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DEVELOPMENT OF 3-D TOPOMETRIC IMAGING METHODS FOR HIGH-THROUGHPUT PHENOTYPING OF SEED RETENTION TRAITS IN PERENNIAL RYEGRASS

T.B. Tubbs and T.G. Chastain

Introduction

Shattering is a widespread natural phenomenon in plants and serves as a mechanism for dispersal of seed to favorable environments. However, in agriculture the loss of seeds resulting from shattering prior to and during harvest can be an important constraint to seed yield. In perennial ryegrass, shattering was observed to cause seed yield losses as great as 700 lb/acre in Oregon (Anderson et al., 2019). Seed lost during shattering is the source of crop volunteers, a major weed problem in perennial ryegrass seed production.

Results from a recent study show some promise in identifying the genes that play a critical role in seed shattering of perennial ryegrass using a comparative genomics strategy (Fu et al., 2018). Identification of phenotypic characteristics associated with shattering is likely to play a key role in reducing seed losses (Elgersma et al., 1988). Methods for field evaluation of plant traits in grasses have long relied on laborious and time-consuming measurements. Moreover, human ratings of plants can be subjective and inconsistent. Since data collection on these characteristics is slow, the quantity of data gathered is generally limited (low-throughput) on these traits (phenotypes).

Current technology has increased the adoption of high-throughput phenotyping (HTP) methodologies in crop physiology and breeding programs, replacing much of the slow and labor-consuming human-based data collection with a variety of sensing devices (Vázquez-Arellano et al., 2016). Advances in topometric scanning and imaging technologies have enabled three-dimensional (3-D) modeling of the physical world. Is it possible to use 3-D topometric imaging methods to provide rapid and repeatable estimates of crop phenotypic characteristics (HTP) under field conditions?

Our objectives were to develop 3-D topometric imaging methods for HTP in perennial ryegrass and to determine the relationship of measurable phenotypic characteristics of spikes and seed shattering.

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. The plant accessions were sourced from seeds acquired from the USDA Western Regional Plant Introduction Station in Pullman, WA, and from commercial cultivars. Each accession was represented by four plants derived from four seeds within the accession in order to characterize the variation within each accession. These plants were used to create vegetative clones of the four genotypic lines (denoted A–D) within each accession or cultivar.

Seeds from each accession were planted in the greenhouse and grown into plants robust enough (multiple tillers) for cloning. Each genotypic line was cloned 4 times to produce a total of 640 transplants for field trials. Transplants were planted in 32 rows with 20 plants per row. Rows were separated by 2-foot-wide access walkways of tall fescue. The experimental design was a randomized block design with four replications, and treatments were the 40 accessions and the 4 genotypic lines within each accession. Standard practices for culture of perennial ryegrass seed crops were employed in the study, with the exception that no plant growth regulators were applied to control lodging.

A portable, hand-held scanner (Artec 3-D Spider) was used to capture 3-D topometric images of perennial ryegrass spikes in the field. The scanner uses blue LED light and has 3-D resolution to 0.1 mm. Unlike a photograph, the 3-D topometric image captured by the scanner can be rotated by the user to reveal all sides.

The Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale was used to assess crop maturity. Representative spikes from the 40 accessions and genotypic lines within accessions were scanned with the 3-D optical scanner at intervals from spike emergence (BBCH 50) to seed maturity (BBCH 80–89). Morphological characteristics of the spike considered in the topometric image analysis included spike length and architecture, number of spikelets/spike, distance between spikelets along the rachis, angle of spikelet attachment to the rachis, and spikelet size.

Once the image of the spike is captured by scanning and saved as a stereolithography (STL) file, the data must undergo several steps before they are ready for

topometric image analysis. The optical imaging system had not been used previously in topometry of plant tissues, so considerable work to develop methods for postprocessing of data was needed to adapt the scanning device. One promising data postprocessing method is known as mesh skeletonization; this approach has the potential to save time and labor needed for data analysis. This process enables the automated measurement of the characteristics found on each spike. The 3-D data from the scan is cropped out of the image, and the surface 3-D polygon mesh is exported to a more usable file format.

The STL files were uploaded into the Slicer program, where 3-D models were incrementally sliced into hundreds of individual "stacked" images. Sliced 3-D images were analyzed by Imagej (image analysis software) for calculation of center points. These center point images were then restacked so that all of the center points were connected by an individual meshed surface (Figure 1).

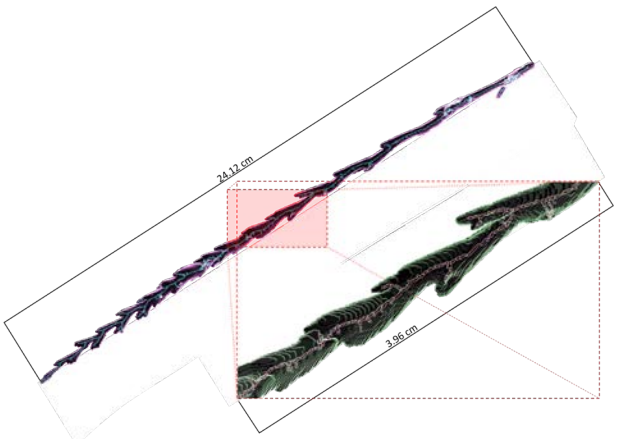

Figure 1. Artec Studio x-ray representation of a scan of a perennial ryegrass spike. This representation depicts two models created from one object and superimposed in Artec Studio. The inner model is the smoothed chordal axis that was created with the Slicer program to convert the 3-D mesh into a stacked image. This stacked image was skeletonized using Imagej software.

