yield in the production years. Spring rate of 40 N ha<sup>-1</sup> applied during starting of growth in the first year of seed harvesting is sufficient, whereas in the second year it should be increased up to 60 or 80 kg N ha<sup>-1</sup>

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## Organization of grass seed research in the Netherlands

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### Abstract

In 2008 it was decided that all research in grass seed would be allocated at the Agricultural Innovation and Knowledge centre Rusthoeve at Colijnsplaat in the Netherlands. Under the flag of this centre a Grass seed Expertise Centre was founded in which Rusthoeve and DLV Plant participate in doing research and trails for the Grass seed sector in general.

The assignment of the Expertise Centre is to gather all necessary information and knowledge to keep up with the latest developments in this specialized crop and also take the initiative to find answers for problems and improvements of the technical and economical aspects of grass seed production. The Expertise Centre operates in commission by a farmers interest working group for grass seed production which, in consultation with the Expertise Centre, determines a yearly research program. The implemented projects are also financed through means of this working group, a farmers contribution to this fund is based on a payment per hectare grass seed production.

To meet up with the above mentioned both Johan Wander and Sam de Vlieger can, due to their long time experience in respectively grass seed research and grass seed production, fall back on a broad network of institutes and specialists. Results of research are published on several websites as [www.kennisakker.nl](http://www.kennisakker.nl) and [www.graszaad.info](http://www.graszaad.info). Currently the Expertise Centre is performing a screening test for herbicides in 20 different grass seed species. Furthermore the research program for 2010 comprises tests with a growth regulator and the testing of remedies against the loss of seed during the ripening.

## Results of herbicide screening on 20 grasses in the Netherlands in 2009

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### Abstract

In 2009 in the Netherlands 26 herbicides and combinations of herbicides were tested in 2 doses on 20 grasses. Most grasses were sprayed at the three leaves stage. A total of more than 1000 plots were observed 3 times. The tested grasses were grass seed crops, weeds or species for mixtures in turfgrasses: black grass (*Alopecurus myosuroides*), perennial ryegrass – lawn and fodder (*Lolium perenne*), Prairie June Grass (*Koeleria pyramidata*), sheep fescue (*Festuca ovina*), orchard grass (*Dactylis glomerata*), brome fescue (*Vulpia bromoides*), rat tail fescue (*Vulpia myuros*), tall fescue – lawn and fodder (*Festuca arundinaceae*), red fescue (*Festuca rubra*), creeping red fescue (*Festuca rubra*), rough stalked meadowgrass (*Poa trivialis*), tufted hairgrass (*Deschampsia cespitosa*), annual bluegrass (*Poa annua*), common bent (*Agrostis capillaris*), smooth stalked meadowgrass (*Poa pratensis*), bentgrass (*Apera spica-venti*), common velvetgrass (*Holcus lanatus*), soft brome (*Bromus hordeaceus*).

The tested product were: carfentrazone-ethyl, clodinafop / pinoxaden, pinoxaden, dimethenamid-P, dimethenamide-P / terbuthylazine, clomazone, diflufenican, ethofumesate, methabenzthiazuron, propyzamide, tepraloxymid, iodosulfuron, jodosulfuron-methyl-Na / mesosulfuron-methyl, nicosulfuron (Accent and Milagro), rimsulfuron and sulfosulfuron.

Several results were promising for controlling weed grasses in grass seed crops or in turfgrasses. A promising result for example: the control of *Poa annua*, *Apera spica-venti* and *Vulpia spp* in *Agrostis capillaris*, *Poa pratensis*, *Festuca ovina* and *F. rubra* with the combination of ethofumesate and rimsulfuron.

# Path Coefficient and Ridge Regression Analysis to Improve Seed Yield of *Psathyrostachys juncea* Nevski

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## Abstract

Based on multi-factor orthogonal designed field experimental blocks, the yield components and their direct and indirect influences on the seed yield of *Psathyrostachys juncea* Nevski. were investigated under variable growing conditions. In each block the yield components: fertile tillers/m<sup>2</sup> (y1), spikelets/fertile tillers (y2), florets/spikelet (y3), seed numbers/spikelet (y4), seed weight (y5) and seed yield were determined by hand in 2003. The results show that in *P. juncea* . seed yield is significantly correlated with yield components y1 (0.749\*\*\*), y2 (0.159\*) and y5 (0.231\*). All of the ridge regression coefficients are >0, which means that increasing any one of the yield components (y1~y5) will increase seed yield, in accordance with biological theory. This study indicates that ridge regression is one of the most promising methods available to unravel the tangled skeins of inter-correlated factors.

## Introduction

*Psathyrostachys juncea* Nevski is a cool-season forage species well adapted to semi-arid climates (Wang et al., 2004). We are interested in investigating the relationships between the seed yield and its components to improve the seed yield of this forage grass.

