11<sup>th</sup> INTERNATIONAL HERBAGE SEED GROUP CONFERENCE



11-18 JUNE 2023 ANGERS CONGRESS CENTRE LOIRE VALLEY • FRANCE

# Proceedings & Abstracts



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# Proceedings of the 11<sup>th</sup> International Herbage Seed Group Conference

Angers, Maine-et-Loire, France

June 11 – 18, 2023

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#### FOREWORD

The Scientific and Organizing Committee and the IHSG Executive Committee are very pleased to see you at the 11th International Herbage Seed Group Conference in France (Angers, Center of France, and the Champagne areas) from 11<sup>th</sup> to 18<sup>th</sup> June 2023. Even though we have been through a worldwide pandemic, we also know that IHSG members have continued their work to obtain a better knowledge of herbage seed production and how research can benefit this production.

The IHSG is an organization dedicated to improving our understanding of the science and technology of grass and forage legume seed production. The IHSG first started meeting in 1987 and has hosted 11 international conferences and 5 workshops at locations across the globe.

The primary missions of the IHSG are:

- To encourage cooperation and communication among those involved with herbage seed in any capacity
- To encourage the interchange of herbage seed research results and publications
- To promote the interchange of ideas and information by meetings and conferences

We are pleased to present here the proceedings of the 11<sup>th</sup> International Herbage Seed Conference where you will be able to hear some of the best herbage grass seed researchers make presentations and show posters, view management results in presentations and posters, participate in field tours, visit breeding stations, and explore some of the most beautiful sites in France. Of course, the most important part of the conference is the discussions and exchange of knowledge between the delegates.

The proceeding consists of keynote addresses, volunteered oral papers, and peer-reviewed poster abstracts presented at the Anger Congress Center, France. The proceeding papers cover a wide variety of topics relevant to the production of herbage seed, from breeding to harvesting, including all the issues affecting production: alternatives to pesticides, irrigation, biocontrol, and improving the competitiveness of production.

This collection of presentations is also available on the www.ihsg2023.org website, under the Conferences tab.

The Local Organizing Committee and their associated organizations have done a tremendously good job preparing this conference. They have managed to put together an exciting program for the 157 delegates participating in the first three days of the conference and 100 delegates that have signed up for the post-tour. The delegates are from 14 different nations, which shows worldwide support for IHSG. Having a conference like this depends on donations from sponsors and we are very grateful for their support, so please take the opportunity to thank the sponsors of the conference during these days.

This occasion is a fantastic opportunity for you to gain knowledge in new area and I am convinced that if you participate in the knowledge sharing you will learn something new. Individually we are specialists but together we are able to make an impact on seed production, seed research, and seed retail.

Finally, I would like to thank the Local Scientific and Organizing Committee and FNAMS, you have throughout the preparation of this conference been highly professional and really eager to make this conference one to remember, please also thank them during these days.

René Gislum President of IHSG Aarhus University, Denmark

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### **KEYNOTE SESSION**

#### PRESENTATION OF SEMAE

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#### 1. SEMAE's Presentation



SEMAE is an interprofessional organisation that facilitates discussions, exchanges, and decision-making. It includes the sector's stakeholders as well as seed consumers. It also promotes the French seed sector inside France and abroad.

#### 1.1. Facilitating discussion and exchanges

Professionals and non-professionals alike are represented in SEMAE's governing board. It brings together nine specialised sections, and

various commissions and working groups. Climate change, market trends, production conditions, and seed marketing are some, but not all, of the topics discussed at SEMAE. These debates help building up useful suggestions for the benefit of everyone, from companies to farmers and gardeners. This space of dialog is essential for all the stakeholders because it's the only place you can find such open discussions.

#### 1.2. Promoting the interests of the French seed sector within France

SEMAE promotes the French seed sector to the following groups: farmers, seed distributors, agricultural advisors, teachers and students, home gardeners, consumers and citizens, policymakers and elected officials and the media.

SEMAE is represented by 238 elected professional members of 48 professional federations members of SEMAE such as the French Seed Growers' Union (FNAMS) and the French Seeds Companies Union (UFS).



#### **1.3.** Key dates in SEMAE's history



Structuration at the beginning of the 20th century. The first French forage seed federation was set up in 1908 in Poitiers. Its aim was to organise itself to meet the food needs of work horses, the primary work power for farmers.

The national interprofessional group of seeds and seedlings was set up to alleviate food shortages and contribute to the war effort during the Second World War.

GNIS was founded in 1962 with a public service mission: the SOC (official seed control and certification service). The aim of the inter-profession is to guarantee the quality and certification of seeds.

#### 1.4 The Direction of Quality and Official Control

Dedicated technical department, responsible for the public service missions entrusted to SEMAE, headed by a civil servant appointed by the Ministry of Agriculture:

- in charge of the control and certification of seeds and seedlings
- in charge of control and authorization to issue plant passports for seeds and seedlings (phytosanitary requirements for movements of plant material within the European union)

Delegated body for controls for the implementation of international phytosanitary legislation for seeds and seedlings (phytosanitary certificate)

National Designated Authority towards:

Activities to support continuous quality improvement:

- Implementation of Scheme « Good Seed and Plant Practices » (GSPP)
- Implementation of Scheme « Végétal local »

Before feeding fodder to an animal, every plant was previously a seed. It takes a whole industry to produce the rights seeds.

#### Certified seed:



The official label and the phytosanitary passport (for species that are subject to it) present on each bag guarantee that the seeds purchased have been controlled and tested to ensure compliance with sanitary and quality standards.



#### 2. Seed production in France for all species

#### Breakdown of sales: Sales France + exports by group of species (wholesale stage - €1000)

Sales in the seeds sector are  $\notin 3.6$  billion (2021/2022 wholesale stage).

By way of comparison, the value of agricultural production (plant products) is  $\notin$ 49.5 billion in 2021 (source: INSEE).

#### Focus on forage seed crops in France.

Approximately 4000 forage seed growers, spread over 50,000 hectares (Grasses 16 010 ha and Fodders leguminous plants 33 803 ha)



#### 3. Conclusion

For 61 years, seed and plant interprofessional action has informed debate, consultation and decision making by all the stakeholders in the sector. SEMAE supports these stakeholders in order to rise together to food production, agricultural, climatic, economic and societal challenges.

In a nutshell, France, strong of its farmers' network and the diversity of its production (including 42 forage species), is the leading European producer and the leading world exporter of seeds. The country has developed a collective strength through very important technical and communication actions within the inter-profession in a highly competitive market where production varies greatly year to year and the number of producers is dropping (age pyramid).

#### PLANT VARIETY PROTECTION IN EU

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**Keywords**: Community plant variety rights system (CPVR), Community Plant Variety Office – CPVO, requirements to grant plant variety rights (PVR), duration of the protection, technical examination of herbage species and varieties.

#### Introduction

The community plant variety rights system (CPVR) is the sole and exclusive form of industrial property protection for plant varieties (sui generis IP system) which is inspired by the model of the 1991 Act of the International Convention for the Protection of Plant Varieties of the International Union for the Protection of Plant Varieties (UPOV). A system for the protection of plant variety rights has been established by the European Commission legislation particularly by Council Regulation (EC) No 2100/94 of 27 July 1994 on Community plant variety rights. The system allows intellectual property rights, valid throughout the European Union, to be granted for plant varieties through a single application.

The Community plant variety rights system is not intended to replace or even to harmonise national systems, but rather to exist alongside them as an alternative; indeed, it is not possible for the owner of a variety to exploit simultaneously a CPVR and a national right or a patent granted in relation to that variety. Where a CPVR is granted in relation to a variety for which a national right or patent has already been granted, the national right or patent is rendered ineffective for the duration of the CPVR.

The object of Community plant variety rights may form varieties of all botanical genera and species, including, *inter alia*, hybrids between genera or species.

Community plant variety rights shall be granted for varieties that are distinct, uniform, stable and novel. Moreover, the variety must be designated by a suitable denomination.

The duration of the protection of plant varieties is 25 years and 30 ears in the case of vine varieties, tree species, potatoes, Asparagus officinalis L., groups of species of flower bulbs, small fruits of woody plants and woody ornamental plants.

The Community Plant Variety Office (CPVO) operates, develops, and promotes an efficient Intellectual Property Rights system providing customer-centric services, thereby supporting innovation and the creation of new plant varieties for the benefit of Society. The CPVO, which is a decentralised European agency, has its own legal status. It is self-financing, mainly based on the various fees paid.

CPVO is based in Angers, France and operates since 1995. The budgetary authority of the CPVO is the Administrative Council, which monitors the CPVO activities and the

management of its President, adopts general guidelines on matters for which the Office is responsible, advises the CPVO management, submits to the European Commission proposals for amendment of Community legislation on Plant Variety Rights and may issue rules on working methods of the Office. The CPVO Administrative Council is composed of a representative of each Member State and a representative of the European Commission, and their alternates.

The application for Community plant variety rights can be filed either online or on paper. Filing online is a service provided by the CPVO, which enables users to apply for CPVR online in English, Dutch, French, Spanish and German languages.

For the technical examinations the CPVO has not created its own technical infrastructure and tests are carried out by the examination offices entrusted by the Administrative Council. The varieties of the main herbage species are tested in Germany, France, Netherlands, Czech Republic, Poland, Slovak Republic, Finland, and Denmark.

The information on the closing dates for applications and the submission requirements for plant material per species and according to the examination offices which are currently entrusted to carry out the technical examination on behalf of CPVO are available in the S2/S3 Publication available on the CPVO website.

If no impediment is found in the application, the Office arranges for technical examination of a variety for distinctness, uniformity, and stability.

If a technical examination has already been carried out or is in the process of being carried out on a variety already benefiting from national plant variety rights or entered for national listing in a Member State of the European Union, the Office may consider the examination reports of the responsible authorities to be a sufficient basis for a decision on the application for a CPVR.

When the Office receives a proposal for a variety denomination, this denomination is checked. If there is an impediment against this denomination the applicant is informed and has the possibility to comment or to submit a new proposal of a variety denomination. In case there is no impediment, it will be published in the Official Gazette. Once the denomination is approved and the variety is granted a right, this variety denomination must be used for all commercial purposes. If a trademark is associated with the denomination, the variety denomination must be easily recognisable as such.

During the period from 1995 until end of 2022 CPVO received 1066 applications for herbage species mainly from applicants originated in Germany, France, Denmark, Netherlands, and United Kingdom. In 2022, 34 candidate varieties were granted, and 21 varieties have been undergoing the examination process.

Most grass applications received were for *Lolium perenne* L., 255 varieties granted, *Lolium multiflorum* Lam., 60 varieties granted, *Dactylis glomerata* L., 29 varieties granted.

Concerning the lucerne and clovers, the highest number of applications belongs to *Medicago* sativa L., 49 varieties granted, *Trifolium pratense* L., 26 varieties granted and *Trifolium repens* L., 22 varieties granted.

In terms of research & development projects, CPVO supports the development of new phenotyping and genotyping tools to improve the efficiency and facilitate the procedures of variety testing. Currently, the Horizon 2020 project INVITE allows to make significant progress on perennial rye grass.

#### A REFLECTION ON THE HISTORY OF THE IHSG, PAST CONFERENCES, WORKSHOPS AND PUBLICATIONS

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#### Abstract

The International Herbage Seed Production Research Group (IHSPRG) was formed 45 years ago in 1978 and became known as the International Herbage Seed Group (IHSG) in 2002. This paper is a reflection by four past presidents of the history and past Conference and Workshops.

#### Introduction

It is 45 years since the IHSG was formed in 1978. In this presentation four past IHSG Presidents share their reflections on the history, past conferences, workshops and publications.

The International Herbage Seed Production Research Group (IHSPRG) was conceived at the 28th Easter School in Agricultural Science held at the University of Nottingham, UK on September 18-22, 1978, with the objective to "encourage co-operation and communication between workers actively engaged in herbage seed production research". Paul Hebblethwaite served as Chairman of the organizing committee, and a constitution was drafted and two types of membership defined: "Research Members who are actively engaged in herbage seed production", and" Subscription Members, which is open to farmers' groups, seed growers' associations and seed companies". It was also agreed that administration of the group should change to another country approximately every three years. In practice, especially in the last 25 years any administration has been undertaken by the President, Vice-President and Treasurer/Secretary.

While the group started as the IHSPRG (International Herbage Seed Production Research Group), this was shortened in 2000 to the IHSG (International Herbage Seed Group) reflecting that the wider membership and conference attendees included not only researchers, but extension specialists, seed company production agronomists and seed growers. Following the name change was the development of a logo selected from designs by a graphic artist commissioned by Bill Young, first used in 2002.

#### Conferences

This Conference in France (June 2023) is the 11<sup>th</sup> Conference. There have been two Southern Hemisphere and 9 Northern Hemisphere Conferences (Table 1). All the Conference Proceedings papers have been scanned and are available on the IHSG website. Conferences are about sharing research but represent an opportunity for members to get to know each other, building relationships that ease ongoing exchanges between conferences. For an International Conference, our numbers are small, typically with 120 to 150 people attending, a reflection of how few people in the world are involved in herbage seed research and extension.

Conference no.	Year	Location
1 <sup>st</sup>	1987	Tune, Denmark
2 <sup>nd</sup>	1991	Corvallis, Oregon, USA
3 <sup>rd</sup>	1995	Halle, Saxony Anhalt, Germany
4 <sup>th</sup>	1999	Perugia, Italy
5 <sup>th</sup>	2003	Gatton, Queensland, Australia with post conference tour in
		Victoria & NSW
6 <sup>th</sup>	2007	Gjennestad, Norway with post conference tour to Denmark
7 <sup>th</sup>	2010	Dallas, Texas, USA
8 <sup>th</sup>	2015	Lanzhou, Gansu Province, China
9 <sup>th</sup>	2017	Pergamino, Buenos Aries Province, Argentina
10 <sup>th</sup>	2019	Corvallis, Oregon, USA
11 <sup>th</sup>	2023	Angers, France

Table 1 - List of IHSG Conferences

#### Workshops

Workshops were less formal than Conferences, often organized around a theme with invited speakers and group discussions and breakout groups. They were linked to the International Grasslands Congress (IGC) and held in years between the IHSG Conferences. This gave a four-year cycle for IHSG Workshops (Table 2), which were usually held two years after each IHSG Conference. However, this routine got out of sync when IGC ran a joint Conference with the International Rangeland Congress (IRC) which required delaying the joint Congress in 2008 (held in Hohhot, Inner Mongolia, China).

Year	Location
1989	Nice, France (linked to IGC in Nice)
1993	Palmerston North, New Zealand (linked to IGC Palmerston North)
1997	Peace River, Alberta, Canada (linked to IGC Saskatoon, Saskatchewan)
2005	Winchester, UK (linked to IGC Dublin, Ireland)
2013	Methven, New Zealand (linked to IGC Sydney, Australia)

In some years the IHSG did not have active membership in the country hosting the IGC e.g. Brazil (2001) and New Delhi, India (2015) meaning arranging a Workshop linked to the IGC was not possible. Mostly, Workshops have been less formal than Conferences, with break-out groups and more field trips. The 2013 IHSG Workshop in Canterbury, New Zealand was the last Workshop run by the IHSG and was linked to the IGC Sydney as a post-conference tour.

#### **Post-Conference Tours**

The post-conference tours have become a "must-not-miss" aspect of IHSG Conferences, as an opportunity to both see field production over a wide geographic area of the host country and for the social interaction with other colleagues. Memorable post-conference tours include Italy 1999 with the tour in northern Italy's Po River basin; Australia 2003 when we flew from Brisbane to Melbourne to view seed production in Victoria and NSW along the Murray River; the Hexi Corridor tour into northern Gansu with its irrigated oasis and fringed by deserts to the east and mountains to the west (2015); Argentina 2017 tour of southern Buenos Aires Province and the unforgettable BBQ meals; Oregon 2019 with the tour of Central and Eastern Oregon seed production areas and the contrasts to the Willamette Valley.

#### Newsletter

During the IHSG first 35 years, a twice annual Newsletter was the main communication tool with members, covering both upcoming, news items of activities by region and research results. A total of 56 Newsletters were produced, starting in 1979. Newsletters 38 (2002) to Newsletter 56 (2017) are on the IHSG website (www.ihsg.org).

Some snippets from the early Newsletters 1 to 11. The first IHSPRG newsletter was published in September 1979 under the leadership of Paul Hebblethwaite, Chairman. This was followed by a second newsletter in March 1980, which announced there were 75 Research Members and 47 Subscription Members from 18 different countries. A third newsletter was published in September 1980, giving notice of the intent to convene a second meeting of the IHSPRG at the IGC venue in June 1981.

The fourth, and final, IHSPRG newsletter under Chairman Paul Hebblethwaite was published in May 1981, which announced that David Chilcote of Oregon State University would assume the Chairmanship and administration of IHSPRG. The IGC Kentucky post-conference tour to the Pacific Northwest featuring grass and legume seed production in Oregon and California had many of the early IHSPRG members toured OSU's Hyslop Crop Science Research Farm in late June of 1981.

Newsletter No. 5 announced the inception of the Journal of Applied Seed Production (JASP), which was being prepared for release in early 1983. The new journal to be published in cooperation with the Oregon Seed Growers League and IHSPRG with Harold W. Youngberg as editor. The IHSPRG newsletter No. 6 was delayed until December 1983, but was accompanied by Vol. I of JASP, which was sent to all IHSPRG members at no additional cost.

IHSPRG newsletter No. 7 was published in January 1985, and was also accompanied by Vol. II of JASP. However, it was announced that to continue a sound financial base for JASP, a US\$15 subscription fee has been implemented. Also, announced in this newsletter was that New Zealand's John Hampton would assume the role of Chairman at the XV International Grassland Congress in Japan in August 1985.

John Hampton's first newsletter (No. 8) was published in January 1986 and was also accompanied by Vol. III of JASP. Minutes of the third meeting of the IHSPRG meeting, held at the IGC Kyoto, Japan on August 28, 1985, were incorporated into the newsletter. There was

a proposal that a fourth meeting of IHSPRG be held during the 1989 XVI IGC in France, and that a plenary paper on seed production with a secession devoted solely to seed production be advocated for. In addition, it was proposed that IHSPRG members should convene at appropriate locations between IGC meetings.

IHSPRG newsletter No. 9 was published in November 1986, and announced the opportunity to gather membership in Denmark in 1987 with the Danish Herbage Seed Production Conference being organized by Sigurd Andersen and Anton Nordestgaard. It was also announced that that papers to be presented in Denmark would be published in Vol. V of JASP. As we're all aware, this event held on June 15-19 at Tune Landboskole, Denmark became the 1st IHSPRG Conference. IHSPRG newsletter No. 10 was published in November 1987 and gave much credit to the Danish hosts.

Chairman John Hampton's last newsletter (No. 11) was published on in February 1989 and reviewed the success of having a specialist section on forage seed production at the XVI IGC in Nice, France, and the opportunity to have a fifth formal meeting of IHSPRG members at that venue. At that meeting, Don Loch was appointed the fourth IHSPRG Chairman, who would serve a term from 1989- 93. Also mentioned in this newsletter was the suggestion of a 2nd IHSPRG Conference in Oregon, USA.

#### Journal of Applied Seed Production (JASP)

The publication of the Journal of Applied Seed Production (JASP) was a major achievement of the IHSG. The Journal ran for 17 Issues (from 1983 to 1999), with Editors Harold Youngberg (1983-1985) and John Hampton (1986-1999). The print runs averaged 200 copies and was mailed to subscription members and some University libraries. JASP brought many seed production related papers into one easy to locate place. Remember this was in the days before online searching for Journal articles was readily available. In the 1990's many Research organizations began to weigh a publications Impact Ranking in accessing staff performance making a small Journal like JASP a less attractive place to publish. The cost of printing and distributing JASP was also becoming an issue. These combined factors led to future JASP hard copy production being suspended. While an e-Journal was considered the problem associated with a small circulation leading to no Impact Factor ranking meant this approach was not adopted. Topics and major species covered in JASP Volume 1 - 10 are listed in Table 3. All 17 Issues of JASP are available on the IHSG website. Today our members publish in a wide range of Journals including Grass and Forage Science, Agriculture (MDPI), Field Crop Research and the Agronomy Journal.

#### Books

The 1997 publication by CABI for IHSG of two books on Forage Seed Production, Vol. 1 Temperate Species (eds Daphne Fairey and John Hampton) and Vol. 2 Tropical and Subtropical Species (eds Don Loch and John Ferguson) were major achievements. Volume 2 sold out. Royalties from the publication were a major revenue source for a 10-year period after publication. The Temperate Species volume is very out of date and needs a champion to take on the editorship of a new edition.

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Topics	Number	Species	Number
Agronomy (fertilizer, managements,	21	Perennial ryegrass	20
row-spacing, sow rates)			
Plant growth regulators	20	White clover	13
Seed Quality (hard seed, dormancy,	12	Lotus spp.	13
germination, vigour)			
Weed control / herbicides	10	Lucerne/alfalfa	7
Environment on seed development and	8	Kentucky bluegrass	7
seed yield			
Floral induction, reproductive growth &	8	Cocksfoot/orchard grass	7
development			
Disease management	7	Bromus spp.	5
Seed development & retention	7	Tall fescue	4
Harvest management	7	Red/fine fescue	4
Insect & nematode pests & control	5	Other legumes (8 species	9
		annual & perennial)	
Yield components and FSU	5	Crop legumes (soybean + 3	7
		species)	
Post-harvest management	5	Tropical grasses (4 species)	6
Seed handling	4	Vegetable seeds	6
Pollinators	2	Cereal crops (wheat, barley,	5
		maize)	
		Other temperate grasses (3	3
		species)	

#### Web Page

The IHSG webpage (<u>www.ihsg.org</u>) was redeveloped in 2017, led by Tom Chastain and is based at Oregon State University. The new web site had scans of all the JASP papers and Newsletters dating from Issue 38, and a Blog page.

#### Seed Grower Engagement

Over recent conferences, a small number of seed growers from Oregon, England, Tasmania and more recently New Zealand have been regular attendees at IHSG Conferences and Workshops. The New Zealand 2013 Workshop and the 10<sup>th</sup> IHSG Conference in Oregon both had one-day field events that were open and targeted to local seed growers. The interaction between growers and IHSG members has been a valuable tool in demonstrating the applied research undertaken by IHSG members.

#### **Past Presidents**

The IHSG has had 11 Presidents, typically serving a four-year term (Table 4). Most Presidents had been Vice-Presidents before taking on the role of President.

Year	President IHSG
1979 – 1983	Paul Hebblethwaite, UK
1983 – 1986	Dave Chilcote, USA
1986 – 1989	John Hampton, New Zealand
1989 - 1993	Don Loch, Australia
1993 - 1997	Daphne Fairey, Canada
1997 - 2001	Mario Falcinelli, Italy
2001 - 2005	William (Bill) Young III, USA
2005 – 2013	Birte Boelt, Denmark
2013 – 2017	Phil Rolston, New Zealand
2017 – 2019	Tom Chastain, USA
2019 – present	Rene Gislum, Denmark

Table 4 - IHSG Presidents, country and years as President.

#### Conclusion

The IHSG has built a loyal following of regular attendees and is a valuable forum for information exchange and building relationships and co-operation between seed researchers in different countries.

## **ORAL PRESENTATION**

#### STATUS AND FUTURE DEVELOPMENT OF THE UROCHLOA AND MEGATHYRSUS HYBRIDS IN GRUPO PAPALOTLA IN THE TROPICAL WORLD

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#### Introduction

*Urochloa* (syn. *Brachiaria*) breeding program was initiated in 1988 at the Centro Internacional de Agricultura Tropical (CIAT), Cali-Colombia. *Urochloa* is a large but poorly delimited genus comprising more than 100 species. The *Megathyrsus* (syn. *Panicum*) genus has about 470 species in the *Poaceae* family and recently, between 2015-2016, a breeding program in *M. maximus* was started. It is a major pantropical grass used throughout the tropics. Both genera are native to tropical regions of the world, with the highest concentration on the African continent, and can be utilized as pasture, fodder in cut-and-carry systems, hay, silage, and mixed grass-legumes, with environmental benefits.

The first comprehensive collecting mission for the *Urochloa* genus was carried out in the mid-1980s in eastern Africa, where approximately 800 accessions were collected. This collection provided a solid base for the development of new *Urochloa* cultivars.

In the last 30 years, agriculture has evolved dramatically. To ensure food to the world and contribute to the quality of the environment, pasture-based animal production systems will also have to undergo more intense evolution. However, the speed of the adoption of new management practices that increase production efficiency in grazing systems is nowhere near what we have seen in agriculture recently. In this scenario, the intensification of tropical grasslands is an important strategy for land utilization in developing countries. This means increasing production and minimizing environmental impact through the best management practices. In this sense, the use of interspecific Urochloa hybrids represents an excellent option, since they combine the best traits of three Urochloa species (U. ruziziensis, U. brizantha and U. decumbens), with higher nutritive value, forage, and seed yield.

#### Keywords

Brachiaria, Panicum, forage yield, nutritive value, carbon sequestration and seed production

#### **Regional evaluations and research in South America**

CIAT's Tropical Forages Breeding Program encompasses three breeding programs: the interspecific *Urochloa*, *U. humidicola* and *Megathyrsus maximus*. These are important forage resources in the tropics and subtropics, with 100 Mha covered by the genus *Urochloa* in Brazil

alone (Pedreira 2015, Jank 2014) and a potential of 3.3 Mha in Africa, where 99% of this area is evenly split between Eastern and Southern Africa (Fuglie 2021).

In addition to agronomic performance, forage quality and tolerance to biotic and abiotic stresses, traits such as carbon sequestration and biological nitrification inhibition (BNI), which are related to climate change mitigation are also gaining importance in these species. However, the mechanisms and genes controlling these traits are not yet clear (Teutscherová 2022, Adnew 2021 Fisher, 1994 and 2007).

#### Agronomic evaluation

In 2003, the seed company, Grupo Papalotla – (GP) began the evaluation of the first 34 interspecific *Urochloa* hybrids selected by CIAT. Since then, more than 500 hybrids have been provided and evaluated for forage production, nutritional value, shade tolerance, waterlogging, pest and disease resistances in different environments. The collections with more than 180 hybrids together with local checks, are being evaluated simultaneously in Brazil and Mexico.

Forage production has reached 34 and 48 t DM ha<sup>-1</sup> after 120 days of growth in the cultivars Mulato II and Cayman, respectively.

The main results in seed production have shown that these hybrids can produce 1 t ha<sup>-1</sup> of pure seed. Seed production doubles when the hybrids are grown at higher altitude locations, from 4 to 750 masl as presented in Table 1. (CIPAT 2013 – 2017).

Table 1 - Effect of altitude on seed production in *Urochloa* hybrids in the states of Chiapas and Oaxaca - Mexico.

Hybrid	Oaxaca 4 masl	Chiapas 750 masl					
	Pure seed grams per plant						
GP- 01	5.8 ± 0.81 c*	12.3 ± 2.71 bc					
GP- 02	11.6 ± 1.58 a	19.0 ± 2.11 a					
GP- 03	8.2 ± 0.86 ab	17.8 ±1.41 a					
GP- 04	4.0 ± 0.02 c	13.3 ± 1.55 b					
GP- 05	5.9 ± 1.02 bc	13.6 ± 2.27 b					
Average	7.1 ± 1.00	15.2 ± 2.01					

\*Significant at P= 0.05 in the same column. Pizarro, E.A. et al., (unpublished data)

In Mexico, GP has evaluated five collections of *U. interspecific* hybrids, from which three cultivars have been released (cvs. Cayman, Cobra and Camello). In addition, evaluations of two advanced collections are currently being carried out. GP has generated data and valuable information, which supports the positioning and credibility of the products in the market (Table 2).

				Trait of interest evaluated													
Hybrid		Waterlogging tolerance		Drought tolerance		Grazing tolerance		Cut and - carry		Shading tolerance		Growth habit		Pest tolerance		Palatability	
Mulato II	* 🌐	۲	NT	١	м	۲	нт	۲	м	۲	м		м		нт		НР
Cayman		۱	нт	1	м		нт		м		м	۱	D	۲	нт		НР
Cobra	(		NT	()))	м	())	м		НР	1	м		E		нт		НР
Camello		۲	NT		нт	۲	нт	۲	м	۲	NT	۲	D	۲	нт		HP
	AND A																

Table 2 - Main characteristics on the released cultivars by Grupo Papalotla. \*\*

\*business logo

\*\* HT= Highly tolerant; M= Middle; NT= Not Tolerant; HP= high performance; D= Decumbent; E= Erect.

Based on the new market demands, GP began to carry out work on hybrids of *U. humidicola* and *M. maximus*. To achieve the objectives, GP is relying on new phenotyping technologies of high throughput data that allow massive and faster evaluations such as UAVs (unmanned aerial vehicles).

GP is also developing partnerships with universities and research institutions to carry out a trial network representing 15 different grassland environments. Those experiments will allow GP to launch hybrids in an even more assertive way, allowing farmers to have the best experience with those products reflecting in benefits for them.

# Preliminary studies on soil factors affecting the sustainability and productivity in integrated crop livestock systems in the Cerrado ecosystem

The adoption of *Urochloa* hybrids might also help farmers to produce in an environmentally friendly manner, due to the potential benefits to soil life enzyme activity and carbon mitigation. The positive impacts are even higher when combined with other species. Diversification of pastures and the incorporation of key functional plant groups (legumes, for instance) generally improve nutrient cycling and often lead to increased carbon sequestration in the soil (Gaviria-Uribe *et al.*, 2020). GP internal data on its *Urochloa* hybrids (Cayman – GP0423 and Camello – GP3025) observed high forage yield, especially under environmental stress conditions such as severe drought (Camello) or poorly drained soil (Cayman) (not published data). Camello, for instance, has demonstrated high carbon accumulation in deep soil layers (Figure 2), suggesting the potential positive impact of its root system to carbon fixation. High forage production combined with good nutritive value led to not just high meat and dairy production, but good digestibility, high carbon stocks in the soil and low methane emission from rumination (Ruden *et al.*, 2018). Furthermore, improved *Urochloa* hybrids can also benefit crops such as corn and soybean by avoiding nematode multiplication (work in progress, not published data), recycling nutrients from deep layers in the soil and adding organic matter to the agriculture system.

Figure 2 - Mean carbon stock (t ha-1) along soil depth levels and genotype. A) Mean carbon stock overall visualization; B) Carbon stock comparison between soil depth levels in each genotype. C) Carbon stock comparison between genotypes along each soil depth level. Colored symbols: adjusted p-values for pairwise comparisons between soil depth levels for each genotype. Black symbols: adjusted p-values for comparison between genotypes in each soil depth level. \*: p-value  $\leq 0.05$ ; \*\*: p-value  $\leq 0.01$ ; \*\*\*: p-value  $\leq 0.001$ .



# Research on plant biotechnology induction of mutations and *in vitro* selection by culture of plant tissues in interspecific hybrids of *Urochloa*

One of the most important advances in the history of genetics was to discover that mutations can be induced by physical and chemical mutagenesis. Mutagenesis has been an important method in the successful generation of a large number of promising varieties in different cultivars. *In vitro* tissue culture techniques have been a valuable tool for the development of regeneration of protocols in forage species.

The biotechnology laboratory of GP is trying to achieve a protocol of regeneration for tropical forage species by culture of plant tissues. Currently, the work carried out in plant biotechnology is focused on generating added value to promising germplasm. At the moment, the main objective is to generate plants with resistance or tolerance to the family of Imidazolinone herbicides, which include imazapyr, imazapic, imazethapyr, imazamox, imazamethabenz and imazaquin. These

molecules control weeds by inhibiting the enzyme acetohydroxyacid synthase (AHAS), also called acetolactate synthase (ALS). The first hybrids with those traits will be launched by 2024. This technology can also be used to develop other traits or even to help to select hybrids with some abiotic tolerances such as drought.

#### Regional evaluation and research with focus on meat and milk production

Studies carried out over almost 20 years show very encouraging results in meat yields and weight gains that can be produced with *Urochloa* hybrids, ranging from 0.650 to 1.07 kg d<sup>-1</sup>, respectively (Torregroza *et al.*, 2015; Suarez *et al.*, 2014; Vendramini *et al.*, 2012).

Various studies show *Urochloa* hybrids as forages with high productive potential and high nutritional value, to increase milk yields in grazing (Pereira *et al.*, 2005) and an important source of minerals, protein and energy for the ideal development of heifers of replacement (Demski *et al.*, 2019, Mutimura *et al.*, 2018, Muinga *et al.*, 2016). These benefits have been considered the best alternative to increase milk production by up to 40% in the African continent and increase the profitability of the livestock systems of small producers (Njarui *et al.*, 2016). Recent studies revealed that diets based on Mulato II hay, in dairy cows, increased their milk yield by 71.6 % compared to hay from native pastures, although there was no effect on milk fat and protein (Mekuriaw *et al.*, 2020).

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### IRRIGATION RESPONSES OF COCKSFOOT SEED CROPS IN NEW ZEALAND

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## Abstract

The soils of the Canterbury plain of New Zealand have a variable topsoil depth which generally overlays gravel. Thus, water holding capacity (the supply) is influenced by the depth of topsoil and the rooting depth of the species being grown. In general, crop water use is determined by the atmospheric demand for water at any given time, multiplied by the ability of the crop/soil to supply that demand via evaporation from leaves (evapotranspiration (ET)). For example, when NW wind conditions prevail in Canterbury, water use is potentially large (6 mm/day) as conditions are hot, windy and of low humidity (i.e. the atmosphere removes water from stomata quickly). During the spring and summer, the accumulated potential deficit of many grass seed crops (ET minus rainfall) commonly exceeds 300 mm. Many soils can only supply between 60 and 120 mm of water before water stress occurs.

Cocksfoot, cultivar Savvy, responses to water stress were investigated over three seasons near Chertsey, Canterbury, New Zealand between 2020 and 2021. The soil was a shallow 'Templeton silt loam' atop of gravels at ~60 cm. The soil supply was ~110 mm of water with an estimated stress point of ~60 mm. Water use was measured in the 0-30 cm layer at hourly intervals using Campbell Scientific CS650 soil reflectometers and in the 30-60 cm layer weekly using a neutron probe. Irrigation was applied weekly to replace the measured water use (MWU), minus rainfall, via a trickle tape irrigation system applying ~12 mm/hour. Treatments aimed to induce periods of drought either prior to, or following, flowering representing either spring or early summer drought.

In all years, the untreated controls achieved seed yields of ~600 kg/ha and accumulated measured water deficits larger than 100 mm. Treatments that replaced MWU weekly achieved seed yields of ~1000 kg/ha with a maximum MWU deficit of ~60 mm. Seed yield was reduced to ~700 kg/ha when MWU deficits of ~75-90 mm were measured, regardless of when the drought occurred. When irrigation was applied to replace 50% of MWU weekly, seed yield was the same as alternative treatments that achieved similar MWU deficits. For example, in the 2020/21 season the treatment supplying 50% of MWU achieved a seed yield the same as the replacement MWU treatment when the maximum deficit was 71 mm. However, in 2019/20, seed yield was reduced by 22% then the MWU deficit was 77 mm.

The results of this study demonstrate that producers need to understand the water supply of their soils and the critical deficit where seed yield decline begins. The subsequent application of irrigation should aim to maintain soil moisture levels above the critical deficit. The critical deficit and yield loss for cocksfoot were similar to other grasses, suggesting that water

extraction is similar between species and cocksfoot is no more tolerant of drought than a ryegrass seed crop.

## Introduction

Water stress during spring and summer is common throughout the seed producing areas of New Zealand's east coast where evapotranspiration commonly exceeds rainfall. The Canterbury plain in New Zealand has varying depths of topsoil, typically covering a layer of gravel (Cox 1978). Consequently, the capacity of the soil to supply water to a specific crop is influenced by both the depth of the topsoil and the rooting depth of the specific plant species. Generally, water used by crops is determined by the atmospheric demand for water, multiplied by the crop/soil's ability to meet that demand through evaporation from leaves, known as evapotranspiration (ET). For instance, when the north westerly winds prevail in Canterbury, crop water use can be significant at around 6 mm/day due to hot, windy conditions with low humidity (Scotter & Heng 2003). During spring and summer, many grass seed crops experience a cumulative potential deficit (ET minus rainfall) exceeding 300 mm. Most Canterbury soils can only provide between 60 and 120 mm of water before water stress occurs (Lilburne et al. 2012). In grasses grown for seed, early spring drought often reduces the number of tillers that produce seed heads, while late season drought reduces seed size and thus the number of saleable seeds harvested (Chynoweth et al. 2012; Huettig et al. 2013; Chastain et al. 2015). Cocksfoot (Dactylis glomerata) is an important seed crop for arable farmers in New Zealand with approximately 1100 ha grown annually for local pasture supply (Stewart et al. 2014) and as a multiplication crops for re-export. This paper reports two seasons results and was setup to investigate the impact of spring drought on cocksfoot seed production.

# Materials & Methods

The trial was set up on a four or five year old stand of 'Savvy' cocksfoot that was established in February of 2016. Plots were reinstated on top of the same treatment as the previous season. Following the previous harvest, the crop residue was removed and volunteer cocksfoot and ryegrass seedlings were controlled using a combination of Hussar<sup>®</sup> (active ingredient (a.i.) 50 g/kg iodosulfuron), Atranex<sup>®</sup> WG (a.i. 900 g/kg atrazine) and Karmex<sup>®</sup> DF (a.i. 800 g/kg diuron) applied between April and July. Autumn nitrogen (N) application was 80 kg N/ha split between March and May. Spring N application consisted of an application of 40 kg N/ha + 16 kg S/ha as ammo<sup>™</sup> 31 in August followed by 80 kg N/ha applied as SustaiN<sup>®</sup> split between September and November. Two applications of 1.5 L/ha of Cycocel<sup>TM</sup> 750 (a.i. 750 g/L chlormequat-chloride) and 0.4 L/ha of Moddus<sup>®</sup> Evo (a.i. 250 g/L trinexapac ethyl) were applied to all plots at growth stage 31 and 33. Two applications of 0.4 L/ha Proline<sup>®</sup> (a.i. 250 g/L prothioconazole), 0.6 L/ha Seguris<sup>®</sup> Flexi (a.i. 125 g/L isopyrazam) and 3 L/ha Tri-Base Blue<sup>®</sup> (a.i. 190 g/L copper as CuSO<sub>4</sub>) were applied at head emergence and flowering.

The soil type was a Templeton Silt Loam with  $\sim 60$  cm of topsoil above free draining gravel (Lilburne *et al.* 2012). The water holding capacity is  $\sim 110$  mm of which approximately half is freely plant available. Irrigation was applied to the in-between row space of each plot via an above ground trickle tape system with drippers spaced approximately 33 cm apart. A single application was applied weekly based on measured water use (MWU) at an application rate of

 $\sim$ 12 mm/hr. Soil moisture was measured in all plots at hourly intervals in the 0-30 cm layer using Campbell Scientific CS650 reflectometers. At weekly intervals, the day prior to irrigation application, soil moisture between 20 and 50 cm was measured by neutron probe to give the weekly measured soil water deficit. Rainfall was measured onsite manually and via a Davies 6466 tipping bucket rain gauge.

Prior to windrowing, a  $0.125 \text{ m}^2$  quadrant was cut from each plot to assess total dry matter production and the number of seed heads produced. All plots were windrowed early/mid January as seed shedding commenced using a modified John Deere windrower. Plots were harvested on mid/late January using a 'Sampo' plot combine following which a sub sample was machine dressed to a 1<sup>st</sup> Generation Seed Certification standard (MPI 2014).

Margin over cost (MoC) was calculated with an irrigation cost of \$2.50/mm applied water and cocksfoot seed grower price at \$4.50/kg. The trial design was a randomised complete block with four replicates, data was analysed by ANOVA using Genstat19 (VSN 2019).

## Weather data

The soil moisture status was considered 'full' at the end of June in each year following early winter rainfall (e.g. Table 1). The accumulated potential moisture deficit increased during spring as evapotranspiration exceeded rainfall. In 2020/21, ~90 mm of rainfall was recorded from flowering (early December) onward which removed the potential for late drought to develop.

Month	Evapotranspiration (mm)	Rainfall (mm)	Potential accumulated deficit (mm)
June	21	80	-
July	28	36	0
August	46	17	29
September	86	37	78
October	106	19	165
November	121	76	210
December	142	69	283
January 1-11 2021	43	23	303
Total	592	356	

Table 1 - Measured rainfall and calculated evapotranspiration from June until windrowing for the experimental location at the FAR Arable Research Site, Chertsey, Mid Canterbury in the 2020/21 season.

# Results

Seed yield was increased by all irrigation treatments compared with the untreated control (Table 2 and Table 3). The October-November drought (treatment 2) reduced seed yield by ~200 kg/ha compared with the fully irrigated treatments. In 2019/20, drought from early seed fill reduced seed yield while in 2020/21 no late season drought developed due to frequent rainfall. Thus, in 2020/21 all post flowering treatments were not different from replacing the MWU, i.e. treatments 4-6 (Table 2).

