PROCEEDINGS OF THE
10TH INTERNATIONAL HERBAGE SEED CONFERENCE

Corvallis, Oregon, USA

May 12–19, 2019

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FOREWORD

The International Herbage Seed Group (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 10 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 10th International Herbage Seed Conference. The proceedings consist of the keynote addresses, volunteered oral papers, and peer-reviewed poster abstracts presented at Oregon State University’s LaSells Stewart Center in Corvallis, Oregon. The proceedings papers cover a wide variety of topics relevant to the production of herbage seed, ranging from breeding and genetics to applications of digital agriculture. The proceedings papers have been edited for style and clarity, but they have not been peer reviewed.

The hosting and organization of the 10th IHSG Conference has been a joint venture of Oregon State University, the U.S. Department of Agriculture’s Agricultural Research Service, and the Oregon seed industry. More than 250 delegates representing 16 countries participated in the conference, with Africa and Antarctica the only unrepresented continents. Of interest is that 12 of the delegates in attendance at this conference also participated in the 2nd IHSG Conference held in Corvallis in 1991, serving as a testament to the longevity of the IHSG and to the commitment of the membership to the mission of our organization.

Thomas G. Chastain
President, IHSG, and co-Chair of the Organizing Committee

Nicole P. Anderson
Co-Chair of the Organizing Committee
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KEYNOTE SESSION
BREEDING IMPROVED COOL-SEASON TURFGRASS WITH IMPROVED SEED PRODUCTION

William A. Meyer1,*, Phillip Vines1, Stacy A. Bonos1, Ronald F. Bara1, Dirk A. Smith1, and Eric N. Weibel1

Abstract
The past 57 years of cultivar development have resulted in the cooperative release of more than 500 cool-season turfgrass cultivars from the turf-breeding program at Rutgers University. Methods have been developed to more efficiently and thoroughly identify parents to be released as high-seed-yielding, superior turfgrasses. Over the years, a large effort has been conducted at Rutgers University to broaden the germplasm base from the countries of origin of 11 cool-season turfgrass species. These new superior cultivars are being made available to consumers throughout the cool-season region.

Keywords: turfgrass, breeding, cultivars, seed yield, disease resistance

History
The New Jersey Agricultural Experiment Station (NJAES) has had a turfgrass-breeding program for cool-season turfgrasses since the early 1960s. The first full-time U.S. turfgrass breeder was Dr. C. Reed Funk. In his effort to develop productive and persistent turfgrasses, he collected clones of all the cool-season species from old U.S. turfgrass areas seeded with material brought from Eurasia hundreds of years earlier by European settlers. These efforts required more than 10,000 hours of work to inspect more than 10,000 hectares of land.

Dr. Funk’s efforts included new clones of perennial ryegrass (Lolium perenne L.) from New York City’s Central Park, which produced ‘Manhattan’ perennial ryegrass in 1967. His work also included the release of ‘Rebel’ tall fescue (Festuca arundinacea Schreb.) from collections from old turfgrass areas ranging from New York to Atlanta, Georgia. It took his team at Rutgers more than 16 years of evaluating parent germplasm and recombination through many cycles of phenotypic recurrent selection to identify the parents of ‘Rebel’ tall fescue (Meyer and Funk, 1989). These two landmark cultivars launched a turf-type perennial ryegrass industry that produced more than 150 million lb per year. Similar levels of turf-type tall fescue were produced annually.

Production of both ‘Manhattan’ and ‘Rebel’ was limited by maturity rate and stem rust disease (Puccinia graminis L.) in the U.S. northwest. Thus, neither of these cultivars was a superior seed yielder. It is now well-known that these species do best in seed production if they are early to medium in maturity and exhibit improved seed yield, turf quality, and tolerance to diseases, insects, drought, and heat.

Current research
In 1996, the Rutgers breeding program began to emphasize a new practice of collecting new sources of germplasm in Europe, where cool-season grass species originated. These collections were increased as clones with summer planting in southwest Netherlands. Each spring, these clone nurseries were

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inspected prior to harvest, and only the superior collections were allowed to produce seed. Over the past 23 years, more than 40,000 collections have been made from more than 30 countries. Approximately 20,000 collections have been shipped to New Jersey as clean seed. All collections are sown into turf plots and evaluated for turf quality. The superior lines are incorporated into various breeding programs, and cultivars are developed in cooperation with more than 30 breeding organizations. Superior germplasm becomes available after Plant Variety Protection is finished when these cultivars are commercialized.

At Rutgers, there are breeding programs on apomictic Kentucky bluegrass (*Poa pratensis* L.) that include intraspecific hybrids and interspecific hybrids with Texas bluegrass (*Poa arachnifera* Torr.). At the NJAES, there are also programs on three bentgrass species (creeping bentgrass, *Agrostis stolonifera* L.; colonial bentgrass, *A. capillaris* L.; and velvet bentgrass, *A. canina* L.), four fine fescue species (strong creeping red fescue, *Festuca rubra* L. subsp. *arenaria* [Osbeck] F. Aresch.; chewings fescue, *F. rubra* L. subsp. *Fallax* [Thuill.] Nyman; slender creeping red fescue, *F. rubra litoralis* subsp. *litoralis* [G.F.W. Meyer] Auquier; and hard fescue (*F. brevipila* Tracey), perennial ryegrass, and tall fescue.

Most of the breeding programs are staged to prepare entries for inclusion in the National Turfgrass Evaluation Program (NTEP), which starts a new trial for each species every 6 years. At Rutgers the 6-year interval was a welcomed notion because it allows for additional evaluation of clonal material as mowed (2.5 inches) and unmowed spaced plantings.

In the bluegrass breeding program, crosses are made in the greenhouse using the nighttime crossing approach, which was developed in the early 1970s (Pepin and Funk, 1971). Using this approach, we make about 70 crosses each spring and grow out the new crosses the following fall and winter. Those plants (usually 100,000 or more) are grown in cool, short-day greenhouses in flats for selection of promising hybrids. These are then planted as spaced plants in the spring to grow to maturity the following year. Those plants that are identified as superior are stripped of around 300 seeds to be seeded in the fall. If they appear apomictic, a total of 48 seedlings are prepared to plant the following spring. The next July, the highly apomictic lines are harvested as promising new cultivars. The better turfgrasses are sent to the U.S. northwest for seed yield evaluation (Meyer et al., 2017).

Usually 8–10 promising cultivars are released after 5–7 years of testing. The cultivar ‘Midnight’ was released in the 1980s. If this cultivar were released today, it probably would not become commercialized because of its low seed yield. It is still a top-15 turfgrass cultivar, however, although not for seed yield. The cultivar ‘Shamrock’ was released in the late 1980s and is an average turf cultivar but a superior seed yielder (Bailey et al., 1995). ‘Shamrock’ is considered the standard for Kentucky bluegrass seed production. A few bluegrass cultivars that are 12.5% Texas bluegrass have superior performance compared to Kentucky bluegrass hybrids.

The 9 open-pollinated species bred at Rutgers are being developed by a combination of clonal evaluation in mowed and unmowed space plantings and progeny testing in mowed turf trials. Resistance to diseases such as brown patch (*Rhizoctonia solani* Kühn), red thread (*Laetisaria fuciformis* [McAlpine] Burds.), and dollar spot (*Clariireedia jacksonii* C. Salgado, L.A. Beirn, B.B. Clarke, J.A. Crouch) are selected in mowed clonal evaluation. Stem and crown rusts (*Puccinia coronata* Corda) resistance is selected in unmowed space plantings when plants reach full maturity. Selecting for resistance to gray leaf spot (*Pyricularia oryzae* Cavara) is most effectively done in seed trials starting during hot summer periods. Superior lines are then evaluated as clones for seed productivity and, in the case of tall fescue, for drought tolerance in a rainout shelter. Recently, much progress has been made to evaluate tall fescue
lines for floret fertility. It has been a surprise to see the segregation of tall fescue clones for seed set at maturity. This approach has resulted in much higher seed yields. In the fine fescue species, it is necessary to evaluate clones in unmowed spaced plantings prior to their inclusion for cultivar development. The creeping fescues and velvet bentgrasses are two species that require thorough evaluation of floret fertility before being used as parents in cultivars.

Current research in the turf-breeding program at Rutgers includes the use of molecular biology tools to complement the well-established, field-based breeding methods. A pseudo-F2 perennial ryegrass mapping population has been developed from a cross between two highly heterozygous parents, I06 and A89. This I06 × A89 population consists of 118 progenies along with the two parent genotypes. Parent I06 has greater salinity tolerance, greater dollar spot susceptibility, lighter green leaf color, and more of a prostrate growth habit compared with parent A89. This population has been used to study salinity tolerance, dollar spot resistance, and growth habit morphology via quantitative trait locus mapping. Future efforts will include developing and implementing marker-assisted selection to improve breeding efficiency for these and other important traits, such as seed yield, in our important cool-season turfgrass species.

Conclusion
Over the past 57 years, much progress has been made to develop improved turfgrasses of the 11 cool-season species. At Rutgers, emphasis has been placed on broadening the germplasm base available to cool-season turfgrass breeders. Efforts are continuing to explore old turf areas and farm pastures in the United States and Europe to locate new sources of parents to develop superior, productive cultivars. Conducting evaluations of lines for turf quality and improved seed set over multiple years has resulted in providing customers around the world with improved turfgrasses. Future efforts will focus on developing and implementing advanced breeding methods to improve the effectiveness of the breeding program.

References
Abstract
The spread and increase in resistant weed populations is of concern for weed management in all conventionally produced crops. However, in seed production the concern also includes the potential for further spread due to contaminated seed lots. The lack of stewardship to prevent or delay resistance has led to widespread resistant weeds in seed crops, and herbicide-resistant weeds now occur in the major herbage-seed production areas. The most prevalent resistant weed species in these areas are *Lolium* species. Some of the other grass weed species reported to be resistant include *Alopecurus myosuroides* Huds., *Apera spica-venti* L. P. Beauv., *Bromus tectorum* L., *Poa annua* L., and *Ventenata dubia* (Leers) Coss. The most frequently reported resistances are to herbicides that inhibit acetolactate synthase (ALS) or acetyl coenzyme-A carboxylase (ACCase). Resistance to other herbicides, including diuron, ethofumesate, flufenacet, glyphosate, glufosinate, paraquat, and pronamide, have also been reported. Over time, populations with cross-resistance and multiple-resistance evolved and now commonly occur. For example, *L. multiflorum* L. populations with cross-resistance and/or multiple-resistance were identified in Denmark, New Zealand, and Oregon. The time and resources required to test for cross- or multiple-resistance within a population make the timely recommendation of an effective herbicide very difficult. The challenge now for managing herbicide resistance in herbage seed production is the lack of herbicides with new mechanisms of action.

Keywords: herbicide resistance, cross-resistance, multiple-resistance, *Lolium multiflorum*

Introduction
Herbicide-resistant weeds are a challenge in most agricultural systems, including herbage seed crops. According to the international survey of herbicide-resistant weeds, there are 499 unique cases of herbicide-resistant weeds globally (http://www.weedscience.org/). This underestimates the actual number, however, because the survey depends on researchers to report the occurrence of resistance, which does not always happen. Resistance has been reported to most of the known herbicide sites of action, especially the herbicides that are now used in crop production. It has been almost 30 years since an herbicide with a new mechanism of action was introduced, so there are no new herbicide options to control resistant weeds. The number of reported resistant species and the number of hectares infested with resistant weeds continue to increase. Although most of the confirmed resistant weeds are not reported specifically from herbage seed production fields, they often are from production systems that include herbage seed crops in the rotation. Therefore, in many cases the resistant species are an issue in the seed crops, too.
Definitions
When discussing a complex topic such as herbicide resistance, it is important for all parties to use the same definitions.

Herbicide resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. Simply stated, a previously effective herbicide is no longer effective. Within a population, one or a few individuals may carry the resistance trait. With repeated use of herbicides with the same mechanism of action, the number of resistant individuals increases until the population is no longer controlled.

Herbicide tolerance is the inherent ability of a species to survive and reproduce after herbicide treatment. In this instance, the herbicide was never effective for control of the species. Tolerance is the basis for selective herbicides. For example, flufenacet controls _L. multiflorum_ without killing _L. perenne_.

The terms herbicide resistance and herbicide tolerance often are used interchangeably, which is incorrect. In addition, researchers sometimes use the term tolerance to refer to plants that have a low level of resistance or to a low frequency of resistant individuals within a population. This confusion about terms can lead to the wrong herbicide being recommended on a particular site.

Cross-resistance is resistance to two or more herbicides with the same mechanism of action. For example, sulfonylurea, imidazolinone, or triazolopyrimidine herbicides all inhibit acetolactate synthase (ALS).

Multiple-resistance is resistance to two or more herbicides with different mechanisms of action. For example, plants may show resistance to both ALS inhibitors and acetyl-coenzyme A carboxylase (ACCase)-inhibiting herbicides.

Cross- and multiple-resistance underscore the need to understand how herbicides are related in order to make the correct recommendations for herbicide rotations in managing herbicide resistance. In many cases, the mechanism of action is listed on herbicide labels, which makes it easier to choose an herbicide with a different mechanism of action.

Herbicide Resistance in Herbage Seed Crops
In Denmark, large-scale surveys have been conducted to assess resistance. _Alopecurus myosuroides_ with ALS or ACCase resistance was identified in a survey conducted from 2013 to 2015. In a 2018 survey of 130 samples of _L. multiflorum_, more than 65% of the populations were resistant to ALS inhibitors, while 38% were resistant to ACCase inhibitors and 32% were resistant to both (S. Mathiassen, personal communication). There are few alternative herbicides available for control of this species, so resistance is a major concern. ALS- and ACCase-inhibitor-resistant _A. spice-venti_ also has been confirmed.

In New Zealand, resistance was confirmed in _L. perenne_ to ALS inhibitors and in _L. multiflorum_ to the ACCase inhibitor haloxyfop (Gunnarsson et al., 2017). Currently, a larger survey is being conducted to test for resistance in _A. fatua_, _Bromus_ spp., and _Lolium_ spp. (P. Rolston, personal communication).

In a survey of _L. multiflorum_ populations conducted in Oregon, resistance to ALS and ACCase inhibitors, flufenacet, glufosinate, glyphosate, paraquat, and pronamide was confirmed (L. Bobadilla, personal communication). Multiple-resistance was found in 52% of 75 samples, and 11% of those populations had
resistance to three mechanisms of action. Cross- and multiple-resistant \textit{P. annua}, \textit{A. fatua}, and \textit{B. tectorum} populations also are present in grass seed crops in Oregon.

In order to determine cross- or multiple-resistance, time- and resource-consuming studies must be conducted to test all of the herbicide combinations to which a population might be resistant. Tests that provide faster results are being developed, but considering the wide range of herbicides and the different mechanisms of action and mechanisms of resistance, this is no small undertaking. The time required to obtain results generally precludes effective alternative in-season herbicide recommendations.

Management of weeds in herbage seed crops has become more difficult because of the number of resistant weed species, the increasing number of species with multiple-resistance, and the lack of herbicides with new mechanisms of action.

**References**


POTENTIAL FOR QUANTUM LEAPS FORWARD IN AGRONOMIC MANAGEMENT OF SEED CROPS

Richard J. Chynoweth

Abstract
Seed yield is generally considered a function of the number of seeds produced per unit area multiplied by their individual weight. Nonlimiting yield can be described by a crop’s ability to capture photosynthetically active radiation (PAR), convert it into biomass, and then partition biomass into seed. In grasses, resource limitations usually influence canopy size and duration, while lodging influences both solar radiation capture and biomass partitioning. Thus, the challenges for improving seed yield are associated with increasing either the number of seeds produced per unit area and/or increasing individual seed weight. In grasses, there is still room to explore stem shortening to aid the capture of PAR and manipulate biomass partitioning, while in legumes information is still limited on flower/flower head promotion and post-flowering assimilate partitioning dynamics. In legumes, a large canopy intercepting PAR is often detrimental to flower and ovule fertility, while production of high flower numbers is a balancing act between promoting and restricting leaf growth. At an individual crop level, increasing individual seed weight can be achieved by increasing either the rate or duration of seed filling.

Keywords: grass seed, legume, partitioning, seed yield, seeds m$^{-2}$

Introduction
Average seed yields of many herbage seed crops have increased over time as growers implement better management practices to promote growth and limit crop stress from nutrient, pest, and disease pressure (Pyke et al., 2004; Chynoweth et al., 2015). Ultimately, plant growth is regulated by canopy expansion and the interception of photosynthetically active radiation (PAR) by green leaf/stem area (Monteith, 1977). The conversion of PAR into carbohydrate and dry matter is affected by canopy architecture, species, water status, nitrogen (N) status, plant population, temperature, CO$_2$ levels, and more. The conversion of dry matter into economically important components is described by harvest index (HI) and in seed crops is quantified as the amount of seed produced in relation to total crop dry matter, e.g., seed yield divided by the total dry matter produced.

Much seed production research has focused on individual inputs or management techniques (e.g., N application) and has described seed yield improvements using components of yield (e.g., spike numbers m$^{-1}$, spikelets per spike, and 1,000-seed weight). Ultimately, however, the major yield components are the number of seeds produced per unit area and their individual seed weight. Thus, understanding the physiological factors that influence the number of seeds produced has greater potential to improve seed yield than describing yield components. For seed yield to be increased, one of two things must change. Either (1) the rate of seed filling per unit area must increase, or (2) the duration of seed filling must be longer.

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Many seed crops have been bred specifically for a single market/end use (e.g., forage or turf), for which seed production is of low importance. Growing these crops requires an enhanced understanding of crop physiology to manipulate them from their target end use into high seed-producing crops.

This paper provides a brief insight into potential areas to advance seed yield in grass and forage legume seed crops.

**Discussion**

**Grasses**

The greatest opportunity to enhance seed yield through management is by increasing harvested seed number. Seed number has been increased by plant growth regulator (PGR) applications, spring N, spring and summer irrigation, rust disease control, and other management practices. Fine-tuning of these inputs has led to the seed yield increases of the past 20 years (Pyke et al., 2004; Chynoweth et al., 2015).

Often, an understanding of plant physiology is used to determine whether crop yield, under a certain set of circumstances, has been limited by the capacity of green tissue to generate assimilate (source limitation) or by the capacity of the harvested organs to accumulate it (sink limitation). Based on their role in assimilate generation and accumulation, organs are commonly defined as either source or sink organs.

Most seed crops have low HI with a relatively low ability to draw assimilate from the stem; thus, they are commonly sink limited. Many crops produce a number of pollinated “true” seeds that are below saleable weights. Therefore, reducing the lag phase of seed growth and extending the duration of growth, or increasing the rate of seed filling, may increase the competitiveness of seeds as a sink and allow a greater number of seeds to reach a saleable weight. Warringa and Marinissen (1997), Clemence and Hebblethwaite (1984), and Chynoweth and Moot (2017) described the stem as changing from sink to source during the “mid seed filling” period for perennial ryegrass (*Lolium perenne*).

Alternatively, the sink size of the stem may be reduced by stem shortening/dwarfing, which may allow greater realization of set seed to saleable seed. Trethewey and Rolston (2009) showed that in perennial ryegrass the seed head itself is a very important source organ during seed fill and should be protected at all times, while Griffith (2000) demonstrated variation among species in their ability to remobilize stored reserve when lodging occurred. Neither study presented data on stem length nor how this could interact with sink strength. Opportunities exist to further our understanding of stem shortening and its influence on dry matter partitioning. For example, the use of three gibberellin (GA) inhibitors in combination can produce severe dwarfing and alter lodging responses, thereby enhancing seed production through increased seed number and more favorable dry matter partitioning (Chynoweth et al., 2014) (Table 1). Opportunities exist around understanding the importance of cytokinins during the cell-division stage of seed development to increase sink size (Jameson and Song, 2015).

Many management practices can increase seed weight but usually only when assimilate supply is low or resource capture is limiting. For example, irrigation increases seed weight when water stress conditions occur. Thus, many management factors may increase individual seed weight when conditions are appropriate.

Grasses produce many tillers of varying ages, which leads to variation in tiller developmental stage near the end of the growing season (e.g., a range of dates at which anthesis and harvest maturity occur).
Variation in tiller age at anthesis has many implications, including difficulties with pollination, seed set, dry matter partitioning, and seed shedding, as well as difficulties in determining agronomic input timings (e.g., crop growth/development stage and harvest timings). Within a single tiller, seed size and weight (nonuniformity) within seed lots results partly from the seed position within the inflorescence and individual seed filling duration (Warringa et al., 1998). An understanding of how individual seeds source assimilate and whether the seed filling duration of younger distal seeds is determined by the older proximal seed would allow research to focus on decreasing nonuniformity in seed size and weight. One method would be to attempt to limit the number of florets per spikelet, which potentially could synchronize anthesis.

The harvesting of mature seeds that are currently lost to shedding would on average add about 400 kg ha$^{-1}$ of saleable seed to the currently achieved seed yields in New Zealand. Future methods to reduce rachilla damage or abscission layer development and prevent shedding may include use of cytokinins to reduce late-season stress and enhance the “stay green” effect, which may reduce seed shattering. Ultimately, plant breeding would provide reduced seed shattering, with investigations by Fu et al. (2018) suggesting some candidate genes for further investigation.

In the future, with reduced shedding, grass seed management would take a quantum leap, with short stems keeping the seed head in the sunshine from anthesis until maturity, thus maximizing potential seed growth.

**Legumes**
Seed yield in legumes, especially clovers, is closely related to the number of flowers or inflorescences (flower heads) produced, provided they are produced within a “tight” flowering period of favorable weather for pollination. Thus, seed production can be considered in three phases: flower head formation, pollination, and post-pollination seed growth.

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**Table 1.** Perennial ryegrass seed yield, cv. ‘Bealey’, following application of seven plant growth regulator treatments at four possible timings when grown at Wakanui, Mid Canterbury, New Zealand, in the 2013–2014 growing season (from Chynoweth et al., 2014).

<table>
<thead>
<tr>
<th>Application date, product, and rate</th>
<th>Seed yield (kg ha$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timing 1 (Oct. 30, 2013)</td>
<td>1,720 a</td>
</tr>
<tr>
<td>Timing 2 (Nov. 4, 2013)</td>
<td>2,100 b</td>
</tr>
<tr>
<td>Timing 3 GS 32 (Nov. 13, 2013)</td>
<td>2,355 bc</td>
</tr>
<tr>
<td>Timing 4 (Nov. 27, 2013)</td>
<td>2,470 cd</td>
</tr>
<tr>
<td>Timing 5 (Dec. 17, 2013)</td>
<td>2,730 d</td>
</tr>
<tr>
<td>Timing 6 (Dec. 17, 2013)</td>
<td>3,360 e</td>
</tr>
<tr>
<td>Timing 7 (Dec. 17, 2013)</td>
<td>Mean 2,567</td>
</tr>
<tr>
<td></td>
<td>P value &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>LSD$_{0.05}$ 283.2</td>
</tr>
</tbody>
</table>

1 TE = Moddus (250 g L$^{-1}$ trinexapac ethyl); CCC = Cycocel (750 g L$^{-1}$ chlormequat chloride); PB = Payback (250 g L$^{-1}$ paclobutrazol)
The timing of flower formation is genetically controlled in response to environmental stimuli, often cool winter temperatures or short days followed by lengthening photoperiod in the spring or summer (e.g., Thomas, 1979), not by a period of “stress” as referred to by many producers. A better understanding of flower formation and emergence will lead to higher seed yields, especially in locations where soil fertility and irrigation can be used to control stress.

Most legumes undergo both reproductive development and growth (defined as leaf production) concurrently. Leaves must emerge for new flowers/flower heads to emerge; therefore, growth conditions must be adequate to keep apical buds active. Following flower emergence from the apical bud, the flower competes with the expanding leaves (both sinks) for assimilate from the stolon until the leaf is large enough to export assimilate, at which time the new leaf becomes a source and supports the young, developing flower head. However, those same leaves shade new flowers and limit the potential of new emerging flower heads. For example, in white clover flower heads that are shaded following emergence, but prior to pollination, individual flowers or ovules tend to abort/become sterile if they remain within the canopy shade for too long (Pasumarty and Thomas, 1990). In extreme conditions, the complete flower head may abort. Shading prior to pollination is more detrimental than shading post-pollination, but both are damaging to seed production (Thomas, 1996).

Thus, to increase the number of flower heads that emerge, agronomists must investigate methods to reduce leaf size, thereby reducing shading, while maintaining active growth on the stolon tips. Currently, many growers use “controlled water stress” to limit leaf size, but better science in this area around nutrient supply or PGRs may improve seed production reliability. Concurrently reducing internode length on branches and/or stolons could allow for a greater number of flowers to develop per unit area. However, reduced internode length means more leaves per unit area and more shading. Thus, in practice, a greater number of branches and/or stolon tips grown in “sparse” canopies (either wider rows or larger internode length) may offer options for increasing flower density.

The relationship between nectar secretion and pollination efficiency remains unclear. However, nectar secretion can be affected by plant nutrient status, particularly phosphorus, potassium, and boron. Understanding volatiles that attract pollinators to flowers may enhance the chances of increased pollination, particularly in marginal environmental conditions. However, many legume seed crops are attractive to insect pollinators (e.g., honey, leaf cutter, and bumble bees), which effectively pollinate crops when weather conditions allow. Even following multiple bee visits, however, many ovules do not produce viable seeds, especially in crops containing multiple ovules per flower, even if pollination per se is optimal (Thomas, 1996). Opportunities exist to combine research on seed provisioning with research on insect pollination to determine whether pollination or seed abortion due to limited assimilate is the cause of suboptimal seed numbers. There is evidence that flower heads compete with each other for assimilate produced by leaves. Thus, reductions in pedicel length may increase the ability of flower heads to draw assimilate from stems and stolons. Post-pollination seed growth is often reduced when solar radiation receipts are reduced or when the rate of photosynthesis is lower due to water or nutrient stress. Growers need to have crops flowering around the longest day, when radiation receipts are at their greatest, and then maintain adequate growing conditions post-pollination.

Thus, legume growers need to adjust the mindset that crops require stress to flower. In reality, they require light at bud level for flowers to emerge, and they require leaves to produce assimilate at optimum levels post-pollination to ensure that seed abortion is minimized and seed size is maximized.
Conclusion
In grasses, the manipulation of lodging/stem length to keep the spike photosynthesizing is key to increasing seed yields toward 4,000 kg ha\(^{-1}\). However, seed shedding must be limited for this strategy to be successful in all environments. To achieve yields beyond 4,000 kg ha\(^{-1}\), advances in assimilate partitioning are required. In legumes, an understanding of the environmental triggers for flowering and ovule abortion, along with assimilate partitioning post-pollination, will drive seed yield advances.

References
Abstract
Digital herbage seed production and research offer new possibilities but also new challenges. Technological, software, and algorithm development proceeds extremely rapidly, while the development of decision support systems and related agronomic and biological interpretation lags. These challenges must not prevent us from exploring new technology and methods in herbage seed production and research. We must recognize, however, that increasing the utilization of applied and available nutrients through the use of sensors, local weather data, and predictions, in combination with application algorithms, may not necessarily increase farmer revenue or reduce negative environmental impacts. Advanced field phenotyping holds great potential and is receiving a great deal of attention, based on publication frequency, and breeders will gain new knowledge from these technologies and methods. The use of advanced phenotyping in combination with crop models is an interesting area that undoubtedly will increase our basic knowledge of plant development and performance, which is necessary in order to better understand the interactions between nutrient utilization, optimal application timing for plant growth regulation, weed control, and other field operations. The conclusion is that digital herbage seed production and research are still in the early development stage.

Keywords: digital farming, image analysis, sensor systems, machine learning, phenotyping

Introduction
Digital herbage seed production and research refers to the creation of value from data. Digital seed production and research therefore takes precision farming/precision agriculture and smart farming a step farther and creates, or at least has the intention to create, value from the large amount of data collected in herbage seed production and research. Digital herbage seed production and research, or “Agriculture 4.0,” should be the next herbage seed production and research evolution.

Herbage seed production and research will probably not pave the way for new digital methods, analytical tools, or machine-learning algorithms due to limited financial resources in both production and research. Instead, we must rely on innovations from other agricultural sectors and adapt them to our herbage grass seed sector. This task is not trivial, and it is complicated by the fact that some of the technology developed under “Agriculture 3.0/precision farming” is not yet fully implemented in herbage grass seed production and research. Undoubtedly, one reason is that herbage seed production and research, like most other agricultural sectors, is part of a complex biological system. Thus, monitoring of crops/plants gives a limited picture that changes rapidly due to changes in weather and other growth factors.

However, these challenges must not prevent us from adapting new technologies, and I have chosen three core areas of interest where I believe herbage seed production and research can benefit from Agriculture...
4.0. The first relates to increased utilization of nutrients, the second to improved phenotyping, and the third to improved crop modeling.

**Increased Utilization of Inputs**

The basis for an optimal nutrient application rate and application strategy to achieve high seed yield is knowledge of nutrient demand. Several results show the economical optimal nitrogen (N) application rate after harvest (*ex post*). However, the farmer needs to know the optimal rate at the time of application, which, for some species, is in the autumn before seed harvest. The drawback to N application is its possible negative environmental impact. Thus, a great deal of research has focused on development of N application strategies based on measurement of factors such as N status, critical N dilution curve(s), and soil information, leading to relatively advanced N-application algorithms.

The potential of this approach is believed to be large. However, a grain crop study by Colaço and Bramley (2018) showed that most studies report N fertilizer savings of 5–40% with little effect on grain yield. Reported impacts on profit ranged from reductions of US$30 ha⁻¹ to increases of US$70 ha⁻¹. In fact, about 25% of studies reported economic losses from sensor-based N application. Colaço and Bramley concluded that sensor-based N applications, despite their benefits in reducing environmental impacts, often were not profitable compared to current N practices. This lack of consistent evidence of economic benefits limits adoption by farmers. Although sensor-based N application doesn’t seem to increase farmer revenue, the present use of yield mapping has a very clear positive effect on adoption of, and intention to adopt, sensing technologies, as the profit increase required to justify adoption is lower among those with yield maps compared to those without maps (Bramley and Ouzman, 2019).

The fact that a positive effect on profit is not necessary for farmers to adopt new technology is interesting, as it shows that the technology in itself is of interest to farmers. The fact that new technology is pushed onto the market via new tractors and implements is of course also an important factor.

The fact that farmers may not increase their revenue through adoption of digital farming has important implications for its implementation. Documentation of practical farm management is needed to push the technology to end users. Development of local decision support systems is probably a key component for implementation of digital farming. This is not surprising, as agriculture, unlike many industries, depends on an unpredictable weather factor, and this factor needs to be incorporated into solutions at a local scale.

**Phenotyping and Digital Farming**

Crop, plant, and seed phenotyping is an important part of digital agriculture that has received a great deal of attention and is especially important for IHSG. According to Costa et al. (2019), the frequency of plant phenotyping and precision agriculture publications was similar in 2012. By 2016–2017, however, the number of plant phenotyping papers was more than 30% of all publications and therefore considerably greater than the number of precision agriculture papers.

Modern crop, plant, and seed phenotyping often utilizes noninvasive technologies and digital technologies to provide essential information on how genetics, epigenetics, environmental pressures, and crop management can guide selection toward productive plants suitable for their environment (Costa et al., 2019). These technologies include sensors mounted on implements and unmanned aerial vehicles (UAVs), automation, robots, storage and management of the large amount of data collected, and sophisticated data analysis such as machine-learning algorithms. Many of these technologies are developed for field use rather than greenhouse measurements, and phenotyping of crops and plants has consequently moved...
to field experiments instead of greenhouse experiments. Interestingly, the technological shift is also associated with a shift in studied species from *Arabidopsis thaliana*, rice, potato, and tomato to *Zea mais*, *Triticum*, barley, and chickpea (Costa et al., 2019). Phenotyping is therefore at the forefront of future plant breeding and is an interesting area.