The advantage of path analysis is that it permits the partitioning of the correlation coefficient into its components, one component being the path coefficient that measures the direct effect of a predictor variable upon its response variable; the second component being the indirect effect(s) of a predictor variable on the response variable through another predictor variable (Milligan et al., 1990). Path analysis has been used by plant breeders to assist in identifying traits that are useful as selection criteria to improve crop yield.

For grass crops, the correlation of economic yield components with grain yield and the partitioning of the correlation coefficient into its components of direct and indirect effects have been extensively reported: e.g. highly significant associations of grain yield were observed with

1000-grain weight and tiller number per plant (Das and Taliaferro, 2009), the number of filled grains per panicle (Wu et al., 2008) and harvest index (Sured et al., 1998).

However, morphological characters influencing yield are often highly inter-correlated, leading to multi-collinearity when the inter-correlated variables are regressed against yield in a multiple-regression equation.

In this study, based on multi-factor orthogonal field experimental design, an attempt was made to study the direct and indirect influences of some important yield components on seed yield in *P. juncea* via correlation, path coefficient and ridge regression analyses with big sample sizes under various growing conditions (field managements).

## Materials and Methods

Using 5 groups of multi-factor orthogonal field experimental designed blocks, the field experiment was set up in Jiuquan (39°37'N, 98°30'E), Gansu Province, China from 2002 to 2003. In total 112 experimental blocks with an area of 28 m<sup>2</sup> were sown in spring (Table 1). Seed yield was recorded in autumn 2002 and averaged 165kg ha<sup>-1</sup>.

According to the orthogonal experimental designs, yearly repeated, under various field managements, conditions from controlled environments, including regimes of fertilizer (experimental factor: X1, X3, and X4), irrigation system (experimental factor: X2), plant density (experimental factor: X5) and plant growth regulators (experimental factor: X6) (Table 1). In each block was measured the yield components: Fertile tillers/m<sup>2</sup> (y1), spikelets/fertile tillers (y2), florets/spikelet (y3), seed numbers/spikelet (y4), seed weight (mg) (y5). Seed yield (kg/hm<sup>2</sup>) (z) was determined by hand in field, randomly based on order, from anthesis to seed harvest in year 2003. The sample size of y1~y5 and z for field experimental block in *P. juncea* are 10, 36, 27, 54, 10 and 4, respectively. Seed weight was measured from 100-grains at a moisture content of 7~10. Seed yield was measured from hand-harvesting a subsample of 1 m<sup>2</sup>. The statistical analysis was performed using Visio FoxPro (Version 6.0) and SAS (Release 8.1, SAS Institute Inc, 1988 (Table 2). Based on Pearson Correlation Analyzed by SAS (Table 3), a QBASIC program was written for path coefficient analysis (Table 4). Furthermore, via SAS, ridge regression analysis was performed on seed yield (z) and its components (y1~y5).

## Results

The results show that in *P. juncea* seed yield is significantly correlated with yield components y1 (0.749\*\*\*), y2 (0.159\*) and y5 (0.231\*). The order of correlation coefficients is: y1>y5>y2>y3>y4 (table 3). The order of direct effects of yield components (y1~y5) on seed yield is y1>y5>y3>y2>y4 (Table 4) by path coefficient analysis, the biggest direct effects to seed yield are the yield component y1 (0.774) and, the biggest indirect effects to seed yield are y3

through  $y_4$  (0.133). Increasing  $y_1$  is the most productive on the seed yield in the components followed by the  $y_5$  and  $y_3$ .

In ridge regression analysis, although the optimal value of  $k$  cannot be determined with certainty, several procedures have been proposed for the selection of  $k$  (Hoerl and Kennard 1970 a, b) have suggested that  $k$  is determined from the ridge trace, with  $k$  selected so that a stable set of regression coefficients was obtained (Newell and Lee, 1981). In this study, according to the ridge trace, for various values of  $k$ , with the values of  $k$  estimated as 0.5, using the method of Hoerl and Kennard (1970a, b), the standard ridge regression models are lined in the figures. The resulting ridge regression coefficients are -892.63, 2.19, 4.61, 15.46, 3.20 and 263.96 for intercept,  $y_1$ ,  $y_2$ ,  $y_3$ ,  $y_4$  and  $y_5$  respectively. Following the ridge regression models is:  $Z = -892.63 + 2.19y_1 + 4.61y_2 + 15.46y_3 + 3.20y_4 + 263.96y_5$  ( $F=33.11$ ,  $Pr<0.0001$ ).

## Discussion

The various field experimental managements ( $X_1 \sim X_6$ ) created a very wide range of seed yields and yield components (Table 2), with a maximum seed yield of 1969.65 kg/hm<sup>2</sup>, and a minimum of 358.41 kg/hm<sup>2</sup> (little irrigation, no fertilizer and low plant density) (table 2).