In 2020/21, seed yield was not reduced when 50% of the MWU was replaced weekly as the soil moisture reserves were maintained above stress point by intermittent rainfall (Table 2). This was in contrast to 2019/20 when drought conditions slowly developed, reducing seed yield by a similar level as other drought treatments. Irrigation scheduling where 50% of the MWU is replaced allows the soil to capture and store rainfall as it occurs during the season, with reduced potential drainage compared with the fully irrigated plots. However, in dry seasons this can lead to drought stress, and yield reductions if the soil moisture deficit becomes large.

Seed yield increases were primarily due to increases in seed head density (Table 2 and Table 3) until  $\sim$ 140 mm of applied irrigation, following which the ultimate number of seeds/head that reached a saleable weight was a determining factor. Following seed cleaning there was no difference in thousand seed weight between treatments (data not shown).

Irrigation had a positive effect on margin over cost (MoC) returning between  $\sim$ \$70/ha in Treatment 2 during 2019/20 up to \$1380/ha in Treatment 4 during 2020/21 (Table 2 and Table 3).

Table 2 - Seed yield of cocksfoot, cultivar Savvy, following the application of seven irrigation treatments based on replacing measured water use (MWU) when grown on a Templeton Silt Loam soil type with a readily available water content of ~60 mm, grown near Chertsey, Mid Canterbury in the 2020/21 season.

	Treatment	Applied water (mm)	Maximum measured deficit (mm)	Seed (kg,	yield /ha)	Mo	C <sup>1</sup> (\$/ha)	Heads/m <sup>2</sup>
1	No irrigation	0	104	580	d	0	С	322
2	Mid drought f.b. <sup>2</sup> MWU	155	77	740	С	600	bc	475
3	MWU until flowering	125	99	840	bc	910	ab	485
4	MWU until early seed fill	210	73	940	ab	1380	а	553
5	MWU until mid seed fill	250	71	910	ab	1110	ab	530
6	MWU	280	66	1020	а	1300	а	499
7	50% of MWU	140	71	910	ab	1190	а	573
			P value	<0.	001		0.05	0.05
			LSD <sub>0.05</sub>	1	36		151	151

<sup>1</sup>Margin over cost, relative to the control. <sup>2</sup>f.b. = followed by

Table 3 - Seed yield of cocksfoot, cultivar Savvy, following the application of seven irrigation treatments based on replacing measured water use (MWU) when grown on a Templeton Silt Loam soil type with a readily available water content of approximately 60 mm near Chertsey, Mid Canterbury in the 2019/20 growing season.

Tre	atment	Applied water (mm)	Maximum measured deficit (mm)	Seed (kg/ha)	yield	MoC¹ (\$	5/ha)	Heads/m <sup>2</sup>
1	No irrigation	0	108	600	е	0	С	460
2	Mid drought f.b. <sup>2</sup> MWU	148	97	710	d	79	с	680
3	MWU until anthesis	115	96	730	cd	283	с	600
4	MWU until early seed fill	138	94	820	bc	762	ab	700
5	MWU until mid seed fill	198	54	890	ab	784	ab	800
6	MWU	263	50	960	а	936	а	810
7	50% of MWU	141	77	780	cd	423	bc	760
			P value	<0.001		0.005		0.014
			LSD <sub>0.05</sub>	103		462		159

<sup>1</sup>Margin over cost relative to the control. f.b.: followed by.

#### Discussion

During spring, grass seed crops require water to produce sufficient seed head numbers to maximise seed yield potential. Following seed head emergence, water is required to maintain green leaf and assimilate supply for seed filling. When the soil water deficit exceeds the site-specific stress point in spring, seed yield reductions occur via seed head abortion compared with late season stress where individual seeds either abort or fail to reach a saleable weight. Similar results were shown for tall fescue (Huettig *et al.* 2013) and perennial ryegrass (Chynoweth *et al.* 2012) where late season drought reduced the number seeds/m<sup>2</sup> that reached a saleable weight. Weekly irrigation rates can be reduced to replacing 50% of measured water use where soil moisture status is monitored and maintained above stress point (~70 mm at this site). Alternatively, the application frequency could be extended if stress point is not reached. Thus, monitoring soil moisture status and an understanding of stress point are essential tools for irrigation and gives an understanding of how rainfall is captured during the growing season.

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### YIELD COMPONENTS AND LIMITING FACTORS OF ALFALFA SEED CROP

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#### Abstract

Surveys in farmers' plots and a network of trials in experimental stations conducted by the FNAMS have improved the understanding of alfalfa seed crop yield development and identified the main limiting factors. Correlation matrices analysis of yield components shows that yield is most strongly correlated with number of seeds per inflorescence, seed weight per inflorescence weight and to a lesser extent with the number of pods per inflorescence. Less data was collected on the components before the beginning of flowering, nevertheless according to the results obtained these components appear little correlated with the yield. Cell multiplication and seed filling are therefore particularly sensitive to limiting factors. Multiple linear regressions were performed on the components most correlated with yield. The limiting factors that significantly impact the key yield components of the crop are water deficit during flowering, lack of wild bees (for pollination) and insufficient control of pests (in particular *Tychius aureolus*, bug).

The expression of these limiting factors is very dependent on the climatic conditions of the year. Based on a case study of one farm in western France, yield variations of 1 to 6 between the worst and the best year were recorded over a 43 year. Excessive water in May, at the time of pre-cutting and during regrowth, is detrimental to the crop. A cumulative rainfall of more than 90 mm will penalize the root system of the crop and cause plant loss. The importance of climatic conditions during flowering is confirmed. To ensure a good pollination and fruit setting, climatic conditions (temperature, rainfall and radiation) during the summer period are essential. Indeed, during this period, an excessive water supply favors the vegetative development, causing lodging and disturbing pollination. Similarly, a lack of temperature or radiation will penalize the yield. On the other hand, excessive temperatures during the summer period will shorten the flowering period and favor pod abortion. For the best yields, stormy rains that may occur in July do not seem to be detrimental to yield. At maturity, alfalfa is susceptible to sprouting in prolonged wet conditions. Several consecutive rainy days between the end of August and the first ten-days of September favor the appearance of this phenomenon.

Climate change is now clearly visible with a rise in the average annual temperature and recurrent heat waves during the summer. In order to optimize the management of alfalfa seed crop, it is therefore necessary to take into account the impact of climate change on the key stages of this species.

### Introduction

Alfalfa is the first forage species cultivated in France for seed production. Historically, production is located in 4 major production areas: the West (Pays de la Loire, Poitou-Charentes), the Centre, the South-West (Occitanie) and, more marginally, the southeast (Rhône Valley of the country. In Europe, alfalfa is mainly produced in three other countries: Italy (with areas larger than France, around 30 to 40,000 ha), Spain (8 to 13,000 ha) and Hungary (4 to 10,000 ha). The French multiplication surfaces represent between 10 and 27 000 ha depending on the year. The national average seed yield is estimated at about 350 kg/ha over the last ten seasons, which is very low compared to the grain potential of this crop, which can reach 1000 to 1200 kg/ha (or even more in some situations). In fact, alfalfa seed production is very irregular in time (high inter-annual variability) and space (high variability between plots in the same production area and between regions). A strong downward trend has been observed over the last ten years. Therefore, the FNAMS has carried out several actions in order to obtain a better understanding of the seed yield build-up and identify the main limiting factors of this crop.

## Materials & Methods

An annual crop survey in farmers' plots was carried out in 2 production areas (Central-West and South-West) for 4 years (2012-2016). In addition, pluriannual experimental trials on 3 FNAMS stations (West, Southwest and Southeast) were conducted from 2013 to 2018. On these 2 experiments, numerous crop observations were carried out (stages, yield components, yield, density and diversity of pollinators, pests, ...). The technical agricultural processes and climate data were also recorded for each farmer plot and trial. All of this information was compiled and formatted in an Excel database. Agro-climatic indicators (water deficit, photothermal quotient, ...) were also calculated for statistical analysis.

Finally, a third set of data is used in this study. This is a complete alfalfa seed yield history recorded on 1 farm in the West of France (Vienne department) from 1978 to 2021. The farmer has been working with the same seed company since 1978 and multiplies one or two varieties each year. The plots remain in place for a minimum of two years and up to three or four years. The producer decides the cutting date according to the climatic conditions of the year and the type of soil (depth and rate of stones). In general, cutting is done between mid-April and mid-May. The harvest takes place over three weeks in September. Thus, the annual averages compile the yields of plots from several production potentials, several production years and several varieties. These records were related to the climatic conditions of the year.

### Results

- <u>Critical period to limiting factors and the main limiting factors.</u>

The yield components (number of fertile stems, number of pods per inflorescence, number of seeds/pods, etc.) measured at key stages of the crop on the farmer plots and station trials have provided a better understanding of the seed yield development. Indeed, correlation matrices analysis of yield components (Table 1) shows that yield is most strongly correlated with number of seeds per inflorescence (average farmer plots: 99 seeds / inflorescence), seed weight

per inflorescence (average farmer plots: 207mg / inflorescence) and to a lesser extent with the number of pods per inflorescence (average farmer plots: 21 pods per inflorescence). Less data was collected on the components before the beginning of flowering, nevertheless according to the results obtained these components appear little correlated with the yield. Cell multiplication and seed filling are therefore particularly sensitive to limiting factors.

Numerous multiple linear regression tests were performed to identify the parameters affecting the components correlated with yield. The limiting factors that significantly impact the key yield components of the crop are water deficit during flowering, deficiency of wild bees (for pollination) and insufficient control of pests, especially the alfalfa seed weevil (in this case Tychius aureolus) and bugs (Figure 1). Depending on the year, the climatic conditions will create situations more or less favorable to the expression of these limiting factors.

Table 1 - Correlation matrix of yield components with the coefficient of determination (Pearson, 5% risk) – 43 seed growers plots. Annual crop survey for 2012 to 2016 in 2 production areas (Central – West and South-West)

Variables	Average	Standard deviation	SY	TSW	SWI	SI	PI	SP	FSM	PHEF
SY	521	261	1							
TSW	2	0	0,016	1						
SWI	207	55	0,189	0,017	1					
SI	99	28	0,205	0,152	0,924	1				
Ы	21	4	0,117	0,004	0,127	0,120	1			
SP	5	1	0,055	0,116	0,601	0,662	0,062	1		
FSM	158	52	0,014	0,006	0,002	0,002	0,024	0,017	1	
PHEF	67	18	0,052	0,032	0,002	0,006	0,041	0,004	0,029	1

In bold: positive significant correlation, in italics: negative significant correlation

**SY**: seed yield (kg/ha), **TSW**: thousand seeds weight (g), **SWI**: Seed weight per inflorescence (mg), **SI**: number of seeds per inflorescence, **PI**: number of pods per inflorescence, **SP**: number of seed per pods, **FSM**: fertile stems per m<sup>2</sup>, **PHEF**: plant height - end of flowering (cm)

Figure 1: Multiple linear regression of the number of seeds per inflorescence, 29 growers plots, model selection: best model – R<sup>2</sup>:0.599. Annual crop survey for 2012 to 2016 in 2 production areas (Central –West and South-West)



F: flowering, GP/BP: green pods / brown pods

- Climate impact on alfalfa – A case study illustration

The analysis of seed yield recorded over 43 years (1978-2021) by a farmer located in western France (Vienne department) in relation to the climatic conditions of the year have made it possible to draw several significant conclusions. On this farm, located in one of the main alfalfa production basins, the seed yield reached an average of 630 kg/ha over 43 campaigns (Figure 2). A strong disparity in results was noted depending on the year, with a variation of 1 to 6 between the worst and best years (Figure 1). Nevertheless, the result of this farm is better than the national average of about 400 kg/ha (Semae 1985-2021).

Rainfall from January to April does not seem to have a major impact on seed yield. On the other hand, too much water in May, at the time of cutting and during regrowth, is detrimental to the crop. A cumulative rainfall of more than 90 mm will penalize the root system of the crop and cause a loss of plants (Figure 3). This phenomenon has been observed during five springs (2013 and 2014 in particular, for the most recent years), with an average yield of less than 500 kg/ha.

The importance of climatic conditions during flowering is indisputable. To ensure a good pollination and fruit setting, the climatic conditions (temperature, rainfall and radiation) during the summer period are essential (Figure 4). Indeed, during this period, an excessive water supply favors the vegetative development, causing lodging and disturbing pollination. This was the case in 2007 (199 mm in June-July), 2014 (166 mm in June-July) and in 2021 (199 mm in June-July).

Similarly, a lack of temperature or radiation will penalize the yield. These limiting conditions have been observed on 6 occasions since 1978 (1978, 1979, 1981, 1985, 1993 and 2007). On

the other hand, excessive temperatures during the summer period will shorten the duration of flowering and favor pod abortion. This was the case in the summer of 2006, and more recently in June 2017. The best yields (> 900 kg/ha in 5 out of 43 campaigns) are obtained in years associated with an average temperature of about 19.8 °C. For these years, the stormy rains that can occur in July (observed in 2010 and 2015) do not appear to be detrimental to yield.

Finally, at maturity, alfalfa is sensitive to the phenomenon of sprouting in prolonged wet conditions. Several consecutive rainy days between the end of August and the first dekad of September (1979, 1993 and 2007) favors the appearance of this phenomenon.

Figure 2 - Average alflafa seed yield on 1 farm in western France from 1978 to 2021 compared to the national average (Semae).



Figure 3: Average alfalfa seed yield on 1 farm in western France in relation to cumulative rainfall in the month of May in mm (Météo France) from 1978-2021.



In bold: good years with annual yield >790 kg/ha Underlined: bad years with annual yield <480 kg/ha

Figure 4: Cumulative rainfall in relation to average temperature in June-July (Météo France). Average alflafa seed yield on 1 farm in western France from 1978 to 2021.



Underlined : bad years with annual yield </80 kg/ha

### **Conclusion and perspectives**

The different actions conducted the last years on alfalfa have shown that the high-risk period to limiting factors impact is during flowering and seed filling. During these phases, water stress, wild bee density and inflorescence pests can significantly reduce yield. Furthermore, the impact

of climate was clearly demonstrated from the case study on 1 farm in the West of France. Depending on the year, climatic conditions will create situations that are more or less favorable to the expression of limiting factors on alfalfa seeds.

Climate change is now clearly visible with a rise in average annual temperature and recurrent heat waves during the summer. In order to optimize the management of alfalfa- seed production, it is now necessary to examine the impact of climate change on the key stages of this species and to identify the levers available.

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#### ORGANIC GRASS AND LEGUME SEED PRODUCTION IN SWEDEN

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#### Abstract

The demand of organic seed for grassland production has brought about partly new and innovative methods of agronomic practices for production of legume and grass seed. Weed regulation, a challenge in white clover (*Trifolium repens* L) seed production, was developed by removal of the herbage with a pasture mower at different time points in the first seed production year, likely has contributed to the sharp increase of certified organic seed in Sweden reaching 6874 hectares in 2022. The technique was developed and investigated experimentally in six field experiments in commercial seed crops of organically farmed white clover, using modern equipment in 2005 – 2007 located in south and central Sweden. The cutting treatments were; 1) no cutting (no C), cutting 2) at budding (CB); 3) at 1-2 flowers (CF); and 4) at full flowering (CFF). Herbage removal by cutting while the buds are just above the soil surface increased seed yield on average by 12%, while cutting at later stages significantly reduced yield. The biomass of *Tripleurospermum inodorum* decreased significantly for all treatments. There was no significant difference between the number of inflorescences between treatments.

Weed regulation by removal of herbage by cutting was soon adopted by organic seed producers, and this agronomic practice is also applied in conventional seed production.

#### Introduction

Grassland production is important in organic farming systems. For this, the availability of organically certified seed is required. Organic seed production was initially disfavoured due to market restrictions, however, from 2004 only organically certified seed are allowed in organic farming. Consequently, a great commitment and a great knowledge building from practical farming started and brought Sweden forward as one of the world leaders in acreage of organic ley seed production (Rhabeck Pedersen, 2006). In 2022, the certified organic seed acreage was 6874 hectares, corresponding to 36 percent of the total seed production acreage in Sweden (Figure 1).



Figure 1 - Acreage of certified seed farmed organically of timothy, red clover, white clover, meadow fescue, perennial ryegrass, tall fescue, and cocksfoot in Sweden 2000- 2022. Source: Swedish Board of Agriculture (SBA)

Seed production of forage crops is multifaceted with several challenges. Timothy (TI; Phleum pratense L), meadow fescue (MF; Schedonorus pratensis Huds.), red clover (RC; Trifolium pratense L) (RC), white clover (WC; Trifolium repens L), perennial ryegrass (PR; Lolium perenne), orchard grass (OG; Dactylis glomerata) and tall fescue (TF; Schedonorus arundinaceus subsp. sechtrizianus) constitute the main individual crops (Figure 1) with specific requirements for management, harvesting, post-management and post-harvest techniques. Requirements for purity and germination must be met for certification, and the same requirements apply here as for conventionally produced seed. A seed lot can be re-cleaned with great costs as a result, and if the lot falls through, it has no value. Partly new and innovative methods concerning establishing the seed crop, weed regulation, strategies for application of organic nitrogen products, post-harvest management to boron application to RC and WC, and swathing prior to harvest were developed in different projects. Techniques for establishing legume and grass seed in spring were developed, as for seeding grass seed in autumn. Time point of seeding grass is species specific and MF required two weeks earlier seeding than TI. Weed regulation, one of the greatest challenges for red clover and white clover seed production was developed by cutting with a pasture mower at different time points.

The acreage of WC seed production has increased the past five years (Figure 1) and we here present an agronomic practise, one of the key techniques for weed regulation, developed in field experiments that likely has contributed to the sharp increase of certified seed. Weed regulation by cutting the herbage of the seed crop in the seed production year was investigated experimentally using modern equipment.

We hypothesize that cutting will i) have an increasing effect on seed yield ii) increase the number of flowers in treatments at budding stage and iii) decrease the biomass of occurring weed species. The objectives of the research were to investigate the effects of cutting the herbage at different development stages on weed occurrence and the number of flowers as well as to find an optimal time point for receiving the highest seed yield of white clover.

## Materials & Methods

Field experiments were established in commercial seed crops of WC farmed organically. Six field experiments were conducted in 2005 – 2007 located in south and central Sweden from latitude 55° N to N 58°. The cultivars of the commercial crops were SW Sonja, SW Hebe, SW Ramona, and Riesling of medium leaf size (intermediate- leaf types) and stand age was the first seed year crop. The WC crops were planted into rows at 12 cm or 24 cm inter-row space.

The cutting treatments were performed 3 cm above the ground surface at various developing stages of the WC plants and was compared with a control without cutting (1, no C). The treatments were cutting 2) at budding (CB); 3) at 1-2 flowers (CF); and 4) at full flowering (CFF). The experiment was arranged in a randomized block design with four replications. The plot size of each treatment was about 30 m<sup>2</sup>, and the harvested plot was at least 15 m<sup>2</sup>. The field experiments were performed by the Field Experimental Divisions of the Rural Economy and Agricultural Societies. The herbage management treatments were applied lengthwise the plot, using a pasture mower, either a mulcher with hammers or a knife mower. The herbage was removed in treatments CF and CFF. Weed biomass was determined three weeks after the CFF cutting. All weeds were collected in four subplots of 0.25 m<sup>2</sup>. The fresh weight of *Tripleurospermum inodorum* and the three most common weed species was determined. The number of WC flowers was counted in 2 x 1 m<sup>2</sup> of each plot. The plots were swathed prior to seed harvest. The whole plot yield was measured, collected, dried, and sent to for cleaning, and percent cleanouts was determined.

### Calculations and statistics

The seed yield is presented as pure seed (yield x percent purity) with 15 % water content. An analysis of Variance (ANOVA) (p = 0.05) with General Linear Model (GLM) followed by post hoc comparison with pare wise t-tests to determine significant differences between the various treatments. Data of yield were transformed by logarithmation, and the square root was used for weight of *T. indorum* before statistical analysis. The software SAS<sup>®</sup> Enterprise Guide was used for all statistical analyses.

### Results

Means of seed yield, number of flowers and fresh weight of *T. inodorum* in the experiments in on average for all six experiments are displayed in Table 1.

Table 1 - Seed yield, number of flowers and biomass measured of *Tripleuropermum inodorum* in the field, in a seed crop of white clover after cutting the herbage at different developmental stages of the white clover plant. Average of six field experiments conducted in cultivars SW Sonja, SW Hebe, SW Ramona and Riesling of medium leaf size in south and central Sweden 2005-2007.

=				
Treatment	Seed yield (kg	Flowers (number	T. inodorum	
	per hectare)	per m <sup>2</sup>	Fresh weight (m <sup>2</sup> )	
1. No cutting (no C)	233 <sup>ab</sup>	<b>471</b> <sup>a</sup>	1326ª	
2. Cutting at	262 <sup>a</sup>	608ª	321 <sup>b</sup>	
budding stage (CB)				
3. Cutting at 1-2	188 <sup>b</sup>	554 <sup>a</sup>	73 <sup>c</sup>	
flowers per m <sup>2</sup> (CF)				
4. Cutting at full	98 <sup>c</sup>	407 <sup>a</sup>	15 <sup>c</sup>	
flowering (CFF)				

Different letters show significance according to Tukeys test, p < 0.05.

Seed yield, numbers of flowers and fresh weight of *T. inodorum* were higher in experiments in south Sweden compared to the experiments in the central part. All cutting treatments showed on average a significant reduced fresh weight of *T. inodorum* compared to the control. Cutting at budding stage slightly increased the seed yield and number of flowers. Cutting at 1-2 flowers m<sup>-2</sup> caused a slight yield reduction, and the cutting at full flowering (CFF) caused a significant decrease in yield. Rainfall after cutting was low all years, hence, the crop stand was shorter after the late cutting (CFF), implying problems at seed harvest. At the experimental sites in south Sweden a yield increase was obtained also in treatment CF, while the yield in the experiments in central Sweden was reduced (results not shown here).

### **Discussion & perspectives**

The results show that removing herbage by cutting at budding had a positive influence on the development of flowers as well as on seed yield of white clover (Table 1). The results were partly in accordance with the hypotheses, *i.e.*, that that cuttings will cause an increase in seed yield due to a decrease the occurrence of *T. inodorum*. All cutting treatments reduced the biomass of *T. inodorum*. Seed yield and the number of flowers increased by the earliest cutting (CB) in south Sweden, where the weed pressure was highest. There was no increase in yield in central Sweden due to the cutting treatments.

To get an even development of flowers, it is important that the crop is not too dense, and that good primary stolon growth is promoted. A moderate water deficiency results in reduced vegetative growth and a higher seed yield (Bissuel-Belaygue, 2002). The crop density can be regulated by cutting, which implies that many new leaves and flower buds will develop and, hence, flowering will be synchronised. Herbage removal did not significantly affect reproductivity in a study reported from Oregon, USA (Medeiros& Steiner, 2000) as flowering was re-established within 4 days.

In conclusion, herbage removal by cutting at budding stage in WC will increase the number of flowers and seed yield, especially in fields with a high pressure of weeds. Cutting in early summer as the buds are just above the soil surface, about 1 June in the south part of Sweden,

was a well-known method to regulate weeds and synchronise flowering prior to introduction of herbicides in seed crops of white clover (Nilsson et al. 1950). Our study shows that cutting will not only reduce weed biomass and improve purity of the seed (data not shown here), but also has a positive influence on seed yield.

The results were implemented and further developed in a project on Participatory Learning and Action Research (Wallenhammar et al., 2016) with a group of growers that met about four times per year, during a period of six years, where weed regulation by cutting in red clover and white clover was considered as one of the most important measures.

Implementation of weed regulation by cutting has further effectively been disseminated to growers throughout the country by collaboration with seed companies, extension service, growers' seed organization, field demonstrations and field days. The rapid expansion of organic legume and grass seed production has brought about nonchemical agronomic practices that are also applied for conventional seed production.

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### CLIMATE AND LANDSCAPE DRIVERS OF AN INVASIVE PEST IN OREGON AND NEW ZEALAND: THE RED CLOVER CASEBEARER MOTH

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#### Abstract

Successful mitigation of invasive pest species depends on integrated pest management (IPM) practices incorporating multiple techniques for effective population management below economically damaging levels. Pest monitoring remains the cornerstone of IPM programs, enabling appropriately timed management action. Phenological models using temperature data and growing degree days (GDD) are commonly used as decision support tools to predict the timing of 'when' economically important life stages are expected to occur. Furthermore, geostatistical models may inform 'where' pest outbreaks are expected to occur along spatial gradients of biotic risk factors. Thus, abiotic and biotic predictors of pest populations can be leveraged to generate spatiotemporal risk assessments. The red clover casebearer moth (Coleophora deauratella; RCCB) is an invasive insect pest in red clover (Trifolium pratense L.) seed production systems in North America and New Zealand. The larval growth stage can inflict crop damage by feeding directly on developing red clover seeds within flower head florets. Recent discoveries of RCCB in Oregon and New Zealand prompted research investigating the seasonal phenology and population dynamics of RCCB to inform management strategies and develop a risk prediction framework to mitigate outbreak severity. As such, the objectives of this study were to (1) develop flight phenology models to predict adult flight timing in three geographic regions with red clover seed production in Oregon (Willamette Valley and Eastern Oregon) and New Zealand, (2) evaluate the dietary history of RCCB in Oregon, and (3) determine landscape-level effects of red clover spatiotemporal dominance and other potential landscape predictors on RCCB population densities. To do this, we sampled 76 site years across the three geographic regions (2013-2014, 2018-2022). An attractant-based trap network was deployed across sampled regions using a female sex pheromone to lure male moths in commercial red clover seed production fields. Remotely sensed temperature and landscape composition data were extracted for phenological and geospatial modeling. Nonlinear logistic regression was used to develop regionally explicit phenology models that predict the unimodal timing of RCCB flights. Median flights of RCCB populations in the Willamette Valley and Eastern Oregon were predicted at 255.6 and 286.2 GDD for each population, respectively (start date = 1 November, lower developmental threshold =  $12.3^{\circ}$ C). For New Zealand populations, the median flight was predicted at 222.3 GDD (start date = 1 November, lower developmental threshold =  $12.3^{\circ}$ C). Molecular gut content analyses revealed the dietary history of early season captures and informed landscape

analysis covariate selection. A spatial Bayesian generalized linear mixed model was developed to test landscape-level effects of landscape composition and configuration predictors on RCCB abundance. The spatiotemporal dominance of clover and grassland land area was positively associated with RCCB populations. These results can be used to forecast RCCB risk across space and time and advise integrated pest management practices.

**Keywords:** Phenological modeling, Population dynamics, Gut-content analysis, Landscape ecology, Red clover casebearer moth

## Introduction

The red clover casebearer moth (*Coleophora deauratella*; RCCB) is an invasive pest in red clover (*Trifolium pratense* L.) seed production in North America and New Zealand. RCCB

larvae inflict crop damage by feeding directly on developing red clover seeds within flower head florets; substantial seed yield loss has been documented in Canada (Evenden et al. 2010) with anecdotal reports of economic loss in Oregon. Although red clover is its primary host, RCCB has also been observed in other clover species. In all geographic regions, RCCB populations cycle through one generation per year and overwinter as mature larvae protected by larval cases on the soil surface or beneath crop residue of infested clover fields in the fall and winter, followed by pupation and adult emergence in the spring and early summer, respectively.

The feeding behavior of RCCB, infestation timing aligned with peak pollination, and limited modes of action registered for chemical control pose challenges for effective RCCB management. Taking advantage of RCCB univoltine life history, research has demonstrated the effectiveness of mating disruption techniques to reduce infestation levels in Canada (Mori and Evenden 2014), and preliminary mating disruption field trials in red clover in western Oregon have been conducted (Kaur et al. 2021). Research has also shown a positive association between increasing stand age and greater RCCB densities (Chynoweth et al. 2018). This phenomenon is often observed in simplified landscapes with high concentrations of source hosts across space and time.

Improved understanding of seasonal flight phenology and population dynamics of RCCB populations in Oregon and New Zealand red clover seed production systems will enable the development of areawide-integrated pest management plans to mitigate associated infestation risks. As such, the objectives of this study were to (1) develop flight phenology models to predict adult flight in three geographic regions with red clover seed production in Oregon (Willamette Valley and Eastern Oregon) and New Zealand and (2) determine landscape-level effects of red clover spatiotemporal dominance and other potential landscape predictors on RCCB population densities.

### **Materials & Methods**

### Field selection and RCCB sampling

Attractant-based trap networks were deployed throughout red clover seed production regions in Oregon and New Zealand to measure RCCB populations weekly using green plastic funnel traps deployed >30 m from field borders and positioned at canopy height. Traps were baited

with a female sex-pheromone lure consisting of a rubber septum injected with a 10:1 ratio blend of Z7-12:OAc and Z5-12:OAc, replaced every three to four weeks; males were used as a proxy for RCCB populations.

Locations selected for monitoring in Oregon included 48 unique commercial red clover seed fields in the Willamette Valley (Western Oregon) sampled across five years (2013-2014, 2020-2022) for a total of 54 site-years (Figure 1). Seven field locations were monitored in three Eastern Oregon counties (2018-2020) across three years, totaling ten site-years. Sampling in the South Island of New Zealand was conducted at eight field locations spanning two territorial authorities and two growing seasons (2019-2020, 2020-2021), totaling 12 site-years (Figure 1).

## Flight phenology model

Temperature data for developing flight phenology models in Oregon were extracted from PRISM Climate Group gridded rasters of daily maximum and minimum temperature. Daily temperature data for New Zealand was extracted from the National Institute of Water and Atmospheric Research (NIWA) National Climate Database and the CliFlo web portal. Seasonal flight phenologies of RCCB were estimated by fitting three-parameter nonlinear logistic regression models with a variance structure for sampling year to predict the cumulative proportion of RCCB counts based on growing degree days (GDDs). A cross-validation procedure was performed to evaluate phenology model predictions for new observations.

### Landscape risk factors

Landscape-scale crop raster was extracted from the National Agricultural Statistics Service CropScape data layer to calculate landscape composition and configuration variables within buffer radii (1 to 3 km) surrounding trap locations. Landscape variables were selected based on gut-content analyses of early captured male RCCB in pheromone traps and prior literature. The spatiotemporal dominance of crop variables was estimated using weighted hectare-years that calculate the area (ha) and proximity of landscape classes to sampled traps over time using inverse distance weighting (IDW) across three years (year of sampling and preceding two years). We assumed crop variables analyzed within buffers closer to traps had a proportionally greater likelihood of contributing to RCCB populations than those further away. Incorporating landscape composition over time is important to understanding the effects of crop dominance on local population densities. A spatial Bayesian hierarchical generalized linear mixed model (GLMM) was used to test landscape predictor covariates.

# Results

# Flight phenology model

Flight phenology of RCCB using GDDs (lower developmental threshold =  $12.3^{\circ}$ C) in the Willamette Valley predicted 25, 50, and 75 percent levels of seasonal flight ( $Y_{25}$ ,  $Y_{50}$ ,  $Y_{75}$ ) at 207.1, 255.6, and 305.7 GDDs for new data, respectively (start date = 1 January) (Figure 2). For Eastern Oregon populations,  $Y_{25}$ ,  $Y_{50}$ , and  $Y_{75}$  levels of seasonal flight were predicted at 216.7, 286.2, and 360.4 GDDs, respectively (start date = 1 January). For the New Zealand model, 181.0, 222.3, and 264.4 GDDs predicted  $Y_{25}$ ,  $Y_{50}$ , and  $Y_{75}$  of RCCB seasonal flight, respectively (start date = 1 November). The average ordinal date (DOY) of peak flight in the

Willamette Valley, Eastern Oregon, and New Zealand was 170 (18 or 19 June), 191 (9 or 10 July), and 9 (9 January), respectively (Figure 2).

# Landscape risk factors

The hierarchical spatial Bayesian GLMM found landscape-level variation in crop composition covariates agriculture, grassland, and clover spatiotemporal dominance, and landscape fragmentation covariates were predictive of RCCB counts in Oregon (Figure 3). Specifically, agriculture and landscape fragmentation negatively influenced RCCB, and grassland and clover were positively associated with RCCB abundance (Figure 3 b-d). Covariate estimates were generated for new points across the sampled regions, and predictions were calculated using model output (posterior distribution) and spatialized using spline interpolation (Figure 3 a).

## **Discussion & perspectives**

Results of this study provide improved phenology models and GDD accumulation thresholds for predicting unimodal RCCB flights in Oregon and New Zealand. These insights are key for continued testing and implementation of reduced application control strategies in these regions (e.g., mating disruption and timing of silage removal). Furthermore, new insights regarding the spatiotemporal dominance of primary hosts clover and grassland, and clover alone, can forecast locations with greater RCCB populations. Complimentary phenological models and landscape analyses present unique insights into local and landscape-level effects of abiotic and biotic factors influencing pest population dynamics and can provide a robust risk prediction framework to bolster integrated pest management.

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Figure 1 – Site-year locations of RCCB pheromone traps in western (Willamette Valley) and Eastern Oregon (left) and New Zealand (right).

Figure 2 – Mean weekly RCCB counts (top row) among site years in each region (columns) throughout the sampling window (DOY) illustrated using spline regression lines; line color represents the sampling year. Cumulative proportion or levels of RCCB seasonal flight (Y<sub>25</sub>, Y<sub>50</sub>, Y<sub>75</sub>) predicted by GDDs (bottom row) using nonlinear logistic regression.



Figure 3 – Interpolation maps of predicted RCCB abundance based on clover spatiotemporal dominance for random points in 2021 (a). Floating bars indicate the posterior distribution of the spatial Bayesian GLMM (b). Simple linear regression of observed and predicted RCCB counts using the complete model (c) and clover alone (d).



## MONITORING AND (BIO)CONTROL OF PESTS IN WHITE CLOVER SEED PRODUCTION IN DENMARK

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### Abstract

The production of white clover seeds in Denmark is threatened by the larvae of weevil pests, Protapion fulvipes and Hypera meles. In recent years, massive damage to yield has been observed in especially organic production. Even in conventional production where insecticides are available, we have seen unsatisfactory pest control to protect seed yield. Thus, use of insecticides is not a stand-alone sustainable long-term solution. Though DLF white clover multiplication, we have access to large amount of field data, which combined with grower surveys and pests monitoring have made it possible to analyse potential correlations between farm practices and seed yields over a three-year period (2020-22) and thereby determine if different farm practices can help limit pest damage. The findings are valuable. The monitored densities of the weevil's P. fulvipes and H. meles show a negative effect on seed yield and growers who made three or more insecticide applications where still unable to maintain sufficient yield. The most promising findings in the organic seed growers' group was that the group that removed the defoliated material from the field prior to flowering in late May/early June, showed a seed yield index improvement by 49 compared with the group that left the cut biomass on field. In addition to this work, DLF focuses on supporting biological control of the weevil pests. At the seed cleaning process, we have developed a method that enable us to separate cocoons of a parasitoid wasp - a natural enemy to H. meles - and releasing them to new clover fields to build-up a stock of biological control insects. The promising effects are monitored by researchers from the Aarhus University, AU-Flakkebjerg.



Adult Protapion fulvipes (right) and Hypera meles (left). Photo: Henrik Bak Topbjerg

### Introduction

The Danish climate is adapted for grass- and clover seed production of high-quality seeds. However, pests are becoming an increasing problem, challenging the future of white clover seed production. The *Curculionidae* (weevils) insects *P. fulvipes* and *H. meles* are the main pests in both organic and conventional white clover seed production. The larvae of both pests feed on the green immature clover seeds (Detwiler, 1923). Previous studies have mention yield loss by more than 40% due to weevil pests (Hansen & Boelt, 2008). In organic production, *P.* 

*fulvipes* where often present in higher densities, why current studies indicate that *P. fulvipes* are the biggest problem in organic production (Lundin *et al.*, 2017). However, the larger pest *H. meles* affects the yield approx. 8-10 times more than *P. fulvipes* (Langer & Rohde, 2005; Topbjerg & Boelt, 2021). In recent years, we have observed several white clover fields, primarily by organic farmers, where seeds have been destroyed by pests' larvae – causing complete harvest failure.

To continue a sustainable seed production, it is essential to widely explore management methods to control these pests. The traditional insecticide applications are not a sustainable long-term solution due to 1) limited active ingredients (AI) approved for seed production in Denmark (AI: lambda-cyhalothrin and acetamiprid) and 2) the risk of developing insect resistance. In addition, we have observed numerous pests despite repetitive insecticide applications at conventional farms. Insecticides may also have a negative effect on natural enemies of insect pests as well as other beneficial insects. Therefore, additional methods and farm practices IPM (Integrated Pest Management) must be considered and implemented for pest control. DLF have through the years made significant investments in standard parcel trials to examine pest and insecticide application strategies. However, small parcel insect trials are challenged due the mobility of pests between parcels and the findings from such trials are often inconsistent. The DLF field production team therefore decided to review "big data" from seed multiplication as an information base and add information from seed growers' surveys. The expectation was that access to background data from several white clover fields could lead to further knowledge about different farm practices and potential effect on weevil pests.

### Materials and methods

Each seed production contract includes information of variety, field size and location, quality, and seed yield. The actual yields were indexed against a five-year average yield reflecting each variety. In addition, each year's average was fixed to 100 to neutralise the difference between varieties and years. We have assumed that the yield differences observed primarily are caused by different pest densities and farm practices, although there might be a high probability that other factors affect the yields.

For the harvest years 2020-22, all conventional white clover growers were asked to respond to a survey about field management e.g., number of insecticide applications and crop defoliation in spring including material handling. In 2021 and 2022 a moderated version of the survey included organic seed growers. The response rate was 60-77% over the 3 years which provide us with a unique set of data. An overview of the yearly responded surveys is shown in Table 1.

Table 1. Total number of responded surveys for each year. Please note that some field data is not available for all the surveys.

	Number of respondents				
Year	Conventional	Organic			
2020	107	-			
2021	104	22			
2022	119	34			
Total	330	56			

The field production team developed a handy method to measure the current level and density of pests in peak flowering white clover ultimo June/primo July: Take a firm grip to a 10-litre bucket and make five fast smooth and wide swings (approx. 0.5 m2) within the clover crop and make a headcount of the pests caught in the bucket. The found densities where then divided into four groups. Pest data were available for 2021 and 2022.

DLF field reps assessed the biodiversity around each of the fields; low (e.g., big homogeny areas) or high (e.g., many hedges, nearby forest etc.). The field reps also assessed the frequency of white clover in the crop rotation. Based on survey feedbacks, yield data was collected and the potential correlations between seed yield index, pest densities and agricultural practices were analysed and some of the most interesting findings are presented in this paper.

The data and results only represent the fields and yields, where the respective growers completed the survey and thus do not represent the entire seed multiplication in DLF. Analysis of variance of the observed data was done using PROC GLM in SAS 9.2.

# Results

For the conventional production, the data are presented as a three-year simple average (2020-22). Findings regarding pest densities will be based on year 2021-22 and data from organic fields are 2-years average (2021-22). A different letter above the bars in a figure, indicates if there is significant difference between the groups (P < 0.05).

Figure 1 shows the observed *H. meles* larvae (A) and *P. fulvipes* adults (B) in conventional white clover seed production fields – grouped by pest densities 2021-22. A: The indexed seed yield was significant higher for the fields where no *H. meles* larvae were monitored in bucket compared to fields were observed above 5 larvae. The seed yield was approximately 35 indexes lower in the group with highest number of larvae. B: For *P. fulvipes*, there was a significant difference in indexed seed yield for fields with more than 5 beetles observed, resulting in a seed yield approximately 20 indexes lower compared to no observed beetles.

Figure 1 A + B. Monitoring of *H. meles* larvae (A) and *P. fulvipes* adults (B) relative to the indexed seed yields 2021-22. Obs. data from conventional fields only.



In Figure 2 conventional yield data 2020-22 are grouped by the number of insecticide applications. There is a tendency that the group of growers that made two applications obtains the highest seed yield, yet this was not significantly higher than the seed yield achieved by growers with one application. The seed yield obtained by the group of growers with three or more insecticide applications was significantly lower than the seed yield achieved for the two-application group.

Figure 2. The number of insecticide applications linked to the indexed seed yield. Data from conventional fields 2020-22.



In Figure 3 data from organic white clover seed production fields are grouped by the handling of biomass following defoliation prior to flowering. The data show a significant yield response in organic fields that we were not able to discover in conventional fields. The organic growers that removed the green material from the field, had a significant higher seed yield by 49 indexes compared to growers that let the clippings on field.

Figure 3. Handling of material after defoliation in May/June relative to the indexed seed yields. Data form organic fields 2021-22.



In Figure 4 the distance to the previous year's white clover field seems to have strong tendency to affect the seed yield for the organic fields, however, not significantly.

Figure 4. Distance to the previous year's white clover field relative to the indexed seed yields. Data form organic fields 2021-22



### **Discussion & perspectives**

In general: All the above analyses and results are conducted through a generalization where the models and graphs are based on single factor of interest. This is, of course, not reflecting the practical case where many factors influence, overlap, and interact within the growing season. However, the large set of data provides us with the possibility to analyse the effect of single factors on yield.