Seed phenotyping is, however, a minor research area, even though high-quality seed is the first step toward resistance to biotic and abiotic stress, rapid and uniform germination, and plant performance. Healthy seeds are furthermore a prerequisite if farmers hope to achieve the highest possible return to costly inputs such as nutrients, water, pesticides, and labor. A review by ElMasry et al. (2019) gives an overview of the latest fully operated multispectral imaging systems for quality assessment of various types of seeds. Seed phenotyping, like other areas in digital agriculture, requires cooperation among specialists from different fields (e.g., seed physiology/anatomy, sensor development and installation, and statistics/machine learning). Only close collaboration among many research areas will unleash the potential within seed phenotyping and digital farming.

**Crop Modeling Is an Important Part of Digital Farming**

With the overall aim of using digital techniques to monitor and optimize agricultural production processes, it is imperative to achieve more information on basic crop development and production. This information can be achieved through crop modeling, and it seems logical to use digital techniques, such as sensors, as supplemental data input. Interesting input could include time-series data on dry matter production based on correlations between ground-truth measurements of dry matter and factors such as crop index. Another possibility would be to use RGB images to estimate plant numbers and to use this input in crop models and in combination with plant growth regulation. This work is not trivial. Although several publications have shown nice correlations between sensor data and ground-truth measurements, most of the work has been based on very local models and, in some cases, there has been poor validation of results. As in other crops, most work in herbage grass seed production has been performed and validated locally.

Sensor data, local weather stations, and local soil information can be very interesting input to existing and future crop models. However, we must be aware that most mechanistic crop models are complex and will, in most cases, never be implemented in practical seed production.

**Conclusion**

Digital herbage seed production and research is expected to expand considerably during the coming years. We will have to rely on techniques and methods developed in other crops and customize them for our purposes. The three areas of special interest discussed here cover both production and research within herbage seeds.

The IHSG has an opportunity to be pioneers in the full research and production chain, from breeding to production of seeds—beginning with in-field phenotyping of new cultivars, continuing through seed production using a combination of new technology and statistics to describe crop development and performance, and ending with phenotyping of harvested seeds for next-year production. Research in these areas will undoubtedly create a great deal of interest among others working in the areas of sensors, data storage, and machine learning. However, to fully develop this potential, there must be a close collaboration among farmers, advisers, and scientists working in the herbage grass seed area. One focus area of IHSG could be to define areas within digital agriculture that are especially interesting for grass seed growers and grass seed research.
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SEED PRODUCTION

ORAL PRESENTATIONS
Abstract
Organic farmers are supposed to use organically produced seed. For more than 20 years, organic cool-season grass and clover seed has been produced in Denmark. For the past 10 years, the average total production area has been approximately 3,000 ha, with the major species in production being perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). Organic seed crops are undersown in a spring barley cover crop using different establishment techniques. Perennial ryegrass seed is sometimes mixed with and sown with the spring barley and can also be established after a mechanical weeding of the spring barley. Obtaining a solid and uniform establishment of the seed crop is considered a very important weed control strategy, which in organic grass seed production also consists of a relatively high nitrogen application. In the seed production year, various new techniques for mechanical weeding are being tested in experiments at Aarhus University and in grower fields. One of the major challenges in organic white clover seed production is insect pests (*Apion* spp. and *Hypera* spp.). Earlier experiments have shown seed yield decreases of 40% in untreated control plots. In years with high incidence of insect pests, the seed grower might choose a late defoliation of the white clover seed crop to reduce the damaging effects of the insects. However, a recent study has shown that biological control using a parasitoid might be promising as a long-term pest management strategy. Over the past two decades, organic seed production practices have been established and, interestingly, are now being tested and adapted in conventional herbage seed production.

Keywords: perennial ryegrass, white clover, organic, establishment, pest management

Introduction
In 1992, the Danish seed industry started the production of organic herbage seed, and, in 1998, funding was obtained for experiments investigating establishment methods in organic grass seed production. Later, the influence of insect pests in clover seed production was also investigated in trials at Aarhus University. In organic farming, organic seed must be used if available. A consultative group consisting of representatives from dairy farmers, seed growers, advisory services, and researchers was formed, and this group drew up a strategy for providing forage mixtures for organic farmers. They agreed to facilitate the use of organic seed in those mixtures, which created a strong incentive to further develop organic herbage seed production. The production area increased from the first recorded plantings in 1998, with 313 ha cultivated, to 2003, when the production area exceeded 3,000 ha (Figure 1). Since then, the 10-year average production area has been approximately 3,000 ha, with the major species being perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). Harvested seed yields in organic herbage seed production are 50–75% of those in conventional production (The Danish Seed Council, 2017). The main reason for lower yields is the difficulty of controlling weeds, diseases, or insect pests. A recent study from Sweden reported organic white clover seed yields as being 42% of those from conventional insecticide-treated plots (Lundin et al., 2017).

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Organic farmers are very interested in producing seed crops, especially clover seed, as it provides nitrogen (N) to the following crop, which often is winter wheat.

Establishment and Weed Control
In Denmark, cool-season grass and clover seed crops are undersown in a cover crop/companion crop. The most common cover crop is spring barley. In organic farming, barley is established at 12-cm row spacing in order to provide as much competition against weeds as possible. Perennial ryegrass seed may be mixed with and sown together with the spring barley, or it may be established after mechanical weeding of the spring barley. Obtaining a good and uniform establishment of the seed crop is considered a very important weed-control strategy, which in organic grass seed production also consists of an increased seed rate and relatively high N application rate.

New equipment for mechanical weeding is being marketed and tested in high-value crops such as vegetables and sugar beet. Currently, various new techniques for mechanical weeding, aimed at both organic and conventional herbage seed production, are being tested in seed crops in experiments at Aarhus University.

Insect Pests
Severe reductions in the seed yield of white clover can occur due to the white clover seed weevil, *Apion fulvipes*; the lesser clover leaf weevil, *Hypera nigrirostris*; and the clover head weevil, *Hypera meles*, which together can reduce seed yield by more than 40%. The white clover seed weevil is usually found in high numbers, but the damage caused by this insect is lower than that from either of the other two insects. In years with a high incidence of insect pests, the seed grower may choose a late defoliation of the white clover seed crop to reduce the damaging effects of the insects. Insect pests overwinter in field edges adjacent to the source field. Therefore, the next clover seed crop is established as far away as possible from the current crop in order to avoid a large infestation of insect pests when they emerge next year from the overwintering sites and immigrate into a new clover crop. This spatial and temporal distribution is considered a long-term strategy to reduce problems with insect pests.
Another long-term strategy is to search for and eventually use natural enemies to reduce insect pest numbers. Several species of larval endoparasitoids within the genus *Bathyplectes* have been used as classical biological control agents to control *Hypera*. A recent study has shown that *Bathyplectes curculionis* (Thomson) (Figure 2) is harvested in large quantities together with white clover seed and brought to the seed-processing plant. These cocoons can be separated from the waste material, and a technique to detect viability of cocoons has been developed (Shrestha et al., 2018). The idea is to bring the viable cocoons into the new clover seed crop and use them as natural enemies against *Hypera*.

Currently, research projects are searching for natural enemies of the most common insect pests, and enemies of insect pests in clover and oilseed rape have been identified. More research is needed to investigate how and to what extent they can be used in the control of insect pests in cropping systems.

We are now seeing that findings and developments from organic cropping systems are becoming increasingly relevant for conventional growers as we, in Europe, experience a rapid decline in pesticides registered in seed crops.

From the beginning, organic seed production seemed very challenging, but during the past 20 years production methods have been developed, and highly committed growers are now capable of producing relatively high yields of good-quality seeds.

**References**


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**Figure 2.** Development of the parasitic wasp *Bathyplectes curculionis* (Thomson) in the clover head weevil (photo: Henrik Bak Topbjerg).
EFFECTS OF SOWING METHODS AND SOWING RATES IN ORGANIC SEED PRODUCTION

Lars T. Havstad1,* and John I. Øverland2

Abstract
Different sowing methods and rates were evaluated in organic seed production of timothy (Phleum pratense L.) (two trials), meadow fescue (Festuca pratensis Huds.) (two trials), and red clover (Trifolium pratense L.) (one trial) at NIBIO Landvik Research Station and on growers’ farms in Vestfold County, Norway, during 2010–2013. The experimental treatments included three sowing methods: (1) broadcast sowing of grass/clover with a cover crop sown at 12-cm row spacing, (2) sowing of cover crop and seed crop in crossed rows, both at 12-cm row spacing, and (3) sowing of cover crop and seed crop in alternating rows (24-cm spacing). The three sowing rates were 5, 10, and 15 kg ha⁻¹ for timothy and meadow fescue and 3, 6, and 9 kg ha⁻¹ for red clover. On average, first-year seed yields were 5–6, 20–25, and 19–25% higher for timothy, meadow fescue, and red clover, respectively, when plots were sown with the cover crop and seed crop in alternating rows compared to plots where the seed crop was broadcast or sown perpendicularly to the cover crop. The corresponding seed yield increases in the second-year seed crops of timothy and meadow fescue were 0–2 and 4–7%, respectively. Sowing method had no effect on weed coverage in the seed harvest years or on weed contamination in the cleaned seed. Increasing the sowing rate usually had a negative influence on seed yield, while weed coverage/contamination was not significantly affected by sowing rate. In conclusion, organic seed crops should be established with cover crops and seed crops sown in alternating rows, the latter with the same low sowing rate as in conventional seed production. However, in organic production systems, even this method will not always meet the requirement for seed crop purity in the first harvest year.

Keywords: establishment, seed yield, plant density, broadcasting, weed control

Introduction
Seed crops of grasses and clovers are normally undersown in a spring wheat or barley cover crop. Traditionally, the standard sowing method has been either (1) to broadcast the forage seed on the surface with special sowing equipment on the cover crop sowing machine, or (2) to plant the cover crop and seed crop in crossed rows at 12-cm row spacing. Deleuran and Boelt (2009) reported better establishment (higher biomass production, more tillers, etc.) of a perennial ryegrass seed crop when the seed crop and cover crop were drilled in every second row, thus reducing competition from the cover crop. Similar row spacing experiments, with seed and cover crop sown every other row, have not been published for the seed crops most commonly grown in Norway.

In conventional seed production, a low seeding rate is normally recommended. However, in the absence of herbicides, a lower plant density will normally increase the amount of weeds due to more open soil space. In cereals, higher seeding rates were found to be an effective tool in reducing the density of weeds, particularly at narrow row spacing (Minkey et al., 1999).

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A main focus of this study was to develop strategies for enhancing the competitive ability of the undersown seed crop against both the cover crop and weeds, in order for certified seed to be harvested from first-year crops.

Methods
Two field trials in timothy (*Phleum pratense* L. cv. ‘Grindstad’), two in meadow fescue (*Festuca pratensis* Huds. cv. ‘Fure’), and one in red clover (*Trifolium pratense* L. cv. ‘Yngve’) were carried out on silty loam or silty clay loam soils at NIBIO Landvik Research Station (58.2 °N) and on seed growers’ farms in Vestfold county (59.4 °N), Norway, during 2010–2013. The experimental layout was a split plot, with sowing methods on main plots and sowing rates on subplots. The sowing methods included:

- Broadcast sowing of a grass or clover seed crop after drilling the cover crop at 12-cm row spacing
- Drilling the cover crop and seed crop in crossed rows, both at 12-cm row spacing
- Drilling the cover crop and seed crop in every other row in one operation (24 cm between rows of the same species)

For each of these three sowing methods, three sowing rates were combined: either 5, 10, or 15 kg ha⁻¹ (timothy and meadow fescue) or 3, 6, or 9 kg ha⁻¹ (red clover). There were nine treatments in total, each trial with three replicates. Plot size was 1.7 m x 8 m.

At each location, sowing was carried out in early spring with a tractor-mounted Øyjord sowing machine with 10 disc coulters. The cover crop was spring wheat except for one timothy trial established in spring barley. In all trials, the cover crop sowing rate was the same (220 or 160 kg ha⁻¹ for wheat and barley, respectively) regardless of row spacing. For sowing methods 1 and 2, the sowing machine had one seed distributor connected to all 10 coulters, while for sowing method 3 it had two distributors connected to 5 coulters each. Sowing depth was 3–4 cm for the cover crop and ca. 1 cm for the seed crop in treatments 2 and 3. In treatment 1, the seed of grass or clover was spread evenly on the surface using an Øyjord distributor constructed for fertilization of plot trials. After sowing, all trials were compacted with a Cambridge roller to improve soil-to-seed contact.

The trials were managed organically, i.e., without mineral fertilizer, pesticides, or chemical growth regulators. Granulated chicken manure (Bina-Grønn NPK 11-1-3 (Binadan AS, Nørre Snede, Denmark) or Marihøne NPK 4-1-12 (Norsk Naturgjødsel AS, Voll, Norway)) was applied at a rate of 100 kg N ha⁻¹ in the sowing year. In the timothy and meadow fescue trials, these fertilizers were also applied in the autumn of the sowing year (30 kg N ha⁻¹ in both species) and in spring in the seed harvest years (50 or 80 kg N ha⁻¹, respectively). No weed control was performed in any of the trials during the experimental period.

Each trial was seed harvested for either 2 years (timothy and meadow fescue) or 1 year (red clover).

Results and Discussion

Sowing method
Sowing of the cover crop and seed crop in every other row resulted in the highest seed yield in the first seed harvest year for all species (Table 1). On average, first-year seed yields were 5–6, 20–25, and 19–25% higher for timothy, meadow fescue, and red clover, respectively, when plots were sown with the cover crop and seed crop in alternating rows compared to plots where the seed crop was broadcast or
sown perpendicularly to the cover crop. This is in concurrence with Deluran and Boelt (2009), who found that optimal establishment of perennial ryegrass occurred when placing the grass seed row 6 or 12 cm from the cover crop row. The positive effect can probably be explained by better light penetration to the undersown seed crop.

Of the two grass species, optimal autumn development is especially crucial for meadow fescue, which requires a long primary induction period in autumn in order to produce flowers and seeds. This may explain why sowing of the cover crop and seed crop in every other row was more beneficial in meadow fescue than in timothy (Table 1), which has no primary induction requirement.

For both timothy and meadow fescue, the increase in seed yield by sowing the cover crop and seed crop in every other row was less pronounced in the second than in the first seed harvest year (Table 1). One possible explanation is that the yield enhancement by this sowing method was more due to less competition from the cover crop than to the row spacing of the seed crop *per se*.

The different sowing methods had no significant effect on weed density in the field or weed contamination of the seed lot after seed cleaning (data not shown).

### Table 1. Main effect of sowing methods and sowing rates on seed yield of timothy, meadow fescue, and red clover.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Timothy (kg ha⁻¹) (Rel.)</th>
<th>Meadow fescue (kg ha⁻¹) (Rel.)</th>
<th>Red clover (kg ha⁻¹) (Rel.)</th>
<th>Timothy (kg ha⁻¹) (Rel.)</th>
<th>Meadow fescue (kg ha⁻¹) (Rel.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of trials</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Sowing method</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broadcast seeding: cover crop sown in rows at 12 cm</td>
<td>496 (100)</td>
<td>263 (100)</td>
<td>318 (100)</td>
<td>565 (100)</td>
<td>573 (100)</td>
</tr>
<tr>
<td>Cover crop and seed crop sown in crossed rows at 12 cm</td>
<td>493 (99)</td>
<td>276 (105)</td>
<td>337 (106)</td>
<td>577 (102)</td>
<td>591 (103)</td>
</tr>
<tr>
<td>Cover and seed crop sown in every other row</td>
<td>521 (105)</td>
<td>330 (125)</td>
<td>398 (125)</td>
<td>578 (102)</td>
<td>612 (107)</td>
</tr>
<tr>
<td><strong>P%</strong></td>
<td>&gt; 20</td>
<td>&gt; 20</td>
<td>2</td>
<td>&gt; 20</td>
<td>&gt; 20</td>
</tr>
<tr>
<td><strong>LSD 5%</strong></td>
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<td></td>
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<tr>
<td><strong>Sowing rate</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5 kg ha⁻¹ (grasses)</td>
<td>527 (100)</td>
<td>302 (100)</td>
<td>374 (100)</td>
<td>577 (100)</td>
<td>595 (100)</td>
</tr>
<tr>
<td>3 kg ha⁻¹ (clover)</td>
<td>503 (95)</td>
<td>289 (96)</td>
<td>358 (96)</td>
<td>600 (104)</td>
<td>589 (99)</td>
</tr>
<tr>
<td>10 kg ha⁻¹ (grasses)</td>
<td>480 (91)</td>
<td>278 (92)</td>
<td>321 (86)</td>
<td>545 (94)</td>
<td>592 (100)</td>
</tr>
<tr>
<td>6 kg ha⁻¹ (clover)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P%</strong></td>
<td>&gt; 20</td>
<td>&gt; 20</td>
<td>17</td>
<td>&gt; 20</td>
<td>&gt; 20</td>
</tr>
</tbody>
</table>
Seed crop sowing rate

Increasing the sowing rate of the seed crop, leading to a higher plant density, normally had a negative impact on seed yield in timothy, meadow fescue, and red clover (Table 1). This is in concurrence with earlier experiments in conventional seed production of the three species (Jonassen, 1976).

In barley, increasing plant density has been shown to suppress weeds while maintaining grain yield (O’Donovan et al., 1999). The same trend has also been observed in wheat, even though wheat is not as competitive with weeds as barley (Xue and Stougaard, 2002). Although significantly less weed cover was found in the establishment year in the timothy trials on plots sown at the highest rate (15 kg ha\(^{-1}\)) than on the plots with the two lower rates, no significant positive effect of higher sowing rates on weed suppression, either in the field or in the seed lot after cleaning, could be detected for any of the species (data not shown). Thus, in terms of sowing rates, the experiments gave no indication that recommendations for organic seed production should differ from those for conventional seed production.

Economic implications

Economic calculations showed that sowing the cover crop and seed crop in every other row gave the highest income in red clover and meadow fescue when sown at the lowest sowing rate (3 and 5 kg ha\(^{-1}\), respectively). In timothy, only small differences in economic income between the three sowing methods were found when sown at an optimal rate of either 5 or 10 kg ha\(^{-1}\).

Conclusion

The results of these experiments suggest that organic seed crops of timothy, meadow fescue, and red clover should be established by drilling the cover crop and seed crop in every other row, using the same low sowing rate for the seed crop as in conventional seed production. However, our results also show that even this favorable method will not always be sufficient to meet the requirement for certified seed in the first harvest year. To avoid problematic weeds and cultural seeds, it is therefore critical to put organic grass and clover seed crops into an agronomically sound crop rotation. For better weed control, a more active approach, e.g., mechanical weed control, may also be considered.

References

Abstract
The forage herb narrow-leaf plantain (Plantago lanceolata L.) has played an important role in New Zealand and some international pastoral systems over the past 24 years. The development of plantain seed production techniques by joint cooperation among researchers, field agronomists, and a group of committed seed producers has resulted in seed yields improving from an average of 300 kg ha\(^{-1}\) in 1995 to more than 1,950 kg ha\(^{-1}\) in 2017, a sixfold increase and an average annual increase of 70 kg ha\(^{-1}\) per year. The forage plantain market has until recently been dominated by the cultivar ‘Ceres Tonic’, and a large proportion of the development work has been conducted on this cultivar. This paper outlines the seed yield performance of certified plantain seed crops from 1995 to 2018. We identify the challenges faced in weed control, seeding rates, nutrient management, pest and disease control, and harvest management. Joint efforts by the field research group and seed growers in conducting small-plot evaluation trials and field-scale evaluations have led to frequent improvements in growing techniques that have been rapidly adopted by seed producers. More recently, the discovery of the role of plantain-based pastures in reducing nitrogen leaching from animal grazing systems in sensitive environments has stimulated further plant breeding efforts to develop new cultivars with the appropriate agronomic and chemical properties desired by the market. In addition to environmental performance testing, these varieties require further testing for seed yield potential.

Keywords: agronomy, plantain, Plantago lanceolata L., seed production

Introduction
In New Zealand, the potential role of plantain as a pasture herb was investigated beginning in the late 1970s, and two cultivars (‘Grasslands Lancelot’ and ‘Ceres Tonic’) were released collaboratively in 1995. ‘Lancelot’ was bred from selected local collections, and ‘Tonic’ from selected Mediterranean germplasm, each providing different seasonal production patterns and levels of forage production. Over 2 years, the forage production level of ‘Tonic’ in Canterbury under a low-irrigation regime was shown to be within 5% of that of perennial ryegrass (Stewart, 1996). The desirable features of ‘Tonic’ were soon recognized by the pasture market, and this cultivar has dominated, occupying approximately 95% of the seed production area until the introduction of ‘AgriTonic’ in 2016.

Agronomic testing and market development initially concentrated on the role of plantain in pasture mixes with commonly used species such as ryegrass, clover, chicory, lucerne, and even forage brassicas. Obvious challenges included control of common weed species in these mixtures, adaptability to different climates and soils, and the effects of animal grazing. A better understanding of optimal climate zone and animal performance data has resulted in the development of specialized intensive pastures with plantain as a dominant component (Moorhead et al., 2002).
New Zealand regional councils are adopting policies to restrict nitrate leaching from agricultural land to protect water quality in river and lakes. The predominant source of nitrate leaching is from the high-nitrogen (N) load of the urine patch, particularly from dairy farming. Researchers at Agricom, Lincoln University, Massey University, and Plant and Food Research have studied the reduced N leaching of mixed sward and plantain-based pastures over the past 10 years. Several mechanisms have been identified, and the published research has recently been summarized by Judson et al. (2018). These mechanisms include dilution as the volume of urine from grazing animals is increased and the concentration of N is reduced. Biological nitrification inhibitors have been identified in both the excreted urine and from the soil environment containing plantain. The role of some secondary plant compounds has been identified; these can be measured and are now incorporated into new plant breeding selection processes. Box and Judson (2018) have also shown that there is significant variation between cultivars and breeding lines in terms of urinary volume and in levels of the secondary compounds. A combination of in vitro laboratory, controlled animal feed, and field studies involving lysimeters has been used to develop an understanding of potential N reductions of 45–89% compared to traditional ryegrass and clover pastures. The environmentally functional plantain cultivars are now commercially represented by the brand Ecotain.

Development of Plantain Seed Production
Although plantain is considered a long-day plant, flowering appears to be driven largely by plant development stage, and this has allowed thermal time equations to be fitted for different cultivars (Payne et al., 2018). Following initial research and production at DSIR Grasslands, Palmerston North, and at Ceres Research Farm, Canterbury, the first field production for commercial seed was sown in March 1994 and harvested in January 1995.

Initial seed yields of 300 kg ha\(^{-1}\) were similar to those achieved in research trials. In parallel with market development, PGG Seeds continued seed production trials and extended this information with intensive advice and monitoring of seed production fields. Achieving crop hygiene and quality standards for markets was initially an essential issue, followed by assessments of crop risks and later by undertaking agronomic studies to improve seed yields and minimize seed loss. These trials were placed in seed growers’ fields and were regularly visited by the group of growers who actively participated in discussions to solve agronomic issues as they arose. The risk of major crop failure was mitigated by selecting and inviting innovative growers with known high standards of seed production and by a geographical spread of crops around Canterbury, New Zealand, to avoid effects of localized climate events. Over time, the value of irrigated crops also became apparent. Once critical mass and geographic spread was achieved, the existing growers took all of the available crop area, so that a core group of 12 experienced growers worked in close contact with research and field agronomists and seed growers.

Concurrent Seed Production Research and Crop Development
Key lessons were quickly learned by direct experience and close interaction with seed growers. Both reactive and preventive protection programs were quickly developed for a range of pests, diseases, and agronomic issues.

Weed-control programs took some time to develop to cover the wide range of arable weeds and volunteer crop species encountered. Initially, this was an essential focus to achieve marketable standards of seed lines for local and international markets. In addition to ensuring crop purity and minimizing seed cleaning losses, smart herbicide programs are a useful tool for crop growth regulation and for concentration of the timing of reproductive development. Some 52 herbicide trials have been conducted over more than
20 years. Key herbicides include diuron (pre- and postemergence), bentazone, dicamba, and paraquat. Some others are used to manage specific weeds, and we have not found any unsurmountable weed issues.

The place of plantain in crop rotation and the use of weed control systems are now well understood. Crops following cereal, clovers, and grass seed are most common; plantain seed following brassica seed is a serious challenge and is not recommended. Sowing time was found to be an important function of soil type and altitude. Plantain is susceptible to frost lift, which greatly affects some soil types, thus requiring early plant establishment, even in coastal areas.

Some diseases are infrequent and are climate influenced, but they can be extremely damaging to seed yield. *Ramularia rhabdospora* (Ramularia leaf spot) often develops on older leaves in mild, damp, spring conditions and again in late summer and autumn. It can be controlled with defoliation by grazing and with fungicides, commonly by an application of triazole plus strobilurin fungicide during flowering. *Phomopsis subordinaria* (stem choke) is more prevalent in wetter conditions from flowering to cutting. It manifests as a constriction of the upper stem immediately below the seed head. The stem turns brown and then black, and the disease prevents nutrient transport to the seed head, resulting in either no seed or incomplete seed fill. This disease can cause complete crop loss in second-year seed crops. Several fungicides provide enough protection in first-year crops, but none of those tested has achieved protection under severe pressure in the second year. Commonly, one or two applications of triazole plus strobilurin fungicide are made during flowering. We have conducted five fungicide trials since 2001.

Some pests are also infrequent but serious problems. The native New Zealand grass grub (*Costelytra zealandica*), a root-feeding coleopteran larva, can be serious even at low populations, and control methods are used at and during crop establishment. Plantain is also very attractive to slugs, notably the prevalent European grey field slug (*Deroceras reticulatum*), which can build to very high populations if unchecked. This pest is especially damaging during crop establishment; well-established crops can withstand high populations until spring, when developing seed heads can suffer damage. Multiyear crops are at serious risk to the native lepidoptera porina (*Wiseana cervinata*) due to surface feeding on the plant crown, which causes crop thinning. This pest is readily controlled in the early larval instars by the insecticide diflubenzuron. More recently, summer pastures of plantain under drought stress have suffered attacks of plantain moths, made up of two native species, *Scopula rubraria* and *Epyaxa rosearia*, the larvae of which feed on plant leaves and crowns. The introduced weevil *Gymnetron pascuorum*, often called “plantain weevil,” can also build to high populations over time and can cause serious damage to seed yield of second-year crops. No insecticides have been registered for these species, but some control has been achieved with chlorpyrifos.

Defoliation of excess vegetative growth is, in New Zealand, referred to as crop “closing.” This can be achieved by animal grazing, topping, or use of a chemical such as paraquat. In six trials since 1998, we have determined that grazing should be completed by the end of September; that plants should not be grazed lower than 50 mm from early September onward; and that paraquat defoliation is an effective tool to combine closing, late weed control, and removal of early seed heads to make flowering more determinate.
Nitrogen (N) input and crop closing time from grazing or defoliation were found to have a large influence on yield potential. Early trials from 1998 indicated that seed yield response to N is similar to that of ryegrass but that N should be applied earlier. Optimum total applied N was 150–200 kg ha\(^{-1}\), with a significant decrease in seed yield above 250 N kg\(^{-1}\). There was no difference in yield response to mid-August and mid-September application, but yields decreased when application was delayed until mid-October. Potassium and chlorine were found to have no impact on seed yield in field trials. In view of the knowledge gained about N storage and soil modification properties, further trials were conducted. In 2017, different forms of N were evaluated, with no significant seed yield differences found between urea and various nitrate forms. It has been observed that N use should also be related to grazing and paraquat closing times. We have conducted six randomized block design fertilizer trials from 1998 to 2017.

The advent of plant growth regulators (PGRs), particularly trinexapac-ethyl, has had a major impact on grass seed yields. Six trials since 2000 have studied the effects of PGRs on plantain seed yield—some in relation to crop “closing” and some in relation to N rate. We have found no significant effect on seed yield from any of the PGRs tested.

High seed shattering can result in large potential seed losses. Cutting times and techniques were initially established based on experience with a range of grass seeds, but substantial differences were soon apparent. Plantain leaves do not readily wilt; both stomata function and antifungicidal properties are believed to play a role in this trait. However, shattering continues in the windrow, and *Phomopsis* stem choke continues activity, thereby restricting seed development. Larger windrows can capture more shattered seed within their bulk but can take 3–4 weeks to dry, exposing them to greater risk from weather events such as rain and winds. Techniques for chemical desiccation of leaf area and application of pod adhesives, combined with protectant fungicides, both pre- and post-windrowing, have been developed in conjunction with innovative seed growers.

As these issues have become understood, the combination of 76 small plot trials and adaptation to large-scale farm techniques has provided a steady improvement in seed yield that has overcome the vagaries of seasonal climatic variation and has provided growers with tools for a reliable crop income.

**Seed Yield, 1995–2018**

‘Ceres Tonic’ and ‘AgriTonic’ have been grown under New Zealand Plant Variety Rights and Seed Certification schemes. Production figures from the New Zealand Seed Certification Bureau, including the Official Seed Testing Station, have been checked against PGG Wrightson Seeds company records to calculate the mean seed yield per hectare as presented in Figure 1. Some differences occasionally occur when field areas are changed or when seed cleaning is not completed in the year of production. Therefore, the 3-year mean of seed yield has also been calculated in the same manner as presented for trends in ryegrass and white clover seed yields in New Zealand (Chynoweth et al., 2015).

**Conclusion**

The domestication of plantain from a weedy, semiprostrate herb to an erect-leaved productive forage and the development of commercial seed yield is an example of the transfer from research to on-farm trials and the value of innovative growers and seed company researchers working together to develop production protocols.
References


**Figure 1.** Plantain seed yield (kg ha⁻¹), 1994–1995 through 2018–2019.

![Graph showing seed production of plantain over 24 years. The graph includes a trend line equation: y = 0.946x + 86.242x + 340.66, with R² = 0.6335.](image-url)
ANNUAL RYEGRASS SEED YIELD RESPONSE TO TRINEXAPAC-ETHYL: NEW ZEALAND AND OREGON

M. Philip Rolston¹*, Nicole P. Anderson², Richard J. Chynoweth¹, Thomas G. Chastain², Murray J. Kelly³, and Bede L. McCloy⁴

Abstract
Previous evaluations of trinexapac-ethyl (TE) on annual and Italian ryegrass (Lolium multiflorum) have shown average seed yield responses of 58% (range 29–122%) in 10 New Zealand (NZ) field trials but generally small (10%) responses in Oregon. The aim of this work was to determine whether these differences in response are due to cultivar or to management and environmental differences. Trials were undertaken in Canterbury, NZ, in 2016–2017 with cv. ‘Hogan’ (a New Zealand cultivar). In 2017–2018, trials were conducted in New Zealand with cv. ‘Gulf’ (an Oregon cultivar) and in Oregon with cv. ‘Gulf’ and ‘Winterstar II’ (a New Zealand cultivar). Seed yield response to TE applied at BBCH 32 was evaluated in replicated plot trials. The plot length was 10 m and 13.7 m in New Zealand and Oregon, respectively. At both locations, trials were windrowed at approximately 40% seed moisture with a small plot windrower and harvested with a plot combine. In New Zealand, a comparison of 0 vs. 400 g TE ha⁻¹ found an increased seed yield in ‘Hogan’ of 1,260–3,330 kg ha⁻¹ (163%) and in ‘Gulf’ of 1,910–2,960 kg ha⁻¹ (55%). In Oregon, 400 g TE ha⁻¹ increased seed yield in ‘Gulf’ from 1,000 to 1,800 kg ha⁻¹ (80%) and in ‘Winterstar II’ from 830 to 1,560 kg ha⁻¹ (88%). Further increases were measured at 600 g TE ha⁻¹ in Oregon. These results suggest that the cultivars used in New Zealand and Oregon are both able to respond to TE treatments. We suggest that crop management practices in spring, particularly spring defoliation, may influence crop response to TE application and that crops with a high biomass at TE treatment time are less responsive to TE.

