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# SEED PRODUCTION RESEARCH

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An Industry Survey of Current Practices, Problems, and Research Priorities in Western Oregon Grass and Clover Seed Cropping Systems
Annual Carbon Balance of a Second-year Tall Fescue Seed Crop
Effects of Straw Removal, Clay Content, and Stand Age on Soil Health in Tall Fescue Seed Production: Final Report
Genetic Variation for Seed Retention in Accessions and Genotypic Lines of Perennial Ryegrass
Spring Mowing and Plant Growth Regulator Effects on First- and Second-year Fine Fescue Seed Crops 21
Effects of Trinexapac-ethyl on Kentucky Bluegrass in the Grande Ronde Valley of Northeastern Oregon (Year 3)
Mobility of the Gray Field Slug
Understanding Billbug Species Complex in Grass Seed Production Systems in Western Oregon
Cutworm and Armyworm Population Dynamics and Investigation of Parasitism in Kentucky Bluegrass Production
Choke Expression in <i>Epichloë typhina</i> Seedling-infected Orchardgrass ( <i>Dactylis glomerata</i> )  Germplasm
Ergot Response of Early-, Middle-, and Late-flowering Kentucky Bluegrass Cultivars in Central and Eastern Oregon
Evaluation of Fungicides for Control of Powdery Mildew on Kentucky Bluegrass in Central Oregon (2020)
Biochar: An Alternative to Activated Carbon in Carbon Seeding for the Establishment of Perennial Ryegrass
Auxin Herbicide Suppression of Italian Ryegrass Seed Viability in Tall Fescue Seed Production
Summer Annual Grass Weed Control During Establishment of Kentucky Bluegrass Grown for Seed 58
Sulfosulfuron for Control of Roughstalk Bluegrass During Kentucky Bluegrass Stand Establishment 60
Evaluation of Experimental Herbicides in Seedling Red and White Clover Grown for Seed
Seed Yield Performance and Flowering Initiation of Twelve Red Clover Varieties (Year 2)
Preemptive Measures to Manage the Red Clover Casebearer Moth in Oregon Clover Seed Crops70
Laboratory Bioassays for Screening Bifenthrin Resistance in White Clover Seed Weevil (Year 2)74

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# AN INDUSTRY SURVEY OF CURRENT PRACTICES, PROBLEMS, AND RESEARCH PRIORITIES IN WESTERN OREGON GRASS AND CLOVER SEED CROPPING SYSTEMS

E.C. Verhoeven and N.P. Anderson

#### Introduction

A series of virtual webinars focused on grass and clover seed cropping systems was conducted in fall 2020 and winter 2021 by western Oregon OSU Extension personnel. Industry attendees at the webinars were surveyed using electronic polling tools to capture the current status of agronomic issues in western Oregon seed production systems. The data collected from the surveys provide valuable information that OSU Extension and research faculty can use to prioritize outreach and education programs and provide guidance as future research programs are developed and implemented.

#### Methods

A total of six virtual webinars were given, three in September 2020 and three in January 2021. Surveying was done through electronic polls following speaker presentations on a variety of production topics. In most cases, two survey polls were conducted during a 1.5-hour webinar. For each survey question, a "Not applicable" or "I do not grow or advise on this crop" response option was included, and those respondents were not included in the final results. The number of respondents for which the questions were applicable is indicated for each question (n) in the summary tables. Survey respondents included both growers and industry representatives who grow or make agronomic recommendations for grass and clover seed crops in the Willamette Valley (Linn, Lane, Benton, Marion, Polk, Yamhill, Washington, and Clackamas counties).

Survey questions and responses were grouped into five categories of issues and priorities: vole damage, liming and soil fertility, grass seed weed management, grass seed insect and slug management, and clover seed production. In some cases, survey responses collected from multiple webinars have been combined. For example, results from liming and soil fertility surveys conducted in fall 2020 and winter 2021, respectively, were combined. Likewise, results for anticipated use of indaziflam (Alion) herbicide (fall 2020 poll) and actual use in grass seed production (winter 2021 poll) were also combined. Individual participants in fall and winter webinars may have differed. Therefore, results should be interpreted as a broad reflection of industry trends and experience.

#### **Results and Discussion**

The multiple survey efforts generated useful information from growers and field representatives in western Oregon (Tables 1–5). Key findings from each group of issues/priorities include:

# Vole damage (Table 1)

- At least some level of vole damage was reported on 85% of acres, with 40% of respondents reporting estimated grass seed yield losses of 200–500 lb/acre.
- 60% of vole-infested acreage was treated with two or more applications of zinc phosphide.
- Overall, only 26% of respondents report satisfactory control of voles with zinc phosphide.
- The top research priority identified was to identify new substrates to improve zinc phosphide baits.

# Liming and soil fertility (Table 2)

- Most respondents indicated they apply lime every 2–3 years (44%) or every 4+ years (40%).
- More than 80% of respondents use the buffer pH test to adjust lime recommendations.
- Nitrogen (N) is typically applied twice in the spring to grass seed fields with growing degree day (GDD) accumulation most commonly used to guide application timing (45%), followed by calendar date (25%).
- The top research priority identified was to develop tools to optimize timing and N rate applications.

# Grass seed weed management (Table 3)

- More than 80% of respondents were satisfied with the efficacy and crop safety of new pyroxasulfone-containing products (Zidua and Fierce).
- Italian ryegrass was reported as the most difficult-tocontrol grass weed species.
- There is strong support for a quick and affordable leaf tissue test for herbicide resistance that could help guide more appropriate herbicide sprays.
- 63% of respondents suspect they have herbicideresistant weed populations on at least 100 acres of land they manage, 18% suspect they have herbicide resistance on more than 1,000 acres they manage.

- (Results include commercial agronomists and individual growers.)
- In September 2020, 88% of respondents planned to use indaziflam (Alion). The following January, 59% of respondents reported having used indaziflam (Alion), with the majority being applied to established tall fescue.
- The majority of people using Alion were satisfied with its performance, while 23% reported some signs of crop injury in perennial ryegrass or tall fescue.

# Grass seed insect and slug management (Table 4)

- The majority of respondents listed worms (armyworms, cutworms, sod webworms) as their top insect pest (63%). Symphylans were listed second (23%).
- Respondents would most like to see insecticide efficacy trials over cultural practices, biocontrols, or host plant resistance.

• Slugs were ranked among the top three pests by most respondents, with 68% reporting that they apply slug bait in the spring.

# Clover seed production (Table 5)

- There was strong support for pursuing a label for saflufenacil (Sharpen) as both a desiccant and an herbicide.
- 39% of respondents reported having small broomrape in the fields they grow or scout, and 84% of respondents would like to see renewed research on small broomrape by OSU.
- Nearly 90% of respondents felt it was important that research also be conducted in some of the more minor clover species (berseem, balansa, arrowleaf, Persian).
- Work on alternatives to chlorpyrifos (Lorsban) should focus on clover seed weevils next.

Table 1. Vole damage and research priorities.

Approximately how many grass seed acres on your farm were damaged by voles this year? (n = 89)

Zero	15%
1-200	37%
201-500	20%
501-1,000	13%
> 1,000	15%

Of your vole-damaged acres, how much yield loss (lb/acre) do you estimate as a result of vole damage? (n = 106)

Zero	8%
1-200	31%
201-500	40%
501-1,000	15%
> 1,000	6%

In fields where activity occurred, on average how many above-ground zinc phosphide applications did you make per grass field between April 30 and September 15, 2020 (n = 113)

Zero	11%
One	30%
Two	49%
Three or more	11%

How satisfied were you with the level of vole control following your zinc phosphide application(s)? (n = 111)

Not at all	9%
Somewhat satisfied	66%
Satisfied	21%
Very satisfied	5%

Of the following researchable topics, which one would you consider your highest priority? (n = 147)

The weatherability of zinc phosphide baits	28%
New substrates to create improved zinc phosphide baits for grass seed crops	41%
Baiting strategies and application methods to improve efficacy of zinc phosphide	32%
Efficacy of other active ingredients (NOT ZP) for possible use in grass seed crops	32%

Table 2. Liming and soil fertility issues and research priorities.

How often do you apply lime to $(n = 135)$	o most of your fields?	What is your main guide for timing your grass seed stands? (n = 179)	of N applications in
Every year Every 2–3 years	10% 44%	Growing degree day accumulation	45%
Every 4+ years	40%	Calendar date	25%
I don't apply lime	5%	Precipitation	8%
Do you use buffer pH to decid	e how much lime to	Appearance of field	5%
apply? $(n = 135)$		Ability to access field	17%
Yes No	83% 17%	What soil fertility research are you $(n = 194)$	most interested in?
How effective do you think SM for estimating the lime require experience? $(n = 135)$		Efficacy and comparisons of new fertilizer products (e.g., coatings, enhanced-efficiency products, etc.)	25%
Very effective	25%	Exploration of biostimulant	13%
Somewhat effective	70%	fertility products	
Not effective	4%	Tools to optimize timing and	29%
How many spring nitrogen applications do you typically make in your grass seed stands? $(n = 177)$		rate of nitrogen applications (e.g., precision ag/image technology, growth models, Nmin soil test)	
One Two	28% 62%	Research on nitrogen leaching	14%
Three	10%	and volatilization of practices	1.70
Greater than three	0%	and/or products	
		Research on micronutrient needs and crop response	19%

Table 3. Grass seed weed management issues and research priorities.

Were you satisfied with the performance and crop safety of the pyroxasulfone products (Fierce and Zidua) in your grass seed crops in 2019–2020? (n = 115)

Yes	86%
No	14%

Which is the most difficult grass weed species to control in grasses grown for seed on your farm or the acres you manage? (n = 140)

Annual bluegrass	29%
Annual ryegrass	44%
Roughstalk bluegrass	20%
Rattail fescue	6%

How many full straw load acres do you manage in an average year? (n = 151)

< 20	38%
20-50	9%
50-100	10%
100-500	25%
500-1,000	9%
> 1,000	11%

If OSU offered a quick and affordable leaf tissue test (< 3-day turn around) for herbicide resistance, would you consider using this to aid herbicide spray decisions? (n = 185)

Yes	97%
No	3%

How many acres that you farm or manage do you suspect have herbicide-resistant weed populations (e.g., ryegrass, annual bluegrass, roughstalk bluegrass, others)? (n = 168)

< 20	13%
20-50	10%
50-100	14%
100-500	28%
500-1,000	17%
> 1,000	18%

With the new indaziflam (Alion) label, do you plan to utilize this product in your grass seed weed management program? (n = 128)

Yes	88%
No	12%

Did you use (or recommend) Alion this year and, if so, on what grass seed species? (check all that apply) (n = 185)

Carbon-seeded tall fescue	7%
Established tall fescue	39%
Carbon-seeded perennial	4%
ryegrass	
Established perennial ryegrass	9%
I did not use (or recommend)	41%
Alion this year	

How satisfied are you with the grass weed control following your Alion applications? (n = 86)

Very satisfied	17%
Satisfied	56%
Neutral (equivalent to other	21%
herbicides used in the past)	
Unsatisfied	6%

If you used Alion this year, have you noticed signs of crop injury? (check all that apply) (n = 109)

120/

%
%
2%
5%

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Table 4. Grass seed insect and slug issues and research priorities.

What is the most challenging insect pest issue for your grass seed production systems? (n = 184)

Aphids	5%
Billbugs	6%
Worms (army and cutworms,	63%
sod webworm)	
Symphylans	23%
Other	3%

What research projects do you want to see more of for addressing insect pest issues in grass seed crops? (n = 193)

Insecticide efficacy trials	46%
Cultural control methods	24%
(e.g., role of fertility or	
residue management)	22%
Exploring biological control	
options	
Investigating host plant	
resistance	8%

Do you spring bait for slugs? (n = 118)

Yes	68%
No	32%

How would you rank slugs as pests? (n = 145)

Worst	3%
Top 3	74%
Top 10	19%
Not in top 10	3%

Table 5. Clover seed issues and research priorities.

Do you think OSU and the clover seed industry should pursue a label for Sharpen (saflufenacil) as a herbicide and/or desiccant? (n = 148)

Yes, herbicide only	11%
Yes, desiccant only	1%
Yes, both herbicide and	86%
desiccant	
No	1%

In the past 10 years, have you had small broomrape (orabanche) in the clover seed field(s) that you grow or scout? (n = 110)

Yes	39%
No	61%

Do you think OSU should renew research efforts to improve understanding and management of small broomrape (orabanche)? (n = 122)

Yes	84%
No	16%

There are some minor clover species that have been increasing in acreage in Oregon (berseem, balansa, arrowleaf, Persian). Is it important for OSU/clover seed industry to conduct agronomic/weed management work on these species? (n = 140)

Yes	87%
No	13%

Lorsban is going away. OSU work in 2020 focused on Lorsban alternatives for aphid control. What is the next most important clover seed insect pest that should be focused on for 2021 research work? (n = 143)

Lygus bugs	4%
Clover seed/leaf weevils	41%
Omnivorous leaftier	3%
Clover casebearer	21%
Clover crown borer	27%
Other	3%

Are you concerned about the red clover casebearer as an emerging insect pest in Oregon red clover? (n = 135)

Yes	53%
Sort of	44%
No	3%

# ANNUAL CARBON BALANCE OF A SECOND-YEAR TALL FESCUE SEED CROP

C.L. Phillips, K.M. Trippe, H. Kwon, B.A. Murphy, W. Creason, C.V. Hanson, and A. Schmidt

#### Introduction

Replacing annual with perennial species is widely recommended as a best practice to increase and maintain soil organic matter in crop and pasture systems. However, there are few data that describe rates of soil carbon accumulation under perennial grass seed production systems (Griffith et al., 2011). Changes in soil organic carbon are slow and can be difficult to detect following changes in cultural practices, in part because multiple factors can have opposing influences on soil organic carbon. Soil organic carbon change is determined by the balance between carbon taken up through photosynthesis and the carbon removed with harvest and lost through plant respiration, soil microbial respiration, and soil erosion. Routine measurements of plant biomass accumulation can describe the amount of carbon taken into a plant-soil system, but additional measurements are needed to fully describe the fluxes of carbon lost and to determine whether a plant-soil system is a net carbon sink or source.

Although it can take years of monitoring soil carbon changes to ascertain whether a plant-soil system is a carbon source or sink, carbon balance can be determined for a single year using the eddy covariance technique. This technique measures the exchange rate of CO<sub>2</sub> between the atmosphere and a plant canopy by means of wind speed and gas sensors affixed to a tower downwind of the study area. The covariance between fluctuations in vertical wind velocity and CO<sub>2</sub> concentration are measured to determine net fluxes of CO<sub>2</sub> entering and leaving the plant canopy. This technique provides nearly continuous measurements of photosynthesis and ecosystem respiration at a field scale. When combined with independent estimates of harvest carbon removal and soil erosional loss, eddy covariance measurements can be used to develop a complete, field-scale annual carbon budget.

In 2015, the eddy covariance technique was used to determine annual CO<sub>2</sub> exchanges for a 2-year-old tall fescue field as part of a regional study on the potential greenhouse gas impacts of bioenergy crops in Oregon (Schmidt et al., 2018). Here we report eddy covariance and ancillary data to evaluate the carbon budget of this second-year tall fescue field.

#### **Materials and Methods**

A 28-acre field in Marion County, OR, was planted with 'Chipotle' turf-type tall fescue in April 2013. Fall nitrogen (N) was applied at 40 lb N/acre and spring nitrogen at 160 lb N/acre. Trinexapac-ethyl plant growth regulator (Palisade) was applied at a rate of 2 pt/acre at the two-node growth stage. Rodenticide, slug bait, herbicide, and fungicide were also applied as needed. The crop was swathed June 25, 2015, and seed and straw were harvested on July 6 and 9, respectively.

Exchanges of CO<sub>2</sub> were measured with the eddy covariance method during the second harvest year, from January through December 2015. Primary instruments included a three-dimensional sonic anemometer (model CSAT3, Campbell Scientific Inc., Logan, UT) and a closed-path infrared gas analyzer (model LI-7200, LI-COR Inc., Lincoln, NE), both measuring at 8.2 feet above ground level. Ancillary meteorological measurements included air temperature and humidity, total solar radiation, and photosynthetically active radiation, ambient air pressure, and volumetric soil water content.

High-frequency (10 Hz) eddy covariance measurements were processed to 30-minute average fluxes following the procedure described by Thomas et al. (2009). Approximately 44% of the initial 30-minute flux data was removed because of limited turbulence development, a wind direction originating outside the tall fescue field, or unusually high CO2 values reflective of vehicle emissions. After removing these data, the flux data were gap-filled using linear interpolation when data gaps were less than 2 hours. For gaps longer than 2 hours, a light-response model and temperatureresponse model were applied separately to the daytime and the nighttime data. Two longer data gaps occurred from May 5 to May 27 (due to power system failure) and from June 28 to July 6 (when equipment was removed for harvest). No meteorological data were available to model these gaps. Therefore, daily-average fluxes were estimated by linear interpolation for the May gap and by extrapolation of postharvest fluxes for the June-July gap.

Net CO<sub>2</sub> fluxes were also partitioned into estimated fluxes for photosynthesis and ecosystem respiration.

These calculations are based on the fact that no photosynthesis occurs at night; nighttime fluxes result from respiration alone. Daytime respiration was estimated by first computing regressions between nighttime fluxes and air temperature, which is the main environmental driver of respiration, and subsequently applying the regression coefficients to daytime air temperatures to estimate daytime respiration. Photosynthesis was computed as the difference between net  $CO_2$  flux and daytime respiration. Uncertainty for annual net  $CO_2$  exchange was estimated as  $\pm$  18%, based on a synthesis of error analysis across the AmeriFlux network (Schmidt et al., 2012).

The amount of carbon exported with harvest was computed from grower-reported seed yield and tons of grass straw removed. A carbon content of 42% was assumed for both straw and seed based on typical values from previous studies (T. Chastain, personal communication). Carbon losses due to soil erosion were not measured, but, based on previous studies showing very low erosion rates for second-year tall fescue (Steiner et al., 2006), these losses were expected to be small

To provide environmental context, long-term average annual precipitation and temperature for the site were retrieved from the Oregon State University PRISM Climate group. Long-term average production statistics for Marion County were also retrieved from Oregon State University Extension.

#### **Results and Discussion**

The tall fescue field had net daily gains of  $\mathrm{CO}_2$  (indicated as a positive flux value, Figure 1A) during the spring growing season and net daily  $\mathrm{CO}_2$  emissions during much of the rest of the year. The plant-soil system began the 2015 calendar year losing  $\mathrm{CO}_2$  to the atmosphere, until daily fluxes began to exceed parity (i.e., photosynthesis exceeded respiration) in mid-February.  $\mathrm{CO}_2$  uptake increased until mid-May and returned to parity by the time the crop was swathed in late June. Following harvest, the field lost  $\mathrm{CO}_2$  on most days. Partitioned fluxes indicate that respiration was relatively consistent throughout the year, and therefore seasonal patterns in photosynthesis dominated  $\mathrm{CO}_2$  exchange rates over the year (Figures 1B and 1C).

High rates of spring CO<sub>2</sub> uptake more than offset emissions during the rest of the year; therefore, the field had a net positive carbon uptake of 1.4 tons C/acre/year (3.2 megagrams (Mg) C/ha/year) when fluxes were

summed for the whole year (Figure 2). Individual fluxes for photosynthesis and ecosystem respiration were estimated to be 8.2 and -6.8 tons C/acre/year (18.4 and -15.3 Mg C/ha/year), respectively.

Grower-reported seed yield for 2015 was 0.9 ton/acre (2.0 Mg/ha), which is within the 15-year range reported for the county but was approximately 35% higher than the county average for 2015 (Figure 3). Unusually warm temperatures in 2015 reduced yields for many of the region's producers, as demonstrated by long-term climate and yield data (Figure 3).

The grower reported 2 tons/acre (4.4 Mg/ha) of straw biomass harvested. Assuming a 42% carbon content in harvested seed and straw provided an estimated carbon removal of 1.25 ton/C/acre (2.8 Mg C/ha, Figure 2). Subtracting harvested carbon from net annual  $CO_2$  exchange provided an estimated carbon balance of 0.19 ton C/acre/year (0.4 Mg C/ha/year, Figure 2). Assuming uncertainties of  $\pm$  18% for net annual  $CO_2$  exchange and  $\pm$  20% for harvested biomass provided an uncertainty range of  $\pm$  0.35 ton C/acre/year (0.8 Mg C/ha/year) for the annual carbon balance. The field was therefore estimated to be carbon neutral to a small

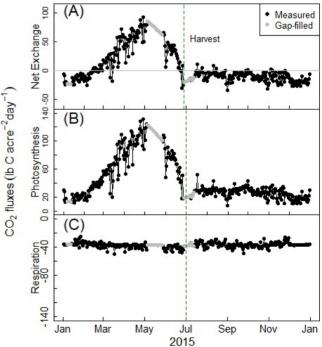


Figure 1. Time series for (A) daily net CO<sub>2</sub> exchange and partitioned estimates for (B) daily photosynthesis and (C) daily ecosystem respiration. Black points show direct measurements, and gray points show fluxes estimated by gap-filling procedures.

carbon sink. Any incremental carbon gain likely took the form of additional root or shoot biomass, as tall fescue plants increase in size over the first several years of stand establishment (Steiner et al., 2006).

The annual carbon balance of tall fescue and other perennial grass stands can be expected to increase with stand age over the first several vears following establishment. This is expected because soil tillage in the establishment year causes microbial respiration of soil carbon and because root and shoot biomass growth over time reduces soil erosion (Steiner et al., 2006). These factors should contribute to lower carbon losses and greater carbon uptake through time. The eddy covariance measurements presented here showed that a tall fescue stand had reached parity between establishment-related emissions and plant CO<sub>2</sub> uptake by the second harvest year. The magnitude of ecosystem respiration that occurs with stand replacement and seedbed preparation has not been examined for perennial grass seed systems, to our knowledge. Several years of plant growth may be needed to compensate for the emissions occurring during the establishment year, and additional research would be needed to assess the carbon balance of a complete 4- to 5-year tall fescue rotation.

In conclusion, a 2-year-old tall fescue stand with straw removal was shown to be carbon neutral or a small carbon sink in 2015. However, the present study did not evaluate carbon balance over a full tall fescue rotation, and it should be stressed that carbon accrued during the rotation may be lost during stand replacement. This study examined only a single tall fescue field for 1 year and did not assess the impacts of management (e.g., postharvest residue management or tillage method) on carbon accrual. However, this study did

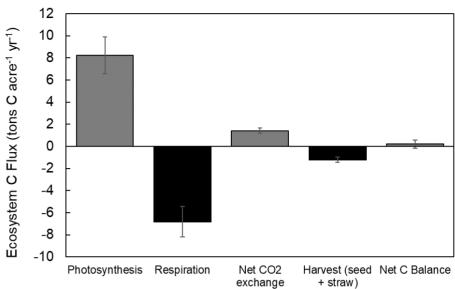


Figure 2. Fluxes contributing to annual carbon (C) balance for a second-year tall fescue stand in Marion County, OR.

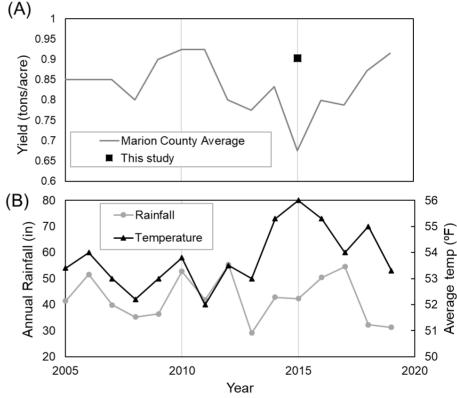


Figure 3. (A) Average grower-reported seed yield in Marion County, OR, compiled by Oregon State University Extension (solid line) and grower-reported seed yield at the study site in 2015 (closed square). (B) Annual cumulative rainfall and average air temperature at the study site.

show that baling straw did not lead to carbon losses for a second-year stand. The near-neutral carbon balance shown here and the lack of change in soil organic carbon shown over time by previous research (Griffith et al., 2011) suggest that tall fescue grown for seed may have a low carbon footprint. This information is useful for establishing the climate and soil health impacts of tall fescue seed production.

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# EFFECTS OF STRAW REMOVAL, CLAY CONTENT, AND STAND AGE ON SOIL HEALTH IN TALL FESCUE SEED PRODUCTION: FINAL REPORT

E.C. Verhoeven, M. Gonzalez-Mateu, A.D. Moore, and D.M. Sullivan

#### Introduction

This report describes our final findings from a soil health project and expands on the dataset first presented in the 2019 Seed Production Research Report (Verhoeven et al., 2020). The aim of this work was to determine whether straw management and stand age affect soil health outcomes in tall fescue stands. In addition, we looked at the effect of soil clay content on measures of soil health.

Soil health describes a soil's ability to maintain productive yields and provide ecosystem services such as reduced nutrient leaching, improved water retention, and nutrient cycling. Generally, there is consensus among the scientific community that soil health test packages should include measurements of the physical, chemical, and biologic status of a soil. For example, measurements of penetration resistance, water-holding capacity, and aggregate stability are commonly used to assess the physical condition of a soil, while measurements of pH, macronutrients, and micronutrients are used to assess chemical properties. Measurements of respiration (CO<sub>2</sub> burst test), organic matter (OM), active carbon (C), soil protein, and potentially mineralizable nitrogen (N) can be used to evaluate the biological status of a soil.

Management practices, such as the frequency and intensity of tillage, crop residue management, rotation sequence, and cover cropping have been shown to influence soil health properties (Awale et al., 2017). However, it is important to remember that many soil health measurements are also affected by inherent site properties that cannot be changed, such as soil clay content, landscape position, and climate. To evaluate soils across soil types and textures, large datasets are needed to establish expected ranges for the different measures and to aid in interpretation of soil health measurements for our regionally important soils and cropping systems. This work is a first step toward establishing such a dataset for our Willamette Valley grass seed systems.

Maintaining soil OM levels is generally considered critical for preserving soil health and function. To maintain or increase soil OM, the inputs of OM into the crop system must be equal to or greater than the

losses of OM in that system. The main practices that reduce OM losses are those that reduce soil erosion and those that reduce the intensity and/or frequency of tillage (Sullivan et al., 2019). Organic matter inputs may originate from a variety of sources, such as manure or compost amendments, crop residues, and increased crop biomass from greater above-ground growth or intercropping.