The growth conditions, as well as the harvest conditions, in the three years (2020-22) have been almost perfect, thus main differences in yields are assumed to be caused by pest's activity. In 2020, we observed a middle pest activity, in 2021 a remarkably high pest activity, and in 2022 a lower activity - confirmed by the average yields for each year. To ensure the strongest database for analysis, we looked at a three-year average, but also single years if relevant.

**Monitoring of** *H. meles* **and** *P. fulvipes*: For *H. meles* (Figure 1A) the yield was highly negative affected when the monitored larvae density exceeded 5 larvae, which could potentially be set as a threshold value. The same threshold could be set for the *P. fulvipes* beetle (Figure 1B) although in previous studies, the damage on yield per induvial larva are found to be less than for the *H. meles* larvae (Langer & Rogde, 2005; Topbjerg & Boelt, 2019; Topbjerg & Boelt, 2021). It is important to note that the larvae of *P. fulvipes* are physically very small and not observed in the buckets, which is why data for the larvae is not available. Monitoring in

both years was carried out almost similar in ultimo June/primo July. In 2022 the monitoring was mainly conducted in primo July, why the densities of larvae can differ between the years. The monitoring was carried out at different locations, changing weather condition, various times of day etc. which may influence the observations. The ratio between observed beetles and larvae of *H. meles* may also vary over time, depending on development stage. The number of observed *H. meles* beetles where in general low, why data is not shown.

**Application with insecticides:** Grouping the fields into one or two insecticide applications gives a clear indication that growers can to some extend prevent massive pest attack in some fields (Figure 2). However, in some fields it is not possible to protect the yield even if the grower sprays three times or more to prevent yield loss.

**Handling of biomass after defoliation in May/June:** By removing the biomass after defoliation by the end of May/early June, it is expected that many pests (insects, eggs, and larvae) are also directly removed from the field. We have observed when the defoliated biomass is removed quickly, the wrapped material contains a lot of weevils. Since these weevils only have one generation per year, the removal of even few beetles directly reduces the damages caused by the larva. If the beetles already laid the eggs in the stem at the time of cutting, the eggs will also be removed with the material. The logical statement would be that the difference in seed yield is a direct consequence of fewer pests where the growers remove the biomass. The results for the organic fields (Figure 3) show a significant seed yield difference between the seed grower group that removes the defoliated biomass, and the group that leave the biomass on field. The difference in favour of removal is a higher yield index by 49 points. The data for the conventional fields show no statistics difference (data not shown). We have not analysed if there is an interaction/correlation between the monitored pests and removal of material, this might require a larger dataset.

**Distance to previous year white clover field:** It is expected that greater distances to previous year white clover field will result in higher seed yield, as it would decrease the transfer of pests into the new field, as found by Hederström et al. (2022). The flying qualities of *P. fulvipes* and *H. meles* are known to be modest, which is why the distance to previous years could be a key factor. However, the results for organic fields (Figure 4) did not show significant evidence when distance between this years and last years' field was below nor above 1000 meters, the same was the case for the conventional (data not shown). Nevertheless, there is a strong tendency that seed yield increases with the greater distance from previous white clover fields. Distance seems to have stronger influence for the organic than conventional fields (data not shown). While ">1000 m" was the maximum distance the growers could choose in the survey, it would have been interesting to analyse distances above 2000 meters as made by Hederström et al. (2022).

**Natural enemies**: In addition to the above results, DLF has also focused on protecting and recovering the natural enemies of weevils. The parasitic wasp Bathyplectes curculionis is a natural enemy to H. meles. When the white clover is harvested, the cocoons of the parasitized *H. meles* larvae flow with the seeds into the warehouse. In the cleaning process, DLF has developed a method to separate these cocoons. In the past years, DLF has isolated many hundred kilos of cocoons, and in collaboration with Aarhus University AU-Flakkebjerg, we have released them into new white clover fields. The preliminary results (not published)

indicate that releasing cocoons improves the parasitoid degree, thus expecting less reproduction of *H. meles* into the following year. These results will be submitted by the Aarhus University, AU-Flakkebjerg (Topbjerg, 2023). In 2021, we made a simple comparison of the seed yield in relation the number of cocoons found in the harvest material (data not shown). The comparison tended to have a negative correlation with yield, indicating that higher numbers of cocoons in general correspond to a high pest pressure. In 2022 we observed fewer cocoons than in 2021, which supports the theory in that we in general observed fewer pests and higher seed yields in 2022.

# Conclusion

The monitored densities of the weevil's pest *P. fulvipes* and *H. meles* show a negative effect on yield, and even growers who applied insecticides three or more times were not able to protect seed yields. Some of the most promising findings is that our data show significant higher yields in the group of organic growers that remove the cuttings of white clover biomass in late May/early June. This group have a 49-point higher yield index compared with growers that leave the clippings on field. The all the above effects will support DLF advisory service significant in further sustainable production of white clover.

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**Keywords**: White clover, pests, monitoring, biological control, natural enemies, farm and field management, biodiversity, crop material handling.

Latin: *Protapion fulvipes* Eng: White clover seed weevil. Latin: *Hypera meles* Eng: Clover head weevil

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#### INTEGRATED APPROACH TO MANAGE YELLOW DWARF VIRUSES IN PERENNIAL GRASS SEED CROPS

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#### Abstract

Oregon's grass seed industry, valued at over \$500 M USD, is the top producer of cool-season grasses worldwide. Perennial ryegrass (Lolium perenne L.), tall fescue [Schedonorus phoenix (Scop.) Holub], and Kentucky bluegrass (Poa pratensis) represent the majority of grass seed land area in the Willamette Valley (perennial ryegrass, tall fescue) and eastern Oregon (Kentucky bluegrass). Oregon reports have attributed seed yield loss and reductions in stand longevity to aphid-transmitted yellow dwarf viruses (YDV). Effective integrated pest management (IPM) strategies have not been thoroughly studied for YDV in grass seed crops. To develop a robust IPM program for this virus-vector complex, a commercial field survey and three-year field trial were initiated in 2021. Field surveys to assess the spatiotemporal distribution and risk factors associated with YDV began in autumn 2021 and continued through autumn 2022. A total of 57 commercial fields (perennial ryegrass, tall fescue, Kentucky bluegrass) across Oregon were monitored weekly for alate aphids using sticky traps (May to November) and sampled for plants and apterous aphids two weeks after the peak spring or autumn aphid flight. Samples were collected by walking four 100 m transects per field. For plant and representative aphid samples, total nucleic acid extractions were conducted, followed by polymerase chain reaction (PCR) to determine YDV genera (Luteovirus or Polerovirus) presence and, if positive, YDV species. Environmental raster data were extracted, and management history metrics were recorded for sampled fields. Across the 57 fields, seven YDV species were detected, and considerable variation in YDV incidence was observed at the field scale. Total aphid alate abundance ranged from less than 100 total aphids across the sampling period to over 2000, with YDV viruses detectable in aphid populations. Environmental and management risk factors associated with aphid abundance and YDV incidence and diversity are being evaluated with spatial models. The field trial was planted with two perennial ryegrass cultivars ('Fastball' and 'Top Gun II'), and the effects of low and high nitrogen (N) rates (135 and 225 kg N ha<sup>-1</sup>) and insecticide spray timing (untreated control, autumn, spring, autumn + spring, or autumn + spring + summer) were evaluated for aphid-YDV suppression. Aphid abundance of alate and apterous morphs were monitored weekly throughout the growing season on sticky cards and within plots using sweep net sampling. Plant samples were collected before insecticide treatments were applied by randomly collecting five leaves per plot. Both aphid and plant samples were evaluated with PCR for YDV genera and species. Although differences in clean seed yield were not detected in the first-year harvest, greater YDV incidence was observed in high N plots and 'Fastball' plots. Moreover, all insecticide application timings were associated with lower YDV incidence compared to the untreated control, albeit insecticide treatments did not lead to complete protection for any treatment. Research findings from regional field surveys, geospatial risk models, and small-plot field trials will be leveraged to optimize management recommendations for aphid-vectored YDV in grass seed production systems.

## Introduction

Oregon's grass seed industry, valued at over \$500 million, is one of the top five agricultural commodities in the state. Perennial ryegrass (*Lolium perenne* L.), tall fescue [*Schedonorus phoenix* (Scop.) Holub], and Kentucky bluegrass (*Poa pratensis*) are known to be susceptible to yellow dwarf viruses (YDV) vectored by aphids. This virus-vector system has been researched extensively in cereal production systems internationally. However, limited studies have observed this pest complex in perennial grasses grown for seed (Fisher and Dreves 2010), and more research is needed to provide effective integrated pest management (IPM) plans for Oregon's grass seed industry. The YDVs present in Oregon are comprised of at least ten strains that are transmitted by at least four aphid species (Parry et al. 2012; Laney et al. 2018). Aphids vector YDVs to grass seed, cereal crops, and non-crop weedy hosts. Once a perennial ryegrass plant is infected, it cannot be cured, complicating pest management strategies. Research is needed to determine the spatiotemporal distribution and prevalence of aphid species and the YDVs they vector in grass seed production regions across Oregon.

To understand the spatiotemporal distribution of the aphid-YDV virus-vector complex and effective management strategies to reduce outbreak severity in perennial grass seed crops, the following objectives were developed:

- 1. Survey commercial perennial ryegrass seed fields for aphid abundance and YDV incidence.
- 2. Evaluate the effects of insecticide timing and nitrogen fertilizer rates on aphid-YDV control in perennial ryegrass seed crops through a replicated seed yield trial.

## **Materials & Methods**

## Field survey

In 2021 and 2022, 57 fields spaced >5 km apart along a gradient of crops identified as potential hosts of aphids and YDV were surveyed across Oregon's Willamette Valley and eastern Oregon. Using sticky cards on the north and south-facing field edge, winged aphids (alates) were monitored and counted weekly from May to November. Aphids on cards were counted and randomly selected for species identification and Polymerase Chain Reaction (PCR; protocol described below). Two weeks after peak flight, plants and sweep net samples were collected. For plant and aphid collections, fields were divided into four quadrants and a 100 m transect was walked, collecting five random leaves every 10 m (n = 40 total samples per field). Leaves were rated for symptoms and processed with PCR as described below. In the same quadrants, four 100-sweep net samples were collected (n = 400 total sweeps). Randomly selected aphids from sticky cards and sweeps were selected for PCR analysis to determine YDV presence.

For PCR evaluation of virus presence in aphids and plants, total nucleic acids from respective sample types were extracted. A multiplex PCR for strain differentiation of yellow dwarf viruses was employed to determine YDV genera, barley yellow dwarf viruses (BYDV) versus cereal yellow dwarf viruses (CYDV) (Malmstrom and Shu 2004). Additional determination of YDV species (9 species detectable) was conducted with additional published primer sets (Laney et al. 2018; Sõmera et al. 2021).

## Field trial

A small-plot field trial was established at Oregon State University's Hyslop Research Farm in the fall of 2021. The trial includes a split-split plot arrangement of treatments and four replications. Each main plot consists of a different genotype of perennial ryegrass to evaluate host resistance, including 'Top Gun II' (Barenbrug USA) and 'Fastball' (Mountain View Seeds). Split plots include two randomized nitrogen rates. Nitrogen was applied in spring 2022 in 16.8 × 15.2 m blocks at a low (135 kg N ha-1) or high rate (225 kg N ha-1). Within each split plot, five  $3.4 \times 16.8$  m subplots were randomly assigned an insecticide treatment: 1) untreated control, 2) autumn only, 3) spring only, 4) autumn + spring, or 5) autumn + spring + summer application. A highly efficacious chemistry was selected for aphid control, flupyradifurone (175 g ha-1; Sivanto, Bayer), to represent the best possible control for foliar application of each treatment time interval. A modified JD small plot swather and Hege combine were used to determine seed yield during the July 2021 harvest. A subsample was collected from each plot to evaluate percent cleanout, thousand seed weight, and clean seed yield.

Winged aphids (alates) were monitored weekly on yellow sticky cards placed on the cardinal edges of the field. Aphids on cards were counted and randomly selected for species identification and PCR (protocol as above). Fall, spring, and summer insecticide spray dates corresponded with leaf collections to evaluate YDV strain presence. For leaf sample collections, five leaves were collected at five random stops along the western-facing plot edge (n = 25 leaves per plot per sampling time). Plants were collected before and one month after spray treatments (n = 6 plant collections). The eastern-facing edge of each plot was scouted for apterous (wingless) and alate (winged) aphid populations weekly for at least eight consecutive weeks to determine aphid species and abundance and natural enemies within sampled plots. Plot centres remained undisturbed for yield evaluation.

# Results

# Field Survey

Aphid counts on sticky cards throughout the Willamette Valley ranged from less than 100 aphids to over 2,000 aphids over the 30 weeks of aphid monitoring (Figure 1). Yellow dwarf virus status of aphids varied throughout the region, with instances of high YDV expression regardless of aphid population density for unique field locations (relatively low aphid populations but high incidence of YDV). Plants had high variability of BYDV incidence, with fields ranging from 0-100% infection. There was less variability in CYDV incidence, with fields ranging from 0% to up to 75% infection but lower infestation overall.

## Field Trial

Based on the 2021-22 growing season, differences were detected in YDV incidence and aphid abundance between cultivars and insecticide application timings. Cultivar 'Fastball' had lower aphid counts in plots with a spring insecticide application compared to the single fall application treatment or the untreated control (Figure 2). Cultivar 'Top Gun II' showed a similar pattern, although no differences were observed. BYDV incidence was greater overall in 'Fastball' compared to the 'Top Gun II' cultivar. CYDV incidence was more consistent in 'Top Gun II,' showing genetic variability in susceptibility to different YDVs. For both cultivars, low nitrogen plots had a lower aphid abundance and YDV incidence and similar seed yield compared to high nitrogen plots in a first-year stand.

## **Discussion & perspectives**

YDV is highly prevalent in Oregon grass seed production. Initial analyses indicate that aphids are widespread, but high aphid counts over time did not directly correlate with YDV incidence. Specifically, high YDV incidence in molecular plant and aphid evaluations were observed irrespective of aphid density in commercial fields sampled across the Willamette Valley. Utilizing risk assessment statistics, preliminary data indicates that growers with a foliar insecticide spray program had a lower risk of YDV incidence, while those planting YDV alternate host crops within 1 km of sampled fields were at higher risk of greater YDV species diversity in commercial grass seed stands. Small-plot research indicates that lower nitrogen rates (135 compared with 225 kg N ha<sup>-1</sup>) in first-year perennial ryegrass crops do not lead to significant seed yield reductions and may help lower aphid and YDV incidence. Plots treated with insecticide sprays in the spring were associated with lowered YDV incidence and increased seed yield. Our small-plot research trial and field survey data will be used to develop best management recommendations for YDV in perennial grasses grown for seed in Oregon.

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## Figures

Figure 1 - (A) Winged aphid count on sticky cards in the Willamette Valley (WV) over 30 weeks of trapping, showing population densities from <100 to over 2000 aphids. (B) Distribution of positive detection of BYDV from sticky card aphids. (C) Distribution of positive detection of CYDV from sticky card aphids. (D) Percentage of plant samples per field that were positive for BYDV in WV and eastern Oregon. (E) Percentage of plant samples per field that were positive for CYDV.



Figure 2 - Small-plot research results after first harvest (2021-22). (A) Mean aphid counts per plot for each cultivar based on insecticide timing. Letters indicate significance groups. (B) Mean clean seed yield for each cultivar by insecticide timing. Plant BYDV (C) incidence and CYDV (D) incidence by cultivar based on insecticide timing. (E) Mean aphid counts per plot for low and high nitrogen rates. (F) Mean clean seed yield for each cultivar by nitrogen rate.



#### TRICHODERMA BIO-INOCULANTS TO ENHANCE GRASS SEED YIELD IN FIELDS AFFECTED BY TAKE-ALL DISEASE

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#### Abstract

Take-all, a serious soil-borne disease of wheat also occurs on several herbage grasses (perennial ryegrass, prairie grass and timothy) and grass weed species (ripgut brome and smooth brome). The disease can affect seed yields both quantitatively and qualitatively. Glasshouse and field experiments were conducted using selected Trichoderma bio-inoculant formulations on prairie grass (Bromus willdenowii) and perennial ryegrass (Lolium perenne) in soils infested with Gaeumannomyces graminis var. tritici (Ggt). In a glasshouse experiment Ggt significantly reduced prairie grass seed yield by 16% by reducing the number of seeds per plant. In the presence of Ggt, Trichoderma atroviride isolates LU132, LU140 and LU584 added to the soil in a prill formulation increased prairie grass seed yield by 13-16%, compared to Ggt controlby increasing seed number per plant. In the field, two different bio-inoculant formulations were used, prills drilled into a second year perennial ryegrass seed crop, and seed coating for a new crop. In general, root quality observations revealed lower infection and disease severity in soils containing Trichoderma which led to a 20% seed yield increase for the prill formulation and 4% increase for the seed treatment. These increased seed-yields were associated with a higher number of reproductive tillers and a small reduction in seed dressing losses. Seed-coating with Trichoderma bio-inoculants as a prophylactic practice to improve grass seed yields will be discussed.

Keywords: Gaeumannomyces graminis var. tritici, Perennial ryegrass, Prairie grass.

#### Introduction

The root-rot pathogen *Gaeumannomyces graminis var tritici* (*Ggt*) causes take-all in cereals and grasses (Chng et al. 2005). In an arable cropping rotation, a perennial ryegrass (*Lolium perenne*) seed crop is often grown after cereals, and this allows the pathogen to persist in soils, providing inoculum to infect subsequent crops (Bithell et al. 2011). In New Zealand, a number of growers have recently had issues of light seed and large dressing losses in irrigated perennial ryegrass seed crops, and diagnostic assessments on some of these fields detected *Ggt* infection of roots. Umar et al. (2019) demonstrated in a glasshouse experiment that *Ggt* infection of perennial ryegrass roots significantly reduced plant root and vegetative shoot dry matter production, and suggested Ggt as the possible cause of the seed production problems being encountered by growers. *Trichoderma* bio-inoculants are widely used for controlling a wide range of pathogens and for promoting growth in various host plants (Samuels and Hebbar 2015), and both Kandula et al. (2014) and Umar et al. (2021) have demonstrated the potential of *Trichoderma* strains to suppress *Ggt* in perennial ryegrass. However, these studies involved vegetative growth. Any impacts on seed production were unknown. The objectives of this study were (i) to assess the impact of *Ggt* on prairie grass (*Bromus willdenowii*) seed production in a glasshouse study and (ii) to determine whether a *Trichoderma* bio-inoculant could increase seed yield in the presence of Ggt (in both a glasshouse and field studies). The hypothèsis was that *Trichoderma* bio-inoculant application would reduce seed yield losses caused by *Ggt*.

## **Materials & Methods**

#### Glasshouse experiment

Prairie grass was used as a model system because it is known to be highly susceptible to Ggt (Chng et al. 2005), and it does not require vernalisation to switch from vegetative to reproductive growth so that seed production was possible within the time available for the experiment. Prairie grass seedlings were transplanted into 9 L pots filled with field soil/pumice (3:1 v/v). Treatments were « soil only control », « pathogen control » and three Trichoderma atroviride isolates applied as prills (for details see Umar et al, 2021). At harvest maturity (between 80-100 days after transplanting) seeds were hand harvested and separated into light and commercial fractions using a Hoffman seed blower (Hoffman Mfg. Inc). Each seed fraction was weighed and thousand seed weight determined (Umar et al. 2021).

## Field experiments

Two field experiments (one in 2019-20 and one in 2020-21) were established as large plots (0.25-0.3 ha) suitable for evaluation by weigh wagon, but were limited to two (2019-20) or three (2020-21) replicates and two treatments (nil versus treated). In 2019-20, prills containing the pasture bioinoculant (PBI) were overdrilled into a second year crop of perennial ryegrass cv. Request on 21 April 2019, while in 2020-21 PBI treated seed of cv. Platform AR37 was sown on 24 March 2020. The prills and treated seed were supplied by Agrimm Technologies Ltd.; PBI is a mixture of four T. atroviride isolates (LU312, LU140, LU584 and LU633). The fields were irrigated and all management inputs were applied by the grower as part of normal seed crop management. The fields were sampled twice for Ggt assessment and dry matter from 2.7 m<sup>2</sup> quadrats per plot. Root disease severity was scored based on root deterioration due to rotting and pathogen colonisation using a scale of 0 (root intact and no runner hyphae) to 5 (totally disintegrated root with hyphae and hyphodia on all roots) (Umar et al, 2019). Seed head and reproductive tillers pre-harvest were assessed from a 0.2 m<sup>2</sup> quadrat in January. At harvest the grower combined each plot separately, the field dressed (FD) seed yield was determined with a weigh-wagon and a sample retained for seed cleaning. The FD seed was cleaned in a laboratory with sieves and blown in a Dakota seed blower to achieve a thousand seed weight (TSW) sample of >1.90 g.

## Results

Treatment	No. of seeds/	FD seed weight	MD seed weight	Thousand seeds
	plant	(g)/plant	(g)/plant	weight (g)
Soil only control	242*	2.36*	2.14*	9.74
Ggt control	201	1.94	1.79	9.61
LU132 + Ggt	234*	2.31*	2.11*	9.85*
LU140 + Ggt	219	2.19*	1.99*	9.77
LU584 + Ggt	238*	2.33*	2.07*	9.76
LSD (5%)	18	0.18	0.16	0.23

#### *Glasshouse* experiment

Table 1 - Effect of take all (*Ggt*) and *Trichoderma* isolates on prairie grass seed yield components and seed yield.

\* indicates significant difference from pathogen control in the same column at P < 0.05.

*Ggt* infection of the prairie grass roots reduced tiller numbers per plant (data not presented) and resulted in 41 fewer seeds per plant, which resulted in a significant reduction in both FD and MD seed yield per plant (Table 1). However Thousand Seed Weight (TSW) was not reduced. In the presence of *Ggt*, treatment with all three *Trichoderma* isolates resulted in seed yields which did not differ from yields where the pathogen was absent (Table 1). However *Ggt* did not significantly increase dressing losses (percentage of light seed; data not presented).

### Field experiments

#### 2019-20

At both assessment times, PBI significantly reduced *Ggt* root infection (Table 2), and at the November sampling had significantly increased tiller numbers and crop dry weight (Table 3). At harvest, the MD seed yield increase (+22%) for the PBI treatment was not significant and thousand seed weight did not differ (Table 4).

	•					
Treatments	2019-20		2020-21			
	Aug '19	Dec '19	Jul '20	Nov '20		
Control	1.46	3.0	0.54	1.00		
PBI	0.62*	1.3*	0.21*	0.42*		
LSD (5%)	0.48	0.3	0.29	0.36		

Table 2 - Effect of PBI on Ggt disease score 1 in field experiments.

\* indicates difference significant at P < 0.05. 1 based on scale of 0 = nil, 5 = Severe infection.

Table 3. - Effect of PBI on reproductive and vegetative tiller number and crop dry weight of 2nd year perennial ryegrass cv 'Request' grown in a Ggt-infected field at Greendale 2019-20 (sampled on 25 November 2019).

Treatments	Reproductive tiller number/m2	Vegetative tiller number/m2	Crop dry weight g/m2
Control	1059	4755	563
PBI	1429*	6772*	645*
LSD (5%)	261	1625	44

\* indicates difference significant at P<0.05

			, 2020).	
	FDS		Dressing	TSW
Treatment	(kg/ha)	MDS (kg/ha)	loss (%)	(g)
Control	1130	1030	8	2.23
PBI	1390	1240	10	2.27
LSD (5%)	NS	NS	NS	NS

Table 4 - Effect of PBI on field dressed seed (FDS) and machine dressed seed (MDS) yield and thousand seed weight (TSW) for 2nd year perennial ryegrass cv 'Request' grown in a Ggt-infected field at Greendale 2019-20 (harvested on 20 January 2020).

### 2020-21

As in the previous year, PBI treatment significantly reduced Ggt root infection (Table 2) and increased reproductive tiller numbers (Table 5). However, vegetative tiller numbers were reduced and crop dry weight did not differ from that of the control. MD seed yield was increased significantly by the PBI treatment and there was a small but significant reduction in dressing loss, but TSW did not differ from that of the control (Table 6).

Table 5 - Effect of PBI on reproductive and vegetative tiller number and crop dry weight of perennial ryegrass cv 'Platform' grown in a Ggt-infected field at Ashton 2020-21 (sampled on 15 January 2021).

Treatments	Reproductive tillers/m2	Vegetative tillers/m2	Crop dry weight (g/m2)
Control	1763	364	958
PBI	2156*	205*	1048
LSD (5%)	383	101	130

\* indicates difference significant at P < 0.05.

Table 6 - Effect of PBI on machine dressed seed (MDS) yield, thousand seed weight (TSW) and dressing loss (%) of perennial ryegrass cv 'Platform' grown in a Ggt-infected field at Ashton 2020-21 (harvested 5 February 2021).

	1		
	MDS yield	TSW	Dressing
Treatments	(kg/ha)	(g)	loss (%)
Control	1745	1.97	21
PBI	1820*	1.98	17*
LSD (5%)	70	0.26	1.2
F.prob	0.047	NS	0.004

\* indicates difference significant at P < 0.05.

# **Discussion & Perspectives**

*Ggt* reduced prairie grass seed yield by reducing seed number per plant but did not reduce TSW or increase the percentage of light seeds produced. In *Ggt* infected perennial ryegrass, seed yield was also reduced, but TSW was not, and seed number data were not recorded. *Ggt* damage to roots reduces the ability of the plant to utilize water and nutrients, either because of a reduction in the size of the root system (Umar et al, 2019), or a reduction in the efficiency of uptake (Umar et al, 2021). Trichoderma treatment significantly reduced *Ggt* root infection in both grass species and increased seed yield. However, the lack of difference in TSW and only small reductions in dressing losses do not support the suggestion that farmer issues with light

seed and large dressing losses in irrigated perennial ryegrass seed crops were directly attributable to *Ggt* root infection.

The *Trichoderma* PBI formulation has previously been shown to provide control of other grass pathogens including *Rhizoctonia*, *Pythium* and *Fusarium* (Kandula et al, 2015) and to promote perennial ryegrass plant growth (Umar et al, 2019). Additionally, Trichoderma spp have been shown to stimulate plant defences against both abiotic and biotic stresses (Woo et al, 2022). The mechanisms by which PBI treatment produced the response recorded in perennial ryegrass seed crops is not yet known and requires further investigation, preferably with more treatment replicates than were available under the on-farm trial system used. However, the results do indicate a beneficial effect of PBI, suggesting that *Trichoderma* seed treatment be considered for grass seed production in New Zealand.

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## DETECTING VOLE DAMAGE IN TALL FESCUE SEED CROPS WITH AERIAL IMAGERY

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## Abstract

This project investigated the feasibility of using an unmanned aerial system (UAS) to measure crop damage caused by gray-tailed voles in tall fescue seed production fields in the Western Oregon, U.S.A. Vole damage to grass seed crops can result in substantial yield losses, and control options are labour intensive. Detecting damage with a UAS would allow researchers to test vole management practices and help bait applicators reduce labour and input costs by targeting areas where voles are active. A UAS was used to collect aerial imagery from two tall fescue seed production fields in March and April 2022. To verify findings from the aerial imagery, ground truth data, including location coordinates, photos, and observations of areas with and without vole damage, were collected from the ground at the time of each flight. In the aerial imagery, patches of vole damage differed from undamaged areas in several ways. Damaged plants were smaller, and soil was more often visible between plants in damaged areas, while canopy closure was more common in undamaged areas. An object based, supervised image classification procedure was used to divide the aerial images into areas of damaged plants, healthy plants, and soil. The accuracy of the classification was evaluated by comparison with the ground truth points. Overall, more than 90% of the combined area surrounding (within 15 cm) ground-truth points was correctly classified. These results show that vole damage can be identified using UAS imagery and highlight the utility of this approach for evaluating vole control practices.

## Introduction

Unmanned aerial systems (UAS) enable agriculturalists to gain aerial views of large crop production fields, providing information that would be difficult and labour intensive to obtain from ground-based observations. Researchers, agronomists, and farmers have used these systems to evaluate crop health, nutrient status, water status and estimate yield (Hassler and Baysal-Gurel, 2019). However, more work is needed before the full potential of UAS will be available in smaller acreage crops such as grass seed.

In Western Oregon, USA grass seed production systems, gray-tailed voles (*Microtus canicaudus*) can cause severe crop damage. Other species of voles are known to damage many crops, beyond the Willamette Valley. In a 2020 survey, growers reported yield losses at least 220 kg/ha on 61% of vole damaged grass seed acres, with > 1120 kg/ha yield losses on 6% of damaged grass seed acres (Verhoeven and Anderson, 2021). Historically, high vole population numbers occurred every 4-8 years, followed by population crashes (Gervais, 2007). Recently, populations have remained elevated, with reports of severely damaged fields every year since 2019. Vole control options are limited to zinc-phosphide baits, and only 26% of growers reported these baits provided satisfactory control (Verhoeven and Anderson, 2021). Between

mid-September and late April, zinc-phosphide baits may only be applied below ground by placing bait in burrow entrances, which requires considerable labour.

There is clear need for improved vole control options, but evaluating new control practices is difficult and costly. Some earlier work has used mark and recapture techniques to measure vole populations, but this method requires labour-intensive live trapping programs. Gervais (2010) studied whether indicators of vole activity such as burrows and fresh droppings could be used to estimate populations, but none of these signs of vole activity were good predictors of vole populations. Vole damage creates large, distinctive patches that are expected to be highly visible in UAS imagery. This study investigated whether UAS aerial imagery can be used to detect vole damage in tall fescue seed production fields to develop a research and scouting tool that will help improve vole management.

## Materials & Methods

*Field sites:* This study was conducted in two established tall fescue fields with severe vole infestations: a turf type field, variety 'Renegade DT', approaching its third harvest, and a forage type, variety 'Goliath', approaching its eighth harvest. The turf type had distinct crop rows, while rows were not visible in the forage type field.

*Data collection:* Two UAS flights were conducted in each field. The turf type field was flown on March 22 and April 27, 2022, and the forage type field was flown on March 23 and April 22, 2022. The UAS used in this study was aDJI Matice 210 v2 quadcopter outfitted with a MicaSense RedEdge MX multispectral camera and a Sony a6000 RGB camera. Flights were conducted at 50 m above ground level, resulting in a ground sample distance of 3.5 and 1.2 cm per pixel, respectively.

Ground-truth data were collected after each flight for 20 patches of vole damage and 20 undamaged areas distributed throughout the study area. Ground-truth points were identified as damaged if there were clear signs of vole activity such as droppings, runways, fresh signs of digging and freshly clipped leaves and stems. Burrows alone were not considered sufficient evidence of vole damage because burrows can persist for several months without vole activity. Undamaged areas had vigorous crop growth with no evidence of vole activity. Observations, photographs, and location information were recorded for each ground-truth point. Evidence of other causes of poor crop growth was looked for, but not observed.

*Georeferencing:* Location information was collected using global navigation satellite system receivers capable of post-processing kinematic positioning (PPK GNSS). A stationary base station receiver (Emlid Reach RS+) was set up at the site prior to data collection and a rover receiver (was used to collect the locations of ground truth observations. Ground control points (markers that are visible in aerial imagery) were placed in the field prior to each flight, and their locations were recorded with the rover receiver. A second rover receiver mounted on the UAS recorded the locations of photos.

*Data analysis:* Photos from the UAS were processed using Pix4D Mapper software to produce orthomosaics of the flight areas. Data outputs also included digital surface models (DSM) which showed the elevation of the crop canopy, single band reflectance images for each band captured by the multispectral camera, and normalized differential vegetation index (NDVI).

ArcGIS Pro software was used for further analysis. Using elevation data from the DSM, relative canopy height was calculated as the difference in elevation between each pixel in DSM and the average elevation of the surrounding area (12.4 m radius). At the location of each ground-truth point, a circular area with a radius of 15 cm was defined, and summary statistics (average and standard deviation) were calculated for pixels in that area for the NDVI and relative canopy height images. ANOVA and Wilcoxon rank sum tests (when assumptions of normality were not met) were used to compare the NDVI and relative canopy height values of damaged vs. undamaged ground-truth points.

Object-based, supervised image classification was performed for each flight using a combination of all multispectral image bands, plus DSM and NDVI, via the image classification wizard in ArcGIS Pro. Training data for the classification model were created by manually marking areas of damaged plants, undamaged plants and soil, based on appearance in the imagery. A previous attempt at image classification did not include a soil category, but did not produce an accurate classification result.

The accuracy of the image classification model was evaluated by assessing whether the computer classification produced by the model was consistent with the human classification made in the field for each ground-truth point. This was done by determining the percentage of the area within a radius of 15 cm of each ground-truth point that was assigned to each classification category.

## Results

Both fields had extensive vole damage that was distributed throughout the study area. Areas with vole damage were easily distinguishable from undamaged areas in imagery from all four flights. Small plants and large areas of visible soil characterized damaged areas, while undamaged areas had larger plants with little to no visible soil. There was notable crop growth between the March and April flights in both fields, resulting in increased canopy closure and a decrease in visible soil in undamaged areas.

Plants in damaged areas were significantly shorter (p < 0.05) than plants in undamaged areas based on relative canopy height. Average NDVI values were lower (p < 0.05), and NDVI standard deviations were higher (p < 0.05) in damaged areas compared to undamaged areas, likely due to the presence of visible soil. Figure 1 shows relative canopy height and NDVI standard deviation measured for both damaged and undamaged ground-truth points.

The image classification results are shown in Table 1. Most of the area surrounding undamaged ground-truth points was classified as undamaged plants by the computer. The majority of the area surrounding damaged ground-truth points was classified as either damaged plants or soil. The aim of this study was to differentiate between damaged and undamaged areas, so accuracy assessments focused on the damaged plant and undamaged plant classification categories. Soil was clearly visible in damaged areas in the aerial imagery, so areas surrounding a damaged ground-truth point that were classified as soil were considered to be correctly classified. Areas classified as soil around undamaged classification points were also determined to be correctly classified based on visual inspection of the imagery. Over all, more than 90% of the combined area surrounding ground-truth points was correctly classified.

## **Discussion & perspectives**

This study demonstrated that there are differences between areas of tall fescue fields with and without vole damage that can be measured in aerial imagery. Image classification can make use of these differences to successfully to identify damaged and undamaged areas in a field. This approach was successful in two tall fescue fields that had marked differences in appearance.

The scope of this study was limited, so more work is needed to build upon these results. Aerial imagery used in this study was collected in the spring, so future research will be needed to test this approach at other points in the crop growth cycle. Crop growth can outpace vole grazing in late spring and early summer, which may make vole damage less visible from the air. Other crop production problems such as pests, diseases and nutrient deficiencies may cause symptoms that appear similar to vole damage in aerial imagery. Future studies may help identify distinct characteristics that differentiate different crop problems. Until that time, aerial imagery should be validated with ground based observations.

This approach may be used by researchers to evaluate alternative vole management practices. As UAS technology and image processing tools become more accessible, aerial imagery has the potential to be used by seed producers as a decision support and scouting tool, allowing them to identify problem areas in fields. Inputs can then be applied only where they are needed, increasing the efficiency of inputs and labour.

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Table 1 – Summary of classification accuracy showing the percentage of the area surrounding damaged (n = 80) and undamaged (n = 80) ground-truth points that was assigned to each classification category by the image classification algorithm.

Ground-truth Classification	Computer Classification							
	Undamaged Plant	Damaged plant	Soil					
Undamaged	91%	7.5%	1.6%					
Damaged	9.4%	54%	36%					

Figure 1 – Aerial imagery values for locations that were observed from the ground. Relative canopy height is derived from the digital surface model and is shown on the y-axis. NDVI standard deviation is shown on the x-axis. Each point represents one ground-truth point that was determined to be damaged (black circles) or undamaged (empty squares). Each panel shows data from one field and flight date.



### **RYEGRASS HARVESTING: A COMPARISON OF CUTTING FRONTS**

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### Abstract

The introduction of high groundspeed windrowing of ryegrass using the John Deere Legacy Air grass front raised questions as to its effectiveness compared with a traditional windrower. The Legacy front uses disc mowers and has foils to move the cut grass into the windrow with reduced shaking and reduced seed loss.

Four large-scale field trials were conducted over three years to assess the performance of different cutting fronts for cutting of ryegrass seed crops. Four cultivars including a forage hybrid type, a forage perennial ryegrass and two turf ryegrasses were used in the trials. Three cutting options were compared: the Legacy front (groundspeed 14.0 kph / cutting 6.5 ha/hour), an auger windrower reciprocating knife cutter bar, (9.4 kph / 4.9 ha/h) and a 2.2 m wide disc mower (11.5 kph / 2.7 ha/h). The crops were cut when the seed moisture content was between 40 and 42%.

The seed yield averaged over the four crops for the Legacy front, auger windrower and disc mower were 3060, 2830 and 2650 kg/ha (LSD 0.05 = 160), respectively. We concluded that although the Legacy front cut at a faster groundspeed there was a seed yield advantage when compared to windrowing. Both were significantly better than traditional disc mowing.

#### Introduction

The introduction of high groundspeed windrowing of ryegrass using the John Deere Legacy Air grass front raised questions as to its effectiveness compared with a traditional windrower. The Legacy front uses disc mowers and has foils to move the cut grass into the windrow with reduced shaking and reduced seed loss. The disc mower has traditionally been used by New Zealand growers to cut ryegrass seed crops for harvest. In previous harvest seed loss studies by the authors, we have shown seed harvest losses in perennial ryegrass seed fields are high, typically representing 16% in windrowed crops and 20% in disc mown crops, representing 400 to 500 kg/ha of seed lost. Most of this seed loss occurs at cutting and is highest on the divide between cuts, and this difference is associated with there being fewer divides on the wider cutting fronts of a windrower (typically 4 m wide) compared to a disc mower (2.2 m wide). Losses in the swath during field drying are weather dependent. Pick-up losses at harvest and losses from poor seed separation at combining were generally low.

#### Methods

Four large-scale field trials were conducted over three years (2017 to 2020) to assess the performance of different cutting fronts for the harvest of ryegrass seed crops in the mid

Canterbury area of New Zealand. Four ryegrass cultivars including a forage hybrid type, a forage perennial ryegrass and two turf ryegrasses were used in the trials (Table 1). Three cutting options were compared: the Legacy front (groundspeed 14.0 kph / cutting 6.5 ha/hour), an auger windrower reciprocating knife cutter bar, (9.4 kph / 4.9 ha/h) and a 2.2 m wide disc mower (11.5 kph / 2.7 ha/h). In the 4<sup>th</sup> trial with cv Hustle (2020/21) the windrower used was a Grasshopper with the cutting knife was replaced by a disc cutting system. The seed crops were cut when the seed moisture content was between 40 and 42%.

The plots were the length of the field (about 300 m long) and 24 m wide. The treatments were randomised and duplicated in each field. The crops were combine harvested by the grower with his combine and a sample was an out and back run with the combine, picking up a single row of the Legacy and auger windrowed treatments and a double row of the disc mower. At the end of each run the harvested seed was augered into a weigh bin, the field dressed yield determined and a sub-sample for seed moisture and seed cleaning was collected. The trial seed yield was calculated from the clean-out percentage and adjusted to 14% SMC.

## Results

The average machine-dressed seed yield adjusted to 14% seed moisture from the Legacy front, auger windrower and disc mower were 3060, 2830 and 2650 kg/ha (LSD 0.05 = 160), respectively (Table 1). We concluded that although the Legacy front cut at a faster groundspeed there was a seed yield advantage when compared with windrowing. Both were significantly better than traditional disc mowing.

One potential disadvantage with the Legacy front is that the windrow has more bulk (wider cutting front) and under wet conditions it may take longer to dry. In damp harvest seasons, disc mowing does offer an opportunity to combine harvest with fewer days between cutting and combining.

Cultivor	Voar	Legacy	Standard	Disc
Cultival	real	Windrower	Windrower	Mower
Shogun (hybrid)	2016/17	3200	2990	2640
DLF 46-600 (turf)	2017/18	3110	2830	n/a
Bokser (turf)	2017/18	2920	2680	2660
Hustle				
(perennial	2019/20	3000	2830*	2640
forage)				
mean		3060	2830	2650
LSD 5%			160	
F.prob			0.005	

Table 1. Seed yield of perennial and hybrid ryegrass in the harvest methods trial, year of trial and seed yield (kg/ha).

\*Grasshopper windrower with disc cutting

## Discussion

In Oregon there have been reports (N. Anderson pers comm) that seed losses are higher and seed yields lower for the Legacy front compared to windrowing. However, in Oregon, perennial ryegrass seed crops are commonly not cut until the seed moisture is <35%, in part because growers are trying to complete tall fescue harvest before starting perennial ryegrass harvest. Our data suggests that if ryegrass crops are cut at higher seed moistures, 40 to 42% the faster groundspeed of the Legacy front is not detrimental to seed yield.