Keywords: annual ryegrass, lodging, Lolium multiflorum, plant growth regulators, seed yield

Introduction
In New Zealand, the stem-shortening plant growth regulator (PGR) trinexapac-ethyl (TE) enhances seed yields in both types of Lolium multiflorum (Lam): annual (Westernwolds) and Italian types (Rolston et al., 2012, 2016; Trethewey et al., 2016). Between 2002 and 2016, 10 trials were carried out at 7 locations in the Canterbury region of New Zealand with 6 cultivars comprised of Italian and Westernmold types of L. multiflorum. The seed yield response to TE averaged 58% with 400 g TE ha⁻¹. The average yield with no TE application was 1,650 kg ha⁻¹; with application of 400 g TE ha⁻¹, seed yield averaged 2,540 kg ha⁻¹. In contrast, previous northern hemisphere studies on seed yield responses from TE application applied to annual ryegrass suggested that responses are generally small, averaging 10% and ranging from 0 to 18% in seven trials (Mellbye et al., 2007), 0 to 11% (Macháč, 2012), and 0 to 18% (Rijckaert, 2010). In New Zealand, the standard management practice for annual ryegrass is to defoliate in winter and early spring, usually with sheep. In Oregon, defoliation is not a standard practice. This paper involves parallel research
in Oregon and New Zealand with two cultivars, one from each region, to understand whether differences in response are related to management, climate, or plant genetics.

**Methods**

In New Zealand, the trial was undertaken at Lincoln in 2017–2018 and compared cv. ‘Gulf’ with no defoliation vs. repeated defoliation through October 25. Plots measured 10 m x 3.2 m with four replicates in a randomized block design. The plots were windrowed and harvested with a plot combine. At another site, annual ryegrass cv. ‘Hogan’ was grazed in winter and spring, with the final defoliation on November 3. TE was applied at four rates (0–600 g ai ha\(^{-1}\)) as single and split applications. In both trials, TE (Moddus Evo) was applied at growth stage BBCH 32.

The Corvallis, Oregon, trial sown in fall 2017 compared cv. ‘Gulf’ with cv. ‘Winterstar II’. Plots were 14.5 m x 3.5 m and sown in adjacent blocks with four replicates. TE (Palisade EC) was combined with three defoliation treatments (nil, one mowing on March 19, 2018, and three mowings on March 19, April 4, and April 13). TE was applied at 0, 200, 400, and 600 g TE ha\(^{-1}\) at BBCH 31–32. Mowing delayed swathing dates, with the three mowing dates swathed on June 15, 19, and 21, respectively.

**Results and Discussion**

In Oregon, there was a large seed yield response in ‘Gulf’ to 400 g TE ha\(^{-1}\) (80% seed yield increase), while in New Zealand there was an 18–55% increase (Table 1). In New Zealand, the final spring mowing on October 25, a date commonly used for New Zealand-bred Italian and annual ryegrass, was too late for ‘Gulf’ and reduced head numbers and therefore yield potential. In Oregon, seed yield increased by 75%, from 1,060 to 1,860 kg ha\(^{-1}\), when the crop was mown in early spring compared to traditional nil mowing (Table 2). There was no seed yield advantage from mowing three times, compared to one mowing, when TE was applied. The 600 g TE ha\(^{-1}\) rate increased seed yield and produced yields that were significantly higher than the 400 g TE ha\(^{-1}\) rate (Table 2).

In a separate New Zealand trial with midspring defoliation by sheep, the response to TE was a 163% increase in seed yield, from 1,260 (no TE application) to 3,330 kg ha\(^{-1}\) (400 g TE ha\(^{-1}\)) (Figure 1). In many New Zealand trials that compare seed yield responses to varying TE rates, the response to TE is described by a linear plateau model (Figure 1). However, in Oregon, we see a linear response to TE for rates from 0 to 600 g TE ha\(^{-1}\) when the crop is not mown, but tending to a linear plateau response when the crop is mown (Table 2). In New Zealand, we see a similar linear TE rate response when the final defoliation is early and a linear plateau when the final defoliation is delayed. This TE response result has been observed

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Type</th>
<th>Site</th>
<th>No TE Application</th>
<th>400 g TE ha(^{-1})</th>
<th>LSD 5%</th>
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<td>Gulf</td>
<td>Annual</td>
<td>Corvallis, USA</td>
<td>1,000</td>
<td>1,800</td>
<td>517</td>
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<tr>
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<td>Annual</td>
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<td>1,910</td>
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<td>1,470</td>
<td>1,740</td>
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<tr>
<td>Winterstar</td>
<td>Italian</td>
<td>Corvallis, USA</td>
<td>830</td>
<td>1,560</td>
<td>182</td>
</tr>
<tr>
<td>Hogan</td>
<td>Annual</td>
<td>Ashburton, NZ</td>
<td>1,260</td>
<td>3,330</td>
<td>297</td>
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</table>

Table 1. Seed yield response (kg ha\(^{-1}\)) of two annual and one Italian ryegrass cultivars to 0 or 400 g trinexapac-ethyl (TE) ha\(^{-1}\) at sites in Corvallis, Oregon, USA, and Lincoln and Ashburton, New Zealand.
in both perennial ryegrass (*Lolium perenne*) and in annual ryegrass when the date of the final mowing is delayed (Rolston et al., 2012). The earlier northern hemisphere TE trials often used 200 g TE ha\(^{-1}\) as the main TE rate evaluated. In the Oregon trial, the seed yield response from 200 g TE ha\(^{-1}\) was small (8%) when TE was applied to unmown plots (Table 2), consistent with previous findings from Europe (Rijckaert, 2010; Macháč, 2012) and Oregon (Mellbye et al., 2007).

These trials suggest that historical differences between results from the northern and southern hemispheres are due not to cultivar/plant genetic or climate differences but rather are strongly influenced by management. Our hypothesis is that defoliation in late winter and early spring, either by mowing or grazing, reduces crop mass at TE application timing (BBCH 31–32), resulting in a greater ryegrass response to TE, possibly mediated by delayed lodging. Many New Zealand seed growers graze livestock, either sheep or yearling cattle, on ryegrass seed crops during the winter and early spring. This practice appears to have a natural fit when stem-shortening PGRs such as TE are used. Growers with no access to grazing livestock make balage or silage to remove bulk and simulate grazing.

### References


### Table 2

<table>
<thead>
<tr>
<th>TE (g ha(^{-1}))</th>
<th>0</th>
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<th>3</th>
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<td>200</td>
<td>830</td>
<td>1,630</td>
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<td>400</td>
<td>1,070</td>
<td>2,120</td>
<td>2,220</td>
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<td>600</td>
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<td>LSD 5% TE</td>
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**Figure 1.** Seed yield response of annual ryegrass cv. ‘Hogan’ at Ashburton, 2017–2018, with the breakpoint calculated in Genstat v19 using split line regression model.
RESPONSE OF MEADOW BROMEGRASS SEED CROPS TO PLANT GROWTH REGULATORS IN CANADA

Calvin L. Yoder1*, Nityananda Khanal2, and Talon M. Gauthier3

Abstract
Meadow bromegrass (Bromus biebersteinii) is one of the major forage seed crops in western Canada. The use of plant growth regulators (PGRs) on grass seed crops is relatively new to Canada. A field trial was conducted in 2017 and 2018 to determine the effect of trinexapac-ethyl (TE) and chlormequat chloride (CCC) and a mixture of TE + CCC at growth stages BBCH 32 or 39 and BBCH 52 on plant height, lodging, seed yield, and seed dockage on a first- and second-year meadow bromegrass seed field. All PGR treatments were effective at reducing plant height, lodging, and percent dockage of meadow bromegrass in both years. In 2017, all PGR treatments increased seed yields in the first-year stand. Application of TE alone and the mixture of TE + CCC at the earlier growth stage were most effective at increasing seed yields. Yield increase from these treatments was 42%. Yield responses from the other PGR treatments were 20% higher than the control. Yield responses to PGR treatments in 2018 were minimal, but CCC applied at BBCH 52 showed a higher yield than the other treatments. None of the treatments applied in either year had any effect on seed germination or 1,000-seed weight. Use of TE and CCC alone or in combination shows potential advantage on meadow bromegrass seed crops in Canada, particularly when crops are harvested by straight combining. Additional studies are necessary to evaluate these PGRs over a number of fields and environmental conditions.

Keywords: meadow bromegrass, trinexapac-ethyl, chlormequat chloride, lodging, seed yields

Introduction
Meadow bromegrass (Bromus biebersteinii) is commonly used throughout western Canada in pasture mixtures. It is a perennial grass adapted to cooler and moist areas on most soil types, but it does not tolerate flooding (Knowels, 1993). Meadow bromegrass is well suited for use in pastures, as it recovers rapidly following grazing. Although a number of cultivars are registered in Canada, the main cultivar grown is ‘Fleet’. The majority of seed produced in Canada is used domestically, although some seed is exported to several northern U.S. states.

Meadow bromegrass is grown for seed across western Canada. Once established, seed crops are generally harvested for 2 or 3 years. Seed yields are higher in the first 2 years and can range from 300 to 1,400 kg ha⁻¹. Meadow bromegrass seed crops are harvested by swathing or by straight cutting.

The use of the plant growth regulators (PGRs) trinexapac-ethyl (TE) and chlormequat chloride (CCC) on grass seed crops is relatively new in Canada. TE (trade name Parlay) is currently registered on perennial ryegrass (Lolium perenne L.) seed crops in Canada, but no other cereal or grass seed crops have been placed on the label. Registration and availability of TE for use on cereals in Canada is expected in 2019 or 2020. CCC is registered on wheat in Canada.

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From 2015 to 2017, a study was conducted at the Agriculture and Agri-Food Canada station at Beaverlodge, British Columbia, to investigate the use of TE at three rates and two growth stages on first- and second-year meadow bromegrass stands (Yoder et al., 2018). The objective of the study was to determine the effect of TE, CCC, and mixtures of TE + CCC on plant height, lodging, seed yield, and seed dockage of meadow bromegrass grown for seed production. In all years, TE reduced plant heights and improved lodging over the check. Seed yield data were variable, and seed yield responses to TE were minimal. First-year meadow bromegrass stands were more responsive to TE than second-year stands. In a study conducted by Anderson et al. (2017), a mixture of TE + CCC reduced lodging in tall fescue but did not have any economic benefit over TE applied alone.

Because of the current availability of CCC in Canada and potential registration of TE on wheat in Canada, PGRs can be an accessible agronomic option for forage seed producers. Additional studies are needed to confirm the previous findings with TE, CCC, and a mixture of both PGRs on grass seed crops in relation to weather variables.

**Methods**
An on-farm trial was conducted in 2017 and 2018 on a field of meadow bromegrass that had been established in 2016. The field was located in the Peace River region of Alberta. ‘Fleet’ cultivar of meadow bromegrass was seeded at a rate of 4 kg ha$^{-1}$ and 30-cm row spacing directly into Roundup Ready canola stubble in the third week of May. The crop was fertilized with 30 kg ha$^{-1}$ of nitrogen (N) and 35 kg ha$^{-1}$ of phosphorus (P) at the time of seeding. The field had received an application of glyphosate + florasulam 3 days prior to seeding to control weeds. A mix of pyrasulfotole + bromoxynil was applied twice during the year to control broadleaved weeds. Precipitation during the establishment year was above average, and stand establishment was excellent. The field was mowed once in the middle of August and fertilized with 110 kg N ha$^{-1}$ in October 2016. In the fall of 2017, following the first seed harvest, the field received N at 70 kg ha$^{-1}$ in September and an additional dose at 70 kg ha$^{-1}$ in mid-October.

The treatments applied are shown in Table 1. Each treatment was applied to an area of 2 x 10 m. The treatments were arranged in a randomized complete block design with four replications. The PGRs were applied with a hand-held plot sprayer with a 2-m boom with four 8001 TeeJet nozzles using a pressure of 270 kPa. Water volume was 100 L ha$^{-1}$. In 2017, the PGR treatments were applied on May 26 at BBCH 39 and on June 3 at BBCH 52. In 2018, treatments were applied on May 23 at BBCH 32 and on June 3 at BBCH 52.

Visual lodging ratings were conducted at flowering and prior to harvest. Lodging was rated on a scale of 1 to 9 with 1 being erect and 9 being flat. Plant heights were measured after flowering by sampling five plants per plot. The plots were harvested by straight combining with a Wintersteiger plot combine. Area harvested was 17 m$^2$ for each plot. The trial was harvested on August 16, 2017 and August 15, 2018. Seed samples were placed in cotton bags, weighed, dried, and then weighed again to determine seed moisture at harvest. Samples were then cleaned and weighed to obtain yield and dockage. Seed quality was assessed in terms of germination percentage and 1,000-seed weight. Results were analyzed using ANOVA and means separated by LSD at 0.05.
Results and Discussion
In 2017, all PGR treatments were effective at reducing plant height and lodging on the first-year stand of meadow bromegrass (Table 1). Trinexapac-ethyl and TE + CCC were more effective in reducing plant height and lodging than CCC alone. Applications made at BBCH 39 (late stem elongation) were more effective than those applied at BBCH 52 (early heading). Seed yields following the application of TE and TE + CCC at the earlier stage were significantly higher than the check. Although the treatment differences were not statistically significant, seed yields from the other treatments were slightly higher than the check. Seed yields were higher when TE and TE + CCC were applied at the earlier growth stage. All PGR treatments reduced seed dockage (Table 1) and had no effect on germination or 1,000-seed weight (Table 2).

Table 1. Effect of trinexapac-ethyl (TE) and chlormequat chloride (CCC) mixes on first-year meadow bromegrass seed stand, 2017.1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stage</th>
<th>Plant height</th>
<th>Lodging</th>
<th>Seed yield</th>
<th>Dockage</th>
<th>Seed moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(kg ai ha⁻¹)</td>
<td>(BBCH)</td>
<td>(cm)</td>
<td>(1–9)</td>
<td>(kg ha⁻¹)</td>
<td>(%)</td>
</tr>
<tr>
<td>TE 0.2</td>
<td>39</td>
<td>126</td>
<td>1.0 a</td>
<td>1.8 c</td>
<td>1,537 a</td>
<td>14.2 bc</td>
</tr>
<tr>
<td>TE 0.1 + CCC 0.56</td>
<td>39</td>
<td>125</td>
<td>1.0 a</td>
<td>1.3 c</td>
<td>1,572 a</td>
<td>13.5 c</td>
</tr>
<tr>
<td>CCC 1.116</td>
<td>39</td>
<td>127</td>
<td>2.8 b</td>
<td>4.3 b</td>
<td>1,282 ab</td>
<td>15.5 b</td>
</tr>
<tr>
<td>TE 0.2</td>
<td>52</td>
<td>128</td>
<td>1.8 b</td>
<td>1.5 c</td>
<td>1,320 ab</td>
<td>15.7 b</td>
</tr>
<tr>
<td>CCC 1.116</td>
<td>52</td>
<td>131</td>
<td>2.3 b</td>
<td>3.0 bc</td>
<td>1,290 ab</td>
<td>14.8 bc</td>
</tr>
<tr>
<td>Check</td>
<td>130</td>
<td>6.0 a</td>
<td>6.3 a</td>
<td>4.5 a</td>
<td>1,092 b</td>
<td>17.7 a</td>
</tr>
<tr>
<td>CV%</td>
<td></td>
<td>3.2</td>
<td>41.3</td>
<td>40.3</td>
<td>11.3</td>
<td>5.1</td>
</tr>
<tr>
<td>LSD P = 0.05</td>
<td></td>
<td>NSD</td>
<td>1.5</td>
<td>1.1</td>
<td>232</td>
<td>1.2</td>
</tr>
</tbody>
</table>

1Numbers followed by the same letter do not significantly differ (P = 0.05 Student-Newman Keuls).

Table 2. Effect of trinexapac-ethyl (TE) and chlormequat chloride (CCC) mixes on germination and 1,000-seed weight of first- and second-year stand of meadow bromegrass.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stage</th>
<th>2017</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(kg ai ha⁻¹)</td>
<td>Germination</td>
<td>1,000-seed weight</td>
</tr>
<tr>
<td></td>
<td>(BBCH)</td>
<td>(%)</td>
<td>(g)</td>
</tr>
<tr>
<td>TE 0.2</td>
<td>32</td>
<td>94.5</td>
<td>5.886</td>
</tr>
<tr>
<td>TE 0.1 + CCC 0.56</td>
<td>32</td>
<td>93.5</td>
<td>6.656</td>
</tr>
<tr>
<td>CCC 1.116</td>
<td>32</td>
<td>96.0</td>
<td>5.715</td>
</tr>
<tr>
<td>TE 0.2</td>
<td>52</td>
<td>96.0</td>
<td>5.793</td>
</tr>
<tr>
<td>CCC 1.116</td>
<td>52</td>
<td>93.5</td>
<td>5.460</td>
</tr>
<tr>
<td>Check</td>
<td>94.5</td>
<td>5.608</td>
<td>97.0</td>
</tr>
<tr>
<td>CV%</td>
<td>4.7</td>
<td>4.4</td>
<td>1.9</td>
</tr>
<tr>
<td>LSD P = 0.05</td>
<td>NSD</td>
<td>NSD</td>
<td>NSD</td>
</tr>
</tbody>
</table>
In 2018, PGR treatments were effective at reducing plant height and lodging of the second-year meadow bromegrass seed crop, especially when applied at BBCH 32 (Table 3). Although the treatment differences were not statistically significant, there was a strong trend for all PGR treatments to have higher seed yields than the check, which was particularly more pronounced in CCC treatments at both stages of application. All PGR treatments reduced dockage and seed moisture content as compared to the check. However, the PGR treatments did not affect seed-quality parameters (Table 2).

The finding that all PGR treatments, particularly TE + CCC or TE alone, applied to a first- and second-year meadow bromegrass stand, reduced plant height and lodging align with findings previously reported on tall fescue by Anderson et al. (2018) and on cocksfoot by Rolston et al. (2014). The effects were stronger at the earlier stage of application. Seed dockage and seed moisture were also lower following the application of PGRs when compared to the check.

Seed yield responses from PGRs were strong on the first-year stand, particularly when TE and TE + CCC were applied at the early growth stage. Growth regulators slightly improved seed yields on the second-year stand. Seed yields following the application of CCC on the second-year stand tended to be higher than those treatments with TE. None of the treatments had any effect on seed germination or 1,000-seed weight.

Conclusion
The results from this study have shown that TE, TE + CCC, and, to some degree, CCC have good potential for use on meadow bromegrass seed crops. They were very effective at reducing lodging in both years, which is particularly important if straight combining is used to harvest the crop. It also appears there may be potential to reduce the rate of TE + CCC mix used in this study and still be effective at reducing lodging on meadow bromegrass seed crops. Additional studies are needed to evaluate various combination of PGRs over a number of fields and environmental conditions.

Table 3. Effect of trinexapac-ethyl (TE) and chlormequat chloride (CCC) mixes on second-year meadow bromegrass seed stand, 2018.1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stage</th>
<th>Plant height</th>
<th>Lodging</th>
<th>Seed yield</th>
<th>Dockage</th>
<th>Seed moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(BBCH)</td>
<td>(cm)</td>
<td>(1–9)</td>
<td>(kg ha⁻¹)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td></td>
<td>July 12</td>
<td>July 12</td>
<td>August 15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TE 0.2</td>
<td>32</td>
<td>33.5 bc</td>
<td>1.0 b</td>
<td>1.0 b</td>
<td>1,016</td>
<td>3.7 bc</td>
</tr>
<tr>
<td>TE 0.1 + CCC 0.56</td>
<td>32</td>
<td>30.6 c</td>
<td>1.0 b</td>
<td>1.0 b</td>
<td>966</td>
<td>3.5 bc</td>
</tr>
<tr>
<td>CCC 1.116</td>
<td>32</td>
<td>37.2 c</td>
<td>1.5 b</td>
<td>1.3 b</td>
<td>1,095</td>
<td>4.4 bc</td>
</tr>
<tr>
<td>TE 0.2</td>
<td>52</td>
<td>35.7 c</td>
<td>1.0 b</td>
<td>1.0 b</td>
<td>1,065</td>
<td>3.3 c</td>
</tr>
<tr>
<td>CCC 1.116</td>
<td>52</td>
<td>39.4 c</td>
<td>1.8 b</td>
<td>1.5 b</td>
<td>1,176</td>
<td>5.2 b</td>
</tr>
<tr>
<td>Check</td>
<td>42.6 a</td>
<td>4.0 a</td>
<td>3.5 a</td>
<td>910</td>
<td>7.2 a</td>
<td>14.1 a</td>
</tr>
<tr>
<td>CV%</td>
<td>6.7</td>
<td>44.4</td>
<td>52.0</td>
<td>13.6</td>
<td>9.7</td>
<td>8.6</td>
</tr>
<tr>
<td>LSD P = 0.05</td>
<td>3.7</td>
<td>1.1</td>
<td>1.2</td>
<td>NSD</td>
<td>1.7</td>
<td>1.6</td>
</tr>
</tbody>
</table>

1Numbers followed by the same letter do not significantly differ (P = 0.05 Student-Newman Keuls).
21 = erect; 9 = flat
References
DELIVERING A SEED PRODUCTION COURSE TO THE WORLD VIA ONLINE DISTANCE EDUCATION

Alyssa S. DuVal1,* and Thomas G. Chastain1

Abstract
Delivery of distance online courses in agriculture has provided many students and industry practitioners access to flexible educational opportunities needed for the fulfillment of degree program requirements and for professional training and advancement in the workplace. Unfortunately, few agronomy courses have been offered to date through online delivery by universities and colleges. We developed “Seed Production,” an online agronomy course offering of Oregon State University, for delivery to both U.S. domestic and global students and practitioners. The objectives in designing this online seed production curriculum were to (1) impart a fundamental knowledge of the modern seed production industry; (2) examine the factors that influence seed yield, seed quality, and the economic viability of seed production operations; and (3) outline the principles and practices required for production of high-quality seed crops. Students in Seed Production learn through a variety of electronic learning modalities in addition to the traditional prerecorded lecture format. These modalities include videos of farm and seed production facilities, student discussion boards, interactive exercises, digital animation, group projects, and more. A key to the success of this course’s design was to weave student interaction and engagement into the learning modules and assignments. Students enrolled in this 11-week course become a part of a learning community through the implementation of active learning techniques, consistent communication, and innovative technologies. The asynchronous online delivery and active learning technologies employed in Seed Production permit learning for students and practitioners who are otherwise unable to fit traditional campus-based agronomy courses into their life schedules.

Keywords: active learning, agronomy, distance education, seed production, student engagement

Introduction
The “Seed Production” course (CROP 460/560) has been offered on campus for decades and delves into the complex management systems for each of the primary seed production industries existing within Oregon. This course is an elective and is not officially required for any degree program at Oregon State University, thereby ensuring that students enrolled in CROP 460/560 historically have had an innate interest in the topic of seed production. Despite the increasing demand for online distance education modalities and agriculture course availability, as of 2015 very few online agronomy courses were available, and none focused on the seed production industry. Acknowledging this deficiency, we acted to adapt and expand upon materials provided in the campus CROP 460/560 to create an online version of the Seed Production course, which became available for undergraduate and graduate students in the fall of 2016. It is important to note that Oregon State University operates on a quarter system, comprised of four 11-week terms, and that CROP 460/560 is offered as an online course each fall term and as an on-campus course each spring term (campus offerings concluded in spring 2018). Staggering the availability of Seed

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Production allowed students to select the offering that met his/her individual preferences, distance status, and term course load.

Several pedagogical strategies and distance education best practices were implemented during the development process for the online Seed Production course. This class is divided into weekly modules (10 modules for content and 1 module for the final exam), each containing measurable learning outcomes, learning content, and assessments. Course learning content includes voice-over PowerPoint lectures with closed-captioning, reading assignments, various multimedia productions, and supplemental resources (websites, journal articles, open-source videos, etc.). Multimedia productions were developed in collaboration with seed production industry professionals and multimedia experts at Oregon State University Ecampus; these productions include several industry video tours, a drag-and-drop seed anatomy practice activity, seed cleaning and screen selection game, and a grass seed harvest animation featuring drone footage. Student achievement of course learning outcomes is evaluated through several assessments—weekly quizzes, discussion boards, assignments, exams, and a group project. Students enrolled in the graduate version of Seed Production (CROP 560) must additionally complete a literature review assignment and a video presentation project relating to his/her thesis research endeavors. Combining the use of traditional graded assessments (homework tasks) with a collaborative group project, discussion boards, graduate student project, and low- and no-stake activities (such as weekly quizzes and multimedia productions) is a tactic for increasing active learning opportunities within the course.

Active learning is a “process where the learner takes a dynamic and energetic role in one’s own education” (Petress, 2008) and is directly related to increased student performance in science, technology, engineering, and mathematics courses (Freeman et al., 2014). Several opportunities for active learning are offered in CROP 460/560. For example, “In the News” research tasks represent half of the assignments in this course, through which activity students peruse current literature and newspaper articles for topics related to seed production. Students then summarize information from three articles and discuss the science and potential industry applications with classmates through a discussion forum. Industry video tours represent another example of active learning in Seed Production. Four separate tours were recorded to immerse students in various aspects of the seed production industry—the Oregon State University Seed Laboratory, helicopter field inspections for Seed Certification, operations at a seed conditioning facility, and grass seed harvest and postharvest practices. Students were required to view each of these videos, questions from which were included in the course examinations. The most in-depth active learning activity in CROP 460/560 is a group project in which students research a seed crop of their choice and develop a detailed report and presentation. The instructor randomly assigns groups, but group progress on the final report and presentation is assessed through nine different graded checkpoint tasks spread throughout the term (including topic selection, group member roles/responsibilities, rough drafts, and a group evaluation). This group project is designed as a hybrid between collaborative and cooperative approaches and assesses both student ability to function responsibly in a group setting as well as mastery of the subject matter.

While student achievement of learning outcomes is the ultimate goal of each course, instructors must consider the recommended best practices for online distance education when designing a course. The recommended best practices for online distance education are based on Quality Matters, an organization that is widely recognized as the world leader in the promotion and improvement of online education and student learning (https://www.qualitymatters.org). The Quality Matters program has established 8 general standards and 42 specific review standards that quality online courses should meet. The fifth general
Quality Matters standard details requirements for student interactions not only with the course content, but also with fellow students and the course instructor. The work of Baker and Moyer (2019) supports this Quality Matters standard and concludes that “students who felt they established a connection with others [both peers and the instructor] had higher levels of engagement, perceived value [of the online course] to career, overall evaluation and preference for online courses, and lower levels of anxiety/frustration.”

Student interactions comprise a large portion of the Seed Production course. Students regularly interact with course content through assessments, interactive multimedia technologies, and voice-over PowerPoint lectures. Student-to-student interactions are exemplified in the group project as well as in weekly discussion board activities where students synthesize information addressed in the learning content or further literature research and discuss, debate, and innovate various topics and applications in seed production. Students are able to interact with the CROP 460/560 instructors through several student- or instructor-initiated avenues: direct email, announcements (instructor-initiated only), question-and-answer discussion board, weekly discussion boards, assignment grading rubrics, and submission comments/feedback on each assessment. Although this level of access to the instructors increases instructor responsibility, it presents a high degree of instructor presence within the course and creates the ability to develop a positive rapport and learning environment (Frisby and Martin, 2010), both of which translate into increased student performance and positive perceptions of the course (Baker and Moyer, 2019).

Methods
The instructional staff facilitating the online Seed Production course recognized a diverse student interest in seed production and primarily positive student experiences with the course design. Hypothesizing that the CROP 460/560 course design is meeting student demands and serving a diverse clientele (student and professional), an initial data analysis was initiated. Student demographic data were collected via student registration records and analyzed for descriptive statistics. Student perceptions of the Seed Production course were obtained through student evaluation of teaching (formal survey), as well as through a student-led discussion board course review (informal survey). Correlations between student demographics, characteristics, perceptions, and course performance were not analyzed, but may be of interest in future studies.

Results and Discussion
The Seed Production course is offered online one term per year, beginning in 2016. Across three years of enrollment records, the majority of Seed Production students were undergraduates majoring in General Agriculture (Table 1), but it is important to note that, during this study period, only General

<table>
<thead>
<tr>
<th>Table 1. “Seed Production” course student demographics.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Student demographics</strong> n = 43</td>
</tr>
<tr>
<td>Undergraduate</td>
</tr>
<tr>
<td>Graduate</td>
</tr>
<tr>
<td>Professional (nondegree seeking)</td>
</tr>
<tr>
<td><strong>Student major</strong></td>
</tr>
<tr>
<td>General Agriculture</td>
</tr>
<tr>
<td>Horticulture</td>
</tr>
<tr>
<td>Crop and Soil Science</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Not declared/nondegree</td>
</tr>
</tbody>
</table>
Agriculture and Horticulture offered online degree programs, which may have influenced student demographics. Professionals enrolled in Seed Production registered as nondegree-seeking students, either as undergraduates or graduates, and were completing the course for professional advancement purposes or were new employees in crop-related industries (especially those new to agriculture). Of all students that completed the Seed Production course, including undergraduate and graduate students, 70% resided within Oregon (Figure 1). However, of the entire course enrollment, only 42% of students identified as Corvallis, Oregon, campus-based students, which demonstrates the increasing need for online distance education. Approximately one-third of the CROP 460/560 students were Oregon residents but were pursuing an online degree either due to distance from the Corvallis campus or because the student received a lower tuition rate as a distance student compared to traditional out-of-state tuition rates. These demographic data support the hypothesis that the Seed Production course serves a diverse student and professional clientele, both in terms of location and educational/occupational interests.

When evaluating the efficacy of course design, it is important to recall that student interactions with the course content, fellow students, and the instructor play a large role in the perceived importance and quality of the course as well as overall student achievements (Baker and Moyer, 2019; Frisby and Martin, 2010). Annual Oregon State University-conducted student evaluation of teaching surveys show that the Seed Production online course consistently achieves high ratings, especially regarding the class environment (data not shown). The survey results demonstrate the immense value that students place on student–instructor interactions. Higher degrees of instructor presence within the course and the development of strong student–instructor rapport lead to improved student comprehension of course materials, achievement of learning outcomes, and overall positive student perceptions of the course and instructor.

To expand upon the student evaluation of teaching survey results, an informal survey of the course design is conducted through a discussion board activity. In one of the final discussion boards for the term, each student is asked to identify his/her favorite element from the course, an element that he/she did not enjoy, and at least one potential improvement to future offerings of Seed Production. After student completion

![Figure 1. Geographic provenance of Seed Production course student enrollees. The geographic distribution of students worldwide is depicted in the chart on the left, and the distribution within Oregon is shown in the chart on the right.](image-url)
of the course, the instructor reviews this discussion board. Typically, students will agree on the top three most enjoyable and least enjoyable course elements within a given term, but these items are generally not consistent across terms.

The most common favorite course elements and top least-enjoyed elements identified by students are presented in Figure 2. Overall, lectures were viewed as beneficial to the course, with only one lecture topic (seed production economics) being selected by a single student as “the least enjoyable element” within the entire CROP 460/560 course. Students identified vegetable seed production and grass seed postharvest residue management as the most enjoyable lectures. All students enjoyed the industry video tours, with a number of students selecting the seed certification and seed conditioning facility tours as their favorite course element, primarily because these multimedia productions provide students with a realistic view of seed production and industry practices, to which many of the students would not have otherwise been exposed. The “In the News” active learning assignments received mixed reviews. Some students preferred these learning opportunities because they allowed for greater research and thought about components of the seed industry, while other students did not enjoy searching through the available literature. Although the relationship was not statistically explored due to small sample size, it is possible that students who did not enjoy these assignments were also not attracted to commercial, conventional seed production and thus experienced difficulty locating scientific seed production publications of interest. The “mini poster” is an assignment in which students briefly research a seed production topic of interest and create an infographic. This assignment was developed in 2017 to address alternative learning styles.