Returning postharvest residues to the field is one method of achieving higher OM inputs to a system. With the phaseout of field burning, most tall fescue seed crop growers have had success with baling and removing straw after harvest. Removing straw can increase the efficacy of soil-active preemergent herbicides, potentially reduce slug and vole damage, and generate immediate farm income from straw sales. The straw is a relatively low-quality organic matter, but it does contain around 100 lb K/acre and on average around 2,175 lb C/acre (5,000 lb/acre biomass x 43.5% C) (Hart et al., 2012). Growers are aware of the need to replace K with potash fertilizer, but the effects of removing C and OM on overall soil health properties are less known. Additional research within western Oregon grass seed production systems is needed to provide producers with reliable information regarding the effects of long-term straw removal on soil health in perennial grass seed production systems and to better understand the effects of soil clay content on measures of soil health

The objectives of this study were:

- To evaluate soil health measurements under bale versus full-straw chop-back management practices in tall fescue seed crops.
- To explore relationships between soil health measures and soil clay content and stand age in tall fescue seed crops.

#### **Materials and Methods**

A total of 34 fields were sampled in either April 2019 (20 fields) or April 2020 (14 fields), resulting in 17 paired fields from 17 locations. Fields were identified prior to sampling and were selected to meet a set of criteria. To evaluate the similarity in soil texture between the paired fields, we compared the

mean percentage of clay and sand between the two fields. Sites with greater than 5% difference in clay content were considered unacceptable pairs and were not included in the analysis. Three sites did not meet this criterion. Year 1 results of the study have been previously published (Verhoeven et al., 2020). The final dataset combined over 2 years includes 14 sites with 28 fields, each consisting of three replicate samples for a total of 84 samples.

All tall fescue seed production fields were greater than 4 years in age (one 3-year-old field pair was included to represent the North Valley). Fields with a history of full-straw chop-back ("full-straw") were paired with similarly aged stands on the same or related soil series in a nearby field (less than 10 miles away) that had a history of continuous straw removal ("baled"). To be considered full-straw management, the field had to have been managed under the full-straw practice for 75% of stand years.

Most fields included in the study had more than one soil series. NRCS soil maps were used to identify sample locations within the dominant soil series type within fields and, to the best of our ability, to match paired fields with soil type. The most commonly sampled soil series was Woodburn, followed by Dayton and Amity. Additional soil series included Quatama, Cornelius-Kinton, Huberly, Aloha, Chehalis, McBee, Malabon, Concord, Bashaw, Helvetia, and Laurelwood.

Sampling occurred over 2 years to break up the field work and allow us to sample enough fields at the same time of year. In most cases, paired fields were sampled on the same day. Three W-shaped transects were sampled per field. Each transect was analyzed separately and was considered a within-field replicate. Transects were placed semirandomly in uniform parts of the field that aligned with soil types in the matching paired fields according to soil maps. Ten soil cores per transect were taken to an 8-inch depth and mixed to form a composite sample. Soil bulk density measurements were collected at two points within each transect (six per field). For the remainder of the analyses, samples were stored at

4°C until laboratory analysis was conducted at OSU's Soil Health Laboratory (formerly the OSU Central Analytical Lab).

In addition to soil health properties (Table 1), soil samples were analyzed for texture (% sand/silt/clay). Soil health analysis followed the methods outlined by Cornell University in the Comprehensive Assessment of Soil Health (CASH, https://soilhealth.cals.cornell .edu/). Soil test potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg) levels were determined by Mehlich-3 extraction. Soil OM was calculated from total C analysis. Other biological soil health indicators included soil respiration and potentially mineralizable N. Both analyses were performed at 23°C on air-dried soil that was rewetted to 50% water-filled pore space. Respiration was measured by CO<sub>2</sub> accumulation at 24 and 96 hours. Potentially mineralizable N was determined using a 28-day aerobic respiration and measuring the accumulation of NO<sub>3</sub><sup>-</sup>.

Data were analyzed using a linear mixed-effects model, with residue management and stand age as independent variables and soil clay content as a covariate. Correlation analysis with stand age and soil clay content was conducted on each management group and across the entire dataset.

# **Results and Discussion**

# Effects of straw management

Results of key soil health parameters are shown in Figure 1. There was no impact of straw management on soil physical properties (bulk density, water-stable aggregates). Among the soil chemical properties, straw management significantly affected soil test K levels, which were higher under full-straw management practices (P = 0.002). This result is not surprising given that the straw contains high amounts of K. Growers typically apply higher rates of potash fertilizer on fields that are baled on a consistent basis. Results from this study suggest that soil K levels are not adequately maintained with potash fertilizer applications in systems with complete straw removal by baling. In addition

Table 1. Soil health parameters measured in this study.

Chemical/nutrient	Physical	Biological
pH Electrical conductivity (EC) Mehlich-3 extractable P, K, Ca, Mg Cation exchange capacity (CEC)	Bulk density Water-stable aggregates (WSA)	Soil respiration (24- and 96-hour) Total C%, total N% Active C (permanganate oxidizable C) Potentially mineralizable nitrogen (PMN)

to higher soil K in full-straw fields, soil P levels were elevated in full-straw fields (P = 0.066). Other soil chemical parameters were not affected by straw management.

Among the biological soil properties measured, we observed elevated respiration in the full-straw fields (96-hour test) (P = 0.051). The 24-hour respiration rate, which is the measure most similar to the commercially available Solvita "burst" test, tended to be higher in the full-straw fields, but differences were not significant. Respiration rates reflect microbial activity, and higher activity may be the result of higher microbial populations and/or increased activity reflective of increased availability of food, such as C from the straw.

Overall, total and active C in soil were not affected by straw management. The lack of straw management effect on soil C may be due to the large size of the soil C pool and/or to more dominant factors that affect total C and OM, such as tillage and below-ground inputs. The soil total C pool is large, and it often takes a long time and significant management changes to detect changes in this pool. We hypothesized that depth stratification may also play a role, as straw C in these systems may

be more concentrated in the surface layers. To help address this question, in 2020 we took subsamples from the 0- to 3-inch depth for analysis of soil C, OM, and active C. However, we still did not see an effect of straw management on soil C. Active C is a subcomponent of total soil C and is thought to be more digestible and utilized quickly by the microbial community. Active C has been reported to be more responsive to management practices (Awale et al., 2017). However, in this study no differences in active C were observed between straw management practices.

# Effect of stand age and clay content on soil health measures

Both stand age and soil clay content were expected to influence soil health measures. The impact of stand age is attributed to the passing of time since the last soil disturbance (e.g., tillage). When soil is disturbed, decomposition of organic matter is accelerated, and soil C is typically lost. Therefore, in the absence of disturbance, soil C levels and microbial communities, especially soil fungi, are more likely to build up. Soil C content often increases with clay content because of the large and negatively charged surface area of clay particles, which absorb OM. In our study, stand age

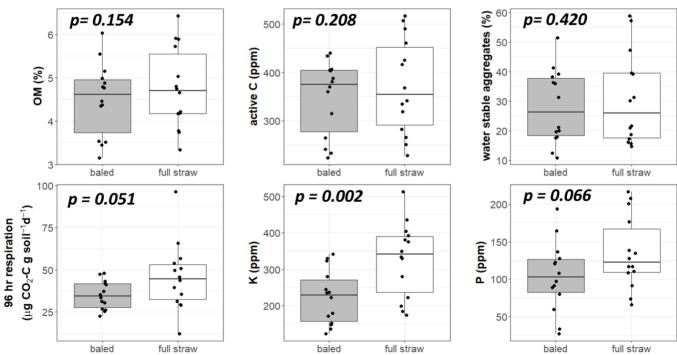


Figure 1. Box plots of key soil health properties in the baled and full-straw fields (n = 14) measured in 0- to 8-inch samples. Each point represents a field and is the average of the three transects sampled within a field. The top of each box represents the 25<sup>th</sup> percentile, while the lower end of the box represents the 75<sup>th</sup> percentile (i.e., 25% of observations were above, and 25% were below, the boxed area). Solid bold lines indicate the mean for each management, respectively.

ranged from 3 to 18 years, with an average of 8 years. Clay content ranged from 15.4 to 47.8%, with an average of 25.7%.

In this study, total soil N and C were significantly affected by stand age (P = 0.018 and P = 0.026, respectively, Figure 2). In the case of soil C, this relationship was likely driven by a correlation between soil C and stand age in the full-straw fields (P = 0.047), as this relationship was not observed in baled-straw fields (P = 0.523). These results demonstrate that with time, straw C additions can contribute to higher soil C.

Soil clay content had a significant effect on most soil health parameters. The only parameters not affected by clay content were K (Figure 2), pH, bulk density, and potentially mineralizable N. Active C, respiration (Figure 2), and Ca were positively correlated with clay content only in full-straw fields. These results indicate an interaction between clay content and straw residue, which improved these soil health parameters. Similarly, P levels declined with increasing clay content, but more so in baled-straw fields.

#### Conclusion

These data show that soil clay content is a powerful driver of many soil health outcomes and that it should be taken into account when analyzing soil health data from Willamette Valley soils. Comparisons between fields are valid only for soils with similar clay content and should be made with caution. Given the strong interaction between soil clay content and soil health parameters, we recommend using soil health parameters to track changes over time within a field rather than to compare fields.

When interactions between soil clay content, stand age, and residue are considered, we saw that total C, microbial activity, and soil K and P increased in fields with full straw loads. As clay content increased, we saw increases in microbial activity (measured by respiration) and active C in the full-straw fields. Soil K, and to a lesser degree soil P, and respiration also increased in full-straw fields regardless of soil clay content. Overall, the results from this study establish important baseline data for typical soils under tall fescue seed production and should help producers, agronomists, and researchers interpret soil health measures in future studies.

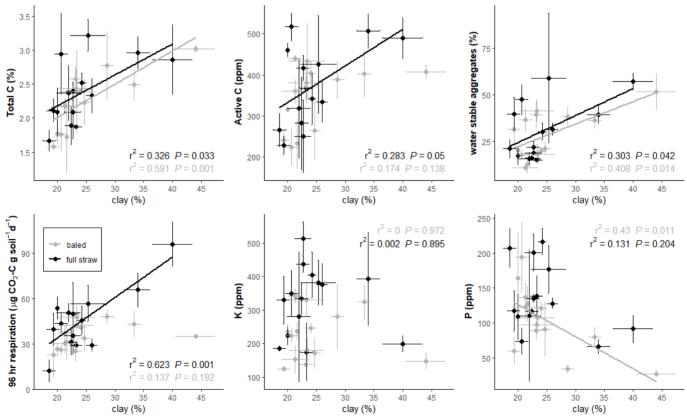


Figure 2. Relationships between soil clay content and select soil health properties for full-straw (black) and baled (gray) fields measured in soil samples from 0 to 8 inches. Each point represents a field and is the average of the three transects. A regression line is shown only when the regression was significant at P < 0.05.

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# GENETIC VARIATION FOR SEED RETENTION IN ACCESSIONS AND GENOTYPIC LINES OF PERENNIAL RYEGRASS

T.B. Tubbs and T.G. Chastain

#### Introduction

Many grasses naturally shed their seeds at maturity as a means of dissemination. This process of seed shedding is known as shattering. Shattering reduces the seed yield of grasses grown for seed crops and is an economic constraint on the profitability of seed-production enterprises. Therefore, retention of seed is an essential trait for improving seed yield in grasses.

Perennial ryegrass (*Lolium perenne* L.) is a coolseason turf and forage grass for which most genetic improvement efforts have focused on end-use quality characteristics, such as dry matter forage yield or turf quality, rather than on traits associated with seed yield, such as seed retention (Stewart and Hayes, 2011; McDonagh et al., 2016). Seed yield in perennial ryegrass is the product of two components: seed number and seed weight. Genetic improvement in seed retention may increase seed yield by significantly increasing the seed number harvested.

There may be viable sources of high seed retention present within germplasm collections of perennial ryegrasses available to plant breeders. Genetic variation for traits in natural populations and cultivars of perennial ryegrass is high, and nonuniformity in these plant materials is the rule rather than the exception. The objectives of this study were to determine whether genetic variation for seed retention was present in readily available perennial ryegrass accessions and to ascertain whether there was variation within these accessions.

#### **Materials and Methods**

Perennial ryegrass plants derived from 40 diverse global accessions were grown for 2 years in field trials at Oregon State University's Hyslop Crop Science Field Research Laboratory, near Corvallis, OR. The plant accessions were sourced from seeds acquired from the USDA Western Regional Plant Introduction (PI) Station in Pullman, WA, from two commercial U.S. cultivars, and from an experimental line from the United States.

Seeds from each accession were planted in the greenhouse and grown into plants robust enough (multiple tillers) for cloning. Each accession was

represented by four plants derived from four different seeds within the accession in order to characterize the variation within each accession. These plants were chosen as the progenitors for creating vegetative clones of the four genotypic lines within each accession or cultivar. Each genotypic line was cloned 4 times to produce a total of 640 transplants for the field trials.

The Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale was used to assess plant maturity for determination of seed retention. Seed retention was determined on three spikes chosen at random from each plant at BBCH 80 (dough development) or greater. Spikes were placed on an aluminum base plate, and a standardized steel bar (0.5 kg) was rolled by hand (about 24 Newtons) over each spike five times (three from tip to base and two from base to tip) in order to subject each spike to a consistent external force. Seeds that were dislodged from the spike in a tray were determined to be viable (carvopsis at least one-half the length of the palea) and were counted and weighed. The seeds retained on the spike were hand stripped from the spike, counted, and weighed.

Measurements of spike length and spikelet number per spike were made by using two-dimensional photogrammetry. Images of the three spikes were captured with a Nikon D5000 camera, and spike images were analyzed for length and spikelet number using Fiji (ImageJ) software. R studio (Version 1.3.1093) was used for computations and data analysis. Years, accessions, and genotypic lines nested within accessions were considered fixed effects in the analysis of variance. Replications were considered random effects.

#### **Results and Discussion**

Only 21 of the 40 accessions survived in sufficient numbers (3 of the 4 genotypic lines representing each accession) across both years for robust statistical analysis (data not shown). None of the plants survived in two of the accessions (231605 and 220179), and another 14 accessions exhibited more than 50% mortality among plants across years. One commercial cultivar did not vernalize the first year; its data are not included in the analysis. This result suggests that not

all of the accessions were adapted to the conditions prevalent at the field site or that some had innately short life spans. The results presented in this report are for the 21 best adapted and longer-lived accessions.

The analysis of variance revealed that there was significant variation among accessions in seed retention and other characteristics of the spike. A large range in seed retention values was observed among accessions. The 21 accessions are ranked from high to low seed retention values in Table 1. The mean seed retention for the 21 accessions was 44.2%, meaning that most of the potential seed would not have been harvested by the seed grower. The seed retention observed in the commercial cultivar 'Cutter' was near the mean of the accessions. Some of the accessions evaluated had superior seed retention compared to that found in 'Cutter' and the experimental cultivar line PR 12.1206. This result is evidence that sources of higher seed retention than that of commercial perennial ryegrass cultivars are available from these accessions and could be used in the breeding of shatter-resistant cultivars.

All spike characteristics measured, including spikelets per spike, seed number per spike, spike length, and seed weight, exhibited high levels of variation among accessions (Table 1). Field studies have indicated that seed yield in perennial ryegrass is most related to the size of the spike and that plant breeders should concentrate on increasing the number of spikelets per spike in order to improve seed yield (Abel et al., 2017).

Some significant differences between years (P = 0.007) were evident and were attributed to environmental factors. However, these differences did not differentially affect seed retention or other characteristics, so values were combined across years.

There is variability in seed retention both among accessions and among the genotypic lines within accessions (Figure 1). This variation among the genotypic lines and within accessions is responsible for much of the large standard deviations in seed retention observed for the accessions in Table 1. One practical consideration for genetic improvement is that this high

Table 1. Mean and standard deviation values for spike characteristics and seed retention in perennial ryegrass accessions in 2018 and 2019.<sup>1</sup>

PI number	Country of origin	Spikelets	Seeds	Seed weight	Spike length	Seed retention
		(no./spike)	(no./spike)	(mg)	(cm)	(%)
231581	Algeria	$21.7 \pm 4.9$	$67.5 \pm 32.5$	$1.2 \pm 0.6$	$13.3 \pm 2.5$	$78.7 \pm 16.8$
231594	Algeria	$26.0 \pm 5.5$	$89.4 \pm 32.9$	$1.6 \pm 0.4$	$19.5 \pm 4.6$	$65.6 \pm 20.0$
598916	Tunisia	$22.9 \pm 3.6$	$116.5 \pm 58.3$	$1.6 \pm 0.5$	$18.1 \pm 3.6$	$62.5 \pm 17.7$
231575	Algeria	$22.7 \pm 5.1$	$69.9 \pm 25.2$	$1.5 \pm 0.4$	$18.2 \pm 8.0$	$60.5 \pm 16.3$
231579	Algeria	$22.3 \pm 3.7$	$87.8 \pm 42.6$	$1.3 \pm 0.6$	$17.6 \pm 4.8$	$58.4 \pm 19.7$
231620	Iran	$26.4 \pm 4.6$	$72.7 \pm 40.4$	$1.9 \pm 0.8$	$21.0 \pm 3.0$	$55.3 \pm 16.1$
231587	Algeria	$21.4 \pm 4.5$	$77.6 \pm 37.1$	$1.9 \pm 0.7$	$19.3 \pm 4.7$	$44.0 \pm 20.2$
Cutter	USA	$23.8 \pm 5.0$	$75.4 \pm 30.5$	$2.0 \pm 0.3$	$19.6 \pm 2.5$	$42.4 \pm 14.6$
418708	Romania	$28.1 \pm 3.6$	$81.9 \pm 33.1$	$2.2 \pm 0.8$	$21.2 \pm 4.7$	$41.0 \pm 25.2$
231584	Algeria	$27.6 \pm 3.9$	$71.4 \pm 37.2$	$2.0 \pm 0.3$	$19.2 \pm 4.7$	$40.0 \pm 16.5$
384479	Poland	$25.0 \pm 3.3$	$71.0 \pm 33.3$	$1.9 \pm 0.5$	$23.3 \pm 3.4$	$38.3 \pm 16.7$
PR 12.1206	USA	$22.4 \pm 3.0$	$67.8 \pm 24.3$	$1.8 \pm 0.6$	$15.8 \pm 2.8$	$38.1 \pm 18.1$
418705	Italy	$24.2 \pm 2.9$	$77.0 \pm 35.9$	$2.0 \pm 0.5$	$20.2 \pm 2.5$	$37.0 \pm 13.5$
231619	Iran	$21.6 \pm 3.0$	$83.2 \pm 21.5$	$1.5 \pm 0.7$	$20.1 \pm 4.4$	$36.9 \pm 21.7$
234779	Germany	$21.5 \pm 3.5$	$79.9 \pm 46.0$	$2.0 \pm 0.5$	$20.0 \pm 3.4$	$35.5 \pm 15.9$
418707	Romania	$22.3 \pm 2.8$	$56.8 \pm 17.1$	$2.6 \pm 0.6$	$19.3 \pm 3.8$	$34.2 \pm 15.1$
220878	Ireland	$24.6 \pm 3.3$	$52.6 \pm 21.7$	$1.8 \pm 0.7$	$17.1 \pm 3.4$	$34.1 \pm 13.7$
238938	New Zealand	$21.4 \pm 3.0$	$62.8 \pm 31.4$	$2.4 \pm 0.8$	$22.4 \pm 4.1$	$33.5 \pm 13.2$
376878	New Zealand	$19.3 \pm 2.7$	$61.7 \pm 30.4$	$2.2 \pm 0.8$	$21.4 \pm 3.9$	$33.5 \pm 15.9$
371952	Bulgaria	$22.1 \pm 3.9$	$64.1 \pm 28.8$	$2.1 \pm 0.8$	$18.8 \pm 4.9$	$32.1 \pm 14.5$
231580	Algeria	$25.9 \pm 3.7$	$54.8 \pm 27.1$	$2.0\pm0.7$	$21.4 \pm 4.0$	$27.1 \pm 14.8$

<sup>&</sup>lt;sup>1</sup>Accessions are denoted by plant introduction (PI) number and country of origin.

variability within accessions means that not all source plants will have the desired level of seed retention. Variation within accessions was also observed for seed weight but not to the same extent as for seed retention (data not shown).

Accession PI 231581 had the highest average seed retention (78.7%) but also had the shortest spike length (13.3 cm). Regression analysis shows that there is a significant relationship between spike length and seed retention for the accessions (Figure 2). Shorter, more compact spikes might be superior in seed retention over longer, more open spikes by physically holding the seed in the spike. Spike length is clearly not the only factor contributing to seed retention, however.

There was a relationship of seed retention with seed weight (Figure 2). Seed weight is an important factor in seed retention, as heavy seed have a greater propensity to be lost in shattering than light seed; accessions tending to produce heavy seed showed greater seed shattering losses. The collective weight of seeds within a spikelet might increase breaking of abscission layers, thereby reducing seed retention (Elgersma et al., 1988). Heavy seeds might be more readily dislodged by forces in the environment, such as wind, than light seeds. Selection of small-seeded types of perennial ryegrass to increase seed retention might not be desirable, however, as some of the accessions had seeds that were lighter than cultivars currently in the marketplace. The

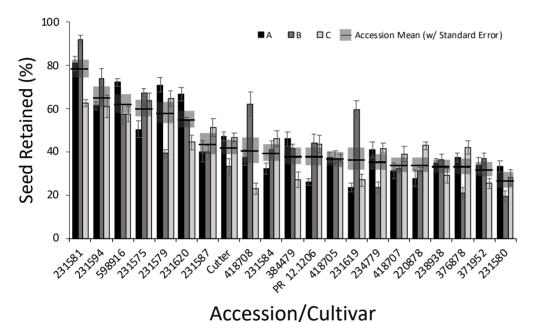


Figure 1. Seed retention among and within accessions or cultivars of perennial ryegrass in 2018 and 2019. Three genotypic lines within accessions or cultivars are denoted A–C.

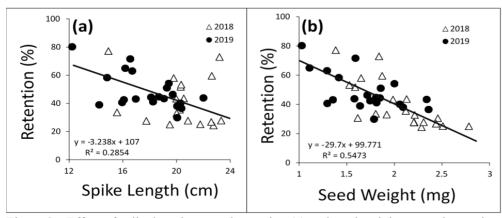


Figure 2. Effect of spike length on seed retention (a) and seed weight on seed retention (b) in accessions or cultivars of perennial ryegrass in 2018 and 2019.

analysis also indicated that seed weight was related to spike length, with long spikes tending to have higher individual seed weight (data not shown). Seed retention was independent of the number of seeds produced per spike or number of spikelets per spike.

This study demonstrates that beneficial variation in seed retention is present in genetic materials accessible to plant breeders and that some plant characteristics influence seed retention in perennial ryegrass. Our work using 3-D topometric imaging techniques for phenotyping has been aimed at further refining our understanding and identification of characteristics related to seed retention (Tubbs and Chastain, 2020). Morphological characteristics of the spike considered in the topometric image analysis included spike architecture, distance between spikelets along the rachis, angle of spikelet attachment to the rachis, and spikelet size. The results of that work will be presented in future reports.

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# SPRING MOWING AND PLANT GROWTH REGULATOR EFFECTS ON FIRST- AND SECOND-YEAR FINE FESCUE SEED CROPS

N.P. Anderson, B.C. Donovan, C.J. Garbacik, and T.G. Chastain

#### Introduction

Fine fescues (*Festuca rubra* L.) comprise a group of three cool-season grass species, including strong creeping red fescue (*F. rubra* ssp. *rubra*), slender creeping red fescue (*F. rubra* ssp. *littoralis*), and Chewings fescue (*F. rubra* ssp. *commutata*). While fine fescues are morphologically similar in many vegetative and reproductive characteristics, strong creeping red fescue and slender creeping red fescue produce rhizomes, while Chewings fescue does not. Their extensive geographic distribution as turfgrasses is due to their wide adaptation to many environmental and management conditions. However, Oregon is the leading producer of fine fescue seed in the United States, with nearly 22,500 acres valued at \$23.3 million in 2019 (Anderson, 2020).

Open-field burning has been an important management tool in Oregon fine fescue seed production fields because removal of postharvest residue maintains high seed yield and quality. The use of the plant growth regulator (PGR) trinexapac-ethyl (TE; Palisade EC) was evaluated over 4 years as a potential alternative to open-field burning for maximizing yield in creeping red fescue (Zapiola et al., 2014). Results of that study indicated that fall-applied TE has no effect on seed yield or seed yield components and cannot be used as a substitute for open-field burning. However, Gingrich and Mellbye (2001) reported a 32–48% increase in fine fescue seed yields from spring-applied TE in a series of on-farm trials conducted in the Silverton Hills.

Clormequat chloride (CCC; Adjust) is another stem-shortening PGR that has been used in other seed-producing areas worldwide, but it has not been examined in Oregon grass seed production until recently. These two PGRs act at different locations in the gibberellin (GA) biosynthesis pathway (Rademacher, 2015). CCC is an onium-type compound, and a label is currently being pursued by the registrant for its use in grass grown for seed. The effect of CCC and TE + CCC mixtures has not been previously examined in fine fescue seed crops grown under Oregon conditions.

Field observations have shown that inflorescence emergence can begin very early in the spring (February–March), and there is concern that early-emerging inflorescences do not make meaningful contributions to seed yield. Spring mowing, with and without combinations of TE and CCC PGRs, was identified as a key research priority in a series of Oregon State University (OSU) focus group meetings held with fine fescue seed growers during the 2017–2018 growing season. There have been no studies to address the effect of spring mowing on seed yield, components of yield, and above-ground biomass production in fine fescues.

The objective of this research is to determine whether a smaller window of inflorescence emergence can be created by mowing in the spring, which could result in more uniform flowering and increased seed yield. We also investigated effects of combining spring mowing with TE, CCC, and TE + CCC PGR applications.