## AUXIN APPLICATION CAN REDUCE SEED SHATTERING WITH NO EFFECT ON SEED PRODUCTION

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### Abstract

Seed shattering is one of main restrictions to seed production in almost all of the warm-season perennial forage grasses. Detaching occurs at seed maturity after an abscission layer is formed by cell differentiation mediated by auxins. In this study we wanted to assess whether seed shattering can be reduced by the external application of auxins. We tested different auxin concentrations on seed retention and on seed production and investigated the best time to apply the hormone in the perennial warm season forage grass Panicum coloratum var. makarikariense. Two experiments were carried out on a pasture established in 2015. The first one in January 2018 (mean temperature 25.4°C, accumulated precipitation during experiment 29.7 mm). Three auxin concentrations (0mM, 0.5mM and 10 mM) were applied at two different times: anthesis (A) and 15 days after anthesis (B). In experiment 2, performed in January 2022, five auxin concentrations were used (0mM, 1mM, 10mM, 50mM, 100mM) only at time B (mean temperature 27.3°C, precipitation 130.3 mm). Tween 20 (1%) was always added before spraying on individual panicles that were then enclosed in seed traps. Seeds were collected and counted at two times, 400 and 800 GDD after anthesis. Shattering was evaluated as the ratio of shed seeds over the total number of seeds during the analyzed period. For data analysis we used generalized mixed linear model with binomial distribution where total number of seeds was used as logit link function. Number of seeds was compared between treatments with ANOVA.

No difference in seed shattering was observed after applying auxin at anthesis, none of the auxin concentrations was able to diminish seed shedding (p=0.28). When auxin was applied after anthesis, in experiment 1, the proportion of shattered seeds decreased from  $0.56\pm0.01$  in control to  $0.36\pm0.01$  and  $0.31\pm0.01$  when adding 0.5 and 10 mM auxin, respectively. In experiment 2, auxin application reduced seed shattering; the concentration with best results was 1mM. High auxin concentration (100 mM) produced an unwanted effect as shattering increased over control. Regarding seed production, the number of seeds per panicle was not affected by auxin application. In addition, the number of empty seeds did not change with auxin treatments. In short, applying auxin increase seed retention when done 15 days after anthesis at concentrations of 50 mM or less with no change in seed production. Higher concentrations reduce retention.

## Introduction

Seed shattering is one of main restrictions to seed production in almost all of the warm-season perennial forage grasses. Abscission is a natural process that facilitates seed dispersion in nature. However, in agriculture, seed shedding is costly to farmers, as it hinders seed harvesting and reduces crop yield. During the process of domestication of different grass species used for grain cultivation such as wheat, rye, barley and rice, a critical change has occurred towards reduction in seed shattering (Dong and Wang, 2015; Vittori et al., 2019). However, forage species have in general a short history of domestication and this ancestral trait is still common in many of them (Thurber et al., 2011).

Detaching occurs at seed maturity and depends on the formation of an abscission zone, a morphologically distinct structure consisting in one or more layers of cells with dense cytoplasm. After cell swelling, the middle lamella between adjacent cell walls dissolves and the organ is released (Yu et al., 2020). The process of abscission is regulated by environmental and developmental cues and among them, hormones such as ethylene and auxin are particularly involved. Ethylene was one of the first identified inductive factors in abscission and auxin has been claimed to perform a negative regulation on the sensitivity of the abscission zone to the action of ethylene (Kućko et al., 2020). For example, Xie et al. (2018) have reported that application of auxin could block the induction of abscission zone in citrus. Although the abscission is common to different organs of the plant, many aspects of the process are still unclear (Kućko et al., 2019).

*Panicum coloratum* is a warm-season perennial grass with a short history of breeding (Armando et al., 2023). As other forage grasses, it still bears seed dispersion characters related with a poorly domesticated species, such as poor seed retention. Efforts to reduce seed shedding in *P. coloratum* included the search of genetic variation of the trait that could eventually allow discrimination of non-shattering genotypes to perform selection. However, seed maturation and shedding seem to be significantly affect by environmental conditions as demonstrated by high levels of genotype x environment interaction and low heritabilities (Tomás et al., 2022).

In this study, we wanted to assess whether seed shattering in *P. coloratum* can be reduced by the external application of auxins. Given the complexity in the interplay between auxin and ethylene actions, we tested different auxin concentrations on seed retention and on seed production and investigated the best time to apply the hormone in the perennial warm season forage grass *Panicum coloratum* var. *makarikariense* 

## Materials & Methods

Two experiments were carried out at INTA EEA Rafaela (31° 12′ S; 61° 39′W). The first one was performed in plants obtained by cloning two genotypes (blocks) during summer 2018 in a completely randomized bock design (mean temperature 25.4°C, precipitation 29.7 mm over the time of the experiment). Anthesis was registered February 21, 2018. Treatments were applied on three plants per block per treatment. Two different factors were evaluated: 1) time of application and 2) auxin concentrations (0mM, 0.5mM and 10 mM). Auxin applications were performed at two moments A) anthesis and B) 208 GGD after anthesis (March 7, 2018). Experiment 2 was performed during summer 2022 (mean temperature 27.3°C, precipitation 130.3 mm) on a pasture established in 2015. Fifty panicles were chosen at random in the pasture, and assigned to one of five levels of auxin concentrations (0mM, 1mM, 10mM, 50mM, 100mM). Anthesis occurred February 18, 2022. Auxins were applied 203 GDD after anthesis (March 3, 2022) in all panicles. In the two experiments, Tween 20 (1%) was added to the auxin solution before spraying on individual panicles. Panicles were enclosed in seed traps consisting

in a steel pole with a cylindrical structure on top covered with a nylon stocking. Seeds were collected in a plastic funnel at the bottom of the structure taken to the lab and counted. In experiment 1 seed were collected at 304, 375, 437, 530, 629, 703 and 803 GDD after anthesis. In experiment 2, seeds were collected at 370, 446, 486, 548 GGD after anthesis. Shattering was evaluated at each collection date as the ratio of the number of shed seeds over the total number of seeds produced by each panicle. For data analysis, we used generalized mixed linear model with binomial distribution where total number of seeds was used as logit link function. Average total number of seeds per panicle was compared between treatments with ANOVA.

### Results

#### Auxin application effects on seed retention

#### Experiment 1:

Auxin application when sprayed at anthesis did not change seed shattering when evaluated before 530 GGD after anthesis (Figure 1A). Significant reduction in seed shattering was observed only for collection dates at 629, 703 and 803 GGD after anthesis. However, although significant, increments in seed retention were very low, representing less than 10% of the retention of the control treatment. When auxin was applied 208 GDD after anthesis seed retention, the effect of the treatment was observed earlier, when the proportion of shattered seeds was still low (Figure 1B). Seed shattering was reduced by approximately 30% after applying auxin 0.5 mM at 437 GDD after anthesis. The improvement in seed retention was higher with auxin concentration of 10 mM (approximately 50%). The best results were registered at 375 GDD, when seed shattering was reduced from  $0.43\pm0.01$  to  $0.19\pm0.01$ . For evaluations after 500 GDD post-anthesis, seed retention in the panicles was higher with auxin application performed at or after anthesis. However, most of the seeds have already shed by the time, so increments in seed retention were too low to account for a considerable advantage at harvest.

Figure 1 – Proportion of shattered seeds at different harvest days in panicles of *Panicum coloratum* in response to auxin application at concentration of 0, 0.5 and 10 mm evaluated along seed maturation. A: auxin applied at anthesis. B: auxin applied 208 GDD post-anthesis. At each time of harvest (GDD after anthesis), means followed by different letters are significantly different at p<0.05





## Experiment 2:

Since no effect was observed when auxin was applied at anthesis in the previous experiment, applications were only essayed 15 days after anthesis. Auxin application significantly reduced seed shattering in panicles of *Panicum coloratum*. The proportion of shattered seeds decreased from 0.7 in control to 0.45 when auxin was applied at concentration of 1 mM. Auxin concentrations over 10 mM were less effective in increasing shattering. Moreover, auxin concentration as high as 100 mM produced an unwanted effect as shattering increased over control.

Figure 2 – Proportion of retained seeds in panicles of *P. Coloratum* at 485 GDD after anthesis in response to spraying of auxin at 203 GDD after anthesis at different concentrations. Means followed by different letters are significantly different at p<0.05



# Number of seeds per panicle

Auxin application did not modify the number of seeds per panicle at any concentration used. The number of empty seeds was monitored in experiment 1. Treating panicles with auxin at different concentration did not affect the number of empty seeds (Figure 3).

Figure 3 – Number of seeds per panicle in plants of *P. coloratum*. Panicles of clones of two genotypes were sprayed with auxin at different concentrations at two different moments: A at anthesis and B, 208 GDD after anthesis. Grey bars indicate the number of empty seeds. Black bars indicate the number of mature seeds and the number of empty seeds. Bars indicate means ± e.e.



## **Discussion & perspectives**

Results from this study showed that auxin is effective in delaying the abscission and hence increasing seed retention in *Panicum coloratum* when applied 208 GGDs after anthesis at concentrations of 50 mM or less. The balance between ethylene and auxin has been recognized to play a central regulatory role in the abscission process (Botton and Ruperti, 2019). The organs that are prepared to shed produce ethylene that is released to the cells in the abscission zone, starting the process of separation and detaching of the organs. Auxin has been suggested to negatively regulate the abscission process by reducing the sensitivity of the abscission zone cells to ethylene's action. In fact, there is evidence supporting the role of auxin depletion in the abscission zone cells as the main signal-leading factor preceding organ detaching (Meir et al., 2015). It has been generally accepted that the transport of auxin from the distal organ towards the abscission zone and makes cells insensitive to ethylene preventing abscission (Kućko et al., 2020). Therefore, the increase in the level of auxin by spraying it over the panicle in *Panicum coloratum* would be responsible for the retard in the abscission process reported here, assuming it would be reducing cell sensitivity to ethylene.

According to our findings, auxin would only affect the formation of the abscission zone since exogenous application of this hormone did not change the number of seeds produced per panicle. Additionally, seed maturation processes was not affected by auxin or the delaying in seed detaching as the proportion of empty seeds did not increase with auxin application.

Given that seed shattering is a common issue in many forage species, evidences presented here give the basis to find possible ways to manage crops to reduce the losses in seed yield due to shattering.

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### DEVELOPMENT OF SEED PRODUCTION AND HARDNESS BREAKING TECHNIQUE FOR LESPEDEZA POTANINII VASS

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#### Abstract

Lespedeza potaninii Vass is a perennial leguminous forage crop with drought, cold and barren tolerance, and plays an important role in livestock nutrition, grassland restoration and soil and water conservation in arid and semi-arid areas of China. However, there are problems such as difficult threshing and high hard seed rate in L. potaninii Vass, and agronomic practices for its seed production have not been reported. We conducted a field study in the Hexi Corridor of Gansu Province, China, from 2020 to 2021. The study showed that plant density, phosphorus fertilizer rate and their interaction significantly influenced the seed yield of L. potaninii Vass. At the plant density of 44,444 plants ha<sup>-1</sup> and phosphate fertilizer rate of 90 kg/ha, the average seed yield over two years reached a maximum of 477.5 kg ha<sup>-1</sup>. The harvest index decreased with increasing plant density and was maximum at 44,444 plants ha<sup>-1</sup>. Among yield components, pods per raceme had the greatest contribution to the seed yield. Further research was conducted on seed threshing and hard seed breaking techniques for L. potaninii Vass. All the persistent sepals were removed when the seeds were treated four times with the seed coat breaking machine and 12 minutes with concentrated sulphuric acid. Although the highest germination rate was above 80.00% by concentrated sulfuric acid treatment for 20~30 minutes, it is a hazardous chemical. The seed coat breaking machine is convenient and safe, 9 times treatment (the germination rate reached 66.33%) was recommended in the large-scale seed production of L. potaninii Vass. Results from this study can serve as a guide for improvement of L. potaninii Vass seed yield.

#### Introduction

Appropriate agronomic practices ensure an adequate supply of economically produced seeds of *L. potaninii* Vass, which is benefit for its various applications. Plant density is one of the main factors affecting plant growth and seed yield (Liu et al., 2014). Low plant density could result in the highest yield per plant, while suitable moderate plant density could result in the highest seed yield per unit area (Li et al., 2018). Optimal plant density facilitates plant growth and seed development, resulting in improved seed production and quality (Han et al., 2013). As a nutrient element, phosphorus (P) is involved in regulating many physiological activities of plants, which in turn affect their growth and development, and ultimately yield (Ayoub, 1999; Gu et al., 2018). Particularly in the legume family, P plays an important role in their biological nitrogen fixation (Abbasi et al., 2010), because the process consumes a large amount of energy, and energy production metabolism depends heavily on the availability of P (Schuize et al., 1999). P in plants promotes flower formation and seed production, increases nitrogen fixation (Limeneh et al., 2020). There are numerous studies to optimize plant density and P fertilization, resulting in optimal seed production for many

species. However, little information is available on seed production of *L.potaninii* Vass. Thus, the objectives of this study were to evaluate the influence of plant density and P fertilization on seed yield and yield components of 'Tenggeli' cultivar of *L. potaninii* Vass, and to determine the main yield components contributing to seed yield.

## Materials & Methods

The field trial was performed Hexi Corridor of in Zhangye City in Gansu Province, China (latitude:  $39^{\circ}04'$  N, longitude:  $100^{\circ}20'$  E, elevation: 1397 m), from 2019 to 2021 growing seasons, on sites with uniform soil fertility. The experiment was a split plot in randomized complete block design with three replications. The main plots had four plant density treatments: (1) D33: 33,333 plants per hectare, with 60 cm within-row spacing; (2) D44: 44,444 plants per hectare, with 45 cm within-row spacing; (3) D66: 66,666 plants per hectare, with 30 cm within-row spacing; (4) D133: 133,333 plants per hectare, with 15 cm within-row spacing. The five subplot fertilization treatments were 0 kg ha<sup>-1</sup> (P0), 45 kg ha<sup>-1</sup> (P45), 90 kg ha<sup>-1</sup> (P90), 135 kg ha<sup>-1</sup> (P135), 180 kg ha<sup>-1</sup> (P180). P was supplied as superphosphate broadcasted at planting. The persistent sepals of *L. potaninii* Vass were removed by seed coat breaking machine and concentrated sulphuric acid. The hardness of the seeds was broken by treatment of seed coat breaking machine, sulphuric acid, high temperature, hot water and liquid nitrogen.

# Results

Plant density, phosphate fertilizer and their interactions significantly affected seed yield of *L*. *potaninii* Vass in both years (Table 1). In 2020, when the plant density was D44 and the phosphate fertilizer was P90, the seed yield was the highest (559.28 kg ha<sup>-1</sup>); and in 2021, the highest seed yield was obtained with the plant density of D44 and the phosphate fertilizer of P135 (412.43 kg ha<sup>-1</sup>). Seed yield was higher in 2020 than in 2021 (Table 2). Taking the values of both the years, the treatments with the highest seed yield were obtained at the plant density of D44 and phosphate fertilizer of P90, which was 477.47 kg ha<sup>-1</sup> (Table 2).

The seed yield components of *L. potaninii* Vass differed in response to main factors and interactions effects (Table 1). Stems per square meter was significantly affected by plant density, phosphate fertilizer and their interactions (Table 1). In 2020 and 2021, stems per square meter increased with increasing plant density, reaching a maximum at D133 of about 200 per square meter, then decreased with increasing phosphate fertilizer rate, reaching a maximum at P90. Both plant density and phosphate fertilizer treatments significantly affected racemes per stem (Table 1), which decreased with increase in plant density, and showed an increasing trend with the increase in phosphate fertilizer rate. Racemes per stem values were highest at D33 and P135, about 65 and 55, respectively (Table 3). Pods per raceme were only significantly affected by plant density, decreasing with increasing plant density, with the most pods per raceme at treatment D44. In addition, there were more pods per raceme in 2020 than in 2021 (Tables 1, 3). Plant density and phosphate fertilizer had a significant effect on seed yield per plant, which decreased significantly with increasing plant density and increased significantly with increasing plant density and increased significantly with increasing plant density and increased significantly with increasing plant density and phosphate fertilizer had a significant effect on seed yield per plant, which decreased significantly with increasing plant density and increased significantly with increasing plant density and increased significantly with increasing plant density and increased significantly increasing plant density and increased significantly with in

The persistent sepals were removed when the seeds were treated four times with the seed coat breaking machine and 12 minutes with concentrated sulphuric acid (Figure 1). All treatments (seed coat breaking machine, sulphuric acid, high temperature, hot water and liquid nitrogen) can significantly increase the seed germination rate, germination potential, germination index and vigor index, and reduce the hard seed rate (P<0.05) (Table 4). The highest germination rate was above 80.00% by concentrated sulfuric acid treatment for 20~30 minutes, while it is a hazardous chemical. The seeds were processed with the seed coat breaking machine for 9 times, the germination rate is the highest (66.33%), this method is convenient and safe.

## **Discussion & perspectives**

Both plant density and phosphorus fertilization significantly affect crop and forage seed yields. Previous research has shown that forage seed yields usually respond curvilinearly to plant density or phosphorus application and reaches a maximum at optimum plant density or phosphorus application (Roques & Berry, 2016). In this study, the seed yield increased and then decreased with increasing plant density, reaching a maximum at D44 and D66. This is the same response to planting density as for maize and oilseed rape crops (Jiang et al., 2018; Hiltbrunner et al., 2007).

In our experiment, the four seed yield components had different responses to the changes in plant density and phosphate fertilizer rate. There was a positive correlation between stems per square meter and plant density. This is consistent with the results of Tao et al. (2019) for *Artemisia sphaerocephala*, which had the fewest stems per square meter at a between-row spacing of 180 cm and a within-row spacing of 100 cm. Furthermore, smaller and medium plant densities have fewer stems per square meter, benefiting the development of individual plant development and increasing seed yield per plant. Askarian et al. (1995) and Dovrat et al. (1969) have shown that with the decrease in plant density, the increase in racemes per stem can be attributed to the production of more primary, secondary and tertiary buds. Similar results were obtained in our study, with 56.05% more racemes in the D33 treatment than in the D133. Therefore, a medium planting density is more desirable for seed production of *L. potaninii* Vass.

Optimizing plant density and phosphate fertilization increased the seed yield of *L. potaninii* Vass. The highest seed yield was achieved at plant density of 4,4444 plants ha<sup>-1</sup> and a phosphorus fertilizer application of 90 kg ha<sup>-1</sup>. Pods per raceme contributed the most to the yield and was the most pivotal yield component. Future researchers should focus on irrigation practices to increase pods per raceme to further improve seed yield in *L. potaninii* Vass.

Source	Seed yield	Stems /m <sup>-2</sup>	Racemes /Stem	Pods/ Raceme	Seed yield per plant
Year (Y)	**	*	**	**	**
plant density (D)	**	**	**	**	**

Table 1 – Analysis of variance for plant density (D), phosphate fertilizer rate (P), and their interaction on seed yield and yield components

Phosphate fertilizer	**	**	**	*	**
(P)					
D×P	**	**	**	NS	**
D×Y	**	**	**	NS	**
Р×Ү	NS	**	**	*	NS
D×P×Y	**	NS	NS	NS	NS

\*: Significantly different at the 0.05 probability level; \*\*: Significantly different at the 0.01 probability level; †NS: not significant at the 0.05 probability level; df: degree of freedom.

Table 2 – Effect of plant density and phosphate fertilization on seed yield in 2020 and 2021.

Treat-	Seed yield in 2020						Seed yield in 2021				Mean seed yield				
ments	PO	P45	P90	P135	P180	P0	P45	P90	P135	P180	PO	P45	P90	P135	P180
			kg∙ha⁻¹					kg∙ha⁻¹					kg∙ha⁻¹		
D33	381.04	395.91	428.88	445.33	452.62	258.66	296.48	307.32	304.4	313.55	319.85b	346.19ab	368.10ab	374.87a	383.09a
D44	338.37	419.31	559.28	538.07	521.71	300.66	315.48	395.66	412.43	400.19	319.52c	367.40b	477.47a	475.25a	460.95a
D66	407.11	462.22	459.91	484.08	451.91	312.95	397.87	384.52	372.03	313.4	360.03b	430.04a	422.21a	428.06a	382.65ab
D133	366.58	448.71	503.47	385.07	425.24	154.28	326.25	316.73	301.92	286.96	260.43d	387.48ab	410.10a	343.49c	356.10bc

Table 3 – Effect of plant density and phosphate fertilization on yield components in 2020 and 2021.

Treatme	Treatments		Stems/m <sup>-2</sup>		Racemes/Stem		Pods/Raceme		Seed yield per plant (g)	
rreatments		2020	2021	2020	2021	2020	2021	2020	2021	
Plant	D33	77.86d	80.04d	61.70a	65.49a	8.04a	7.43b	12.62a	8.82a	
density	D44	103.12c	100.63c	47.61b	57.99b	8.14a	7.60a	10.70b	8.21b	
(plants ha-	D66	133.14b	130.84b	47.50b	49.07b	7.99a	7.16b	6.80c	5.34c	
1)	D133	213.56a	198.63a	40.52c	41.11c	6.84b	6.43c	3.19d	2.08d	
	P0	118.84c	120.96b	46.04c	49.18b	7.64a	6.77c	6.98c	5.09b	
Phosphate	P45	140.56a	127.55ab	46.05c	52.26b	7.72a	7.06abc	7.90b	6.10a	
rettilizer	P90	139.36a	133.06a	48.96bc	56.73a	7.72a	7.41a	9.03a	6.40a	
1)	P135	136.53a	128.42ab	51.34ab	56.67a	7.69a	7.35ab	8.90a	6.45a	
,	P180	124.21b	131.44ab	54.27a	52.33b	7.95a	7.05bc	8.82a	6.32a	

Table 4 – Effects of seed coat breaking machine, sulfuric acid, high temperature, hot water and liquid nitrogen treatments on seed germination of *L. potaninii* Vass

Treatments		Percentage of	Germination	Germination	Germination	Vigor
		hard seeds (%)	energy (%)	rate (%)	index	index
Seed coat breaking machine	СК	96.67a	2.00f	2.67f	0.72g	0.02f
	7	38.67ef	56.00ab	60.00ab	28.36b	0.56b
	8	36.33f	57.33a	61.33a	31.68ab	0.87a
	9	33.00fg	61.67a	66.33a	33.14a	0.75a
Sulfuric acid	СК	96.00a	4.00d	4.00e	1.93e	0.07d
	15 min	16.67d	67.33b	74.67b	26.94c	0.81b
	20 min	14.67d	74.67ab	82.67a	35.8ab	1.13a
	25 min	17.33d	72.67ab	81.33ab	34.12b	1.14a
	30 min	13.33d	80.00a	84.00a	38.73a	1.03ab
High temperature	СК	96.00a	4.00e	4.00g	1.93e	0.07e
	80°C,1.5 h	96.00a	6.67de	7.33efg	4.39de	0.15de
	90°C,1.5 h	87.33b	10.00d	12.00de	7.66d	0.40a
	100°C,1.5 h	53.33d	32.67a	35.33a	21.75a	0.47a
Hot water	СК	96.00a	4.00c	4.00d	1.93d	0.07d

	70°C	95.33a	4.00c	4.67d	1.70d	0.08cd
	80°C	82.00b	10.00b	15.33c	4.66c	0.21b
	90°C	60.00c	22.67a	38.00a	12.19a	0.42a
Liquid nitrogen	СК	96.00a	4.00b	4.00b	1.93b	0.07b
	3 min	85.00b	8.00ab	14.00a	5.02ab	0.19a
	5 min	84.66b	8.00ab	13.33a	5.05ab	0.16ab
	10 min	81.33b	14.00a	16.67a	6.78a	0.17ab

Figure 1 – Effect of the seed coat breaking machine and concentrated sulfuric acid treatment on the persistent sepals of Lespedeza potaninii Vass



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## BREEDING AS A LEVER TO IMPROVE SEED YIELD OF FORAGE SPECIES

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#### Abstract

Breeding for seed yield is a lever that is not widely used. Indeed, the evaluation of seed yield components requires specific experimental conditions that come in addition to the ones used for the evaluation of forage-related traits. In addition, the genetic resistance to pests that are particularly damaging the plants at reproductive stages has not been much studied. Current knowledge is quickly reviewed and suggestions to implement molecular breeding are proposed. Selection for both forage and seed traits could be managed in cost-effective breeding schemes.

#### Introduction

Genetic improvement of forage species mainly targets forage yield, quality and stress resistances. Seed yield is a key trait for a variety to access the market (Boelt *et al.* 2015). As any other trait, seed yield can be selected to create new varieties (Wilkins and Humphreys 2003; Boelt *et al.* 2015). Seed yield results from components that start from flowering date and pollination to seed harvest. In addition to seed yield components, pests and diseases that are specific to reproductive stages, may alter seed yield potential. Knowledge of the genetic determinants of seed yield components is useful to create improved varieties. However, the selection for seed yield requires additional designs to those devoted to forage yield. Molecular markers could also help to introduce the selection of seed yield components in breeding schemes. The paper reviews current knowledge on seed yield of herbage species and possible changes in breeding schemes to improve genetic gain for seed production.

#### Why is it difficult to select for both forage and seed yield components?

For forage species, breeding for the traits that compose forage production such as forage yield and quality, are the main objectives. Recurrent breeding schemes are based on a breeding pool that is enriched with new introductions and produce candidate varieties and improved families that form the breeding pool for the next cycle of selection. The breeding traits are also evaluated during the Value for Cultivation and Use (VCU) tests that compose the process of variety registration on Official catalogues of varieties. The evaluation of forage related traits requires management practices and cutting schemes that are not those of seed production. Basically, plants are cut before or at the beginning of flowering. Consequently, seed yield potential is not fully targeted in the breeding schemes, and conversely, breeding for seed yield components requires specific managements within the breeding schemes.

Forage as well as seed productions are also submitted to pests and diseases. Some of them are common to both types of production while additional pests and diseases are specific to reproductive stages. Pesticides have been selected to fight against several pests that are detrimental to seed production (Bouet *et al.* 2021; Gaier *et al.* 2022) but active substances are now banned one after the other because of their negative effects on human and environmental health. While management options may be available to limit pest damages (as an example, for Hypera on lucerne (Pellissier *et al.* 2017)), the genetic option is also an important lever to activate but this breeding objective is very recent.

Because of these constraints, breeding for seed production has not been invested much, as requesting dedicated efforts that come in addition to those devoted to forage traits improvement.

## Seed yield components

Seed yield, as the seed weight per m<sup>2</sup> in dense canopy, is measured in small plot trials that mimics seed production fields; in a breeding scheme, it can be evaluated when enough seeds are produced, in the latest stages only. Breeding is the most efficient when the selection is applied in the earliest stages of a breeding scheme. The decomposition of seed yield into components is useful to identify traits that are easy to measure in a cost-effective way. Such traits must be correlated to seed yield. Seed yield components are defined at a biological scale, plants, stems, inflorescences, pods and seeds in legume species (Boelt et al. 2015), and plants, stems, inflorescences, spikelets, florets, seed set and seeds in grass species (Abel et al. 2017). Seed retention (grasses) and pod indehiscence (legumes) have been selected in modern germplasm. An efficient breeding criterion is a trait that shows a high genetic variation, a high genetic correlation with seed yield measured in plots and that is easy to measure, preferably in individual plants. The correlation between traits measured on spaced plants designs (the nursery in the early stage of a breeding scheme) and seed yield measured in dense canopy must also be high (Wilkins and Humphreys 2003). In several forage legume (Bolaños-Aguilar et al. 2000; Vleugels et al. 2016) and grass (Robins et al. 2015) species, seed traits measured at the inflorescence level have been shown to be an accurate breeding criteria. Comparison of varieties for seed yield to assess a genetic progress is not frequent. However, on perennial ryegrass, no genetic progress for seed yield has been reported among varieties bred for turf (Sampoux et al. 2013) or forage (Sampoux et al. 2011).

# Pest and disease resistance

During legume seed production, several insects attack vegetative (*Agriotes* sp., *Apion* sp., *Colaspidema* sp., *Hypera* sp., *Sitona* sp., *Bemisia tabaci*) or reproductive (*Tychius* sp., *Contarinia medicaginis*, *Dasineura ignorata*, *Lygus* sp., *Adelphocoris* sp., *Bruchophagus* sp., *Cydia medicaginis*) parts of the plants while aphids (*Acyrtosiphon* sp.) suck sap of all green organs. Their occurrence varies depending on the environment, their impact on forage production is usually low or neglected and their negative impacts on seed production may be major. For some insects such as aphids and *Hypera*, protocols for variety testing are available (Girousse and Bournoville 1994; Ratcliffe 2022), by using insects raised in controlled conditions (aphids) or insects collected in fields (*Hypera*). In the USA, lucerne varieties are described for resistance to three aphid species and potato leafhopper (NAFA 2023). Insect damages on grasses are not frequent in Europe but well described in other regions of the world (Wilkins and Humphreys 2003). Generally speaking, more knowledge is needed on the biology
of pests to rise insects, develop protocols for variety testing and identify genetic diversity for insect resistance. Then, mechanisms of genetic control of resistance can be studied, possibly involving physical barriers such as trichomes (Gonzalez-Garcia *et al.* 2000) or biochemical compounds (Yan *et al.* 2023).

Disease occurrence is generally not specific to seed production but, as the growing period is longer than for forage production, their impact may be more important. For the main diseases in each species such as *Sclerotinia trifoliorum* on red clover, *Verticillium alboatrum* on lucerne, anthracnose (*Colletotrichum trifolii*) on both red clover and lucerne, rusts on grasses, efficient selections are already conducted, aiming at a genetic progress. Disease tests are carried out under controlled conditions and resistant plants or progeny are introduced in the breeding scheme. In addition, candidate varieties that have been improved for forage yield components may be further improved for resistance to specific diseases before they are deposited for registration.

### Implementation of molecular breeding

Molecular breeding has shown its efficiency to improve genetic gains in major crops. Recently, with genome-wide association studies (GWAS), loci (QTL) that explain more than 10% of the variation have been identified in forage crops too, mostly for forage-related traits (Keep *et al.* 2020; Pégard *et al.* 2021). Genomic selection seems promising, with predictive ability ranging from 20 to 70% depending on the studies and the traits (Annicchiarico *et al.* 2015; Keep *et al.* 2020; Pégard *et al.* 2021). Again, seed yield traits have been less studied than forage related traits or disease resistances. However, QTL have been detected in a bi-parental population of red clover for seed yield components (Herrmann *et al.* 2006). In a collection of perennial ryegrass, the QTL detected for spike density were explained by difference in earliness only and no QTL was detected for the other seed yield components (Keep *et al.* 2020). However, predictive ability for seed yield components was moderate to high (39% to 86%) (Keep *et al.* 2020).

Quantitative genetics, including GWAS and genomic selection, may also be efficient for resistance to diseases, as shown on red clover for anthracnose resistance (Frey *et al.* 2022). Resistance genes have been identified (Yang *et al.* 2022), they could be introduced through crossings but also by transgenesis (Tohidfar *et al.* 2013). No report for GWAS nor genomic prediction for resistance to pests on herbage crops has been identified so far in the literature.

### Conclusion

Seed yield of herbage species results from seed yield potential and, especially for legume species, pest resistances. Both types of traits are complex to study and select for, and knowledge is lagging behind. Combination of selection for forage traits and seed traits in breeding programmes is costly while the seed market of forage species has a relatively low profitability. In these conditions, the development of tools for molecular assisted selection could help to improve seed yield. Such genetic progress could be acknowledged by the inclusion of seed yield in the evaluation of varieties for registration.

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### GENETIC DETERMINISM OF SEED YIELD COMPONENTS IN LUCERNE

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#### Abstract

Seed production is a major component of the commercial development of a variety but breeding for seed yield is expensive. A set of 400 cultivated lucerne accessions were phenotyped for seed yield traits in two locations and genotyped with GBS markers. A large diversity was found among accessions but the heritability of the traits was moderate. Quantitative trait loci (QTLs) were found for one seed yield component. Genomic prediction was moderate to high, depending on the traits and the locations. Molecular breeding could be effective to assist lucerne selection for seed yield, in addition to forage-related traits.

#### Introduction

Forage legume varieties are commercialized as seeds that are produced in dedicated production fields. When the seed production is low, the cost of production and maintenance of the variety increases. Seed production is affected by management practices but also depends on the variety. Considering the importance of seed yield on the commercial development of a variety, breeding efforts for seed yield of forage crops are generally insufficient. Indeed, scoring seed yield components implies dedicated evaluations that come in addition to forage yield and quality tests, strongly increasing the breeding costs. In these conditions, markers associated to seed yield components and equations of genomic prediction could be useful to select the most promising individuals for the next breeding generation. The experiment aimed at evaluating genetic diversity for seed yield components in a set of 400 cultivated accessions of lucerne, detect quantitative trait loci (QTLs) in a genome-wide association study (GWAS) analysis and test genomic prediction.

#### **Materials & Methods**

A set of 400 lucerne accessions was studied in field conditions in two locations (France and Serbia) for seed yield components in 2018 in an augmented design. The allele frequency for more than 200K SNPs was available (Pégard et al., 2023). Adjusted values were estimated in each locations and over the two locations, taking into account the spatial effects in the trials. Heritability of traits was estimated. In a GWAS, QTL detection was carried out, taking into account the genetic structure of the material (Pégard et al., 2023). Genomic prediction was tested with a training population of 270 accessions, with a GBLUP option.

## Results

After spatial adjustment, a large genetic variation was evidenced for seed yield and seed yield components (pod number per inflorescence, thousand seed weight) (Figure 1). The heritabilities were moderate in both locations and over the locations (Table 1).



Figure 1 – Distribution of seed yield traits in Lusignan and Novi Sad Lusignan:

Table 1 – Heritability of seed yield traits in two locations and over the two locations (global)

Traits	Location	Heritability
Pod Number per Inflorescence	Lusignan	0.274
	Novi Sad	0.224
	Global	0.153
Thousand Seed Weight	Lusignan	0.321
	Novi Sad	0.685
	Global	0.206
Seed Yield	Novi Sad	0.349

With a GWAS, two QTLs were found for pod number per inflorescence in Lusignan only, explaining 0.086% (on chromosome 3) and 0.074% (on chromosome 7) of the variation.

Genomic prediction showed promising predictive ability (Figure 2).



Figure 2 – Predictive ability (%) for seed yield traits in two locations and over the two locations (global)

### **Discussion & perspectives**

A large diversity for seed yield traits was evidenced in the set of 400 cultivated accessions. QTLs were found for a single trait, pod number per inflorescence, and the two QTLs explained a total of 16% of the variation. With genomic prediction, a correct predictive ability was obtained. These results suggest that markers could be used to select more intensively for seed yield. Breeding schemes should be revised accordingly.

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# THE USE OF NITROGEN NUTRITION INDEX IN HERBAGE GRASS SEED PRODUCTION

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### Abstract

Sufficient nitrogen (N) is fundamental to obtaining a high seed yield but unfortunately, also a nutrient that can have a negative environmental impact through leaching and run-off. The critical N dilution curve and the N nutrition index are seen as a method to secure sufficient but not excess N for agricultural crops. We have tested the use of this method in perennial ryegrass and red fescue for seed production under Danish growing conditions and regulations. Our conclusion is that the standard Danish N application rates for the two crops are sufficient to secure adequate N for the crops and NNI values above 1 will not necessarily increase seed yield. The use of NNI is interesting and could be combined with the the standard Danish N application rates.

# Introduction

Nitrogen (N) application and utilization have been intensively studied for many years and numerous field experiments have been performed to estimate the optimum N application rate. Application of N in agriculture has, however, also had a negative environmental impact in some production areas. This has sharpened our interest to test new application strategies and algorithms. Another important cause for testing new strategies and algorithms has also been the development and implementation of different technologies in agriculture like satellites, unmanned aerial vehicles (UAV), and different sensors mounted on tractors and implements.

Denmark is due to many reasons one of the countries where there is a lot of focus on the environmental aspects of N application. This has undoubtedly forced us to test new application strategies and algorithms to continue our cost-effective production and protect our ground and surface water from nitrate pollution.

The use of the critical N dilution curve (CNDC) and subsequently the nitrogen nutrition index (NNI) is probably the most used strategy to optimize the utilization of applied N, defined as avoiding N to be the limiting factor for a high seed yield and concurrently lower excess application of N. The nitrogen nutrition index (NNI) defined by Lemaire (1997) is shown in Figure 1 and indicates insufficient N supply (NNI<1) and excessive N supply (NNI>1).

Several studies have been published in peer-reviewed journals using CNDC and NNI and most show promising results e.g., Wang et al. (2019) and Gislum et al. (2021). Unfortunately, most of the published results are based on data from experimental plots and/or have not been validated on an external dataset. This will inevitably lead to overoptimistic results with limited practical use for seed growers. Jiang et al. (2022) concluded that more studies are needed to

further optimize their in-season wheat N diagnosis using machine learning and develop UAV remote sensing-based N topdressing methods under diverse on-farm conditions. The use of novel machine-learning methods might be the solution we are looking for, however, there are also drawbacks when machine-learning methods are introduced like the fact that much more data is needed and the risk of overfitting the models is high. Based on this we suggest that the focus is on a better understanding of the CNDC and NNI methods in relation to grass seed production and especially if and how the method can be used by the farmers.

The aim of this study is to test the use of NNI in experimental plots and discuss if the method can be implemented by farmers.

Figure 1. The concept of the critical nitrogen (N) concentration curve and the nitrogen nutrition index (NNI). The abbreviation for plant dry matter is DM.  $N_c$  is the  $N_c$  according to the  $N_c$  dilution model and  $N_a$  is the actual measured N concentration in a sample. The parameters a and b in the  $N_c$  dilution curve are the critical N concentration when DM is 1 t ha<sup>-1</sup> and the slope of  $N_c$ .



#### **Materials & Methods**

The dataset used originates from five field experiments, one field experiment with perennial ryegrass (*Lolium perenne* L.) in 2019 and two field experiments with perennial ryegrass or red fescue (*Festuca rubra* L.) in 2020 and 2021, respectively. The spring N application rates were 0, 80, 120, or 160 kg ha<sup>-1</sup> in perennial ryegrass and 40, 80, or 120 kg ha<sup>-1</sup> in red fescue. The N was applied at the initiation of spring growth and applied as ammonium nitrate. Three plant cuts (12.5 cm x 25 cm) were taken in each plot (2.5 m x 8 m) and analysed for dry matter and N concentration using the combustion method whereafter N accumulation was calculated (table 1). The NNI was calculated according to the CNDC in Gislum and Boelt 2009. Growing degree days (GDD) were calculated for each plant cut using a start date of 1<sup>st</sup> January and a base temperature of 0°C. The grass seed crop was harvested with a trial combiner, and yields are shown at 12 % moisture. The straw was weighed for each plot. A sub-sample of the seeds and straw was taken per plot and analysed for total N.

# Results

The results from the plant cuts during the spring growing season have considerable variation in %N, dry matter production, and N uptake (table 1). The corresponding NNI, seed yield, and the final N uptake in seeds and straw show likewise an expected variation (table 1).

Variable	Ν	Mean	Minimum	Maximum
% N in plant samples	190	2.6	0.21	5.4
Biomass in plant samples, T ha <sup>-1</sup>	190	5.8	2.2	15
N uptake in plant samples, kg N ha <sup>-1</sup>	190	144	10	314
NNI	190	1.4	0.10	2.8
N uptake in seed, kg ha <sup>-1</sup>	92	40	9.0	65
N uptake in straw, kg ha <sup>-1</sup>	192	46	13	83
Total N uptake (seed plus straw), kg ha <sup>-1</sup>	92	86	22	133
Seed yield, kg ha <sup>-1</sup>	190	1819	522	2475

Table 1 - Number of observations, mean, minimum and maximum values of selected variables.

The calculated NNI during the spring growing season from the first sampling at 442 GDD and the last sampling at 993 GDD is related to the spring N application rate through what seems to be a second-degree polynomial (Figure 2). The NNI is furthermore closely related to the N uptake in the plant cuts and the intersection for NNI equal to one is close to 100 kg N ha<sup>-1</sup> (Figure 2). The final seed yield is closely related to total N uptake in seed and straw and to seed N uptake alone (Figure 2).

Figure 2 - Relationships between nitrogen nutrition index (NNI) and nitrogen (N) application rate (A), NNI and N uptake in plant cuts (B), Seed yield and NNI (C), and seed yield and N uptake in seed and straw (D).



### **Discussion & perspectives**

The use of NNI to optimize the utilization of applied N is extensively studied and tested. Most results are promising, however, some argued that the method and results are not sufficiently applicable to be used by farmers (Gislum et al. 2021; Vleugels et al., 2017). The present results show that an NNI of 1 can be achieved by the application of 40 kg N ha<sup>-1</sup> for red fescue and 80 kg ha<sup>-1</sup> for perennial ryegrass and this is equivalent to approximately 100 kg N ha<sup>-1</sup> in the crop during the spring growing season. Based on the relation between NNI and seed yield, NNI must be above one to achieve a sufficiently high seed yield while the seed yield does not change when NNI is above one.

The use of NNI to evaluate N status in experimental plots is straightforward and easy to implement. However, the most important question is if the method will be implemented by farmers. The standard Danish N application rates for red fescue and perennial ryegrass are 70 to 80 kg ha<sup>-1</sup> and 160-170 kg ha<sup>-1</sup>, respectively. The NNI will consequently be above one and the crop will have a sufficient N status. Based on this the standard Danish N application rates are sufficient to secure adequate N for the crop. We have used the published CNDC (Gislum and Boelt, 2009) and it would be interesting to test if some of the other published CNDC models will give similar results.