Figure 2. Student perception of Seed Production course elements.
and capitalize on student creativity; however, the students that did not enjoy this assignment (Figure 2) noted a personally perceived “lack of creativity” as being the primary reason for dissatisfaction with this task.

Students are often regarded as resistant to participation in online discussion boards as a learning modality. As reported by Baker and Moyer (2019), “students ranked discussion forums as most helpful in achieving a sense of social presence...[but] discussion forums were ranked last in contributing to their learning.” However, only one student across all 3 years of CROP 460/560 identified discussion boards as his/her least enjoyable course element. Perhaps this discrepancy with the literature results from differences in student demographics, interest in the course materials (especially considering that Seed Production is an elective course and not a requirement), or the individual discussion board prompts.

In addition to discussion board activities, students also tend to resist group projects, both in traditional and online modalities (Smith et al., 2011). Students in CROP 460/560 typically followed this trend, with 9 of 43 students describing the group project as the least enjoyable course element (Figure 2). Similar to discussion boards, when informally surveyed further regarding student experience with the group project, most students recognized group dynamics and previous negative experiences with group projects as factors biasing their view of collaborative work, both in face-to-face courses and in the online setting. Instructions for the CROP 460/560 group project were improved for the 2017 course offering, which led to an improvement in student performance and perception of the value of group work. Surprisingly, the number of students selecting this course element as the least enjoyable has been declining each year, which may be the result of differing group dynamics, more detailed group project instructions, or the increasing presence of group projects across online courses, thereby working to reduce student hesitation toward online collaborative work and to provide increasingly positive group experiences.

In addition to elucidating students’ most and least enjoyable activities within the Seed Production course, the informal discussion board survey also provided students with an opportunity to brainstorm future improvements in course design. With the exception of student suggestions to modify the course group project requirement into an individual project, student recommendations have provided insight into student interests in the seed production industry. The most widely student-recommended improvements include developing additional seed industry video tours, expanding upon the vegetable seed production content, and creating a module addressing global seed crop production (comparison of scope and production practices with the Oregon-based industry). This type of student-led course review activity helps students think critically about not only the course content but also how the class is designed. Instructors are able to perceive content areas that students value and others that may not meet student expectations.

**Conclusion**

Through the years of teaching an online seed production course, it is apparent that a strong, expanding, and diverse clientele exists in both the academic and professional settings (i.e., more individuals are interested in a seed production course). Additionally, when designing online agronomic courses, instructors should integrate best practice standards for student–content, student–student, and student–instructor interactions. The success of each online course relies not only on the quality of course content...
and student achievement of course learning outcomes, but also on consistent instructor presence within the course that drives the development of a strong, positive student perception of the course and, ultimately, a long-lasting course and instructor reputation.

References
SEED DEVELOPMENT
AND HARVEST EFFICIENCY

ORAL PRESENTATIONS
IS RED CLOVER SEED SET POTENTIAL INFLUENCED BY POLLEN QUALITY?

Shuxuan Jing¹, Per Kryger¹, and Birte Boelt¹,*

Abstract
The commercial use of high-forage-yielding tetraploid red clover (Trifolium pratense L.) is limited due to its low seed yield, but, surprisingly, the seed set potential of individual florets of diploid and tetraploid red clover cultivars is rarely studied. Hand pollination experiments were conducted in two diploid and one tetraploid red clover cultivars to investigate (1) the correlation between seed set and the number of pollinated florets per flower head, and (2) how seed set is influenced by the different sources and amounts of pollen deposited on the stigmas. The results demonstrate that an increase in the number of pollinated florets (from 0 to 80 florets) increased the number of harvested seeds. However, the seed set rate increased only up to approximately 20 pollinated florets. No significant difference in seed set was found between the florets pollinated with a low (on average 43 pollen grains) or high (on average 84 pollen grains) number of pollen grains, except for the red clover cultivar, which has the highest pollen viability. We suggest that the amount of viable pollen deposited on the stigma influences seed set in red clover. Furthermore, results showed that pollen from cultivars with different ploidy levels might disturb seed set. In conclusion, results indicate the importance of pollen quality (pollen viability and pollen source) to seed set of diploid and tetraploid red clover. We found hand pollination an excellent method/tool for investigating the underlying causes of low seed set in red clover, provided that at least 20 florets per flower head are pollinated.

Keywords: red clover, hand pollination, pollen viability, ploidy level, seed production

Introduction
The limitation for commercial exploitation of tetraploid red clover is its low seed yield. The seed yield of red clover generally represents a small fraction of theoretical seed yield (Dennis and Haas, 1967), indicating a potential for significant improvement. However, red clover seed set potential per floret is rarely studied. Each floret of red clover has the potential to produce two seeds, as each ovary usually has two ovules, but ovule site utilization is reported to be only 25% (Lorenzetti, 1993).

It is not known if this low site utilization is caused by inadequate pollination. Overall, we would expect a higher number of harvested seeds when additional florets are pollinated. Further, the amount of pollen deposited on the stigma may also influence seed set. Pollen quality refers to pollen from different sources for the outcrossing plants, based on whether pollen grains are from the same plant or not. In sunflower (Helianthus L.), a joint effect of pollen quantity and pollen quality was found to influence grain set (Chamer et al., 2015). Pollen viability is an integral part of pollen quality. Surprisingly, the effects of pollen quantity and quality on seed set of diploid and tetraploid red clover have not been studied. The current study aimed to explore the seed set potential of red clover cultivars with different ploidy levels based on the following hypotheses: (a) there is a correlation between seed set and the number of pollinated florets per flower head, and (b) the sources and amounts of pollen deposited on the stigma influence red clover seed set.

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Methods
Experiments were performed at AU-Flakkebjerg, Denmark (55°19′52″N, 11°24′29″E) during the summer of 2018 with three red clover cultivars: ‘Rajah’ and ‘Suez’ (both 2n or diploid) and ‘Amos’ (4n or tetraploid). Hand pollination experiments were conducted in a climate chamber set with 14 hours of daylight (08:00–22:00) and with temperature of 20°C (day) and 15°C (night). Pollen from the pollen donor plants was collected by tripping the pollen from the anthers to a petri dish with a toothpick. Pollen grains on the toothpick were collected every 10 florets from the petri dish, and a new toothpick was used for pollinating each flower head by depositing the pollen on the stigmas.

Hypothesis (a) included treatments of 0, 10, 20, 40, and 80 pollinated florets per flower head. All of the available florets were pollinated when the maximum number of florets per flower head was below 80 florets, and the seed set rate was calculated according to the actual number of pollinated florets. For the calculation of seed set rate, we assume that each red clover ovary contains two ovules and thus has the potential to produce two seeds per floret (Lorenzetti, 1993). Seed set (%) = the number of harvested seeds/(number of pollinated florets per flower head × 2) × 100%. Pollen donors were the red clover plants from the same cultivar.

To test hypothesis (b), we conducted both self-pollination and cross-pollination experiments. The same plant was used as the pollen donor for self-pollination, while pollen donors for cross-pollination included (1) plants from the same cultivar other than the pollen recipient plant and (2) plants from other cultivars. In addition, low and high amounts of pollen were applied, and the average pollen grain amount per floret was determined in the preliminary study: 43 pollen grains for the low amount and 84 pollen grains for the high amount. For each treatment except the no pollination treatment, 10 florets per flower head were pollinated. Three flower heads per plant were used as replicates, and in total five plants were used in each red clover cultivar. Seed set for hypothesis (b) was presented as the harvested seed number per flower head among the 10 pollinated florets. The 2,3,5-triphenyl tetrazolium chloride (TTC) test was used for quantification of the pollen viability of each red clover cultivar. Pollen viability was measured as the percentage of stained pollen grains among the total amount of pollen grains.

In all hand pollination experiments, five plants were used for each red clover cultivar, with three flower heads per plant used as replicates. After hand pollination, plants were moved to the semi-field, and pollinated flower heads were carefully bagged using light-transparent and air-permeable nonwoven fabrics. The number of seeds from the bagged flower heads was counted after the flower heads were harvested, air dried, hand threshed, and cleaned in October 2018. The generalized linear mixed model (GLMM) was used in R version 3.5.0 to compare pollen viability, harvested seed number, and seed set rate (%) of the three red clover cultivars.

Results and Discussion
Harvested seed number increased with an increasing number of pollinated florets in three red clover cultivars (Figure 1A). However, the increasing number of pollinated florets only increased the seed set rate up to a level of 20 pollinated florets (Figure 1B), indicating the limitation of seed set ability per flower head in the current study. Notably, when 40 florets were pollinated, the seed set rate of ‘Suez’ (2n) was significantly higher than that of ‘Rajah’ (2n) and ‘Amos’ (4n) (Figure 1B), which may be related to the higher fertility of the pollen recipient plants of ‘Suez’ (2n). No significant differences in seed number per flower head or seed set rate were found between ‘Rajah’ (2n) and ‘Amos’ (4n) in any of the
treatments, indicating their similar seed set ability. In addition, the pollen viability of ‘Rajah’ (2n) and ‘Amos’ (4n) was not significantly different (data not shown). Therefore, diploid and tetraploid red clover may not differ in seed set potential in the current hand pollination experiment.

Red clover has a high degree of self-incompatibility, especially for diploid red clover (Julén, 1950). However, an increase in pollen amount did not significantly increase seed set of either diploid or tetraploid red clover cultivars in the self-pollination experiment (Figure 2). For cross-pollination, treatments with

![Figure 1](image1.png)  
**Figure 1.** Effect of pollinated floret number on seed number per flower (A) and seed set rate (B) in three cultivars: ‘Rajah’, ‘Suez’, and ‘Amos’. Error bars indicate the 95% confidence interval of the estimated marginal means (EMMs).

![Figure 2](image2.png)  
**Figure 2.** Effect of self-pollination with different amount of pollen (no pollination, low pollen amount, high pollen amount) on seed number per 10 pollinated florets in three cultivars: ‘Rajah’, ‘Suez’, and ‘Amos’. Error bars indicate the 95% confidence interval of the estimated marginal means (EMMs). EMMs sharing a letter are not significantly different at 0.05 significance level (Tukey adjusted comparisons).
pollen from the same ploidy level showed a higher seed set compared to pollen from the different ploidy level (Figure 3). Julén (1950) suggested that the presence of haploid pollen (from diploids) might disturb seed set of tetraploid red clover, and the current study supports this observation. With ‘Suez’ (2n) as the pollen donor (Figure 3B), an increase in pollen amount significantly \((P < 0.05)\) increased seed set in ‘Rajah’ (2n) and ‘Suez’ (2n). However, the increased pollen amount did not increase seed set when the pollen donor was ‘Rajah’ (2n; Figure 3A) or ‘Amos’ (4n; Figure 3C), reflecting the higher pollen viability of ‘Suez’ (2n) as seen in the pollen viability test (data not shown). Aizen and Harder (2007) suggested that experiments with pollen supplementation may overestimate the effect of pollen amount because pollen quality may also influence plants.

In the experiment for hypothesis (b), 10 florets per flower head were pollinated. However, the 10 pollinated florets showed rather low seed set rate (Figure 1), and we recommend that at least 20 florets per flower head should be pollinated in hand pollination experiments.

**Conclusion**

We suggest that seed set of red clover was not directly limited by the amount of pollen deposited on the stigma but rather by the amount of viable pollen. These results indicate the importance of pollen quality (pollen viability and pollen source) to seed set in diploid and tetraploid red clover.

**References**


![Figure 3](image.png)

**Figure 3.** Effect of cross-pollination on seed number per 10 pollinated florets in pollen recipient plants receiving the low and high pollen amount from pollen donor ‘Rajah’ (A), ‘Suez’ (B), and ‘Amos’ (C). Error bars indicate the 95% confidence interval of the estimated marginal means (EMMs). EMMs sharing a letter are not significantly different at 0.05 significance level (Tukey adjusted comparisons).
HARVEST EFFICIENCY AND SEED QUALITY IN ORCHARDGRASS SEED PRODUCTION

Federico Bertuch1,*, Santiago N. Tourn2, M. Belén Mermoz3, and Pablo Grehan4

Abstract
Orchardgrass (Dactylis glomerata L.) is a high-yielding forage grass with good palatability and high digestibility (Christie and Mowat, 1968). Its botanical characteristics make incomplete threshing an important issue during seed harvest. Compared to other temperate grass species, there are a greater number of doubles; thus, dressing losses are high, and redressing is often needed. Consequently, the gap between potential and obtained seed yield is large. The objective of this work was to evaluate harvest efficiency (HE) and seed purity (SP) (percentage of single seed, doubles, groats, and inert matter) when harvesting at different combine speeds and with different threshing aggressiveness. Two independent trials were conducted in a first-year orchardgrass seed paddock with the cultivar ‘Porto’. The first trial evaluated two combine speeds: 3 km h⁻¹ and 6 km h⁻¹. The other evaluated threshing aggressiveness: covering 50% or 0% of the concave. Both experiments had a completely randomized design with three replications. The studies were conducted in January 2018 in the southeastern part of Buenos Aires province, Argentina. Harvest was accomplished by swathing at 32% seed moisture content (SMC) and combining with a John Deere 1175 combine. There was a significant difference in HE when harvesting at 3 km h⁻¹ (70%) and at 6 km h⁻¹ (37%), but there were no significant differences between these treatments in SP or in the percentage of doubles. There was a significant difference in HE when covering 50% of the concave (77%) vs. covering 0% of the concave (65%), but there were no significant differences between these treatments in SP or in the percentage of doubles. These results suggest that, in this environment, the best harvest settings tested were a combine speed of 3 km h⁻¹ and a 50% covered concave. These settings increased orchardgrass seed yields and maintained SP standards without increasing the percentage of doubles and subsequent dressing losses.

Keywords: orchardgrass, harvest efficiency, combine speed, threshing aggressiveness, dressing losses

Introduction
Orchardgrass (Dactylis glomerata L.) is a high-yielding forage grass with good palatability and high digestibility for livestock (Christie and Mowat, 1968). It is an important species in Argentina’s domestic market, as it is the fourth forage species in market share, behind lucerne, tall fescue, and annual ryegrass. From 2014 to 2017, orchardgrass was the only temperate forage species that had a positive double-digit interannual variation in market share (Cámara de Semilleristas, 2014–2018).

Its botanical characteristics make incomplete threshing an important issue when harvesting orchardgrass grown for seed. Compared to other temperate grass species, there are more doubles; thus, dressing losses are high, and redressing is often needed. Consequently, the gap between potential and obtained seed yield is large.

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IHSG 2019 – Corvallis, Oregon, USA
Seed yields differ greatly between production regions in South America. Castaño et al. (2001) evaluated seed production of nine orchardgrass cultivars in Pergamino (-33.891° S, -60.575° W) and Balcarce (-37.847° S, -58.255° W) and found no genotype–environment interaction. Seed yields were significantly higher in Balcarce than in Pergamino: 699.4 vs. 136.6 kg ha	extsuperscript{-1} and 521.5 vs. 294.7 kg ha	extsuperscript{-1} in 1997 and 1998, respectively. From the second year onward, growers’ yields are usually 150–250 kg ha	extsuperscript{-1} in Balcarce (Castaño, 2005). Balcarce is located in the southeastern part of Buenos Aires province. This area is a subhumid-humid region, according to Thornthwaite’s (1948) classification, with seasonal water deficits. Average annual rainfall during the period 2000–2017 was 945 mm, with the maximum in March and the minimum in July (Figure 1). The maximum monthly mean temperature was 21°C in January, while the minimum monthly mean temperature was 8°C in July. The average date of first frost is April 24, while the average date of last frost is November 10 (INTA, 2019).

The increasing demand for orchardgrass seed in South America makes it necessary to identify the regions with the greatest potential for seed production and to develop technologies that guarantee good yields and quality seed. The objective of this work was to identify the potential seed yield of orchardgrass in the southeastern region of Buenos Aires province and to evaluate harvest efficiency (HE) and seed purity (SP), including the percentage of single seed, doubles, groats, and inert matter, when harvesting at different combine speeds and with different threshing aggressiveness.

**Methods**

Two independent trials were conducted in a first-year orchardgrass seed paddock with the cultivar ‘Porto’. The first trial evaluated two combine speeds: 3 km h	extsuperscript{-1} and 6 km h	extsuperscript{-1}. The second trial evaluated threshing aggressiveness treatments: covering 50% and 0% of the concave. Both experiments had a completely randomized design with three replications.

The studies were conducted in January 2018 in Coronel Vidal county, in the southeastern region of Buenos Aires province, Argentina. The crop was sown on May 6, 2017 with a sowing rate of 5 kg ha	extsuperscript{-1}. The soil was a Thaptoargic Hapludoll with a land capability classification of IIws. The height above sea level was 18 meters.

![Figure 1. Annual precipitation and monthly average high and low temperature for the southeastern region of Buenos Aires province, 2000–2017.](image-url)
The harvest method was swathing at 32% soil moisture content (SMC) and combining with a John Deere 1175. The combine settings were a 3-mm clearance between the concave and the cylinder, 900-rpm cylinder speed, 6-mm gap in the top sieve, 4-mm gap in the bottom sieve, and 350-rpm fan speed. Potential field-dressed (FD) seed yield, real FD seed yield, and harvest losses were measured for all treatments and replications. Potential FD seed yield was measured by collecting 2 m of the windrow and threshing it at the laboratory; seed shattered to the ground was not taken into consideration in potential yield. Real FD seed yield was obtained by collecting the seed from the combine grain tank. Harvest losses were measured with loss pans. All samples were packed, labeled, and weighed. Harvest efficiency was calculated for all treatments and replications. Laboratory working samples for purity analysis were obtained with a soil divider. The pure seed definition used for *Dactylis glomerata* L. was that established by ISTA (International Seed Testing Association), in which all of the following are considered pure seed: florets with lemma and palea enclosing a caryopsis, with or without awn (single seed); caryopsis or pieces of caryopsis larger than one-half the original size (groats); and multiple seed units consisting of spikelets or parts of spikelets with more than one floret, with or without glumes (doubles). The percentage of single seed, groats, and doubles was identified during purity analysis.

The results were analyzed by ANOVA and Tukey’s test at the 0.05 level of significance.

**Results and Discussion**

Results of ANOVA are shown in Tables 1 and 2. There was a significant difference in HE when harvesting at 3 km h\(^{-1}\) (70%) and at 6 km h\(^{-1}\) (37%), but there were no significant differences in SP or in the percentage of doubles between these treatments. Likewise, there was a significant difference in HE when covering 50% of the concave (77%) vs. covering 0% of the concave (65%), but there were no significant differences in SP or in the percentage of doubles between these treatments.

**Table 1.** Potential field-dressed (FD) yield, real FD yield, and harvest efficiency (HE) when harvesting with different combine speeds and different threshing aggressiveness.  

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment</th>
<th>Potential yield (kg ha(^{-1}))</th>
<th>Real yield (kg ha(^{-1}))</th>
<th>Harvest efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combine speed</td>
<td>3 km h(^{-1})</td>
<td>616 a</td>
<td>430 a</td>
<td>70 a</td>
</tr>
<tr>
<td></td>
<td>6 km h(^{-1})</td>
<td>569 a</td>
<td>207 b</td>
<td>37 b</td>
</tr>
<tr>
<td>Threshing</td>
<td>0%</td>
<td>622 a</td>
<td>403 a</td>
<td>65 a</td>
</tr>
<tr>
<td>aggressiveness</td>
<td>50%</td>
<td>561 a</td>
<td>432 a</td>
<td>77 b</td>
</tr>
</tbody>
</table>

\(^{1}\)Means with a common letter are not significantly different (\(P > 0.05\)).
Table 2. Seed purity (SP), inert matter, single seed, doubles, and groats (%) when harvesting with different combine speeds and different threshing aggressiveness.\(^1\)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment</th>
<th>Seed purity (%)</th>
<th>Inert matter (%)</th>
<th>Single seed (%)</th>
<th>Doubles (%)</th>
<th>Groats (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combine speed</td>
<td>3 km h(^{-1})</td>
<td>96.63 a</td>
<td>3.37 a</td>
<td>72.10 a</td>
<td>17.83 a</td>
<td>6.70 a</td>
</tr>
<tr>
<td></td>
<td>6 km h(^{-1})</td>
<td>93.20 a</td>
<td>6.80 a</td>
<td>71.17 a</td>
<td>14.10 a</td>
<td>7.93 a</td>
</tr>
<tr>
<td>Threshing aggressiveness</td>
<td>0%</td>
<td>95.83 a</td>
<td>4.17 a</td>
<td>74.53 a</td>
<td>10.03 a</td>
<td>11.27 a</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>94.87 a</td>
<td>5.17 a</td>
<td>65.40 b</td>
<td>12.97 a</td>
<td>16.50 a</td>
</tr>
</tbody>
</table>

\(^1\)Means with a common letter are not significantly different (\(P > 0.05\)).

**Conclusion**

These results suggest that, in the environment in which the trials were conducted, the best harvest settings tested were a combine speed of 3 km h\(^{-1}\) and a 50% covered concave. These adjustments improved HE without affecting SP. The absence of significant differences in the percentage of doubles between treatments may ensure that dressing losses will not increase with these combine settings.

Considering that average orchardgrass seed yields in the southeastern region of Buenos Aires province have been between 150 and 250 kg h\(^{-1}\) and that potential FD seed yields can reach 561–622 kg h\(^{-1}\), it can be concluded that correct harvest settings, such as combine speed and threshing aggressiveness, can have a positive impact on HE and real seed yields.

In addition, the SMC recommended for swathing orchardgrass is between 42 and 46%. Thus, it can be expected that swathing at 32% SMC might have had a negative impact on potential FD seed yield because of high seed shattering.

**References**


Thornthwaite, C.W. 1948. *Thornthwaite Climate Classification*. 
SEED YIELD AND SEED SHATTERING WITH DIFFERENT WINDROWERS IN OREGON GRASS SEED CROPS

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Abstract
The loss of viable seed as a result of shattering during harvest can be an important constraint to achieving high grass seed yields. Seed moisture content (SMC) is currently the only reliable tool available to determine harvest timing and reduce seed loss during the windrowing process. Harvest losses from shattering in perennial ryegrass (Lolium perenne L.) averaged 24% in New Zealand. The objective of this study was to compare seed yield and shattering losses among four windrowers (John Deere (JD) double auger 890, John Deere rotary disc 995, MacDon (MD) single auger A40DX, and MacDon draper DX115) in commercial perennial ryegrass and tall fescue (Schedonorus arundinaceus [Shreb.] Dumort.) seed fields. Field trials were conducted at four on-farm sites with a plot size of 7.6 m x 150 m. The experimental design was a randomized block with three replications. Shattered seed was collected by vacuuming three 0.74-m² areas in each plot 1 week after windrowing. Plots were harvested with grower combines, and seed yield was determined by using a weigh wagon. In tall fescue, the JD double auger and the MD draper windrowers harvested 11.3% and 15.3% greater yield than the JD rotary disc, respectively. There were no differences in seed shattering losses in tall fescue among windrowers, with losses ranging from 7.7 to 10.5%. In perennial ryegrass, the MD single auger and MD draper harvested 13 and 15.2% greater yield than the JD double auger, respectively. Seed losses in perennial ryegrass ranged from 18 to 34.9%, with the MD draper windrower producing lower seed loss compared to other windrowers. Results suggest that windrowers perform differently in tall fescue and perennial ryegrass and that the risk of losing seed yield to shattering at harvest is greater in perennial ryegrass compared to tall fescue under Oregon conditions.

Keywords: seed shattering, harvest timing, perennial ryegrass, tall fescue, seed moisture content

Introduction
Obtaining maximum seed yield in grass seed crops is dependent on strategies to improve upon biological and management inefficiencies. Several biological processes negatively affect seed yield potential of grass seed crops. Many more florets are produced by grass seed crops than are pollinated or fertilized, and, as a result, few florets become seed. Of the seed produced by grass seed crops, many are aborted or shattered. As a consequence, seed loss from shattering is an important constraint to improving seed yield that has not been adequately quantified in Oregon grass seed crops. Shattering is a natural phenomenon that aids in the dispersal of seed. In grass seed crops, most seed shattering occurs before and during the windrowing process (Elgersma et al., 1988). Rolston and Chynoweth (2010) reported that yield losses as a result of harvest activities averaged 24% in perennial ryegrass (Lolium perenne L.) grown in New Zealand. Losses of this magnitude can result in a considerable loss of production profit.

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The lack of uniformity in crop development in grass seed crops leads to variation in seed maturation within a field and among cultivars, making optimum harvest timing difficult to determine. Measuring seed moisture content (SMC) is the most reliable tool currently available to growers to determine the timing of windrow harvest and to minimize seed loss during harvest (Silberstein et al., 2010). Andrade et al. (1994) showed that seed shatter in tall fescue (Schedonorus arundinaceous [Shreb.] Dumort.) ranged from 12 to 27% when windrowed at approximately 400 g kg\(^{-1}\) SMC. The objective of this study was to compare seed yield and shattering losses among four windrowers in commercial tall fescue and perennial ryegrass seed fields.

**Methods**
Large-scale field trials were conducted at four on-farm sites in western Oregon. Two sites were dryland turf-type tall fescue (cv. ‘Rambler’ and ‘Rebel Advance’), and two sites were irrigated turf-type perennial ryegrass (cv. ‘ASP0116EXT’ and ‘Stellar 3GL’). Plots were 7.6 m wide x 150 m long. The experimental design was a randomized block with three replications. The four experimental windrower treatments were a John Deere (JD) double auger 890, John Deere rotary disc 995, MacDon (MD) single auger A40DX, and MacDon draper DX115. Seed moisture was measured at the time of windrowing according to methods described in Silberstein et al. (2010) and Silberstein and Anderson (2011).

Seed loss from shattering was determined by vacuuming three 0.74-m\(^2\) areas in each plot 6–7 days after windrowing. Within the vacuumed area, 50% was under the windrow, and the remaining 50% was outside of the windrow. A durable plastic board with smooth surfaces was gently inserted under the windrow and was used to raise it in order to prevent shattered seed from reaching the soil surface during the process of moving the windrow. Viable seed was separated from nonviable seed, dirt, and straw with several seed-cleaning techniques, including use of an air blower, hand screens, a gravity table, a tabletop air-screen cleaning machine, and manual removal with forceps.

Each of the four windrowers was used to cut a strip (width = windrower header width) from the center of each plot on the same day that the seed grower cut the field outside of the trial area. Seed moisture content ranged from 24.7 to 30.5% for the tall fescue and from 14.6 to 28.2% for the perennial ryegrass. Windrows were dried in the field for 7–10 days until the harvested crop material was ready to be threshed with a commercial combine harvester. A weigh wagon was used to determine bulk seed weight from individual plots. Clean seed yields were determined on subsamples of the harvested seed processed through an air-screen cleaning machine. Analysis of variance was conducted to test windrower treatment effects on shattered seed losses and clean seed yield. Treatment means were separated by Fisher’s protected LSD values at the 5% level of significance.

**Results and Discussion**
Seed yield and seed shattering losses varied among species, cultivar, and SMC. The grass seed crops at all four locations were windrowed at SMC lower than that recommended by Silberstein et al. (2010) to minimize seed shattering losses. The JD double auger and the MD draper windrowers harvested 11.3% and 15.3% greater seed yields than the JD rotary disc, respectively, across the two tall fescue sites (Table 1). In perennial ryegrass, the MD single auger and MD draper harvested 13 and 15.2% greater seed yields than the JD double auger, respectively (Table 2).

There were no significant differences in seed shattering losses in tall fescue among windrowers, with losses ranging from 7.7 to 10.5% (Table 1). Seed losses in perennial ryegrass were higher, with losses...
ranging from 18 to 34.9% (Table 2). The MD draper windrower produced lower seed losses compared to the other three windrowers tested. While the seed yield of the ‘Stellar Advanced’ field was high, there was significantly higher seed loss compared to the ‘ASP0116EXT’ field even though its SMC was almost twice as high.

**Conclusion**

These results suggest that windrowers perform differently in tall fescue and perennial ryegrass and that the risk of losing seed yield to shattering at harvest is greater in perennial ryegrass than in tall fescue under western Oregon conditions. Performance of the windrowers might have been different if the fields had been windrowed at a higher SMC. This could be especially true for the JD rotary disc, which is operated at a much higher ground speed compared to the other machines included in this study. Nevertheless, windrowing at SMC values lower than recommended is common when growers fall behind in their harvest schedules. Genetic differences among cultivars also appear to play a role in seed shatter susceptibility of the crop.

While proper windrower selection and seed moisture testing are important harvest management tools that help reduce seed loss at harvest, they are limited in their ability to eliminate the problem altogether. Identification of phenotypic characteristics and gene traits associated with seed shattering is more likely to play a key role in reducing seed losses due to shatter in the future. Genes involved in the seed shattering process have been isolated and characterized in several monocot crop species. The shattering trait has been largely bred out of domesticated grasses such as cereals, but it has persisted in grasses used

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**Table 1.** Mean clean seed yield, shattered seed loss, and percent yield loss with four windrower treatments across two dryland turf-type tall fescue seed fields in western Oregon.\(^1\)

<table>
<thead>
<tr>
<th>Windrower type</th>
<th>Clean seed yield (kg ha(^{-1}))</th>
<th>Seed loss (kg ha(^{-1}))</th>
<th>Yield loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MacDon single auger A40DX</td>
<td>1,588 ab</td>
<td>166</td>
<td>10.5</td>
</tr>
<tr>
<td>John Deere double auger 890</td>
<td>1,691 bc</td>
<td>177</td>
<td>10.5</td>
</tr>
<tr>
<td>MacDon draper DX115</td>
<td>1,751 c</td>
<td>135</td>
<td>7.7</td>
</tr>
<tr>
<td>John Deere rotary disc 995</td>
<td>1,519 a</td>
<td>158</td>
<td>10.4</td>
</tr>
</tbody>
</table>

\(^1\)LSD (0.05)

**Table 2.** Mean clean seed yield, shattered seed loss, and percent yield loss with four windrower treatments across two irrigated turf-type perennial ryegrass seed fields in western Oregon.\(^1\)

<table>
<thead>
<tr>
<th>Windrower type</th>
<th>Clean seed yield (kg ha(^{-1}))</th>
<th>Seed loss (kg ha(^{-1}))</th>
<th>Yield loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MacDon single auger A40DX</td>
<td>2,763 b</td>
<td>798 b</td>
<td>28.9 b</td>
</tr>
<tr>
<td>John Deere double auger 890</td>
<td>2,446 a</td>
<td>855 b</td>
<td>34.9 b</td>
</tr>
<tr>
<td>MacDon draper DX115</td>
<td>2,820 b</td>
<td>508 a</td>
<td>18.0 a</td>
</tr>
<tr>
<td>John Deere rotary disc 995</td>
<td>2,641 ab</td>
<td>706 b</td>
<td>26.7 b</td>
</tr>
</tbody>
</table>

\(^1\)LSD (0.05)
for forage and turf. Results from a recent study show some promise in identifying the genes that play a critical role in the seed shattering of perennial ryegrass using a comparative genomics strategy (Fu et al., 2018). Collaborative efforts between seed crop agronomists and plant breeders are needed to further address the seed shattering problem.