With multi-factor orthogonal experimental designs and big sample statistics analysis in field experiment, the significant correlation coefficients (at  $P=0.0001$  and 0.01) show the results are reliable. Ridge regression effectively overcome the problem of highly multi-correlated predictor variables like yield components (Hoerl and Kennard, 1970a,b).

In this study all of ridge regression coefficients are  $>0$ , that means increasing any one of the yield component ( $y_1 \sim y_5$ ) will increase seed yield, in accordance with biological theory. In the result of the ridge regression model, increasing every 1 unit of yield components ( $y_1 \sim y_5$ ), respectively will increase seed yield ( $Z$ ) by 2.19, 4.61, 15.46, 3.20, 263.96 kg/hm<sup>2</sup>, respectively; The component  $y_2$  may develop in a oversaturated situation or from reasons of climate; By comparisons, it is in average 90.2 spikelets/fertile tillers in this study, whereas *P. juncea* in the second year of growth the spikelets/fertile tillers ( $y_2$ ) are 20~36, 27.5~34.5 and 53.6~60.2 in Xinjiang Weiwuer Autonomous Region (44°31'N, 87°5'E), northwest of China (Zhang et al, 2002), in Yuershan Farm (41°44'N, 140°16'E), Hebei province, east of China (Mao et al, 2000), and in Pulandian Farm in Dalian city (39°47'N, 121°54'E), Liaoning province, northeast of China (Fang et al, 2001),, respectively. So, it is needed to clarify the dynamic effect of weather factors such as temperature, rainfall and hours of sunshine, etc. on yield components of *Psathyrostaehys juncea* Nevski. in the period of the grass development. However, the order of the yield components:  $y_1 > y_5 > y_2 > y_3 > y_4$  of correlation and  $y_1 > y_5 > y_3 > y_2 > y_4$  of path coefficients, imply that  $y_1$  and  $y_5$  is most important yield components for improving seed yield, and  $y_4$  the least.

## **Conclusion**

Combined path coefficients and ridge regression model analyses, show that  $y_1$  is most important yield components for improving seed yield,  $y_5$  and  $y_3$  comes next, and  $y_4$  the least. All of ridge regression coefficients are  $> 0$ , that theoretically means increasing any one of yield component ( $Y_1 \sim Y_5$ ) will increase seed yield. Additionally, evidence from this study and others indicates that ridge regression is one of the most promising of the methods available to unravel the tangled skeins of inter co-related factors. Inter-correlated variables are very prevalent in the arrays of measured yield components and other agronomic characters used by herbage breeders trying to enhance the seed yield efficiency of crops.

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**Table.1** Field Experimental design and factors (*P. juncea*)

Field experimental design groups	Factors	Repeat	blocks
1.2-D-optimum orthogonal design(1)*	2( $X_3, X_4$ )	3	18
2.2-D-optimum orthogonal design(2)*	2( $X_3, X_4$ )	1	6
3.Quinque-factor orthogonal design	5( $X_1 \sim X_5$ )	1	36
4.Bin-factor orthogonal contract blocks	2( $X_2, X_3 + X_4$ )	1	22
5.Tri-factor orthogonal design	3( $X_1, X_3, X_6$ )	1	23
CK	--	7	7
total	6( $X_1 \sim X_6$ )	--	112

Note: \*: Applied N and  $P_2O_5$  differently between design (1) and (2);  $X_1 \sim X_6$ : is time of fertilizing, quantity of irrigation, applied nitrogen, applied  $P_2O_5$ , planted density, amount of spray plant regulator Paclobutrazol(PP333), respectively.

**Table.2** Simple Statistics of  $Y_1 \sim Y_5$ ,  $Z$  (*P. juncea*)

Variable	N	Mean	Std-Dev	Std-Error	Minimum	Maximum	Pr> t
y1	112	205.6	70.7	6.9	76.1	415.1	<.0001
y2	112	90.2	2.7	0.265	79.7	96.960	<.0001
y3	112	4.6	0.7	0.074	3.01	6.25	<.0001
y4	112	2.1	0.3	0.033	1.500	3.05	<.0001
y5	112	3.5	0.18	0.018	2.9	3.8	<.0001
z	112	964.4	336.93	32.9	358.4	1969.6	<.0001

**Note :**  $Y_1$  : Fertile tillers/m<sup>2</sup> ;  $Y_2$  : Spikelets/Fertile tillers ;  $Y_3$  : Florets/spikelet ;  $Y_4$  : Seed numbers/spikelet ;  $Y_5$  : Seed weight(mg) ;  $Z$  : Seed yield(kg/hm<sup>2</sup>).