# **Materials and Methods**

Two years of field trials were conducted on 'Survivor' Chewings fescue and 'Wendy Jean' creeping red fescue at OSU's Hyslop Research Farm near Corvallis, OR, from 2018–2020. The soil type at the site is a Woodburn silt loam. Plot size was approximately 11 feet x 45 feet. Plots were established with conventional tillage during spring of 2018. Routine fertilizer and herbicide applications were made as needed to manage fertility and weed pests. Mowing treatments were implemented using a standard flail mower set to a cutting height of 2–3 inches. Postharvest residue was managed by straw removal and flailing (no field burning). The experimental design was a randomized complete block with a split-plot arrangement of treatments and four replications. Main plots were spring mowing timings, and subplots were PGR treatments. Subplots were randomly allocated within spring mowing main plots. All PGR treatments were applied at BBCH 32 growth stage (two-node).

Spring mowing main plots included the following timings:

Untreated control (no mowing)							
Early mow	Early mow Year 1: March 31 Year 2: February 12						
Late mow Year 1: April 18 Year 2: March 5							

PGR subplots include the following active ingredients and application rates:

- Untreated control (no PGR)
- Trinexapac-ethyl (TE): 1.4 pt/acre (Chewings) or 2.8 pt/acre (creeping red)
- Clormequat chloride (CCC): 1.34 lb/acre
- TE: 1.4 pt/acre (Chewings) or 2.8 pt/acre (creeping red) + CCC: 1.34 lb/acre

At peak flowering (BBCH 65), three 0.1 m<sup>-2</sup> samples were harvested (cut to 2 cm above ground level) at random from each plot to determine inflorescences m<sup>-2</sup>, tillers m<sup>-2</sup>, stem length cm<sup>-2</sup>, and above-ground biomass. Inflorescences m<sup>-2</sup> were determined by counting all inflorescences within each sample, and stem length was determined by measuring ten stems chosen randomly from each sample. Samples were placed in a dryer at 65°C for approximately 48 hours and were then weighed to determine the above-ground biomass.

Plots were swathed with a modified John Deere 2280 swather and combined with a Hege 180 plot combine. Subsamples of harvested seed were collected from each plot and cleaned using a Clipper M2B cleaner to determine cleanout percentage and clean seed yield. Seed weight was determined by counting two 1,000-seed samples with an electronic seed counter and weighing these samples on a laboratory balance. Harvest index (HI), the ratio of seed yield to aboveground biomass, was also quantified.

#### **Results and Discussion**

Results from the first year of the study (2019) indicate that both early- and late-spring mowing treatments decreased seed yield in first-year stands of both Chewings fescue (Table 1) and creeping red fescue (Table 2). The decrease in seed yield can likely be attributed to a reduction in fertile tillers and seed number. There was an increase in seed weight from both the early and late mowing in both fine fescue varieties. There were mixed effects on HI among varieties. Above-ground biomass and tiller height were reduced with both mowing treatments in both varieties.

In the second year of the study (2020), mowing treatments were applied approximately 1 month earlier, and the seed yield response differed from year 1. Results indicated no difference in seed yield between mowing treatments for either Chewings fescue (Table 3) or creeping red fescue (Table 4) when PGRs were not applied. However, seed yield increased in Chewings fescue with early mowing when TE + CCC was applied

and for late mowing when both TE and TE + CCC were applied (Table 3). Conversly, seed yield in creeping red fescue was negatively affected when late mowing and TE + CCC were combined, but all other treatments were the same (Table 4). There were no differences in fertile tiller number, above-ground biomass, or HI between mowing treatments for either variety in year 2. Unlike year 1, neither mowing treatment reduced fertile tiller height in either variety.

In the first year, PGR effects on seed yield differed between the two fine fescue species and mowing treatments. In Chewings fescue, all PGR treatments increased seed yield with no mow and early mow but remained unchanged with late mow (Table 1). PGRs increased creeping red fescue seed yield only with the no-mow treatment (Table 2). PGR treatments containing CCC increased seed weight with no-mow and earlymow treatments in Chewings fescue (Table 1). In creeping red fescue, effects of PGR on seed weight varied, with an increase in seed weight with all PGR treatments in the no-mow treatment and a decrease in seed weight when TE + CCC and late-mow treatments were combined (Table 2). PGR treatments did not affect fertile tiller number in either variety, but the TE and CCC treatments decreased tiller height when combined with early mowing in creeping red fescue. There were mixed effects on biomass but no effects from any of the PGR treatments on HI in Chewings fescue (Table 1). All CCC PGR treatments decreased above-ground biomass in creeping red fescue with no-mow and early-mow treatments (Table 2). The TE + CCC PGR treatment increased HI across all mowing treatments.

PGR effects on seed yield differed between the two fine fescue species in year 2. All PGR treatments, except CCC combined with early mow, increased seed yield in Chewings fescue (Table 3). In creeping red fescue, TE and TE + CCC treatments increased seed yield, except for TE + CCC with late mow (Table 4). Seed weight was often, but not always, increased in both varieties when TE-containing PGR treatments were applied. The TE + CCC PGR treatment decreased tiller number in no-mow Chewings fescue (Table 3) and with all mowing treatments in creeping red fescue. All PGR treatments decreased tiller height and above-ground biomass across all mowing treatments in Chewings fescue (Table 3), but only TE-containing PGR treatments decreased tiller height in creeping red fescue (Table 4). The TE + CCC PGR treatment decreased above-ground biomass in creeping red fescue across all mowing treatments (Table 4). TE and TE + CCC also

Table 1. Effects of spring mowing and plant growth regulators on seed yield, yield components, and above-ground biomass of first-year 'Survivor' Chewings fescue, 2019.

		Seed yield	Cleanout	Seed weight	Seed no.	Biomass	Tiller no.	Tiller height	$\mathrm{HI}^2$
		(lb a <sup>-1</sup> )	(%)	(mg)	(no m <sup>-2</sup> )	(kg ha <sup>-1</sup> )	(no m <sup>-2</sup> )	(cm)	(%)
No mow	No PGR TE CCC TE + CCC	785 c 865 cd 907 de 1,007 e	5.61 e 5.57 e 4.65 d 3.71 ab	0.8865 a 0.8820 a 0.9150 b 0.9132 b	99,673 cd 110,132 de 111,172 de 123,807 e	8,223 ef 9,849 g 8,811 fg 9,654 g	325 cd 350 cd 320 c 375 d	77.9 e 77.5 e 74.4 e 70.6 de	10.8 bc 10.0 b 11.7 bc 11.0 bc
Early mow	No PGR TE CCC TE + CCC	554 b 714 c 729 c 739 c	4.25 bcd 3.71 bc 2.97 a 3.70 ab	0.9817 c 0.9947 c 1.0025 cd 1.0032 cd	63,409 b 80,486 c 81,901 c 82,573 c	5,578 abc 7,588 def 6,188 bcd 6,775 cde	161 b 205 b 166 b 192 b	57.5 bc 61.1 bcd 62.1 cd 51.8 ab	11.4 bc 10.9 bc 13.4 c 12.6 bc
Late mow	No PGR TE CCC TE + CCC SE =	157 a 172 a 162 a 174 a 62	4.09 bcd 4.57 d 4.46 cd 4.79 d 0.49	1.0295 def 1.0485 f 1.0310 ef 1.0240 de 0.0136	17,128 a 18,472 a 17,699 a 19,081 a 7,799	5,104 abc 3,879 a 4,211 a 4,651 ab 607	62 a 58 a 69 a 85 a 19	47.3 a 44.2 a 43.4 a 43.6 a 3.4	3.6 a 5.4 a 4.6 a 4.3 a 1.2

<sup>&</sup>lt;sup>1</sup>Numbers followed by the same letter are not significantly different at LSD (P = 0.05).

Table 2. Effects of spring mowing and plant growth regulators on seed yield, yield components, and above-ground biomass of first-year 'Wendy Jean' creeping red fescue, 2019.<sup>1</sup>

		Seed yield	Cleanout	Seed weight	Seed no.	Biomass	Tiller no.	Tiller height	$\mathrm{HI}^2$
		(lb a <sup>-1</sup> )	(%)	(mg)	(no m <sup>-2</sup> )	(kg ha <sup>-1</sup> )	(no m <sup>-2</sup> )	(cm)	(%)
No mow	No PGR TE CCC TE + CCC	1,187 d 1,416 f 1,297 e 1,370 ef	3.61 abc 3.19 ab 3.23 ab 2.99 a	1.0496 a 1.0960 bc 1.0997 bc 1.1387 d	124,215 d 144,786 f 132,249 de 134,787 ef	10,371 e 8,977 d 9,852 de 7,057 c	389 g 378 g 391 g 358 g	68.0 h 61.3 g 67.9 h 49.8 ef	13.1 abc 18.0 de 15.1 bcd 21.9 f
Early mow	No PGR TE CCC TE + CCC	872 c 904 c 826 bc 769 b	4.53 cd 4.23 bcd 4.54 cd 4.94 d	1.1387 d 1.1381 d 1.1320 cd 1.1148 bcd	85,663 bc 88,975 c 81,705 bc 77,028 b	7,272 c 4,866 b 6,409 c 4,886 b	234 f 180 de 171 cde 187 ef	53.1 f 40.7 bc 48.4 de 33.2 a	13.7 abc 21.7 ef 15.7 bcd 17.5 d
Late	No PGR TE CCC TE + CCC SE =	445 a 441 a 395 a 426 a 68	6.62 e 7.18 ef 7.14 ef 8.06 f 0.46	1.1268 cd 1.0851 ab 1.1097 bcd 1.0887 b 0.0165	44,108 a 45,237 a 39,722 a 43,870 a 6,519	4,218 b 4,057 b 4,242 b 2,803 a 553	103 ab 126 bcd 117 abc 75 a 20	44.5 cd 38.7 b 43.1 bc 31.0 a 1.6	11.7 ab 12.1 ab 10.6 a 16.7 cd

<sup>&</sup>lt;sup>1</sup>Numbers followed by the same letter are not significantly different at LSD (P = 0.05).

<sup>&</sup>lt;sup>2</sup>Harvest index (HI) is the ratio of seed yield to above-ground biomass.

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Table 3. Effects of spring mowing and plant growth regulators on seed yield, yield components, and above-ground biomass of second-year 'Survivor' Chewings fescue, 2020.<sup>1</sup>

		Seed yield	Cleanout	Seed weight	Seed no.	Biomass	Tiller no.	Tiller height	HI <sup>2</sup>
		(lb a <sup>-1</sup> )	(%)	(mg)	(no m <sup>-2</sup> )	(kg ha <sup>-1</sup> )	(no m <sup>-2</sup> )	(cm)	(%)
No mow	No PGR TE CCC TE + CCC	897 ab 1,155 cd 1,026 bc 1,517 e	14.67 cd 14.04 abcd 14.30 bcd 13.84 abcd	0.8755 b 0.8763 b 0.8910 bcd 0.9040 cde	114,878 ab 147,769 de 129,183 bc 188,092 f	10,967 c 9,814 bc 8,990 abc 8,315 ab	330 bcd 308 abcd 258 abc 252 a	76.0 ef 62.9 d 63.4 d 49.7 ab	9.5 a 13.7 ab 13.2 ab 20.8 c
Early mow	No PGR TE CCC TE + CCC	880 a 1,276 d 1,101 c 1,789 f	15.30 d 14.13 bcd 14.70 cd 13.09 abc	0.8553 a 0.9098 def 0.8880 cb 0.9148 efg	115,436 ab 157,196 e 138,863 cd 219,299 g	10,030 bc 9,589 abc 10,822 c 9,176 abc	302 abcd 304 abcd 347 d 308 abcd	84.4 g 73.3 e 77.4 ef 52.5 bc	10.6 a 15.2 b 11.4 ab 22.3 c
Late	No PGR TE CCC TE + CCC SE =	838 a 1,487 e 1,059 c 1,543 e	14.16 bcd 12.92 abc 12.27 ab 11.99 a 0.49	0.9011 cde 0.9305 g 0.9030 cde 0.9263 fg 0.0065	106,265 a 179,028 f 131,413 bcd 186,788 f 5,784	9,773 bc 8,175 ab 9,823 bc 7,207 a	326 abcd 284 abcd 337 cd 256 ab	82.6 fg 59.0 cd 74.1 e 44.0 a 2.4	9.9 a 21.0 c 12.1 ab 24.3 c

<sup>&</sup>lt;sup>1</sup>Numbers followed by the same letter are not significantly different at LSD (P = 0.05).

Table 4. Effects of spring mowing and plant growth regulators on seed yield, yield components, and above-ground biomass of second-year 'Wendy Jean' creeping red fescue, 2020.1

		Seed yield	Cleanout	Seed weight	Seed no.	Biomass	Tiller no.	Tiller height	HI <sup>2</sup>
		(lb a <sup>-1</sup> )	(%)	(mg)	(no m <sup>-2</sup> )	(kg ha <sup>-1</sup> )	(no m <sup>-2</sup> )	(cm)	(%)
No mow	No PGR TE CCC TE + CCC	1,015 abc 1,187 de 1,002 ab 1,156 d	17.29 ab 17.97 ab 17.54 ab 18.70 bc	0.9886 ab 1.0198 cde 0.9761 a 0.9943 abcd	115,007 abc 130,846 de 115,294 abc 130,228 de	8,249 ef 6,506 bcde 7,182 cdef 5,418 abc	315 e 255 bcde 288 cde 208 ab	58.2 d 48.4 bc 55.2 d 38.1 a	14.2 a 22.8 cd 16.4 ab 24.7 de
Early mow	No PGR TE CCC TE + CCC	1,022 abc 1,277 e 1,073 bcd 1,175 de	17.53 ab 16.81 ab 17.06 ab 17.40 ab	1.0091 bcde 1.0369 ef 0.9925 abc 1.0361 ef	114,299 abc 138,626 e 120,488 bcd 127,143 cde	8,652 f 6,463 bcde 8,171 ef 4,616 ab	304 de 240 abcd 291 cde 169 a	55.7 d 44.0 b 53.0 cd 35.6 a	13.4 a 22.7 cd 15.2 a 29.3 e
Late	No PGR TE CCC TE + CCC SE =	931 a 1,127 cd 1,015 ab 1,008 ab	16.78 ab 16.46 a 16.32 a 20.79 c 0.92	1.0079 bcde 1.0661 f 1.0021 abcd 1.0232 de 0.0101	103,629 a 122,182 bcd 113,408 abc 110,506 ab 5,279	7,383 def 5,777 abcd 6,570 cde 4,160 a	319 e 230 abc 260 bcde 225 abc 30	56.0 d 43.5 b 53.6 cd 33.9 a 2.0	14.7 a 22.1 bcd 17.4 abc 27.8 de 2.2

<sup>&</sup>lt;sup>1</sup>Numbers followed by the same letter are not significantly different at LSD (P = 0.05).

<sup>&</sup>lt;sup>2</sup>Harvest index (HI) is the ratio of seed yield to above-ground biomass.

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had the greatest effect on HI in both varieties. Overall, PGR treatments appear to have had similar effects in first- and second-year stands.

The differences in seed yield response to mowing treatments between years 1 and 2 can likely be attributed to differences in mowing timing in relation to growing degree day (GDD) accumulation. In year 1, early and late mowing treatments were carried out at 461 GDD and 648 GDD, respectively. Due to the negative effect of spring mowing on seed yield in the first year, mowing treatments were applied at 291 GDD (early) and 423 GDD (late) in the second year. The differences in the resulting seed yield response are an indication that components of seed yield are being set prior to the accumulation of 399 GDDs; therefore, any spring mowing practices should occur before that time. Results also suggest that only an early-spring mowing treatment should be considered in both species because a late mowing is likely to be detrimental to achieving high seed yields.

These data reinforce results from previous studies indicating that TE PGR use in fine fescue seed crops increases seed yield. This was evident in both Chewings and creeping red fescue in both the first- and second-year stands. Interestingly, CCC had a positive effect on seed yield, both alone and in combination with TE, in Chewings fescue but not in creeping red fescue. If this product receives a grass-grown-for-seed label, its use should be considered in Chewings fescue seed crops.

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# EFFECTS OF TRINEXAPAC-ETHYL ON KENTUCKY BLUEGRASS IN THE GRANDE RONDE VALLEY OF NORTHEASTERN OREGON (YEAR 3)

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#### Introduction

A 3-year study was initiated in the spring of 2018 to evaluate the effects of trinexapac-ethyl (Palisade EC) plant growth regulator (PGR) on seed yield of Kentucky bluegrass. Trinexapac-ethyl (TE) is a stem-shortening PGR that inhibits the action of a key enzyme in the gibberellic acid biosynthesis pathway, thereby preventing cell elongation and resulting in shortened internodes. PGRs are widely utilized in grass seed production systems worldwide to increase seed yield potential via reduced lodging, improved pollination and fertilization, and improved swathing.

Plant growth regulator research in Kentucky bluegrass (KBG) seed production is limited (Butler and Simmons, 2012), whereas extensive research has been conducted in perennial ryegrass, tall fescue, and fine fescue (Chastain et al., 2015; Silberstein et al., 2002). Results from these previous studies show that crop response to PGR application rates and timing varies among grass seed species. However, there is overwhelming evidence that TE effectively increases seed yield in grass seed crops under Oregon conditions.

The objective of this multiyear study is to evaluate the effect of TE application on seed yield of three different classes of KBG cultivars during first-through fourth-year harvest stands. Classes of Kentucky bluegrass include a BVMG type ('Baron'), a Midnight type ('Skye'), and a Shamrock type ('Gaelic'). The classes are based on pedigree, turf performance, and

morphological (PTM) attributes (Shortell et al., 2009). The 2020 results presented in this article reflect the third and final year of the study.

#### **Materials and Methods**

The final year of the study was initiated in spring 2020 in the Grande Ronde Valley of northeastern Oregon by establishing trials in irrigated, commercial Kentucky bluegrass seed production fields previously utilized for each variety in each year of the study. The experimental design at each site was a randomized complete block with three replications. Plot dimensions were 29 feet x 300 feet. Standard crop management practices were provided by grower cooperators with the exception of the TE application, which was applied by the investigator using a tractor-mounted R&D research sprayer with a 27-foot boom delivering 16 gal/acre spray volume. Crop growth stage, stand planting date, and environmental conditions at application time are shown in Table 1. Trinexapac-ethyl PGR treatments included an untreated control (no TE), 0.8, 1.4, and 2.8 pt TE/acre.

Two 1-square-foot above-ground biomass samples were collected from each plot in late June prior to mature seed development (BBCH 80–87) to determine above-ground biomass dry weight/acre, tiller height, panicle number, and ergot infection levels. Twenty-five panicles/plot were collected from swathed windrows to further evaluate ergot infection levels. Seed was harvested with grower-owned equipment, and a weigh

Table 1. Crop growth stage and environmental conditions at time of trinexapac-ethyl (TE) plant growth regulator (PGR) application on three varieties of Kentucky bluegrass grown for seed.

	Baron KBG	Skye KBG	Gaelic KBG
Application date KBG growth stage	May 7, 2020 two-node (BBCH 32)	May 7, 2020 two-node (BBCH 32)	May 4, 2020 two-node (BBCH 32)
Stand planted	Spring 2017	Spring 2016	Spring 2017
Air temperature (°F)	68	60	64
Relative humidity (%)	38	55	31
Cloud cover (%)	Clear	Clear	40
Wind velocity (mph)	0–4 from NW-W	0–5 from NW	3–7 from SE
Soil temperature, surface (°F)	70	59	68
Soil temperature, 1 inch (°F)	68	52	63
Soil temperature, 2 inch (°F)	62	52	60
Soil temperature, 4 inch (°F)	54	44	57

wagon was used to measure dirt weights in the field. Subsamples of seed were collected from each plot and were cleaned twice with a small-capacity three-screen cleaner (Westrup LA-LS) to determine clean seed yield. Purity of clean seed was determined with a seed blower (Hoffman model 67HMC-LK). Other crop/weed seeds and inert matter were not quantified. Seed weight was determined by weighing two 1,000-seed samples with an electronic seed counter located at the Oregon State University Agriculture and Natural Resource Program at Eastern Oregon University, La Grande, OR.

Seed quality samples for each treatment/site were collected by combining 50 grams of seed from each replicated treatment at each site to determine seed germination and vigor. Twelve bulked seed samples were submitted to the OSU Seed Lab for viability and vigor testing. A standard germination test was conducted with 4 replications of 100 seeds/rep for each treatment. Seeds were chilled at 50°F for 7 days, then transferred to a growth chamber set for alternating daily temperature and illumination cycles consisting of 16 hours dark at 59°F and 8 hours light at 77°F for 2 weeks. Tetrazolium tests (TZ) were performed to determine viable seeds on 2 replications of 100 seeds/rep with seeds moistened for 16 hours. Pierced seeds were soaked in 1% TZ solution overnight and then microscopically examined to determine number of normal seedlings. Accelerated aging tests (AAT) were performed on 4 replications of 50 seeds/rep. These seeds were subjected to stress at 106°F for 48 hours. They were then allowed to germinate at 59-77°F for 2 weeks to assess seed vigor based on the number of normal seedlings that emerged after exposure to stress.

#### **Results and Discussion**

# Seed yield and lodging

'Baron' Kentucky bluegrass: TE did not increase seed yield, and seed yield was suppressed at the 2.8 pt TE/acre rate (Table 2). Lodging did not occur in any of the treatments and was consistent with observations made for 'Baron' in 2019 (Walenta and Anderson, 2020). Differences were observed for biomass but did not correspond with TE rate. TE applied at 1.4 and 2.8 pt/acre reduced tiller height by 15 and 33%, respectively. No differences were observed for seed weight, panicle number, or clean seed purity.

'Skye' Kentucky bluegrass: Seed yield was not increased with TE; however, the lowest seed yield was observed at the 2.8 pt TE/acre rate (Table 3). Lodging was reduced by 46% and 100% at the 1.4 and 2.8 pt TE/acre rates, respectively. The 0.8 pt TE/acre rate did not reduce lodging. Tiller height was reduced by 3.6 inches at the 2.8 pt TE/acre rate. TE did not affect above-ground biomass, panicle number, seed weight, or seed purity.

'Gaelic' Kentucky bluegrass: Seed yield was not increased with TE application, and yield was suppressed at the 2.8 pt TE/acre rate (Table 4). Lodging was reduced at all TE application rates. Tiller height was reduced by TE and was dependent upon application rate. TE did not affect above-ground biomass, panicle number, seed weight, or purity.

The number of spikelets per panicle differed by variety (Table 5). 'Gaelic' KBG was the only variety in which TE rate affected the number of spikelets/panicle. There

Table 2. Effect of trinexapac-ethyl (TE) plant growth regulator (PGR) on seed yield, yield components, and growth characteristics of third-year-harvest Kentucky bluegrass var. 'Baron'.<sup>1</sup>

PGR treatment	Seed yield	Cleanout	Biomass	Tiller height	Panicles	1,000-seed weight	Purity	Lodging
(pt TE/a)	(lb/a)	(%)	(ton/a)	(in)	(no./ft²)	(g)	(%)	(%)
Control 0.8 1.4 2.8 LSD (0.05)	439 a 387 a 375 ab 252 b 134	51.5 b 53.3 ab 53.2 ab 58.9 a	5.4 ab 5.0 b 6.0 a 5.4 ab 0.9	22.1 a 21.3 a 18.7 b 14.9 c 1.9	365 364 497 339 NS	0.372 0.358 0.357 0.360 NS	97.1 95.1 95.1 95.8 NS	0 0 0 0 NS

<sup>&</sup>lt;sup>1</sup>Means followed by the same letter are not different at LSD (P = 0.05).

was an 18% reduction in spikelet number at 2.8 pt TE/acre, compared to the untreated control.

#### Ergot infection

Ergot infection was detected at all sites in 2020, with the highest infection level recorded in 'Skye' (Table 5). Ergot incidence and severity levels were low in 'Baron' and 'Gaelic' varieties. Ergot spore monitoring utilized both Burkhard and roto-rod spore traps at the 'Gaelic' trial site. Spore trap results indicate peak airborne spore activity occurred early- to mid-May 2020, which was approximately 9 days prior to the start of flowering for 'Gaelic' (data not shown). Thus, less disease inoculum was available for initial infection as the 'Gaelic' flowering stage advanced. Ergot spore trap results from the 'Skye' site indicate peak spore activity occurred early- to mid-June, which directly coincided with flowering period (June 5–25). Thus, the high levels of airborne spores led to high infection levels.

# Seed viability and vigor

Standard germination and TZ test procedures showed no effect of TE on seed viability (Table 6). Varying levels of dormancy existed for all three KBG varieties, as indicated by the TZ test results being higher than those of germination. The AAT results were very low for the three varieties, even after 2 weeks of germination. However, 'Skye' and 'Gaelic' exhibited better seedling vigor compared to 'Baron'. This result can be attributed to the physiological quality of each variety and the varieties' responses to stress conditions (high temperature and high humidity) in the AAT test.

The low vigor results observed in all three varieties are not attributed to TE applications but rather to the tolerance of each variety to the stress conditions of the AAT test. Overall, TE application at any rate did not affect seed viability or vigor measurements.

Table 3. Effect of trinexapac-ethyl (TE) plant growth regulator (PGR) on seed yield, yield components, and growth characteristics of fourth-year-harvest Kentucky bluegrass var. 'Skye'.

PGR treatment	Seed yield	Cleanout	Biomass	Tiller height	Panicles	1,000-seed weight	Purity	Lodging
(pt TE/a)	(lb/a)	(%)	(ton/a)	(in)	(no./ft²)	(g)	(%)	(%)
Control	584	46.5	6.3	29.6 a	256	0.430	99.3	99 a
0.8	620	49.7	6.2	28.5 b	242	0.436	99.5	95 a
1.4	662	45.2	6.6	27.6 ab	229	0.428	99.4	53 b
2.8	545	43.5	6.3	26.0 b	240	0.424	99.6	0 c
LSD (0.05)	NS	NS	NS	2.2	NS	NS	NS	16

<sup>&</sup>lt;sup>1</sup>Means followed by the same letter are not different at LSD (P = 0.05).