References


Abstract
Seed moisture content (SMC) is the most reliable indicator of seed maturity and harvest timing in grass seed crops. Present SMC testing methodologies are slow, and, as a result, it is difficult to make timely harvest decisions. Delaying harvest past the point of physiological maturity reduces seed yield and increases shattering losses. The objective of this study was to determine the feasibility of using portable near-infrared reflectance spectroscopy (NIR) as an alternative to the oven method for determination of SMC in perennial ryegrass (*Lolium perenne* L.), tall fescue (*Schedonorus arundinaceus* [Shreb.] Dumort.), and orchardgrass (*Dactylis glomerata* L.). Field trials were conducted over a 3-year period. Daily testing of SMC began when experimental crops were at BBCH 69 and continued until seed harvest. Seed samples were collected by stripping 30 inflorescences into airtight containers from each crop until ready for NIR determination of SMC in the field or by laboratory air-oven (130°C). In perennial ryegrass, NIR determination of SMC over the course of seed development was strongly related to the oven method in all years ($r = 0.948$, $P < 0.01$). The SMC values provided by NIR also showed a good relationship with the oven method in orchardgrass ($r = 0.927$, $P < 0.01$). Differences in SMC measurement were observed in tall fescue; the relationship of NIR SMC to oven SMC in turf-type tall fescue was similar to other species ($r = 0.905$, $P < 0.01$), but NIR SMC in forage-type tall fescue was more variable in relation to oven SMC ($r = 0.828$, $P < 0.05$). Spring agronomic practices, including plant growth regulators, foliar fungicides, and nitrogen (urea) had no influence on NIR determination of SMC compared to untreated controls. The portable NIR is a promising tool for determination of harvest timing in grass seed crops by SMC.

Keywords: seed shattering, digital agriculture, harvest timing, physiological maturity, seed moisture content

Introduction
Seed moisture content (SMC) is the most reliable indicator of seed maturity and harvest timing in grass seed crops (Silberstein et al., 2010). Harvesting within the correct range of SMC values (physiological maturity) for each species maximizes harvestable seed yield and minimizes seed shattering losses. Seed shattering is lowest at high SMC and highest at low SMC. Thus, seed growers need to cut at high SMC near physiological maturity of the seed to maximize seed yield (Andrade et al., 1994).

Portable electric seed moisture testing devices currently available for use in grass seed crops utilize the electrical properties of seeds (conductivity or capacitance) to measure SMC (Silberstein et al., 2010). The SMC measurements made by these portable electric devices are rapidly available; however, they are accurate only within a range of SMC values that are much below physiological maturity and thus are too low for determining timing of seed harvest. As a consequence, grass seed growers predominantly use...
air-oven methods or microwave oven methods that are more time-consuming and are difficult to conduct in the field (Silberstein et al., 2010). Since the most widely adopted SMC testing methodologies are slow, it is difficult to make timely, field-based decisions about harvest maturity of crops. A rapid and reliable method is needed to measure SMC in the field for determination of harvest timing in grass seed crops.

Near-infrared reflectance spectroscopy (NIR) has been widely used for the determination of moisture content in a wide range of agricultural products, including cereal grain and oilseed crops (Armstrong, 2006). Technological advances now make it possible to use portable NIR spectroscopy for farm-level forage and silage analysis. However, no information is available on the testing of grass seed crops for SMC under field conditions using portable NIR devices. Since SMC testing by NIR is a secondary method, calibration against a primary SMC testing method such as the laboratory air-oven is needed before this technology can be used as a tool for the determination of harvest timing in grass seed crops (Benjamin and Grabe, 1988).

Our objective was to determine the feasibility of portable NIR as an alternative to the oven method for determination of SMC in cool-season grass seed crops.

Methods
The SMC testing methodologies were examined with four grass seed crops in field trials that were conducted at OSU’s Hyslop Research Farm near Corvallis, Oregon, in three harvest seasons: 2016, 2017, and 2018. These four seed crops were perennial ryegrass (*Lolium perenne* L.), turf-type and forage-type tall fescue (*Schedonorus arundinaceus* [Shreb.] Dumort.), and orchardgrass (*Dactylis glomerata* L.). Together, these four grass seed crops represent more than 60% of the total seed crop acreage in Oregon. The influence of agronomic practices on SMC and harvest maturity in the grass seed crops was also assessed. These practices included plant growth regulator (PGR) application (trinexapac-ethyl), nitrogen (N as urea) application rate, and fungicides (propiconazole + azoxystrobin = DMI + QoI fungicide; benzovindiflupyr = SDHI fungicide) for stem rust control.

Daily testing of SMC began when the experimental crops were at BBCH 69 (a couple of days past peak flowering) and continued until seed harvest by swathing of the standing crop. Testing of SMC in the seed crops was conducted by use of a Digi-Star Moisture Tracker NIR and by the laboratory air-oven method. Additional SMC measurements were made on seed taken from the swath up until the time of combine harvest and on seed that was past harvest maturity in unharvested plots.

Seed samples were collected by stripping 30 inflorescences into airtight containers from each crop until ready for NIR determination of SMC in the field or by laboratory air-oven. The samples were taken in mid- to late morning to ensure that dew was no longer present, and the seed was stored under cool conditions until ready for NIR SMC determination. Operation of the NIR was conducted per the manufacturer’s recommendations, with slight modifications as needed to accommodate grass SMC determination.

Procedures for the reference air-oven included taking three 10-gram subsamples from the same sample used to determine NIR SMC (Benjamin and Grabe, 1988). Each subsample was placed in a metal sample container and weighed prior to oven-drying to obtain the wet weight of the seed. Subsampled seeds and containers were placed in the laboratory air-oven and were dried at 130°C for 2 hours. After drying was complete, covers were placed on each sample container, and samples were cooled to room temperature prior to weighing in a desiccant-containing chamber to obtain the dry weight of the seed. The calculations
of SMC were made as follows: the dry weight of seed was subtracted from the wet weight, the difference was divided by the wet weight, and the resultant value was expressed as a percentage.

The SMC values measured by NIR were regressed against SMC values determined from the laboratory air-oven method for the same sampling date, year, and seed crop by using PROC REG from SAS (SAS Institute, 2012). The linear regression models for the relationship of NIR SMC and laboratory air-oven method in each of the four grass seed crops were tested for fit and significance.

**Results and Discussion**

In perennial ryegrass, NIR determination of SMC over the course of seed development was strongly related to the oven method in all 3 years ($r = 0.948$, $P < 0.01$). There was a linear relationship of NIR SMC to oven SMC in perennial ryegrass over the 3 years of the study (Figure 1). While foliar DMI + Q,1 and SDHI fungicides delayed crop maturity for a short period (< 2 days) in perennial ryegrass, there was no effect of fungicides on the ability of the NIR device to measure SMC as compared to the untreated control in all 3 years of testing (data not shown).

The SMC values determined by NIR showed a very good relationship with the oven method in orchardgrass ($r = 0.927$, $P < 0.01$). Over the 3 years, NIR SMC values and oven SMC values exhibited a linear relationship (Figure 2). Obtaining timely and accurate readings of SMC in orchardgrass is especially important since the crop is highly susceptible to seed shattering losses prior to and during harvest. Trinexapac-ethyl PGR and N application are two of the important agronomic practices in grass seed production. The results revealed that the use of this PGR and the rate of N application had no effect on the ability of the NIR device to measure SMC.

![Figure 1. Relationship of near-infrared reflectance spectroscopy (NIR) seed moisture content (SMC) values with air-oven SMC values in perennial ryegrass over a 3-year period. Control represents perennial ryegrass not treated with a fungicide, while DMI + Q,1 fungicide (propiconazole + azoxystrobin) and SDHI fungicide (benzovindiflupyr) indicate fungicide application for rust control.](image1)

![Figure 2. Relationship of near-infrared reflectance spectroscopy (NIR) seed moisture content (SMC) values with air-oven SMC values in orchardgrass over a 3-year period. Two rates of spring nitrogen (N), with and without trinexapac-ethyl plant growth regulator (PGR), were examined.](image2)
Differences in SMC measurement were observed in tall fescue; the relationship of NIR SMC to oven SMC in turf-type tall fescue was similar to that for perennial ryegrass and orchardgrass ($r = 0.905$, $P < 0.01$), but NIR SMC in forage-type tall fescue was more variable in relation to oven SMC ($r = 0.828$, $P < 0.05$). A linear relationship between NIR-measured SMC and oven SMC was evident in both turf-type tall fescue (Figure 3) and forage-type tall fescue (data not shown). Andrade et al. (1994) also noted differences in SMC measurement between forage-type and turf-type tall fescue. Trinexapac-ethyl PGR had no effect on SMC measurement by NIR in tall fescue (data not shown).

Variability was evident among individual seed moisture tests, but this variability was much less than that observed for other portable electric moisture meters that have been tested for use in grass seed crops (Silberstein et al., 2010). Some of the variation in SMC is most likely the result of the high degree of natural variability in maturity that is typical for grass seed crops. Moreover, there was no direct correspondence of the oven test results with the SMC reading on the NIR device. In other words, a harvest recommendation of 35% SMC for perennial ryegrass as measured by the oven would correspond to an NIR SMC value of 25.6%. Nevertheless, the NIR SMC values were highly related to the official oven test results (Figures 1–3).

Deviations of NIR SMC from the oven SMC ranged from 0% to 5.3% in the SMC increment from 10 to 20% but ranged from 13.2% to 25.3% in the 50–60% SMC increment (data not shown). Measurement of SMC with the NIR was closest in agreement to the oven at low SMC, while at high SMC the values diverged most from the oven. In both tall fescue seed crops, SMC measured by NIR diverged quickly with increased SMC. For perennial ryegrass and orchardgrass, this deviation in NIR SMC from the oven test results was markedly less in magnitude. The most important observation is that the deviations are systematic in nature; therefore, the deviations between the two SMC testing methodologies can be easily corrected for with a calibration for each of the individual grass seed crops and a software update to reflect these calibrations in the NIR.

**Figure 3.** Relationship of near-infrared reflectance spectroscopy (NIR) seed moisture content (SMC) values with air-oven SMC values in turf-type tall fescue over a 3-year period. Trinexapac-ethyl plant growth regulator (PGR) and untreated control were examined.
Conclusion
The portable Digi-Star NIR device is a promising digital agriculture tool for rapidly measuring SMC in grass seed crops and has demonstrated utility as a method for ascertaining harvest maturity. Spring agronomic practices, including PGR, foliar fungicide, and N application, had no influence on determination of SMC by the NIR. Specific calibration of the device and measurement protocols are needed before the commercial application of this device in grass seed moisture testing.

References
PEST MANAGEMENT

ORAL PRESENTATIONS
SOIL TILLAGE STRATEGY TO REDUCE SEED SURVIVAL OF TWO IMPORTANT ANNUAL GRASSES

Peter K. Jensen¹,*

Abstract
In grass seed production, physical purity of the product is very important. Many annual grass weeds are difficult or impossible to control chemically in grass seed crops. At the same time, seed characteristics make it difficult to separate weed seeds from some cultivated grass seeds. It is therefore important to use all other tools to reduce problems with annual grass weeds in crop rotations with grass seed production. Annual grasses typically have seeds with low persistence. Seed survival is typically very low at the soil surface but increases when the seeds are incorporated into the soil. Infestation levels can be reduced by applying strategies that reduce seed production and seed incorporation into the soil. This study investigated the influence of different tillage strategies on the survival of seed of two annual grasses: blackgrass (Alopecurus myosuroides Huds.) and annual ryegrass (Lolium multiflorum L.). Seed survival was studied in two parallel trial series. One series used full-scale implements, and the other simulated soil tillage using small samples and a known number of seeds. Both series included four replicates and were repeated for 2 years. The lowest viability was found in seeds left at the soil surface, and there was no significant effect on viability of shallow “finger” harrowing. Increasing viability was generally found with increasing depth. This study indicates that shallow soil tillage has a limited influence on incorporation of seeds from the soil surface into the soil. Thus, we concluded that it is possible to do a shallow soil cultivation and still maintain a reduction in seed survival comparable to that achieved by leaving the seeds undisturbed on the soil surface.

Keywords: soil tillage, soil depth, seed survival, blackgrass, annual ryegrass

Introduction
Annual/Italian ryegrass (Lolium multiflorum L.) is grown for seed production, but it is also considered a troublesome weed in other grass seed crops in some areas. Blackgrass (Alopecurus myosuroides Huds.) is primarily a winter annual grass weed typically found in crop rotations with a high proportion of winter cereals in areas of the country with clayey soil types. Blackgrass is considered an increasing problem in grass seed production. Herbicide resistance is a significant problem, and a robust and resilient control strategy has to rely on a combination of chemical and nonchemical control methods. Efficient handling of seeds of the two annual grasses can contribute to this goal. The purpose of this study was to test the influence of different stubble treatments on the longevity of newly shed seeds of these two annual grasses.

Methods
Two types of experiments were conducted: field experiments using normal tillage implements and small-plot field experiments simulating the influence of various tillage treatments on placement of seeds in the soil profile. Both types of experiments investigated the influence of primary tillage immediately following harvest of a cereal crop.

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In the full-scale experiments, straw was removed from the field at harvest, and seeds of the two investigated annual grasses were dispersed on the soil surface at a rate corresponding to 200 seeds m\(^{-2}\) prior to primary tillage. The primary tillage treatments included no tillage, shallow tillage to 1–2 cm or to 2–4 cm with a finger weeder, or a deeper stubble harrowing to 5 or 10 cm. Following an interval of 1.5–2 months, depending on the year, all germinated plants were counted and controlled chemically, and a second tillage was conducted. This tillage was uniform in all treatments and consisted of a seedbed harrowing to 2–4 cm. Approximately 2 months after this treatment, germinated grass plants were counted. The result is taken as an indication of the effect of primary tillage on seed survival.

In the experiment simulating tillage treatments, samples of seeds were placed at distinct soil depths, and the longevity of the seed samples following these treatments was assessed. The small-plot field experiment was carried out using seeds from the same seed lot as those used in the full-scale trials. Samples of 400 seeds were counted and placed either at the soil surface or buried at increasing soil depths to simulate different kinds of soil tillage. This was typically done around August 1 at the same time as primary tillage in the full-scale trial. Two treatments included placement of the seeds at the soil surface. These treatments were carried out in small pots. In the first treatment, seeds were left directly at the soil surface, whereas in the second treatment, a shallow harrowing was carried out with the fingers to mimic shallow soil tillage. The treatments including burial at different depths were carried out using samples with seeds mixed with soil and placed in fabric mesh bags. By the end of September, all samples were collected from the field, and a germination test was carried out in the laboratory. During the germination test, soil samples were kept moist to ensure optimal conditions for germination. Germinated seedlings were counted when emergence ceased, and the result is taken as an indication of the influence of the various field treatments on the longevity of seeds of the two annual grasses.

**Results and Discussion**

The results of the small-plot experiments were very similar for the two grasses. The results from two of the experiments are shown in Figures 1 and 2.

![Figure 1](image.png)

**Figure 1.** Germination of seeds of annual/Italian ryegrass from samples kept at different soil depths in the field from the beginning of August to the end of September 2015. The figure shows the number of plants in the germination test as a percentage of the original seed sample. LSD = 4.75.
The result with annual/Italian ryegrass varied between the 2 years. In 2015 (Figure 1), the lowest viability was found in seeds left at the soil surface, and finger harrowing had no influence on viability. With increasing depth, increasing viability was generally found. Obviously, it seems that finger harrowing had a limited influence on seed placement and hence longevity. In 2016 (not shown), there was no significant difference between seeds left at the surface and seeds incorporated to 5 cm depth. However, greater viability was seen for seed samples incorporated to 10 and 25 cm depth.

Results for blackgrass seeds in the small-plot experiments were very similar between the 2 years. Figure 2 shows the results from the second year. The lowest viability was found in seeds left at the surface, and finger harrowing had no significant influence on viability. Viability was generally much higher in incorporated seeds compared to seeds at the soil surface, and seeds incorporated to just 1 cm depth had a much higher viability than seeds placed at the soil surface. Obviously, it seems that finger harrowing had a limited influence on seed placement and hence longevity.

The influence of seed burial and soil depth seen in these experiments is in accordance with results for other important annual grasses such as *Poa trivialis* and *Vulpia myuros* (Jensen, 2010a and 2010b).

The primary role of the full-scale trials was to support the results from the small-plot experiments. Soil depth cannot be controlled as precisely with full-scale implements as in the small-plot experiments. Furthermore, the tillage depth does not determine the placement of the seeds in the soil profile. It was a general observation in all 4 years that stubble harrowing reduced the number of germinated seedlings of both annual/Italian ryegrass and blackgrass following the primary tillage. This indicates a deeper burial of seeds by stubble harrowing than by any of the other treatments. Seeds that are nondormant will germinate, but if they are buried too deeply they are not able to establish seedlings. The full-scale field experiments thus indicate that stubble harrowing incorporates a fraction of the seeds deeper into the soil and hence into the more persistent seed bank. A superficial tillage using a flex-tine weeder, on the contrary, did not influence germination and persistence of seeds of the two annual grasses compared to the treatment in which seeds were left undisturbed at the soil surface.

**Figure 2.** Germination of blackgrass seeds from samples kept at different soil depths in the field from mid-July to the end of September 2018. The figure shows the number of plants in the germination test as a percentage of the original seed sample. LSD = 3.6.
Conclusion
The rapid decrease in longevity of seeds at the soil surface is an efficient way to deplete the soil seed bank, as annual grasses typically depend on this annual seed rain. Leaving the seeds at the soil surface for a period following seed shedding therefore helps to reduce the impact of annual grasses in crop rotations with grass seed production.

References
ADVANCES IN GRASS WEED MANAGEMENT 
IN GRASSES GROWN FOR SEED IN OREGON

Andrew G. Hulting¹*, Daniel W. Curtis¹, Kyle C. Roerig¹, and Carol A. Mallory-Smith¹

Abstract
Annual bluegrass (Poa annua L.), roughstalk bluegrass (Poa trivialis L.), and other grass weed species invade newly established and established cool-season grasses grown for seed in Oregon, causing significant production and economic challenges for growers. Field experiments were conducted from 2007 to 2018 to determine the potential for fall-applied applications of indaziflam, pyroxasulfone, and a commercial premix of pyroxasulfone + flumioxazin to control grass weed species and volunteer crop plants in perennial ryegrass and tall fescue seed crops. A range of application rates and timings was compared to current industry standards, including applications of diuron, metolachlor, dimethenamid, and flufenacet + metribuzin. Weed control efficacy, crop injury, and crop yield were evaluated. Indaziflam applications (14–44 g ai ha⁻¹) resulted in excellent annual bluegrass control (> 90%), but the higher rates injured perennial ryegrass and tall fescue. Tall fescue was more tolerant to indaziflam than perennial ryegrass. A single application of indaziflam (14–28 g ai ha⁻¹) during the life of a grass seed stand may be appropriate to manage annual bluegrass. Indaziflam applications over multiple years may reduce stand life, particularly in perennial ryegrass. Pyroxasulfone applications resulted in excellent annual bluegrass control (> 90 %) and were less injurious to tall fescue and perennial ryegrass than indaziflam. Application of 50–100 g ai ha⁻¹ resulted in little crop injury and no yield loss. Applications of the pyroxasulfone + flumioxazin premix at 80–160 g ai ha⁻¹ provided excellent annual bluegrass control. These studies suggest that these active ingredients provide good weed control and adequate crop safety when applied to established perennial ryegrass and tall fescue, making them reasonable alternatives to current soil-applied herbicides. Additional trials to build efficacy and crop-safety data sets are ongoing.

Keywords: annual bluegrass, soil-applied herbicides, perennial ryegrass, roughstalk bluegrass, tall fescue, weed management

Introduction
Grass grown for seed has been the principle field crop in the Willamette Valley, Oregon, in terms of value and acreage for many years. The decrease in open field burning and shifting tillage practices to more conservation-tillage-based methods has increased weed management problems on much of the grass seed acreage. Besides direct crop losses due to weed competition, weed seed contamination increases the cost of seed conditioning and affects certification of the seed and, thus, the subsequent value of the crop. Grower costs from not meeting seed certification requirements can be appreciable. Herbicide and labor inputs for weed management add substantially to the cost of grass seed production, making continued development of viable weed management strategies imperative. This research specifically addresses the ongoing management priorities of weed control and herbicide resistance management. Weed management research benefits growers by helping to ensure return on investments made when purchasing herbicides or changing production practices.

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While significant progress has been made in recent years, annual bluegrass (*Poa annua* L.) still reduces crop yield and quality in many production areas, and roughstalk bluegrass (*Poa trivialis* L.) populations are increasing. Other weeds, such as annual ryegrass (*Lolium perenne* spp. *multiflorum* L.) (including herbicide-resistant biotypes), bromes (*Bromus* spp.), and many broadleaf weeds, are continual problems. Developing management solutions for these weed species is the result of a long-term commitment to evaluating, implementing, and refining the applications of herbicides and other control practices. Therefore, the general and ongoing objective of this research was to evaluate herbicides and application techniques for crop tolerance and for control of annual bluegrass, roughstalk bluegrass, and other difficult-to-control weeds in grasses grown for seed.

**Methods**

Field experiments were conducted from 2007 to 2018 to determine the potential for using fall-applied applications of indaziflam, pyroxasulfone, and a commercial premix of pyroxasulfone + flumioxazin to control grass weed species and volunteer crop plants in perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.) grown for seed. All studies were conducted with standard weed science small-plot research practices. Preliminary herbicide screening trials were conducted at Oregon State University research stations, with more well-developed management concepts and efficacy experiments conducted in cooperating growers’ commercial production grass seed fields. A range of application rates and timings (generally September–January) of these products was compared to current industry standards, including applications of diuron, metolachlor, dimethenamid, and flufenacet + metribuzin, in established grasses grown for seed. Weed-control efficacy (visual rating), crop injury (visual rating), and crop yield (small-plot swather and combine) were evaluated each year.

**Results and Discussion**

Indaziflam applications at rates ranging from 14 to 44 g ai ha\(^{-1}\) resulted in excellent annual bluegrass control (> 90%) but injured perennial ryegrass and tall fescue at the higher application rates. However, tall fescue was more tolerant to indaziflam than perennial ryegrass. Application rates of 14–28 g ai ha\(^{-1}\) of indaziflam used once during the life of the grass seed stand may be appropriate to manage annual bluegrass. Indaziflam applications over multiple years may reduce the life of the stand, particularly perennial ryegrass stands. Indaziflam is a Group 29 (WSSA Herbicide Site of Action Classification) herbicide representing a site of action that is not currently used in grasses grown for seed, and its potential registration represents a significant opportunity to manage herbicide-resistant biotypes of grass weeds. Pyroxasulfone applications also resulted in excellent annual bluegrass control (> 90%) and were less injurious to both tall fescue and perennial ryegrass than indaziflam applications. Application rates ranging from 50 to 100 g ai ha\(^{-1}\) resulted in little crop injury and no yield loss. Applications of the pyroxasulfone + flumioxazin premix at rates of 80–160 g ai ha\(^{-1}\) provided excellent annual bluegrass control.

**Conclusion**

These studies suggest that these active ingredients provide good weed control as well as adequate crop safety when applied to established perennial ryegrass and tall fescue, making them reasonable alternatives to current soil-applied herbicides used in grass seed production systems. Additional trials are ongoing to build efficacy and crop safety data sets with these herbicides, which will be needed should the industry choose to pursue uses of these materials in grasses grown for seed. The commercial premix of pyroxasulfone + flumioxazin (Fierce) was registered for use in Oregon in 2019 in part as a result of this research.
References


Abstract

*Claviceps purpurea* causes ergot, a floral disease of grasses, including Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.) grown for seed. The fungus infects flowers of grasses and transforms ovaries into sclerotia, which germinate to release airborne ascospores that serve as primary inoculum the following spring. A mixture of conidia and plant sap, called honeydew, can be exuded from florets after infection and can serve as secondary inoculum. A series of interdisciplinary studies was performed in Hermiston and Madras, Oregon, between 2012 and 2018 to determine the relative importance of sclerotia, ascospores, and conidia in the epidemiology of ergot in Pacific Northwest grass seed production. Spatial patterns of ergot were characterized by significant autocorrelation and clustering within fields, suggesting that the disease was not uniformly or randomly distributed in fields. Primary inoculum in the form of sclerotia and ascospores were found in large numbers (3.8–119.4 sclerotia m⁻² and 79–114,525 ascospores per season, respectively) in first- and second-year fields, and analysis of molecular variance (AMOVA) suggested that airborne inoculum could be dispersed from field to field. Population genetics analyses using 12 microsatellite markers at different spatial scales (within and between production regions, fields, and plants) revealed moderate levels of genotypic diversity ($H = 3.43–4.23$) and gene diversity ($H_{exp.} = 0.45–0.57$) within fields. Clonal fractions of *C. purpurea* populations in Madras and Hermiston ranged between 27 and 49%, respectively, and most clonal isolates were collected from the same seed head, suggesting that conidia-laden honeydew contributed to secondary spread within seed heads. Significant correlations between ergot incidence and the presence of insects, specifically *Botanophila* spp. ($r = 0.89$, $P = 0.0068$), indicated that insects can spread ergot conidia via honeydew. These results provide important insights into ergot epidemiology that can be used to inform future integrated disease management strategies.

**Keywords**: ergot, Kentucky bluegrass, perennial ryegrass, plant disease, population genetics

Introduction

Cool-season grass seed production is an important agricultural industry in the U.S. Pacific Northwest, and Oregon is a leading world producer. In 2017, more than 254,000 metric tons of grass seed were harvested from more than 148,000 hectares in Oregon, with a value of $393,899,000. Besides their economic value, cool-season grass seed crops are important rotation crops in the cropping systems of central and eastern Oregon.
Ergot is an important seed replacement disease in cool-season grass seed production systems of Oregon. The fungal pathogen *Claviceps purpurea* has a wide host range among grasses, including Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.). The fungus infects unfertilized flowers and results in the production of sclerotia instead of seed. Sclerotia serve as the overwintering structures of the pathogen and germinate to produce fruiting bodies called capitula, which release primary inoculum in the form of airborne ascospores in the spring. Infected flowers exude a substance called honeydew, which contains a mixture of plant sap and asexual spores called conidia and can spread the disease if splash- or insect-dispersed to uninfected flowers. Economic losses due to ergot occur at various stages of grass seed production.

A series of interdisciplinary studies was performed in central and eastern Oregon between 2012 and 2018 to better understand the epidemiology and population biology of ergot in irrigated grass seed production systems of central and eastern Oregon. The spatial patterns of ergot epidemics were elucidated over time in perennial production fields, and sclerotia, the main source of primary inoculum, were quantified in fields after harvest. The relative importance of ascospores and conidia as primary and secondary inoculum was determined by investigating the genetic and genotypic diversity of *C. purpurea* at different spatial scales using a hierarchical sampling approach. Finally, a 2-year survey was undertaken in 2014 and 2015 to quantify the incidence of contamination of potential insect vectors by *Claviceps* conidia and to determine whether any association exists between insect populations and ergot epidemics in the lower Columbia Basin of Oregon and Washington.

**Methods**

**Spatial patterns of ergot in commercial fields**

Three first-year 50-ha commercial perennial ryegrass fields near Hermiston, Oregon, were surveyed about 1 week prior to swathing in 2012. Sample points were located along wheel tracks and consisted of quadrats approximately 1 m$^2$ in size spaced 10 m apart. Quadrats were located 2 m away from the wheel track and were mapped using a GPS unit. Ten seed heads were arbitrarily collected from each quadrat, and the number of sclerotia was counted to determine incidence and severity at each quadrat. Moran’s *I* and the SADIE indices of aggregation, patch clusters, and gap clusters were used to infer spatial autocorrelation and aggregation of disease severity. These statistics were used to determine whether ergot was evenly distributed throughout the field or tended to occur in patches, or foci. The same three fields were sampled again in 2013 during the second year of production.

Samples of crop debris, soil, sclerotia, and seed were collected following harvest and residue management operations in 2012 and 2013. A commercial vacuum-sweeper, towed at approximately 1.6 kph, was used to collect samples from 20 to 24 plots per field. Each plot was approximately 2 m wide and 5 or 10 m long. Seed and sclerotia were separated from soil and plant residues using an air screen machine, indent cylinder, air column separator, and hand screens. A stereo microscope was used to identify and count sclerotia.

**Population genetics of *C. purpurea***

Two perennial ryegrass and two Kentucky bluegrass seed production fields were sampled in 2017 using a hierarchical sampling strategy. Five transects were randomly established 72 m apart, and at least 10 diseased plants were randomly sampled from 8 or 9 quadrats located 10 m apart. Two sclerotia were randomly sampled from each seed head. A total of 102 and 124 isolates were obtained from the two perennial ryegrass and Kentucky bluegrass fields, respectively.
Isolates were characterized genotypically using 12 previously published microsatellites (Gilmore et al., 2016). Capillary electrophoresis (fragment analysis) of microsatellite amplicons was performed at the Oregon State University Center for Genome Research and Biocomputing. Population genetics analyses (clone correction, identification of multilocus genotypes, heterozygosity) were conducted using the package “popprr” (Kamvar et al., 2014) in R. Pairwise population differentiation and gene flow were analyzed using GenAlEx v6.5, and analysis of molecular variance (AMOVA) was performed with ARLEQUIN v3.5.2 as previously described (Dung et al., 2013).

Role of insects in ergot epidemiology

Insect abundance was monitored in three 50-ha commercial perennial ryegrass fields in Washington and Oregon during the 2014 and 2015 growing seasons. A 0.4-ha experimental field plot at the Hermiston Agricultural Research and Extension Center (HAREC) in Hermiston, Oregon, was also included in both years. Insect traps were situated in four quadrants in each commercial field approximately 3 to 5 m from the field perimeter. In the smaller experimental field plots at HAREC, a single trap site was used. Insects were captured using yellow sticky cards, sweep nets, and black light traps. Sticky cards and sweep net samples were collected weekly, and captured insects were sorted to family and identified to species when possible.

Ergot incidence was estimated at crop maturity and was based on the number of infected seed heads containing sclerotia out of 100 seed heads collected randomly from each quadrant of each field. Spearman correlation coefficients were calculated to identify associations between ergot incidence and the number of insects in each field. Correlation coefficients were also calculated for ergot incidence and the number of insects collected by family, order, and species (when identified).

Representative insects from both Diptera and Lepidoptera were chosen for molecular examination for Claviceps conidia based on their abundance and ease of handling during DNA extraction. A high-fidelity polymerase chain reaction (HF-PCR) assay with Claviceps-specific β-tubulin primers was developed to detect Claviceps conidia from insect samples. A subset of PCR products was cloned and sequenced at the Oregon State University Center for Genomic Research and Biocomputing. Sequence data were assembled and compared to sequences of Claviceps species previously submitted to the GenBank database.

Results and Discussion

Spatial patterns of ergot in commercial fields

Surveys and plots of disease intensity suggested that disease severity was not evenly distributed throughout the fields, with significant \( P \leq 0.002 \) clustering of disease severity observed in all three fields using Moran’s \( I \) values and SADIE indices of aggregation (Table 1). Variance-to-mean ratios of ergot severity suggested that ergot sclerotia were clustered among quadrats and among infected seed heads within each quadrat (Table 1). Previous studies have also found that sclerotia tended to occur in clusters on infected seed heads of Kentucky bluegrass rather than being randomly distributed (Alderman and Barker, 2003). The clustered patterns of ergot severity observed prior to harvest could be caused by concentrated sources of sclerotia and ascospores in the field, multiple or prolonged exposures to ascospores during anthesis, secondary spread within and among plants via honeydew, or contributions from both types of inoculum.

Large numbers of sclerotia were collected from fields after harvest and postharvest residue management in both the first year of production (3.8–15.3 sclerotia m\(^{-2}\)) and second year of production.
Although *C. purpurea* sclerotia survive for only 1 year under field conditions, sclerotia left in fields after harvest can potentially serve as important sources of inoculum in subsequent years of perennial production. Soil-applied fungicides that target overwintering sclerotia can potentially be used to reduce sclerotial germination and subsequent ascospore production in perennial production systems (Dung et al., 2018).