**Table.3** Pearson correlation coefficients of  $Y_1 \sim Y_5$ ,  $Z$  (*P. juncea*)

	y1	y2	y3	y4	y5	z
y1	1.000	0.309**	0.106	0.131	-0.008	0.749***
y2		1.000	-0.071	-0.128	-0.021	0.195*
y3			1.000	0.927***	0.158	0.127
y4				1.000	0.122	0.110
y5					1.000	0.231*

\*\*\*, \*\* and \* significant at  $P=0.0001$ , 0.01 and 0.05, respectively

**Table.4** Path analysis showing direct and indirect effect of  $Y_1 \sim Y_5$ ,  $Z$  (*P. juncea*)

	y1→z	y2→z	y3→z	y4→z	y5→z
y1	<b>0.774</b>	-0.011	-0.012	-0.036	-0.006
y2	0.058	<b>-0.052</b>	0.002	-0.028	-0.001
y3	-0.051	-0.014	<b>0.207</b>	0.133	0.041
y4	-0.063	0.009	0.057	<b>-0.219</b>	0.025
y5	0.033	-0.013	0.019	0.029	<b>0.230</b>

## Relative humidity, seed moisture content, storage temperature and seed longevity in Zoysiagrass (*Zoysia japonica* Steud.)

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### Abstract

Zoysiagrass, *Zoysia japonica* Steud., is a native warm-season grass in China. It is characterized as a deep coat-imposed dormancy and with limited seed longevity. The objective of this study was to explore zoysiagrass seed longevity related to storage conditions. The equilibrium moisture contents of zoysiagrass seed by 20% NaOH dormancy breaking treatment at 60%, 70% and 80% relative humidity stored at 25 °C were 10.39%, 12.34% and 15.03% with dry weight basis. Based on the equilibrium moisture content, zoysiagrass seeds (original germination percentage of 84% and viability of 89%) with one of four moisture contents (7.9%, 12.11%, 15.8%, 19.36%) were stored hermetically at one of four constant temperatures (3, 14, 25, 35 °C) for up to 217 d, and changes in final germination percentage (FGP), mean germination time, viability and vigor index were determined at 5d interval during first 30 day period, and tested at 15 to 30 d interval afterward. At the highest seed moisture content of 19.36% and storage temperature of 25 and 35 °C, after 30 d and 50 d respectively, FGP decreased to 50% of original germination percentage. At the seed moisture content of 15.8% and 35 °C, after 42 d FGP decreased to 50% of original germination percentage, but at 25 °C, after 31 weeks FGP decreased to 52% of original germination percentage. At the seed moisture content of 12.34% and 35°C, after 31 weeks, FGP decreased less than 10%. At the lowest seed moisture content of 7.9%, no significant FGP changes at all of four temperature conditions, however, the vigor index results indicated a consistent deterioration occurred during storage. Our results suggest at ambient temperature condition the optimum storage conditions for zoysiagrass seed as following, atmospheric relative humidity is not higher than 60%, seed moisture content is less than 8.0%.

# Effects of depth and duration of burial on seed seasonal germination, dormancy and viability of 3 desert plant species

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## Abstract

Dynamics of seed fate in the soil is very important for prediction of future plant community, land restoration and conservation. Present study investigated seasonal germination, dormancy and longevity of three desert plant species seeds which were subjected to different burial depth and duration in the field. Seeds of *Lespedeza potaninii*, *Nitraria tangutorum* and *Peganum multisectum* were buried at different depths (0, 2, 5 and 10 cm), and exhumed at bimonthly intervals from April 2008 to October 2009 in Alxa desert region, China. The exhumed seeds were classified as 'field germination', 'enforced dormancy', 'innate dormancy' and 'decayed seeds' (Harper, 1957). The results showed the field germination of 3 species increased with the buried depth rising. After 18 months burial, the maximum field germination of *L. potaninii*, *N. tangutorum* and *P. multisectum* are 15%, 26% and 12%, respectively. The innate dormancy percentage of *L. potaninii* seeds decreased from initial 98% to 64% on the soil surface (0 cm), but less change was observed when buried at 2 cm, 5 cm and 10 cm. Although a high level dormancy in *N. tangutorum* when seed were harvested, its innate dormancy percentage decreased from 94% to 1% after 4 months burial in the field. Contrary to above two species, *P. multisectum* seeds exhibited seasonal pattern (dormancy cycle) during experimental period. The maximum points of innate dormancy in two years were observed in October, and then decreased. The decayed seeds decreased with the buried depth rising, the maximum decayed seeds percentage of 3 species are 3.5%, 31% and 12%, respectively. These results indicated that the innate dormancy of 3 desert plant species decreased in different degree, but their field germination were all few, most of seeds lost innate dormancy were in an enforced dormancy state and it is an important strategy in the desert regions. The loss of seeds indicated that the 3 species all behaved as persistent soil seed bank according to Thompson's system (1979).