Table 4. Effect of trinexapac-ethyl (TE) plant growth regulator (PGR) on seed yield, yield components, and growth characteristics of third-year-harvest Kentucky bluegrass var. 'Gaelic'.<sup>1</sup>

PGR treatment	Seed yield	Cleanout	Biomass	Tiller height	Panicles	1,000-seed weight	Purity	Lodging
(pt TE/a)	(lb/a)	(%)	(ton/a)	(in)	(no./ft²)	(g)	(%)	(%)
Control 0.8 1.4 2.8 LSD (0.05)	1,290 1,242 1,372 950 NS	30.8 31.9 26.5 41.1 NS	5.8 5.6 5.3 5.3 NS	30.3 a 26.3 b 23.5 c 20.1 d 2.0	329 413 428 457 NS	0.381 0.384 0.379 0.399 NS	96.0 97.7 94.1 96.8 NS	78 a 7 b 0 b 0 b 33

<sup>&</sup>lt;sup>1</sup>Means followed by the same letter are not different at LSD (P = 0.05).

Table 5. Effect of trinexapac-ethyl (TE) plant growth regulator (PGR) on spikelet number and ergot infection frequency/severity of three Kentucky bluegrass varieties.<sup>1</sup>

PGR treatment	Spikelets/panicle	Ergot-infected panicles	Ergot sclerotia/panicle
(pt TE/a)	(no.)	(%)	(no.)
		var. Baron	
Control	147	11 a	1.6 a
0.8	146	8 ab	1.0 a
1.4	141	0 b	0.0 b
2.8	132	0 b	0.0 b
LSD (0.05)	NS	11	0.7
		var. Skye	
Control	188	42	2.6
0.8	187	40	1.9
1.4	177	31	3.6
2.8	173	50	2.2
LSD (0.05)	NS	NS	NS
		var. Gaelic	
Control	215 a	0	0
0.8	192 a	4	1
1.4	221 ab	0	0
2.8	175 b	2	1
LSD (0.05)	35	NS	NS

<sup>&</sup>lt;sup>1</sup>Means followed by the same letter are not different at LSD (P = 0.05).

Table 6. Effect of trinexapac-ethyl (TE) plant growth regulator (PGR) on seed viability and vigor of three Kentucky bluegrass varieties.<sup>1</sup>

PGR treatment	Standard germination test	Tetrazolium test	Accelerated aging test
(pt TE/a)	(%)	(% viable seed)	(% germination at 2 weeks)
_		var. Baron	
Control	77.5	84.6	9.0
0.8	72.0	83.2	6.5
1.4	70.0	83.7	5.5
2.8	66.0	81.4	5.0
LSD (0.05)	NS	NS	NS
		var. Skye	
Control	87.0	95.2	23.0 ab
0.8	87.0	96.2	34.0 a
1.4	86.0	96.2	22.5 ab
2.8	86.5	95.7	18.0 b
LSD (0.05)	NS	NS	12.7
_		var. Gaelic	
Control	78.5 a	90.0	24.0 b
0.8	79.8 a	86.2	10.5 c
1.4	59.8 b	82.4	16.5 bc
2.8	84.8 a	89.1	47.5 a
LSD (0.05)	8.2	NS	9.9

<sup>&</sup>lt;sup>1</sup>Means followed by the same letter are not different at LSD (P = 0.05).

Overall, results for the third year of the study indicate that TE application did not increase seed yields for any of the three varieties. The 2.8 pt TE/acre rate resulted in the lowest seed yield for each variety and is consistent with year 2 results. Seed viability and vigor were not affected by any TE treatments. Cleanout percentages were extremely high due to cleaning seed samples twice to achieve adequate purity levels.

Kentucky bluegrass varieties differ in susceptibility to lodging as stands age. For example, 'Baron' lodged in the first harvest year without application of TE but did not lodge in the second or third years of the study. 'Skye' experienced severe lodging at zero, 0.8, and 1.4 pt/acre TE rates in all 3 years of the study. 'Gaelic' lodged at 0, 0.8, and 1.4 pt TE/acre rates in the first 2 years of the study, but lodging was not observed in the third year. The 2.8 pt TE/acre rate eliminated lodging in each of the three varieties in all 3 years. However, no benefit can be realized since this treatment resulted in decreased seed yield.

The results of this study will be investigated further to identify any interactions and trends between stand age, variety, and TE rate. In addition, efforts will continue to determine optimal TE application rates for Kentucky bluegrass varieties grown in the Grande Ronde Valley.

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#### MOBILITY OF THE GRAY FIELD SLUG

R.J. Mc Donnell, N.P. Anderson, A.J. Colton, and B.C. Donovan

#### Introduction

Slugs are among the most important pests of the grass seed industry in Oregon, and the gray field slug (Deroceras reticulatum) is the most damaging species. Although it is one of the best studied species worldwide, to the best of our knowledge no information exists on its mobility within crops, which is surprising given its status as a significant pest of global agriculture. Such knowledge would not only provide important insights into the ecology of this slug, but from a practical standpoint the data could also be used to inform the size of plots needed to perform more meaningful field trials by ensuring that plots are at least the size of the slug's home range. Thus, the aim of this study was to generate the first data on the mobility of the gray field slug, with the ultimate goal of using the information to design a spring baiting trial.

#### **Materials and Methods**

#### Field sites

This study was carried out in established perennial ryegrass fields in both the south and the north Willamette Valley. The southern site was located near Shedd, OR, and the northern site was located near Banks, OR. Visual surveys at each site prior to setup showed that both locations were infested with *D. reticulatum*.

#### Tagging slugs

To investigate mobility in a species, it is crucial to recognize and track individual specimens. Wallin and Latty (2008) successfully marked slugs (Ariolimax columbianus) by injecting a small, colored elastomer, i.e., a polymer with elastic properties (Northwest Marine Technology, Shaw Island, WA), just below the surface of the foot. This resulted in a highly visible colored tag on the underside of the body. An added advantage of these tags is that they fluoresce under ultraviolet (UV) light. To determine whether these elastomers would be a suitable approach for marking D. reticulatum, ten adult slugs were injected with the tagging material using a 29-gauge, 0.3-ml syringe (BD Microfine Plus U0100 insulin syringe). For the most part, we followed the procedure outlined by Wallin and Latty (2008), although some modifications were made. First, the test slugs were not anesthetized, as

it was deemed too time-consuming to do so. Second, to increase the longevity of the elastomer prior to injection, it was not mixed with the curing agent. Although the slugs did eject some of the material immediately after tagging, a sufficient amount remained inside the foot to enable identification. However, there was some migration of the elastomer inside specimens, so individual slugs could not be given a specific identification mark.

After tagging, the slugs were placed individually in plastic Tupperware containers with a perforated lid, damp paper towel, and a piece of organic carrot. The slugs and marks were checked daily for 14 days. At that time, there was 100% slug survival, all of the tags were clearly visible, the slugs appeared to crawl normally, and there were no indications of atypical behaviors, e.g., reluctance to move.

# Mobility study

At each field site, we deployed a standard slug blanket trap (DeSangosse, Agen, France) about 75 m from the field edge on November 8, 2019. This trap was termed the central trap. Radiating out from this blanket, we set up concentric rings of traps at 3 m (4 traps total), 5 m (8 traps total), 10 m (12 traps total), 25 m (18 traps total), and 50 m (30 traps total). Traps were roughly equidistant from each other in each ring. A 75-m ring (50 traps total) was set up at the South Valley site only on December 15, 2019. In addition to these traps, we deployed 50 blankets about 450 m away from the rings and adjacent to the field edge on November 8, 2019. These traps were termed the satellite traps and were used to supplement the total number of slugs tagged per month.

On November 13, 2019, all slugs underneath the central trap were marked with one of the tags, and additional slugs were collected under the satellite traps to ensure that a minimum of 100 slugs were tagged monthly. A different colored tag was used each month (Table 1). Slugs were tagged in the foot because there are no vital organs in this part of the slug's body and the brightly colored tags are also hidden from potential predators. After tagging, the slugs were released just north of the central trap. The central trap and all traps in the concentric rings were then checked monthly until

May 2020 for previously tagged slugs, and a new batch of slugs was tagged and released each month (Table 1). A UV light was used to confirm the presence/absence of tags in ambiguous specimens. All slugs were tagged only once with a single color, i.e., previously tagged slugs were not retagged.

#### **Results and Discussion**

A total of 2,380 slugs were tagged (1,190 at each site). Of these, 12 live, previously tagged slugs were recovered (Table 2). Eleven were found underneath the blanket traps, and one was found crawling on the grass about 14 m from the central trap. Such low recapture rates are not unusual for mark-recapture studies involving invertebrates (e.g., Birley and Charlwood, 1989). For our study, the low recapture rate is likely due to some of the tagged slugs moving underground and others not finding a trap under which to take refuge. Nevertheless, the mean (+ SE) distance moved by recaptured specimens was 6.4 m (+ 1.3 m) (Table 2). At the South Valley site, the slugs moved 6.3 m (+ 1.3 m), and at the North Valley site they moved 6.8 m (+ 3.2 m). Thus, the mean distance moved by the recaptured D. reticulatum over the 7 months was comparable between the two locations.

The distance moved per slug per month is also presented in Table 2. It varied from 1.7 m to 10 m with a mean ( $\pm$  SE) of 4.5 m ( $\pm$  1.1 m). At the South Valley site, the slugs moved an average of 4.5 m ( $\pm$  1.3 m) per month, and at the North Valley site they moved an average of 4.4 m ( $\pm$  2.1 m) per month. Thus, as above, the mean distance moved by *D. reticulatum* per month was comparable between the two locations.

To the best of our knowledge, these data are the first estimates of mobility for *D. reticulatum*. The data will be useful for informing plot size for future field trials for researchers and fieldmen throughout the Willamette Valley. For example, for a month-long baiting trial, the minimum plot size used for treatments and controls should be at least 10 m x 10 m because our data suggests that slugs appear to be able to move a maximum of 10 m per month.

Limitations to this study include the low number of recaptures and the lack of data on juvenile and neonate slugs, which may be more active dispersers than adults. Future mobility studies with *D. reticulatum* should consider utilizing micro-RFID tags, as these would enable individual slugs to be tracked both on the surface and in the soil and thus would likely yield more accurate data on the mobility of this species.

Table 1. Total number of gray field slugs (*Deroceras reticulatum*) tagged per month and the corresponding tag color.

Month	Tag color	Number of slugs tagged
November 2019	Orange	200
December 2019	Green	115
January 2020	Pink	250
February 2020	Yellow	250
March 2020	Black	250
April 2020	Blue	125

Table 2. Distance moved by tagged gray field slugs (*Deroceras reticulatum*) from November 2019 to May 2020 in established perennial ryegrass fields at the North (white background) and South (shaded background) Willamette Valley study sites.<sup>1</sup>

Slug number	Distance moved	Months elapsed	Distance moved per month
	(m)		(m)
1 2 3 4	0 3 10 14	1 1 1 3	0 3 10 4.7
5 6 7 8 9 10 11 12	0 5 10 10 5 5 5 10 5	1 1 1 1 1 3 3 4	0 5 10 10 5 1.7 3.3 1.3
Mean (± SE) <sup>2</sup>	6.4 ( <u>+</u> 1.3)	_	4.5 (± 1.1)

<sup>&</sup>lt;sup>1</sup>Slugs that were recovered under the central trap were assumed to have moved 0 meters.

<sup>&</sup>lt;sup>2</sup>At the North Valley site, the mean ( $\pm$  SE) total distance moved and the mean distance moved per month by recaptured tagged slugs was 6.8 m ( $\pm$  3.2) and 4.4 m ( $\pm$  2.1), respectively. At the South Valley site, the mean ( $\pm$  SE) total distance moved and the mean distance moved per month by previously tagged slugs was 6.3 m ( $\pm$  1.3) and 4.5 m ( $\pm$  1.3), respectively.

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# UNDERSTANDING BILLBUG SPECIES COMPLEX IN GRASS SEED PRODUCTION SYSTEMS IN WESTERN OREGON

N. Kaur, B.C. Donovon, L. Van Slambrook, D.L. Walenta, and N.P. Anderson

#### Introduction

Billbugs (Sphenophorus spp.) are complex weevil pests affecting cool-season grasses across the United States (Dupuy and Ramirez, 2016). In the Pacific Northwest (PNW), at least four species of this genus are known to cause significant damage to grass seed crops. The billbug species complex that is commonly found in Oregon grass seed production systems is comprised of the bluegrass billbug (Sphenophorus parvulus), Denver billbug (Sphenophorus cicatristriatus), orchardgrass billbug (Sphenophorus venatus confluens), and Sphenophorus sayi (no common name) (Walenta et al., 2004). There are four life stages: egg, larva, pupa, and adult. Adults feed on spring regrowth, chewing through young, folded leaves near the plant crown. As leaves extend, the characteristic damage of paired holes through leaf interiors is observed (Salisbury and Anderson, 2020).

Adult damage is not of major concern, as it has little effect on yield. Billbugs do most of their damage while in the larval stage and can cause significant damage by feeding on stems, roots, and crowns, causing severe discoloration and eventual plant death. Heavy larval feeding compromises the root system, and stems of severely damaged turf break and pull away easily from the soil. Often, a sawdustlike frass is present in hollowed-out stems (Salisbury and Anderson, 2020). Due to the cryptic nature of larvae and similarity of damage symptoms, i.e., weak crowns or presence of sawdustlike frass material, billbug damage in western Oregon grass fields is sometimes misdiagnosed as sod webworm damage.

Bluegrass billbug (*S. parvulus*), the main pest of Kentucky bluegrass grown for seed, was initially thought to be limited to eastern Oregon production systems until the recent report of this species occurring in commercial tall fescue fields in western Oregon at levels causing visible crop damage (Anderson, personal communication). Therefore, monitoring efforts to better understand the billbug species complex occurring in western Oregon are warranted.

The key morphological characteristics that can help distinguish between adults of at least three important billbug species of concern to grass seed crops are



Figure 1. Adults (left to right): Denver billbug (S. cicatristriatus), orchardgrass billbug (S. venatus confluens), and bluegrass billbug (S. parvulus). Photo: Babu Panthi, OSU Field Crop Entomology Program.

presented in Figure 1. The Denver billbug is about 10–12 mm in length and has small, even dimples on the thorax. The orchardgrass billbug is about 7–8 mm long, the thorax has many tiny indentations that look like pinholes, and the wing covers are coarsely grooved. The bluegrass billbug has even dimples covering the thorax and is 5–7 mm in length. No such characteristics exist for larval identification of these three species, which further complicates the understanding of their biology and seasonal phenology, as regional billbug pest complexes can co-occur in the same agroecosystems and are difficult to find during sampling.

# **Materials and Methods**

#### Sampling

Monitoring efforts were conducted in five commercial tall fescue fields, including three in Yamhill County and two in Washington County, OR. Weekly sampling for billbugs was initiated in September 2019 and continued through mid-November. Sampling resumed in January 2020 and continued until seed harvest. Monitoring efforts included nondestructive linear pitfall sampling of ground-active billbug adults and destructive sampling

using soil cores for larval stages in the soil. Two linear pitfall traps (a slit PVC pipe allowing linear movement of insects into a collection cup containing killing agent) were installed at each commercial field site. Specimens collected from pitfall traps were stored in plastic containers, brought to the laboratory, counted, and forwarded to the OSU Field Crops Entomology Lab for confirmation of species identification using molecular techniques.

Sod-soil samples were also collected from each site on a biweekly basis to target the presence of immature stages. Samples were taken with a shovel, collecting at least a 4-inch (total) sample according to sampling techniques modified from Walenta et al., 2004. Sod samples were subjected to either Berlese funnels or manual dissections in the laboratory.

# Molecular studies

DNA-based identification methods were employed, adapted from Duffy et al. (2018), to confirm the species identification of billbugs collected in western Oregon. PI Walenta collected Denver billbug samples from a commercial Kentucky bluegrass field in eastern Oregon to be included in this study for a comparative analysis.

Following manufacturer protocols, genomic DNA was extracted using a DNeasy blood and tissue kit (Qiagen, Valencia, CA) from whole-body homogenizations of the thorax and abdomen for three species: *S. venatus*, *S. parvulus*, and *S. cicatristriatus*. DNA was quantified using a ThermoScientific NanoDrop 2000

spectrophotometer. Three loci—COI, 18S, and ITS2—covering mitochondrial (mtDNA), ribosomal (rDNA), and nuclear ribosomal (nrDNA) DNA, respectively, were amplified using polymerase chain reaction (PCR). The PCR products upon cleanup using Qiagen kits were submitted to the Center for Genome Research and Biocomputing (CGRB) at OSU for Sanger sequencing. The NCBI website's BLAST search function was used to identify insect DNA sequences based on their similarity to archived sequences.

#### **Results and Discussion**

This study revealed that the bluegrass billbug and orchardgrass billbug were found to co-occur in western Oregon tall fescue fields, as indicated by the number of adult captures in the pitfall traps at all five field sites in 2019–2020 (Figure 2). The first adult beetle captures corresponded to *S. parvulus* in the pitfall traps and occurred in late February 2020 at two field sites (Figure 2), indicating resumption of bluegrass billbug adult foraging activity after the overwintering period.

The first and only larva capture occurred in late May at only one sampling site, corresponding to the bluegrass billbug, based on its 100% similarity to the sequence reads of *S. parvulus* isolate AD01 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence corresponding to GenBank Accession (MG385047). Weekly sampling efforts targeted for larval and pupal stages were not able to detect these life stages throughout the crop harvest period.

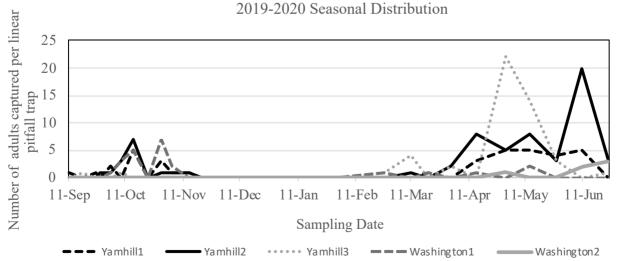


Figure 2. Cumulative adult capture of the bluegrass billbug (*S. parvulus*) and orchardgrass billbug (*S. venatus*) in linear pitfall traps each week at five commercial tall fescue seed fields in western Oregon, 2019–2020.

Additional scouting efforts in early August through September 2020 in commercial fine fescue seed fields (not included in this study) revealed the presence of larval and pupal stages corresponding with 100% identity to the orchardgrass billbug isolate AD06 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence GenBank Accession (MG385050).

The sequence read based on CO1 primers obtained from Denver billbug corresponded to the CO1 region of *Sphenophorus* spp. BYU-CO246 mitochondrion, complete genome with 100% identity to GenBank Accession (GU176342.1).

The sequence data for all loci—COI, 18S, and ITS2—are being processed for submission to the GenBank for the billbug species complex found in Oregon surveys for future referencing. The data collected during 2019–2020 on insect phenology of the bluegrass billbug is indicative of only one generation per year in western Oregon. This closely aligns with the earlier phenology model proposed for the bluegrass billbug in eastern Oregon by Rondon and Walenta (2011).

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# CUTWORM AND ARMYWORM POPULATION DYNAMICS AND INVESTIGATION OF PARASITISM IN KENTUCKY BLUEGRASS PRODUCTION

J. Green, L. Van Slambrook, N. Kaur, and D.L. Walenta

#### Introduction

Damage from cutworms and armyworms (Lepidoptera: Noctuidae larvae) can occur quickly, and the potential for stand loss is of particular concern. This project builds on previous work by OSU Extension faculty to assess the impact of cutworms and armyworms in Kentucky bluegrass (KBG) seed production systems. Butler and Hammond (2001) used light traps to sample adult moths. They identified a suite of about ten species that could be problematic for regional KBG production. The primary objective of our work is to gain a better understanding of flight timings and relative abundance of noctuid moths relevant to KBG production systems. Second, we aim to assess the impact of parasitoids, predators, and naturally occurring entomopathogens, because these beneficial agents can help mitigate damage from cutworm and armyworm larvae in fields.

Our study focused on three species:

- Winter cutworm (*Noctua pronuba*), a relatively new concern in Oregon grass production (Landolt et al., 2015)
- Glassy cutworm (*Apamea devastator*), a known grass pest in this region (Kamm, 1990)
- Snowy-veined Apamea (*A. niveivenosa*, previously *Protagrotis niveivenosa*), which was noted in prior KBG studies by OSU (Butler and Hammond, 2001)

#### **Materials and Methods**

### Adult moth activity

Seasonal abundance of adult moths was tracked using green UniTrap buckets baited with pheromone lures. Lures were developed by a local supplier (AlphaScents, West Linn, OR). The winter cutworm lure is commercially offered by the supplier. The glassy cutworm lure, however, had to be custom formulated, based on pheromone ratios for snowy-veined Apamea that had been identified but never field tested (Keith, 1965). An intoxicant strip was placed within each bucket to effectively and quickly kill captured moths within the trap. Traps were placed near commercial KBG production fields in eastern Oregon and near ryegrass and fine fescue fields in western Oregon as comparisons. Traps were placed in late May and checked every 2 weeks through September. Catch

collections of all adult moths (species of interest and nontargets) were bagged, labeled, and shipped to OSU for identification

#### Lab evaluation of larvae

Noctuid larvae were collected from grass seed production fields in western Oregon from November 5 to December 7. Larvae were reared individually on artificial diet, which is a combination of powdered protein (wheat germ, soybean), nutrients and vitamins, agar, and other ingredients. Percent mortality was evaluated, with a specific interest in detecting parasitoid emergence or symptoms of parasitoid development.

A rating scale was established to assess overall "viability" of each cutworm, with 0 = death within 2 weeks; 1-2 = an intermediate continuum of distinct tissue damage, lethargy, darkening of the cuticle, and eventual death; 3 = continued feeding with no notable reduction in activity; and 4 = the cutworm was able to successfully molt.

### **Results and Discussion**

#### Adult moth activity

A total of 1,048 noctuid moths were collected during the 2020 season. Unfortunately, 45% of all captured moths were cabbage looper (*Trichoplusia ni*), and another 10% were true armyworm (*Mythimna unipuncta*).

Snowy-veined Apamea moths were detected in eastern Oregon only, mainly from one site (278 of 375 total). The first trap catch occurred on June 11 (Figure 1a). A total of 81 glassy cutworms were trapped from both eastern and western Oregon, with a first capture date of June 30 (Figure 1b), which matches timing previously reported (Kamm, 1990). Surprisingly, only four winter cutworms were trapped throughout the entire season (data not shown). The low trap catch of *N. pronuba* may be reflective of low densities during the sampling period, sampling methodology, or both.

Custom lures developed for trapping *Apamea* species were successful. We were able to detect both glassy cutworm moths and snowy-veined moths using the lures. This result is important because a commercially available lure does not yet exist. Knowing that the

lure formulation works is a crucial aspect for future investigation of the *Apamea* moth "complex" that has been reported previously from bluegrass production systems (Keith, 1965).

# Lab evaluation of larvae

The first challenge of the lab assay was to ensure that glassy cutworm and winter cutworm could be reared in the laboratory; to our knowledge this has never been attempted for either species. Artificial diet was accepted by all larvae, and we now have methodology in place to continue these research efforts. Observed emergence of parasitoids (Diptera: Tachinidae) occurred in two glassy cutworms and one winter cutworm (Table 1). Our sample sizes were small (n = 20 glassy cutworms and 7 winter cutworms), but this work is ongoing. Both species can be found in western Oregon in the winter (Green, 2018), yet there has been some dispute about whether they are actively feeding or just persist as diapausing larvae. In our trial, 35% of glassy cutworms were able to complete a molt. Most of the winter cutworms, on the other hand, perished soon after being brought into the lab environment. Further investigation is needed to elucidate the causes of mortality and how parasitism plays a role.

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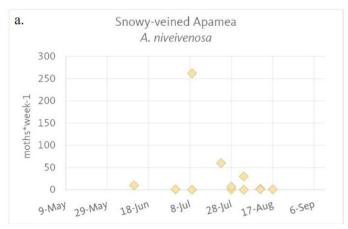
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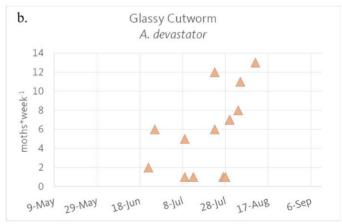


Figure 1. Activity trends of snowy-veined Apamea (a) and glassy cutworm (b), as measured by bucket traps baited with a custom-formulated lure.

Table 1. Assessment ratings of field-collected larvae from western Oregon, November–December 2020.

No. of individuals tested	Species	es Behavioral and physiological ratings <sup>1,2</sup>				Actual emergence of parasitoids	
		0	1	2	3	4	
				(%)			(count, %)
20	Glassy cutworm (Apamea devastator)	20	5	20	20	35	2, 10
7	Winter cutworm (Noctua pronuba)	57	0	0	29	14	1, 14

<sup>&</sup>lt;sup>1</sup>0 = died within 14 days; 1 = localized tissue damage, eventual death; 2 = cuticle darkening, lethargy, eventual death; 3 = continued feeding with no change in activity; 4 = successful molt <sup>2</sup>Mortality does not necessarily indicate parasitism; see text.

# CHOKE EXPRESSION IN *EPICHLOË TYPHINA* SEEDLING-INFECTED ORCHARDGRASS (*DACTYLIS GLOMERATA*) GERMPLASM

L. Merlet, B.S. Bushman, J.E. Dombrowski, and R.C. Martin

#### Introduction

Choke disease caused by *Epichloë typhina* (Pers.) Tul. & C. Tul. was first reported in Oregon in 1997 in an orchardgrass (*Dactylis glomerata* L.) stand grown for seed production (Alderman et al., 1997), and by 2003 it was reported to be present in 90% of the orchardgrass seed production fields surveyed in Oregon (Pfender and Alderman, 2006). While the fungus is innocuous most of the year, as the reproductive tillers elongate just prior to flower head emergence, a rapid and dense growth of fungus within and among leaf sheaths forms a sexual reproductive structure (stroma) that destroys, or "chokes," the host flower. This disease is estimated to cause up to 30% losses in seed yield (Pfender and Alderman, 2006).