Our conclusion is that the Danish standard N application rates are sufficient to secure adequate N for red fescue and perennial ryegrass. We furthermore conclude that an increase in NNI from

one to two will not necessarily increase the seed yield. Based on this we conclude that it would be interesting to combine the use of NNI with the current Danish N application system.

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## PHENOTYPING OF HERBAGE SEED FIELD PLOTS USING UAV-MOUNTED SENSOR SYSTEMS

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#### Abstract

Phenotyping using unmanned aerial vehicles is used to describe the development of plants and crops and is consequently also used in herbage grass seed research. Some crop parameters such as dry matter production, % nitrogen (N), and N uptake would be very interesting to estimate if the errors are sufficiently low. Our results are based on 1024 samples of grasses collected during the spring growing season from 2017 to 2021. Dry matter, %N, and N uptake were measured and correlated to weather data, four raw wavelengths, and associated crop index. Two algorithms namely partial least squares regression and support vector machines (SVM) were used to build models and the lowest root mean square error of prediction for dry matter, %N and N uptake was 1.12 tons DM ha<sup>-1</sup>, 0.36 %N, and 33 kg N ha<sup>-1</sup>, which were achieved using the SVM algorithm. Our conclusion is that it is up to the farmer and the specific field to decide if this is sufficiently precise to be implemented in the management.

#### Introduction

The use of unmanned aerial vehicles (UAV) for phenotyping in field plot experiments is widely used and integrated at breeding stations, companies, and universities performing field plot experiments. Phenotyping using UAV is especially useful as it can cover a large area and different crop parameters can be measured at the same time. Data can be extracted on a plot level and used for further calculation, such as classification and regression. Biomass, nitrogen (N) status as the percentage of N in the crop, and crop N uptake are three crop parameters that give valuable information on crop development and performance. The information can also be used to optimize for example additional N application rates and application of plant growth regulators.

Lussen et al. (2022) were able to predict the aboveground dry matter of temperate grassland using random forest or support vector machine algorithms with a median root mean square error (RMSE) of a cross-validated model by 0.197 to 0.206 tons ha-1, N% was estimated with a median RMSE of 0.31 to 0.33%, while N uptake was estimated with a median RMSE of 7 kg ha-1. The range in dry matter, %N, and N uptake were 0.158 to 3.376 tons DM ha-1, 1.67 to 5.06 %N and 5 to 108 kg N ha-1. Amorim et al. (2022) were able to estimate the biomass of spring wheat with an RMSE of 0.83 tons ha-1. The dry matter of winter wheat was predicted with an RMSE of 0.43 to 0.61 tons ha-1 (range from 0.6 to 7.27 tons ha-1) and N uptake by an RMSE of 13 to 17.5 kg ha-1 (range from 13.6 to 219 kg ha-1) (Jiang et al., 2022). If these results are sufficiently precise and accurate is difficult to discuss and it must be up to the individual farmer and advisor to decide. It is however indisputable that a method that can

estimate biomass, N%, and N uptake is valuable for achieving high utilization of applied and available N, reducing the N leaching from the crop, and better applying the right amount of plant growth regulators.

The aim of the current paper is to show our set-up for the phenotyping of herbage seed field plots, our results from the prediction of the different response variables, and the possibilities and challenges of using UAN sensor readings for phenotyping in herbage seed production.

### **Materials & Methods**

The data consist of 1023 grass plant cuts taken at 0.5 meters \* 0.5 meters in field plots of approximately 20 m<sup>2</sup>. The sampling was performed from 2017 to 2021. The plant material was dried and weighed whereafter the amount of dried biomass in tonnes per hectare was calculated. A subsample was taken for total nitrogen (N) analysis using the combustion method. The total N uptake was afterward calculated and expressed in kg per hectare. There were three different grass species included in the study namely perennial ryegrass, red fescue, and smooth-stalked meadow grass. Multispectral images of the cuts were acquired prior to cutting. The images were acquired using the Sequoia camera with four monochrome sensors for capturing images at four different narrow bands: green (550 nm  $\pm$  20 nm), red (660 nm  $\pm$  20 nm), red-edge (735  $nm \pm 5 nm$ ) and near-infrared (790 nm  $\pm 20 nm$ ). Values were averaged for each cutting area and 18 different crop indices were calculated based on two or more of the bands. For example, the Normalized Difference Vegetation Index was calculated by: (790-660) / (790+660). Weather data was collected as growing degree days, precipitation, and global radiation. All weather data were accumulated values starting from the first of January, the first of February, or the first of March, and all ending at the days of cutting the samples. This gave a total of 4 (narrow bands) + 18 (crop index) + 9 (weather data) = 31 variables for each biomass (response)variable). All variables were pre-processed using auto-scaling which means mean-centering followed by a division of the standard deviation of that variable. The partial least squares regression (PLSR) and support vector machine (SVM) models were developed using %N, kg N/ha, and dry matter (DM) as response variables and the 31 variables as explainable variables. Test set validation was used to evaluate the robustness of the models. The test set consisted of every fourth sample after the data were ordered from lowest to highest values for each variable. The PLSR and SVM modeling then consisted of training and validating a calibration model followed by a test of the trained model. All calculations were made with MatLab (2021) Natick, Massachusetts: The MathWorks Inc.

#### Results

The prediction of all three variables showed a considerable variation (Table 1 and Figure 1). The best results were obtained for the prediction of %N and DM while a precise estimation of N uptake was difficult, and this goes for both PLSR and SVM results. An initial principal component analysis of the data (data not shown) did not show any grouping of the data and based on this, we decided to use the chosen validation method instead of an e.g., yearly-based validation model.

Variable	Ν	Average	Minimum	Maximum
%N	1024	2.72	0.21	5.40
DM	1024	5.23	0.66	15.8
Kg N	1024	133	10	373

Table 1. The number of plant cuts (N), average, minimum, and maximum values for % nitrogen (%N) in the dry matter, dry matter production in tons per hectare (DM), and kg N uptake per hectare.

The relation between measured and predicted %N, N uptake, and dry matter production is shown in Figure 1. There was a considerable variation in the relationship which was most probably due to the variation in year and species but of course also due to an error we can't explain.

Figure 1. Predicted versus measured percent nitrogen (%N) of dry matter for PLSR (a) and SVM models (d), N uptake per hectare for PLSR (b) and SVM models (e), and tons of dry matter production (DM) per hectare for PLSR (c) and SVM models (f). Calibration and validation data are marked as '+' and 'o' respectively.



The most interesting part of the analysis from a farmer's and adviser's point of view must be the RMSEP which shows the prediction error on a sample that is not part of the calibration model. The predictive errors for %N, Kg N, and DM were 0.52 %N, 42 Kg N per hectare, and 1.61 tons DM per hectare for the PLSR model and 0.36 %N, 33 Kg N per hectare, and 1.12 tons DM per hectare for the SVM model.

Table 2. Results from training, validating, and testing the PLSR and SVM models on the three variables: percent nitrogen (%N) in dry matter, N uptake in kg (Kg N) per hectare, and dry matter (DM) production in tons per hectare. Results are given as the number of principal components (#PC), regression coefficient (R<sup>2</sup>), root mean square error of calibration, cross-validation, and prediction (RMSEC, RMSECV, and RMSEP), together with the bias.

Variable	Model	Training set		Validation	set	Test set		
		#PC	R <sup>2</sup>	RMSEC	R <sup>2</sup>	RMSECV	R <sup>2</sup>	RMSEP
%N	PLSR	5	0.51	0.51	0.50	0.52	0.51	0.52
Kg N	PLSR	4	0.33	39	0.32	40	0.22	42
DM	PLSR	5	0.52	1.6	0.50	1.7	0.51	1.6
%N	SVM	747	0.85	0.28	0.78	0.35	0.75	0.36
Kg N	SVM	654	0.70	26	0.57	31	0.52	33
DM	SVM	597	0.82	0.99	0.73	1.2	0.75	1.12

### **Discussion & perspectives**

Being able to estimate crop dry matter, N%, and N uptake during the growing season using images from UAV or even better satellites would be very beneficial for farmers and probably also for the environment and climate. There are no technical or mathematical obstacles for this to be implemented as part of the farmer's decision support system, it is solely a question if the estimates are sufficiently precise and accurate. The information from the images consisted of four wavelengths and based on these several crop indexes were calculated. We decided to include weather data in our modelling as these data are easy to get and available for all farmers in Denmark.

Even though we included weather data in our prediction, the errors were still considerably higher compared than results from Lussen et al. (2022), Amorim et al. (2022), and Jiang et al. (2022). The methods used in all three studies were comparable to ours while there was a difference in the number of samples and the range of measured crop parameters. It is difficult to state if this was the reason but a higher range in dry matter, %N and N uptake of course had an impact on our results. The main question is still if the farmers are satisfied with an error in dry matter production of ~1.12 tons ha-1 when he or she applies plant growth regulators, and this can only be answered by the farmer and the specific field. In any cases, we suggest that local models are developed to reduce the error that undisputedly will be part of a global model.

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# UNDERSOWING TALL FESCUE IN FORAGE COVER CROPS AND EFFECT OF THE DATE OF FORAGE COVER CROP CUTTING ON THE TALL FESCUE ESTABLISHMENT AND SEED YIELD

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#### Abstract

Grass seed productions in France are undersown in a cover crop to provide a longer establishment period. This methodcan also be used for weed control and to increase purity and seed yield. Various cover crops have been tested and used in France, and considerable differences in grass seed yield have been registered depending on the area, the date of sowing and the cover crop species. Seed growers are concerned by both the cover crop income and grass seed yield and breeder seed growers are also interested by the potential forage value of the cover crop.

Different research projects about forage seed production or pasture establishment have been conducted in the last decade in a partnership of Fnams with the chamber of agriculture of "Les Pays de la Loire" forage experimental network. Recently (2019-2022), during the project named "Procerherb", new types of forage cover crops have been tested for tall fescue seed production.

In a field experiment at Brain sur l'Authion (France) and two successive tests, tall fescue for seed production was undersown simultaneously with different cover crops. The three tested forage cover crops were based on a mixture of cereal (triticale or oat) and legumes (pea, forage pea, common vetch, faba bean depending on the modality) with early cutting (April) or late cutting (June) of green hay bales. The seed production reference cover crop was wheat which was harvested later in July.

The forage cover crop cut in April allowed a better tall fescue establishment than the later one in June; at the end of summer, the tall fescue soil cover was significantly higher with the early forage cover crop cut than with the late cut. This provides an advantage by possible mechanical weeding between the rows of tall fescue.

Forage yield (t of dry matter/ha) was less regular and sometimes lower with the early cover crop cut vs late cut; but the forage value of the plant protein-enriched cover crop is of interest for the breeders in compensation for a possible loss of tonnage.

Sowing forage cover crop with early or late cut had no significant effect on the tall fescue seed yield in 2020. Significant effect was observed in 2022: late cutting of a legume-enriched cover crop negatively affected seed yield contrary to early cutting due to better establishment.

These results showed the impact of forage cover crop on tall fescue establishment and its seed yield. They suggest that mixed legume-cereal cover crops are new possible alternative cover crops with potentially positive effects on tall fescue seed yield depending on the date of cutting.

Keywords: tall fescue, forage cover crop, establishment, seed yield

# Introduction

Grass seed productions in France are mainly undersown in a cover crop to provide a longer establishment period, and a better weed control to increase purity and seed yield. Various combinations of cover crop have been tested and used in France, the more traditional way is to establish grass seed crop in winter wheat crop either in autumn or in spring by direct sowing. However, cover crop could penalize first year seed yield of tall fescue by a lower number of fertile tillers due to the competition of the cover crop (spring cereal) and less radiation intercepted by the undersown tall fescue plants (Chastain & Grabe 1989, de Ruiter & Hare 1993). In France, differences in establishment quality and seed yield have also been recorded depending on the location, the date of seeding, the cover crop species used, the density of the cover crop and the occurrence of lodging. The cereal cover crop density should be adapted to avoid too much competition between the two species. Other successful spring cover crops are also potentially used in France such as spring barley, spring pea, spring field bean, sunflower, forage maize, flax, soybean (Fnams 2003, 2020).

In the objective to obtain high seed purity, and with few chemical herbicide solutions, cover crop choice should primarily relate to weed control opportunities (mechanical or herbicide solutions in the cover crop) and security of seed crop establishment.

Delaying the seeding of grass seed crops into the cover crop generally increases weed control opportunities, but as a result, competition from cover crops could be stronger. For example, delaying the date of tall fescue sowing in forage maize cover crop or sunflower provides the opportunity to an early mechanical weed control and wider herbicide solutions (Poirier & Bouet 2020); Cover crop species adapted to a late date of sowing in spring such as black wheat in the West of France is interesting to avoid some grass weed such as ryegrass, brome grass, vulpia in tall fescue seed production; but the undersown grasses may develop poorly in case of a late cover crop harvest.

The choice of cover crop should also consider the interest of legumes as cover crop for the following grass seed production. The beneficial impact of a legume crop in the previous year on nitrogen uptake of tall fescue seed crop is demonstrated. Nitrogen uptake is higher with legumes sequence vs cereal cover crop even with a lower N supply with legumes according to balance method calculating; and this effect is increased in case of dry spring conditions for the seed production (Bouet 2021). Spring pea is an interesting tall fescue cover crop but does not permit to avoid ryegrass weed, this cover crop was also affected in the last decades in France by pest damage, climate conditions and economic constraints of the farmer. Soybean could be a substitute legume crop but is not well developed yet.

For breeder seed grower the choice of the cover crop must be explored. Seed growers are concerned by both the cover crop income and grass seed yield and breeder seed growers are also interested by the potential forage value of the cover crop. It is related to the farmer's strategy, which is looking for yield or in the last few years for the best quality of forage.

Different research projects about forage seed production or pasture establishment have been conducted in the last decade in partnership Fnams with the chamber of agriculture of "Les Pays de la Loire" forage experimental network. Triticale cover crop is an alternative solution to establish tall fescue. Winter peas compete too much with tall fescue to obtain a satisfactory yield. Mixed cover crop with cereal and legumes can possibly affect the seed yield of tall fescue in case of lodging favoured by a high density of legumes and late cutting (Fnams 2019).

Recently (2019-2022), the purpose of the research project named "Procerherb" was to study new type of forage cover crops for tall fescue seed production.

The objective of this study is to evaluate the influence of different mixed forage cover crops harvested with an early or late cut on tall fescue seed crop establishment and seed yield.

## Materials and Methods

The trial was undertaken at Brain sur l'Authion - France (47° 26' 46" N et 00° 24' 39" O) for two couples of years (2019-2020 and 2021- 2022) ; tall fescue for seed production (CALINA or KIOWA tall fescue cultivars) was undersown by direct drill simultaneously with cover crops in a randomized block design with four replicates (plots measured 10 m x 2.5 m). Tall fescue was sown at 50 cm row spacing and the cover crops at 16.6 cm.

The tested forage cover crops are based on mixed crop of cereal (triticale or oat) and protein legumes plant (pea, forage pea, common vetch, field bean depending on the treatment) with early green hay bale (April) or late one (June). The seed production reference cover crop was wheat harvested later in July. These cover crop treatments are described in table 1 with cover plant density and their date of forage cut.

No herbicide was applied to the mixed cover crop due to the difficulties related to the number of species, but the wheat cover crop received a broadleaf herbicide. The forage yield of the cover crop (dry matter production) was obtained from manually collected samples from each replicated plot treatment. Tall fescue N fertilisation was the same for all the treatments based on the lower requirement treatment. Tall fescue establishment quality (visual grading with a scale from 0 to 10) and % of tall fescue soil cover was registered for each treatment following the harvest of cover crops, and each trial was for a couple of years and the seeds were harvested at the end using a plot combine.

### **Results and discussion**

The first harvests of the mixed forage cover crop B and C occurred in the second half of April, while late harvests were delayed for about 1.5 months in the first half of June. The forage yield of the mixed cover crop (t dry matter /ha) (tab 2) was variable depending on the cut date and the year. Dry matter (DM) tonnages produced are well differentiated between the two successive tests, ranging from almost simple to triple for the "early" April cut (approximately 2 t DM/ha in 2019 versus over 7 t in 2021) (tab 2). At this time of the early spring, we observed that the export DM production may be much lower depending on the year, as was the case in 2019 in contrast to 2021. "Late" harvests (June) were less dependent on the year and were more regular in terms of exported tonnage (around 7.5 t DM). But this occured at the expense of forage quality on the total nitrogen matter (N) compared to early cuts (Source: Procerherb) with

a loss of 19 % N with the late cutting vs early one for Mixed forage crop B and C. The reference A with a high proportion of triticale and with a late cutting obtained a very low N level with 48 % less compared to early cutting C (tab 3). The forage value of the cover crop enriched in legume species may be of interest sought by breeder farmers in compensation for a potential loss of tonnage.

After the forage crop cut, establishment quality of the tall fescue was registered in summer and in autumn (tab 4). We can see that delaying the cutting in June for the mixed forage cover crop B and C tended to influence the quality of tall fescue implantation. The forage cover crop with cutting in April allowed a better tall fescue establishment compared to the later one in June. After forage harvest, the tall fescue soil cover in late of summer was significantly higher with the early forage cover crop cut vs late cut due to a stronger tall fescue tillering and development. It also gave an advantage by an advanced possible mechanical weeding between the clearly visible rows of tall fescue (conducted until this date without herbicides).

On the contrary, the tall fescue after a late cutting of the mixed cover crops is less developed.

The late cutting in June led to increased irregularity in the tall fescue rows, which can generate later a greater weed infestation in the least established areas. It suggests that the competition with the mixed forage cover crop is too strong even with no lodging for tall fescue in case of a late cutting.

In these trials, tall fescue seed yield (tab 3) has been obtained under different crop management concerning irrigation. The 2020 yield level was somewhat impacted by the absence of irrigation and hot weather compared to 2022 conducted with irrigation (25 mm in May).

Sowing forage cover crop with early or late cut had no significant effect on the tall fescue seed yield in 2020 and significantly effect in 2022: late cut for a legume rich cover crop affected seed yield with the cover crop (same tendency in 2020). And higher tall fescue seed yield was obtained with the same forage cover crop C and an early cut due to a better establishment. This suggest that the mixed covercrop with more legumes is an interesting cover crop with an early cutting. But the risk to penalise seed yield is present in case of late cutting.

### Conclusion

These results show the impact of forage cover crop on tall fescue establishment and its seed yield. They suggest that mixed cereal-legume cover crop are new possible alternative cover crops with potentially positive effects on tall fescue quality establishment and seed yield depending on the date of cutting. Mixed legumes cover crop give interesting results in terms of weed control without any herbicide. They provide interesting forage quality results and could be solutions for farmers seeking ways to increase the self-sufficiency of their forage and protein systems. In this study "Procerherb", they also showed a way to better establish pasture with 1 to 2 earlier forage harvests.

Table 1 - The sowing density in mixed forage crop tested and actual density one month after sowing, and date of harvest on the two successive trials with undersown tall fescue (8 kg/ha) at Brain sur L'Authion.

			Sowing density (grain/m <sup>2</sup> )				Actu	ial densit	y (grain,	/m²) - A	verage 2	2019 and 2	2021			
N°	Cover crop	Harvest date (actual date for the 2 successive trials)	Winter Wheat	Triticale	Winter Oat	Winter pea	Winter Field pea	Winter Common vetch ( <i>Vicicia</i> <i>sativa</i> )	Winter Field bean	Winter Wheat	Triticale	Winter Oat	Winter pea	Winter Field pea	Winter Common vetch (Vicicia sativa)	Winter Field bean
т1	Réf. winter wheat/ grain harvest at maturity	Grain harvest at maturity (09-21 July)	300							254						
T2	Mixed forage crop A	Late forage cutting (triticale BBCH 77-83 *) (13-16 June)		250			15	15			149			16	13	
тз	Mixed forage crop B	Early cutting forage (17-24 april)		125			15	15	20		88			14	12	14
т4	Mixed forage crop B	Late cutting forage (06-16 June)		125			15	15	20		66			11	11	17
T5	Mixed forage crop C	Early cutting forage (17-24 april)			40	40	15	15	10			39	31	8	12	9
т6	Mixed forage crop C	Late cutting forage (06-16 June)			40	40	15	15	10			38	36	9	14	8

\* : late milk- early dough

Table 2 - The dry matter (T/ha) for forage cutting on the two successive trials with undersown tall fescue at Brain sur L'Authion.

			Dry matter forage o	r (t/ha) for cutting
N°	Cover crop	Harvest date (actual date for the 2 successive trials)	Procer A 2019/20	Procer B 2021/22
T1	Réf. winter wheat/grain harvest at maturity	Grain harvest at maturity (09-21 July)	-	-
Т2	Mixed forage crop A	Late forage cutting (triticale BBCH 77-83) (13-16 June)	Réf. winter wheat/grain harvest at maturity	6,5
Т3	Mixed forage crop B	Early cutting forage (17-24 april)	Mixed forage crop A	7,8
т4	Mixed forage crop B	Late cutting forage (06-16 June)	Mixed forage crop B	7,6
Т5	Mixed forage crop C	Early cutting forage (17-24 april)	Mixed forage crop B	6,6
тб	Mixed forage crop C	Late cutting forage (06-16 June)	Mixed forage crop C	7,5
	p-value		0,000	0,46

(Average wheat yield : 70,5 q/ha)

Table 3. Forage quality according to the date of cutting and the forage cover crop (8 trials Procerherb 2019-2021).

N°	Cover crop	Harvest date (actual date for the 2 successive trials)	Total N (g/kg DM)	Milk forage unit (mfu/kg DM)
T1	Réf. winter wheat/ grain harvest at maturity	Grain harvest at maturity (09-21 July)	-	-
Т2	Mixed forage crop A	Late forage cutting (triticale BBCH 77-83) (mid -June)	78d	0,80 .b
Т3	Mixed forage crop B	Early cutting forage (early May)	128.b	0,85 ab
Т4	Mixed forage crop B	Late cutting forage (mid-June)	102c.	0,82 ab
Т5	Mixed forage crop C	Early cutting forage (early May)	153 a	0,90 a.
Т6	Mixed forage crop C	Late cutting forage (mid-June)	122 .bc.	0,83 ab
P-value		0.00	0.02	

Table 4. Effect of the cover crop on the establishment quality and seed yield of the tall fescue (1st year of production) on the two successive trials at Brain sur L'Authion.

			After the cover crop harvest					
		Establi quality c fes (scale 0 opti	shment of the tall cue to 10 = mal)	% soil cov fes	ver by the cue	Tall fescue seed yield (kg/ha)		
N°	Cover crop	Harvest date (actual date for the 2 successive trials)	Procer A 2019/20 09/10/19	Procer B 2021/22 15/07/21	Procer A 2019/20 09/10/19	Procer B 2021/22 15/07/21	Procer A 2019/20	Procer B 2021/22
T1	Réf. winter wheat/ grain harvest at maturity	Grain harvest at maturity (09-21 July)	10	-	26 abc	-	515	655 .b
т2	Mixed forage crop A	Late forage cutting (triticale BBCH 77-83) (13-16 June)	10	10	28 abc	23 .b	428	821 ab
тз	Mixed forage crop B	Early cutting forage (17-24 april)	10	10	31 ab.	56 a.	420	827 ab
T4	Mixed forage crop B	Late cutting forage (06-16 June)	9,4	8	18c	13 .b	491	760 ab
Т5	Mixed forage crop C	Early cutting forage (17-24 april)	10	10	34 a.	58 a.	677	1013 a.
т6	Mixed forage crop C	Late cutting forage (06-16 June)	9	7	23 abc	33 ab	571	641 .b
	p-val	ue	-	-	0,000	0,000	0,160	0,004

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# INTEGRATING FORAGE SEED CROPS IN CROPPING SEQUENCES FOR PROFITABILITY AND SOIL HEALTH: EXPERIENCE FROM WESTERN CANADA

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Agroecological pathway to agricultural sustainability integrates measures of resource use efficiency, substitution of ecofriendly inputs and practices, and transformative redesign of agricultural landscape. Crop rotation overlaps through all those measures of agricultural transformation. A study examined the agroecological performance of cropping sequences with annual food crops and perennial forage seed crops at Beaverlodge Research Farm in western Canada from 2013 to 2020. An eight-year field experiment comprised eight different cropping sequences generated from four annual crops - barley (Hordeum vulgare L.), canola (Brassica napus L.), pea (Pisum sativum L.) and wheat (Triticum aestivum L.), and four perennial forage seed crops - alsike clover (Trifolium hybridum L.), creeping red fescue (Festuca rubra L. ssp. rubra), meadow bromegrass (Bromus riparius Rehmann) and red clover (Trifolium pratense L.). We compared canola equivalent yield (CEY), gross margins (GM) and various soil health parameters among cropping sequences. The soil health index and fall NH<sub>4</sub><sup>+</sup>-N: NO<sub>3</sub><sup>-</sup>-N ratio were highly correlated (r = 0.78; P = 0.02). Eight-year cropping sequences comprising two perennial phases of creeping red fescue seed crops, separated by canola and pea on fourth and fifth years, produced significantly higher CEY than corresponding clovers-based sequences. Annual crop-based sequences mostly remained on par with perennial-based sequences for the CEY. Lower cost of production due to replacement of N fertilizer by clovers improved the comparative gross margins of the clover-based sequences. However, the cost saving could not fully compensate for their lower CEY. The CEY and the soil-health index were highly correlated (r = 0.84; P = 0.008), suggesting the profitability and soil health go together. Furthermore, the study results and experience suggested the need of refinement in individual crop agronomy, especially of perennial forage legumes on the aspect of crop establishment, intercrop compatibility, weed control, and management of preceding crop volunteers in the sequential cropping. Further studies should consider quantification of legume N-fixation, mineralization, plant uptake, N-transfer from legumes to non-legume companion crop, N-loss through NO3<sup>-</sup> leaching and N2O emission to guide appropriate nutrient management, cropping sequence and inter-crop compatibility.

#### Introduction

Cropping system diversification can improve environmental and economic performance and enhance resilience in the face of weather extremes and unpredictable climate. Simplified systems based on annual crops often have high reliance on agrochemical inputs and are sensitive to pest pressures and climate variability (Bowles et al., 2020; Petersen-Rockney et al., 2021). Climate change typified by increases in temperature, precipitation and frost-free periods could be favourable for crop diversification and agricultural intensification in the Peace region (Motha & Baier, 2005; Weber & Hauer, 2003). Farmers are motivated to adopt various climate-adaptive practices (Davidson et al., 2019). Diversification of cropping systems is more ecologically rational strategy than agricultural expansions in the newer frontiers (Klöffel et al., 2022). Enhanced resource use efficiency substituting part of the nitrogen input and perenniality in the cropping systems can contribute to reduction in greenhouse gas emissions (GHG) and building soil carbon (C) stock (Liu et al., 2016; Smith & Lampkin, 2019). Rapid increase in fertilizer and other input prices further justifies the quest of options for capitalizing on the nature-based N credits through crop diversification. Cropping systems design constitutes a central strategy to improve agroecological and economic efficiencies.

The Peace River region of western Canada is globally reputed for perennial forage seed production and export, which historically accounts for one-third to one-half of the forage seed production within and exported from Canada (Wong, 2017). Prevailing cropping systems in the region are relatively simple such as the yearly alternation of wheat and canola, and more diversified cropping systems use peas, barley, and oat at varied frequencies. Over 600 producers grow forage seed crops mostly as niche commodities within those annual crop rotations. Forage seed crops are mostly established as monocrops, and as a result, do not have any economic production during the year of establishment. Due to the lack of recommendations based on proven innovations, farmers have adopted cropping sequences based on their own experiences and information shared among fellow farmers. Some farmers occasionally inter-seed an annual crop as a companion crop with the forage seed crop in the establishment year. Establishment of a uniform competitive stand of perennial forages is important for the suppression of weeds and protect the crop yields. Studies are lacking on the aspect of optimizing the cropping systems through integration of forage seed crops and annual field crops for resource-use efficiency, economic profitability, and soil health. This study attempted to examine the agroecological performance of cropping sequences with major annual food crops and perennial forage seed crops prevalent in the Peace region of western Canada.

### **Materials and Methods**

The field study was established at Beaverlodge Research Farm of Agriculture and Agri-Food Canada (55°12' N 119°24' W) near the town of Beaverlodge, Alberta in 2013. Continental boreal climate prevails with average monthly temperatures ranging from 15 °C to -11 °C from the warmest month of July to the coldest month of January, and annual total precipitation of 434 mm. The soil is classified as Dark Gray Luvisol.

Experiment was laid out in split plot design with 4 replicates. Treatments included eight main plots of different cropping sequences (Table 1) and three sub-plots of nitrogen rates (0, 45, 90 kg N ha<sup>-1</sup>). The sub-plot size measured 2.5 m by 8 m area with 8 rows spaced 30 cm apart. Crop management included zero-till air-seeding on 30 cm-row spacing; pre-seed and post-emergence herbicide application; nitrogen treatment (0, 45, 90 kg ha<sup>-1</sup>), phosphorus, potassium, and sulphur at 40, 30

and 10 kg ha<sup>-1</sup> respectively as band application in the spring for annuals and as broadcast application in the fall for perennial seed crops. Major phenological events, plant health, weed composition, canopy coverage, crop biomass, seed yield were recorded. Crop yield samples were taken from two central rows of 6 m length excluding the border effect.

Soil samples for the physico-chemical analyses were collected prior for 0 to 60 cm depth prior to seeding in the spring and following crop harvest. The samples of 0-15, 15-30, and 30-60 cm were crumbled, air-dried, and passed through an 8-mm screen and then ground to pass through a 2-mm sieve for analyzing soil attributes. Established protocols were followed to analyze soil quality parameters such as bulk density, aggregation, available water capacity, infiltration, soil pH, electrical conductivity, organic matter, and soil nutrient stratus in various years. Soil samples for the soil biological analyses was collected following crop harvest. Soil microbial biomass C was measured using the substrate-induced respiration method (Horwath & Paul, 1994), and the activities of extracellular enzymes  $\beta$ -glucosidase (C cycling), N-acetyl-  $\beta$ -glucosaminidase (C and N cycling), acid phosphomonoesterase (P cycling) and arylsulphatase (S cycling) was quantified using microplate fluorimetric assays (Marx et al., 2001). Active C (permanganate oxidisable C) was determined using potassium permanganate extractant (Weil et al., 2003).

The costs for field operations applicable to the test crops and their output prices were collected from various sources for the gross margin (GM) analysis (gross revenue – total partial variable cost) and deriving canola equivalent yield (CEY = price ratio of canola to non-canola crop multiplied by non-canola seed yield) for each crop as the indicator of relative profitability of long-term cropping sequences and nitrogen fertility regimes. The yearly average prices of canola and non-canola seeds (\$/kg) were used to calculate the CEY. The soil health variables were standardized by dividing the mean values of soil health variables of each sequence by the corresponding values of wheat-canola sequence. A standardized values based on the nature of the variables and summing the values up for each cropping sequence. Soil bulk density, penetration resistance (compaction), fall NO<sub>3</sub> content were considered negative soil health variables, hence assigned negative signs, while soil organic carbon, permanganate oxidizable C, microbial biomass C, total N, NH4<sup>+</sup>-N, spring NO<sub>3</sub><sup>-</sup>-N, and various soil enzymes activities were considered positive variables, hence assigned positive signs.

All quantitative data were analyzed by using SAS GLIMMIX (SAS 9.4, SAS Institute, 2013). A two-way analysis of variance (ANOVA) with crop sequences and nitrogen rates as factors was used in PROC GLIMMIX model to determine the significant difference among treatment means and correlations between variables. The denominator degrees of freedom method (ddfm) option used the Kenward-Roger method. Multi-treatment comparisons were made using the Tukey's studentized range test at the probability level of  $P \le 0.05$  to establish statistical significance.

### Results

Cumulative CEY: The CEY of different cropping sequences was significantly different (p < .0001) (Figure 1). The highest CEY was recorded from the creeping red fescue-based cropping sequence, which is statistically on par with meadow bromegrass and annual crop-based sequences.

Cumulative GM over partial variable costs: The GM showed significant differences among the cropping sequences (Figure 2). The GM were higher for the forage grasses and annual crops-based sequences than those of alsike clover-based sequences, while red clover-based sequences stood on par with the high performing sequences.

Changes in soil physical properties: The diverse cropping sequences and nitrogen fertilization affected the soil penetration resistance over the years (Table 2). The creeping red fescue and alsike clover-based sequences (sequence 2 and 4) showed higher resistance to penetration in 2022 And higher mean diameter of soil aggregates measured in 2017after 5 years of cropping sequence practice. However, there were no significant differences in soil bulk density and water content at field capacity between the cropping sequence treatments over the years of experimentation.

Changes in soil carbon and nitrogen fertility status: Soil organic carbon content was influenced by cropping sequences over the years of experimentation. The clover-based sequences had consistently higher soil organic carbon content (Table 3). Similarly, an increased amount of total nitrogen content was observed from the clover-based rotation. The  $NH_4^+$ -N and  $NO_3^-$ -N levels were highly variable.

Changes in soil enzyme activities and carbon fractions: Cropping sequences showed differential soil carbon and nutrient cycling enzyme activities (Table 4). The fescue and alsike clover-based sequences showed higher levels of microbial biomass C,  $\beta$ -glucosidase, and acid phosphomonoesterase activities. Fescue-based sequence also had higher arylsulfatase activity.

Correlations among SSHI, CEY and post-harvest  $NH_4^+$ -N:  $NO_3^-$ -N in the fall: The CEY and SSHI were highly correlated (r = 0.84; P = 0.008). The high correlations were also observed between the post-harvest fall  $NH_4^+$ -N:  $NO_3^-$ -N ratio and the SSHI (r = 0.78; P = 0.02) and CEY (r = 0.67; P = 0.07) (Table 5).

# **Discussion and Perspectives**

Some cropping sequences in this study appeared superior to others in terms of economic values expressed as CEY and GM. Creeping red fescue seed crop-based cropping sequence produced the highest cumulative CEY and GM, and superior soil structure as evident from the highest mean weight diameter of soil aggregates. This sequence also had consistently higher level of permanganate oxidizable C, microbial biomass C and various soil enzyme activities, suggesting the positive effect on soil health. Past studies on perennial-integrated and diversified cropping systems in the Peace region and other parts of western Canada provide the evidence for multiple ecosystems services such as enhancing economic competitiveness, soil carbon (C) sequestration, greenhouse gas (GHG) emissions and mitigating other externalities of prevailing synthetic input-

intensive crop rotations in the Peace region (Khanal, 2022). Diversified cropping systems with the inclusion of perennial turfgrass and forage crops provide living roots for soil binding and generating soil microbial substrates (Sanford et al., 2021), enhance soil C stock and organic matter (Bolinder et al., 2007; Kätterer et al., 2011) and contribute to the development of discrete granular structures (Pawluk, 1980) with greater aggregate stability (Broersma et al., 1997).

In contrary to the expectation, the CEY of the clover seed crops-based sequences were lower than creeping red fescue and annual crops-based sequences. However, due to their lower cost of production associated with N credits, the clover-based sequences in general had similar level of gross margins to other cropping sequences. Based on the study by Rice (1980) at Beaverlodge in the Peace region, alsike and red clover fixed nitrogen in the range of 21–143 kg ha<sup>-1</sup> & 15–77 kg ha<sup>-1</sup> respectively in a single growing season. Continuous 16 years' (1968-1984) sequence of red clover had 10-fold higher N mineralization rate than the non-legumes crop sequences (Broersma et al., 1996). However, the clover-based sequences had inconsistent effects on soil health variables in our study.

It was interesting to observe high correlations among SSHI, CEY and post-harvest  $NH_4^+$ -N:  $NO_3^-$ -N. High correlation between CEY and SSHI suggests that soil health improving cropping systems can be economic profitable. High correlations between post-harvest fall  $NH_4^+$ -N:  $NO_3^-$ -N ratio and the SSHI as well as the CEY suggests the importance of managing N dynamics through agronomic and cropping systems approach for balancing the trade-offs in the production system.

Despite numerous agro-ecological merits of perennial grasses and legumes (Khanal, 2022), poorer performance of forage legume seed crops-based sequences in this study calls for further studies on the following areas:

- refinement in individual crop agronomy, especially of perennial forage legumes on the aspects of crop establishment, intercrop compatibility, weed control, and management of volunteer plants of the preceding crops
- understand interactions such as allelopathy, competition, and bio-stimulation between potential preceding, succeeding and intercrop crop species
- mitigation of soil compaction and challenges in stand establishment under long-term the zero-tillage system with the integration of proper residue management and bio-tillage regime
- strategic intervention of primary and/or secondary tillage along with soil aerating cover crops to balance the profitability and soil health trade-offs
- transitioning from forage seed crop stand to forage feed biomass system leading to the annual crop phase for capitalizing on the multi-year biomass production potential of perennial forage crops leading to effective termination and transitioning to the annual crop phase

• quantification of N-fixation, mineralization, plant uptake, N-transfer from legumes to nonlegume companion crop, and N-loss through nitrate leaching and N<sub>2</sub>O emission to guide appropriate nutrient management, cropping sequence and inter-crop compatibility.

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Main plot	2013-2016	2017-2020
S1	C-C-C-C-	P-MB-MB-MB-
S2	CF-CF-CF-C-	P-CF-CF-CF-
S3	RC-RC-W-C-	RC/W-RC-W-C-
S4	AC-AC-W-C-	AC/W-AC-W-C-
S5	inRC-RC-W-C-	RC/W-RC-C-W-
S6	inAC-AC-W-C-	AC/W-AC-CF-CF-
S7	P-B-W-C-	P-W-C-P-
S8	W-C-W-C-	W-C-W-C-

Table 1 - Main Plots of cropping sequence treatments.

Note: S1....S8 refer to the cropping sequences; the slash sign ' / ' refers to the intercrop and dash sign ' - ' refers to the sequential crop; AC = alsike clover, B = Barley, C = canola, CF = creeping red fescue, in = inoculated with bio-fertilizer, Mb = meadow bromegrass, P = pea, RC = red clover, W = wheat

Cropping sequence	Bulk de (g cm-	ensity 3)	Penetration resistance (PSI)		MWD of aggregates (mm)	Field capacity (%)
	2018	2022	2017	2022	2017	2022
S1	1.31	1.51	164	564ab	1.27ab	45.0
S2	1.38	1.41	135	609a	2.15a	48.6
S3	1.37	1.45	146	571ab	1.20b	47.0
S4	1.30	1.46	158	639a	1.06b	45.5
S5	1.44	1.42	176	493b	1.60ab	46.8
S6	1.35	1.44	168	553ab	1.13b	48.6
S7	1.40	1.48	138	579ab	1.20b	46.1
S8	1.32	1.41	122	586ab	1.68ab	47.4
p-values	0.211	0.156	0.055	0.012	0.007	0.080
SEM	0.041	0.037	13.01	27.28	0.194	1.043

Table 2 - Soil physical health indicators of diverse cropping system at 0-15 cm depth with variable rates of nitrogen inputs

Table 3 - Soil organic carbon and nitrogen fertility status of diverse cropping systems with variable rates of nitrogen inputs at 0-15 cm depth

Cropping	Organic carbon (%)		Available N (mg kg-1)				Total N (%)	
sequence			NO3N	NO3N		NH4+-N		
	2018	2020	2018	2020	2018	2020	2018	2020
S1	2.46bc	2.90ab	8.32ab	0.93bc	4.28	4.48b	0.22b	0.26ab
S2	2.41c	2.94ab	9.48a	0.85c	5.54	5.00ab	0.22b	0.28ab
S3	3.18a	2.73ab	3.16d	3.26bc	4.43	5.33a	0.29a	0.27ab
S4	2.88a-c	2.53b	4.48cd	2.92bc	4.57	4.90ab	0.27ab	0.24b
S5	2.80a-c	3.08a	3.71d	4.38b	4.97	5.01ab	0.27ab	0.29a
S6	2.91a-c	3.16a	4.46cd	11.43a	4.73	5.38a	0.27ab	0.29a
S7	3.00ab	2.90ab	8.25ab	3.46bc	4.15	4.78ab	0.27a	0.26ab
S8	2.83a-c	2.97ab	6.38bc	2.39bc	4.80	4.38b	0.26ab	0.28ab
p-values	0.001	0.009	<.0001	<.0001	0.082	0.002	0.002	0.007
SEM	0.129	0.133	0.539	0.761	0.408	0.203	0.013	0.013

Note: Soil sampling date: May 17, 2018, and October 02, 2020

Cropping	POXC	MBC	Soil enzyme	oil enzyme activities (pmol g-1 h-1)					
sequence	(mg	(mg kg-	β-	β-N-acetyl-	Acid	Arylsulfatase			
	kg-1)	1)	glucosidase	glucosaminidase	phosphomonoesterase				
S1	587c	930abc	1048b	284ab	2477ab	24.1b			
S2	682b	1023a	1641a	294ab	2696a	37.5a			
S3	661bc	592d	1327ab	207b	2441ab	21.3bc			
S4	591c	692cd	1030b	208b	2186b	14.6d			
S5	723ab	636d	1108b	276ab	2516ab	16.1cd			
S6	766a	986ab	1532a	383a	2515ab	21.2bc			
S7	665bc	664cd	1025b	274ab	2467ab	21.8bc			
S8	682b	718bcd	1074b	234b	2655a	20.5bc			
p-values	<.0001	<.0001	<.0001	0.0016	0.0026	<.0001			
SEM	18.18	68.47	52.65	30.23	99.61	1.42			

Table 4 - Effect of cropping sequences on permanganate oxidizable C (POXC, mg kg-1), microbial biomass C (MBC, mg kg-1) and soil enzyme activities (pmol g-1 h-1) in 2022 post-harvest.