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## Ergovaline contents in grasses from semi-natural grasslands in Poland

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### Abstract

The aim of following study was to investigate presence of endophyte (from *Neotyphodium* genera) and ergovaline contents in grasses from semi-natural grasslands in Poland. More than 610 ecotypes from grasslands in Central and North – Eastern Poland were tested for endophyte presence and ergovaline contents. More than 30% of tested ecotypes were colonised by endophytes (further marked as E+) and the majority of them (66%) produced ergovaline with different intensities.

All E+ tall fescue ecotypes, originating from Central Poland, were found to produce the highest amount of ergovaline (average = 1.86 ppm). Majority of perennial ryegrass and meadow fescue E+ ecotypes were also found to produce ergovaline. However, mean alkaloid contents in mentioned species were lower than for tall fescue (0.166 ppm and 0.122 ppm, respectively). Red fescue and sheep's fescue produced only traces of ergovaline (0.07 ppm, and 0.06 ppm, respectively). Ergovaline was not detected in E+ ecotypes of tufted hairgrass, italian ryegrass and Kentucky bluegrass. It can be concluded that due to multi-species nature of Polish semi-natural grasslands and rather low amount of ergovaline, current animal threat associated with alkaloid intake during feeding is rather low. Tall fescue in Poland is rarely consumed by animals as compared to meadow fescue or perennial ryegrass and probably has never been sown alone for feeding purposes or hay production. However, in case of highly productive short rotation meadows, especially were only a few species as perennial ryegrass or meadow fescue were sown, it is possible the high concentration of alkaloid in forage. Therefore, intensive research and monitoring of existing grasslands is still needed.

### Introduction

Permanent grasslands in Poland cover ca. 3 mill ha (approx. 30% of the whole country area). The most important and valuable grass species in grassland swards are: perennial ryegrass (*Lolium perenne* L.), meadow fescue (*Festuca pratensis* Huds.), red fescue (*Festuca rubra* L.) and tall fescue (*Festuca arundinacea* Schreb.). Mentioned species are also known to host symptomless endophytic fungi, from *Neotyphodium* genera (Petroni *et al.* 1986). For agricultural practice, endophyte – plant symbiosis is both positive and negative. Endophyte colonized (E+) grasses express range of adaptations to abiotic (drought, mineral imbalance, soil acidity) and biotic (disease, pest or animals) stress (Funk *et al.* 1994). As a result, E+ grasses are more compatible than non colonized grasses and thrive better in presence of limited resources. However, in certain

circumstances endophytes may produce toxic alkaloids (ergovaline, lolitrem B, etc.) that have been linked with animal production and health problems. “Ryegrass staggers syndrome” and “fescue toxicosis” are the most common animal diseases caused by E+ grass. Ryegrass staggers syndrome (decrease of milk production, weight loss, disorders of nervous system) is caused by presence of large quantities of lolitrem B in animal forage. Ergovaline production is connected with fescue toxicosis, which is manifested by reduced forage intake, excessive salivation and reduced reproductive performance. In case of both animal diseases described above, high mortality was also noted (Siegel *et al.* 1985; Reed *et al.* 2000).

In Poland, endophyte research is rather scarce; however endophytes were found both in seeds and in plants of common grass species in Polish grasslands (Pańska and Żurek, 2005; Wiewióra *et al.*, 2008). Endophyte colonization (from 20% to 60%) was also noted in Polish grasslands by Pfanmüller *et al.*, (1994) and by Lewis (2000). Positive effect of endophyte colonization on persistency and aesthetic value of *Festuca rubra* cultivars was noted (Pańska and Żurek, 2005; Prończuk and Prończuk, 2000). It was also concluded that endophyte colonization increase along with grass plantation age (Wiewióra *et al.*, 2006). However, nothing has been done so far, concerning ergovaline contents in Polish E+ grasses. The aim of current study was to identify endophyte colonization on semi-natural grasslands in different regions of Poland and to estimate potential sward toxicity due to ergovaline contents.

## Materials and Methods

Ecotypes were collected from semi-natural grassland in a form of living plant. From 5 to 10 plants were collected per one ecotype in each locality. Plants were further planted in field collection in Radzików and analysed for endophyte presence with rapid staining method according to Saha *et al.* 1988. Further, during laboratory work, grass samples of ecotypes with endophytes were prepared for analytical treatment and HPLC analyses of ergovaline after descriptions given by Craig *et al.* (1994) with Hovermale and Craig (2001) modifications. Jordi RP column (100A, 150 x 4.6 mm, 5 µm) with BRP-1 pre-column was used for mentioned toxin analyses.