**Population genetics of *C. purpurea***

The 226 *Claviceps purpurea* isolates collected in this study were assigned to one of four subpopulations based on the host/geographical region (Madras/Kentucky bluegrass or Hermiston/perennial ryegrass) and fields (two fields for each region) *a priori*. The presence of two genetic clusters (Δ*K* = 2) best explained the population structure of the isolates, and the two suggested clusters corresponded to the two geographical regions/host origins of the isolates. Discriminant analysis of principal coordinates also indicated that two genetic clusters were represented among the isolate collection (Figure 1), and pairwise population differentiation (*F*<sub>s</sub>) indicated significant (*P* < 0.05) differentiation between isolates from Madras/Kentucky bluegrass and Hermiston/perennial ryegrass.

Moderate levels of genotypic diversity (*H* = 3.43–4.23) and gene diversity (*H*<sub>exp</sub> = 0.45–0.57) were observed among the isolates, and clonal fractions of *C. purpurea* populations ranged between 27 and 49%. Approximately 21% of the variation was due to genetic differences between isolates from Madras/Kentucky bluegrass and Hermiston/perennial ryegrass, but the two groups were not significantly different (*P* = 0.32), and approximately half of the genetic variation (49%) was attributed to differences among isolates collected from plants within the same quadrat (Table 2). The remaining variation was explained by genetic differences among quadrats within the fields.

Within a geographical region, none of the genetic variation could be explained by differences between subpopulations from the two fields (*P* = 0.74) (Table 2), and significant differentiation was not observed.

**Table 1.** Spatial autocorrelation, aggregation analysis, and variance-to-mean (VTM) ratios of ergot severity in three perennial ryegrass seed fields during the first (2012) and second (2013) years of production.

<table>
<thead>
<tr>
<th>Field</th>
<th>Year</th>
<th>Observed</th>
<th>Expected</th>
<th>Z</th>
<th>I&lt;sub&gt;a&lt;/sub&gt;</th>
<th>v&lt;sub&gt;j&lt;/sub&gt;</th>
<th>v&lt;sub&gt;i&lt;/sub&gt;</th>
<th>Among plants</th>
<th>Among quadrats</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2012</td>
<td>0.0708</td>
<td>-0.0020</td>
<td>16.2</td>
<td>3.0</td>
<td>-3.0</td>
<td>2.9</td>
<td>1.6</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>0.0303</td>
<td>-0.0021</td>
<td>7.0</td>
<td>1.5</td>
<td>-1.4</td>
<td>1.5</td>
<td>1.9</td>
<td>5.4</td>
</tr>
<tr>
<td>B</td>
<td>2012</td>
<td>0.0557</td>
<td>-0.0021</td>
<td>12.6</td>
<td>3.2</td>
<td>-3.3</td>
<td>2.9</td>
<td>1.9</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>0.0638</td>
<td>-0.0021</td>
<td>14.0</td>
<td>3.8</td>
<td>-3.8</td>
<td>3.7</td>
<td>2.1</td>
<td>6.0</td>
</tr>
<tr>
<td>C</td>
<td>2012</td>
<td>0.0519</td>
<td>-0.0022</td>
<td>11.3</td>
<td>2.9</td>
<td>-2.9</td>
<td>2.8</td>
<td>2.1</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>0.0360</td>
<td>-0.0022</td>
<td>7.9</td>
<td>2.2</td>
<td>-2.0</td>
<td>2.1</td>
<td>2.0</td>
<td>6.5</td>
</tr>
</tbody>
</table>

1Observed and expected Moran’s *I* values and corresponding *Z* values. Positive *Z* values indicate positive autocorrelation. All values significant at *P* < 0.0001.

2The SADIE (Spatial Analysis of Distance IndicEs) method calculates an index of aggregation, *I*<sub>a</sub>, where values of *I*<sub>a</sub> > 1 represent clustering and values < 1 represent a regular spatial pattern. Above-average patch clusters or below-average gap clusters of ergot were determined using the SADIE index *v*<sub>i</sub>, where *v*<sub>i</sub> > 1.5 indicates above-average clustering, *v*<sub>j</sub> < -1.5 indicates below-average gaps, and *v* values between -1.5 and 1.5 indicate a random spatial distribution.

3A variance-to-mean (VTM) ratio > 1 indicates aggregation of ergot severity, and a VTM ratio < 1 indicates a uniform distribution of ergot severity. A VTM ratio close to 1 suggests a random distribution of ergot severity.
among populations between fields within a location ($F_{st} = 0.0–0.01$). These results suggest that gene flow, probably in the form of ascospore dispersal, occurs between fields in a production area. Negative binomial regression analysis of spatial distribution data collected in 2012 and 2013 also indicated that ergot severity significantly ($P < 0.006$) decreased with increasing distance from established nearby fields and especially fields located upwind, suggesting field-to-field spread of ergot inoculum. These results are especially important for perennial production systems, where established seed fields may harbor important sources of inoculum for newly planted fields nearby. However, most clonial isolates were collected from the same seed head, suggesting that secondary spread occurs at smaller spatial scales (e.g., within seed heads) via conidia in honeydew.
Role of insects in ergot epidemiology

Overall, the insects collected from commercial perennial ryegrass seed fields were grouped into 6 orders and 13 families, with Thysanoptera the most abundant insect order, followed by the order Diptera. One muscid, *Fannia canicularis*, the lesser housefly, and two anthomyiids, *Delia radicum* and *Botanophila discreta*, were most abundant. Noctuid and pyralid moths were the predominant lepidopteran insects. Two occasional pests of grass seed crops in Oregon and Washington, the armyworm (*Pseudaletia unipunctata*) and cutworm (*Agrotis ipsilon*), were also observed in this study.

HF-PCR detected *Claviceps* in 39 and 36% of dipteran samples collected in 2014 and 2015, respectively. Similarly, 44 and 17% of lepidopteran insects tested positive for the presence of *Claviceps* in 2014 and 2015, respectively. Sequence data corresponded to the β-tubulin gene sequences of *C. purpurea* and *C. humidiphila* isolates present in the National Center for Biotechnology Information (NCBI) database.

Ergot incidence data from perennial ryegrass seed fields in both years indicated significant positive correlations between ergot incidence and both the total number of insects trapped ($r = 0.93$, $P = 0.0025$) and the number of *B. discreta* trapped ($r = 0.89$, $P = 0.0068$). Insects, and specifically *B. discreta*, may contribute to ergot infections in grass seed production fields by spreading honeydew from infected flowers to healthy flowers within and between fields. Insects harboring *Claviceps* conidia may serve as vectors for secondary inoculum when foraging within or among grass seed production fields. Additionally, earlier flowering grass hosts such as cereal rye (*Secale cereale*), which usually become infected and produce honeydew prior to anthesis in grass seed fields, may serve as sources of primary inoculum to be carried by insects and dispersed to healthy plants.

Table 2. Analysis of molecular variance for *Claviceps purpurea* isolates grouped by geographic location/host.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum of squares</th>
<th>Variance component</th>
<th>% of variation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between sampling locations/hosts</td>
<td>1</td>
<td>95.21</td>
<td>0.82</td>
<td>22.44</td>
<td>0.32</td>
</tr>
<tr>
<td>Between fields within location/hosts</td>
<td>2</td>
<td>6.92</td>
<td>0.00</td>
<td>0.00</td>
<td>0.74</td>
</tr>
<tr>
<td>Among plants within fields</td>
<td>109</td>
<td>421.63</td>
<td>1.03</td>
<td>28.11</td>
<td>0.01</td>
</tr>
<tr>
<td>Within samples</td>
<td>113</td>
<td>205.00</td>
<td>1.81</td>
<td>49.65</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>225</td>
<td>728.76</td>
<td>3.66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Isolates were collected from two fields of Kentucky bluegrass in Madras, Oregon ($n = 102$) and two fields of perennial ryegrass in Hermiston, Oregon ($n = 124$).

Conclusion

The spatial patterns of ergot epidemics in Pacific Northwest grass seed production are characterized by significant autocorrelation and clustering, and the disease does not exhibit random or uniform distribution within fields. Many factors contribute to ergot epidemiology, including the timing, duration, and amount of primary and secondary inoculum production, the timing and length of host anthesis, environmental and cultural conditions, and other factors. The population genetics of *Claviceps* populations from grass seed fields suggest that the pathogen exhibits mixed modes of reproduction and that both ascospores and conidia can be important sources of inoculum at different spatial scales. Established grass seed fields that are infested with sclerotia of *Claviceps* may serve as potential “green bridges” for ergot, providing sources of inoculum for newly planted fields. Conidia-laden honeydew may serve as either primary or secondary inoculum for ergot, and insects may play a role in spreading honeydew from infected flowers to healthy flowers.
References
DISCOVERY AND BIOLOGICAL CONTROL POTENTIAL
OF SLUG-KILLING NEMATODES IN OREGON

Rory J. McDonnell¹*, Andrew J. Colton¹, Dana K. Howe², Anh D. Ha², and Dee R. Denver²

Abstract
Nematodes in the genus *Phasmarhabditis* are lethal to many species of slugs and snails (gastropods) and have important biological control potential. In fact, in Europe, *Phasmarhabditis hermaphrodita* (Schneider) is currently used as a commercially available biological control agent (Nemaslug) to protect a wide range of crops from gastropod damage. The recent discovery of multiple species of *Phasmarhabditis* in California has resulted in renewed interest in these nematodes as biological control agents of slugs and snails in North America. This provided the incentive for this study, which aimed to (1) determine whether nematodes in the genus *Phasmarhabditis* are present in Oregon, and, if so, (2) assess the lethality of select species to *Deroceras reticulatum* (Müller), which is the most damaging slug pest in the region. To this end, surveys were completed throughout the state, and nematodes recovered from dead slugs were identified using the 18S ribosomal gene. To date, three species of *Phasmarhabditis* have been collected from multiple locations throughout Oregon. Infectivity trials with two of these species were completed in 16-oz plastic containers containing 25 g sterilized, damp topsoil to which 20,000 mixed-stage nematodes and 6 *D. reticulatum* were added. Control containers contained no nematodes, and all treatment and controls were replicated five times. These trials showed that both nematode species were lethal to *D. reticulatum*, but the extent of mortality and the speed with which the nematodes caused death varied between species. Future research needs for this promising pest control system are highlighted.

Keywords: slug pests, *Deroceras reticulatum*, nematodes, *Phasmarhabditis*, biological control

Introduction
Slugs are among the most damaging pests of agricultural production in Oregon. A diverse range of crops are damaged by these invertebrates, particularly in the agriculture-rich Willamette Valley, including seed crops (e.g., annual ryegrass, perennial ryegrass, radish, and white clover), a wide range of vegetables (e.g., brassicas), legumes (e.g., peas), and fruits (e.g., strawberries). Ornamental production is also heavily impacted because slugs and snails represent a significant contamination problem when infested plants are shipped out of state. The problem with slugs is compounded by the fact that many species help vector both plant (e.g., choke grass) and human (e.g., *E. coli*) pathogens, and they lower crop quality through fecal and mucus contamination.

Despite the economic losses caused by these organisms (e.g., in recent years, slug damage has accounted for nearly $100 million in damage to the $500 million grass seed industry), control measures are focused overwhelmingly on the use of chemical molluscicides. However, considerable variation in efficacy of the most widely used active ingredients (metaldehyde, iron phosphate, and sodium ferric EDTA) is reported.

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by growers. Basically, when slugs are problematic, none of the commercially available baits suppress populations below damaging levels. Other traditional slug management strategies now have limited use in Oregon. For example, in the early 2000s, the practice of burning straw residue after seed harvest was gradually phased out in the Willamette Valley, and this straw residue in fields now provides an ideal microhabitat for slugs. Additionally, farmers in the region adopted no-till production for soil conservation purposes, and this change in cultivation practice also benefited slug population growth. Thus, there is an urgent need to identify and develop alternative control practices for growers in the region. One such option is biological control, which is the use of natural enemies to control a pest.

Slugs and snails have a diverse range of natural enemies (Barker, 2004), but their biological control potential has been largely overlooked in the United States. In Europe, a nematode called *Phasmarhabditis hermaphrodita* (Schneider) is currently used as a commercially available biological control agent called Nemaslug (Rae et al., 2007). This nematode is lethal to a wide range of pest slugs, including many of the key species impacting Oregon agriculture. Furthermore, the product has been used in Europe to significantly reduce slug populations and damage in a wide range of crops, including ornamental plants, brassicas, and strawberries. However, Nemaslug is not currently available in the United States because until recently the nematode was not found here. Then, in 2012, *P. hermaphrodita* was discovered from invasive slugs in multiple locations in northern California (Tandingan DeLey et al., 2017). In addition to this nematode, the authors discovered two additional slug-killing species of *Phasmarhabditis*, one of which (*P. californica*) was new to science.

However, to the best of our knowledge, no systematic survey of the nematode fauna associated with gastropods in Oregon has taken place. This is surprising given the extent of the slug problem in the state and the fact that the first recorded discovery of *Phasmarhabditis* in the United States was made in California, less than 70 miles from the Oregon border. This suggests that these nematodes are also very likely present in Oregon. Thus, the aims of this study were to (1) determine whether nematodes in the genus *Phasmarhabditis* are present in Oregon, and, if so, (2) assess the lethality of select species to *Deroceras reticulatum* (Müller), which is the most damaging slug pest in the region.

**Methods**

**Surveys**

Field surveys for slugs and snails focused on agricultural and horticultural areas where gastropods are likely to be abundant. Thus, we collected specimens from plant nurseries, garden centers, perennial ryegrass, tall fescue, white clover, Christmas trees, brassicas, strawberries, and mint. In total, 50 sites throughout the state have been surveyed. At each site, gastropods were collected either by hand (e.g., nurseries) or by using refuge traps (e.g., strawberry crops). Collected specimens were placed into plastic containers, stored in a cooler, and returned to the laboratory. Identifications were made to species level using Burke (2013). Specimens of the same species collected from the same site were maintained together in plastic containers (12 inches x 8 inches x 4 inches) lined with moist paper towels and organic carrot for food. A maximum of 25 specimens were kept per container. The food and towels were replaced twice weekly. Each box was checked for cadavers three times weekly. When found, cadavers were used for nematode screening.
Screening for nematodes
Dead gastropods were examined under light microscopy, and M9 buffer was also pipetted onto dead specimens and then transferred to a petri dish to look for the presence of nematodes. Nematodes were pipette-transferred to Nematode Growth Medium (NGM) agar plates and allowed to grow at 20°C, along with any bacteria that cocultured with the nematodes. Nematode cultures were subcultured to new plates every 2 weeks to ensure that lab populations did not die out due to overgrowth. After nematode cultures were established for about 2 weeks in the lab, we performed DNA-based species identification of nematodes in culture. This was achieved by polymerase chain reaction (PCR) amplification and sequencing of a region of the 18S rDNA gene. DNA sequences were compared to sequences present in the GenBank database to find species matches.

Infectivity trials
Infection trials were run in 16-oz plastic containers that contained 25 g sterilized, damp topsoil. The soil in each container was inoculated with either (1) a species of *Phasmarhabditis* (20,000 mixed-stage nematodes) or (2) a deionized water control. The treatment and control containers were replicated five times and repeated for two *Phasmarhabditis* species. After the soil was inoculated, six *D. reticulatum* were added to each container. The specimens were monitored daily for 2 weeks for symptoms of infection (e.g., swelling of the mantle, emaciation of the body, exposure of the internal shell, nematodes visible on the body) and mortality.

Results and Discussion
Our surveys throughout Oregon have resulted in the discovery of *Phasmarhabditis* for the first time in the state. Three different species were recovered, but, since genetic analysis is ongoing, we are not in a position to reveal the species names. Infectivity trials with two of these species demonstrated that both were lethal to the key slug pest, *D. reticulatum*, but the extent of mortality (67–100%) and the speed with which peak mortality occurred (9–12 days) varied between species. Future research needs include determining the infectivity of these nematodes to nonpest native species such as banana slugs (e.g., *Ariolimax columbianus* Gould), developing methods for mass rearing the various species, and elucidating the extent of interspecific competition.

Conclusion
In conclusion, *Phasmarhabditis* nematodes will likely be an important tool for managing gastropod pests in Oregon in the future. In Europe, *P. hermaphrodita* has demonstrated efficacy for protecting a wide range of field crops from slug damage, so there is a precedent for this system. Also, the wet weather and low light levels that promote high slug populations during the fall in western Oregon are also optimum conditions for these nematodes, so the climate in the Willamette Valley is likely to support biological control efforts during times when slugs are most active. Furthermore, our recent survey of grass seed growers throughout the Valley showed that more than 95% would be willing to try biological slug control using nematodes (McDonnell and Anderson, 2017), thus confirming the strong demand for such an approach and the high likelihood for widespread use. Ultimately, the implementation of an effective biological control agent will help reduce gastropod damage in grass seed crops and improve crop yields throughout Oregon.
References
GENETICS AND BREEDING

Oral Presentations
GENETIC VARIATION OF ALFALFA SEED YIELD IN THE ESTABLISHMENT YEAR

Đura Karagić, Dragan B. Milić, Snežana M. Katanski, Branko R. Milošević, Miroslav Z. Zorić, and Bernadette Julier

Abstract
In Serbia, alfalfa (Medicago sativa L.) is grown on about 140,000 ha, out of which 3–7% are intended for seed production. Climatic factors (amount and distribution of precipitation) are the main determinants of alfalfa seed yield. Average alfalfa seed yield in Serbia is about 250 kg ha\(^{-1}\), and seed production is characterized by huge variation (15–800 kg ha\(^{-1}\)). The most challenging year for seed production is the establishment year, i.e., the first year of plant life. To be successful on the market, new alfalfa varieties are selected for high forage production, quality of forage, and persistence. However, outstanding seed-yield potential is also needed. Even if breeding for seed yield is not prioritized, genetic variation among cultivars is known. In order to explore genetic variation for alfalfa seed yield and its components in the establishment year, a trial with 400 accessions was established at the experimental field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia, on May 21, 2018. The genotypes were tested in a partially replicated design. For the purpose of this study, we extracted model best linear unbiased predictions (BLUPs) for 20 populations, which represent a part of the European alfalfa core collection. The plot size was 6 m\(^2\). Seed yield and its components were recorded for each plot. The 2018 field season was not favorable for alfalfa seed production in Serbia, but the results clearly demonstrate differences among varieties for total seed yield and its components in the establishment year. Seed yields varied from 31.8 kg ha\(^{-1}\) for the variety ‘Tereza’ to 96.7 kg ha\(^{-1}\) for the variety ‘Etincelle’. Higher seed yields were obtained with less dormant varieties (dormancy ratings 5–6), while lower yields were recorded with more dormant varieties (dormancy ratings 3–4). Analyses showed that, even under unfavorable conditions, genetic variation could be important for alfalfa seed production in the year of establishment.

Keywords: alfalfa, seed yield, variety, genetic variation

Introduction
In Serbia, alfalfa (Medicago sativa L.) is grown on about 140,000 ha, out of which 3–7% are intended for seed production. Climatic factors (amount and distribution of precipitation) are the main determinants of alfalfa seed yield. Average alfalfa seed yield in Serbia is about 250 kg ha\(^{-1}\), and seed production is characterized by a huge variation (15–800 kg ha\(^{-1}\)). The most challenging year for seed production is the establishment year, i.e., the first year of plant life. To be successful on the market, new alfalfa varieties are selected for high forage production, high quality of forage, and persistence. However, outstanding seed-yield potential is also needed. Even if breeding for seed yield is not prioritized, genetic variation among cultivars is known.

The main goals of the research were to: (1) evaluate seed yield and its components in one part of the European alfalfa core collection in the year of establishment, (2) analyze phenotypic correlations among...
the main seed yield traits, and (3) compare distribution of the examined traits in total genetic variation.
The probability of exploring genetic variation in breeding programs is considered.

Methods
In order to explore genetic variation for alfalfa seed yield and seed-yield components in the establishment
year, a trial with 400 accessions was established at the experimental field of the Institute of Field and
Vegetable Crops, Novi Sad, Serbia on May 21, 2018. The experimental site was located in northern
Serbia, at 45°20’ N, 19°51’ E, at 80 m above sea level. This area has a continental semiarid to semihumid
climate, a mean monthly air temperature of 11°C, annual total precipitation of around 600 mm, and a
highly uneven distribution of precipitation. The genotypes were tested in a partially replicated design. The
design grid size was 10 rows by 44 columns. The proportion of replicated genotypes was 0.14. The data
on the measured traits were analyzed by the ASReml-R package mixed model approach (Butler et al.,
2009).

Furthermore, the subsets of best linear unbiased predictions (BLUPs) from the model were used for
visualizations by means of simple scatter plot graph and multivariate principal component analysis (PCA)
technique. The biplot graph was used as visual display of the genotype-by-trait relationship.

For the purpose of this study, we extracted model BLUPs for 20 populations, which represent a part
(‘SW Nexus’), and 1 from Denmark (‘Creno’), with dormancy ratings of 3 to 6. The plot size was 6 m².
Seed yield (SY, kg ha⁻¹) and its components (plant height in full flowering, (PHF, cm), stems m⁻²(SM),
fertile stems m⁻²(FSM), number of inflorescences per stem (IS), number of pods per inflorescence (PI),
seeds per pod (SP), and 1,000-seed weight (TSW)) were recorded for each plot.

Results and Discussion
The results clearly demonstrate the influence of weather conditions on alfalfa SY and its components in
the establishment year. Average SY in the trial was 64.4 kg ha⁻¹. The highest seed yield was recorded with
variety ‘Etincelle’ (96.7 kg ha⁻¹), while the lowest yields were found in variety ‘Tereza’ (31.8 kg ha⁻¹).
A huge SY variation was recorded in the trial, from 7.8 kg ha⁻¹ within varieties ‘Lusiante’ and ‘Tereza’
to 140.2 kg ha⁻¹ within the variety ‘Sanditi’. There is a strong correlation between alfalfa seed yield and
unfavorable agroecological conditions. The average number of SP was 2.6, while the mean value for PI
was 7.6. The average number of FSM was 254.7. Mean PHF was 79.3 cm, ranging from 69 cm to 93 cm.

The highest positive phenotypic correlations were found between SY and PI, FSM, and SP (Table 1) in
the year of establishment. Also, the highest positive correlations in the trial were found between SM and
FSM (0.78), as expected. Similar phenotypic correlations between seed yield and seed yield components
were reported by Karagić (2004), with one main difference regarding climatic conditions; Karagić’s
observations were reported for the year 2000, which was favorable for alfalfa seed yields in Serbia in
terms of weather conditions.

Figure 1 depicts the relationship between SY and PI. These two traits are the most important for
successful alfalfa seed production. Bolaños Aguilar et al. (2000) reported strong high positive correlations
between these two traits in alfalfa. Figure 1 clearly shows that populations were divided into four groups.
The upper right quadrant contains the accessions that have high SY and a high PI (‘Etincelle’, ‘Mezzo’,
The lower left quadrant includes genotypes with the lowest SY and PI in the year of establishment. The PCA biplot in Figure 2 shows the relationship between genotypes and SY and its components. The biplot explains about 60% of the total phenotypic variation. Most of the populations are concentrated on the right side of the first dimension. These genotypes (‘Galaxie’, ‘Nijagara’, ‘Mezzo’, ‘Holyna’, ‘Sanditi’) were characterized by high values of highly correlated traits (SY, IS, SP, FSM, SM, and TSW), based on the biplot pattern. On the negative side of the first dimension are the genotypes ‘Tereza’, ‘SW Nexus’, ‘Milky Max’, and ‘Creno’, which present more dormant varieties (dormancy ratings 3–4) in the trial. Moreover, the biplot shows overall negative correlations among PHF and the groups of highly correlated traits. The lack of relationship between PHF and other traits is due to the unfavorable weather conditions during the trial, as well as the relation between plant height and lodging in alfalfa.

Table 1. Phenotypic correlations (Pearson coefficient) between seed yield and seed yield components.\(^1\)\(^2\)

<table>
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<tr>
<th></th>
<th>PHF</th>
<th>SY</th>
<th>SM</th>
<th>FSM</th>
<th>IS</th>
<th>PI</th>
<th>SP</th>
<th>TSW</th>
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<tr>
<td>PHF</td>
<td>1.000</td>
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<td></td>
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<tr>
<td>SY</td>
<td>-0.055</td>
<td>1.000</td>
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<tr>
<td>SM</td>
<td>-0.115</td>
<td>0.491*</td>
<td>1.000</td>
<td></td>
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<td></td>
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<tr>
<td>FSM</td>
<td>-0.054</td>
<td>0.589**</td>
<td>0.781***</td>
<td>1.000</td>
<td></td>
<td></td>
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<tr>
<td>IS</td>
<td>-0.137</td>
<td>0.406</td>
<td>0.155</td>
<td>0.253</td>
<td>1.000</td>
<td></td>
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<tr>
<td>PI</td>
<td>0.382</td>
<td>0.649**</td>
<td>0.249</td>
<td>0.441</td>
<td>0.522*</td>
<td>1.000</td>
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<tr>
<td>SP</td>
<td>-0.128</td>
<td>0.575**</td>
<td>0.270</td>
<td>0.457*</td>
<td>0.599**</td>
<td>0.516*</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>TSW</td>
<td>-0.108</td>
<td>0.205</td>
<td>0.116</td>
<td>0.154</td>
<td>0.228</td>
<td>-0.167</td>
<td>0.218</td>
<td>1.000</td>
</tr>
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</table>

\(^1\)PHF = plant height in full flowering; SY = seed yield; SM = stems m\(^{-2}\); FSM = fertile stems m\(^{-2}\); IS = inflorescences per stem; PI = pods per inflorescence; SP = seeds per pod; TSW = thousand-seed weight

\(^2\)Significance levels: *0.05; **0.01; ***0.001

The PCA biplot in Figure 2 shows the relationship between genotypes and SY and its components. The biplot explains about 60% of the total phenotypic variation. Most of the populations are concentrated on the right side of the first dimension. These genotypes (‘Galaxie’, ‘Nijagara’, ‘Mezzo’, ‘Holyna’, ‘Sanditi’) were characterized by high values of highly correlated traits (SY, IS, SP, FSM, SM, and TSW), based on the biplot pattern. On the negative side of the first dimension are the genotypes ‘Tereza’, ‘SW Nexus’, ‘Milky Max’, and ‘Creno’, which present more dormant varieties (dormancy ratings 3–4) in the trial. Moreover, the biplot shows overall negative correlations among PHF and the groups of highly correlated traits. The lack of relationship between PHF and other traits is due to the unfavorable weather conditions during the trial, as well as the relation between plant height and lodging in alfalfa.
Conclusion
The relationships between SY and SY components presented in this paper are in line with previous research related to alfalfa seed yield performance. Breeding objectives should take into account alfalfa SY *per se*, but also PI and SP. To an extent, genetic variation in alfalfa is likely to be very dependent on climatic conditions in a particular year.

Higher SY was obtained from the less dormant varieties (dormancy ratings 5–6), while lower yields were recorded in the more dormant varieties (dormancy ratings 3–4). Overall, the analyses showed that, even under unfavorable conditions, genetic variation could be important for alfalfa seed production in the year of establishment. Further analyses are needed to reach a final conclusion and carry out tandem selection for both forage yield traits and seed yield.

Acknowledgments
This research was supported by the Breeding Forage and Grain Legumes to Increase EU’s and China’s Protein Self-sufficiency (EUCLEG) project no. 727312 and is funded by the European Union under the Horizon 2020 Programme.

References
STEM RUST RESISTANCE IN USDA PERENNIAL RYEGRASS GERMPLASM

Ryan J. Hayes¹*, Mark D. Azevedo¹, Lori M. Evans-Marks¹, and Dana D. Moore²

Abstract

Stem rust on perennial ryegrass (Lolium perenne L.) is a damaging disease that is currently controlled with fungicides; host resistance could reduce fungicide use. Resistant germplasm developed by the U.S. Department of Agriculture (USDA), Corvallis, Oregon, is more useful if the resistance is consistently expressed in diverse environments, but nothing is known about the stability of resistance in this germplasm. The objective of this research was to determine the stability of resistance in 52 clones from 18 accessions or crosses used for stem rust resistance breeding by the USDA. Germplasm was chosen based on previous selection for resistance, genebank data indicating resistance, or from known susceptible cultivars. Clones were transplanted in replicated field experiments in Aurora, Corvallis, and Crabtree, Oregon, in 2016 and 2017 and rated biweekly for disease severity (percent diseased foliage) from April to June the following year. The second-year plants from the 2016 plantings were also reevaluated in 2018, resulting in nine separate environments. Disease occurred from naturally occurring inoculum. The standardized area under the disease progress curve (sAUDPC) was calculated using disease severity data. Disease levels varied across environments, with four environments having a median sAUDPC of 0.0 and three environments having extensive disease (sAUDPC > 5.0). The other environments had median sAUDPC between 0.3 and 1.0. Clones previously selected for resistance generally had lower disease (clone median sAUDPC range: 0 to 0.14) than known susceptibles (clone median sAUDPC range: 0 to 1.28). Rank correlations among all environments that tested first-year plants and second-year plants in Crabtree and Corvallis were significant (P < 0.05) and ranged from 0.3 to 0.82. Correlations involving second-year plants in Aurora were less than 0.27 and not significant. Significant agreement between clone rankings across environments was found using Kendall’s coefficient of concordance (W = 0.42; P = 0.006), indicating consistent performance in this germplasm across the nine tested environments.

Keywords: disease, resistant germplasm, durability, breeding, genetics

Introduction

Stem rust of perennial ryegrass grown for seed can cause total crop loss, and most cultivars need two to five fungicide applications annually to produce a crop. Two large-effect and pathotype-specific resistance quantitative trait loci (QTL), qLpPg2 and qLpPg1, were found on linkage groups 1 and 7, respectively, in the cultivar ‘Kingston’ (Pfender and Slabaugh, 2013). Breeding these resistance QTL into new cultivars could reduce fungicide use, and subsequent work developed breeding materials based on ‘Kingston’ resistance using greenhouse experiments and pathotypes that select for each QTL. Little is known about the genetic variability of the stem rust pathogen (Puccinia graminis f. sp. lolium, Pgl) in western Oregon, which makes ascertaining the potential for the pathogen to defeat new resistance genes difficult. Since the ‘Kingston’ QTLs are pathotype-specific, it is plausible that they could be quickly defeated by preexisting pathotypes in western Oregon. Breeding with genes that are easily defeated by new strains of the pathogen

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makes little sense, and prior knowledge of the potential durability of ‘Kingston’-derived resistance can aid decision making on whether to use ‘Kingston’-derived germplasm in a resistance breeding program. The objective of this research was to determine the stability of stem rust resistance in breeding material developed by the USDA.

**Methods**

The material evaluated were clones selected from cultivars, crosses, and genebank accessions: 24 clones from the cultivar ‘Kingston’ or crosses involving ‘Kingston’, 19 clones sampled across 8 different accessions, and 9 clones from the susceptible cultivars ‘Jet’ and ‘Morningstar’. The ‘Kingston’ germplasm was previously selected for resistance in greenhouse experiments using two Willamette Valley derived Pgl pathotypes that select for resistance conferred by qLpPg2 and qLpPg1 (Pfender and Slabaugh, 2013). Clones were propagated from vegetative tillers, grown in the greenhouse, and transplanted during the fall into replicated field experiments in Aurora, Corvallis, and Crabtree, Oregon, in 2016 and 2017. Clones were arranged as a randomized complete block with three blocks and a single plant per clone in each block. Disease occurred from naturally occurring inoculum, and plants were evaluated biweekly for disease severity (percent diseased foliage) from April to June the following year. The second-year plants from the 2016 plantings were also reevaluated in 2018, resulting in nine test environments. The standardized area under the disease progress curve (sAUDPC) was calculated using disease severity data as a method to summarize season-long disease progression (Simko and Piepho, 2012). Data distributions were non-normal and exhibited nonconstant variance, and data were summarized using nonparametric statistics. Disease severity in each environment was compared using median and maximum values. Dependence between environments was determined using Spearman’s rank correlation. Kendell’s concordance ($W$) was calculated to measure and test for agreement of ranks across all environments (Madden et al., 2007). The statistic varies from 0 (no agreement of ranks) to 1 (complete agreement of ranks). Detection of significant differences between clone sAUDPC was conducted using analysis of variance type statistics of ranked data (Shah and Madden, 2004).