Efforts to control this disease have focused on insect control and fungicide application to prevent fertilization/growth of the fungal stromata or to slow the growth of the fungus, stubble removal by propane burning after straw removal, and fertility management and plant growth regulators to help plants outgrow the fungus (Alderman et al., 2008). While most of these methods had no significant effect for controlling choke, burning stubble after straw removal and nitrogen fertilization efforts seemed to show some benefits. However, burning stubble did not significantly reduce choke incidence, possibly due to the high variability present in those studies.

With the high prevalence of choke and lack of control methods for this disease, Bushman and coworkers (2019) initiated efforts to identify choke-resistant orchardgrass germplasm to provide an alternative longterm approach to disease management. In the current study, we focused on screening progeny of their most promising choke-resistant germplasm using traditional methods and supplemental trials using preinoculated seedlings. Progeny from 23 of the most resistant maternal lines and two susceptible and resistant control cultivars were inoculated with E. typhina at a very early seedling stage (Feekes 1.0). These inoculated seedlings were used to search for tolerant germplasm lines that may be able to escape expression of choke symptoms even when infected with E. typhina. The noninoculated plants were used to establish flowering times for the accessions.

#### **Materials and Methods**

## Plant and fungal materials

Hand-inoculated seedlings of 23 accessions identified in previous trials as having better resistance/tolerance to choke, plus 'Potomac' and 'Baraula' as susceptible cultivars and 'Killarney' and 'Barlegro' as resistant cultivars, were utilized in this study. Accessions were assigned an ID number from #1 to #28 (#13 missing) to facilitate tracking. Based on earlier data (Bushman et al., 2019), parental lines 1–5 are early-heading lines, 6-18 are medium-heading lines, and 19-24 are lateheading lines. Of the reference varieties used, 'Baraula' (#25), 'Barlegro' (#27), and 'Killarney' (#28) are late heading, and 'Potomac' (#26) is an early-heading cultivar. Three infected plants (G4, G5, and G6) removed from two commercial orchardgrass fields were used as sources of *E. typhina* for inoculating seedlings. Epichloë typhina was isolated each week by placing surface-sterilized leaves from each infected source plant onto potato dextrose agar (PDA) plates. Cultures were transferred 9 days later and were used for inoculations the following week only in order to provide fresh cultures for all inoculations.

#### Inoculation protocol

For seedling inoculation, 15–20 surface-sterilized seeds were placed on 3% water agar in 100 mm square x 15 mm Petri dishes and were germinated at 24°C in the dark on vertically positioned plates. When seedlings were 2–5 cm long and the meristem at the junction of the mesocotyl and hypocotyl was visible under a stereo microscope, they were inoculated using a modified version of Latch and Christensen (1985). A sterile scalpel was used to make a superficial 2-3 mm longitudinal slit above the meristem. A small piece of mycelia scraped from a fresh E. typhina culture was introduced carefully into the slit near the meristem, trying to not tear the tissue. Plates were placed vertically in the dark at 24°C for at least 48 hours to facilitate growth of the fungi and were then transferred to 24-hour light/24°C conditions for about 2 days. Seedlings were transferred to Cone-tainers (Stuewe and Sons, Albany, OR) prefilled with moist Sun Gro Professional potting mix (Sun Gro Horticulture, Hubbard, OR). Seedlings were watered from below and

lightly fertilized with Technigro 20-18-20 all-purpose fertilizer (Sun Gro Horticulture, Hubbard, OR) every other week.

#### Examination for presence of Epichloë typhina

When plants were 6–7 weeks postinoculation, one of the older sheaths was examined microscopically for the presence of hyphae. Small forceps and a razor blade were used to peel a strand of the inner epidermis, which was put on a microscope slide in a drop of acidified aniline blue. Samples were mounted in a drop of water and immediately examined for presence of *E. typhina* hyphae using a binocular microscope at 200x magnification. Plants were transplanted into the field in October 2018 when they were between 2 and 3.5 months old.

# Variety trial using hand-inoculated plants (ongoing study)

A field trial was planted to evaluate the susceptibility of progeny from select genetic accessions that were previously identified as more resistant to choke. Two locations (A and B) near Corvallis, OR, were used, and each plot was divided into one section for hand-inoculated plants and one section for noninoculated plants. Within each section, there were three replicates of 23 genetic accessions and 4 control cultivars randomly arranged. In total, there were 18 preinoculated plants and 30 noninoculated plants for each of the 27 genotypes, duplicated at two locations. The two plots were planted in October 2018 and maintained following the recommended cultural practices for orchardgrass seed production.

#### Field assessment

Scoring for choke expression was performed on June 5, 2019, when all noninoculated plants were flowering. Each plant was scored (+/-) for choke incidence and anthesis: "choked" if stromata were observed on at least one tiller and "flowered" when at least one flower that would produce some seeds, either perfectly healthy or partially choked, was observed. Each plant was categorized as belonging to one of four groups: only flowered, both flowers and choked heads, only choked, or not headed.

#### **Results**

#### Infection of hand-inoculated plants

In February 2018, seven 'Potomac' plants were hand-inoculated with *E. typhina* and transferred to the greenhouse. One month later, stem sheaths of inoculated

plants were stained, and microscopically examined for the presence of hyphae. All seven plants were confirmed positive for the presence of *E. typhina*. Six months later, all tillers were examined by microscopy for the presence of hyphae. In total, 107 tillers were tested, 12–17 per plant. All tillers were positive for the presence of hyphae. Four months later, after vernalization in a cold chamber, all seven plants headed and produced only choked heads.

# Hand-inoculation success varied depending on genetic background

Overall, 2,017 out of 2,775 inoculated seedlings were still alive 6–7 weeks postinoculation, with an average survival rate of 73%. For the 27 accessions, the survival rate ranged from 55% to 94%. Among the survivors, fungal hyphae were observed in 1,228 plants when examined microscopically, which represents a 44% infection rate on average, ranging from 23% to 85% among accessions. An overview of the response of different lines to inoculation is shown in Figure 1. The infection rate among the surviving plants ranged from 34% to 90%, with an average of 61%. These plants were maintained in Cone-tainers in the greenhouse until field plots were planted.

# Characteristics of field trial locations

In October 2018, 907 inoculated plants, instead of the 972 planned ( $36 \times 27 = 972$ ), were planted at two locations, as some accessions were recalcitrant to infect and not enough plants were obtained in time. In spring 2019, 843 plants had survived and were scored. Because not enough plants could be obtained in time, some were missing at location B. Out of the 162 plants that should have been planted in each replicate, 21 were missing from the second replicate (R5) and 40 from the third replicate (R6). Coincidently, location B was flooded in April 2019, and R6 was the most affected replicate, with 40 plants in that replicate dying (a 33% mortality rate). The other 24 plants that died were distributed evenly across the remaining 5 replicates, leading to a low mortality rate (3%) in absence of the prolonged flood.

# Heading date of noninoculated plants and heading defect in inoculated plants

Most genetic lines were classified the same as their progenitors for heading class, except for lines 8, 9, 14, and 18, which were classified as early instead of medium, and 22, which was classified medium instead of late. In spring 2019, 15% of the inoculated plants showed no sign of heading. Although 91–92% of the plants headed in the first two replicates at each location,

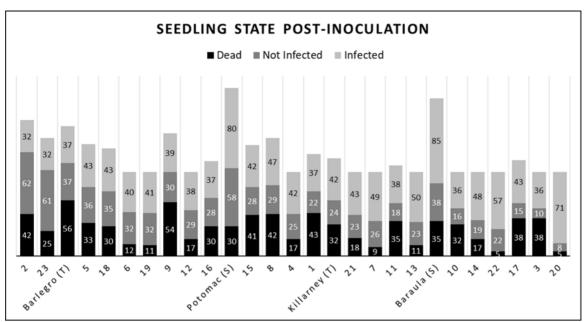


Figure 1. Response of seedlings to inoculation with *Epichloë typhina*. Number of seedlings that died (black), number of seedlings that survived but were not infected (dark gray), and number of surviving seedlings that were infected.

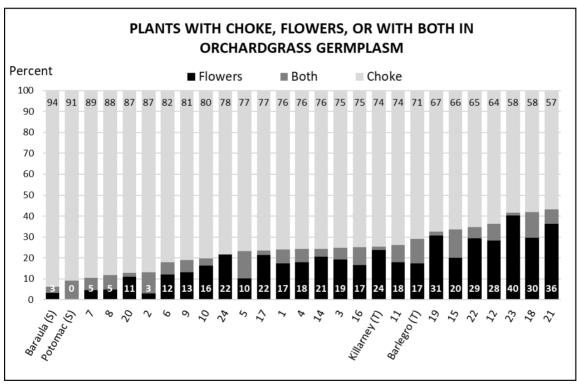


Figure 2. Percentage of hand-inoculated plants producing flowers only (values in white at the base of the columns), flowers and choke, or only choke (values in black at top of the columns) for each genetic accession (average over 2019 and 2020).

only 66% headed at location A replicate 3 (driest area of the plot) and 72% at location B replicate 6 (in the flooded area). The probability of heading was also dependent on the time of heading. Twelve percent of the plants classified as "early" did not head, while that proportion was 20% for plants from the "late" group. It is possible that some late-flowering accessions may not have finished flowering at the time of scoring in the driest (R3) and the flooded (R6) replicates.

# Choke expression in artificially inoculated plants with true systemic infection over 2 years

The overall distribution of plants that exhibited only choke, only flowers, or both is depicted in Figure 2. Among the plants that headed, 85% showed at least some sign of choke, and 24% had at least one flower head with potential for seed production. Overall, an average of 76% of all plants were completely choked, with a range of 57–94% of plants in the different accessions. Six percent of the plants, overall, showed both healthy flowers and choked heads, ranging from 0–14% in the different accessions. Interestingly, over the 2 years, an average of 18% of the plants showed only healthy flowers with no sign of stromata, with a range of 0-40% in the different germplasm lines. Early-flowering genotypes were more fully choked (81%), included more plants with both choke and flowers (9%), and had significantly fewer fully flowered heads (10%) than the medium-flowering (77%, 6%, and 17%, respectively) and late-flowering genotypes (75%, 5%, and 20%, respectively). As expected, the two susceptible cultivars were among the lines having plants with the most choked tillers, while the tolerant checks were among lines that had fewer plants with choked heads and more plants with only flowers.

#### **Discussion**

Because of the asymptomatic nature of the *E. typhina* endophyte in orchardgrass infections, the link between infection (whether hyphae are present in the plant) and expression (whether stromata are visible) is not straightforward. In a field trial, lack of visible symptoms on a plant is not sufficient to determine its infection status. Using artificially inoculated plants bypasses this issue, as it creates plants that have fungi in all of their above-ground parts, similar to vertical transmission. In the case of orchardgrass and *E. typhina*, although this artificial infection is different from natural infections, it simplifies observations. If a flower appears, it emerged from an infected tiller and was able to escape choking. This technique is ideal to detect conditions and/or tolerant genotypes that promote flower escape.

However, it is important to note that this does not necessarily equal field resistance, as overall resistance likely includes other parameters that are bypassed by hand inoculation. As an example, germination and survival of spores on the plant may differ in a greenhouse setting compared to field conditions, as well as penetration or growth of the fungus in the plant toward the meristem. In this greenhouse trial, both the hand-inoculation failure rate, which may represent the resistance of plants to fungal infection, and the appearance of fertile flowers in the field, which represents the resistance/tolerance to the fungi starting its sexual cycle, were dependent on the genetic line. This confirms that an artificial inoculation approach can be used to complement the field resistance/ tolerance trials by identifying traits impacting symptom expression in different genotypes.

The appearance of flowers in artificially infected plants was dependent on both flowering class, with more escaped flowers on later-flowering groups, and location (P = 0.06), with more escaped flowers at location B. It is possible that moist environments and later-flowering types improve the ability for flowering. Since the early 1960s, the hypothesis has been that lack or presence of choke in infected plants depends on whether or not they are able to escape infection (Kirby, 1961). The speed of growth of the fungus and its host during a precise time period was suggested to determine the probability of escape. In this experiment, plants were confirmed positive about a month before planting. However, it is possible that some plants managed to completely suppress the fungus in the year that followed. Interestingly, the area with the most escaped flowers was R6, which was the most affected by the flood. It is possible that adverse abiotic stresses (e.g., flooding) led to loss of the fungus in some plants.

When plants were artificially inoculated to have the fungus in all their tillers, most of the plants (78%) had no sign of normal flowering. This suggests that each tiller's status as choked or nonchoked depends mainly on its infection status and that conditions may have only a marginal impact on choke expression. However, the probability to choke did depend on the genetic line, with the percentage of escape ranging from 4 to 42% for the best genetic lines. This indicates that there may be a genetic basis for tolerance to *E. typhina* and the ability to flower despite its presence in orchardgrass. If these results are consistent over the years, these tolerant genotypes will prove useful toward breeding for tolerance to choke.

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# ERGOT RESPONSE OF EARLY-, MIDDLE-, AND LATE-FLOWERING KENTUCKY BLUEGRASS CULTIVARS IN CENTRAL AND EASTERN OREGON

J.K.S. Dung, Q. Cheng, and K.E. Frost

#### Introduction

Ergot can be an important disease in irrigated Kentucky bluegrass (KBG) seed production systems of central and eastern Oregon. The disease is caused by *Claviceps purpurea*, a fungus that survives as sclerotia in seed and soil. Sclerotia germinate in the spring and produce airborne ascospores, which infect grass flowers prior to fertilization and result in the formation of sclerotia instead of seed.

In some years, the timing and duration of ascospore production by the pathogen may not coincide with anthesis (the only period of host susceptibility), limiting epidemics. Kentucky bluegrass cultivars that flower before or after periods of typical peak spore production and thus escape infection could be grown as part of an ergot integrated pest management program. The objective of this study was to evaluate KBG cultivars with different anthesis periods for ergot incidence and severity under central and eastern Oregon growing conditions.

#### **Materials and Methods**

Plots of 16 KBG cultivars were established at the Central Oregon Agricultural Research and Extension Center (COAREC) and Hermiston Agricultural Research and Extension Center (HAREC) in August 2019. Cultivars were grouped into early-, mid-, and late-flowering cultivar groups according to information provided by the seed suppliers. Plots were 30 feet x 6 feet and were planted at a seeding rate of 8 lb/acre. Each plot was replicated four times, and cultivars were arranged in a randomized complete block design. The borders of the experiment area were artificially infested in October 2019 with ergot sclerotia collected from Oregon seed lots representative of the production region. Plots were irrigated, fertilized, and maintained using standard production practices for each region. A fungicide application was made at HAREC in March to manage powdery mildew, but fungicides were otherwise not applied, especially immediately before or during anthesis.

Disease incidence (the number of infected panicles) and severity (the number of sclerotia in each panicle) were determined from a random sample of 100 panicles collected from each plot at harvest. A Burkard 7-day

recording volumetric spore sampler was used to monitor airborne ascospore levels at COAREC and at a commercial perennial ryegrass seed field near Echo, OR. Burkard tape samples were analyzed using quantitative PCR (Dung et al., 2018) to determine the number of ascospores captured each day. Incidence data were subjected to a square-root transformation prior to ANOVA, and multiple comparisons were made using Tukey's test. Severity data were subjected to a Kruskal-Wallis test, and multiple comparisons were performed using a Bonferroni adjustment.

#### **Results and Discussion**

In general, most cultivars exhibited greater ergot incidence and severity at COAREC compared to corresponding cultivars at HAREC (Table 1). Significant differences in ergot incidence and severity were observed among KBG cultivars at HAREC but not at COAREC (Table 1). Differences in anthesis length, which were not recorded, may have contributed to the variation among cultivars within early-, mid-, and late-flowering groups. Further research would be needed to determine if the differences in ergot levels among cultivars within a flowering group were due to genetic/physiological resistance to ergot, environmental conditions during anthesis, or other factors.

Cultivars were grouped according to flowering period (early, mid, or late) for analysis. At COAREC, the later-flowering cultivars exhibited more infected panicles than did mid-flowering cultivars (Table 2), and most ascospores (82%) were detected at COAREC in the latter third of the spore monitoring season. At HAREC, late-flowering cultivars exhibited lower ergot incidence and severity than mid-flowering cultivars (Table 2), and spore trapping in the area indicated that fewer ascospores were present during the last third of the cropping season compared to earlier in the year. A previous study evaluating klendusity (disease escape) in perennial ryegrass cultivars at HAREC found a negative correlation between anthesis initiation date and ergot incidence (Kaur et al., 2016), also suggesting the potential for disease escape in perennial ryegrass cultivars that flower later in the season.

It should be noted that physiological resistance was not evaluated in this study and may also play a role

Table 1. Ergot incidence and severity for early-, mid-, and late-flowering Kentucky bluegrass cultivars grown in artificially infested plots in Madras and Hermiston, OR.<sup>1</sup>

Anthesis		Madras, OR	(COAREC)	Hermiston, O	R (HARAC)
period	Cultivar	Incidence	Severity	Incidence	Severity
		(%)	(no.)	(%)	(no.)
Early	A	28.3	77.5	6.3 cd	7.3 b
	В	25.5	61.0	33.3 a	71.5 a
	C	20.3	70.3	5.0 cd	6.5 b
	D	32.0	126.5	2.5 d	2.8 b
	E	20.8	86.5	17.0 abcd	23.8 ab
	F	24.0	91.5	10.0 abcd	11.8 ab
Mid	G	22.8	56.5	20.5 abcd	34.3 ab
	Н	20.3	38.0	8.8 abcd	11.0 ab
	I	29.8	117.8	35.5 abc	69.8 a
	J	15.8	50.3	30.8 ab	62.3 ab
	K	28.0	65.0	12.5 abcd	20.5 ab
Late	L	32.3	69.5	8.5 abcd	11.8 ab
	M	27.5	88.5	7.8 abcd	9.5 ab
	N	35.3	141.0	6.0 bcd	7.0 b
	O	42.8	137.3	2.5 d	3.3 b
	P	32.3	91.8	10.8 abcd	22.0 ab
	P-value	0.6773	0.718	< 0.0001	0.0009

<sup>&</sup>lt;sup>1</sup>Disease incidence (the number of infected panicles) and severity (the number of sclerotia in each panicle) were determined from a random sample of 100 panicles collected from each plot at harvest. Incidence data were subjected to a square-root transformation prior to ANOVA, and multiple comparisons were made using Tukey's test. Severity data were subjected to a Kruskal-Wallis test, and multiple comparisons were performed using a Bonferroni adjustment. Column means followed by the same letter are not significantly different at LSD (P < 0.05).

Table 2. Ergot incidence and severity for early-, mid-, and late-flowering Kentucky bluegrass cultivars grown in artificially infested plots in Madras and Hermiston, OR, and the percentage of the total number of ergot ascospores captured at one representative site in each production region.<sup>1</sup>

	Mad	ras, OR (COAF	REC)	Hermis	Hermiston, OR (HARAC)			
Anthesis period	Incidence	Severity	Spores captured at one site	Incidence	Severity	Spores captured at one site		
	(%)	(no.)	(%)	(%)	(no.)	(%)		
Early (n = 6) Mid (n = 5) Late (n = 5) <i>P</i> -value	25.1 ab 23.3 b 34.0 a 0.036	85.5 65.5 105.6 0.115	0.6 17.2 82.1	12.3 b 21.6 a 7.1 b 0.003	20.6 ab 39.6 a 10.7 b 0.006	24.6 59.9 15.6		

<sup>&</sup>lt;sup>1</sup>Disease incidence (the number of infected panicles) and severity (the number of sclerotia in each panicle) were determined from a random sample of 100 panicles collected from each plot at harvest. Incidence data were subjected to a square-root transformation prior to ANOVA, and multiple comparisons were made using Tukey's test. Severity data were subjected to a Kruskal-Wallis test, and multiple comparisons were performed using a Bonferroni adjustment. Column means followed by the same letter are not significantly different at LSD (P < 0.05).

in reducing ergot; however, ergot resistance is likely a complex and quantitatively inherited trait (Gordon et al., 2020). Regardless, the results from this and other studies suggest that flowering period could be an important trait contributing to ergot susceptibility or klendusity in KBG cultivars and that this factor could be exploited by plant breeders and grass seed growers to reduce ergot risk in grass seed crops.

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# EVALUATION OF FUNGICIDES FOR CONTROL OF POWDERY MILDEW ON KENTUCKY BLUEGRASS IN CENTRAL OREGON (2020)

J.K.S. Dung and H. Downing

#### Introduction

Powdery mildew caused by *Blumeria graminis* f. sp. *poae* is a common foliar disease in Kentucky bluegrass (KBG) seed production systems of the Pacific Northwest. In central Oregon, the disease usually appears in early spring, causing white, powdery spots on leaves that eventually turn necrotic (Butler et al., 2002). Powdery mildew is favored by relatively cool and humid conditions, and the disease tends to be more prominent in older leaves and first-year stands. Powdery mildew is primarily controlled with fungicides, of which many are labeled for use in KBG seed production (Pscheidt and Ocamb, 2020). A study was initiated in the spring of 2020 to compare the efficacy of currently labeled fungicides and fungicides that show potential for labeling in grass grown for seed.

#### **Materials and Methods**

The trial was established in a first-year commercial KBG seed production field (cv. 'Rhythm') in Madras, OR. The field was planted in August 2019 in 2.5-footwide beds (three rows of plants per bed) and was furrow irrigated. Plots were 30 feet x 10 feet with 5-foot buffers. The experimental design was a randomized complete block with four replicates and eight treatments, including a nontreated control.

Fungicides were applied when plants were 2–5 inches in height on April 8 and again at late boot to early heading on May 7. Applications were made using a CO<sub>2</sub>-charged spray boom configured with six TP8002VS flat fan nozzles spaced 18 inches apart delivering 20 gal/acre at 28 psi.

Powdery mildew severity was evaluated in five  $10\text{-ft}^2$  subplots located in the center two beds of each plot using a 0–5 ordinal scale, where 0 = no disease present, 1 = 1-10% of subplot exhibiting mildew, 2 = 11-30% of subplot exhibiting mildew, 3 = 31-70% of subplot exhibiting mildew, 4 = 71-90% of subplot exhibiting mildew, and 5 = 91-100% of subplot exhibiting mildew.

The sum of the ordinal ratings from the five subplots was used for analyses. Repeated ratings were used to calculate area under disease progress curve (AUDPC) values using the following formula:  $\sum_{i}^{n-1} ((Y_i + Y_{i+1})/2) (t_{i+1} - t_i)$ , where  $Y_i$  = cumulative disease severity at the  $i^{th}$  observation,  $t_i$  = time (days) at the  $i^{th}$  observation, and n = number of observations. The center two beds of each plot (30 feet x 5 feet) were swathed on July 10, 2020 and harvested on July 28. The seed was cleaned and conditioned using laboratory-scale seed-cleaning equipment to assess seed yield.

Disease rating and AUDPC data were subjected to analysis of variance using PROC MIXED in SAS 9.4, and treatment means were compared using Tukey's honest significant difference test ( $\alpha = 0.05$ ). Yield data were subjected to the Kruskal-Wallis test using PROC NPAR1WAY in SAS.

#### **Results and Discussion**

A significant effect of fungicide treatment was observed at all evaluation dates and for AUDPC values (P < 0.0001; Tables 1 and 2). Quilt Xcel SE, Aproach 2.08 SC, Aproach 2.08 SC + PropiMax EC (both rates), and Tilt 3.6E significantly reduced powdery mildew at all evaluation dates compared to the control. All of the fungicide products significantly reduced AUDPC values compared to the nontreated control, with the exception of Headline SC.

Although not statistically significant, fungicide treatments increased numerical yields by 41.3-73.4% compared to the nontreated control (Table 1). Interestingly, Headline SC, which did not significantly reduce AUDPC values compared to the nontreated control, exhibited the second-highest yields overall. Significant (P < 0.05) negative correlations were observed between yield and powdery mildew ratings collected on April 23, April 29, May 6, and May 14, as well as between yield and final AUDPC values, indicating reduced yields with increasing powdery mildew severity (Table 2). Plans are in place to repeat this trial at two locations in 2021.

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Table 1. Powdery mildew disease severity ratings, area under disease progress curve (AUDPC) values, and seed yields of Kentucky bluegrass following fungicide treatments.<sup>1</sup>

Treatment (rate) <sup>2</sup>	Apr. 18	Apr. 23	Apr. 29	May 6	May 14	AUDPC	Yield <sup>3</sup>
							(lb/a)
Nontreated	8.0 a	8.8 a	11.5 a	11.5 a	15.8 a	183.1 a	1,151.8
Quilt Xcel SE (14 oz/a)	3.0 bc	2.5 bc	1.3 c	0.5 c	0.8 c	31.1 bc	1,997.0
Aproach 2.08 SC (9 oz/a)	2.3 c	3.0 bc	3.8 bc	3.8 b	8.0 b	59.6 bc	1,627.6
Aproach 2.08 SC (9 oz/a)	2.8 bc	2.0 c	1.5 c	0.3 c	0.0 c	28.5 c	1,810.4
+ PropiMax EC (4 oz/a)							
Aproach 2.08 SC (6 oz/a)	3.0 bc	1.5 c	1.3 c	0.0 c	0.0 c	23.9 c	1,811.6
+ PropiMax EC (4 oz/a)							,
TebuStar 3.6L (8 oz/a)	5.3 ab	5.5 ab	4.5 bc	3.0 bc	2.3 c	83.1 b	1,805.6
Headline SC (12 oz/a)	5.3 ab	6.8 a	8.5 ab	9.3 a	10.3 b	137.9 a	1,826.7
Tilt 3.6E (4 oz/a)	4.3 bc	2.5 bc	0.5 c	0.0 c	0.0 c	27.6 c	1,747.3
<i>P</i> -value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0762

<sup>&</sup>lt;sup>1</sup>Disease rating and AUDPC data were subjected to analysis of variance using PROC MIXED in SAS 9.4 (SAS Institute, Cary, NC), and treatment means were compared using Tukey's honest significant difference test ( $\alpha = 0.05$ ). Column means followed by the same letter are not significantly different at LSD (P < 0.0001).

Table 2. Correlations and corresponding *P*-values between yield and in-season powdery mildew ratings and between yield and final area under disease progress curve (AUDPC) values.

	Apr. 18	Apr. 23	Apr. 29	May 6	May 14	AUDPC
Yield	-0.23339 $P = 0.1986$	-0.45054 $P = 0.0097$	-0.50698 $P = 0.0031$	-0.45134 $P = 0.0095$	-0.55664 P = 0.0009	-0.49051 $P = 0.0044$

<sup>&</sup>lt;sup>2</sup>All products were applied with Induce, a nonionic surfactant, at 0.25% v/v.