## Results and Discussion

Grass ecotypes were collected on 58 localities from semi-natural grasslands in different regions of Poland. Total number of 618 ecotypes were gathered in a form of living plant, mostly of fescues (red – 172 ecotypes, meadow – 130, tall – 31 and sheep’s - 11) but also of perennial ryegrass (146 ecotypes), tufted hairgrass (*Deschampsia cespitosa* L. – 68) and Kentucky bluegrass (*Poa pratensis* L. – 56 ecotypes). Only few ecotypes of giant fescue (*Festuca gigantea* (L.) Vill.) and Italian ryegrass (*Lolium multiflorum* Lam.) were also collected.

The highest endophyte colonization was noted for meadow fescue – from 81 to 75% of all ecotypes collected (tab. 1). Tall fescue colonization ranged from 8.3% (ecotypes from Central East) to 47.4% (ecotypes from Central Poland). Red fescue endophyte colonization ranged from

11.8 % (Central East) to 40.0% (North East Poland). Perennial ryegrass ecotypes were colonised at lower level, despite of ecotype origin (range from 17.9 to 25.8%). Only traces of colonization were detected in tufted hairgrass ecotypes (4.4%) and in one ecotype of giant fescue. Kentucky bluegrass ecotypes were not colonized at all. Tufted hairgrass or giant fescue were infrequently reported as grass species hosting endophytes.

Ergovaline contents were not detected in all ecotypes colonised by endophytes. Only in 19.5% from all ecotypes collected, ergovaline contents ranging from 0.005 up to 2.63 ppm were detected. The highest ergovaline contents were found in ecotypes of tall fescue from Central Poland (range from 1.21 up to 2.63 ppm). Such amounts are claimed to be extremely toxic for animals (Bony and Delatour, 2000). Chronic toxic amounts causing weight loss, milk production decrease, daily weight increase reduction etc. are 0.2 – 0.4 ppm, disease symptoms may occur at 0.3 – 0.5 ppm for horses or at 0.4 – 0.7 ppm for cattle.

Generally, in more than one-third of ecotypes with ergovaline, toxic amounts (more than 0.2 ppm) were detected. However, due to specific nature of Polish grasslands, possible animal threat associated with ergovaline content in sward is rather low. In swards of majority of Polish grasslands up to 25 – 30 plant species as grasses with dicotyledonous herbs grow together. Therefore, even high concentration of toxins in only a few plants per grassland will be ‘diluted’ in green sward or in hay. Tall fescue is not very common on pastures and therefore it is rarely consumed by animals. However, due to increasing area of highly productive short rotation meadows, the possibility of toxic concentration of ergovaline or other alkaloids become quite real. Very important are also local stress factors as drought, which may induce significant increase of alkaloids in grasses (Zabalgogezza and Bony, 2005). Therefore, if our grassland will suffer from summer drought periods, percentage of endophyte infected plants and average amounts of alkaloids may increase. This will directly influence animal health.

**Table 1.** Endophyte presence and average ergovaline contents in grass ecotypes collected in Poland

Country region	Genus, species	Ecotypes collected	Frequency (%) of ecotypes with:		Ergovaline contents (ppm):	
			endophyte	endoph.+ ergovaline	mean	range (min. - max.)
Central East	<i>Desch. cespitosa</i>	68	4.4	0.0	-	-
	<i>F. arundiancea</i>	12	8.3	8.3	1.298	1,298
	<i>F.pratensis</i>	69	81.2	49.3	0.069	0,005 - 0,749
	<i>F.rubra</i>	102	11.8	7.8	0.144	0,026 - 0,545
	<i>Lolium perenne</i>	76	23.7	15.8	0.023	0,011 - 0,080
	Average for region			25.9	16.2	0.383
North East	<i>F.ovina</i>	11	27.3	9.1	0.076	0,076
	<i>F.pratensis</i>	29	75.9	58.6	0.289	0,044 - 1,545
	<i>L. multiflorum</i>	1	1.0	0.0	-	-
	<i>F.rubra</i>	25	40.0	8.0	0.028	0,016 - 0,040
	<i>L. perenne</i>	31	25.8	22.6	0.121	0,065 - 0,192
Average for region			34.0	19.7	0.129	
Central	<i>F. arundiancea</i>	19	47.4	47.4	1.924	1,213 - 2,630
	<i>F.ovina</i>	1	100.0	0.0	-	-
	<i>F.gigantea</i>	2	100.0	50.0	0.112	0,112
	<i>F.pratensis</i>	32	75.0	59.4	0.097	0,024 - 0,433
	<i>F.rubra</i>	45	20.0	13.3	0.042	0,050 - 0,089
	<i>L. perenne</i>	39	17.9	15.4	0.595	0,162 - 1,706
	<i>Poa pratensis</i>	56	0.0	0.0	-	-
Average for region			51.5	26.5	0.554	
Average for all ecotypes			38.5	21.4	0.37	