**Results and Discussion**

Environments differed for disease progression. All environments had clones with zero disease, but environments varied widely for median and maximum disease. This likely resulted from the reliance on natural infection and inherent differences between environments in conduciveness for disease development. The least amount of disease was observed in first-year stands at Aurora and Hyslop in 2017 and in second-year stands at Hyslop and Crabtree evaluated in 2018. All of these experiments had median sAUDPC of 0, and disease development on susceptible checks was present but limited. The heaviest disease generally occurred in first-year stands in 2018; all locations with first-year stands in 2018 had a median sAUDPC of 5 or more. Heavy disease was observed on susceptible clones in five environments having sAUDPC higher than 17. Disease levels for individual clones were generally consistent across environments. Thirty-two of the 36 possible rank correlation coefficients between environments were significant ($P < 0.05$), ranging from 0.3 to 0.82. The four nonsignificant correlations all involved the second-year stand in Aurora. Concordance, which summarizes the degree of agreement of clone ranking across all nine environments, was significant ($W = 0.42$; $P = 0.006$). This is further evidence that clone performance was consistent across environments; more specifically, ‘Kingston’-derived germplasm consistently demonstrated resistance in all environments.

The sAUDPC of each clone was summarized across all environments and indicated differences in resistance (Figure 1). Clones from susceptible cultivars were largely heavily diseased. The exception
Figure 1. Maximum and median standardized area under the disease progress curve (sAUDPC) for 52 perennial ryegrass clones derived from the cultivar ‘Kingston’, accessions from genebanks, and susceptible cultivars ‘Jet’ and ‘Morningstar’. Data is from nine replicated experiments. *Indicates ‘Kingston’ germplasm with significantly less disease than all susceptible checks determined through analysis of ranked data (Shah and Madden, 2004).
was clone 11-5, which appears to be less susceptible than other ‘Jet’ and ‘Morningstar’-derived clones. Excluding clone 11-5, the susceptible material had median sADUPCs ranging from 0 to 1.8 and maximum sAUDPCs from 9 to 26. The germplasm previously selected for resistance and derived from the cultivar ‘Kingston’ had zero to limited amounts of disease. The maximum sAUDPC for ‘Kingston’-derived material, or the greatest amount of disease observed in at least a single experiment, was 5.8. The median sAUDPC of this group ranged from 0 to 0.1. Six clones from the Kingston germplasm (R4, M05, M11, F02, F03, and F06) had significantly less disease than all susceptible checks. Clones derived from accessions had variable levels of disease, including some clones with minimal disease. However, none of these clones had significantly lower disease than susceptible checks.

**Conclusion**
These experiments demonstrate that ‘Kingston’-derived germplasm can exhibit stem rust resistance across a range of western Oregon environments and appears to be suitable material to use as parents in resistance breeding.

**References**
Abstract
Kentucky bluegrass (*Poa pratensis* L.) seed production in Washington and Oregon periodically suffers from poor seed set due to incomplete vernalization. As a polyploid, facultative apomict, Kentucky bluegrass has a complicated genetic system that impedes selection in plant breeding. To improve Kentucky bluegrass selection for easier vernalization and seed production, a more robust method to find the genes that affect vernalization is necessary. To this end, we are conducting full-genome sequencing of a Kentucky bluegrass polyhaploid plant, and characterizing the number and arrangement of vernalization genes within its genome. For the main gene, Vrn1, we found three copies of the gene, each with a unique arrangement that included a duplicated exon. All varieties tested thus far had the duplicated exon, but the ancestral species did not.

Keywords: *Poa pratensis*, Kentucky bluegrass, vernalization, polyploid, Vrn1

Introduction
Kentucky bluegrass (*Poa pratensis* L.) is a cool-season amenity (turf) and forage grass distributed widely across the world. Its uses include highly maintained golf course fairways, lawns, parks, livestock pastures, and roadside soil stabilization (Bonos and Huff, 2013). There are hundreds of Kentucky bluegrass cultivars and collections throughout the world, with extensive variation for phenology, morphology, turf quality, and abiotic stress responses (Funk, 2000). In the United States, Kentucky bluegrass seed is produced primarily in central Washington and Oregon, where climate and irrigation provide high seed yields and minimal disease pressure. However, seed yields periodically suffer in central Washington due to inadequate vernalization caused by insufficient duration of cold winter temperatures. To understand how Kentucky bluegrass responds to low temperatures, we have initiated a study to examine the vernalization-responsive genes and find variants that correspond to a lesser vernalization requirement.

The Kentucky bluegrass genome and breeding system pose challenges to identifying functional genes that affect vernalization (Bushman et al., 2013). As a facultative apomict, Kentucky bluegrass can be fully fertile and produce seed despite odd chromosome numbers or the presence of aneuploidy. As a high polyploid, there are multiple redundant copies of each gene in the Kentucky bluegrass genome. Thus, finding useful molecular markers to help in selection is challenging. These challenges necessitate a reference genome sequence. To this end, we have sequenced a Kentucky bluegrass reference plant using PacBio platform. We use this reference genome to identify different genes in the vernalization pathway.

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Methods
A polyhaploid Kentucky bluegrass hybrid from the cultivar ‘Hampton’ was sequenced on a PacBio Sequel platform at the Brigham Young University DNA sequencing core facility. Raw sequence “reads” were corrected, trimmed, and assembled using the CANU pipeline on the USDA-ARS Ceres high-performance computer servers. Candidate genes affecting vernalization were extracted based on homology to a perennial ryegrass (Lolium perenne L.) Vrn1 sequence in the NCBI Genbank. The BLASTn search against the KBG genome used an e-value of -20, and was conducted separately for the different exons of the LpVrn1 gene. Putative gene families were aligned in Geneious Prime (Biomatters, Inc., Newark, NJ, USA), and primers from Vrn-1 and FT-1 were designed using Primer3. Identity of the Vrn-1 gene pieces was tested with PCR on agarose gels, testing a set of cultivars and ancestral diploid species.

References
DEVELOPMENT OF NEW PCR METHODS TO DISTINGUISH MORPHOLOGICAL SIMILARITIES IN HERBAGE SEEDS

Yuguo Wu1, Dong Luo1, Shuheng Shen1, Lichao Ma1, Xiaowen Hu1, 0anrong Wang1,* and Zhipeng Liu1,*

Abstract
Unlike many crop seeds that are large in size, herbage seeds are relatively small. Moreover, more similarities in color, shape, size, and weight are shared among some herbage seeds within the same genus, and even among seeds of different genera. To distinguish similar-looking seeds from one another, traditional morphological testing is experience-dependent, time-consuming, and often inaccurate. Therefore, there is a need for an accurate and low-cost alternative method to distinguish herbage seeds. This work was aimed at developing reliable, accurate, and simple polymerase chain reaction (PCR) methods to distinguish similar herbage seeds. DNA was extracted from dry seeds, and the sample concentration of DNA was adjusted to 25 ng μl⁻¹. To distinguish a pair of similar seeds, a same pair of PCR primers was designed based on the differences in a pair of homologous genes, such as plastid genes and nuclear genes. Amplification of PCR was carried out in a 20-μl reaction volume. Finally, PCR products (5 μl per lane) were separated on 10% nondenaturing polyacrylamide gels. Morphologically similar seeds were identified based on the electrophoretic patterns of different sizes (or existence and nonexistence) of PCR-amplified products. This experimental procedure can be completed within 2 days. This method has been successfully used to distinguish eight pairs of similar herbage seeds of different species: alfalfa (Medicago sativa) and white sweet clover (Melilotus albus), alfalfa and yellow sweet clover (M. officinalis), alfalfa and Medicago polymorpha, alfalfa and dodder (Cuscuta spp.), perennial ryegrass (Lolium perenne) and annual ryegrass (Lolium multiflorum), Elymus sibiricus and Elymus dahuricus, Medicago truncatula ecotype A17 and Medicago truncatula ecotype R108, sorghum (Sorghum bicolor) and Sorghum × S. sudanense grass. In conclusion, these methods have good potential to be utilized in herbage seed genus identification and herbage seed purity testing, when distinguishing between morphologically similar seeds.

Keywords: morphologically similar, herbage seeds, PCR methods, seed genus, seed purity

Introduction
Each herbage species has its own unique life cycle and agricultural purposes. However, some herbage seeds share a similar morphology—for example, alfalfa (Medicago sativa) and sweet clovers (Melilotus albus and M. officinalis), alfalfa and Medicago polymorpha, alfalfa and dodder (Cuscuta spp.), perennial ryegrass (Lolium perenne) and annual ryegrass (Lolium multiflorum), Elymus sibiricus and Elymus dahuricus, Medicago truncatula ecotype A17 and Medicago truncatula ecotype R108, sorghum (Sorghum bicolor) and S. sudanense grass (Figure 1). It is difficult to distinguish these seeds from one another, and, when they are confused or mixed, inestimable losses to agriculture and livestock may result.

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The traditional method for evaluating seed purity is morphological comparison. However, replication of seed observations is inconclusive and time-consuming. The similarities of these seeds increase the difficulty of identification by morphological methods, even for experienced plant taxonomists. A one-step polymerase chain reaction (PCR) protocol could be developed to distinguish a pair of similar seeds by designing a same pair of PCR primers and then examining the different amplicon sizes. This method is particularly suitable for this application because of its reliability, fast protocol, sensitivity, and accuracy.

**Methods**

DNA was extracted from dry seeds, and the sample concentration of DNA was adjusted to 25 ng μl⁻¹. To distinguish between a pair of similar seeds, a same pair of PCR primers was designed based on the differences in a pair of homologous genes of species A and species B from the National Center for Biotechnology Information (NCBI), such as plastid genes and nuclear genes. Amplification of PCR was carried out in a 20-μl reaction volume. The designed primers with different sizes of amplicons needed to be reconfirmed in more germplasm of species A and species B. Finally, PCR products (5 μl per lane) were separated on 10% non-denaturing polyacrylamide gels. Morphologically similar seeds were identified based on the electrophoretic patterns of different sizes (or existence and nonexistence) of PCR-amplified products. This experimental procedure can be completed in 2 days.

**Figure 1.** Seven pairs of similar herbage seeds of different species: (A) *Lolium perenne* and (B) *Lolium multiflorum*; (C) *Elymus sibiricus* and (D) *E. dahuricus*; (E) *Medicago sativa* and (F) *Melilotus albus*; (G) *M. sativa* and (H) *M. polymorphha*; (I) *M. sativa* and (J) *Cuscuta*; (K) *M. truncatula* ecotype A17 and (L) *M. truncatula* ecotype R108; (M) *Sorghum bicolor* and (N) *S. sudanense*. Scale bars = 1 mm.
Results and Discussion

This method has been successfully used to distinguish eight pairs of similar herbage seeds of different species, including alfalfa (Medicago sativa) and white sweet clover (Melilotus albus), alfalfa and yellow sweet clover (Melilotus officinalis), alfalfa and Medicago polymorpha, alfalfa and dodder (Cuscuta spp.), perennial ryegrass (Lolium perenne) and annual ryegrass (Lolium multiflorum), Elymus sibiricus and Elymus dahuricus, Medicago truncatula ecotype A17 and Medicago truncatula ecotype R108, Sorghum bicolor and S. sudanense grass.

We can use the identification of annual and perennial ryegrass seeds as an example to better demonstrate the accuracy and speed of this method. DNAMAN sequence analysis and resequencing results clearly show that annual ryegrass lacked the 20bp fragment (Figure 2). This primer pair has the ability to distinguish between other ryegrass accessions. The accuracy and sensitivity of the primer pair was further demonstrated by mixing perennial and annual ryegrass DNA in predefined proportions (Figure 3). These results revealed that perennial and annual ryegrass accessions could be clearly distinguished using Lolium-F and Lolium-R primers.

Conclusion

In conclusion, these new PCR methods have good potential to be utilized in herbage seed genus identification and herbage seed purity testing when distinguishing between morphologically similar seeds.

![Figure 2. Sequencing results. (A) DNAMAN analysis results of partial plastid region sequences in perennial and annual ryegrass. The vertical black arrow indicates the region that lacked 20 bp in annual ryegrass. The region is present twice in the perennial ryegrass sequence between 97 and 136 bp, whereas the region is present only once in the annual ryegrass sequence between 97 and 116 bp. The horizontal black arrows indicate the location of the primers used for PCR amplification. (B) The sequencing results confirm the DNAMAN analysis results. The vertical arrow indicates the repeat region in perennial ryegrass.](image)
Figure 3. PAGE results of perennial (*Lolium perenne*) and annual (*Lolium multiflorum*) ryegrass accessions. (A) Lanes 1–26 of perennial ryegrass, 26 perennial ryegrass accessions. Lanes 1–34 of annual ryegrass, 34 annual ryegrass accessions. (B) PAGE results of DNA mixtures of perennial and annual ryegrass at the predefined proportions. CK(-) = negative control; M = molecular markers (200 and 150 bp).

References
POSTER ABSTRACTS
EFFECTS OF SOWING METHODS AND COVER CROPS ON TALL FESCUE SEED YIELD

Serge Bouet1,*, François Deneufbourg1, and Guenaëlle Hellou2

Abstract
Traditionally, grass seed crops in France are commonly established in a winter wheat cover crop. A multiyear experimental study was established at the Fédération Nationale des Agriculteurs Multiplicateurs de Semences (FNAMS) experimental station near Angers. The objective of this work was to measure the impact of different sowing methods and cover crops on tall fescue (Schedonorus arundinaceus Schreb.) seed yield. The effect of undersowing forage grass seeds on both cover crop and tall fescue seed yield was investigated over five seasons. Plots were 10 m long x 6 m wide. The experiment was arranged in a randomized block design with four replications. Treatments included tall fescue sown as follows: (1) undersown in a winter wheat crop in autumn, after maize (control), (2) undersown in a winter wheat crop in autumn, after red clover, (3) undersown with red clover in forage maize in spring (“triple sowing”), (4) sown after a wheat crop (first year: maize harvest; second year: red clover; third and fourth years: tall fescue seed production), and (5) sown simultaneously with red clover on bare soil in late summer after a winter wheat crop. Impact on the quality of seedling establishment, weed control, vegetative growth (dry matter and root growth in 2018), nitrogen (N) uptake, fertile tiller number, and seed yield was evaluated. Undersowing tall fescue with red clover in the spring in forage maize significantly increased seed yield (on average by 50%) compared to the control treatment. The long establishment period and the presence of a legume in the crop rotation resulted in more fertile tillers and consequently increased seed yield. Nitrogen uptake by the tall fescue crop was also higher with this treatment. This research indicates that cover crops can affect tall fescue and that these effects should be investigated further.

Keywords: tall fescue, cover crop, seed yield, quality establishment, nitrogen absorption
TOPPING EFFECT ON WHITE CLOVER SEED PRODUCTION IN ARGENTINA

Ezequiel De Bárbara¹,* and Martín Borini¹

Abstract

One of the main performance components in white clover (Trifolium repens L.) seed production is inflorescence number, which depends on the number of active growing points. Shading of growing points by plant foliage can produce abortions of developing buds, thus reducing inflorescence number. Mechanical canopy removal when the crop has one inflorescence m⁻² is a common practice among Argentine growers, but its seed yield effect has never been measured. Previous trials conducted in New Zealand showed that topping 14 or 21 days after first inflorescence m⁻², or double cutting (at first inflorescence m⁻² and 14 days later), generated a significant seed yield increase compared to not topping. The objective of this work was to evaluate the effect of topping on seed yield and dry matter (DM) on a first-year irrigated white clover ‘Apolo’ cultivar in Balcarce, Argentina. The study design was a randomized complete block with three replications. Plot size was 5 m x 3 m. Seven topping treatments included: (1) control (no topping), (2) topping 2 weeks before the appearance of the first inflorescence m⁻², (3) topping at first inflorescence m⁻², (4) topping 14 days after first inflorescence m⁻², (5) topping 21 days after first inflorescence m⁻², (6) double topping at first inflorescence m⁻² and 14 days later, and (7) chemical topping using paraquat (828 g ai ha⁻¹) at first inflorescence m⁻². Seed yield was not significantly different between treatments. However, treatments 3, 4, and 6 presented lower DM volume by 1,895 kg ha⁻¹, 1,355 kg ha⁻¹, and 1,811 kg ha⁻¹, respectively. In addition, these three treatments showed significant positive correlation (P < 0.05) between total inflorescence m⁻² and seed yield (one mature inflorescence m⁻² = 0.88 kg seed ha⁻¹). These results show that there are no significant seed yield differences between topping and no topping. However, mechanical canopy removal can help the crop reach harvest time with lower DM volume, thereby facilitating desiccation and harvest.

Keywords: white clover, topping, seed yield, dry matter, inflorescence

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EFFECT OF GRAZING ON KERNZA GRAIN YIELD AND LODGING IN DUAL-USE WHEATGRASS

Jeremie R. Favre¹*, Jacob M. Jungers², Mitch C. Hunter², Craig C. Sheaffer², and Valentin D. Picasso¹

Abstract
Kernza is the first commercial perennial grain crop in the world and was developed with conventional breeding to increase seed yield of intermediate wheatgrass (Thinopyrum intermedium [Host] Barkworth & D.R. Dewey). When grown in the upper Midwest, forage production of intermediate wheatgrass is abundant and offers potential for dual use (grain and forage). The objective of this work was to determine the impact of grazing timing on Kernza grain yields, lodging, and annual forage production of intermediate wheatgrass managed for dual use. Plots were established in Lancaster, Wisconsin, and Morris, Minnesota, with intermediate wheatgrass planted in monoculture and in mixture with a legume (red clover or alfalfa). Four grazing treatments were applied: no grazing, spring grazing, fall grazing, and spring and fall grazing. Grain yields, lodging scores, and forage dry matter yields were assessed during the 2017 and 2018 growing seasons. In both years, grazing intermediate wheatgrass in the spring and fall increased total harvested forage and did not reduce grain yields. In Lancaster, first-year grain yields were greater for the intermediate wheatgrass monoculture compared to the red clover intercrop, with 938 versus 780 kg ha⁻¹, respectively. Grain yields declined in the second year, with 319 kg ha⁻¹ and no vegetation effect. Grain yields in Morris were similar across treatments; they averaged 297 kg ha⁻¹ in the first year and decreased to 41 kg ha⁻¹ in the second year. In 2018, in Lancaster, the incidence of lodging was reduced from 41 to 12.5 on a scale of 100 by the application of early spring grazing. These results show that intermediate wheatgrass can be managed for dual use without impacting grain yield.

Keywords: Kernza, intermediate wheatgrass, legume intercropping, grazing timing, dual-use

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Abstract
Tall fescue (Schedonorus arundinaceus [Schreb.] Dumort.) is an important grass seed crop in Oregon. Trinexapac-ethyl (TE) is a plant growth regulator (PGR) that consistently increases grass seed yield through lodging control. Combinations of TE and chlormequat chloride (CCC), another PGR, increased seed yield in studies conducted in New Zealand. Objectives of this study were to investigate potential effects of PGR combinations on seed yield, yield components, and canopy characteristics in forage-type (‘Fawn’) and turf-type (‘Spyder’) tall fescue. Trials were conducted at Oregon State University’s Hyslop Farm near Corvallis, Oregon, in 2017 and 2018. The experimental design was a randomized block with a split-plot arrangement of treatments and four replications. Seven PGR treatments were applied at BBCH 32: an untreated control, 210 g TE ha\(^{-1}\), 1,500 g CCC ha\(^{-1}\), 105 g TE ha\(^{-1}\) + 750 g CCC ha\(^{-1}\), 210 g TE ha\(^{-1}\) + 1,500 g CCC ha\(^{-1}\), 210 g TE ha\(^{-1}\) + 750 g CCC ha\(^{-1}\), and 105 g TE ha\(^{-1}\) + 1,500 g CCC ha\(^{-1}\). Seed yield was greater in ‘Spyder’ than in ‘Fawn’. Yield was governed by an interaction of cultivar and PGR in 2018, but not in 2017. Yield was increased by 35.5% in 2017 with 210 g TE ha\(^{-1}\) + 750 g CCC ha\(^{-1}\) compared to the control, and by 31% in 2018 with 210 g TE ha\(^{-1}\) + 1,500 g CCC ha\(^{-1}\). In 2018, combinations of 105 g TE ha\(^{-1}\) + 750 g CCC ha\(^{-1}\) and 210 g TE ha\(^{-1}\) + 1,500 g CCC ha\(^{-1}\) increased yield in ‘Spyder’ over the control and TE or CCC alone, but not in ‘Fawn’. Seed yield was not increased by CCC alone. Results from this study indicate that PGR combinations produce inconsistent effects on tall fescue seed yield under Oregon conditions.

Keywords: trinexapac-ethyl, chlormequat chloride, plant growth regulators, seed yield, lodging
SEED CROP AGRONOMY OF CREEPING RED FESCUE IN CANADA’S PEACE REGION

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Abstract
Creeping red fescue (Festuca rubra L. ssp. rubra) is one of the pioneer crops and an export commodity of the Peace Region of Canada. However, relatively few studies have attempted to analyze its responses to environmental and management variables. The knowledge and technology gaps are manifested by the crop’s stagnant seed yield and by the sharp decline in seed yield in perennial harvests. This presentation synthesizes the results of agronomic studies on creeping red fescue in the Peace Region and proposes potential avenues for breaking through seed yield barriers. Of the 135 fescue cultivars registered in Canada, ‘Boreal’ has been one of the most predominant in terms of acreage and volume of seed production. A creeping red fescue seed crop fits well as a break crop in the prevalent crop sequence of peas, wheat, and canola; however, effective termination for efficient crop rotation is yet to be devised. The crop can be established under minimum tillage condition in canola stubble. Plant densities from 12.5 to 50 plants m⁻², with row spacing between 20 and 40 cm, produce similar yields. The higher densities produce higher yields the first year, with sharp declines in subsequent years. Moderate densities produce more stable yields over years. The crop responds to medium levels (65 kg ha⁻¹) of nitrogen fertilizer application, but different timing and methods of application produce inconsistent results. Application of plant growth regulators does not have significant effects on plant height, seed yield, seed weight, or germination. The interaction between crop management and weather variables obscures the crop response to management factors. A wide range of herbicides are compatible for weed management. Pests and diseases, such as silver-top, rust, snow mold, and stem eye-spot are observed, but yield losses have not been quantified.

Keywords: creeping red fescue, seed, agronomy, plant growth regulators, Peace Region

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ROW SPACING INFLUENCES SEED YIELD AND YIELD COMPONENTS IN ALFALFA SEED PRODUCTION

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Abstract
Alfalfa (Medicago sativa L.) seed yield can be influenced by many factors, including climatic conditions and field management practices. Plant density is one important factor for alfalfa seed production. However, little is known about how row spacing influences seed yield and yield components in an alfalfa plant stand for long-term seed production. Field experiments located in the Inner Mongolia autonomous region (western China) were carried out from 2008 to 2017 with different row spacing treatments (30, 60, 90, and 120 cm) to investigate the effect on seed yield and seed yield components. The results showed significant effects of row spacing on seed yield and shoots m⁻² with increasing stand age from 2009 to 2017. The highest seed yield was harvested at 60-cm row spacing from 2009 to 2013 and at 30-cm row spacing from 2014 to 2017. Narrower row spacing treatments (30 and 60 cm) achieved higher seed yield than wider row spacing treatments (90 and 120 cm) during the entire experimental period. For seed yield components, the number of shoots m⁻² was highest at 30-cm row spacing and significantly (P < 0.05) decreased with increased row spacing. Inflorescences per shoot and flowers per inflorescence achieved the highest density at 120 cm and gradually decreased as the stand aged. The changes in pods or seeds per inflorescence significantly correlated with harvest year but not with row spacing. The path analysis showed a significant and positive seed yield effect of pods per inflorescence from 2009 to 2013 and of shoots m⁻² from 2014 to 2017.

Keywords: alfalfa, seed yield, yield component, row spacing, seed production

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ACCUMULATION AND PARTITIONING OF NUTRIENTS BY HYBRID CARROT GROWN FOR SEED

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Abstract

Information on the accumulation of nutrients in various parts of the plant is often lacking for minor crops, such as hybrid carrot seed (Daucus carota). The objective of this study was to evaluate the uptake of agronomic nutrients in roots, shoots, and flowers of hybrid carrot plants grown for seed. This study was conducted on two irrigated grower fields on a Madras loam in Madras, Oregon, planted to hybrid carrot variety ‘Nantes’ type 969 in early August 2017. Sixteen plots were established in each field, with four replicated plots within each field randomly selected for nutrient uptake evaluations. Plants from female rows were sampled by plot on a monthly basis from October 2017 to August 2018. At each sampling event, whole plants were removed from three separate transects (0.9 m long) for each plot. Plant samples were separated by hand into roots, tops, and flowers. Separated samples were weighed (fresh/green weight), dried, weighed (dry weight), ground, and analyzed for total nitrogen (N), phosphorus (P), and potassium (K) concentration. Mean total biomass over the two fields was 8,570 kg ha⁻¹ on a dry weight basis. Approximately 7, 65, and 28% of the biomass accumulated in the roots, tops, and flowers, respectively. Mean total N uptake was 200 kg N ha⁻¹. Similar to biomass, approximately 6, 65, and 29% of the N accumulated in the roots, tops, and flowers, respectively. Mean total P uptake was 50 kg P₂O₅ ha⁻¹. Approximately 6, 54, and 40% of the P accumulated in the roots, tops, and flowers, respectively. Mean total K uptake was 340 kg K₂O ha⁻¹. Approximately 5, 76, and 19% of the K accumulated in the roots, tops, and flowers, respectively. Amounts and rates of accumulation measured in this study can provide general guidelines for hybrid carrot seed production systems.

Keywords: carrot, seed, partition, biomass, nutrient

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TRINEXAPAC-ETHYL TIMING AND RATE EFFECTS ON CRIMSON CLOVER SEED PRODUCTION

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Abstract
Oregon is the world’s leader in crimson clover (Trifolium incarnatum L.) seed production. Seed yield increase of red clover (T. pratense L.) has been reported with the application of trinexapac-ethyl (TE), a plant growth regulator (PGR) that reduces plant height, but the effects have not been investigated in crimson clover. The objective of this study was to determine the effects of TE rate and timing on canopy characteristics, seed yield, and seed yield components in crimson clover seed crops. Field trials were conducted at two on-farm locations (Banks and St. Paul, Oregon) in 2012 and 2013 and at Hyslop Experimental Farm (Corvallis, Oregon) in 2015 and 2016. Treatment applications were made at 0, 105, 140, 210, 280, 420, and 560 g a.i. TE ha⁻¹ and at two timings (stem elongation, BBCH 32; inflorescence emergence, BBCH 51). Canopy height and soil water content were affected by TE application, but stem number and aboveground biomass were not influenced by applications of this PGR. Seed yield was increased in 2012 and 2013 with ≤ 280 g a.i. TE ha⁻¹ application at BBCH 32; however, TE applications did not increase seed yield in 2015 and 2016 field trials due to dry spring weather. Seed weight was inversely proportional to TE rate; high TE application rates resulted in low seed weight. Seed yield was correlated with seeds m⁻² (r = 0.998***). Where increases in yield were manifested, this yield component offset the TE-induced losses in seed weight. Results indicate that TE is an effective tool for crop canopy manipulation and lodging control in crimson clover seed crops and that applications of the PGR can increase seed yield in the absence of spring drought conditions.

Keywords: plant growth regulators, seed yield, seed number, seed weight, crimson clover

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SEED PRODUCTION OF ENDOPHYTE-FREE AND INFECTED TALL FESCUE UNDER TWO DEFOLIATION FREQUENCIES

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Abstract
Tall fescue, Schedonorus arundinaceus (Schreb.) Dumort., is a perennial C₃ forage and turfgrass species widely used in most temperate regions of the world. This species is sometimes associated with the endophyte fungus, Epichloë coenophiala, which enhances plant tolerance to biotic and abiotic stresses and produces alkaloids that are toxic to grazing animals. The objective of this work was to evaluate the effect of two defoliation frequencies on the seed yield of tall fescue plants originating from a naturalized population infected with Epichloë coenophiala (FNE+) and from a commercial endophyte-free cultivar (FCE-, cv. ‘Taita’). On May 2, 2016, micropastures were sown at a density of 200 plants m⁻². The experimental factors included tall fescue micropasture (FNE+ and FCE-) and frequency of defoliation (HF = high frequency and LF = low frequency, with defoliation every 450 ± 100°C day and every 850 ± 100°C day, respectively, during the period between October 20, 2016 and August 16, 2017). Eight cuts were carried out in HF treatments and four cuts in LF treatments. A completely randomized experimental design with a factorial arrangement of two factors and five replicates was used. Seed yield was higher in FNE+ than in FCE- plants (316.44 ± 35.03 and 183.53 ± 18.19 seeds per plant, respectively; P = 0.0033). Seed yield was not affected by defoliation frequency (P = 0.2581), although seed production of the FNE+ tended to decrease under the highest frequency of defoliation. Based on these results, it would be advisable to apply a frequency of defoliation that is high enough to maintain the pasture in a vegetative state without compromising plant survival. This management will allow grazing animals to consume mostly leaf blades (which have a lower ergoalkaloid content than pseudostems and stalks) and will tend to decrease the production of endophyte-infected seed.

Keywords: Schedonorus arundinaceus, Epichloë coenophiala, seed production, defoliation frequencies, endophyte

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EFFECTS OF PLANT GROWTH REGULATORS AND NITROGEN ON KENTUCKY BLUEGRASS SEED PRODUCTION

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Abstract

Plant growth regulators (PGRs) are widely used to control lodging and increase the yield of grass seed crops. However, information on use of PGRs for Kentucky bluegrass (Poa pratensis L., KBG) is limited. In this study, a field trial was conducted at the Oregon State University Hermiston Agricultural Research and Extension Center to determine the effect of trinexapac-ethyl (TE) PGR on KBG. A KBG elite ‘Midnight’ variety was planted in Adkins sandy loam soil under center pivot irrigation in September 2017. A total of 307 kg ha⁻¹ nitrogen (N) was split-applied in fall and early spring. In early May of 2018, when the KBG was at the early stem elongation stage (BBCH 32), eight TE treatments were applied, including combinations of four TE rates (0, 0.9, 1.6, and 2.8 L ha⁻¹ Palisade EC) and two N rates (0 and 56 kg ha⁻¹). Treatments were arranged in a randomized complete block design with three replications. In total, there were 24 plots, with plots measuring 1.5 m x 10 m and 7 rows per plot. Field measurements indicated that all PGR treatments reduced lodging, with the high TE rates showing a 25% reduction. The 56 kg ha⁻¹ N application slightly increased lodging (by 11%) over the 0 kg ha⁻¹ N treatments. Stem height of KBG was reduced slightly at a TE rate of 1.6 L ha⁻¹ or higher. KBG seed yields were increased by 29 to 74% by N application in most cases. The TE application tended to increase seed yields in the plots with additional N applications. However, in general, no significant differences were found in this experiment because of the large standard deviations. Additional data are needed to further evaluate the interactive effect of PGR and N applications on the growth and production of KBG.