<sup>&</sup>lt;sup>3</sup>Yield data were subjected to the Kruskal-Wallis test using PROC NPAR1WAY in SAS. The Kruskal-Wallis chi-square statistic and corresponding *P*-value are presented.

# BIOCHAR: AN ALTERNATIVE TO ACTIVATED CARBON IN CARBON SEEDING FOR THE ESTABLISHMENT OF PERENNIAL RYEGRASS

K.M. Trippe, K.M. Meyer, D. Watts, J.M. Novak, and M. Garcia-Jaramillo

#### Introduction

The ability to produce seed crops that are free of weed seed is vital to meet consumer demand, phytosanitary restrictions, and certification requirements. In the Willamette Valley, the ongoing challenge to produce weed-free grass seed is often addressed by two strategies. The first strategy delays planting until spring, when soil temperatures warm beyond a threshold that limits germination of most weedy grasses but still favors germination of the crop. However, limited moisture and vernalization can create uneven stands or delay harvest. The second strategy, carbon seeding, allows producers to establish crops in the fall by applying a narrow band of activated carbon (AC) directly over the seed furrow, followed by treatment with a broadcast preemergent herbicide (Lee, 1973; Curtis et al., 2018). The AC provides crop safety by absorbing the herbicide, essentially deactivating it within the planting row. While this method is generally effective, the combined cost of the AC and the herbicide limits its feasibility. Methods that reduce the cost—but not the efficacy—of weed control in grass seed crops are needed.

Biochar, or charcoal that is added to soil, is produced from the combustion of low-value feedstocks, including poultry litter, forest and agricultural residues, anaerobic digestate, and others. A growing interest in the use of biochar has prompted studies that evaluate its potential to condition soil, improve crop yields, and increase soil pH. The ability of biochar to bind environmental contaminants, including aromatic compounds, heavy metals, and pesticides, has also been widely examined (Khalid et al., 2020).

In general, studies have determined that the physicochemical properties of the biochar, which are determined primarily by the feedstock and production conditions, have a profound effect on biochar performance (Khalid et al., 2020). Additionally, the adsorption of pesticides depends on the chemical properties and environmental behavior of the pesticide. For example, a previous study determined that biochar produced from macadamia nut shells at high temperatures binds indaziflam and that the binding efficiency increases as the biochar ages. However, in the same study, the binding coefficient ( $K_d$ ) for the herbicide terbuthylazine decreased with biochar

age (Trigo et al., 2014). Because biochar:herbicide interactions vary according to the physicochemical properties of the biochar and the herbicide, it is important to evaluate these interactions on a case-by-case basis (Trigo et al., 2014; Khalid et al., 2020). In some instances, the surface area and the functional capacity of the biochar approach or exceed those of AC; as such, this study evaluated whether biochar could be a substitute for AC in carbon seeding applications.

In this report, we present the preliminary results of a greenhouse study that evaluated the level of crop safety provided by barley, juniper, and a mixed-conifer biochar toward three herbicides used in carbon banding practices: diuron, indaziflam (Alion), and a mixture of flumioxazin + pyroxasulfone (Fierce). The efficacy of these biochars to provide crop safety is compared to AC. Further studies that examine the binding capacities, mechanisms, and efficacy of these biochars under field conditions are currently underway.

#### Materials and Methods

### Soil

The soil used in the study is a fine, silty, mixed, superactive, mesic Aquultic Argixerolls classified in the Woodburn series, which was collected from 0–20 cm at Hyslop Farm (Oregon State University, Corvallis, OR). The soil was air dried at room temperature for 10 days, sieved to 10 mm, and added to 25 cm x 25 cm x 6 cm black, high-density polyethylene plastic flats lined with landscape fabric to prevent soil loss. Soil was initially added to a depth of 4 cm. After seeding, 0.65 cm of the same soil (sieved to 5 mm) was placed on top of the seeds, for a final soil depth of 4.65 cm.

#### Biochar production

Barley-based biochar was produced from postharvest residue. Barley straw was collected from Hyslop Farm in the fall of 2018, baled, and transported to Florence, SC. There, the straw was mechanically reduced to pass through a 6-mm sieve. The biochar was produced by pyrolysis using a Lindburg oven with a retort at 350°C. Juniper-based biochar was produced from air-dried mill ends and kiln-dried edge strips of western juniper (*Juniperus occidentalis* Hook.), which was pyrolyzed using the flame cap method in an open-topped

trapezoidal "Oregon" kiln (Wilson Biochar Associates), according to methods previously described (Phillips et al., 2020). Previous measurements estimated that maximum temperatures in the Oregon kiln reach 650–700°C (Kelpie Wilson, personal communication). Mixed-conifer biochar (Rogue Biochar) was obtained from Oregon Biochar Solutions (White City, OR). This biochar is produced at 750–950°C in a wood-fired power plant that provides energy to a local power grid. AC (Darco GroSafe) was obtained from Nutrien Ag Solutions. Biochar properties were measured as previously described (Phillips et al., 2020). Prior to application, all charcoals (biochar and AC) were dried at 70°C for 48 hours and ground using a NutriBullet grinder to < 150 μm.

Prior to the application of biochar or AC, perennial ryegrass (*Lolium perenne* L. var. 'Morningstar', 96% germination rate) was sown at a rate of 49 seeds per flat in a 7 x 7 grid pattern (3-cm spacing) and covered with 0.65 cm soil as described above. Immediately after seeding, biochars and AC were applied as a slurry (2.21 g charcoal in 35.3 mL water) with an electric spray gun (Rexbeti 700-watt high-power paint sprayer). Charcoal was evenly sprayed over the entire soil surface of the soil flat. This amount corresponds to a rate of 300 lb/acre broadcast or 25 lb/acre in-row application. Soil flats without any charcoal treatments and flats without any herbicide treatment were seeded and plants evaluated to serve as controls. Four replicate flats were included in each treatment.

Label rates of a flumioxazin + pyroxasulfone mixture (0.104 kg Fierce/ha), indaziflam (0.07 kg Alion/ha), or diuron (2.69 kg a.i./ha) were applied to the flats in a spray booth at a 187-L/ha equivalent with the following spray booth parameters: target 25 inches below nozzle tip, fan nozzle Tee Jet 8003EVS, travel rate 2.8 mph, track path 6 feet, air pressure 40 psi. After herbicide application, the flats were distributed in a randomized block design across four benches in a glass greenhouse (18–20°C). No supplemental light was provided.

The day after herbicide treatment, each flat was watered with approximately 750 mL. For the remainder of the experiment, the flats were watered twice weekly to saturation.

The experiment started (first watering) on December 18, 2020 and ran for 45 days. Plant safety was evaluated by monitoring seedling emergence (defined as the presence

of a cotyledon above the soil surface) and mortality two or three times per week and on the day before harvest. Newly emerged or dead seedlings were counted and marked with a color-coded toothpick inserted into the soil about 1 cm from the sprout. At the end of the experiment, the surviving plants from each flat were cut at the soil surface, combined into a paper bag, dried for 48 hours at 50°C, and weighed.

Data were analyzed separately for each herbicide by analysis of variance. When effects were significant, means were separated at the 5% level using Tukey's honestly significant difference test. In the no-carbon, herbicide-treated data sets, few, if any plants survived the herbicide treatment. Because little to no variation was present in this data set, they were excluded from the statistical analysis.

#### **Results and Discussion**

Our study evaluated the potential for three biochars to provide plant safety when treated with three commonly used herbicides. In general, the survival and biomass data indicated that mixed-conifer biochar provided plant safety equivalent to that provided by AC, regardless of the herbicide tested (Figure 1A). Juniper-origin biochar provided significantly less plant safety in comparison to AC when the flumioxazin + pyroxasulfone mixture was applied. While this trend was also true for diuron and indaziflam, the reduced plant safety provided by the juniper-origin biochar was not different in comparison to AC. Barley-origin biochar provided substantially less plant safety in comparison to AC, regardless of the herbicide tested. Barley-origin biochar was particularly ineffective against indaziflam and the flumioxazin + pyroxasulfone mixture.

Few plants germinated or survived when no charcoal was applied to the soil surface prior to herbicide application (Figure 1B); conversely, germination and survival rates were similar across all treatments when no herbicide was applied (Figure 1A). These results indicate that the herbicide treatments were effective and that charcoal itself was not phytotoxic. At the end of the experiment, the biomass of the surviving plants was measured. Overall, the trends observed in the biomass data reflect the trends observed in the survival data (data not shown) and indicate that, in all cases, the performance of mixed-wood biochar was similar to AC, the performance of juniper-origin biochar depended on the herbicide, and barley-origin biochar provided substantially reduced plant safety.

Biochar has been widely studied for its ability to bind herbicides; however, most studies have evaluated biochar:herbicide interactions in the context of reducing the transport, environmental impact, or residual activity of herbicides. To our knowledge, this is the first study to evaluate whether biochar can act as a substitute for AC in carbon banding applications. The preliminary results of this study suggest that some, but not all, biochars are suitable replacements for AC.

Previous studies have determined that the binding efficiency of indaziflam to biochar is likely influenced by pH, specific surface area (SSA), and the presence of organic films on the surface of fresh biochar (Trigo et al., 2014). Similar trends for diuron:biochar interactions have also been described. However, the mechanism driving biochar sorption of diuron is thought to be related to organic carbon content of the biochar. The pH of the biochars is 10.4, 10.6, and 9.2 for mixed-conifer-, juniper-, and barley-origin biochar, respectively. Thus, biochar pH is not correlated to the ability to provision safety from the effects of indaziflam or flumioxazin + pyroxasulfone herbicides. However,

SSA correlates well with safety for all herbicides tested. The SSA of the biochars are 6.9, 79.7, and 418.3 m²/g for barley-, juniper-, and mixed-conifer-origin biochar, respectively. During the production process, the mixed-conifer biochar is exposed to high temperatures and steam, which may result in substantially higher SSA than is typical of other biochars. Indeed, the SSA of the mixed-conifer biochar begins to approach that of most ACs (approximately 1,000 m²/g), which may explain their similar behavior. A more complete characterization of the physicochemical properties of the charcoals is underway and may provide insight into their activity and behavior.

There are few viable options that reduce the cost of carbon seeding practices. Biochar is substantially less expensive than AC; at the time of publication, the cost of mixed-conifer biochar was about 55% of the cost of AC (Scott Culver and Karl Strahl, personal communication), but this biochar was as effective as AC. Future studies evaluating the efficacy of biochar in carbon seeding applications in the field are ongoing. Additional analytical studies that quantify binding

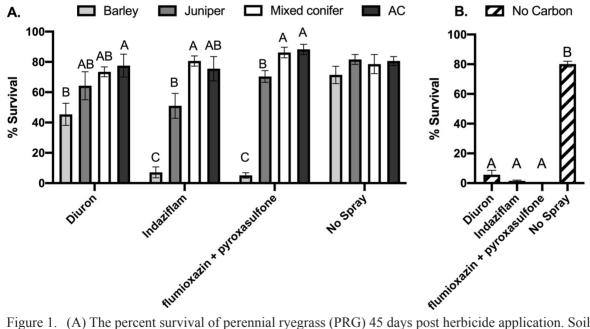


Figure 1. (A) The percent survival of perennial ryegrass (PRG) 45 days post herbicide application. Soil flats were planted with PRG and oversprayed with biochar produced from barley, juniper, or a mixed-conifer feedstock or with activated carbon (AC). Label rates of the herbicides diuron, indaziflam, and a mixture of flumioxazin + pyroxasulfone were subsequently applied. Charcoals were also applied to planted flats that did not receive an herbicidal treatment (no spray). (B) The percent survival of PRG planted without carbon (biochars or AC) 45 days post herbicide treatment. In both plots, the bars represent the mean percentage of plants surviving after 45 days (n = 4) ± standard error. Means not sharing any letter are significantly different by the Tukey HSD test at the 5% level of significance.

capacities of these herbicides to biochar are also underway. Collectively, these studies will determine which biochars are suitable for use in carbon seeding practices in the Willamette Valley.

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# AUXIN HERBICIDE SUPPRESSION OF ITALIAN RYEGRASS SEED VIABLITY IN TALL FESCUE SEED PRODUCTION

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#### Introduction

Grass seed growers in Oregon have questions regarding the control of late-season Italian ryegrass (Lolium multiflorum L.) escapes in tall fescue (Schedonorus arundinaceus (Schreb.) Dumort.) grown for seed. Once Italian ryegrass populations become established. especially multiple resistant populations, control and management are challenging in seed production cropping systems. Italian ryegrass produces 2,000– 6,000 seeds per plant, many of which enter the seed bank, intensifying the problem over time. Weed seed banks are the major source of weed population persistence. Control and management of the seed bank can reduce populations in a field; therefore, preventing seed production on late-season Italian ryegrass escapes could help to reduce the seed bank and manage herbicide resistance.

Previous research on weed species in rangelands showed that applications of synthetic auxin herbicides affected seed viability and could be used as a management tool to reduce seed production of invasive annual grasses such as downy brome (Ball, 2014; Rinella et al., 2010, 2013). In addition, annual grasses were more susceptible than perennial grasses to synthetic auxin herbicides, and sensitivity varied within and among species.

Therefore, we tested the hypothesis that an application of a synthetic auxin herbicide applied late in the growing season could reduce Italian ryegrass seed viability before entering the weed seed bank, while at the same time maintaining tall fescue seed yield and viability.

### **Materials and Methods**

#### 2017 sites

Two studies were conducted at OSU's Hyslop Experimental Farm near Corvallis, OR. One site was planted with Italian ryegrass ('Florida 80'), and the other site was in an established 2-year-old turf-type tall fescue ('Rebel XLR') stand. Average annual precipitation was 43 inches, with an average annual temperature of 54°F. A third study was conducted in a commercial 3-year-old turf-type tall fescue field ('AST 5112') infested with Italian ryegrass located north of

Dallas, OR. At this site, average annual precipitation ranged from 40 to 45 inches, with an average temperature of 54°F. Plot size was 10 feet x 34 feet at all three sites.

#### 2018 sites

Two studies were conducted at OSU's Schmidt Experimental Farm near Corvallis, OR. One site was a 3-year-old tall fescue ('Rebel XLR') stand, and the other site was planted with Italian ryegrass ('Florida 80'). The average annual precipitation is 43 inches, with an average annual temperature of 54°. A third study near Gaston, OR, was conducted in a commercial 4-year-old tall fescue ('Penn RK4') field infested with Italian ryegrass. The average annual precipitation was 45 inches, with an average annual temperature of 57°F. Plot size was 6 feet x 12 feet at all three sites.

Six synthetic auxin herbicides were applied to Italian ryegrass and tall fescue at two growth stages, BBCH 49 (boot) and BBCH 59 (anthesis) (Table 1). For the Hyslop and Schmidt Farm trials, a strip split randomized block design was used, with herbicides as the primary treatment and BBCH growth stage as the secondary treatment. For the Gaston and Dallas trials, a complete randomized block design was used. Four

Table 1. Synthetic auxin herbicides applied to Italian ryegrass and tall fescue.

Herbicide <sup>1</sup>	Trade name	Rate
		(lb ae/a)
Dicamba acid	Vision	0.89
2,4-D acid	Unison	0.98
Aminopyralid	Milestone	0.45
Dicamba + 2,4-D	Latigo	0.71 + 0.98
2,4-D + clopyralid	Unison + Stinger	0.98 + 0.27
Dicamba acid <sup>2</sup>	Vision	1.96
2,4-D acid <sup>2</sup>	Unison	1.96
Halauxifen-methyl + florasulam <sup>2</sup>	Quelex	0.36

<sup>&</sup>lt;sup>1</sup>All treatments sprayed with nonionic surfactant 0.25% v/v.

<sup>&</sup>lt;sup>2</sup>Applied only on 2018 trials.

replications were used for each treatment combination. Seeds were harvested at recommended moisture levels (Silberstein et al., 2010).

Seed viability and speed of germination were evaluated using standard seed germination tests with four replications for both Italian ryegrass and tall fescue. Viability was considered the sum of seeds that germinated after each count plus the seeds that were viable in the tetrazolium test. Seed weight of 1,000 seeds was measured with two subsamples for each replication for each species. OSU research farm trials and growers' on-farm trials were analyzed separately because of the differences in study design.

#### **Results and Discussion**

Aminopyralid was the only herbicide included in the study that reduced seed viability and seed weight (Tables 2 and 3). However, the average effect of aminopyralid on seed viability was greater in tall fescue than in Italian ryegrass (Table 2). Aminopyralid reduced seed viability of Italian ryegrass by 55% when applied at anthesis, while at the boot stage the reduction was 46%. Tall fescue had a greater sensitivity to the treatment when aminopyralid was applied at anthesis, with an average seed viability reduction of 79%, compared to 59% when applied at boot stage.

Table 2. Seed viability (%) after synthetic auxin applications to Italian ryegrass and tall fescue at boot and anthesis stages in field trials.<sup>1</sup>

		OSU research f	farm trials	
	Italian ryegras	ss seed viability	Tall fescue s	seed viability
	Boot	Anthesis	Boot	Anthesis
	(%	⁄ <sub>0</sub> )	(	%)
Control	85.9 c	85.6 cd	97.2 de	97.2 e
2,4-D	89.1 cde	86.2 cde	96.5 cde	97.8 e
Dicamba	87.5 cde	85.2 cd	96.2 cde	95.9 cde
Aminopyralid	53.8 b	42.0 b	48.5 b	23.8 a
2,4-D + clopyralid	86.0 cd	87.6 cde	94.9 cde	96.0 cde
Dicamba $+2,4-D$	87.5 cde	89.0 cde	95.5 cde	94.6 cde
Dicamba $(2x)^2$	89.0 cde	91.5 cde	93.5 cde	94.8 cde
$2,4-D(2x)^2$	96.5 e	89.8 cde	94.8 cde	94.5 cde
Halauxifen-methyl + florasulam <sup>2</sup>	94.2 cde	92.8 cde	95.8 cde	97.5 cde
		Grower fie	ld trials	
	Italian ryegra	ss seed viability	Tall fescue	seed viability
	Boot	Anthesis	Boot	Anthesis
	(%	(6)	(	(%)
Control	94.5 d	94.5 d	94.8 d	95.4 d
2,4-D	94.6 d	94.6 d	93.9 d	92.8 d
Dicamba	94.1 d	94.0 d	92.0 d	92.8 d

38.2 bc

94.4 d

95.4 d

97.2 d

98.0 d

97.0 d

43.5 c

93.1 c

95.4 d

960 d

99.5 d

99.0 d

Halauxifen-methyl + florasulam<sup>2</sup>

Aminopyralid

Dicamba  $(2x)^2$ 

 $2,4-D(2x)^2$ 

2,4-D + clopyralid

Dicamba + 2.4-D

17.8 a

91.6 d

93.1 d

95.0 d

95.5 d

96.2 d

30.0 ab

92.8 d

92.5 d

94.5 d

91.8 d

96.8 d

<sup>&</sup>lt;sup>1</sup>Means followed by the same letter within either the OSU research farm trials or within the grower field trials are not different at HSD Tukey (P < 0.05).

<sup>&</sup>lt;sup>2</sup>Applied only on 2018 trials.

Similar results were documented for seed weight reduction with aminopyralid application; however, there were no differences between species (Table 3). The average seed weight reduction in Italian ryegrass after application of aminopyralid was 42% at anthesis and 39% at boot stage. For tall fescue, the average reduction of seed weight was 47% and 46% for anthesis and boot stages, respectively. Aminopyralid affected the speed of germination by 1 or 2 days (data not shown); however,

these results were not different among the herbicides, nor were they consistent among the different trials.

Even though Italian ryegrass seed viability was reduced with aminopyralid applications, the reduction in tall fescue seed viability precludes its use for control of late-season Italian ryegrass escapes in tall fescue seed production fields. These results are in contrast to previous research, which reported that perennial grasses

Table 3. Seed weight (g/1,000 seed) after synthetic auxin applications to Italian ryegrass and tall fescue at boot and anthesis stages.<sup>1</sup>

antificsts stages.						
-		OSU research f	arm trials			
_	Italian ryegra	ss seed weight	Tall fescue	seed weight		
_	Boot	Anthesis	Boot	Anthesis		
_	(g/1,00	00 seeds)	(g/1,0	00 seeds)		
Control	2.1 f	2.2 f	2.3 f	2.4 f		
2,4-D	2.1 df	2.1 f	2.3 f	2.3 f		
Dicamba	2.1 bdf	2.1 f	2.3 f	2.3 f		
Aminopyralid	1.2 ac	1.2 ace	1.3 ace	1.2 ab		
2,4-D + clopyralid	2.1 bdf	2.1 f	2.3 f	2.2 ef		
Dicamba + 2,4-D	2.1 df	2.1 f	2.2 f	2.3 f		
Dicamba $(2x)^2$	2.0 bdf	2.0 bdf	2.0 bdf	2.1 cef		
$2,4-D(2x)^2$	2.0 bdf	2.0 bdf	2.1 bdf	2.0 cef		
Halauxifen-methyl + florasulam <sup>2</sup>	2.0 bdf	2.1 bdf	2.1 bdf	2.0 cef		
	Grower field trials					
_	Italian ryegras	s seed viability	Tall fescue se	ed viability		
_	Boot	Anthesis	Boot	Anthesis		
_	(g/1,00	00 seeds)	(g/1,00	00 seeds)		
Control	2.1 c	2.1 c	2.2 c	2.2 c		
2,4-D	2.1 c	2.1 c	2.2 c	2.2 c		
Dicamba	2.1 c	2.0 c	2.2 c	2.2 c		
Aminopyralid	1.4 ab	1.2 a	1.3 a	1.2 a		
2,4-D + clopyralid	2.1 c	2.1 c	2.2 c	2.2 c		
Dicamba $+ 2,4-D$	2.1 c	2.1 c	2.2 c	2.2 c		
Dicamba $(2x)^2$	2.0 c	2.0 bc	2.0 c	2.0 bc		
$2,4-D(2x)^2$	2.0 c	2.0 bc	2.0 c	2.0 bc		
Halauxifen-methyl + florasulam <sup>2</sup>	2.0 c	2.0 bc	2.0 c	2.0 bc		

<sup>&</sup>lt;sup>1</sup>Means followed by the same letter within either the OSU research farm trials or within the grower field trials are not different at HSD Tukey (P < 0.05).

<sup>&</sup>lt;sup>2</sup>Applied only on 2018 trials.

were generally less susceptible to synthetic auxin treatments than annual grasses (Rinella et al., 2010, 2013).

Results of the study indicate that aminopyralid applications, when properly timed, can reduce seed size and viability of Italian ryegrass, but tall fescue seed is too sensitive to permit its use during the crop season. Aminopyralid is currently registered for use in rangelands, pastures, and some noncrop areas and was previously shown to reduce the viability of invasive grass species, so it may have some applications to reduce Italian ryegrass seed production on sites other than grass seed production fields.

Because aminopyralid was the only herbicide in this study to reduce seed viability and seed weight, these results raise questions about why some synthetic auxins affect seed development while others do not and why our results do not agree with previous reports that perennial species are more tolerant to these herbicides. Different synthetic auxin molecules may have different auxin receptors involved in the mechanism affecting seed viability. Additional studies should be conducted with synthetic auxin herbicides that were not tested in this study.

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The full results of this research can be found in the following publication: Bobadilla, L.K., A.G. Hulting, D.W. Curtis, C.A. Mallory-Smith. 2020. Application of synthetic auxin herbicides to suppress seed viability of Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) in tall fescue seed production. Weed Tech. 34:489–497.

# SUMMER ANNUAL GRASS WEED CONTROL DURING ESTABLISHMENT OF KENTUCKY BLUEGRASS GROWN FOR SEED

D.L. Walenta and B. Merrigan

#### Introduction

Summer annual grass weeds, such as witchgrass (*Panicum capillare*) and green foxtail (*Setaria viridis*), are persistent problems during the establishment year of Kentucky bluegrass (KBG) seed production fields in the Grande Ronde Valley of northeastern Oregon. Herbicide options are limited for control of summer annual grass weeds in new KBG stands, in particular, for use during the early stage of stand establishment (before KBG develops six or more tillers). Mesotrione (Callisto) and flucarbazone-sodium (Everest 2.0) are registered for use in seedling KBG, providing two options for control of various broadleaf and grass weed species.

Mesotrione provides both selective contact and residual control either as a pre- or postemergence application but is limited to a maximum rate of 6 oz/acre per application and a maximum rate of 9 oz/acre/year. Previous research in seedling KBG indicates that mesotrione provides good postemergence control of witchgrass (WG) at 3- or 6-oz/acre application rates (Ball and Bennett, 2009).

Flucarbazone provides only postemergence control, and, due to soil residual concerns, its use is restricted in annual KBG seed production systems. East of the Cascade Mountain Range it is restricted to use only during the year of KBG establishment. Only one application is allowed per year at a maximum rate of 1 oz/acre. Previous research indicates fair to good control of WG with pre- and/or postemergence applications of flucarbazone

at 0.3 or 0.6 oz/acre (Ball and Bennett, 2009).

A trial was conducted in spring/ summer 2020 to evaluate crop tolerance and potential for longterm WG control in seedling KBG by using split applications (pre- and postemergence) of mesotrione and flucarbazone. Pendimethalin (Prowl H2O) at 5 pt/acre was also evaluated for preemergence WG control later in the growing season after KBG seedlings had developed six or more tillers. Mesotrione and flucarbazone split application timings and rates utilized in this study were for experimental purposes only and should not be considered recommendations for commercial use.

#### **Materials and Methods**

The trial was located in an irrigated commercial field of 'Wildhorse' KBG in the Grande Ronde Valley, Union County, OR. The new stand of KBG was seeded at a rate of 3.5 lb/acre on February 28, followed by a spring pea cover crop at 70 lb/acre. Preemergence (PRE) treatments were applied prior to WG emergence, when KBG seedlings were in the three- to five-leaf stage. Postemergence (POST) treatments were applied to WG seedlings when KBG seedlings were in the 5+ tiller growth stage. Environmental conditions at the time of herbicide application are summarized in Table 1, and herbicide rates and timings are provided in Table 2.