Table 5 - Correlations among standardized soil health index, canola equivalent yield and post-harvest NH4+-N: NO3--N in the fall

Soguence	Standardized soil Canola equivalent yield (CE		Fall
Sequence	health index (SSHI)	ha-1)	NH₄⁺-N: NO₃⁻-N
S1	0.50	12074	4.82
S2	0.64	13126	5.88
S3	0.50	11108	1.63
S4	0.42	9238	1.68
S5	0.44	10519	1.14
S6	0.28	8936	0.47
S7	0.46	12554	1.38
S8	0.50	11956	1.83
Correlation with SSHI r = 0.84; P = 0.008 r = 0.		r = 0.78; P = 0.02	
Correl	ation with CEY	-	r = 0.67; P = 0.07

Figure 1 - Eight-year cumulative canola equivalent yields (CEY) of eight cropping sequences at Beaverlodge, AB from 2013 to 2020. Error bars represent the standard error of mean (SEM, n=4).



Figure 2 - Eight-year cumulative gross margins of eight cropping sequences at Beaverlodge, AB from 2013 to 2020. Error bars represent the standard error of mean (SEM, n=4).



#### **IMPROVING SEED PRODUCTION RELIABILITY IN URUGUAY**

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#### Abstract

Uruguay has a well develop and successful herbage seed industry. Over the last 20 years there have been significant improvements in both the yields and quality. Major advancements have been achieved in seed crop agronomy: crop nutrition, grazing management, weed and disease control as well as harvest techniques. Growers have played a key part in this process, moving from being opportunistic (i.e., harvesting the seed in 'good' years) to becoming professional seed growers. This mindset change has driven investment in their seed production capabilities, such as, windrowing and harvesting equipment, seed drying technologies, and processing capabilities. The challenge for Uruguayan seed producers is consistency because the climate is challenging, resulting in variable yields and qualities. The adoption of irrigation is key to improving the competitiveness of the Uruguayan seed industry. In recent years, there has been a significant increase in irrigation in Uruguay. There is now more than 38,500 ha of irrigated farmland and this has increased by 30,000 ha in the last 10 years. However, only 3% of this area is used to irrigate pastures and herbage seed crops. Growers need to be convinced that irrigating herbage seed crops is as good an option as irrigating other winter crops.

In 2021, the La Niña weather system created drought conditions all over the country. A survey of dryland and irrigated yields from Italian ryegrass cv. Magno (*Lolium multiflorum* L.), showed an average yield of 422kg/ha for dryland crops compared to 1946kg/ha for irrigated fields. This survey indicated that there was a fourfold advantage in seed yield with irrigated crops. During 2022, the irrigated and non-irrigated areas of Magno ryegrass were evaluated to determine the components of yield that explained the large differences measured in 2021. The analysis of yield components showed increased numbers of heads/m<sup>2</sup> (1,010/m<sup>2</sup> vs. 735/m<sup>2</sup>), greater length of the spike (28.4 vs 21.1 cm), more florets/spikelets (8.47 vs. 6.28/spikelet), and greater numbers of full seeds per spike (152 vs 110) for irrigated than dryland areas. No difference was found in the number of spikelets/spike (28/spike) and thousand seed weight (2.7grs).

From a grower's perspective, the yield advantage with irrigated crops also creates a more efficient production system, where direct production costs are significantly lower on a per kg basis, e.g., US\$1.93/kg, compared to US\$1.4/kg for non-irrigated seed crops. Irrigation not only improves the profitability of ryegrass seed production, but also the consistency of yields for seed growers, therefore, enhancing the reputation of the Uruguayan seed industry. The irrigated fields of Mango ryegrass produced a crop of higher profitability when compared to other winter crop options, such as, canola, wheat or barley.

#### Introduction

In Uruguay, the market of temperate forage seeds amounted to 51.4 thousand tons (average between 2017-2021), representing 26% of the total market share of Uruguayan seeds (INASE). The main destination of the seeds produced is the local market (54%), 25% is exported, and 21% is used by farmers themselves. Annual ryegrass is the main forage species produced (18 thousand tons - average between 2017-2021), with a higher exported percentage (45% of the total seed produced) than other species. In the last two years (2021 and 2022), spring drought has affected most of the country, compromising the annual ryegrass seed production, mainly in late flowering cultivars like Italian ryegrass Mango. Ryegrass seed production under irrigation would improve reliability for growers and stabilise the production base for seed companies. The adoption of irrigation is key to improving the competitiveness of the Uruguayan seed industry.

The aim of this study was (i) determine the difference in ryegrass seed yield between dryland and irrigated conditions; (ii) determine what yield components explained the yield variation, and (iii) determine the profit of each system.

#### Materials & Methods

The experiment was located in the San José, Uruguay. During 2022, a paddock of Italian ryegrass (*Lolium multiflorum L.*) cv. Magno was evaluated under dryland and irrigated conditions. The seed crop was planted on 13 May 2022 at 19 kg/ha of seed rate with non-tillage method, the preceding crop was maize. Broad leaf weeds were controlled in August with herbicide. The crops were grazed between on August 1<sup>st</sup> to September 19<sup>th</sup> (closing date). Nitrogen was applied at 146 kg N/ha in three times: July 23<sup>rd</sup>; September 20<sup>th</sup> and October 12<sup>th</sup>. Fungicide and insecticide were applied at Zaddock 3.2 stage (November 1<sup>st</sup>), and a second application of fungicide was made 25 days after. Trinexapac-ethyl (Moddus®25% a.i.) plant growth regulator was applied under irrigated conditions at 0.7 l/ha at Zaddock 3.2 stage (November 1<sup>st</sup>). The dryland crop (18 ha) was windrowed on November 29<sup>th</sup> and harvested on December 9<sup>th</sup>.

Rainfall between closing date and windrowing time was 43 mm, this was just 20% of normal rainfall for this period. Figure 1 provides a summary of the mean climate data for Uruguay and also highlights the drought conditions experienced in 2022. Irrigation totalling 180 mm was applied with a centre pivot irrigator from a surface water dam which was a sustainable water harvesting resource. Before the windrowing time, six quadrats 0.5 m long were cut by hand in the dryland and irrigated parts of the field, the dryland areas were the corners of the field that the irrigator could not reach. In each one it was measured: total biomass (kg DM/ha) and, the number of seed heads/m<sup>2</sup>. The harvest index was calculated as the ratio of seeds produced, kg/ha, relative to total biomass. Samples of 10 spikes were taken in each cut quadrate. The length of the spike, the number of spikelets/spike, the number of florets/spikelet, the number of full seeds/spike and the thousand seed weight (TSW) was measured. The percentage of concretion of floret was calculated between the ratio of the number of full seeds relative to total number of florets. Seed crop yield was measured in the seed processing unit, all the seed harvest was trucked and weighed (field dressed seed kg/ha). The seed was processed with a sequence

of blowers, screens and indent cylinders until the commercial seed purity standard was achieved (97%). Cleaned seed was then weighed and yield estimated by dividing weight by the harvest surface.

Direct production cost was calculated including cost of inputs, services and irrigation.

# Results

Seed yield was increased under irrigated condition (239%) vs dryland (Table 1). Crop total dry matter at windrowing time was double under irrigated condition vs. dryland (p=0.0009) and, the harvest index was 8,2% and 4,8% respectively. The main components that explain the difference in yield were the number of seed head (p=0.0009), and the number of full seeds/spikelet (p=0.0421). Under the irrigated condition the length of the spike was greater than dryland (p=0.0035). There was no difference in the number of spikelets/spike (p=0,783), the percentage of concretion of floret (p=0.444) and the TSW (p=0.791). There was a tendency for higher numbers of seeds/spike in the irrigated condition 152, compared to 110 in dryland areas (p=0.062).

The direct production cost per kilogram of clean seed was higher under the dryland condition vs irrigated crop (2.37 USD/kg vs 1.2 USD/kg).

Irrigated ryegrass crops cost per hectare was greater (53% more) than other winter crops options such as barley, wheat or canola (Ryegrass: USD 1,286; Wheat: USD 840; Barley: USD 897; Canola: 782 USD/ha). However, when the net income was calculated, the profitability of irrigated ryegrass crops was 60% higher than the winter crops average (Table 2). The financial comparison is for the seed component of the ryegrass seed crop. Grazing is both an important tool and source of income for Uruguayan seed growers. Uruguay's mild winters allow the production of large amounts of high-quality grass that is grazed in situ, often using rotational grazing. The integration of animals with cropping systems is feature of Uruguayan farms.

# **Discussion & perspectives**

During 2022 drought conditions affected most of Uruguay's seed production region, mainly in the South-west of the country. There was only 200 mm of rain during the ryegrass crop development (between May to December) or 40% of average rainfall for this period. This situation has become more frequent in the country through the effects of climate change and ryegrass seed production has been struggling with these conditions. Irrigation of forage seed crops is not common, despite the good results highlighted in this work. This study shows that irrigated seed crops yielded more than three times those under dryland conditions and moreover, the profit of the irrigated crops was much higher. The results showed the positive effect of irrigation in the number of head/m2, the length of the spike and the numbers of full seeds/spike. However, in this trial, there was no statistical difference in the TSW, which is contrary to what has been reported in other countries (FAR, 2010).

Irrigation of ryegrass seeds crops improve yield, reduces the cost per kilogram of seed produced and also improves the stability of yields for seed growers. These results should encourage an increase in the area of seed crops under irrigation, due to the higher profitability in comparison to other winter crop options, such as, canola, wheat or barley (Table 2). The improvement in yield stability will allow Uruguayan seed producers opportunities to reach new markets and enhances the reputation of the Uruguayan seed industry.

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#### Appendices

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Yield Components	Dryland	Irrigation	p-value
Yield seed (kg/ha)	326	1.075	
Total biomass (kg DM/ha)	6.8 a	13.1 b	0.0009
Harvest index (%)	4.8	8.2	
Heads/m <sup>2</sup>	735 a	1000 b	0.0047
Spike length (cm)	21.1 a	28.4 b	0.0035
Spikelets/spike	28.8 a	28.1 a	0.7283
Florets/spikelet	6.3 a	8.5 b	0.001
Full seeds/spikelet	3.8 a	5.5 b	0.0421
TSW (gr)	2.5 a	2.5 a	0.7918
Concretion (%)	58.4 a	64.3 a	0.4443



Figure 1 – Uruguayan Climate Data (date average between 1972 to 2023 – INIA Las Brujas)

Table 2 – Comparative financial analysis between irrigated ryegrass and winter crops

	Ryegrass	Wheat	Barley	Canola
Average yield (kg/ha)	1075	4251	4252	1687
Direct crop cost (USD/ha)	\$ 1,286	\$ 840	\$ 897	\$ 782
Net income (USD/ha)	\$ 488*	\$ 393	\$ 294	\$ 230

\* Excludes income from beef production

# A GROWER'S PROSPECTIVE OF CHALLENGES OF HERBAGE SEED PRODUCTION, FROM THREE DIFFERENT REGIONS OF PRODUCTION

David Birkett (New Zealand), Troels Prior Larsen and Ulrik Lunde (Denmark), Mark Simmons (Oregon)

#### Abstract

The past ten years have seen significant developments in the growing of herbage seed crops through the continued research by the many factions of our herbage seed industry. This research is vitally important to the industry but has had the impact of masking some of the outside factors that have both a direct and indirect impact on growers' ability to produce and our profitability within the industry. This presentation will look at the different challenges that exist in three regions of production, Denmark, Oregon and New Zealand.

The challenges that each region faces are often different, due to different regulatory rules and laws, while other challenges are common and have been present for some time but are exacerbated by the global crises of pandemics, climate change and war. Some of the challenges that have been around for some time now, are access to some of our agri-chemical inputs due to the removal of their registration or the banning of them from our markets, input limitations (especially N) and land use change to more perennial and profitable crops. While other challenges are more recent, including inflation pressure on crop inputs and climatic shifts.

The presentation will look at how research has allowed us to manage some of these challenges, to ensure that we maintain our productivity with reduced access to products and limited inputs, but also highlights challenges that are beyond the ability of research to rectify.

Research has generally been aimed at increasing productivity (usually through yield) to compensate for increased input costs or post farm gate costs that continue to rise. This approach is acceptable while yield gains can keep up with inflationary pressure, but this hasn't been the case over the last ten years and has been of major concern with the global inflation that we have all experienced over the last two years. We have made gains through research on the areas that have the greatest impact on yield but as we move forward, the new research will probably deliver smaller gains which places more pressure on grower profitability. Are there areas of research that we have missed that would change this outcome?

Some of the challenges are specific to the individual production regions and a presenter from each region will highlight these during the presentation. It is probable that some solutions will be generic across the global industry, while others will need to be designed for each region of production.

A collective approach to these challenges, especially the global ones, that can be facilitated through the likes of the IHSG is a logical way of addressing the issues before they negatively impact the whole herbage seed industry.

# New Zealand

There are many challenges we face in seed production in NZ, but most can be categorized into the following areas.

- Climate changes are forcing growers to look at ways to reduce the impact of weather events on our crops, particularly at harvest time when the crop is most vulnerable to losses. While adjustment to climate during the growing season can be managed with tools such as irrigation and disease management, the challenges at harvest time are more difficult. The options of increased harvesting capacity and drying facilities help to alleviate the challenge but cannot eliminate it. These options are also very capital intensive which requires a profitable crop to allow investment.
- Input restrictions on both fertiliser and agri-chem inputs are becoming more of a challenge. While we are not as restricted as the Northern Hemisphere growers, we generally follow them in relation to access to agri-chem. We have manged to negotiate that any N restrictions only apply to the grazing phase of the crop, therefore not limiting our seed yield potential.
- Profitability of herbage seed production has been on a slow decline over the past 10 years. It was not until the impact of covid and the subsequent large increases in input costs that the issue was brought to a head. While research has continued to provide an increase in seed yields within a variety (see graph below), it doesn't compensate for the increases in production costs and a lower seed yield that we are seeing from some of the newer forage ryegrass genetics in NZ. Detailed costs of production that are openly shared with all parties in the industry are essential to solving this issue.





This decline in profitability has seen a reduced investment in infrastructure, both on and off farm which the industry is currently having to address too.

The solutions to these challenges are many but a common one is better communication between growers and seed companies at both the domestic and global level, while other solutions will require new research approaches.

# Denmark

In Denmark there is a group of dedicated seed growers to herbage, vegetable and flower seeds.

At the same time, we have dedicated politicians; they are unfortunately not dedicated in ensuring a robust frame for seed production. It is not easy for growers to find the way to grow profitably seeds.

#### **Political pressure**

It is especially problematic in Denmark (and EU) with these weak tolerances, where the number of different pesticides is decreasing in a fast number and have been for several years. One special reason in Denmark, is the Danish way of using unfiltered groundwater for drinking water. In Denmark the water is only filtered through a sand filter before used as drinking water. Because of this, Denmark has a particularly strict regulation of pesticides. It takes for example special documentation for the risk of leaching to the ground water before pesticides can get an approval. The low amount of active ingredients used on seeds when producing coated seeds, is taken out of use. Here the use of pesticides is safe for the user and the doses per ha is low. This means that the numbers of available pesticides are lower than in other countries and even countries in the EU.

And at the same time, often the Danish approval of pesticides is only for main crops and not for the special seed crops. This means that the seed growers organisation together with the chemical companies must apply for Off Label use or dispensation. For both of which, the Danish environmental authorities are well paid.

At the same time, the regulation on nutrients means that Danish farmers have a lot of catch crops, of which some can be problematic for the growing of for example different Brassica seeds. And EU have come with new regulation to ensure untouched soil in the autumn if no crops is sown. This means the possibility to do some autumn mechanical weed and pest control is removed. Together with low numbers of pesticides, this is problematic.

This political pressure is increasing on the grower and make the risk of reduced yield and quality of the seed, since it can be very difficult to fight weed and pests.

The growers are together with seed companies, consultants and universities looking for new ways to reduce these new political created growing problems. This is difficult to find science-based solutions on these issues. The seed industry is yearly using money on trials to find solutions. In this, there are using time on searching for new smart technology to be part of the solution.

More seed crops are facing pest problems than have been seen for many years. One reason is the lack of efficient pesticides but it could also be an effect of rising temperatures. The Danish seed sector have applied for funds to run a project to find alternative solutions for the pest problems in seed production.

Every year, the Danish seed sector is using large amount of funds on science-based trials to solve issues in the seed production.

## Special risk when growing vegetable seeds.

In addition to the political issues for growing vegetable seeds in Denmark, we see as growers some challenges in the terms for growing the seeds.

Here we just want to explain the main issue. As grower, you are offered a contract for growing a vegetable seed, with both a number of hectares (ha) and how many tons you are supposed to produce. At the same time, the contract states that you get reduced pay pr. kg seeds, for seeds you produce more than for example 115 % (varies between 100-150 %) of the number of tons seed, in the right size spectrum, as issued in the contract. The grower's opinion is that, if the contract states ha and tons, the price must be the same for all produced seeds.

With rising general risk of producing vegetable seed due to the Danish and European political situation and more unreliable weather conditions, it is problematic that we see difficulties in ensuring the profitability in the seed growing.

#### **Dialog with multiplication companies**

There is a good dialog about these issues between seed growers and the multiplication companies in Denmark. What we sense from the dialog, is that the terms originate earlier in the value chain that the multiplication companies we have the contract with. We hope that the whole value chain will open to new discussions of the risk for seed production under the new political and climatic conditions. The risk has to be placed more evenly over the whole value chain.

# Oregon: Biggest challenges facing cool season turf grass seed growers in the United States

**Cost of Production plus a fair profit must be covered in the price paid to farmers for herbage seed** or their land will be planted to other crops. Farmers here have options and will not grow seed if it is not profitable. To help farmers understand the true "break even" cost of production, we are preparing a detailed cost analysis of 9 herbage crops that are routinely grown in Oregon. This information is made available to any who ask for it so that both dealers and farmers have objective information to use in making planting decisions.

The cost of producing grass seed has gone up dramatically the last several years and many fields that formerly were producing grass seed have been converted to hazelnuts, berries, grapes and other crops. If grass seed production is not going to continue to decline, the return to farmers must be competitive with other crops. Our seed dealer partners must recognize cost of production or they will need to find other areas to produce seed.

Accurate Data for use in making planting recommendations to growers: In order to do our part in balancing supplies with market demand, good information is required. This information needs to include accurate information on seed consumption by end users, export volumes, the amount of seed carried forward from prior years, the number of acres that will be harvested in the coming season and a rough anticipation of expected yield. The information we have today is at best, a guess. We must have a committed partnership between growers and dealers to gather and share credible seed industry data. This leads to much greater price stability at profitable levels which benefit everyone.

#### **INCREASING SEED YIELD IN OREGON'S ANNUAL RYEGRASS CROPS**

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#### Abstract

Annual ryegrass [Lolium perenne L. ssp. multiflorum (Lam.) Husnot] is an important forage seed crop grown in Oregon on approximately 50,000 ha annually. In recent decades, successful efforts have been made to increase grass seed yields but research activities have primarily focused on other cool-season species, including tall fescue [Schedonorus phoenix (Scop.) Holub] and perennial ryegrass (Lolium perenne L.). Meanwhile, seed yield trends in Oregon's annual ryegrass crops have remained relatively unchanged. The aim of this work was to determine whether more intensive crop management, including the use of spring defoliation and plant growth regulators (PGR), can be used to further increase annual ryegrass seed yield under western Oregon conditions. Four studies were conducted to evaluate seed yield and yield components response to trinexapac-ethyl (TE), chlormequat chloride (CCC), and TE + CCC combinations applied at BBCH 31-32, with and without mechanical defoliation. Field trials were undertaken at Oregon State University's Hyslop Farm from 2018 - 2021. Trials were sown to 'Gulf' annual ryegrass and individual plot size was 14.5 m x 3.5 m. In each study, spring defoliation and PGR treatments were arranged in a split-plot design with four replications. In the first study (2018-2019), TE (0, 200, 400, and 600 g ha<sup>-1</sup>) applied at BBCH 31-32 was combined with three spring defoliation treatments (no mow, single mow, and triple mow). Seed yield increases from TE ranged from 30-123%, with the highest seed yield occurring with 600 g TE ha<sup>-1</sup> applied to either single mow or triple mow plots. In the second study (2020-2021), TE (0, 400 g ha<sup>-1</sup>), CCC (0, 600, 1200, and 1800 g ha<sup>-1</sup>), and TE + CCC were combined with two defoliation treatments (no mow and double mow). Treatments containing TE increased seed yield by 61% and 90% with no defoliation and double mowing, respectively. There was no effect on seed yield from CCC alone and further increases were not provided by combining CCC and TE PGRs. There was a positive correlation (r=0.9728) between spike length and seed yield which reveals an advantage to producing a shorter spike with less space between spikelets. These results suggest that annual ryegrass seed yields in Oregon are able to respond to more intensive spring crop management. Seed yield improvements are likely to occur with grower adoption of spring defoliation and TE PGR applications at 400 g ha<sup>-1</sup> or higher. Further investigation is needed to better understand the role of carbon portioning and seed shatter susceptibility when spike length is shortened by these management practices.

#### Introduction

Forage grass seed crops, including annual ryegrass (Lolium multiflorum L.), are a vital part of seed production enterprises in Oregon. Like other cool-season grasses, annual ryegrass only produces 15 to 33% of its potential seed yield. Lodging of the crop during flowering is one of the

major factors limiting maximum seed yield. Making better use of management practices that reduce stem length and decrease lodging is one area that should be further explored to address seed yield losses.

Plant growth regulators (PGRs) are widely used in many temperate grass species, to reduce lodging and increase seed yield (Chastain et al., 2014; 2015). Seed yield is reduced by lodging during anthesis and early seed fill as a result of self-shading in the canopy and reductions in pollination. While seed yield increases from use of trinexapac-ethyl (TE) have been well documented in perennial ryegrass, its use patterns and potential effects on the seed yield of annual ryegrass is relatively understudied in Oregon. Previous work in the northern hemisphere suggests seed yield responses of annual ryegrass to TE are generally small (Mellbye et al, 2007; Rijckaert, 2010; Macháč, 2012). However, studies conducted in New Zealand report seed yield increases of 30 to 50% when TE is applied (Trethewey et al., 2016).

Plant growth regulators containing CCC have not previously been registered for use in grass seed crops in Oregon, but a federal registration is being sought. Early work by Hebblethwaite et al. (1978) examined the effect of CCC on perennial ryegrass and found that it had little effect on tiller length or lodging. However, seed yield was increased in some years, likely due to improved assimilate transfer to the seed. Hampton (1986) also evaluated effects of CCC on perennial ryegrass and found that neither tiller length nor lodging was reduced, but seed yield increases resulted from improved survival of tillers.

In addition to PGR use, defoliation by grazing or mechanical mowing are also standard practices used to reduce tiller height, decrease lodging, and increase seed yield in annual ryegrass seed crops across the globe. Historically, spring defoliation is carried out at the appearance of the first node on reproductive stems (BBCH 30-31), although more recent research has demonstrated higher seed yields when defoliation occurs slightly later (BBCH 32-33) (Rolston et al., 2010). Effects of spring grazing on annual ryegrass seed crops were evaluated in Oregon during the late 1970's (Young et al., 1996), but no work has been done since the introduction of PGRs.

Recent research shows that even greater seed yield increase in annual ryegrass crops are possible when PGR applications are strategically timed with spring defoliation. Rolston et al. (2012) reported seed yields of 3015 lbs/acre when 200 g ai TE ha-1 was applied to annual ryegrass that was defoliated at GS 32-33. This is a 35% increase over the treatment with the same TE rate applied to annual ryegrass that was defoliated once at BBCH 30-31 and a 123% increase over the no PGR treatment. The aim of this work was to determine whether more intensive crop management, including the use of the use of spring defoliation and plant growth regulators, can be used to further increase annual ryegrass seed yield under western Oregon conditions.

# **Materials and Methods**

In both studies, field trials were conducted on 'Gulf' annual ryegrass at Oregon State University's Hyslop Research Farm over two harvest years, 2018 to 2019 (Study 1) and 2021 to 2022 (Study

2). Plot size was approximately 14.5 x 3.5 m. Spring defoliation by sheep grazing was simulated using a flail mower. The experimental design was a randomized complete block with a split-plot arrangement of treatments and four replications.

In Study 1 (2018 and 2019), main plots were spring defoliation timings and subplots were TE rate and application timings. Subplots were randomly allocated within defoliation main plots. Defoliation treatments included an untreated control (no mowing), a single mowing at BBCH 32 and a double mowing at BBCH 32 and again when regrowth was at BBCH 32. Subplots included the following TE treatments: untreated control (no PGR), TE subplots include 0, 200, 400, and 600 g TE ha-1 applied at BBCH 31-32.

In the Study 2 (2021 and 2022), main plots were spring defoliation and subplots were PGR treatments. Subplots were randomly allocated within defoliation main plots. Defoliation treatments included an untreated control (no mowing) and a single mowing at BBCH 32. Subplots included TE (0, 400 g ha-1), CCC (0, 600, 1200, and 1800 g ha-1), and TE + CCC mixes (600 g CCC ha-1 + 400 g TE ha-1, 1200 g CCC ha-1 + 400 g TE ha-1, 1800 g CCC ha-1 + 400 g TE ha-1). All PGR applications were made at BBCH 31-32, except for a single CCC treatment which was split over two timings (1200 g CCC ha-1 at BBCH 32 + 600 g CCC ha-1 at BBCH 51).

In both studies, two 0.3 m quadrat samples of above-ground biomass were collected from each plot near crop maturity to determine total dry matter and seed yield components. Lodging ratings were recorded in Study 2 on a scale of 0-100%.

Seed was harvested by a small-plot swather and combine, and seed was cleaned to determine seed yield. Seed weight was determined by counting two, 1000-seed samples with an electronic seed counter and weighing these samples on a laboratory balance. Harvest index (HI), the ratio of seed yield to above-ground biomass, was also calculated.

Analysis of variance was conducted to test spring defoliation and PGR treatment effects and their interaction on seed yield, seed weight, seed number, and other characteristics for each trial. Spring defoliation and PGR treatment means for each trial were separated by Fisher's protected LSD values at 5% level of significance. Regression analyses were conducted to elucidate the nature of relationships behind spike length and seed yield.

# **Results and Discussion**

In Study 1, there was an interaction of spring mowing and PGR for seed yield and seed number in both years (Table 1 and Table 2). The combined effects of these two management practices further increased seed yield and seed number over individual treatment (spring mowing or PGR) effects. While seed weight was reduced in one of two years, the increase in seed number still allowed for a greater overall seed yield.

In Study 2, there was an interaction on spring mowing and PGR for seed yield, seed number, HI, and final lodging in both years (Tables 3 and 4). All PGR treatments containing TE resulted in

increased seed yield in both years. Applications off CCC alone had no effect on seed yield in either year, with and without spring mowing. There was a positive correlation (r=0.9728) between spike length and seed yield (data not shown) which reveals an advantage to producing a shorter spike with less space between spikelets. Annual ryegrass suffers from seed yield losses as a result of shattering before and during the harvest process. It may be possible that the decrease in spike length and more compact placement of spikelets results in less seed loss.

These results suggest that annual ryegrass seed yields in Oregon are able to respond to more intensive spring crop management. Seed yield improvements are likely to occur with grower adoption of spring defoliation and TE PGR applications at 400 g ha-1 or higher. Further investigation is needed to better understand the role of carbon portioning and seed shatter susceptibility when spike length is shortened by these management practices.

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			Seed	Seed
Treatment	Yield	V	Veight	Number
	kg ha	<sup>.1</sup> mg	g seed <sup>-1</sup>	no m <sup>-2</sup>
No Mow				
Untreated Contr	rol 777 a	a 3	.1755	24693*
400 g TE ha⁻¹	825 a	a 3	.0273	27339
800 g TE ha <sup>-1</sup>	1065 a	. 2	.8537	37703
1200 g TE ha <sup>-1</sup>	1585 k	oc 2	.7533	58157
Single Mow				
Untreated Contr	rol 1242 a	ıb 3	.0165	41647
400 g TE ha <sup>-1</sup>	1625 b	)c 2	.8413	57153
800 g TE ha <sup>-1</sup>	2121 c	le 2	.7468	77181
1200 g TE ha <sup>-1</sup>	2432 e	2 2	.7410	88832
Triple Mow				
Untreated Contr	rol 979 a	a 2	.8395	34577
400 g TE ha <sup>-1</sup>	1886 (	d 2	.6413	71435
800 g TE ha <sup>-1</sup>	2218 (	de 2	.6195	84895
1200 g TE ha <sup>-1</sup>	2450 e	e 2	.5908	94591
LSD=0.05	P = 0.0497	0.1468	0.0296	

Table 1 - Interaction effects of spring mowing and trinexapac-ethyl (TE) plant growth regulators on seed yield, seed weight, and seed number of 'Gulf' annual ryegrass, 2018.

\* homogenous group format cannot be used because of pattern of significant differences

Table 2 - Interaction effects of spring mowing and trinexapac-ethyl (TE) on seed yield, seed weight, and seed number in 'Gulf' annual ryegrass, 2019.

			Seed	Seed
Treatment	Yield	1	Weight	Number
	kg ha	-1	mg seed <sup>-1</sup>	no m <sup>-2</sup>
No Mow				
Untreated Contro	ol 866 a	b	3.345 h	25956 ab
400 g TE ha <sup>-1</sup>	1179 a	bcd	3.286 h	36111 abcd
800 g TE ha <sup>-1</sup>	1200 0	cde	3.188 g	37728 cde
1200 g TE ha <sup>-1</sup>	1421 (	de	3.043 ef	46936 de
Cincle Manu				
Single Wow				
Untreated Contro	ol 1204 l	bcd	3.096 fg	38922 bcd
400 g TE ha <sup>-1</sup>	1031 a	abc	2.987 de	34578 abc
800 g TE ha <sup>-1</sup>	1495 d	def	2.862 c	52217 def
1200 g TE ha <sup>-1</sup>	1572 e	ef	2.750 b	57240 ef
Triple Mour				
Triple Now				
Untreated Contro	əl 820 a	а	2.923 cd	28129 a
400 g TE ha <sup>-1</sup>	1852	fg	2.664 b	69578 fg
800 g TE ha <sup>-1</sup>	2001	gh	2.531 a	79008 gh
1200 g TE ha <sup>-1</sup>	2192 ł	า	2.546 a	86082 h
LSD=0.05	P = 0.0002	0.0202	0.000	)

Table 3 - Interaction effects of chlormequat chloride (CCC) and trinexapac-ethyl (TE) plant growth regulators on seed yield, components of yield, harvest index and lodging in 'Gulf' annual ryegrass with and without spring mowing, 2021.

		Seed	Seed	Harvest	
Treatment	Yield	Weight	Number	Index	Lodging
	kg ha⁻¹	mg seed <sup>-1</sup>	no m <sup>-2</sup>	%	%
No Mow					
Untreated Control	1536 a	2.966	51968 a	12.0*	96*
400 g TE ha <sup>-1</sup>	2399 bcd	2.843	84936 bcdef	11.5	73
600 g CCC ha <sup>-1</sup>	1531 a	3.091	50000 a	7.6	90
1200 g CCC ha <sup>-1</sup>	1423 a	3.132	45751 a	7.8	90
1800 g CCC ha <sup>-1</sup>	1297 a	3.024	43038 a	8.0	93
600 g CCC ha <sup>-1</sup> + 400 g TE ha <sup>-1</sup>	2541 cdefg	2.777	92050 defg	14.1	74
1200 g CCC ha <sup>-1</sup> + 400 g TE ha <sup>-1</sup>	2453 cde	2.744	89395 cdef	14.3	66
1800 g CCC ha <sup>-1</sup> + 400 g TE ha <sup>-1</sup>	2471 cdef	2.636	94343 efg	17.4	61
1200 g CCC ha <sup>-1</sup> + 600 g CCC ha <sup>-1</sup>	1638 a	3.136	52295 a	10.9	89
2X Mow					
Untreated Control	1670 ab	2.953	56698 ab	17.3	96
400 g TE ha <sup>-1</sup>	2944 defg	2.676	109908 fg	28.8	73
600 g CCC ha <sup>-1</sup>	1856 abc	2.932	63639 abcde	17.4	90
1200 g CCC ha <sup>-1</sup>	1564 a	2.925	53748 ab	15.4	90
1800 g CCC ha <sup>-1</sup>	1796 abc	2.981	60261 abc	15.3	93
600 g CCC ha <sup>-1</sup> + 400 g TE ha <sup>-1</sup>	3229 efg	2.617	123392 gh	32.1	74
1200 g CCC ha <sup>-1</sup> + 400 g TE ha <sup>-1</sup>	3247 fg	2.570	126645 h	25.4	66
1800 g CCC ha <sup>-1</sup> + 400 g TE ha <sup>-1</sup>	3282 g	2.559	128693 h	28.2	61
1200 g CCC ha <sup>-1</sup> + 600 g CCC ha <sup>-1</sup>	1803 abc	2.892	62312 abcd	15.0	89
LSD=0.05	P= 0.0294	0.2319	0.0066	0.0006	0.0003

\* homogenous group format cannot be used because of pattern of significant differences

Table 4 - Interaction effects of chlormequat chloride (CCC) and trinexapac-ethyl (TE) plant growth regulators on seed yield, components of yield, harvest index and lodging in 'Gulf' annual ryegrass with and without spring mowing, 2021.

			Seed	Seed	Harvest	
Treatment		Yield	Weight	Number	Index	Lodging
		kg ha⁻¹	mg seed <sup>-1</sup>	no m <sup>-2</sup>	%	%
No Mow						
Untreated Control		1397 bc	2.851 f	49401 bc	9.0*	93 c
400 g TE ha <sup>-1</sup>		1823 e	2.723 cde	66958 efg	10.0	90 c
600 g CCC ha <sup>-1</sup>		1379 bc	2.824 ef	48801 bc	7.4	91 c
1200 g CCC ha <sup>-1</sup>		1579 cd	2.840 ef	55722 cd	9.2	95 c
1800 g CCC ha <sup>-1</sup>		1557 cd	2.846 f	54758 cd	8.8	94 c
600 g CCC ha <sup>-1</sup> + 400 g TE ha <sup>-1</sup>		1693 de	2.661 c	63838 def	9.8	93 c
1200 g CCC ha <sup>-1</sup> + 400 g TE ha <sup>-1</sup>		1576 cd	2.688 cd	59192 de	8.7	91 c
1800 g CCC ha <sup>-1</sup> + 400 g TE ha <sup>-1</sup>		1577 cd	2.755 cde	57584 cd	8.8	91 c
1200 g CCC ha <sup>-1</sup> + 600 g CCC ha <sup>-1</sup>		1213 ab	2.827 ef	43003 ab	8.1	90 c
2X Mow						
Untreated Control		1251 ab	2.788 cdef	44883 ab	14.8	95 c
400 g TE ha <sup>-1</sup>		1858 ef	2.501 b	74470 gh	17.0	75 b
600 g CCC ha <sup>-1</sup>		1179 ab	2.794 def	42240 ab	8.9	89 c
1200 g CCC ha <sup>-1</sup>		1154 ab	2.840 ef	40842 ab	9.8	95 c
1800 g CCC ha <sup>-1</sup>		1066 a	2.759 cdef	38715 a	8.1	94 c
600 g CCC ha <sup>-1</sup> + 400 g TE ha <sup>-1</sup>		1745 de	2.445 ab	71307 fgh	18.6	58 a
1200 g CCC ha <sup>-1</sup> + 400 g TE ha <sup>-1</sup>		1880 ef	2.412 a	78068 h	15.6	61 a
1800 g CCC ha <sup>-1</sup> + 400 g TE ha <sup>-1</sup>		2087 f	2.384 a	87693 i	19.7	61 a
1200 g CCC ha <sup>-1</sup> + 600 g CCC ha <sup>-1</sup>		1217 ab	2.772 cdef	44042 ab	10.5	93 c
LSD=0.05	P=	0.0000	0.0000	0.0305	0.0014	0.0000

\* homogenous group format cannot be used because of pattern of significant differences

#### SEED PRODUCTION OF RED CLOVER

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#### Abstract

Warmer and more dry summers drive an increased interest to include red clover in clover-grass mixtures, and since the tetraploid form has a higher dry matter production there is a particular focus on providing high quality seed of those. In general, the area of red clover seed production has declined in Denmark corresponding to the decreasing seed yields. Various courses may affect the seed yield potential like lack of adequate pollinators, seed eating pests, adverse weather conditions during maturation and harvest.

In a three-year period, we have compared seed yield between two diploid and one tetraploid type of red clover under field conditions in Aarhus University, Denmark. The red clover was established undersown in a spring barley crop. Pests were controlled by insecticides according to good experimental practice. Honey and bumble bees were available for pollination and the harvest procedure was swathing followed by combining 3 to 11 days later.

Flowering phenology was recorded from medio June to medio August. In two experimental years flowerheads were collected prior to harvest for the registration of floret number and the number of seed per flowerhead and from those registrations the seed set was calculated.

Overall seed yields were high between 782 - 1.022 kg ha-1 in average of three varieties over the three years, however, the tetraploid variety only yielded 50% of the diploid variety. This corresponds with findings from other experiments where pollination has been studied in detail.

This extended abstract is summarising results from the publication "Seed production of Red Clover (*Trifolium pratense* L.) under Danish Field Conditions" by Shuxuan Jing and Birte Boelt (2021).

#### Introduction

There is likely to be an increasing emphasis on the role of forage legumes in the production of high-quality meat and milk, combined with the increasing requirement to reduce the environmental footprint of grassland agriculture (Abberton *et al.*, 2008). High seed yield in forage legumes is a prerequisite to meeting the market requirements for new, improved cultivars and hence harness the economic impacts of modern plant breeding for a better livelihood and environment. Reproductive traits that do not impair vegetative growth and quality have been identified, and they should be used as selection criteria on individually spaced plants. These traits are inflorescence density and seed yield per inflorescence. However, seed yield is genetically complex and in the perennial, insect-pollinated forage legumes it is further highly influenced by environmental conditions and crop management factors.

In seed production experiments, 90-110 florets per flower head have been recorded (Puri and Laidlaw, 1983). The ovary normally contains two ovules, but usually only one develops into a seed (Lorenzetti, 1993). Only few data are available regarding the actual number of developed seed per floret and hence our information on pollination success is very limited. The current study aim to collect data on these seed yield components in two types of red clover.

# Materials & Methods

Three red clover cultivars were undersown in a lodging-resistant spring barley in the spring of 2012, 2013, and 2014, in a completely randomized experimental design. 'Rajah' (diploid or 2x), cultivar 'Suez' (2x) and cultivar 'Amos' (tetraploid or 4x) in the field experiment. 'Rajah' and 'Amos' are the two most commonly grown diploid and tetraploid red clover cultivars for seed production in Denmark. To compare cultivars with similar flowering patterns, 'Suez' was included since, like 'Amos', it is an early/intermediate-flowering type, while 'Rajah' is an intermediate/late-flowering type. For each cultivar, there were four plots as replicates (each plot size:  $8 \times 2.5$  m). The clover and the cover crop were drilled in alternating rows. Seeds of red clover cultivars were sown at 1.5 kg ha<sup>-1</sup> regardless of the cultivar. Weeds and insects were controlled chemically when necessary in accordance with suitable experimental practice. No irrigation treatment was applied in the experiment. Flowering intensity was recorded during the flowering period using a visual score-based judgment where 0 equals 'no florets open' and 100 equals 'one or more florets open on any flower head'. One honey bee (Apis mellifera L.) hive was placed in the vicinity of the experimental plot, and wild bumble bees (Bombus spp.), such as Bombus pascuorum Scopoli, Bombus muscorum L., and Bombus hortorum L., were observed during the flowering period. The crop from each plot was swathed and left for drying before combine-harvested according to the maturity of each cultivar. Seeds were air-dried and cleaned before determining the yield (kg ha<sup>-1</sup>). Seed yields are expressed as 100% pure seed.

Detailed registrations of seed yield components were performed in 2014 and 2015. The number of seeds per flower head were registered in 15 flower heads per plot and the number of florets per flower head were registered in four flower heads per plot. Each red clover ovary produces two ovules and hence seed set (i.e., the percentage of ovules developed to seeds) was calculated as the following: seed set (%) = number of seeds per flower head / (number of florets per flower head × 2) ×100. seed yield was adjusted to 100% pure seed.

For information on statistical analysis see Jing and Boelt (2021).