Keywords: Kentucky bluegrass, lodging, stem height, trinexapac-ethyl, seed yield

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Abstract
Kleingrass (Panicum coloratum var. coloratum) is widely adapted to the Argentinian central La Pampa region, with desirable traits including persistence and high nutritional value. Nonetheless, kleingrass utilization has been limited due to seed scarcity and erratic success in pasture establishment. The objective of this study was to compare seed yield components and forage remaining after harvest under different rates of nitrogen (N) fertilization in a farmer’s field. Kleingrass pastures were sown October 1, 2017 under direct tillage, with a density of 10 kg ha\(^{-1}\) uncoated seed without classification. The study was conducted on an 80-ha paddock located in the northeast of La Pampa province, with a soil type of Entic Haplustoll. The farmer utilized two fertilization strategies: natural condition (N0) and N application at 75 kg ha\(^{-1}\) (N75). Fertilizer was applied with a commercial Yomel fertilizer spreader to half of the area (40 ha) in the tillering stage after the paddock was closed to grazing (October 26, 2018). Samples were collected 5 days before mechanical harvest (January 30, 2019) to reduce losses due to seed shattering. Samples were collected in 1 m\(^2\) samples (n = 3). Panicles and forage samples were separately cut with sheep shears and weighed. We present means of each treatment and 95% confidence intervals (CI). Pasture height was increased by N (N0 = 104 cm, CI: 98–110, vs. N75 = 126 cm, CI: 122–129). Seed yield was increased by N (N0 = 33 kg ha\(^{-1}\) pure clean seed, CI: 17–50, vs. N75 = 107 kg ha\(^{-1}\), CI: 39–174). Samples contained a higher proportion of glumes in the N0 treatment. Fertilization increased panicle number (N0 = 242 panicles m\(^{-2}\), CI: 201–284, vs. N75 = 421 panicles m\(^{-2}\), CI: 232–610) and increased 1,000-seed weight (N0 = 0.23 g, CI: 0.18–0.28, vs. N75 = 0.37 g, CI: 0.31–0.42), probably indicating a different stage of maturity between fertilization treatments. Number of seeds per panicle (N0 = 57 seeds panicle\(^{-1}\), CI: 46–69, vs. N75 = 66 seeds panicle\(^{-1}\), CI: 51–81) was comparable. Remaining forage was increased by N (N0 = 2,226 kg DM ha\(^{-1}\), CI: 1,127–2,756, vs. N75 = 4,431 kg DM ha\(^{-1}\), CI: 4,190–4,672) with a lower leaf:stem relationship (N0 = 0.52, CI: 0.34–0.71, vs. N75 = 0.33, CI: 0.29–0.36). We report data suggesting that N fertilization may increase both forage and seed production in Panicum coloratum. These preliminary results support the need for further studies with a solid experimental design and stronger sampling effort, including other issues related to fertilization, such as timing.

Keywords: South America, tropical grasses, nitrogen fertilization, seed producers
PERCEPTION OF FACTORS AFFECTING TROPICAL FORAGE GRASS SEED PRODUCTION IN ARGENTINA

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Abstract
In an extended region of Argentina, tropical pastures have shown adaptation to diverse stresses and climate change. Despite their potential role in livestock systems, tropical pastures have been underutilized, mainly because of limited seed availability and quality. To evaluate the perception of people involved in tropical grass seed production, we conducted a survey identifying issues related to management and markets. Results were compared with a similar survey carried out 20 years ago by Hacker and Loch (1997) in Australia. The questionnaire, adapted to species and peculiarities in Argentina, was sent to 45 experts, including seed producers, breeders, researchers, and seed company managers. There were 32 questions with four answer choices (not corresponding to expertise, do not agree, agree, strongly agree) for 10 tropical grass species produced for seed in Argentina. Fifty-one percent of the surveys were returned, and 50% of the collected answers were “not corresponding to expertise.” Responses varied among species: 68% of the surveys were completed for Panicum coloratum but only 27% for Paspalum spp. There was agreement that more research is needed for seed production (57%), while there was less agreement that burning the crop is needed to promote growth (33%). A great lack of awareness was found related to plant growth regulators and their ability to increase seed yield (88%). Although results generally were similar to the Hacker and Loch survey, “not corresponding to expertise” answers were remarkably higher in this survey (50% vs. 17%), presumably due to a gap in knowledge and/or higher specialization in a few species. As in the work in Australia, respondents in Argentina agreed that technical advice from experts and more research are needed, but they were most concerned about management of weeds, diseases, and insect pests. Awareness of seed certification as beneficial for the industry was identified in both studies.

Keywords: tropical grass species, South America, survey, seed producers

Reference
Abstract
The red clover casebearer moth (CBM), Coleophora deauratella (Lepidoptera: Coleophoridae), is a native European species first reported in 1989 to be a pest in eastern Canada red clover (Trifolium pretense L.) seed production (Landry, 1991). CBM later spread to red clover seed production in the Peace River region of Alberta in western Canada and, since 2006, has become a pest of economic concern (Otani, unpublished). Recent monitoring in Oregon’s Willamette Valley, the primary growing region for clover seed in the United States, detected CBM in at least five western Oregon counties (Anderson et al., 2014). The objectives of this study were to conduct a pheromone-based monitoring program to determine the presence/absence of CBM east of the Cascade mountain range and to determine potential for larval feeding damage to developing red clover seed. Sex-pheromone-baited traps (Evenden et al., 2010) were placed in two commercial red clover seed production fields on May 25, 2018 to attract male moths. The two fields, a 1-year-old and a 2-year-old stand, were selected due to close proximity (< 0.4 km). Traps were monitored weekly for 10 weeks. Monitoring ceased August 3, 2018. Weekly monitoring activities included (1) collecting moth specimens for identification and quantification, and (2) evaluating red clover seed heads for larvae presence and damage. Monitoring efforts successfully detected CBM adult moths in both 1- and 2-year-old stands of red clover grown for seed (1,575 and 83 moths per season, respectively) in the Grande Ronde Valley of northeastern Oregon. The frequency of CBM larvae damage to red clover seed heads was only 2% (14 of 623 seed heads) in 2018. Based on this study, a priority need was established to increase pheromone-based monitoring efforts to further delineate CBM distribution, population dynamics, and potential impact on seed yield in all eastern Oregon clover seed production areas.

Keywords: casebearer moth, Coleophora deauratella, pheromone, red clover, Trifolium pretense

References
Abstract

Trinexapac-ethyl (TE) is a plant growth regulator used to delay lodging in perennial ryegrass (*Lolium perenne* L.) seed crops. Previous research and farmer experiences suggest that a single application at GS 29–30 and split applications at GS 29–30 and 36–39 are more effective at increasing yield than a single application at GS 31–32. Research was conducted in The Netherlands to compare the effects of rates, timing, and single vs. split applications of TE on lodging and seed yield. Experiments consisted of replicated field tests for three varieties: ‘Melspring’ (diploid forage-type), ‘Venice’ (turf-type), and ‘Valerio’ (tetraploid forage-type). Four Moddus TE rates (0.6, 0.9, 1.2, and 1.7 (split) L ha\(^{-1}\)) were applied as a single application at GS 31–32 and as split applications at GS 29–30, 31–32, 36, or 39. A single application of Trimaxx TE (0.8 L ha\(^{-1}\)) was applied at GS 31–32 to evaluate differences in TE formulation. Lodging decreased as rate increased for all three varieties. However, the 1.2 L ha\(^{-1}\) rate decreased seed yield by 12% and 6% in ‘Melspring’ and ‘Venice’, respectively. In ‘Melspring’, splitting 0.9 or 1.2 L ha\(^{-1}\) produced higher lodging, but seed yield increased (7%). This treatment had no effect on ‘Venice’ and decreased seed yield (7%) in ‘Valerio’. Split applications (GS 31–32 and 36) did not affect lodging but increased seed yield in ‘Melspring’ (13%). A triple split application (GS 29–30 + 31–32 + 39) had no additional effect on lodging or seed yield in any variety. Lodging and seed yield response with Trimaxx applied at 0.8 L ha\(^{-1}\) was comparable to 0.6 L ha\(^{-1}\) of Moddus applied at GS 31–32. These results indicate that effects of TE rate and timing vary among varieties. This work will be repeated in 2019.

Keywords: trinexapac-ethyl, lodging, seed yield, application timing, *Lolium perenne*

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EFFECT OF COMBINE SPEED ON WHITE CLOVER HARVEST EFFICIENCY

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Abstract
Direct combining is becoming the standard harvest method for white clover (Trifolium repens L.) seed production in South America. Past research done by Instituto Nacional de Investigación Agropecuaria (INIA) in Uruguay (García et al., 1991) indicates that growers underestimate the relevance of combine settings as factors that may limit seed yield. It is important to better understand harvest efficiency (HE) and to identify combine settings that have positive effects on seed yield but are independent of combine models. Optimum combine speed (CS) is one of the settings that needs more investigation. The objective of this work was to evaluate the effect of CS on white clover HE in South American seed production systems. The trial was arranged in a randomized complete block design with five combine speed treatments (1 km h⁻¹, 2 km h⁻¹, 3 km h⁻¹, 4 km h⁻¹, 5 km h⁻¹) and three replications. Each plot was 0.1 ha. The study was conducted in January 2019 in a first-year crop located in the southeastern part of Buenos Aires province, Argentina (38°23ʹ27.11ʺ S; 58°12ʹ13.54ʺ W). The harvest method was direct combining after desiccation with paraquat. The relationship between CS and HE was explained by a quadratic function (\(y = -0.0114x^2 + 0.0284x + 0.6786\)) with a high coefficient of determination (\(r^2 = 0.98\)). The economic analysis suggests that an optimum CS would be between 1 km h⁻¹ and 2 km h⁻¹ when direct combining white clover crops, with potential seed yields (machine dressed) of 800 kg ha⁻¹ or higher in South American production systems.

Keywords: white clover, harvest efficiency, combine speed, direct combining, seed yield

References

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PERENNIAL RYEGRASS SEED LOSS AT HARVEST

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Abstract
In New Zealand, perennial ryegrass (Lolium perenne L.) seed loss was measured by vacuum sampling a minimum of three quadrats, with each quadrat area measuring 0.25 m² per swath after combine harvesting, to determine the importance of harvest technique on seed loss. The vacuumed samples were cleaned by sieving, rubbing, and blowing to remove soil, straw, and light/empty seed, leaving a sample similar to a machine-dressed seed sample. In eight ryegrass seed growers’ fields where the seed crops were cut with disc mowers, the average seed loss was 650 kg ha⁻¹ (range 270–1,300 kg ha⁻¹). The average seed yield for the fields was 1,610 kg ha⁻¹ (range 790–2,500 kg ha⁻¹), giving an average loss of saleable seed available for harvest of 29% (range 18–45%). In six ryegrass seed fields cut with a windrower, the average seed yield loss was 440 kg ha⁻¹ (range 190–560 kg ha⁻¹). The average seed yield for the fields was 2,430 kg ha⁻¹ (range 1,660–3,100 kg ha⁻¹), giving an average loss of seed available for harvest of 16% (range 6–20%). The lowest loss (6%) was windrowed on a damp morning. Of the total seed lost during harvest, 49% occurred at cutting (range 32–74%), while loss during swath drying averaged 43% (range 15–75%). Losses at combine pickup averaged 11% (range 7–12%), while losses of seed not being separated from straw in the offal row averaged 5% (range 2–6%). Our observations suggest that cutting losses were highest on the crop divide. In conclusion, we have shown that seed losses at harvest can be large and that seed yield increases of 10–20% can be achieved by cutting with the correct equipment under favorable weather conditions.

Keywords: seed harvesting, perennial ryegrass, swathing, seed loss, windrowing

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Abstract

*Bromus auleticus* Trinius is a perennial grass native to South America with high forage value attributed to good palatability, spring-summer forage biomass production, digestibility, and crude protein content. This species is resistant to drought, high temperatures, and heavy grazing, which makes it a valuable pasture grass for restrictive environments. However, sowing in no-till production systems is difficult because of low seed weight and long awn length, which cause seed to clog in the hopper. The objective of this work was to develop seed-processing methods to improve establishment of *Bromus auleticus* without affecting its seed germination rate (SGR). Two seed-processing methods (single de-awning, SDA; double de-awning, DDA) and a 50 and 100% seed weight increase (50SWI and 100SWI), achieved by coating with calcium carbonate, were evaluated as methods to improve sowing success. Six treatments with six replications were carried out: (1) SDA w/o SWI, (2) SDA + 50SWI, (3) SDA + 100SWI, (4) DDA w/o SWI, (5) DDA + 50SWI, and (6) DDA + 100SWI. Seed germination rate was determined from 100 seeds following International Seed Testing Association (ISTA) protocol for *Bromus* sp., and sowing improvements were tested using a six-row no-till experimental sower with a trickle dispenser of the Chevron type and independent hoppers for each row. The processed seed was loaded into an individual hopper at a 25 kg ha⁻¹ sowing rate, and the amount of seed that passed through the sower over 1 meter was collected and weighed. Double de-awning + 50% SWI increased the final seed weight to 54.98 g m⁻¹ and significantly improved sowing capability (*P* < 0.05) without affecting germination (85%). In conclusion, sowing efficiency can be improved in *Bromus auleticus* by de-awning and increasing seed weight with coating without affecting the germination rate. These are encouraging results for future commercialization of this forage species.

**Keywords**: *Bromus auleticus*, seed processing, germination, seed coating, native forage
Abstract
Production of high-quality seeds is of fundamental importance for successful crop production. However, knowledge of the effects of increased temperature resulting from global warming on seed quality of alpine species is limited. We investigated the effect of maternal environment on seed quality of three cultivars of the leguminous forage species *Vicia sativa*, giving particular attention to temperature. Plants of ‘Lanjian 1’, ‘Lanjian 2’, and ‘Lanjian 3’ were grown at 1,700 and 3,000 m elevation. Seed weight, germination, electrical conductivity (EC) of seed-soaking leakage, and seed longevity were determined for mature seeds over 3 years. Seeds of all three cultivars produced at the 1,700 m elevation had significantly lower seed weight and seed longevity but higher EC of leachate than those produced at 3,000 m elevation, suggesting that higher air temperatures decreased seed quality. However, seed viability did not differ between elevations for any of the cultivars. Environmental effects on seed germination strongly depended on cultivar and germination temperature. At 10 and 15°C, seeds of ‘Lanjian 3’ produced at 3,000 m elevation had a higher percentage germination than seeds produced at 1,700 m. The opposite result was observed at 20°C. Elevation had no significant effect on germination of seeds from ‘Lanjian 1’ or ‘Lanjian 2’ at either temperature. Our results indicate that the best environment for the production of high-quality *V. sativa* seeds is one in which air temperatures are relatively low during seed development.

**Keywords:** maternal environmental effect, temperature, seed quality, seed longevity, seed germination
EFFECT OF SEED COAT SEMIPERMEABLE LAYER ON ELECTRICAL CONDUCTIVITY SEED VIGOR ASSESSMENT

Yanrong Wang¹*, Yanyan Lv¹, Xueqing He¹, and Xiaowen Hu¹

Abstract
A semipermeable layer (SPL) of seed coat is present in some plant families, including Poacea. The SPL restricts the exchange of solutes, while allowing movement of internal and external water and gas. Previous research indicated that this SPL does not affect seed germination. However, it is not known whether it affects seed vigor as measured by electrical conductivity (EC). Several studies were conducted to determine the presence and location of the SPL and its relation to seed vigor assessment with EC tests in seeds of 10 forage grass species: Avena sativa, Bromus inermis, Elymus dahuricus, Elymus nutans, Festuca arundinacea, Festuca sinensis, Leymus chinensis, Lolium perenne, S. bicolor × S. sudanense, and Sorghum sudanense. A lanthanum nitrate tracer and X-ray dispersive energy analysis showed that the SPL was present in 8 of 10 test species. The two species found to have no SPL were Festuca arundinacea and Avena sativa. This is the first time a member of the Poaceae family has been found to have no SPL. The SPL was located within the testa in the majority of the test species but was found in the endosperm of S. sudanense, and S. bicolor × S. sudanense. Results of these studies showed that the SPL restricts seed solutes from moving out. Therefore, the EC test does not accurately measure seed vigor among seed lots with different quality. However, we found that once the seed coat was slightly pierced, the EC result was significantly negatively related to germination and/or field emergence ($P = 0.01$); thus, it can accurately predict seed vigor in these forage grass species. Development and chemical components of the SPL, and its relation to the seed viability tetrazolium chloride test, are also discussed.

Keywords: seed vigor, forage grass, semipermeable layer, electrical conductivity test

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EFFECT OF $^{12}\text{C}^{6+}$ HEAVY ION IRRADIATION ON GERMINATION OF *Vicia unijuga* SEEDS

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Abstract

*Vicia unijuga* is a native perennial forage legume found in the Qinghai-Tibet Plateau grassland. The biological mutagenesis effect of heavy ion irradiation is strong, with high mutation frequency and easy-to-stabilize mutants. Heavy ion irradiation has received more and more attention as a new source of mutagenicity for plants, and breeding based on heavy ion radiation has become one of the important ways to develop new varieties and genetic resources. At present, there is no report on the irradiation of *V. unijuga* by heavy ions. In this study, $^{12}\text{C}^{6+}$ was used to irradiate the seeds of *V. unijuga* to study the effects of heavy ions on seed germination and seedling growth. This experiment systematically analyzed germination percentage, germination index, and seedling growth with $^{12}\text{C}^{6+}$ heavy ion irradiation under irradiation doses of 50, 100, 200, 300, 400, and 500 Gray (Gy). In general, the results showed that seed germination and seedling growth of *V. unijuga* were inhibited by $^{12}\text{C}^{6+}$ irradiation. Germination percentage and seedling biomass were significantly lower for seeds treated with 400 Gy than for those of the control ($P < 0.05$). Plumule and radical length of seedling plants was 26.45% and 62.48% of the control, respectively. Therefore, this study suggests that the 400 Gy dose is the optimal irradiation dose, thereby providing a theoretical basis for mutagenic breeding and innovation of germplasm resources in *V. unijuga*.

Keywords: *Vicia unijuga*, $^{12}\text{C}^{6+}$ heavy ion, germination percentage, germination index, seedling growth

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Abstract
Contemplating whether growers merely manage for preexisting weed problems or whether weaknesses inherent in available options generate worsening outcomes over time should improve future research planning. Sorting out the extent to which both processes occur simultaneously may help growers slow the speed at which tools for controlling weeds become obsolete. The carbon-band system for fall planting of tall fescue (*Schedonorus arundinaceus* Schreb.) and perennial ryegrass (*Lolium perenne* L.) was developed nearly half a century ago to produce high-yielding, clean grass seed crops, when the major preexisting problem was the large soil weed seed bank of Italian ryegrass (*Lolium multiflorum* L.) and annual bluegrass (*Poa annua* L.) in fields used to grow grass seed crops. Nearly all biotypes were susceptible to diuron and ethofumesate. Over time, the weaknesses of relying on small numbers of herbicides to control weeds became apparent, leading to the widespread distribution of biotypes with resistance to both of these herbicides as well as to other chemistries. Not all weaknesses in crop rotation options can be blamed on the past success and near universal adoption of systems such as carbon-band planting. For example, abandonment of open field burning in the 1990s, and the corresponding increased reliance on herbicides to control seedling weeds, was a deliberate societal trade-off between health and safety of the general public and availability of an effective, nonherbicidal method for suppressing weeds. The introduction of preemergence herbicides, such as metolachlor, dimethenamid-P, pendimethalin, and flufenacet, and early postemergence herbicides, such as oxyfluorfen and glufosinate, solved the problem of a high density of fall-emerged weed seedlings in the absence of field burning. However, it simultaneously increased the selection pressure on weeds, eventually leading to increased herbicide resistance. Complex cropping sequences imply that many growers are still searching for better rotations.

**Keywords:** cropping sequences, rotations, resistance, preemergence, postemergence
GREEN LEAF VOLATILES PRIME FOR WOUND RESPONSE IN GRASSES

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Abstract
Forage and turf grasses are routinely cut and grazed throughout their life cycle. When grasses are cut or damaged, they rapidly release a volatile chemical cocktail called green leaf volatiles (GLV). Previously, we have shown that mechanical wounding or exposure to GLV released from cut grass activated a Lt 46 kDa mitogen-activated protein kinase (MAPK) within 3 minutes and a 44 kDa MAPK within 15–20 minutes in the model grass species Lolium temulentum L. (Lt, Darnel ryegrass). Currently, very little is known concerning the perception, signaling, or molecular responses associated with wound stress in grasses. Since GLV are released during wounding, we investigated what genes and signaling pathways are induced in undamaged plants exposed to GLV. RNA-Seq generated transcriptome of Lolium plants exposed to GLV identified 4,308 up- and 2,794 distinct down-regulated differentially expressed sequences (DES). Gene ontology analysis revealed a strong emphasis on signaling, response to stimulus, and stress-related categories. Transcription factors and kinases comprise over 13% of the total DES found in the up-regulated dataset. The analysis showed a strong response within the first hour of GLV exposure, with over 60% of the up-regulated DES being induced. Specifically, sequences annotated for enzymes involved in the biosynthesis of jasmonic acid and other plant hormones, MAPKs, and WRKY transcription factors were identified. Interestingly, 11 DES for ferric reductase oxidase, an enzyme involved in iron uptake and transport, were exclusively found in the down-regulated dataset. Twelve DES of interest were selected for quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis; all displayed rapid induction 1 hour after GLV exposure and were strongly induced by mechanical wounding. The information gained from this analysis and previous studies suggests that GLV released from cut grasses transiently primes an undamaged plant’s wound stress pathways for potential oncoming damage and may play a role in both inter- and intraplant signaling.

Keywords: green leaf volatiles, kinases, Lolium temulentum, RNA-sequencing, transcription factors, wounding

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A METHOD TO SELECT FOR ROOT CHELATION ACTIVITY IN ANNUAL RYEGRASS

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Abstract
Acidic soils reduce plant nutrient availability and increase uptake of cations such as aluminum (Al³⁺) at toxic concentrations. Acidic soils can occur in areas where grasses are grown for seed, forage, or cover crops. Acid-tolerant grass species produce root exudates with higher concentrations of chemicals that chelate Al³⁺ than do acid-sensitive grass species. The objective of this research was to develop a high-throughput assay to measure root exudate chelation capacity (CC) in order to facilitate breeding grasses tolerant to acidic soils. The color indicator dye chrome azurol S (CAS) is bound to metal ions, and the loss of these ions due to chelation by a test chemical causes decreased absorption at 595 nm. Chelation capacity is calculated as absorbance of test samples divided by absorbance of a rooting solution. An optimized assay using the copper form of CAS with 2.0 mM CaCl₂ in 10 mM MES buffer (pH under 5.5), with or without addition of Al³⁺ as AlCl₃ (30 µm), gave rapid and consistent results. Seeds of the annual ryegrass cultivar ‘Gulf’ were germinated in 48 tissue cluster wells (1 seed/well) with 300 µl rooting solution. Rooting solutions sampled from the wells with germinating seedlings were tested for chelation capacity in a plate reader, with results ranging from 0.3 to 1. Seedlings were grown into three-tiller plants and retested for CC. Measurements were correlated to the seedling assay \( r = 0.43, P = 0.04 \). Plants were divided into low (CC > 0.78) and high (CC < 0.5) chelation groups of nine plants each and allowed to intermate. Mean CC of progeny seedlings from low (0.93) and high (0.78) groups was significantly different \( t = 2.6, P < 0.01 \). These results indicate that this colorimetric assay is valuable for determining ryegrass root exudate chelating activities and that CC appears to be a heritable trait.

Keywords: fragipans, abiotic stress, breeding, acidic soils, selection

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GENETIC DIVERSITY OF *PUCCINIA GRAMINIS* F. SP. *LOLII* FROM THE WILLAMETTE VALLEY

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Abstract
Stem rust disease caused by *Puccinia graminis* f. sp. *lolii* (*Pgl*) can cause complete crop failure in perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Schedonorus arundinaceus* Schreb.) grown for seed. Genetically diverse pathogens have a greater chance of overcoming control measures and may require multitactic disease control approaches. The objective of this study was to develop simple sequence repeat (SSR) markers that can be used to detect *Pgl* genetic diversity in the Pacific Northwest. Bulk urediniospores were collected from each grass species from locations near Corvallis, Oregon, and DNA was extracted. Eighty-five SSR markers previously used to study stem rust on wheat (*Puccinia graminis* f. sp. *tritici* (*Pgt*)) were tested for amplification of *Pgl* DNA by thermal gradient polymerase chain reaction (PCR) and agarose gel electrophoresis. It was found that *Pgt* primers amplified products in *Pgl* and are therefore useful to study *Pgl* diversity. A library of 46 SSR primers that amplified *Pgl* DNA has been created, and 11 of these generate SSR amplicons in only tall fescue *Pgl*. Additionally, banding pattern differences observed between the tall fescue bulk and the perennial ryegrass bulk indicate genetic differences. Two hundred, twenty-four single infection pustules from six fields throughout the Willamette Valley were collected. In future research, amplifications with select primers will be carried out as a multiplex using these samples.

Keywords: stem rust, *Puccinia graminis*, microsatellite, SSR, disease

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DEVELOPMENT OF 3-D TOPOMETRIC IMAGING FOR HIGH-THROUGHPUT PHENOTYPING IN PERENNIAL RYEGRASS

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Abstract
Advances in topometric scanning and imaging technologies have enabled 3-dimensional (3-D) modeling of the physical world. Is it possible to use this technology to provide rapid and repeatable estimates of crop phenotypic characteristics under field conditions? Our objective was to develop 3-D topometric imaging methods for high-throughput phenotyping (HTP) in perennial ryegrass (Lolium perenne L.). A portable hand-held scanner (Artec 3D Spider) was used to capture morphological characteristics of the spike, including spike length and architecture, number of spikelets per spike, distance between spikelets along the rachis, angle of spikelet attachment to the rachis, and spikelet size. Representative spikes from 40 diverse global accessions of perennial ryegrass were scanned at intervals from emergence of the spike to seed maturity. The scanner uses blue LED as the light source, and, as it has 3-D resolution to 0.1 mm, many fine details of the spike were captured by the device. Unlike a photograph, the 3-D image of the spike can be rotated to reveal all sides. The work to date indicates that multiple measurements can be made in the field at sequential stages of development, and the scanner has the ability to capture a 3-D representation of the spike and store that data for subsequent analysis. The experimental material taxed the resolution threshold for the scanner, but it was able to record the diversity of characteristics and architecture of spike morphology observed among accessions. The topometric data will be used to map structural changes throughout development of the spike and key differences among accessions. Our investigation suggests that the topometric scanner has promise as a HTP tool, potentially replacing slow and laborious human-based data collection in studies of the inflorescence in grasses.

Keywords: topometric scanning, digital agriculture, high-throughput phenotyping, perennial ryegrass, spike morphology

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SUSCEPTIBILITY OF GRASS CULTIVARS TO BARLEY YELLOW DWARF VIRUSES

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Abstract
Most cool-season grass seed in the United States is grown in the Willamette Valley, Oregon. In recent years, reduced stand performance has been observed in grass seed production fields. Many of these grasses are susceptible to Barley yellow dwarf viruses (BYDVs), which can cause reddening or yellowing of leaf tips, stunting, reduced root mass, and yield losses, although plants often are asymptomatic. The BYDVs are considered one of the most widespread and economically damaging virus diseases of major cereal crops. Previously, BYDVs have been reported to cause poor stand establishment, reduced competitiveness, and reduced productivity in perennial ryegrass (\textit{Lolium perenne} L.) pastures. Recently, surveys of Willamette Valley seed production fields were performed to determine whether viruses might be contributing to stand decline. Three strains of BYDVs were found in perennial ryegrass fields. BYDV-PAV and Cereal yellow dwarf virus (CYDV)-RPV were reported in fescue (\textit{Festuca} sp.) fields, and CYDV-RPV was found in orchardgrass (\textit{Dactylis glomerata} L.) fields. Cultural practices, such as altering planting times to avoid aphids during early growth stages, applying systemic insecticides at planting, and applying foliar sprays to control aphids during peak flight, may reduce the impact of BYDVs. However, insecticides are costly, environmentally unfriendly, and sometimes ineffective. The best long-term solution to disease management is arguably through host genetic resistance or tolerance to BYDVs or their vectors. Twenty-five varieties each of tall fescue (\textit{Festuca arundinacea} Schreb.), fine fescue (\textit{Festuca} sp.), perennial ryegrass, and orchardgrass are being evaluated for resistance or reduced susceptibility to these viruses. Identification of cultivars with resistance or reduced susceptibility to these viruses would be valuable for increasing stand longevity during seed production and would also decrease potential virus reservoirs in grasses where major food crops are grown.

Keywords: Barley yellow dwarf virus, orchardgrass, perennial ryegrass, fescue

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CHOKE SPREAD AND EVALUATION OF ORCHARDGRASS GERMPLASM FOR CHOKE RESISTANCE

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Abstract
Choke in orchardgrass (Dactylis glomerata L.), caused by Epichloë typhina (Pers.) Tul & C. Tul, was first reported in western Oregon in 1997. The pathogen quickly spread within and between fields, and, by 2003, 90% of tested fields were infected. Reported yield losses in orchardgrass seed production fields were as great as 30%. Epichloë typhina infects orchardgrass systemically, with a low-density of hyphae found in leaves and stems. Infected plants remain asymptomatic during most of the year. As reproductive tillers elongate, and just prior to seed head emergence, a rapid, dense growth of the fungus within and among leaf sheaths effectively blocks the emergence of seed heads. In most grasses, E. typhina typically infects host ovaries during flowering, resulting in infected seed and subsequent infected plants. However, in orchardgrass, E. typhina has not been found in, or transmitted through, seed, and very little is known about how infections occur in the field. Currently, there is no effective management for choke in orchardgrass seed production fields. The best long-term solution to disease management includes host genetic resistance. Using a seminatural inoculation method to look at Epichloë transmission in orchardgrass, we showed that infection can occur in young plants. Furthermore, disease spread does not seem to rely on wounding during harvest, although wounding might improve transmission. Artificially inoculated plants were included to detect tolerant cultivars, which produce fewer or no stromata upon infection. Recent cultivar and germplasm trials identified lines and plants that appear to demonstrate potential resistance to the disease. Currently, we are screening 27 orchardgrass lines in a multiyear field trial to identify cultivars resistant to infection under field conditions.

Keywords: choke, Epichloë typhina, germplasm, orchardgrass

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IDENTIFICATION OF HSP70 FAMILY STRESS RESPONSE GENES IN CLEISTOGENES SONGORICA

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Abstract

Cleistogenes songorica is a native perennial grass of the desert grasslands of northwest China, as well as Kazakhstan, Kyrgyzstan, Russia, Turkmenistan, and Uzbekistan. It is tolerant of large temperature fluctuations ranging from –40 to 41°C. Therefore, C. songorica could play an important role in vegetation restoration of desert ecosystems. C. songorica is an allotetraploid grass with a karyotype of 2n = 4x = 40. Members of the heat shock protein 70 (HSP70) family function as molecular chaperones to maintain cellular homeostasis and help plants cope with environmental stress. To identify C. songorica HSP70 genes, the C. songorica genome database was analyzed and 25 HSP70 genes were identified. C. songorica HSP70 genes can be grouped into five subclasses based on comparisons to HSP70 families in Arabidopsis thaliana, Brachypodium distachyon, Oropetium thomaeum, and Oryza sativa. In this study, we used Illumina-based RNA-seq to obtain the root (R) and shoot (S) transcriptomes of young C. songorica plants subjected to high temperature stress (HT) and low temperature stress (LT) treatments. HSP70 family members showed not only similar but also distinct transcriptions when treated by different stresses. A total of 10, 13, 8, and 8 C. songorica family genes showed significant up-regulation in S-HT, S-LT, R-HT, and R-LT. Among them, CsHSP70-5, CsHSP70-18, and CsHSP70-24 showed the most significant expression change at the transcriptional level in different tissues and stresses. The information presented in this study provides a detailed understanding of the HSP70 protein family of C. songorica.

Keywords: Cleistogenes songorica, HSP70, gene family, stress response, transcription factor

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