Plots were 8 feet x 25 feet and were arranged in a randomized complete block design with four replications. All herbicide treatments were applied with a hand-held CO<sub>2</sub> sprayer delivering 21 gpa at 32 psi. To minimize drift potential, TeeJet air-induction extended-range (AIXR) 11002 nozzle tips were used for all applications. Nonionic surfactant at 0.25% v/v was added to all POST treatments except treatment 8 (pendimethalin). All mesotrione treatments received an herbicide activator/deposition aid/water conditioner (Hel-fire) at 2 qt/100 gal water. A general broadleaf herbicide cover spray including pyrasulfotole +

Table 1. Crop growth stage and environmental conditions at time of herbicide application to seedling Kentucky bluegrass.

	Apr. 28, 2020	Jun. 5, 2020
Application timing	Preemergence (PRE)	Postemergence (POST)
KBG growth stage	1.5 to 2 leaf	5–10 tillers
Witchgrass growth stage	None emerged	$1-4 \text{ leaf}$ , $2\frac{1}{2}$ to $3\frac{1}{2}$ in
Air temperature (°F)	62	62
Relative humidity (%)	61	60
Cloud cover (%)	Zero	80
Wind velocity (mph)	0–3 mph from NW	0–2 mph from NE
Soil temperature, surface (°F)	62	77
Soil temperature, 1 inch (°F)	60	73
Soil temperature, 2 inch (°F)	60	72
Soil temperature, 4 inch (°F)	64	65

bromoxynil (Huskie) at 15 oz/acre + bromoxynil (Maestro) at 8 oz/acre was applied to the entire trial site on June 17. Visual evaluations of crop injury and WG control were made June 17, June 27, July 16, and August 13. Seed yield was not determined.

#### **Results and Discussion**

WG control evaluation results are summarized in Table 2. Mesotrione applied as a PRE- plus POSTemergence split application provided 87-100% WG control throughout the duration of the trial (10 weeks). Similarly, mesotrione applied PRE to WG, followed by pendimethalin applied at the POSTemergence application timing, also provided 93-100% WG control that lasted until trial termination on August 13. Regardless of application rate, stand-alone mesotrione treatments provided 84-100% WG control through late June, but control declined by August 13. No discernable differences were observed between the 5- and 6-oz/acre mesotrione rates applied preemergence to WG. All mesotrione treatments provided excellent broadleaf weed and cover crop pea control. Split application of flucarbazone did not provide acceptable WG control in this trial, a result that differs from previously reported results (Ball and Bennett, 2009). Crop injury was not observed from any treatments during the course of this trial, which is consistent with

previously reported results (Ball and Bennett, 2007; Ball and Bennett, 2009). Further investigation of mesotrione split application rates is warranted to optimize summer annual grass weed control in spring-seeded stands of KBG.

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Table 2. Witchgrass control with pre- and postemergence herbicide applications in seedling Kentucky bluegrass in the Grande Ronde Valley of northeastern Oregon, 2020.<sup>1</sup>

			Witchgrass control				
Treatment	Application rate <sup>2</sup>	Timing	Jun. 17	Jun. 27	Jul. 16	Aug. 13	
	(product/a)			(0/	(o)		
Control	_	_	0 c	0 c	0 c	0 c	
Mesotrione //	5 oz	PRE	100 a	99 a	88 a	87 ab	
mesotrione	5 oz	POST					
Mesotrione +	6 oz	PRE	100 a	94 a	68 a	78 ab	
bromoxynil	2 pt						
Mesotrione	6 oz	PRE	100 a	85 a	56 ab	71 ab	
Mesotrione	5 oz	PRE	100 a	84 a	63 ab	55 b	
Flucarbazone //	0.5 oz	PRE	67 ab	35 b	24 bc	0 c	
flucarbazone	0.5 oz	POST					
Mesotrione //	6 oz	PRE	100 a	97 a	93 a	97 a	
pendimethalin	5 pt	POST					
LSD (0.05)	<u> </u>		35	22	41	40	

<sup>&</sup>lt;sup>1</sup>Numbers followed by the same letters are not significantly different by Tukey's HSD all-pairwise comparisons test

<sup>&</sup>lt;sup>2</sup>Application rates for mesotrione and flucarbazone = fluid ounces/acre.

# SULFOSULFURON FOR CONTROL OF ROUGHSTALK BLUEGRASS DURING KENTUCKY BLUEGRASS STAND ESTABLISHMENT

J.F. Spring and R.P. Affeldt

#### Introduction

Kentucky bluegrass and roughstalk bluegrass (Poa trivialis) have both been successful seed crops in central Oregon for decades, despite the fact that most Kentucky bluegrass markets tolerate little or no seed contamination from roughstalk bluegrass. While volunteer roughstalk bluegrass is a common weed in seedling Kentucky bluegrass in the region, it has been successfully controlled with the ALS-inhibitor (Group 2) herbicide primisulfuron, formulated as Beacon and as a premix with dicamba as NorthStar. Production of primisulfuron was discontinued by the primary registrant Syngenta in 2018 and has been unavailable for several years. Registration of a replacement herbicide for primisulfuron is necessary to maintain the viability of high-value Kentucky bluegrass seed production in many fields in central Oregon and is an important need for other production regions as well. Sulfosulfuron (Outrider), also an ALS-inhibitor herbicide, was identified as a likely candidate for this use in preliminary greenhouse (Jeliazkova et al., 2019) and field (Jeliazkova et al., 2020) experiments. Field trials were established in newly seeded irrigated Kentucky bluegrass stands in Jefferson County, OR, for the 2020 crop year to replicate these findings, to test a wider range of use patterns, and to generate further data to support 24c SLN registration efforts for this use in Oregon.

#### **Materials and Methods**

Field trials were established in four commercial Kentucky bluegrass seed production fields in Jefferson County, OR, with varieties 'Kelly', 'Wildhorse', 'Shamrock', and 'Rockstar' (same order as sites in Figure 1). Stands were established with standard production practices in August 2019. After bluegrass emergence, trials were established in a randomized complete block design with four replications and individual plot size of 10 feet x 30 feet. Sites were chosen in three fields with roughstalk bluegrass populations and in a fourth known to be free from the weed. Outrider (sulfosulfuron) was applied at several rates and at three application timings: fall, spring, or split-applied in both fall and spring (Figure 1). Results were compared to the industry standard use of splitapplied Beacon (primisulfuron). Applications were made with a CO<sub>2</sub>-powered backpack sprayer delivering 15 gpa through four 110025 Greenleaf TurboDrop air induction nozzles at 32 psi. All treatments were applied with MSO at 1% v/v and liquid AMS at the equivalent of 8.5 lb AMS/100 gal.

Fall herbicide applications were made in late October to early November, when Kentucky bluegrass was at the two- to four-tiller stage and roughstalk bluegrass had three to six tillers. Spring applications were made in early April, within a week of the first irrigation of the year. All other management matched standard production practices in the rest of the field.

Crop safety and weed control were rated in late April and at Kentucky bluegrass heading in early June. Crop injury and weed control in April were rated on a percent scale from 0 to 100, with no effect at 0 and complete plant death at 100. In June, assessment of individual roughstalk bluegrass plants was extremely challenging, so ratings were made on a categorical abundance scale. At crop maturity, a 6-foot x 27-foot portion of each plot was swathed, allowed to dry in the field for 2 to 5 days, and threshed with a plot combine. Samples were further processed with experimental-scale cleaning equipment (stationary thresher, brush debearder, air screen) to clean seed yield at 22-23 lb/bu and 98-99% purity. Data were analyzed with ANOVA (yield) in base R software or via beta regression (injury and control) with the R package betareg. Consistency with model assumptions was confirmed via examination of residual and quantile-quantile plots. Tukey's multiple comparison procedure was conducted with emmeans, and letter displays were generated with *multcomp*. Data plots were generated with ggplot2, and summaries were derived with ggpubr.

#### **Results and Discussion**

#### Roughstalk bluegrass control

Roughstalk bluegrass was present at three of the four sites. In late April, a fall-only application of Outrider at 0.76 oz/acre controlled roughstalk bluegrass at all three infested sites as well as the standard split application of Beacon (Figure 1, bottom). Control was comparable to Beacon with 0.38 oz/acre of fall-applied Outrider at two of these sites and more variable (resulting in lower average control) at the third. Outrider applied in the

spring did not control large, overwintered roughstalk bluegrass plants. Split applications of Outrider provided good control at all but the lowest rate, matching or exceeding the performance of Beacon.

However, by crop heading in early June, some severely injured roughstalk bluegrass plants recovered and produced seed heads. This result was unexpected, and by the time it became apparent, accurate evaluation was not possible due to inability to confidently identify

individual plants in mature grass stands. From the extremely limited data that were collected, it appears that control from fall-only Outrider was probably slightly less than from Beacon, and control from split applications was slightly greater (data not shown). No treatment (including Beacon) entirely prevented roughstalk bluegrass head production at any site. It is emphasized that this observation is tentative at best and requires further confirmation before confident conclusions can be made.

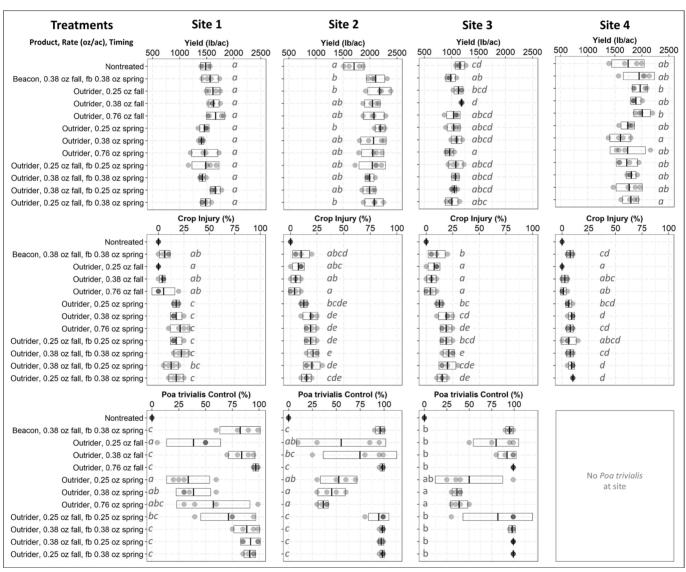


Figure 1. Experimental treatments and results for four trial sites in new stands of irrigated Kentucky bluegrass in central Oregon, 2020. Yield is in pounds clean seed per acre (approximate bushel weight 23 lb, 98–99% purity). Kentucky bluegrass crop injury and roughstalk bluegrass (*Poa trivialis*) control were evaluated in late April (crop in early boot stage) on a percent scale from 0 (no visible injury) to 100 (complete plant death). Individual observations are plotted as points; treatment mean (cross hatch) and accompanying 89% confidence intervals (box outline) are also indicated. Within a plot, treatments followed by the same number are not significantly different by Tukey's multiple comparison procedure (α = 0.05).

## Kentucky bluegrass crop safety

In April, safety on Kentucky bluegrass was good for fall applications of Outrider at all sites and rates. Crop injury was less than injury from Beacon at two sites and equivalent at the others (Figure 1, middle). In contrast, spring applications of Outrider resulted in numerically higher levels of injury at three of the four sites, particularly at higher rates, although injury was statistically greater than that with Beacon at only one site. Split applications of Outrider had injury similar to spring applications. At three of the four sites, mean crop injury reached 20% or more from spring-only and split applications of Outrider. While injury for all treatments declined over the remainder of the growing season, relative patterns remained the same through crop heading (data not shown).

# Kentucky bluegrass yield

Yield data were somewhat variable (Figure 1, top), and meaningful statistical separation was not evident between treatments. At three of the four sites, inspection of the raw data indicate yields with fall-only Outrider treatments similar to the Beacon standard. At two of these sites, yield of spring-only and split applications was numerically lower than for the Beacon/fall Outrider group of treatments; at the third it was similar. At site 3, no clear pattern is evident.

# **Conclusions**

Fall + spring applications of Outrider provided the best control of roughstalk bluegrass but with excessive risk for crop injury. Spring-only applications of Outrider also appear to have excessive crop injury potential, as well as poor control of roughstalk bluegrass. Given the injury observed from these treatments, it is unlikely that they will be suitable for general use.

When applied in the fall only, Outrider at 0.76 oz/acre provided roughstalk bluegrass control nearly equivalent to Beacon, and 0.38 oz/acre gave slightly lower, but still useful, control. Crop safety was good with both rates. At this time, it appears that fall applications of Outrider at 0.38–0.76 oz/acre offer a workable replacement for Beacon for control of roughstalk bluegrass in irrigated seedling Kentucky bluegrass. In fields with heavy weed pressure, split applications may represent a useful salvage treatment when expected economic loss from contamination exceeds the possible yield reduction (about 20%) from herbicide injury. Discussion is ongoing with the registrant (Valent USA) and the Oregon Department of Agriculture regarding the potential for 24c SLN labeling for this use.

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Note: The rights to primisulfuron and associated products were purchased by Gowan USA in late 2020, but it is unclear when—or even if—24c Special Local Needs labels will be renewed for use of new primisulfuron products in Kentucky bluegrass in Oregon. The original SLN labels were approved prior to the EPA requirement for established federal feeding tolerances for all products labeled in grass seed crops. Primisulfuron does not have established feeding tolerances, which could prevent, or substantially delay, the return of primisulfuron to the Kentucky bluegrass market. As sulfosulfuron already has established feeding tolerances, it could quite feasibly receive 24c labeling prior to renewal of primisulfuron labels and serve at the very least as a valuable interim substitute, if not an outright replacement. Continued development and registration efforts for Outrider remain pertinent at the time of writing and are ongoing.

# EVALUATION OF EXPERIMENTAL HERBICIDES IN SEEDLING RED AND WHITE CLOVER GROWN FOR SEED

C.A.C.G. Brunharo, K.C Roerig, A.G Hulting, and C.A. Mallory-Smith

#### Introduction

Clover grown for seed is an important crop in Oregon's Willamette Valley. Oregon produces approximately 89% and 68% of the nation's red clover and white clover seed, respectively (USDA-NASS, 2020). Weed interference may reduce crop yield. Furthermore, for certified product, seed quality and purity dictate the marketability of the product, as stringent certification requirements must be met. In this context, weed control becomes crucial to prevent crop loss, weed seed contamination, and depreciation of the clover seed.

Weed control in clover seed crops is achieved with a combination of pre- and postemergence herbicide applications, with the former typically applied in late fall/early winter and the latter applied during winter dormancy. This approach allows the use of postemergence, burn-down herbicides, which can be very effective for control of most weed species. It also allows for the recovery of the clover plants, as herbicide translocation and crop damage are minimized due to reduced metabolism during dormancy. Postemergence herbicide applications, after clover crop growth resumes, may have additional weed-control benefits such as targeting summer annual species.

The dormant application is particularly attractive when winter annual weed species germinate November—February and have the potential to set seed during the summer if not controlled. It also is beneficial to choose a burn-down herbicide that could provide some residual activity for the remainder of the growing season, particularly for control of broadleaf weed species.

The objective of this study was to test potential postemergence burn-down herbicides in seedling clover seed crops in western Oregon. We also included a preemergence herbicide that has shown potential to control plantain species (*Plantago* spp.). We focused our efforts on comparing herbicide products that are currently registered for clover seed crops, but also identified new products to evaluate efficacy and possible candidacy for future registrations. Of the herbicides tested in this work, only flumioxazin and paraquat are currently registered for use in clover grown for seed, and the results from this research should not be used as recommendations for commercial use.

#### **Materials and Methods**

The field experiments were performed in the 2019–2020 season at the Oregon State University Schmidt Research Farm, near Corvallis, OR. New clover stands were planted on September 25, 2019 at a planting rate of 13.5 lb/acre on 7.5-inch row spacing. Plots were 8 feet x 35 feet. Study design was a randomized complete block with four replications.

Several herbicides were selected for these two studies lin seedling red clover (Table 1) and white clover (Table 2). An application timing factor (Tables 1 and 2) was also included in the treatments to quantify the response of clover. Herbicide treatments were applied with a customized bicycle sprayer equipped with air induction nozzle tips (AIXR 11003) calibrated to deliver 20 gal/acre. Application dates were November 19 (A), January 20 (B), March 19 (C), and May 11 for red clover and April 20 for white clover (D). In the red clover trial, the last application timing occurred immediately after chopping the red clover for forage.

Weed-control evaluations were performed in January and March for red clover and in May for white clover, using a 0–100% scale, where 0% represents absence of weed control and 100% represents complete control. Crop injury was also evaluated using the same scale. Red clover was harvested on August 19, and white clover was harvested on August 13, 2020 with a small-plot combine. Harvested seed was conditioned to determine clean seed yield.

#### **Results and Discussion**

# Seedling red clover

Seedling red clover exhibited excellent crop tolerance to most of the herbicide treatments tested. We observed increased injury for the May application, when some of the treatments exhibited as high as 35% crop damage (Table 1).

We were not able to analyze the crop yield statistically even after data transformation, as data did not meet ANOVA assumptions; thus, data are provided as means without multiple comparisons. The crop yield data seem to reflect the injury observed on June 23, in that

Table 1. Weed control and crop safety of experimental herbicides applied between November 19, 2019 and May 11, 2020 in seedling red clover.<sup>1</sup>

					ntrol 6, 2020)	Red c	lover
Treatment	Rate	Unit	Date applied	Shepherd's purse	Desert rock purslane	Injury (Jun. 23)	Seed yield (Aug. 19)
				(%)	(%)	(%)	(lb/a)
Untreated	_		_	0 e	0 f	0 b	437
Norflurazon	0.49	lb ai/a	Nov. 19	28 d	18 ef	0 b	453
Norflurazon	1	lb ai/a	Nov. 19	63 bc	45 cde	0 b	426
Norflurazon	1	lb ai/a	Jan. 20	25 d	25 ef	0 b	443
Norflurazon	1.97	lb ai/a	Jan. 20	25 d	18 ef	0 b	463
Pyridate	0.94	lb ai/a	Jan. 20	20 d	25 ef	0 b	450
+ COC	1	% v/v	Jan. 20	20 u	23 C1	0 0	430
Paraquat	0.5	lb ai/a	Jan. 20	58 bc	73 bc	0 b	434
+ NIS	0.25	% v/v	Jan. 20	30 DC	75 00	0 0	7.7
Saflufenacil	0.23	lb ai/a	Jan. 20	93 a	83 ab	10 b	325
+ MSO	1	% v/v	Jun. 20	)5 u	05 40	10 0	323
+ AMS	1.67	lb ai/a					
Flumioxazin	0.128	lb ai/a	Jan. 20	100 a	100 a	0 b	424
+ MOS	0.25	% v/v	Jun. 20	100 u	100 a	0 0	727
Pyridate	0.23	lb ai/a	Mar. 19	0 e	0 f	5 b	471
+ COC	1	% v/v	iviui. 17	0 0	0 1	5 0	7/1
Paraquat	0.5	lb ai/a	Mar. 19	68 b	68 bcd	8 b	370
+ NIS	0.25	% v/v	17141. 19	00 0	00 000	0 0	370
Saflufenacil	0.0445	lb ai/a	Mar. 19	63 bc	63 bcd	10 b	350
+ MSO	1	% v/v	17141. 17	03 00	03 0 <b>cu</b>	10 0	330
+ AMS	1.67	lb ai/a					
Flumioxazin	0.128	lb ai/a	Mar. 19	43 cd	43 de	3 b	423
+ NIS	0.25	% v/v	17141. 17	15 64	15 40	5 0	123
Pyridate	0.94	lb ai/a	May 11	_		0 b	463
+ COC	1	% v/v	1,14,5			0 0	.02
Paraquat	0.5	lb ai/a	May 11			10 b	454
+ NIS	0.25	% v/v	1,14,5			100	
Saflufenacil	0.0445	lb ai/a	May 11	_		35 a	401
+ MSO	1	% v/v	1,14,5			20 4	.01
+ AMS	1.67	lb ai/a					
Flumioxazin	0.128	lb ai/a	May 11	_	_	5 b	377
+ NIS	0.25	% v/v					- , ,
LSD P = 0.05	<del></del>	—	_	16	21	6	
CV			_	26	34	87	

 $<sup>^{1}</sup>$ Means followed by the same letter are not different at LSD (P = 0.05, Student-Newman-Keuls). Multiple comparison analysis was not performed for crop yield. Weeds were not evaluated for treatments 14–17, as the crop canopy closed and weeds were suppressed.

treatments unaffected by the herbicide applications yielded approximately 450 lb/acre (Table 1).

The predominant weed species in the red clover site were Shepherd's purse and desert rock purslane. Norflurazon (Solicam), which is a preemergence herbicide, exhibited poor control of both weed species.

Treatments containing saflufenacil (Sharpen) and flumioxazin (Chateau) were superior for Shepherd's purse and desert rock purslane control.

# Seedling white clover

More variation in crop response was observed in the seedling white clover trial (Table 2). For this

Table 2. Weed control and crop safety of experimental herbicides applied between November 19, 2019 and April 20, 2020 in seedling white clover.<sup>1</sup>

					White	clover
Treatment	Rate Unit Date appl	Date applied	Prickly lettuce control (May 20, 2020)	Injury (Jun. 23)	Seed yield (Aug. 13)	
				(%)	(%)	(lb/a)
Untreated	_	_	_	0 d	0 c	392 ab
Norflurazon	0.49	lb ai/a	Nov. 19	0 d	0 c	397 ab
Norflurazon	1	lb ai/a	Nov. 19	0 d	0 c	416 ab
Norflurazon	1	lb ai/a	Jan. 20	25 cd	0 c	505 a
Norflurazon	1.97	lb ai/a	Jan. 20	0 d	0 c	394 ab
Pyridate	0.94	lb ai/a	Jan. 20	23 cd	0 c	422 ab
+ COC	1	% v/v				
Paraquat	0.5	lb ai/a	Jan. 20	95 a	0 c	367 b
+ NIS	0.25	% v/v				
Saflufenacil	0.0445	lb ai/a	Jan. 20	100 a	38 a	307 bc
+ MSO	1	% v/v				
+ AMS	1.67	lb ai/a				
Flumioxazin	0.128	lb ai/a	Jan. 20	95 a	0 c	386 ab
+ MOS	0.25	% v/v				
Pyridate	0.94	lb ai/a	Mar. 19	65 ab	0 c	427 ab
+ COC	1	% v/v				
Paraquat	0.5	lb ai/a	Mar. 19	100 a	3 c	367 b
+ NIS	0.25	% v/v				
Saflufenacil	0.0445	lb ai/a	Mar. 19	100 a	25 b	245 c
+ MSO	1	% v/v				
+ AMS	1.67	lb ai/a				
Flumioxazin	0.128	lb ai/a	Mar. 19	88 a	0 c	397 ab
+ NIS	0.25	% v/v				
Pyridate	0.94	lb ai/a	Apr. 20	45 bc	0 c	415 ab
+ COC	1	% v/v				
Paraquat	0.5	lb ai/a	Apr. 20	96 a	0 c	375 b
+ NIS	0.25	% v/v				
Saflufenacil	0.0445	lb ai/a	Apr. 20	98 a	23 b	296 bc
+ MSO	1	% v/v				
+ AMS	1.67	lb ai/a				
Flumioxazin	0.128	lb ai/a	Apr. 20	90 a	3 c	362 b
+ NIS	0.25	% v/v			_	
LSD P = 0.05	_			27	9	76
CV	_	_	_	31	114	14

 $<sup>^{1}</sup>$ Means followed by the same letter are not different at LSD (P = 0.05, Student-Newman-Keuls).

crop, high injury levels were observed in the March evaluation (data not shown). However, plants recovered for the most part before the June visual assessment. Interestingly, visual crop injury did not reflect the differences observed in seed yield. Treatments with saflufenacil reduced crop yield.

Most of the treatments controlled wild carrot, except those containing norflurazon (data not shown). In addition, reduced wild carrot control was observed for pyridate (Tough) regardless of application timing.

#### Conclusion

This study highlights the potential of new herbicide chemistries to be assimilated into clover seed production systems in western Oregon. For example, flumioxazin is currently registered only for established red and white clover. However, our results suggest flumioxazin should be considered for registration for dormant

applications to seedling red and white clover. Similarly, we observed that saflufenacil has excellent crop safety when used as a postemergence burn-down herbicide in dormant seedling red clover. The OSU Weeds program is currently repeating these experiments, and additional data will help support future herbicide registration efforts in Oregon clover grown for seed.

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[USDA-NASS] U.S. Department of Agriculture National Agricultural Statistics Service. 2020. Oregon Agricultural Statistics. https://www .oregon.gov/oda/shared/Documents/Publications /Administration/ORAgFactsFigures.pdf.

#### Acknowledgments

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# SEED YIELD PERFORMANCE AND FLOWERING INITIATION OF TWELVE RED CLOVER VARIETIES (YEAR 2)

N.P. Anderson, R.L. Wilson, and B.C. Donovan

#### Introduction

Forage legume seed crops, such as red clover (*Trifolium pratense* L.), continue to be a vital part of seed production enterprises and valuable rotation crops for grass seed and cereal crops grown in Oregon. Red clover, a biennial seed crop, is the most widely grown legume species in Oregon. According to OSU Extension seed crop estimates, the estimated value of red clover seed produced in Oregon in 2019 was approximately \$12.5 million, with 17,770 acres harvested (Anderson, 2020). Consistent seed sales and relatively stable prices have allowed this crop to be a profitable rotation in Oregon field cropping systems for many years.

The most commonly grown clover variety in Oregon is 'Medium Red'. While its origins are speculated, this variety has not been recognized as a certified variety for many years. It has high yield potential, possibly due to environmental adaptation, but does not always fulfill the highest quality and performance characteristics desired by end users. Breeding efforts in the U.S. and elsewhere have resulted in the release of new genetic material, but seed yield potential for many of these varieties is unknown, and seed growers are hesitant to plant them.

The objective of this 2-year study was to measure the seed yield potential of 12 red clover varieties. 'Medium Red' and another historically common variety ('Kenland') were used as control treatments. We also evaluated percent bloom from early inflorescence emergence to harvest in order to better understand flowering length and crop maturity differences among varieties. Results from the second year of this study are presented in this report.