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# Long-term Evaluation of Annual Ryegrass Cropping Systems for Seed Production

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## Abstract

A long-term field trial was established in 2005 to evaluate the economics of reduced tillage systems in continuous annual ryegrass seed production, and to measure their impact on soil properties. In Oregon, a majority of the annual ryegrass seed crop acreage is managed with conventional tillage and planting systems. Preliminary results after four years indicate that alternating a conventional tillage system with a no-till or volunteer method of establishment can provide seed yields comparable to continuous conventional tillage, but at a lower cost of production. Seed yields under continuous no-till production were the lowest among treatments to date due to slug damage. Soil carbon levels in the 0-20 cm depth were comparable among treatments, but in the continuous no-till system stratification of soil carbon was observed. This study demonstrated that there are alternatives to annual conventional tillage that reduce costs while maintaining seed yields.

## Introduction

Annual or Italian ryegrass (*Lolium multiflorum* Lam.) is grown on approximately 50,000 ha in the southern Willamette Valley of Oregon. Production has occurred mostly on soils too poorly drained for cereal and vegetable crops, and on soils less productive than needed for higher value perennial grass and clover seed crops. This led to systems of continuous seed production of annual ryegrass, and some fields have been in production for over 40 years without any crop rotation.

Annual ryegrass seed production systems have changed significantly from the time when open field burning and no-till planting was a common and important practice. Prior to the 1990, over 50% of the acreage was open field burned. In recent years, only about 20% of the acreage was open field burned. Legislation in 2009 further restricted burning in Oregon and essentially eliminated open field burning for annual ryegrass seed production.

Currently, a majority of the annual ryegrass seed crop acreage is successfully managed with conventional tillage and planting systems. However, the cost of tillage is expensive; thus, alternative no-till and volunteer systems are being tested and used. In the volunteer system, a seed crop is produced from seed shattered from the previous crop and is essentially a no-till and no-plant system of production for un-certified seed production (less than 5% of Oregon annual ryegrass is certified). In the volunteer system, grazing and strip or row spraying with herbicides are used to control stand density. When no-till planting is used, a sprout of volunteers and weed seeds are first sprayed with glyphosate herbicide. These systems offer a way to reduce tillage and fuel expenses, and reduce concerns about dust and air quality. Previous field work demonstrated that seed yields under the volunteer and no-till systems decline significantly over time if used more

than one year in a row (Young et al., 1997). In the first year of production though, no-till and volunteer methods have been comparable to conventional methods of planting, suggesting a system of alternate year tillage may be a feasible way to maintain seed yields over time. This study was designed to evaluate the long-term economics of these various cropping systems in a continuous annual ryegrass monoculture over multiple years. A secondary objective was to measure the impact on soil properties, especially carbon sequestration, of reduced tillage systems of seed production.

## **Material and Methods**

This study was established at the Hyslop Crop Science Farm near Corvallis, Oregon in the fall of 2005. The field had been planted to ‘Gulf’ annual ryegrass the previous two years, and we continued with the same variety. Soil was a moderately well-drained silt loam soil with a pH of 5.4 and soil test levels of P, K, Ca, and Mg above levels considered adequate for seed production. Six treatments were included in a Randomized Complete Block design, and replicated three times with plots 7.6 m x 38 m. The resulting treatments included:

1. Continuous conventional tillage and planting system.
2. Continuous no-till planting system
3. No-till/conventional tillage rotation (alternate year tillage)
4. Volunteer/conventional tillage rotation (alternate year tillage)
5. Burn and no-till/ conventional tillage rotation (alternate year tillage)
6. Volunteer/no-till/conventional tillage rotation (tillage every 3<sup>rd</sup> year)

In all except the burn treatment, residue from the previous year’s crop was flail chopped and left on the field. Tillage included plowing to a depth of 20-25 cm, disking, and pulvi-mulching. A final seedbed was prepared by harrowing and rolling. All treatments except the volunteer included at least one preplant application of glyphosate to control volunteer seedlings. A preplant fertilizer of 200 kg/ha of 16-16-16 was applied to all treatments. A Great Plains no-till drill was used to seed all treatments except the volunteer at a planting rate of 19 kg/ha. The volunteer plots were established by allowing the seeds left on the surface the previous year to germinate and grow. Rows in the volunteer plots were established by spraying out 18 cm of every 25 cm of crop with glyphosate at 3.9 L/ha. All herbicide use, pest control and spring fertilization were performed according to OSU recommendations and industry standards.

Plots were harvested by swathing in late June, using a modified John Deere 2280 swather (1.83 m cutting width) and combined in mid-July with a Hege 180 plot combine. Seed was cleaned using a Clipper M2B cleaner and clean seed yields, cleanout percentage and seed weight determined. Seed yield results were analyzed as a Randomized Complete Block using treatment means over 4 years as replications or blocks.