# **Results and discussion**

In the current study, the three cultivars obtained high seed yields compared with the general seed yield of 400-500 kg ha<sup>-1</sup> for diploid red clover (Taylor *et al.*, 1996; Vleugels *et al.*, 2019). In two of the three years, seed yields in 'Rajah' and 'Suez' were higher than 1000 kg ha<sup>-1</sup> and seed yield in 'Amos' was around 500 kg ha<sup>-1</sup> in 2014. These findings are in line with seed production from western Oregon in the United States, where the typical red clover seed yields can reach 600 kg ha<sup>-1</sup> and where over 1000 kg ha<sup>-1</sup> were reported for some seed growers (Anderson *et al.*, 2018). Remarkable is that the tetraploid cultivar 'Amos' obtained only around half of the seed yield of the two diploid cultivars in 2014 and 2015 (**Figure 1**), which is in agreement with previous studies (Amdahl *et al.*, 2016; Jing *et al.*, 2021b).

Figure 1 - Comparison of seed yield (kg ha-1) for red clover cultivars 'Rajah' (2x), 'Suez' (2x), and 'Amos' (4x) during the seed production years 2013-2015. Error bars indicate the 95% confidence interval of the estimated marginal means (EMMs).



There was a significant interaction effect of 'Cultivar  $\times$  Year' in seed number per flower head. In both 2014 and 2015, diploid cultivars had higher seed number per flower head compared to tetraploid red clover (Figure 2). The seed number per flower head in 'Rajah' was significantly higher in 2014 than in 2015, whereas 'Suez' and 'Amos' showed no difference in the seed number per flower head between the two years.

Figure 2 - Comparison of seed number per flower head among three red clover cultivars 'Rajah' (2x), 'Suez' (2x), and 'Amos' (4x) during the seed production years 2014 and 2015. Error bars indicate the 95% confidence interval of the Estimated marginal means (EMMs).



'Amos' had a slightly lower number of florets per flower head, however this was adjusted in the calculated seed set:

Figure 3 - Comparison of seed set (%) among three red clover cultivars 'Rajah' (2x), 'Suez' (2x), and 'Amos' (4x) during the seed production years 2014 and 2015. Error bars indicate the 95% confidence interval of the Estimated marginal means (EMMs).



The overall yield difference corresponds to a lower seed number per flower head and calculated seed set in 'Amos' (Figure 2, 3).

#### Perspectives

A number of studies have identified the number of flower heads per unit area as an important seed yield component (Oliva *et al.*, 1994; Vleugels *et al.*, 2015). Management practices like high seeding rates, irrigation, and no or early forage removal provide seed producers with tools to obtain high flower head numbers (Steiner *et al.*, 1995). We did not quantify the number of flower heads, which may limit the comparisons among the years. It should also be mentioned that although we registered seed yield components in detail, we only analyzed 15 flower heads per plot. This may be a too low number to represent the whole plot/crop. The calculated seed set from 2014 and 2015 showed a lower seed set in 'Suez' in 2014, whereas no difference was found in 'Amos' (**Figure 3**). The specific mechanisms underlying the reduced floral fertility are yet to be studied. Studying meiotic aberrations and embryo abortion (Vleugels *et al.*, 2019) and investigating the pollination process that directly linked to the seed setting process (Jing *et al.*, 2021a,b) may improve our understanding for future breeding activities.

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# **POSTER ABSTRACTS**

#### SEED PRODUCTION OF ENDOPHYTE-FREE AND ENDOPHYTE-INFECTED TALL FESCUE IN REPONSE TO PARTIAL SUBMERGENCE AT TWO ONTOGENETIC STAGES

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#### Abstract

Tall fescue, Festuca arundinacea Schreb. [syn. Schedonorus arundinaceus (Schreb.) Dumort, Lolium arundinaceum (Schreb.) S.J. Darbyshire], is a perennial C3 forage widely used in most temperate regions of the world. Tall fescue is an invasive species that dominates plant communities of pastures and grasslands through natural reseeding. This species occupies an important area in the Flooding Pampa (Argentina) and it is commonly infected with asexual endophytes of the Epichloë coenophiala species. The endophyte fungus only spreads through seeds, a process that is known as "vertical transmission". The relationship between Epichloë endophytes and host grasses is mutualistic. The maintenance cost of the endophyte would be counterbalance by increasing plant vigor and by protecting plants against invertebrate and vertebrate consumers, including domestic herbivores, through the production of toxic alkaloids. The particular consequence for livestock production systems based on endophyte-infected tall fescue as main forage resource, is that high economic losses can occur due to animal disorders caused by the ergot alkaloids produced by endophytes. Under the flooding conditions of the region, the proportion of endophyte-infected plants is usually high - which suggests an endophyte-mediated advantage over the endophyte-free counterparts. The objective of this work was to evaluate the endophyte effect on seed production of tall fescue plants subjected to partial submergence in two ontogenetic stages. Seeds from four tall fescue materials were used: endophyte-free naturalized population and -infected with wild type E. coenophiala, and cv. Taita (Gentos S.A) endophyte-free and -infected with safe endophyte AR584 (Grasslanz Technology Limited, Palmerston North, New Zealand). The experiment was carried out in a glasshouse in Balcarce (Unidad Integrada Balcarce, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata - Estación Experimental Agropecuaria INTA Balcarce, 37°45' S, 58°18' W). Three seeds of the same tall fescue material were sown in 3 L plastic pots containing a mixed substrate (1:1 v:v) of river sand and topsoil from the horizon A of a Typical Argiudol and fertilized with urea and triple superphosphate to provide doses equivalent to ~50 kg N ha<sup>-</sup> <sup>1</sup> and 20 kg P ha<sup>-1</sup>. After 15 days, seedlings were thinned to leave one per pot. Both, at 5-leaf stage and at the beginning of the elongation of internodes, two submergence treatments were applied for 14 days: control (plants kept at field capacity) and partial submergence (PS, a 50 mm layer of water above ground level). For this purpose, two black polyethylene bags and an extra pot were fit outside each PS pot in order to hold the water layer fixed. At the internode elongation stage, half of the pots that had been subjected to each submergence treatment at the 5-leaf stage were assigned to the same previous treatment and the other half to the opposite treatment. After treatments, the plants were kept at field capacity until the reproductive phase was completed, to estimate seeds production. The effects of the experimental factors and their possible interactions on the response variables considered were analysed using ANOVA. Partial submergence at the 5-leaf stage (P < 0.001) had a negative effect on plant seed production that was irrespective of the plant material and endophyte presence. On average, plants subjected to submergence at 5-leaf stage produced fewer seeds than those kept at field capacity (1080±58 and 1636±72 seeds/plant, respectively). The former plants also had a lower number of panicles (8.3±0.5 vs 12.2±0.6, P<0.001). No differences were recorded between these two group of plants in the 1000-seed weight  $(2.00\pm0.05 \text{ vs. } 1.99\pm0.04)$  (P=0.871). These results suggest that fungal endophytes would not confer a differential advantage to tall fescue plants facing flooding conditions. Given that young plants showed high sensitivity to water excess, further studies should evaluate the effect of flooding on critical stages of seed germination and seedling emergence of endophyte-infected and endophyte-free materials.

Keywords: Festuca arundinacea, Epichloë coenophiala, seed production, flooding.

# HOW WILL THE FUTURE CLIMATE IMPACT THE PRODUCTION OF RED CLOVER SEEDS IN FRANCE ?

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#### Abstract

Red clover seed production has become more complex in recent years. Strong pest pressure, heat waves and summer droughts, the yields of this crop are decreasing steadily... 2019 was a particularly catastrophic year in France with an average yield of 2.3 q/ha and many plots with yields less than 1 q/ha or not even harvested.

Climate change is now clearly visible with, in particular, a rise in the average annual temperature and recurring episodes of heat waves during the summer period.

It is now urgent to better understand its impact on the variability of the red clover seed yields, what effects it could have in the future and what levers the sector must activate today to reduce or avoid its effects.

A tool named "CLIMAXXI" was used for this study, it was developed by the Chambres d'Agriculture<sup>1</sup> and UniLasalle Rouen<sup>2</sup>. It makes it possible to model the evolution of agroclimatic indicators over three periods: the near past (1976-2005), the near future (2021-2050) and the distant future (2071-2100) at a local scale. The input data comes from the "ALADIN" model developed by MétéoFrance<sup>3</sup> and is provided by the DRIAS<sup>4</sup>.

The simulations were initially carried out on the data from the city of Châteauroux in the center of France, at the heart of the historic red clover seed production area. A little over forty agroclimatic indicators were modeled for this study, such as the dates of appearance of certain stages, water deficits or accumulation of rain, high temperatures reached during certain periods, etc.

The objectives of these simulations are to try to answer some of the following questions:

- How is the climate risk changing for late summer sowing or spring sowing of the red clover?

- Are the current recommendations for pre-cut dates in spring still appropriate?
- What will be the evolution of the flowering date? and the harvesting date?

- How does climate risk change during flowering? During ripening?

- Will we still be able to grow red clover seeds in historical areas? which sectors would be more suitable in the future?

<sup>1</sup> Chambres d'agriculture: public agricultural establishments

<sup>2</sup> Uni La Salle Rouen: a higher education establishment in agriculture

<sup>3</sup> MétéoFrance: the official service of meteorology and climatology in France

<sup>4</sup> DRIAS: the national meteorological research center

Keywords: red clover seeds, climate change

#### TALL FESCUE CULTIVARS RESPONSE TO SALINITY AS INFLUENCED BY INOCULATION WITH NON-LIVESTOCK-TOXIC ENDOPHYTE AR584

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#### Abstract

Tall fescue, Festuca arundinacea Schreb. [syn. Schedonorus arundinaceus (Schreb.) Dumort, Lolium arundinaceum (Schreb.) S.J. Darbyshire], is a perennial C<sub>3</sub> forage and turf grass species widely used in most temperate regions of the world. The main agronomic qualities of this species are its high productivity, rusticity, plasticity and perennity. An important plant-microbe symbiosis occurs between tall fescue and the asexual Epichloë fungal endophyte which can provide tolerance to biotic and abiotic stress to the host, although in some Epichloë strains can cause toxicity to grazing livestock due to the production of ergot alkaloids. The endophyte fungus only spreads through seeds (vertical transmission). The infection is asymptomatic and the growth coordination between the two partners is perfect as almost all the produced seeds by a host plant are endophyte-infected. Although endophyte-free fescue cultivars are commonly selected to avoid toxicity in grazing animals, using seeds inoculated with non-livestock-toxic endophytes provide an alternative management strategy. Salt stress is a very prevalent adverse plant condition in Argentina. The negative impact caused by salinity increases as a result of inadequate management of agroecosystems. For this reason, in these environments, it is necessary that the species that make up the cultivated pastures have the capacity of establishment and high persistence. An experiment was carried out in a germination chamber with the objective of comparing the salinity tolerance in cultivars of tall fescue endophyte-free seeds and seeds inoculated with non-toxic fungal endophyte AR584 (Grasslanz Technology Limited, Palmerston North, New Zealand) during germination. A randomized complete block design with three replicates in time with factorial arrangement was used. The experimental factors were: 1) cultivars of tall fescue, six levels: Malma, Taita and Royal Q200, endophytefree (E-) and inoculated (E+) with the non-toxic fungal endophyte AR584; and 2) saline condition, three levels: 0 (control), 120 and 200 mM NaCl. Seeds were placed in rolls of paper soaked in the appropriate saline solution and the following variables associated with germination were evaluated by International Seed Testing Association (ISTA) rules: germination energy (seed vigour, 7 days), germination power (14 days), coleoptile and radicle length, fresh weight and dry weight of seedlings. First, four paper towels were stacked and were moistened with a water sprayer (approx. 25 ml). Second, the 50 seeds were homogeneously distributed and then covered with another set of four towels on top (also moistened with 25 mL). Finally, it was carefully rolled up and vertically placed in an independent glass jars (height: 15 cm, and diameter: 12 cm). Then the rolls were incubated in a germination chamber
at constant 25°C and 8:16 light:dark photoperiod. The effects of the experimental factors and their possible interactions on the response variables considered were analysed using ANOVA. Detrimental effects were observed for germination power and seedling fresh weight (P<0.001) under 200 mM NaCl. Non-toxic endophyte reduced germination energy by 40% and germination power by 37% in cv Royal (P<0.001) and decreased both radicle and coleoptile length by15% in cvs Malma and Royal. The non-toxic endophyte also reduced seedling dry weight in cv Taita (P<0.001) by 18%. No differences were found in radicle length among cultivars under 120 mM NaCl. However, non-toxic endophyte increased coleoptile length in cv Malma in 120 mM NaCl by 10%. Endophyte reduced root length by 25% and coleoptile length by 40% under 200 mM NaCl in cvs Taita and Royal. Malma presented the lowest seedling dry weight regardless of its infection level (P<0.05). These results would indicate the existence of great variability in salinity tolerance among tall fescue cultivars evaluated and that the presence of the endophyte would generate an additional cost in the host genotype at germination stage.

**Keywords:** *Festuca arundinacea, Epichloë coenophiala*, seed, germination energy, germination power.

# GENETIC DIVERSITY FOR GERMINATION IN RESPONSE TO TEMPERATURE IN A SET OF LUCERNE VARIETIES

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## Abstract

A large genetic diversity has been evidenced in the *Medicago sativa* complex for germination in response to temperature (Ghaleb et al., 2020). Here, we focused on a large set of 391 varieties cultivated worldwide. Their germination at 5, 15 and 34°C has been tested. The variation has been compared to the geographic origin of the varieties, their adaptation to climatic conditions (autumn dormancy) and their registration date. A significant genetic diversity has been evidenced. Non dormant varieties had a good germination at 34°C while dormant varieties were able to germinate at 5°C. The results offer prospect to improve the germinability of lucerne varieties to adapt this crop to variable temperature conditions that happen during sowing times.

Keywords: Medicago sativa, genetic diversity, lucerne, alfalfa

# SPRING MOWING AS A TOOL FOR CEREAL VOLUNTEER CONTROL IN RYEGRASS

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## Abstract

The most frequent preceding crop for Italian Ryegrass and Perennial Ryegrass in the Czech Republic are winter barley or winter wheat. Cereal volunteer is that common "weed" in Ryegrass seed crop and thus decreased the Ryegrass seed yield or threatens the certification of seed. In field trials conducted on Grassland Research Station at Zubří in 2013-15 the methods for cereal volunteers control in ryegrass seed crops were evaluated. In trial was assessed the mechanic way of volunteer control - mowing with low stubble (3 cm) in two terms, compared with pesticide applications (glyphosate, metribuzin). The plots seeded by winter barley and winter wheat compared with plots without cereal contamination were used in trials. The best method for cereal volunteer control was mowing in first term - 10 days after spring growth revival, when was achieved 90-95% effectiveness in volunteers control and no effect on Ryegrass seed yield. Mowing at end of tillering has best effectiveness on volunteer control (98-100 %), but also caused the seed yield decreasing. From herbicides the best volunteers control was achieved in metribuzin in dose 560 g a.i. per hectar, whose effectiveness reached 68–73 %. On plots treated by glyphosate in dose 300 g a.i. per hectar or metribuzin in dose 350 g a.i. per hectar was effectiveness only 60-68 %. The seed yield decreasing of ryegrasses caused by cereals volunteers amounting to 25-30 % was observed. When the mowing was provided in first term, the seed yield of Italian ryegrass was higher about 6-10 % compared with untreated control without cereals volunteers and on control level in perennial ryegrass. Mowing on end of tillering caused seed yield decreasing of Italian Ryegrass and Perennial Ryegrass for 15 % and 30 % respectively, compared with control plots without cereals. However, seed yield of both Ryegrasses, which were mowed at end of tillering, on plots contaminated by cereal volunteers were on the same level compared with control plots with cereal volunteers. Treatment by metribuzin or glyphosate caused Ryegrasses seed yield declination for 17-27 % or 60-63 % respectively. Based of trial results, the mowing of Ryegrasses in early spring is effective tools for cereal volunteer control in Ryegrasses seed crop in the czech conditions. This method is widely used in practice now.

Keywords: wheat, barley, mechanical control

## IPM STRATEGIES TO REDUCE WEED INFESTATIONS IN GRASS SEED CROPS

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## Abstract

Many annual grasses are difficult or impossible to control chemically in grass seed crops. At the same time seed characteristics make it difficult to separate the seeds from some of the cultivated grasses in the cleaning process. A main issue in an integrated strategy is therefore to reduce or eliminate the seedbank of weed species that possesses the above mentioned characteristics before the grass seed crop is established. This article gives an overview of occurrence of important annual grasses as weeds in grass seed production. 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 allows the volunteer seeds to be left as long as possible at the soil surface or in stubble favours a fast decrease of the soil seedbank of the investigated annual grasses.

Keywords: weed control, grass weeds, IPM strategy, seed survival

## TOLERANCE OF ESTABLISHED TIMOTHY (*PHLEUM PRATENSE*) SEED CROP TO HERBICIDE CIRPREME XC IN THE PEACE RIVER REGION OF CANADA

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## Abstract

Timothy is the third largest grass seed crop grown in western Canada. Timothy seed growers and companies require registration of herbicides that are safe on stands to ensure a high quality seed production. Cirpreme XC (florasulam+halauxifen+ clopyralid) is a Corteva Agriscience herbicide for annual and perennial broadleaf weed control in wheat and barley. Cirpreme XC is a mixture of two Group 4 (halauxifen+clopyralid) and one Group 2 (florasulam) active ingredients providing a wide spectrum of weed control. The active ingredient halauxifen is relatively new and works well across variable weather conditions and weed stages. It is particularly strong on cleavers even at later growth stages. Cirpreme XC provides control of many hard-to-kill broadleaf weeds including Canada thistle and scentless chamomile, and also provides suppression of night-flowering catchfly and white cockle. Cirpreme XC also controls barnyard grass. The addition of MCPA ester to Cirpreme XC increases the number of broadleaf weeds controlled.

Five field research trials were conducted on growers fields in the Peace River Region of Alberta from 2018 to 2022 to evaluate the tolerance of established timothy seed crops to Cirpreme XC with and without MCPA ester. The experimental design was a randomized complete block design with four replications. Cirpreme XC was applied at 1x and 2x registered rate used in wheat and barley. Cirpreme XC 1x was also applied with MCPA ester. Spring applied Cirpreme XC at 1x and 2x registered rates used in wheat and barley crops applied to established timothy did not result in any visual damage at all five sites. There were no significant differences in seed yields between the check and both rates of Cirpreme XC with or without MCPA ester at all five sites. Although a non-significant variation in seed yield between Cirpreme XC+MCPA ester and the check were observed, there was a strong trend of seed yield reduction with the addition of MCPA ester to Cirpreme XC at four of the five sites. None of the Cirpreme treatments with or without MCPA ester affected seed germination and 1000 SWT.

In conclusion, Cirpreme XC alone shows good potential for use on timothy grown for seed production and should be considered for a User Requested Minor Use Label Expansion. The addition of MCPA ester to Cirpreme XC 1x appeared to lower timothy seed yields and should likely be avoided unless necessary to control specific weeds.

Keywords: Timothy, Cirpreme XC, MCPA ester, seed yield

## INTEGRATED APPROACH TO MANAGE YELLOW DWARF VIRUSES IN PERENNIAL GRASS SEED CROPS

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#### Abstract

Oregon's grass seed industry, valued at over \$500 M USD, is the top producer of cool-season grasses worldwide. Perennial ryegrass (Lolium perenne L.), tall fescue ([Schedonorus phoenix (Scop.) Holub]), and Kentucky bluegrass (Poa pratensis) represent the majority of grass seed land area in the Willamette Valley (perennial ryegrass, tall fescue) and eastern Oregon (Kentucky bluegrass). Anecdotal reports have attributed seed yield loss and reductions in stand longevity to aphid-transmitted yellow dwarf viruses (YDV). Effective integrated pest management (IPM) strategies have not been thoroughly studied for YDV in grass seed crops. To develop a robust IPM program for this virus-vector complex, a commercial field survey and three-year field trial were initiated in 2021. Field surveys to assess the spatiotemporal distribution and risk factors associated with YDV began in autumn 2021 and continued through autumn 2022. A total of 57 commercial fields (perennial ryegrass, tall fescue, Kentucky bluegrass) across Oregon were monitored weekly for alate aphids using sticky traps (May to November) and sampled for plants and apterous aphids two weeks after the peak spring or autumn aphid flight. Samples were collected by walking four 100 m transects per field. For plant and representative aphid samples, total nucleic acid extractions were conducted, followed by polymerase chain reaction (PCR) to determine YDV genera (Luteovirus or Polerovirus) presence and, if positive, YDV species. Environmental raster data were extracted, and management history metrics were recorded for sampled fields. Across the 57 fields, seven YDV species were detected, and considerable variation in YDV incidence was observed at the field scale. Total aphid alate abundance ranged from less than 100 total aphids across the sampling period to over 2000, with YDV viruses detectable in aphid populations. Environmental and management risk factors associated with aphid abundance and YDV incidence and diversity are being evaluated with spatial models. The field trial was planted with two perennial ryegrass cultivars ('Fastball' and 'Top Gun II'), and the effects of low and high nitrogen (N) rates (135 and 225 kg N ha<sup>-1</sup>) and insecticide spray timing (untreated control, autumn, spring, autumn + spring, or autumn + spring + summer) using 175 g flupyradifurone ha<sup>-1</sup> were evaluated for aphid-YDV suppression. Aphid abundance of alate and apterous morphs were monitored weekly throughout the growing season on sticky cards and within plots using sweep net sampling. Plant samples were collected before insecticide treatments were applied by randomly collecting five leaves per plot. Both aphid and plant samples were evaluated with PCR for YDV genera and species. Although differences in clean seed yield were not detected in the first-year harvest, greater YDV incidence was observed in high N plots and 'Fastball' plots. Moreover, all insecticide application timings were associated with lower YDV incidence compared to the untreated control, albeit insecticide treatments did not lead to complete protection for any treatment. Research findings from regional field surveys, geospatial risk models, and small-plot field trials will be leveraged to optimize management recommendations for aphid-vectored YDV in grass seed production systems.

**Keywords:** Grass for seed, yellow dwarf virus, integrated pest management, aphid-vectored plant viruses, risk description

# THE CLOVER HEAD WEEVIL (*HYPERA MELES*) A PEST IN WHITE CLOVER SEED

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Keywords: White clover, Hypera meles, Bathyplectes curculionis

#### Abstract

In Denmark white clover seed production is challenged by two weevil pests, the white clover seed weevil (*Protapion fulvipes*) and the clover head weevil (*Hypera meles*), combined the two pests can reduce seed yields significantly.

Through a number of years the later pest and its naturally occurring parasitoid *Bathyplectes curculionis* has been studied with the intent of evaluating if the parasitoid can be used as a biological control agent. An initial project focused on sorting and storage of the parasitoid cocoons, with subsequent release of the parasitoid in the forthcoming seed field. A later project focused on collecting more data on the parasitation rate when releasing the parasitoid and if the effectiveness of the adult parasitoid could be enhanced.

In 2019 the pest was found in quantities not previously experienced. This led to activities trying to map the sensitivity of populations of the clover head weevil to the primary utilised insecticide (a.i. lambda-cyhalothrin). Populations were collected within traditional areas of white clover seed production and in areas with sparse production.

We would like to present our results from working with the challenges and management opportunities of the clover head weevil in white clover seed production.

#### **Summary of findings**

The initial project showed that it was possible to sort, store and reintroduce the cocoons of the native parasitoid. In the initial project, parasitation rates were found to increase 11% when the parasitoid cocoon was released, compared to a control without release. Later releases from Aarhus University (AU) 2020 showed parasitation to increase by 9%. Combined results from AU and DLF seeds, which began releasing cocoons on their own in 2021, showed an increase in parasitation of 7% and 2% for 2021 and 2022 respectively.

It seems that the parasitoids host range is limited to the genus *Hypera* and therefore in Denmark associated with white clover seed fields. Samples of the harvested material from 143 white clover fields (2019) showed a linear correlation (R2 0.5, P-value  $<0.01^{**}$ ) between the number

of parasitoid cocoons to the number of fields per region (postal code) i.e. a high density of fields resulted in a high number of cocoons. Currently analysis of samples from the 2021 and 2022 harvest are under way. In areas with high concentration of white clover seed production the background population of the parasitoid is high. In regards to how releases increase the parasitation, the result becomes difficult to interpret as location and field history become factors of importance.

The response of adult clover head weevil to lambda-cyhalothrin was in 2021 and 2022 evaluated based on the IRAC method 011. Populations were collected from 54 fields of which 12 were organic. Populations from conventionally grown fields showed 93% to be resistant or highly resistant, 5% were moderate resistant and 2% were susceptible. For organic fields 58% were resistant or highly resistant, 33% moderate resistant and 8% susceptible. The results indicate that the pest cannot be effective controlled with the insecticide most commonly used.

# ESTABLISHMENT OF PERRENIAL RYEGRASS (Lolium perenne) WITH GLUFOSINATE TOLERANT CANOLA (Brassica napus) IN WESTERN CANADA

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## Abstract

Perennial ryegrass (Lolium perenne) is grown for seed production in annual crop rotations throughout western Canada. Traditionally it is seeded with wheat in year one, harvested for seed in year two and then taken out of production and the field is placed back into an annual crop. Canola has always proven to be an excellent annual crop for establishing grasses as canola is less competative than cereals. In addition volunteer canola is easier to control than volunteer cereals the following year. Unfortunately, herbicides compatibility with both the canola and seedling grass is limited. Glufosinate tolerant canola varieties make up 65% of the canola acres seeded each year in western Canada. The objective of the study was to evaluate the effects of different rates and timing of glufosinate applications on perennial ryegrass seeded with glufosinate tolerant canola. One trial in each year of 2020, 2021 and 2022 was conducted in growers fields. Growers seeded glufosinate tolerant canola at a seeding rate of 12.5 kg/ha and turf type perennial ryegrass at a seeding rate of 25 kg/ha. The perennial ryegrass and canola was seeded in the same row. The experimental design was a randomized complete block design with four replications. Various rates of glufosinate were applied when canola was at the 2 to 4 leaf stage and perennial ryegrass at the 1-2 leaf stage. Treatments requiring a 2<sup>nd</sup> application of glufosinate were applied 14 days after the first application just prior to the canola bolting and the perennial ryegrass at the 2-3 tiller stage. Visual percent injury ratings were taken 28 DAT, following canola harvest and once again the following spring. Visual percent stand reduction ratings were taken after canola harvest and the following spring. Seed yield data was not collected. Single applications of glufosinate at 410 g ai/ha or 480 g ai/ha caused some very slight leaf burn but did not visually reduce plant populations of seedling perennial ryegrass. Glufosinate applied at the 2x rate of 960 g ai/ha did cause significant visual injury and visual stand reduction to perennial ryegrass. Two applications of glufosinate at 410 g ai/ha or 480 g ai/ha caused slight injury and visual plant stand reduction to seedling perennial ryegrass, but the stand fully recovered by harvest. Results from this study show perennial ryegrass can be established successfully with glufosinate tolerant canola if a single application and lower rates of glufosinate are used. A second application of glufosinate should be avoided unless necessary for controlling weeds. The majority of perennial ryegrass grown in western Canada is now established using this method.

Key Words: perennial ryegrass, canola, glufosinate, visual percent stand reduction

## **PRODUCTION QUALITY ASSESSMENT - DODDER IN ALFALFA SEED CROP**

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#### Abstract

Forage (grasses and legumes) and turf seed production take place in France under a contractual framework with an obligation to produce results in terms of seed batch quality. The seeds growers deliver the post-harvest raw seed batch to the company. A representative sample of this batch is then submitted for approval to verify that the standards (after sorting) laid down in the multiplication contract are met. The approval analysis determines the quality of the raw seed batch from a representative sample according to three criteria: purity, determination (of certain weeds) and germination capacity.

The farmer's income is determined by these results. Only the net quantity of seed brought back to standards is paid. The standards for batch certification are referenced by SEMAE, French Seed Interprofessional Technical Regulation. More restrictive standards may also be set by the company in the multiplication contract, and they are usually valued by quality bonuses added to the remuneration. Seed batch approval are performed by companies in their own accredited analytical laboratory, or by independent laboratories such as LABOSEM.

Each year LABOSEM analyses about 3500 forage seed samples of all species (mainly 12). The compilation of the approval results for the period 1996-2021 represents nearly 100 000 samples. As part of its inter-professional technical program, FNAMS analyses these data to identify and quantify the evolution of the main weeds present in forage seed batches in each production areas (8 in France).

Here we propose to focus on the presence of dodder in alfalfa seed batches. This unwanted species, that grows upon alfalfa among other plants, is regulated with a "0" standard during plant growing (0 plant with dodder in the plot) and in the sorted batch (0 dodder seed on two 100G sample). The chemical field control method is very limited, with only one effective active substance, pendimethalin (+imazamox) (PROWL 400, NIRVANA S). An adapted seed sorting process by the laboratory or the seed company is essential. During sorting, LABOSEM performs a count of *Cuscuta epithymum* L. (seed diameter < 1mm) and *Cuscuta campestris* Yuncker (seed diameter  $\leq 1.3$ mm) on a 500G sample.

After sorting, no certified batch exceeds the "0" (mandatory) standard. The presence of dodder in seed batches is visually assessed during sorting, after passing through the separator. An average of 9% of the harvested batches (farmer plot) present at least 1 dodder seed. *Cuscuta epithymum* is detected on average in 2% of batches with a variation of 0 to 5% depending on the year. A downward trend is recorded since 2010. *Cuscuta campestris* is present on average in 6% of the batches with a variation from 3 to 8% depending on the year. The two species of dodder are very regionalized: *Cuscuta epithymum* is mainly present in west of France (Poitou-Charentes while it is in the southwest that *Cuscuta campestris* is most frequently found. This analysis makes it possible to draw up a qualitative assessment of production and to study the changes over time in relation to the conditions of production among farmers, in order to assess precisely the difficulties and prospects of production.

Keywords: seed production, alfalfa, analysis grading, dodder

# ANALYSIS OF EXPLANATORY FACTORS FOR GRAIN YIELD OF ALFALFA (MEDICAGO SATIVA L.) IN FRANCE

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# Abstract

Seed alfalfa (*Medicago sativa* L.) is an emblematic crop of forage legume seed production in France. However, this species has experienced production difficulties in recent years, particularly in terms of grain yield. Several factors are involved to explain yield, in particular the presence of insect pests, which are generally quite numerous on this crop. In order to help alfalfa seed producers improving their production and reasoning out the use of insecticides, a national network for monitoring insect pests in fields has existed in France for more than 30 years. This network collects data on the presence of insects in fields over a period between April and August each year, i.e. between the spring vegetative growth and the ripening of the pods.

The data collected between 2006 and 2019 were compiled, representing a database of more than 900 sampled plots. Statistical analysis has been carried out by comparing different models of linear or random forest types, in order to highlight which factors would best explain variations in grain yield. Among the insect pests, mirid bugs (*Lygus* spp. and *Adelphocoris lineolatus* Goeze), tychius (*Tychius aureolus* Kiesenwetter) and weevils larvae (*Hypera postica* Gyllenhal) seemed to be the most damaging. Other factors affecting yield include variety, weather conditions (temperature and rainfall) and farmers' technical skills. Nevertheless, despite the large number of explanatory variables available for the analysis (31 variables), the predictive quality of the different statistical models used remains average. This indicates that other explanatory factors not available in the dataset are likely to be important in understanding the variation of alfalfa grain yield.

Keywords: Alfalfa, seeds, grain yield, insect pests

# STEM RUST OF PERENNIAL RYEGRASS, HOW EFFECTIVE ARE BIOCONTROL AND ALTERNATIVE SOLUTIONS AGAINST THIS DISEASE?

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## Abstract

Stem rust, caused by *Puccinia graminis*, is a detrimental disease to the yield of seedling perennial ryegrass. In France, the management of this disease is currently ensured by a biomonitoring network (established in the most important production areas) and fungicide protection. The latter is based on the application of triazoles or a combination with a strobilurin or an SDHI as soon as the first pustules appear on the stems and renewed if necessary. With a view to reducing the use of conventional fungicides, FNAMS has evaluated various biocontrol and alternative solutions against stem rust in ryegrass. During four campaigns (between 2018 and 2021) & 2 sites, 8 trials have been conducted on perennial ryegrass with sensitive stem rust cultivar (BARILLON 2018-2019-2020), and medium sensitive (ETERLOU 2021); six products were evaluated on four replicated plots. Disease pressure was variable between years and sites. As a result, depending on the year and site, three products (Veg'lys, LBG-01F34 and 030-P-3-D) showed statistically efficacy results but lower effects compared with the chemical reference. This could open up the possibility of introducing these products into the perennial ryegrass protection program against stem rust on low stem rust sensitive cultivars.

Keywords: ryegrass, biocontrol, stem rust

# ALFALFA SEED PRODUCTION WITHOUT CHEMICAL PESTICIDE

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## Abstract

Less and less pesticides are approved for seed production in France. In the aim of testing seed production without pesticide and evaluating the consequences, a 'system experimentation', a long-time experimental trial named AgroSem, is conducted by FNAMS (French seed growers association). This trial (from 2019 to 2026) is located on 3 places (Castelnaudary and Condom in the south-west of France and Brain-sur-l'Authion in west-Center of France). It's based on a long crop rotation (8 years) with 8 different seed crops: wheat; spring barley or buckwheat depending on the site; sugar beet or garden beet; carrot, onion or bean; tall fescue and alfalfa. Seed crops are grown on one replication long strip (10-12 m wide to 120 m long). To help auxiliary insects' development, flower strips and grass strips has been sown between the crops. Only biocontrol or organic pesticides are allowed in this experiment.

In spring 2019 to 2022, alfalfa was undersowned with a cereal cover crop (wheat or spring barley) before the cereal joint stage. But this one was too competitive on 8/12 crops for the young alfalfa due to cold temperature, water and light competition, and alfalfa had to be resown on bare soil in late summer.

In order to manage weeds, the cereal cover has been helpful, but only when alfalfa succeed to install in the cereal crop. Hoeing was necessary in autumn and spring depending on the number of weeds. Two vibrashank shallow pass after cutting were also efficient. *Helminthotheca echioides, Sonchus oleraceus* and *Rumex crispus* were the most frequent and potentially problematical weeds. Some manual weeding was used to take off *Rumex* or *Helminthoteca* (between 5 to 21 h/ha). In general, mechanical weeding and alfalfa shredding were successful to avoid weed seeds in the final alfalfa harvest sample (all the purity analysis were below the 97% standard).

To prevent pests *Apion pisi* and *Hypera postica* pullulations, alfalfa was often cut lately, between mid-May and early June. And due to a good water holding capacity of the soils, alfalfa regrowed correctly after cutting. Depending on the area, the *Tychius aureolus* populations could be important or not during the blooming period. In 2 cases, they were killed by a Spinosad application (registered on French organic production). For the 7 other crops, no pesticide was used. Rust appeared rarely, at the end of the cycle on 2/9 cases thanks to low sensitive cultivar. The alfalfa seed yields were pretty good and most of the time upper than the French national mean yield (AgroSem's mean yields: 563 kg/ha in 2020, 475 kg/ha in 2021, 329 kg/ha in 2022). For 9/9 alfalfa seed production, the germinations were upper than the 80% standard (91.2 % in average).

AgroSem economical results were compared to FNAMS production cost reference on nonorganic alfalfa seed production (surveys made on the 2019 harvest). They show a labour time higher on AgroSem's alfalfa seed crop compared to the reference costs: 13h/ha for AgroSem's average (from 6 to 32 h/ha when manual weeding against *Helminthotheca* is important) vs 7h/ha average in the surveys (chemical production). The AgroSem's production costs will be presented.

Keywords: system experimentation, zero pesticide, alfalfa seed production

# ALTERNATIVE WEED CONTROL IN PERENNIAL RYEGRASS SEED CROP

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## Abstract

In seed grass production, the certification standards are increasingly difficult to reach in view of the growing difficulties of weeding (few herbicides available, resistant weeds...). The example of perennial ryegrass seed crop is developed here, a species for which there are few effective chemical solutions against black grass (*Alopecurus myosuroides*) (seeds not sortable at the plant in the seed lots), but also against some dicotyledons.

For this crop carried out at low inter-rows, mechanical weeding such as hoeing is not easy to set up. A study aims to look at alternative solutions such as: undersowing in a spring cover crop, association with companion crops (for faster ground cover) and mechanical weeding (hoe, weeder harrow, etc.).

This type of trial was set up for 2 years on a single site. The trial included 6 modalities without repetition, with plots of approximately 300 m<sup>2</sup>. Two first modalities concerned undersowing in a spring cover crop: peas or buckwheat. The two others are sowed early in bare soil in summer (July-August) in association with a no frost-resistant plant: mustard (year 1), fenugreek (year 2) and foxtail millet (*Setaria italica*) (both year). These four modalities are coupled with passages of mechanical weeding tools. The last two modalities are sowed in bare soil at the end of summer (September), one will be managed only with mechanical weed control and the other with chemical herbicides and mechanical weeding.

Soiling during cultivation is observed on these 6 plots, as well as selectivity on perennial ryegrass. At the beginning of spring, dry matter is carried out on the crop and on the weeds. Seed yield was measured by harvesting each plot, cleaning at the laboratory, with sample for specific purity analysis and germination.

The main conclusions of this trial are:

- the difficulty of carrying out a passage of the hoe at 12.5cm spacing;
- the method under cover allow a significant limitation of weeds, in particular black grass, but the yield of ryegrass seems to be strongly impacted by this practical, especially with a cover crop of buckwheat.

**Keywords:** perennial ryegrass, black grass (*Alopecurus myosuroides*, alternative weed control, mechanical weeding

# GENETIC DIVERSITY OF *PUCCINIA GRAMINIS* F. SP. *LOLII* CAUSING STEM RUST ON PERENNIAL RYEGRASS IN THE WILLAMETTE VALLEY OF OREGON.

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### Abstract

Stem rust disease caused by Puccinia graminis f. sp. lolii (Pgl) can cause complete crop failure in perennial ryegrass grown for seed. The disease consistently occurs in the Willamette Valley of Oregon, the primary seed growing region in the U.S., and is effectively controlled with fungicides. Genetic resistance is known and is a viable control measure when it is bred into commercially available cultivars. Genetically diverse plant pathogens have a greater chance of overcoming any control measure and adapting to changes in the production environment. Variable plant pathogens may require multi-tactic disease control approaches. Little is known about the genetic variation of Pgl, and the objective of this study was to assess the genetic diversity of Pgl in the Willamette Valley of Oregon. Ninety-one single infection stem rust pustules were collected from perennial ryegrass at six field sites spread from the North to the South Willamette Valley. Sample sizes from each site ranged from five to 48. The DNA from each pustule was extracted and used to amplify 21 simple sequence markers in six multiplexed PCR reactions of up to four markers per multiplex. Amplification fragments were sized on an AB 3730 capillary DNA sequencer and alleles were scored manually using Geneious Prime software v. 2022.0.2. Data was analyzed using the Poppr macro in R. Nei's gene diversity index was calculated for each marker as a measure of diversity. Clones were identified within the population to determine the number of multi-locus genotypes. Rarefaction curves were used to determine the expected number of multi-locus genotypes (eMLG) from each site, a number adjusted for differences in site sample size. A dendrogram was drawn using Nei's genetic distance with 200 replications on 20 random samples to evaluate clustering by collection site. The 21 markers generated 3 to 7 alleles each, with Nei's gene diversity ranging from 0.02 to 0.63. The mean number of alleles for this group of markers was four with a genetic diversity of 0.39. Thirty-two multi-locus genotypes were identified across all sites and the eMLGs for each site were similar, ranging from three to 6.45. The dendrogram clustered most isolates into a single group, with a few genetically distinct isolates separating from the main cluster. Pgl samples from a common site did not group together on the dendrogram. These results demonstrate that the Willamette Valley population of Pgl from perennial ryegrass has genetic variation. Most of the genetic variation is within collection sites and there is no evidence of unique subpopulations of Pgl occupying specific Willamette Valley locations.