Results from year 1 of this study indicated that four varieties ('Redomon', 'Secretariat', 'Dynamite', and 'DLFPS-102/3') produced significantly higher seed yields compared to 'Kenland', while 'Dynamite' was the only variety that produced a significantly higher seed yield (14%) than 'Medium Red' (Anderson et al., 2020). All other varieties produced seed yields equal to or lower than the controls.

### **Materials and Methods**

The field trial was established at OSU's Hyslop Crop Science Research Laboratory in the fall of 2018. The first- and second-year seed harvests occurred in 2019 and 2020, respectively. Plot size was 8 feet x 40 feet. The experimental design for this trial was a randomized complete block with four replications. In addition to the two controls, ten proprietary varieties were entered from seven different seed companies.

The following red clover varieties were included as treatments:

- 'Medium Red' (control)
- 'Kenland' (control)
- 'Blaze'
- 'Vulcano'
- · 'Freedom! MR'
- · 'Redomon'
- · 'CISCO'
- · 'Relish'
- · 'FS3662'
- · 'Secretariat'
- · 'Dynamite'
- · 'DLFPS-102/3'

Routine herbicide, molluscicide, and insecticide treatments were applied to manage pests as needed. Spring nitrogen was applied to plots at a rate of 20 lb N/acre. All plots were flailed to a height of 2–3 inches on May 7 and in the reverse direction on May 15. When regrowth reached the two-node growth stage (BBCH 32), trinexapac-ethyl plant growth regulator (Palisade EC) was applied at a rate of 2.5 pt/acre. Four inches of irrigation water was applied on June 18. Pollination was aided by honeybee hives placed nearby and by the presence of native bumblebees.

Above-ground biomass samples were taken from each plot near crop maturity, and dry weight of the standing crop was determined. Inflorescence number and number of florets/inflorescence were determined from the above-ground biomass samples.

Plots were swathed with a modified John Deere 2280 swather and combined with a Hege 180 plot combine. Subsamples of harvested seed were collected from each plot and were cleaned using a Clipper M2B

cleaner to determine cleanout percentage and clean seed yield. Seed weight was determined by counting two 1,000-seed samples with an electronic seed counter and weighing these samples on a laboratory balance. Harvest index (HI), the ratio of seed yield to aboveground biomass, was also quantified.

#### **Results and Discussion**

In this second-year trial, seed yields from 'Medium Red' and 'Kenland' were 847 and 674 lb/acre, respectively (Table 1). Two varieties, 'Secretariat' and 'Dynamite', produced higher seed yields compared to 'Kenland'. Both of those varieties also produced seed yields that were significantly equal to 'Medium Red', but no varieties produced higher yields than 'Medium Red'. All other varieties produced seed yields equal to or lower than the controls.

The two varieties with the highest seed yields, 'Secretariat' and 'Dynamite', had significantly equal seed numbers compared to the two controls (Table 1). There were mixed effects on seed weight, with some varieties producing lower seed weights compared to the controls and some having higher seed weights. Inflorescences from all varieties contained floret numbers that were equal to or less than 'Kenland'. Three varieties, including 'Vulcano', 'Redomon', and 'Secretariat', produced a greater number of florets compared to 'Medium Red'. There were no differences among varieties in cleanout, above-ground biomass, inflorescence number, or HI.

Flowering initiation varied among varieties (Table 2). There was no obvious trend that would indicate a relationship between flowering initiation and seed yield. There were some differences in percent flowering near the end of bloom; however, only one variety, 'Secretariat', reached full bloom earlier than all other

varieties. 'Vulcano' and 'Redomon' had a longer bloom period, but this did not appear to aid in achieving higher seed yield compared to either control.

Results of this 2-year study indicate that there are several varieties, including 'Secretariat' and 'Dynamite', that have seed yield potential equal to or better than 'Medium Red', in both the first and second years of production. This study provides some evidence that, while local adaptation to environment might be a factor in the historically strong yield performance shown by 'Medium Red', there are newer proprietary varieties that can perform just as well. These results are encouraging and suggest that the Oregon clover seed industry may have opportunities to produce varieties with improved end-use quality and performance characteristics, without giving up seed yield potential.

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The authors thank the Oregon Clover Commission and the seed companies for the funding provided to carry out this work. We especially appreciate the collaboration between private and public entities who participated in the project.

Table 1. Second-year seed yield, yield components, and growth characteristics of 12 red clover varieties.<sup>1</sup>

Variety	Seed company	Seed yield	Cleanout	Seed Cleanout weight		Biomass	Inflorescences	Florets/ inflorescence	Harvest index
		(lb/a)	(%)	(mg/seed)	(no./m²)	(kg/ha)	(no./ft²)	(no.)	(%)
Medium Red		847 e	2.3	1.71 f	55,702 f	10,931	91.5	109 ab	9.1
Kenland	_	674 bcd	1.7	1.66 ef	45,608 cde	9,750	65.4	115 bc	8.0
Blaze	Mountain View	604 b	1.8	1.63 e	41,387 abc	8,034	63.1	116 bc	8.4
Vulcano	Gentos	626 bc	1.8	1.62 de	43,362 bc	10,554	80.6	121 cd	6.9
Freedom! MR	Barenbrug USA	632 bc	1.7	1.94 g	36,498 a	8,122	57.4	100 a	8.9
Redomon	Van Dyke Seed	693 cd	1.9	1.58 cd	49,363 e	9,837	70.0	129 d	8.0
CISCO	Van Dyke Seed	614 b	2.0	1.56 c	44,111 bcd	9,917	74.4	120 bcd	7.2
Relish	PGG Wrightson	524 a	2.3	1.50 b	39,138 ab	7,328	77.9	109 ab	8.2
FS3662	PGG Wrightson	505 a	2.4	1.43 a	39,630 ab	8,156	83.5	112 abc	7.3
Secretariat	PGG Wrightson	790 e	1.5	1.57 cd	56,480 f	10,014	91.9	121 cd	9.6
Dynamite	Grassland	837 e	2.3	1.67 ef	56,155 f	11,203	91.5	114 bc	9.1
DLFPS-102/3	DLF Pickseed	710 d	2.0	1.64 e	48,582 de	10,367	76.5	111 abc	7.8
<i>P</i> -value		0.0000	0.1724	0.0000	0.0000	0.0837	0.0776	0.0042	0.3718

<sup>&</sup>lt;sup>1</sup>Numbers followed by the same letter are not significantly different at LSD.

Table 2. Percent bloom from flowering initiation to full bloom in 12 red clover varieties.<sup>1</sup>

Variety	Seed company	June 20	June 28	July 5	July 12	July 19	
		(% bloom)					
Medium Red	_	2.8 a	16.3 cde	50.0 c	83.8 ab	100.0	
Kenland	<del></del>	2.0 a	17.5 de	50.0 c	88.8 bcd	100.0	
Blaze	Mountain View	1.8 a	13.8 bc	37.5 b	88.8 bcd	100.0	
Vulcano	Gentos	8.8 b	15.0 cd	36.3 b	86.3 abc	100.0	
Freedom! MR	Barenbrug USA	2.0 a	16.3 cde	48.8 c	91.3 cd	100.0	
Redomon	Van Dyke Seed	1.8 a	10.0 a	28.8 a	81.3 a	100.0	
CISCO	Van Dyke Seed	1.8 a	11.3 ab	36.3 b	86.3 abc	100.0	
Relish	PGG Wrightson	2.0 a	13.8 bc	52.5 c	90.0 cd	100.0	
FS3662	PGG Wrightson	10.0 c	17.5 de	60.0 d	97.3 e	100.0	
Secretariat	PGG Wrightson	10.0 c	18.8 e	48.8 c	91.3 cd	100.0	
Dynamite	Grassland Oregon	1.8 a	16.3 cde	50.0 c	90.0 cd	100.0	
DLFPS-102/3	DLF Pickseed	2.0 a	18.8 e	50.0 c	92.5 de	100.0	
P-value	_	0.0000	0.0001	0.0000	0.0003		

<sup>&</sup>lt;sup>1</sup>Numbers followed by the same letter are not significantly different at LSD.

# PREEMPTIVE MEASURES TO MANAGE THE RED CLOVER CASEBEARER MOTH IN OREGON CLOVER SEED CROPS

N. Kaur, B.A. Mori, J. Otani, W.R. Cooper, D.L. Walenta, K.C. Tanner, L. Van Slambrook, B. Panthi, and N.P. Anderson

#### Introduction

The red clover casebearer moth, Coleophora deauratella (Lepidoptera: Coleophoridae), is an invasive insect species predominantly occurring in the red clover (Trifolium pratense L.) seed-growing regions of North America. Since its first detection in Oregon in 2011, intermittent monitoring of this pest using pheromone traps in red clover seed fields from 2014 to 2019 has indicated high moth activity that can inflict economic damage to the crop by reducing seed yield (Walenta et al., 2020). Although high adult moth captures occurred, corresponding larval populations were not detected, potentially indicating a limiting biotic factor in Oregon. We speculated that the number of larvae might be regulated by the mechanical disruption of the insect life cycle due to silage cutting of the red clover crop prior to seed production, by presence of a natural enemy (egg or larva parasitoids), or by the presence of an additional favorable host plant. Since previous monitoring efforts were limited to commercial red clover seed fields, and the host range of this insect in Oregon is currently unknown, we employed a DNA-based method to determine whether other seed crops might be at risk of infestation by the red clover casebearer moth and to identify noncrop host plants of this insect

Adult moth flights occur during May through August, and eggs are laid directly on newly set red clover heads. Highly concealed first- to third-instar larvae feed on developing seeds within florets. The fourth instar is easier to detect, as it constructs a portable case while feeding. During harvest in the late summer/early fall, the mature larvae crawl onto the soil surface, where they overwinter in sealed cases within crop residue until the following spring. The red clover casebearer moth has only one generation per year.

Larvae are capable of consuming two to three developing seeds per day and can cause up to 80% seed loss in red clover stands, especially in the second year of the crop (Walenta et al., 2020). Red clover seed crops in western Canada have been modified to single-year seed production systems because of the extensive damage caused by the pest by year 2 (Evenden et al., 2010). The establishment of this pest in Oregon's

red clover seed fields poses a risk to seed growers' profitability in the world's largest clover seed growing region. This pest may also impede grass seed growers from utilizing an important crop in their rotation. Therefore, it is imperative to formulate a management plan before this pest reaches its damaging potential in Oregon.

Foliar-applied insecticides with contact modes of action can be ineffective at killing the highly concealed larvae. The adult flight is protracted (i.e., moths fly for several weeks), so repeated insecticide applications may be required to effectively manage this pest and reduce its effects on seed yield. Pollinators are critical for maintaining high seed yield, making multiple insecticide applications a cause of concern for pollinator health. A phenology model to predict adult flights will be a valuable tool for growers to make informed management decisions. Targeting adults with a pheromone-based mating disruption technique has been demonstrated to be effective for adult moth suppression, significantly reducing larval numbers and increasing seed yield in an earlier study conducted in Canada (Mori and Evenden, 2015).

The objectives of this study were to test mating disruption methods, develop phenology models, examine whether additional hosts may exist, and identify the presence of natural enemies. Knowledge generated from this work will help formulate a management plan against the red clover casebearer moth in Oregon clover seed crops.

### **Materials and Methods**

A sex-pheromone bait utilizing a pheromone blend of female *C. deauratella* (10:1 Z7-12:OAc to Z5-12:OAc, Evenden et al., 2010) was utilized to capture adult male moths in 15 commercial red clover seed production fields and 1 experimental red clover site at OSU's Hyslop Research Farm in western Oregon. A single green UniTrap was placed in each field at least 100 feet from the field edge and at crop canopy height. A septum baited with the pheromone lure was placed in the pheromone housing at the top of the trap, and the bucket on the bottom contained an insecticide vapor strip to euthanize captured moths. Pheromones were

replaced after the first 30 days. Traps were monitored weekly for 8–10 weeks. Monitoring efforts ended in late July to mid-August, depending on harvest schedules at each location. Weekly monitoring activities included: (1) collecting adult moth specimens from each trap for identification and quantification, and (2) evaluating red clover heads for larvae presence and/or feeding damage.

#### Phenology model

The number of moths per trap per sampling date was converted to a cumulative proportion of total trap catches over the season. Accumulation of growing degree days (GDD) began on January 1 and ended on August 4, 2020. The weather data to calculate degreedays, using daily maximum and minimum temperatures, were obtained from weather stations corresponding to the nearest field site (http://pnwpest.org/dd/model\_app). An air temperature of 53°F was used as the base temperature to calculate GDD. Three-parameter nonlinear regression models (Weibull, Gompertz, and logistic) were fit using the cumulative proportion of trap catches as the dependent variable (y) and cumulative degree-days as the independent variable (x) in JMP 15 Pro (SAS Institute Inc.).

# Mating disruption technique

In 2020, a mating disruption experiment was conducted on a commercial third-year red clover field site in Marion County, OR. In this study, X-mate disruption devices (Alpha Scents, West Linn, OR) were used at the recommended rate of 20 devices/acre to test whether pheromone-mediated mating disruption can suppress *C. deauratella* damage. Two green UniTraps were placed in each plot to evaluate communication disruption, at 13–14 inches above the soil surface and 41 feet from the center of the plot. Four treatment plots contained mating disruption devices that were placed 13–14 inches above the soil surface. The four control plots remained untreated. Each 164-foot x 164-foot plot was separated by 82-foot buffers.

Traps were checked weekly for adult captures, and data on larval densities were recorded from the third week onwards. Twenty-five flower heads were randomly collected weekly from each plot to determine larval infestation and feeding injury. Data were analyzed using generalized linear repeated-measures mixed-effects models (GLMM), where the pheromone treatment was specified as a fixed effect, and replicate (site) within the week was treated as a random effect (SAS Institute Inc.).

## Gut content analyses

A DNA-based method allows detecting host-plant DNA signals to infer an insect pest's dietary history. Representative insect samples (very first captures of the season) collected from seven different western Oregon sites were subjected to gut content analysis to examine the crop or weed hosts on which the insect may have fed or developed. DNA was extracted from insects using DNAeasy Blood & Tissue Kit (Qiagen). Polymerase chain reaction (PCR), using universal primers for the chloroplast gene trnL and the ribosomal gene ITS, were used to amplify regions of plant DNA from the guts of insects according to methods described in Cooper et al. (2019). PCR products were direct sequenced using a PacBio platform. The NCBI website's BLAST search function was used to identify plant sequences based on similarity to archived sequences.

### Natural enemy survey

Several species of parasitoid wasps are known to be associated with Coleophora species in Europe and other countries of their origin. Discrepancy in the numbers of male moths collected using pheromone-baited traps and the number of larvae found upon dissection of individual florets have led us to speculate that natural enemies may exist in our red clover seed cropping systems. While sampling red clover heads for larval presence and/or damage at the mating disruption experiment site (Marion County), larvae-infested florets were brought to the laboratory and reared until the later instars successfully pupated, to determine whether any parasitism occurred.

#### **Results and Discussion**

#### Phenology model

A three-parameter logistic model described the relationship between cumulative proportion of trap catches and cumulative GDDs in western Oregon (Figure 1). According to the model, the median flight of red clover casebearer moth occurs after 323 GDDs have been accumulated after January 1. In 2020, these GDDs were accumulated between June 17 and June 23.

### Mating disruption

Overall, male captures in assessment traps positioned with mating disruption pheromone-treated plots were reduced by  $38 \pm 17.8\%$  compared to captures in traps in the control plots ( $\chi 2 = 10.15$ , df = 1, P = 0.0014)

over the 5-week trapping period (Figure 2). The subsequent decline of damage (72.6  $\pm$  8.3%) (t = 4, df = 3, P = 0.028) in terms of the feeding holes and larval density in treated plots compared with untreated plots indicated promising potential for using this mating disruption technique for pest suppression (Figure 3).

# Gut content analyses

The data in both ITS and tnrF sequences corresponded to plants in the Fabaceae family, with 47% of the sequence data corresponding to *Trifolium* species (*T. repens, T. pratense, T. occidentale*), 7% to *Medicago sativa*, and 1% to *Vicia* species. The rest of the sequences corresponded to plants in the Poaceae family, including weed species that commonly occur within red clover fields or field borders, suggesting that adult moths seek out additional food resources while foraging. In the future, sampling of additional plant species (identified in this study) in field stands, along with laboratory testing, can elucidate whether these plant species are suitable hosts for this insect by allowing its successful development.

# Natural enemy survey

Two parasitoid wasps, belonging to the Pteromalidae and Ichneumonidae families, that are known to attack lepidopteran larvae were found during sweep net sampling and while dissecting the collection of seed heads from the western Oregon fields. At least 2% of field-collected larva were associated with a parasitoid wasp in the family Pteromalidae (*Catolaccus aeneoviridis*), and identification was further confirmed by the Insect Pest Prevention and Management Program, Oregon Department of Agriculture. Further confirmation of the biocontrol potential of this parasitoid wasp species is needed.

In summary, the first-year data using a mating disruption technique are promising and warrant additional testing to provide growers with a viable control option to help mitigate damage caused by *C. deauratella*. The phenology model will be further validated for adult flight activity during the 2021 growing season.

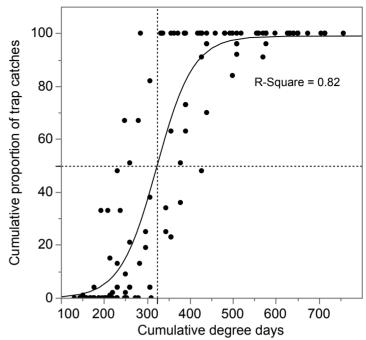


Figure 1. Cumulative proportion of trap catches of male *C. deauratella* in sex-pheromone-baited traps during 2020 plotted against cumulative degreedays (base 53°F from January 1). The vertical dotted line indicates the degree days accumulated corresponding to median moth flight.

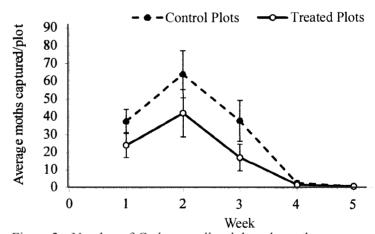


Figure 2. Number of *C. deauratella* adult male moths captured per plot per week in assessment traps using mating disruption pheromones.

6

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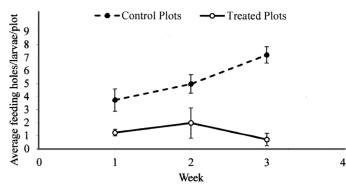


Figure 3. Number of *C. deauratella* feeding holes and/ or larva detected per plot per week in seed head samples randomly collected in the mating disruption pheromone experiment.

# LABORATORY BIOASSAYS FOR SCREENING BIFENTHRIN RESISTANCE IN WHITE CLOVER SEED WEEVIL (YEAR 2)

N. Kaur, L. Van Slambrook, B. Panthi, S.J. Dorman, and N.P. Anderson

#### Introduction

Clover seed weevil (CSW), Tychius picirostris Fabricius (Coleoptera: Curculionidae), is a key insect pest in white clover seed production systems, and it requires control in western Oregon. Besides white clover, CSW is also known to attack other clover species, including alsike, arrowleaf, and Ladino clovers (Anderson, 2020). CWS is a small, gray weevil about 0.1 inch in length. It has a characteristic long snout, and its body is covered with gray and white hair (Reeher et al., 1950). CSW has the potential to cause significant yield loss because larvae feed on developing clover seeds for a prolonged period during the growing season. In recent years, crop advisors in western Oregon have reported several cases of failed CSW control with bifenthrin (Brigade) insecticide. Testing for bifenthrin-resistance had not been investigated prior to a preliminary study conducted by Kaur et al. (2020). The objective of the present study was to further examine the extent to which bifenthrin insecticide resistance exists within CSW populations found in western Oregon white clover seed production systems.

### **Materials and Methods**

In 2020, two laboratory studies were conducted to expose four field-collected adult CSW populations using two bioassays to test for the development of resistance against contact insecticides: (1) adult vial assay and (2) Potter spray tower assay.

#### Adult vial assay

In May 2020, four populations of CSW were collected from four different commercial white clover seed fields in Linn County, OR. Collected CSW adults were placed in separate, large, ventilated chambers for 24 hours prior to conducting bifenthrin dose-response assays according to methods described in Miller et al. (2010). Adult CSW were exposed to increasing bifenthrin rates (0, 17.5, 68.5, 112.5 g a.i./ha; 0, 1, 3.9, 6.4 fl oz a.i/acre) by treating the inside surface of 20-ml glass vials with 0.5 ml of commercially formulated Brigade in acetone. The treated vials were placed on a vial roller to dry and ensure uniform product distribution on each vial's interior surface. Ten field-collected CSW adults were then introduced to each treatment, with ten adults per vial. Vials were closed with a perforated stopper to

allow air exchange. Each treatment was replicated five times (n = 50 adults per treatment).

Vials were then inspected for mortality (dead insects, often lying on their backs) after 12, 24, and 36 hours of bifenthrin exposure. Mortality data were subjected to log dose probit analysis using JMP Pro 15 (SAS Institute Inc., 2019) to generate estimates of a lethal dose that provides mortality to 50% (LD50) of the target populations. LD50 is the amount of test substance that is sufficient to kill 50% of a test population.

### Potter spray tower assay

One field population of CSW adults was collected in late July. This method of screening simulated the exposure of the test population to bifenthrin by using a Potter spray tower (Burkard Manufacturing Co. Ltd., England). This method delivers the insecticide solution as a fine mist, which forms a uniform layer on the insects without creating any visible droplets. To expose the test population, adult insects (n = 10)were immobilized with a flash chilling treatment and placed in a 140-mm plastic petri dish. Each petri dish containing ten adult weevils was placed on the Potter spray tower stage and sprayed with 2 ml of the insecticide treatment (10 and 100 ppm concentration of bifenthrin ( $\leq 17.5$  g a.i/ha;  $\leq 1$  fl oz a.i./acre). Water was used as untreated control or 0-ppm treatment. Treatments were applied using an air pressure of 47 kPa (6.8 psi) and a spray distance of 22 cm. Each spray treatment was replicated three times (n = 30 adults per treatment).

Insect mortality was recorded hourly for 8 hours. Total mortality was calculated at the end of the experiment for each replicate petri plate in all treatment and control groups. Kaplan-Meier log rank survival analysis was performed using GraphPad Prism Version 8.0.2 (GraphPad Software, San Diego, CA).

#### **Results and Discussion**

### Adult vial assay

All four CSW populations exposed to bifenthrin in the adult vial assays resulted in 83.3 to 94.6% mortality after 36 hours of exposure (Figure 1). The dose probit

analysis showed that a dose of 38.68–76.49 g a.i./ha caused 50% mortality (LD50) in 12 hours (Table 1). The LD50 value further decreased to 16.18–25.67 g bifenthrin/ha after 24 hours and to 8.79–16 g bifenthrin/ha after 36 hours. The subsequent decline of LD50 value as the exposure time progresses indicated a delayed mortality effect of bifenthrin on the adults.

The LD50 value can be used to establish baseline susceptibility of target CSW populations. In the future, these data can be used to determine whether the susceptibility of the target population has shifted. Actual LD50 values can be compared among populations by examining the 95% confidence intervals. If the upper

and lower limits do not overlap, then it is likely that the population has experienced a significant change in susceptibility; in some situations this is an indication of developed resistance.

#### Potter tower assay

Control survival was  $\geq 80\%$  for the duration of the study (Figure 2). A log-rank test indicated significant differences in insect survival among experimental groups (Kaplan Meier, log rank test  $\chi 2 = 239$ , df = 2, and P < 0.001). The 10- and 100-ppm treatments did not significantly differ. After the 8-hour exposure period,

10% and 0% of adults survived the 10- and 100-ppm treatment, respectively.

In summary, these results from both the 2019 and 2020 studies did not indicate resistance development to bifenthrin. However, steps should be taken to avoid or delay the development of resistance in the future. We recommend managing crop canopy height and density to promote effective spray coverage and defoliating the crop to remove early-season inflorescences for better synchronization of bloom. Insecticide applications should be timed to coincide with economic thresholds for CSW, as listed in the *PNW Insect Management Handbook* (Anderson, 2020).

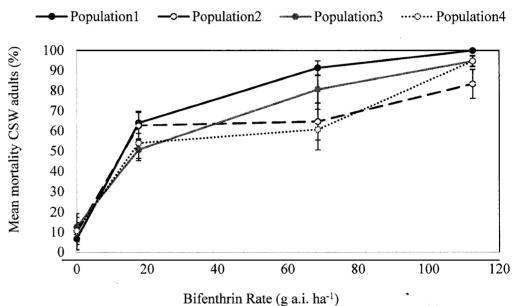


Figure 1. Mean mortality (%) of clover seed weevil adults exposed to different rates of bifenthrin in the adult yial test.

Table 1. The bifenthrin dose (g a.i./ha) that caused 50% mortality (LD50) of clover seed weevil adults within four field populations 12, 24, and 36 hours after exposure to treatment.

	12 hours				24 hours		36 hours			
	95%	95% confidence interval			95% confidence interval			95% confidence interval		
Population	LD50	Lower CI	Upper CI	LD50	Lower CI	Upper CI	LD50	Lower CI	Upper CI	
	(g a.i./ha)									
1 2 3 4	38.68 75.26 70.33 76.49	32.53 64.88 62.24 67.34	45.72 87.56 78.95 87.21	16.70 23.56 16.18 25.67	10.02 3.87 6.51 13.01	23.21 37.80 24.44 36.22	10.37 8.79 11.60 16.00	4.57 -13.71 3.69 6.15	15.82 23.56 18.46 24.26	

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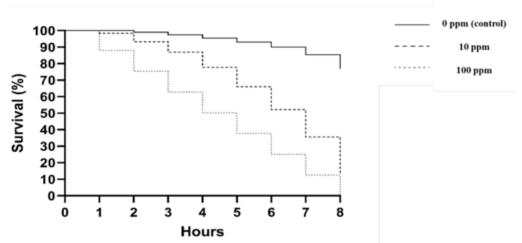


Figure 2. Clover seed weevil adult survival during the 8-hour observation period during the Potter spray tower experiment when exposed to different concentrations of bifenthrin.

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