## **Results and Discussion**

The seed yields obtained during the first four years of this long-term study ranged from a high of 2308 kg/ha in 2009 to a low of 1261 kg/ha in 2008. Seed yields in 2008 were significantly affected by slug and vole damage. For this reason, composite yields for the treatments over the four years were below normal for annual ryegrass seed fields in the Willamette Valley. However, there were significant differences between treatments ( $p = 0.10$ ) averaged over years (Table 1). The continuous no-till treatment had the lowest yield of the six different systems of production. The burn and no-till planting method alternated with conventional tillage had the greatest mean

seed yield. All systems of establishment that included alternate year tillage provided 4-year mean seed yields comparable to or greater than the conventional tillage method of establishment.

Among the six systems of establishment, the continuous conventional tillage and planting approach had the highest cost of production, based on the Oregon State University Enterprise Budget for annual ryegrass (Eleveld et al., 2007). The conventional system was \$112 to \$223/ha more than methods of establishment that used reduced tillage. Continuous no-till provided the lowest cost of production, but also had the lowest seed yield and the highest risk of establishment under Western Oregon conditions. Stand reduction due to slugs was a major reason for poorer yields in the continuous no-till treatment. Slug damage to seedling crops in the region is a common problem and a significant economic risk. Slug numbers in no-till annual ryegrass fields can be 14 to 29 times greater than in plowed and conventionally worked plots (Fisher et al., 1996). Systems that alternate no-till or volunteer methods with tillage have less risk of damage from this widespread and common pest, and over the course of this study to date, the alternate year tillage systems were more profitable than continuous conventional tillage.

**Table 1.** Seed yields and economic comparisons of annual ryegrass establishment systems.

<b><u>Preliminary 4-Year Results</u></b>	Total Cost	Seed Yield	Seed Yield
Establishment System (2006-2009)	(US \$/ha)	4-year average (kg/ha)	4-year average (% conventional)
1.Continuous conventional tillage	1512	1866 bc	100
2.Continuous no-till	1289	1777 c	95
3.No-till / conventional tillage rotation	1400	1858 bc	100
4.Volunteer / conventional tillage rotation	1304	1938 ab	104
5.Burn and no-till / conventional tillage rotation	1586	2026 a	109
6.Volunteer / no-till / conventional tillage rotation	1300	1904 bc	102
p level 0.0802			
LSD (0.10)		129	

\* means followed by the same letter do not differ significantly

One of the reasons for using reduced tillage systems is to maintain soil organic matter levels and potentially increase carbon storage in the soil. After three years, soil samples taken in this study showed that soil organic matter and soil carbon levels were similar under conventional or alternate year tillage systems in the 0-20 cm depth (Table 2). Soil organic matter and carbon were stratified under continuous no-till due to accumulation of soil organic matter in the surface layer (0-5 cm depth). Below 5 cm, soil carbon in the continuous no-till was significantly less. Soil organic matter and nutrient stratification have been observed in previous no-till trials (Mellbye et al., 1999). Despite differences in tillage and organic matter distribution, total accumulation of soil carbon among treatments to date was similar (assuming similar soil bulk density). Results may change over time, but these data suggest alternate year tillage or plowing in annual ryegrass cropping systems can maintain soil carbon at levels similar to those achieved with continuous no-till, at least over a short period of time.

**Table 2.** Soil organic matter, soil carbon, and soil nitrogen levels from selected annual ryegrass establishment systems (May 2009).

Selected Treatments	Soil Depth (cm)	Soil Organic Matter (%)	Soil Carbon (%)	Soil Nitrogen (%)
1.Continuous tillage	0-20	3.83	1.59	0.090
2.Continuous no-till	0-20	3.35	.62	0.107
Surface layer to 5cm	0-5	4.32	1.92	0.103
Below 5cm	5-20	3.61	1.48	0.077
4.Volunteer /conventional (Tillage alternate years)	0-20	3.95	1.63	0.083
6.Volunteer / no-till / conventional (Tillage every third year)	0-20	3.58	1.64	0.080
p level		0.0002	0.0001	0.0102
LSD (0.05)		0.27	0.10	0.017

Preliminary results after four years demonstrate that alternating a conventional tillage system with a no-till or volunteer method of establishment can provide seed yields comparable to continuous conventional tillage, but at a lower cost of production. In addition, alternate year tillage appears to maintain soil carbon levels comparable to continuous no-till. This trial is designed to last a minimum of 9 years, and a more thorough economic analysis of results will be presented in the future. The take-home message at this time is alternatives to annual conventional tillage exist in annual ryegrass production that reduces costs while maintaining yields.

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