Keywords: Stem Rust, Perennial ryegrass, simple sequence repeat, diversity

# EFFECT OF STORAGE TIME ON GERMINATION AND SEED-BORN FUNGI OF FORAGE OAT SEED

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# Abstract

Oat (Avena sativa L.) is one of the main forage crops in northwestern China, usually used to make hay and silage. The content of crude protein and fatty acid in oat seed is much higher than that in other cereal crops, indicating its higher nutritional value. But this also affects the storage time of the seed. In this paper, seed of three oat varieties (L2, B4 and B7) harvested in the same field of Laozhan Village (2350m above sea level), Huajialing Township, Tongwei County, Gansu Province of China and stored for 1, 2, 3 and 5 years at room temperature in the village were used to study the effect of storage time on seed germination and seed-born fungi. The result showed that with the increasing storage time, the germination rate declined and B7 had significantly higher germination rate (91.5% vs. 80.65 of L2 and 67.50% of B4) among the 3 varieties after 5 year storage, despite no significant differences were observed after 1-, 2- and 3-year storage. The seed electrical conductivity showed the similar results with B7 had the least  $(46.67 \,\mu\text{S/cm}, p < 0.05)$  value after 5 years storage. The seed-born fungi were isolated after seed surface sterilization with 1% NaOCl and identified by plate culture, morphological identification, and rDNA-ITS sequence analysis. A total of 28 species from 13 fungal genera were detected, the most commonly detected were Alternaria sp and Aspergillus sp, and the dominant species varied with storage time and varieties. As many as 10 species were isolated from L2 stored for 2 years, while only 4 species from B7 stored for 5 years. The most aboundant species in L2 seeds under the four storage treatments was Alternaria alternate, with 35.08% isolation rate. Drechslera avenae and Pyrenophora avenae were only detected in L2 seeds stored for 5 years. Alternaria brassicae and Alternaria porri had higher aboundance in B4 seeds after one year storage, and when stored for 5 years, Aspergillus flavus had Cladosporium cladosporioides were the most aboundant. When the seeds of B7 were stored for one and two years, Aspergillus niger was the dominant with 32.86% and 37.92% isolation rate. Aspergillus fumigatu (25%) and Lecanicillium aphanocladii (16.67%) had higher aboundance in the seeds of B7 stored for 5 years. This study demonstrated that the differences of varieties and storage time are the main factors affecting seed germination and changes of seed-born fungi of oat.

Keywords: oat seed, storage time, seed germination, seed-born fungi

# THE IMPACT OF DIFFERENT NON-THERMAL POST HARVEST RESIDUE MANAGEMENT PRACTICES ON A TALL FESCUE (*Festuca arundinacea*) SEED CROP

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## Abstract

Tall fescue seed crops require post-harvest residue management to prepare the crop for the following season. Previously farmers relied mostly on burning residue, but due to a change in social license around air quality and public safety concerns, there has been a reduction in this management practice. The objective of this research was to investigate non-thermal postharvest residue management practices required to maximise seed yield for a tall fescue seed crop with full post-harvest straw load retained. The experiment was conducted on a crop of continental tall fescue (cv. Volupta) entering its third year of seed production based in Barrhill, Methven, New Zealand. Plots were either grazed using store lambs and calves or mown to replicate balege cuts. In the absence of grazing, the most effective method of residue management was to leave the crop stubble at a post-harvest height of 14 cm, and to mow the crop either under a frequent (monthly) or lax (once at closing) regime. The frequency of the mowing treatments had no effect on seed yield. These treatments produced an average seed yield of 435 kg/ha. This suggests that a grower could take monthly balage cuts to diversify income from the seed crop without negatively effecting seed yield. Under grazing, seed yields were highest in plots left with 14 cm of stubble after harvest, with an average seed yield of 327 kg/ha. The initial hypothesis was that opening up the sward, would allow more light into the canopy and therefore increase the final seed yield. The results contradicted this in that the plots cut to 7 cm produced significantly lower seed yields than the plots cut to 14 cm after the inital seed harvest. The lower seed yield in the cutting to 7 cm and the nil cut treatments, was a result of a lower number of fertile tillers. For the former, cutting removed all of the leaf tissue, while in the latter, plants were shaded. Both reduced the leaf surface area for photosynthesis and therefore the ability to support tiller initiation and growth.

Keywords: seed production, tiller production, grazing, cutting, stem reserves

# EVALUATION OF PRE-HARVEST DESSICATION STRATEGIES IN CLOVER SEED PRODUCTION

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## Abstract

Desiccation with diquat about one week before seed harvest has up to now been the common practise in Norwegian clover seed production. However, after withdrawal of diquat in 2020, clover seed growers no longer have any desiccators available to dry down their crops before harvest. In 2019 and 2020, six field trials in red clover and two field trials in white clover was carried out to evaluate various alternative chemical products at different rates and at two different spraying dates, either early at 50% mature heads and / or late at 65% mature heads, i.e. about 14 and / or 7 days before seed harvesting. Products included in the study, either for one or two years, was Spotlight Plus (carfentrazonethyl), Beloukha (pelargonic acid), Glypper (glyphosate), Gozai (Pyraflufen-ethyl), Harmonix LeafActive (acetic acid), Harmonix FoliaPlus (pelargonic acid), Flurostar (fluroxypyr) and Saltex (sodium chloride) and liquid fertilizers (e.g. urea). In addition, swathing was examined as an alternative to desiccation in two red clover trials in 2020. Despite none of the tested chemical products considered being superior to diquat, the most promising overall alternative desiccants was the two Harmonix-products, either FoliaPlus and LeafActive in red clover or FoliaPlus in white clover, that after an early and late spraying visually wilted the plants most in the experiments in 2020. Although normally less effective than the two Harmonix-products, also Beloukha alone, especially when sprayed in two rounds (early and late), showed an acceptable desiccation effect. Another promising strategy was the combination of an early spraying with Glypper (glyphosate) followed by Beloukha a week later. However, as the glyphosate spraying affected germination negatively in some of the trials, the method seems too uncertain to recommend. None of the other products (Gozai, Spotlight Plus, urea, Flurostar, vinegar or Saltex) desiccated the plants sufficient to be consider for use in clover seed production. It is also concluded that swathing before harvest, using finger bar cutters, is an effective drying method under favourable weather conditions in clover seed production.

Keywords: chemical desiccation, lodging, seed harvest, seed yield, swathing

# SEED HARVESTING DATE PREDICTION IN Urochloa spp. BASED ON GROWING DEGREE-DAYS ACCUMULATION

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#### Abstract

Seed shattering is a natural process affecting seed harvesting management in tropical grasses. Anthesis prediction for tropical Urochloa species is of great importance in order to increase seed production and carry out effcient seed harvesting with uniform seed maturity and quality. The objective was to define the starting moment as well as the maximum peaks for seed shattering in Urochloa species, based on growing degree days (GDD) information. The study was carried out in the experimental area of Semillas Papalotla SA de CV in Chiapas, México, during 2020's growing season. Climate records were obtained using an Oregon Scientific Weather Station (Mod. WRM 300) located within the experiment station. The study was carried out in 2 x 5 m experimental units sown in furrows for Urochloa hybrids (GP3025, GP0423) and a cv. Humidicola grass, all stablished during 2019. Experimental units were cut and ten days later fertilized with nitrogen, phosphorus and potassium at 150-80-50, in accordance with management protocols recommended for the research area. Plants were enclosed using nylon sieves for daily seed collection during 35 days. Data was registered from 1 linear meter and 1  $m^2$  for hybrids and humidicola, respectively. Harvested seed was sun dried and cleaned using a forced air seed blower (DE LEO) and weighed on analytical balance. Growing degree days were calculated using the fornula (Zolom et al., 1983):  $GDD = \sum [(T - Tb) - C];$  Where; T = mean temperature; Tb = base temperature (10 °C; Jensen et al., 2013), C = correction coefficient, when temperature is above 40 °C. A completely randomized experimental design was used with three replicates and mean comparisons using Tukey (P < 005). Different GDD requeriments were observed among plant materials as consequence of phisyological processes (P < 0.05). Humidicola started seed shattering at 749.3±2 GDD and the maximum fall seed ocurred at 837.1±2, the Urochloa hybrid GP3025 started seed shattering at 658.5±3 GDD and the maximum seed shattering was recorded at 749.3±3 GDD; GP0423 hybrid started seed shattering at 1275.1±4 GDD with maximum seed shattering in the same date. Variability for seed shattering may improve seed harvesting management for this species. Delayed shattering indicates longer vegetative stage for GP0423, important as forage cultivar. Different GDD requirements were recorded for crops evaluated and maximum seed shattering was observed within five days after seed shattering start.

Keywords: Urochloa hybrids, seed production, growing degree days

# YIELD VARIATION, YIELD COMPONENTS AND YIELD LOSSES IN RAINFED TALL FESCUE AND ANNUAL RYEGRASS SEED PRODUCTION IN SUBTROPICAL URUGUAY

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#### Abstract

Rainfed field seed yields for tall fescue and annual ryegrass in Uruguay are estimated to be close to 0.33 and 0.98 tt seed/ha, respectively. These values are far lower than those reported for other regions worldwide, what might reflect the subtropical climate, less adjusted crop management or greater losses during harvest. To better understand these differences, we quantified seed yield of 20 tall fescue and annual ryegrass commercial crops in southwestern Uruguay to assess what yield components explain yield variation, and to determine the magnitude of losses between yield estimated by manual harvest at optimal cutting time (OCT) and effectively harvested field yield. In this survey of more than 800 has of rainfed commercial crops, yields estimated at OCT were as high as 1.4 and 4.1 tt seed/ha for tall fescue and annual ryegrass, respectively. These are values comparable to those achieved in more temperate climates, particularly for annual ryegrass. Variation in yield at OCT was largely unrelated to variation in thousand's seed weight, which ranged between 2.0 and 2.8 g. Therefore, the main determinant of yield was the number of seeds per m<sup>2</sup>. We found no evidence of an inverse relationship between these two yield components. As a consequence, yield appears to be largely determined by management factors that determine both the number of panicles (tall fescue) or spikes (ryegrass) per m<sup>2</sup>, and the number of seeds per panicle or spike, rather than by conditions during the filling period affecting individual seeds' weight. High numbers of seeds per m<sup>2</sup> were associated with crops that attained some 500 panicles or 800 spikes per m<sup>2</sup> and 160 seeds/panicle or 200 seeds/spike. Despite the comparatively high generated yields, effectively harvested field yields were far lower, averaging 0.4 tt seed/ha in tall fescue and 1.3 tt/ha in annual ryegrass, close to the national average for the 2018-2021 period of 0.33 and 0.98 tt seed/ha for tall fescue and annual ryegrass, respectively. Therefore, a substantial loss of yield was observed between yield estimated manually at OCT and the effective mechanically harvested field yield: between 40 and 60% in tall fescue, and between 30 and 60% in annual ryegrass. What caused such losses is unclear, but could involve the effect of delayed timing in cutting (windrowing) and in harvesting the swath, which would both lead to dehiscence associated with low seed humidity. These processes would determine loss of the earlier maturing, heavier seeds. Further losses could have happened inside the combine, which would involve blowing off lighter seeds. Once causes are identified and solved, there is a chance to increase the competitiveness of seed production of tall fescue and annual ryegrass in subtropical Uruguay by reducing yield losses.

**Keywords:** yield loss, yield gap, yield components, tall fescue, annual ryegrass, subtropical environment.

#### DOES TRINEXAPAC-ETYL IMPROVE LODGING CONTROL AND SEED YIELD IN KENTUCKY BLUEGRASS CROP IN NORTHEASTERN OREGON?

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#### Abstract

The utilization of trinexapac-ethyl (TE) plant growth regulator (PGR) is a common management practice to control lodging in cool season grass seed crops. However, limited research is available on the effect of TE on Kentucky bluegrass (KBG) (Poa pratensis) lodging, seed yield, yield components, and seed quality. The objective of this three-year study was to evaluate the effects of TE application on seed yield and quality of three different KBG cultivars, representing different turf-quality classes (BVMG, Midnight, and Shamrock), in northeastern Oregon. Application rates of TE included 0, 112, 196, and 392 g a.i. ha<sup>-1</sup> were applied at the second node growth stage (BBCH 32). The study was conducted in commercial seed production fields to evaluate tiller height, lodging control, panicles m<sup>-2</sup>, 1000 seed weight, seed yield, and seed viability. Results indicate that TE can effectively reduce tiller height by up to 34%. Lodging was completely controlled at the 392 g a.i. TE ha<sup>-1</sup> rate in all cultivars, but responses at lower rates varied by cultivar and year. The effects of TE rates on seed yield and panicles m<sup>-2</sup> were not consistent across cultivars or years. Seed quality (seed viability and 1000 seed weight) was not affected by TE. Seed yield increases were not achieved with TE application as previously reported for other species of cool season grasses grown for seed in Oregon, and elsewhere globally. Application of TE effectively reduces lodging in KBG, however, response varied by KBG cultivar. Midnight and Shamrock elite turftypes were more susceptible than BVMG types to lodging at standard use rates of 196 g a.i. TE ha-<sup>1</sup> or less, which indicates that additional study is needed to determine optimal TE applicate rates for these cultivars. The application of TE in KBG seed production is more suitable to optimize harvest efficiency, rather than increase seed yield, by reducing costs (time, labor, machinery maintenance) associated with the difficulties of swathing lodged KBG.

**Keywords:** Plant growth regulator, trinexapac-ethyl, Kentucky bluegrass, *Poa pratensis*, seed yield, seed quality, harvest efficiency.

# ALTERNATIVE PRE-HARVEST DESICCATION STRATEGIES FOR WHITE CLOVER SEED CROPS

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## Abstract

In New Zealand (NZ) white clover (*Trifolium repens* L) is usually direct-harvested following the application of a chemical desiccant. Common treatments are based on a single or double application of diquat (200 g/L Reglone<sup>®</sup>) and/or the phenoxy herbicide 2-methyl-4-chlorophenoxyacetic acid (MCPA) as a pre-desiccant. The aim of MCPA application is to fold/twist leaves to open the clover canopy and allow greater penetration of the diquat into the canopy prior to harvest.

In well-managed white clover seed crops, these desiccation treatments are very effective. However, some crops grow in excess of 6000 kg dry matter (DM)/ha and crop re-growth can occur following treatment, especially if it rains. In these 'bulky crops', the collapsing petioles can drag flower heads into a wet-decaying leaf mat. In addition, diquat has been banned in parts of Europe, and is amongst the chemicals under consideration for review by the Environmental Protection Agency in NZ. Therefore, there is a need to identify alternative crop desiccants for direct harvesting, and to find alternative options for use when environmental conditions are less favourable.

In five trials conducted throughout Canterbury, spanning 2017-2021, diquat applied alone consistently produced the highest yield of white clover seed or was among the highest yielding group of pre-harvest treatments. Over the five-year period the yield using diquat ranged from 384 - 850 kg/ha.

Alternative products including glufosinate, glyphosate, clopyralid and tribenuron-methyl resulted in similar seed yields to diquat alone or when using MCPA followed by diquat. However, such products reduced crop re-growth, with up to a 93% decrease in post-harvest DM production. Reduced DM is a constraint for integrated livestock systems where post-harvest re-growth is used for finishing lambs.

The fatty-acid organic desiccant, GreenMan<sup>TM</sup>, showed promise as an alternative to diquat. GreenMan<sup>TM</sup> was not an effective desiccant at the recommended 2-4 % dilution rates on clover, possibly limited by the water volume sprayed and the resulting coverage. However, when applied at 16% dilution rate, GreenMan<sup>TM</sup> became comparable with diquat during good harvest seasons.

Windrowing white clover is not common practice in NZ and in three field trials resulted in a 20-27% reduction in seed yield compared with direct-harvest following desiccation. However, if diquat is removed from the NZ market windrowing may be an option, particularly in crops with high vegetative bulk or during unfavourable harvest seasons.

This research reiterated the importance of diquat to the NZ white clover seed growing system, while identifying potential herbicide options for desiccation in the lead up to harvest.

Keywords: white clover, harvest, desiccation, diquat

# **OVERSEEDING A CEREALS AND PROTEIN CROP MIX IN ESTABLISHED ALFALFA: WHICH IMPACTS ON WEEDS AND PRODUCTION?**

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# Abstract

Alfalfa is a sensitive crop to weed soiling during the winter. This study investigates the impact to overseeding a mix of cereals and protein crop at the autumn to weed development during the winter and to the alfalfa annual production.

In 2022, three trials were carried out in west of France on established alfafa. Two trials were on forage fields and one trial was on seed production field. The two forage trials were conducted with 3 to 5 forage harvests, and the seed production trial with 1 forage harvest at the spring (cutting) followed by the seed harvest at the end of summer. This last one had also large inter row (50 cm) of alfalfa seedlings to allow seed production.

All of these 3 trials compared the effect of overseeding or not in two different harvest strategies: an early harvesting strategy based on alfalfa vegetative stage, and a classic harvesting strategy based on late bud stage (20 days later). Also, two different mixes were overseeded in early harvesting strategy.

One trial did not show any effect of overseeding on weed development and annual production. The two different overseeded mixs also show similar results, so the mix does not seem to be major factor.

However, the two other trials displayed interesting effect. The effect was not significant on weed quantities, but overseeding a mix seems to reduce weed quantities. On the seed production trial, there was a significant reduction weed population allowed by the overseeding. The harvest strategy did not impact weed development. On the first spring harvest for these two trials, the mix of cereals and protein crop also allowed to produce between 1.2 to 2.2 tons of dry matter per hectare more in early harvest strategy and 2.5 to 2.7 tons of dry matter per hectare more in classic harvest. These differences are statistically significant.

The gain of production earned during the spring was conserved in the annual production for the forage trial and did not impact the seed production for the second trial.

To conclude, this practice seems to be promising to enhance forage production and avoid weed concurrence. The trial which did not show any effect had a high forage yield of alfalfa and a lot of white clover, so they may not permit the weed and overseeding development.

To complete this first year of observation, three new trials are carried again in 2023.

Keywords: Alfalfa, overseeding, weeding

# SIMULATED ENVIRONMENTAL AND CLIMATIC EFFECTS OF PERENNIAL RYEGRASS SEED PRODUCTION

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## Abstract

As a part of the green transition of agriculture, grass production for protein refinement or bioenergy is a type of cropping system with the potential to combine agricultural intensification with reduced environmental and climatic impact. If such production systems increase in popularity, the demand for grass seed is likely to increase, and this production should also be carried out in a sustainable and environmentally friendly way. Since environmental and climatic effects are difficult to measure and take place over long periods, we used computer simulations to evaluate the effects of introducing perennial ryegrass (PR) seed production in a typical grain based cropping system under Eastern Danish soil and climatic conditions. The simulation model was the Danish mechanistic system model DAISY, which can track the movement of nitrogen (N), carbon (C) and water in the agroecosystem.

As a reference scenario, we defined a five year grain based cropping system of oilseed rape – spring barley – winter wheat – spring barley – winter wheat. As alternative scenarios, we replaced either a single wheat crop by PR (undersown in barley) or two subsequent wheat and barley crops by PR (undersown in barley). For these three cropping systems, we defined two strategies for wheat straw management – it was either harvested for use elsewhere (bioenergy/bedding) or incorporated into the soil. Each of these six scenarios was simulated with historic weather records for 40 year (i.e. eight repetitions of the five year cropping sequence) and with nitrogen application according to the Danish regulation.

We found that the introduction of PR for seed increased annual average nitrate leaching by roughly 3-7 Kg N/ha when cultivated for one season only, but reduced leaching by up to 5 Kg N/ha when grown for two consecutive years (depending on wheat straw management). The amount of organic matter returned to the soil with crop residues was higher in the grass seed production systems, especially when PR was cultivated for two consecutive years. The increased organic matter input resulted in an increased N mineralization, which is a good proxy for microbial activity and soil fertility. The soil C stock declined throughout the simulation period in the reference scenario. Introduction of PR ameliorated this trend, but the C stock still declined, primarily in the topsoil (0-20cm). Only when PR was cultivated for two consecutive years, C was sequestered in both the topsoil and subsoil throughout the simulation period, especially when the wheat straw was also incorporated. In these scenarios, the increase in soil C stock flattened towards the end of the simulation indicating the emergence of a new equilibrium state after which no more C will be sequestered with this type of management.

In summary, PR for seed has the potential to reduce both the environmental and climatic impact of agricultural practice relative to a conservative grain based cropping system, but *only* when grown for two consecutive seasons. When incorporated into a crop rotation as an annual crop, PR for seed caused increased N leaching and soil fertility was not sustained in the long run.

**Keywords:** Perennial ryegrass, Computer simulation, Nitrogen leaching, Soil organic carbon, Environmental effect, Climate effects.

# FROM THE IHSG WORKSHOP IN METHVEN TO THE FORMATION OF THE TASMANIAN SEED INDUSTRY GROUP (TSIG)

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# Abstract

The origins of the Tasmanian Seed Industry Group (TSIG) began back in 2013 at the IHSG Workshop in Methven, New Zealand. Both Robert Dent and Bramwell Heazlewood attended and were particularly interested in the research into Moddus which had previously been tried in Tasmania with varying responses.

Following the workshop Bramwell started putting some of this research into practice. Robert, who had a joint program with the Tasmanian Institute of Agriculture (TIA) looking at new and novel pasture cultivars, began looking into running a pasture seed conference in Tasmania with a couple of speakers from New Zealand. This coincided with Tony Butler being employed by TIA as an Herbage Development Officer, who accepted the challenge of organising the conference.

At the same time a new government body, Tasmanian Irrigation, was working to create irrigation schemes to help mitigate climate change in Tasmania. They enthusiastically took the opportunity to sponsor an overseas speaker who turned out to be Tom Chastain from Oregon. With Tom, Richard Chynoweth and Murray Kelly from New Zealand, and some local speakers, the seed conference was held in Launceston, Tasmania in late 2014. The second day involved a workshop with all the speakers and selected members from the Tasmanian seed industry participating.

From this workshop it was agreed to start a not-for-profit industry organisation involving the whole Tasmanian seed production industry, from farmers to agronomists, seed companies to seed cleaners, university and government. TSIG was formally formed in early 2015. Later, the vegetable seed industry was included, making TSIG the peak industry body representing all of the seed industry in Tasmania.

The goal of TSIG is to identify opportunities to support the continued growth of the seed industry in Tasmania and remove any barriers to expansion. To date we have encouraged increased storage, drying and cleaning facilities in the state, promoted and ensured continued operation of the Tasmania Government's Seed Laboratory and conducted trials to support minor use permits for a range of pesticides.

Most importantly, TSIG is conducting research to improve the competitiveness of the industry, particularly looking into Moddus, nitrogen, irrigation and grazing in ryegrass seed crops. This has demonstrated that less nitrogen and Moddus can be used and higher yields, culminating in a demonstration area and a 70 hectare commercial annual ryegrass crop which yielded 4.72 tonnes per hectare. This has been achieved using a voluntary levy from seed producers.

TSIG has recently been awarded funding to look into white clover seed production from AgriFutures Australia. Tasmania is now regarded as a reliable place to grow temperate seed crops. The industry is worth > AU44 million gross farm gate value and is the second highest value non-food agricultural crop in Tasmania. Production has expanded, growers have become more professional and more production companies are being attracted to Tasmania.

Keywords: Tasmania, Australia, Profitability, Seed, Industry, Research, IHSG

## CAN WE ACHIEVE RYEGRASS SEED YIELDS OF 5 TONNES PER HECTARE?

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#### Abstract

The Tasmanian Seed Industry Group Inc. (TSIG) have been conducting research to improve the competitiveness of the Tasmanian pasture seed industry by improving the profitability of pasture seed crops. Since attending the IHSG Workshop in Methven, New Zealand, in 2013, perfecting Nitrogen and Moddus inputs in ryegrass seed crops has been a major focus of the group.

Moddus (trinexapac-ethyl) and Nitrogen usage is widespread in the Australian seed production industry. With rising input costs and increased environmental awareness, TSIG realised that there was considerable work required to determine the optimum application rates of Nitrogen and Moddus inputs in the Tasmanian environment, while still achieving high seed yields.

Using a voluntary levy from seed producers, TSIG have conducted two seasons of small-plot replicated field trials and paddock-scale demonstration site looking into optimising Moddus and nitrogen usage in ryegrass seed crops.

Small-plot replicated field trials were conducted by TSIG in the 2018-19 and 2019-20 seasons, testing a range of Moddus and nitrogen rates on ryegrass seed yields. Results from both years were similar, suggesting that more modest input rates would achieve similar yields to crops with higher inputs. As well as being more profitable, this would also better meet environmental and community expectations. The small-plot trials suggested a Moddus strategy of 1.6 L/ha over 2 applications combined with 120-156 kg/ha N (260-340 kg/ha of urea) applied over 2 or 3 timings would deliver the most profitable result. While the results looked good on paper, it was difficult for growers to see how this more modest usage would translate to a commercial paddock scale.

In the 2021-22 season, a demonstration trial was conducted on a 70-hectare commercial ryegrass seed crop in the northern-midlands area of Tasmania. A 1.3-hectare area of the crop was treated with the lower Nitrogen and Moddus input rates. The inputs of 147 kg N/ha over 3 timings and 1.60 L/ha of Moddus over 2 applications were used. The remainder of the commercial crop received 172 kg N/ha over 3 timings and 2.4 L/ha of Moddus over 2 applications.

The results of the demonstration trial were very exciting. The lower input section had the same clean seed yield as the rest of the commercial higher-input crop under centre-pivot irrigation – the demonstration site and surrounding commercial crop both yielded very high, averaging 4.72 tonnes of clean seed per hectare.

The lower Nitrogen usage is more environmentally friendly and the lower trinexapac-ethyl usage meant rates used were within the use pattern on the Australian Moddus label. With its lower inputs, the demonstration site saved growers approximately AU\$133.00 per hectare, with the same high yield.

With further research, 5 tonnes per hectare may be possible!

Keywords: Nitrogen, N use, Moddus, Profitability, Yield, Trinexapac-ethyl, Ryegrass

#### **CONTRASTING TALL FESCUE SEED PRODUCTION SYSTEMS: CASE STUDIES**

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#### Abstract

Two tall fescue seed production fields (A and B) with some similarities but with important differences on the technology used were contrasted. Similarities: same cultivar (Rizar), an early, rhizomatous, with good seed potential production; new crops (two- and three-years old field areas); grazed from January to June; free of pests; 100 kg/ha of nitrogen in August/September and windrowed at the middle of November. Differences: 185 (A) vs 80 (B) kg/ha of nitrogen during fall and winter; rainfed (A) vs 165 mm of irrigation in 14 times form middle of August to windrowing (B), and harvest five days after windrowing with 15% seed humidity and artificial drying (A) vs harvest 14 days after windrowing with less than 9% seed humidity (B). Seed yield was evaluated in 12 main plots of 0.24 to 0.31 ha size in each field (field seed yield - FSY) using a commercial harvester, and in small plots (potential seed yield – PSY) taken before windrowing (three samples in each main plot), where seed yield components (seed weight - TSW and inflorescence/m<sup>2</sup> - infl/m<sup>2</sup>) were evaluated. Seed losses were registered in each main plot at different places and moments: among windrows before harvest (before and during windrowing seed losses), within windrows after harvest (windrow losses) and among windrows after harvest (harvester losses). Total forage dry matter (DM t/ha) before windrowing was assessed in each main plot. FSY was 759 kg/ha (A) and 896 kg/ha (B) and PSY was 751 kg/ha (A) and 1148 kg/ha (B). The main reason for this difference was seed losses, 73 kg/ha (A) and 332 kg/ha (B). Each field constructed the yield differently; the number of inflorescences were higher in A (723 inf/m<sup>2</sup>) than in B (436 inf/m<sup>2</sup>), but B had higher TSW (2.54 g) than A (2.16 g), and higher number of seeds/inflorescence, 104 in B and 48 in A. Total forage at harvest was high, 10.8 DM t/ha in A and 18.8 DM t/ha in B, with a harvest index respect to PSY of 0.070 (A) and 0.061 (B). With these results we hypothesize that the route for 1.0 t/ha seed production in Uruguay (0.33 t/ha national yield average) could be an adequate nitrogen rate in fall/winter to ensure enough new tillers, irrigation to obtain better seed set and seed weight, and seed drying to be able to harvest earlier with high seed humidity and thus reduce seed losses. However, the low number of inflorescences in the irrigated field was caused only by applying less nitrogen during fall/winter? Or we must improve management of irrigated crops, closing the grazing later? Or irrigate in a way that promotes tillers differentiation instead of new tillers, generating at the end of the process a crop with less total forage dry matter and higher harvest index? Could it be possible to combine the better yield components of these two fields (723 infl/m<sup>2</sup>, 2.54 g TSW, 104 seeds/infl) to reach a 1.910 kg/ha seed tall fescue yield?

Keywords: tall fescue, seed production, irrigation, seed losses

## **BIOCHAR AS CARBON ALTERNATIVE**

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## Abstract

In Oregon, late summer and early autumn establishment of perennial grass seed fields have often relied upon the use of carbon banding technologies that overlay the seed furrow with a band of activated carbon of approximately 2.54 cm in width that is followed by a preemergent herbicide application to the entire field. The carbon band absorbs the herbicide and acts as a safener for the germinating grass seed in the seed furrow. While this technique is effective, there is interest in seeking activated carbon alternatives to reduce costs. Biochar is a carbon-rich soil amendment derived through pyrolysis of organic residues. Some biochars have physicochemical characteristics that may be as effective as activated charcoal for carbon banding. Since some biochar is produced in the Pacific Northwest, this regionally local source of biochar may be less expensive as a carbon banding option in Oregon.

Results from a previous greenhouse experiment suggested that biochar derived from a mixed-conifer source provided the same level of herbicide safety as activated carbon. To elucidate whether a mixedconifer source biochar is as effective as activated carbon for crop safety in a grass seed field production setting, a randomized complete split plot design experiment replicated four times was implemented on October 20, 2022 at the Oregon State University Hyslop Field Lab in Corvallis, Oregon. Whole plot factors included three sources of carbon: mixed-conifer source biochar, activated carbon, and a non-treated control mixed in a slurry comprising of 2.27 kg of product in 37.9 liters of water applied at a rate of 28.0 kg per hectare. Sub-plots included six preemergent herbicides applied at the following rates: rimsulfuron (0.053 kg a.i. ha<sup>-1</sup>); pronamide (0.289 kg a.i. ha<sup>-1</sup>); diuron (2.692 kg a.i. ha<sup>-1</sup>); EPTC  $(4.908 \text{ kg a.i. } ha^{-1})$ ; a combination of flumioxazin  $(0.035 \text{ kg a.i. } ha^{-1})$  + pyroxasulfone (0.045 kg a.i.)ha<sup>-1</sup>); and indaziflam (0.015 kg a.i. ha<sup>-1</sup>) along with a non-treated control. The carbon sources were applied directly above the furrow using fixed spray nozzles attached behind the seeder applying 'Prominent' perennial ryegrass at a rate of 13.2 kg of seed per hectare on a 30.5 cm row spacing. Preemergent herbicide applications were applied the day after seeding (October 21, 2022) using a CO<sub>2</sub> pressurized backpack sprayer with a handheld boom comprising of four TeeJet XR11001VS flat spray nozzles. The carrier volume was 187 liters per hectare with a boom pressure of 2.1 bars. No irrigation was applied because rain was forecasted for the following five days (rainfall from October 22<sup>nd</sup> to October 26<sup>th</sup> totaled 2.24 cm).

By the 9<sup>th</sup> of November 2022, perennial ryegrass was apparent in most of the seed furrows and dicot plants began to emerge in some plots. Ratings occurred weekly starting on the 9<sup>th</sup> of November. Preliminary results suggest that there are no differences in safety between the biochar and activated charcoal for any of the herbicides tested and both indaziflam and a combination of pyroxasulfone + flumioxazin suppress between row plant emergence for a longer duration than the other herbicides tested.

Keywords: Carbon banding, biochar, preemergent herbicides, perennial ryegrass

# NEW REFERENCES FOR REASONING P-K-MG FERTILIZATION IN LEGUMES AND GRASSES SEED CROPS

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## Abstract

Phosphorus (P<sub>2</sub>O<sub>5</sub>), potassium (K<sub>2</sub>O) and magnesium (MgO) are essential elements for plant growth and seed formation and filling. The reasoning of phosphorus-potassium fertilization developed by COMIFER (French Committee for the Study and Development of Reasoned Fertilization) is based on 4 criteria: the crop's requirements, the soil's P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O content, the recent history of fertilization (number of successive years without fertilization since the last fertilization) and the management of crop residues from the previous crop. By combining these 4 criteria, COMIFER has developed export multiplier coefficient tables. Depending on the situation, a temporary impasse, a maintenance manure or a reinforcement of manure will be recommended. These tables have been simplified and adapted for legumes and grasses seed crops.

The dose to be applied is the result of the multiplication of 3 components:

 $P_2O_5$  or  $K_2O$  dose to be applied = Export multiplier coefficient x Yield of harvested products (forage cuttings, seeds) and crop residues x  $P_2O_5$  or  $K_2O$  content in exports.

An important collection of measurements carried out during four years (2016-2019) on FNAMS trials in the different production basins, has made it possible to update and complete the references of  $P_2O_5$ ,  $K_2O$ , MgO contents in exports (harvested products: forage cuttings, seeds and crop residues) for six legumes and grasses seed crops: orchard grass, tall fescue, perennial ryegrass, Italian ryegrass, alfalfa and red clover. For these six species, exports calculated on the basis of a reference yield with the export of harvested straw and a forage operation (excluding perennial ryegrass), are estimated on average at: 25 to 50 kg  $P_2O_5$ /ha, 100 to 260 kg  $K_2O$ /ha, 10 to 30 kg MgO/ha.

The magnesium requirement for legumes and grasses seed crops, even with a forage operation, is relatively low: it is around 30 kg MgO/ha at most. In most cases, the bioavailability of this element in the soil is sufficient to ensure a non-limiting supply to the crops for their production. Nevertheless, in soils with a low content of this element, a contribution can be justified to satisfy the needs of the crops.

Legumes and grasses seed growers now have a method adapted from the COMIFER reference method to reason their phosphorus-potassium and magnesium fertilization and thus ensure optimal crop nutrition and preserve soil fertility.

Keywords: fertilization management, phosphorus, potassium, magnesium

# AN INNOVATIVE CROP ASSOCIATION: PRODUCING RED CLOVER SEED CROP UNDERSOWED IN CRIMSON CLOVER SEED CROP

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#### Abstract

Red clover seed yield have been decreasing in France in the last decade, mainly due to forstrong pest pressure (*apion trifolii*) with the loss of insecticide (nicotinoïd) and bad climatic conditions; weed control is also difficult for seed growers when red clover is sown in bare soil due to its slow establishment in autumn and its low competitiveness against weeds. Mechanical weeding could be a solution (weeder harrow, hoeing,), but the implementation is often difficult in the early stage of red clover and in wet climatic fall conditions. Combining red clover seeds with cover crop or companion crop are interesting to cover the inter-row and limit the development of weeds. A lot of Fnams trials have been conducted and showed interesting results with mustard, niger, buckwheat, millet as companion crop and with spring barley, forage maize, buckwheat as harvested cover crop followed by red closer seed harvest the year after. But why not go further with these associations? why not harvest two successive seed crops in the same year?

Different trials have been conducted by Fnams (2020 to 2022) to test new associations in this way with annual clovers as *Trifolium incarnatum* or *Trifolium Squarrosum*. The seed weight of these annual clovers tested is different from red clover, so the cleaning of each species is possible for seed production in case of potential mixture.

With more vigor at the beginning of the cycle than red clover, they could cover the rows more quickly against weed. The two successive seed harvests require early annual clover cultivars to partly avoid as much as possible summer heat waves and droughts.

The trials (with 1 to 4 replications) were carried out in two locations: in Bourges in central France and in Brain sur l'Authion in the West. Red clover and crimson clover are sown the same day at the end of summer (late august), one row out of two each. The harvesting of the annual clover seeds occurred in late May or early June for crimson clover and later for Squarrosum and served as cutting for the following red clover seed crop. Once the annual clover seed crops are harvested, the red clover seed crop needs sufficient water supply for the regrowth and flowering. Interesting results have been obtained with irrigation in 2020 and 2021, depending on the location. Crimson clover performed better with an early harvest compared to *T. squarrosum* with no mixing of seeds in the harvested samples in both cases. But with too hot summer conditions (2022), red clover seed yield was strongly penalised or with zero grain yield. The delay in the flowering date of red clover indirectly allowed better management of *Protapion trifolii* populations with a delay in the sensitive stage compared to the population peak. Impact on weed control was also interested.

This technique to harvest the two successive seed crops the same year is encouraging, but strongly dependent of climate summer conditions and/or water supply. It must be confirmed by further trials.

#### Keywords: red clover seeds, crimson clover seeds, association, weeding

# IMPACT OF CHANGES IN CROPPING PRACTICES ON THE ECONOMIC ASSESSMENT OF GRASS AND LEGUME FORAGE SEED GROWING

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## Abstract

Due to political and regulatory considerations, farming practices are changing. Many chemical solutions have been banned, and farmers can no longer use them. For example, it was common to use diquat to dry alfalfa crop before harvesting the seeds. Now farmers swath the plant and wait 5-10 days before harvesting the dry seeds.

Every year we observe the impact of input price evolution on the calculation of production cost. The 2022 harvest was exceptionally with a 20% increase in the cost production of tall festuc seed due mainly to fertilization. The rising reach up to 8% for alfalfa. These estimations are based on French index IPAMPA (purchase price index for agricultural inputs) measured by INSEE (French statistics institute) and minimum legal French salary evolution. We project a further increase in the cost of feed seed production in 2023 due to the impact of energy prices. Cultivation practices are frequently studied. In 2014, a large study with a field survey was done. This study made it possible to define a crop itinerary reference for each species. This reference is generally higher than the average, with safe cultivation practices. However, it reflects field observations. To define the reference, FNAMS makes a territory/species pair. Tall festuc is growing all over France, but most of the production is concentrated in the Champagne region. Alfalfa is also grown throughout France, with a high concentration of production in western France. The reference is therefore defined for the Champagne region for tall festuc and in the West for alfalfa. In 2019, we noticed a gap between the references and the practices, so FNAMS worked on the reference according to the experts' recommendations. In 2022, a new field survey will be launched in February, with the first results expected in autumn 2023.

When the effect of input prices is removed, the impact of changes in cultural practices on the assessment of the cost of production is observed. Chemical solutions withdrawn must be replaced by others, by a new technology or by a change in practice.



In the 2019 revision, the main changes we see on tall fescue relate to the crop protection programme. The cost of production has increased by 15% only because of the change in practice between the 2014 reference and the new 2019 reference.



In the 2019 revision, the main changes we see in alfalfa are in mechanisation costs, mainly due to harvesting and the replacement of chemical desiccation with swathing. The production cost has increased by 18% only due to the change in practice between the 2014 reference and the new 2019 reference.

Keywords: Economy, input, technical program

# INVESTIGATING THE OPTIMUM NITROGEN APPLICATION RATE AND THE USE OF THE POTENTIALLY MINERALISABLE N (PMN) TEST, FOR ESTIMATING N MINERALISATION, IN PERRENIAL RYEGRASS SEED CROPS

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## Abstract

Improvements in fertiliser management is critical to the economic and environmental sustainability of New Zealand's agricultural production systems. Effectively forecasting fertiliser N requires the ability to predict the supply of plant-available N from soil and the demand for that N during crop growth. The N released by mineralisation of soil organic matter (SOM) can contribute a large amount of plant-available N and varies widely depending on soil type and land use history (ranging from <40 to >300 kg N/ha/y). Accurately predicting the supply of N from mineralisation is one of the greatest limitations to: 1) correctly forecasting the amount and timing of N fertiliser to meet, but not exceed, crop demand and 2) minimising the risk that excess N may be lost from arable and vegetable production systems via nitrate leaching and/or gaseous emissions.

This study evaluated if soil test data (mineral N and potentially mineralisable N (PMN)) can be utilised to improve N fertiliser forecasting to maintain ryegrass seed yield while reducing applied nitrogen. The industry guideline for spring applied N (kg/ha) is 172 minus mineral N 0-30 cm. The main N applications are split between closing (date of last defoliation, early stem extension, GS30-31), and about five weeks later; i.e. plant growth regulator timing to flag leaf emergence. Grass growth is very responsive to N; excessive, or poor timing of N will cause excessive vegetative bulk, increased disease susceptibility, lodging, light interception and ultimately reduced seed yield.

The PMN test has proved hard to predict nitrogen availability in ryegrass seed crops. Research conducted by FAR from 2003-2013 concluded the test was inadequate to accurately predict nitrogen. The modern hot water extractable organic nitrogen (HWEON) test can quickly analysis PMN and calculate when nitrogen may become available to the plant.

Two trials were conducted at the Foundation for Arable Research site in Chertsey, mid Canterbury, New Zealand. 12 treatments and 4 reps were evaluated under dryland and irrigated management. A first year cv 'Nui perennial ryegrass seed crop was sown 8 April in the 2021/22 growing season in a Templeton 9a.1 silt loam. Initial soil mineral N results were low at 18 kg N/ha (0-30 cm) at 3 August 2021. The HWEON soil test concluded that 75 kg/N/ha (dryland) and 85 kg/N/ha (irrigated) would become available to the crop between October to December. The application of 170 kg total N significantly yielded  $\geq$  compared with other nitrogen treatments except one. The post winter application of 40 kg/N on 1 September 2021 significantly (p=0.001) increased irrigated seed yield (170 total N) by 13% (1580-1815 kg/ha), however this response was not repeated in the dryland trial.

Using the PMN or HWEON test to reduce nitrogen application would have resulted in reduced seed yield as the total predicted N would not be available during early stem elongation, reducing

seed head number. During October (stem elongation) only 15 kg/N/ha (dryland) and 30 kg/N/ha (irrigated) would have become available to the plant from mineralised N. Hence supplying inadequate nitrogen to reach yield potential. The data further supports the industry guideline of 172 kg total N (applied + mineral N), an average of 28 trials from 2003-2013.

Further results on the economic benefits and trade-offs of including soil N supply in fertiliser decisions will be presented including results from the 2022/23 trial.
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## LIST OF DELEGATES

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