Field-based sampling for the 40 accessions was done to validate the 3-D scanning methodology. Each scanned spike was collected along with two other representative spikes from the accession. Accessions and cultivars were tested for shatter resistance (seed retention) by placing spikes on an aluminum base plate and rolling a standardized steel bar (0.5 kg) by hand (approximately 24 Newtons) over each spike five times (three times from tip to base and two times 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 competent (caryopsis at least one-half the length of the palea) and were counted and weighed. The seeds retained on the spike were then hand-stripped from the spike, counted, and weighed. Each spike was photographed with a camera. The number of spikelets per spike and the length of the spike were measured per established methods (Chastain et al., 2014).

Results and Discussion

Perennial ryegrass spikes taxed the resolution threshold for the scanner, but it was able to record the diversity of characteristics and architecture of spike morphology observed among accessions. The scanner was not able to consistently distinguish individual florets on the spike, but other structural components of the spike were distinguishable at the device's resolution. The work to date indicates that multiple measurements can be made in the field at sequential stages of development and that the scanner has the ability to capture a 3-D representation of the spike and store that data for subsequent topometric analysis.

The 40 accessions and genotypic lines within accessions were found to differ greatly in plant mortality, crop architecture, maturity, spike size, and seed shattering. Some of the accessions and genotypic lines were observed to be short lived as perennials and did not survive more than 1 year. Since the mortality was generally consistent across replications within accessions, the poor survivorship was likely the result of lack of adaptation to the environment by the accession or innate short life span.

There was evidence of large differences in seed retention in the spike among accessions, as shown in Table 1. An accession from Tunisia (PI 598916) showed excellent seed retention (low shattering) compared to a commercial cultivar ('Cutter') and a shattering-susceptible accession from Poland (PI 384480). These accessions also differed in spikelets/spike, seed number/spike, and seed weight.

There was considerable variation in seed retention, both among accessions and cultivars and within accessions (genotypic lines A–D) and cultivars (Figure 2). This was expected because of the genetic nature of perennial ryegrass; phenotypic and genotypic variation among plants within accessions or cultivars is common and can be quite large. Nevertheless, some accessions

had much higher seed retention than others and in general had greater seed retention than that found in commercial cultivars such as 'Accent'. This is evidence that sources of higher seed retention are available from these accessions and could be used in the breeding of shattering-resistant cultivars.

Our investigations suggest that the topometric scanner has promise as an HTP tool, potentially replacing slow and laborious human-based data collection in studies of the inflorescence in grasses. With this device, data can be collected quickly, and measurements can be made multiple times on live plants in the field at sequential stages of development without destructive removal. The 3-D images are available after the user leaves the field for additional examination and future comparisons. Nevertheless, requirements for postprocessing currently delay the availability of estimates of crop phenotypic characteristics under field conditions using 3-D topometric imaging.

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Table 1. Spike characteristics and seed retention in perennial ryegrass accessions or cultivars.

Accession or cultivar	Spikelets (no./spike)	Seeds (no./spike)	Seed weight (mg)	Seed retention (%)
PI 598916	21.7	46	1.50	76.1
Cutter	20.9	30	1.49	32.0
PI 384480	31.4	57	2.24	23.9

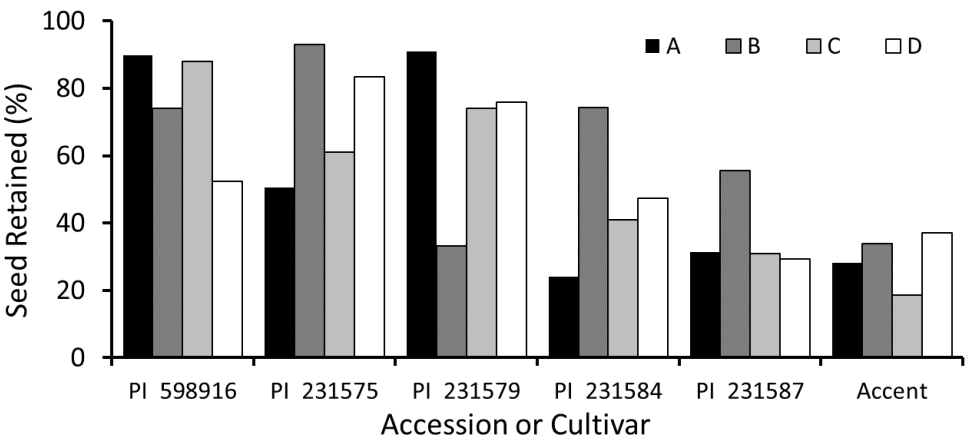

Figure 2. Seed retention among and within accessions or cultivars of perennial ryegrass. The four genotypic lines within accessions or cultivars are denoted A–D.

ARE HIGHER YIELDS POSSIBLE IN ANNUAL RYEGRASS SEED CROPS? (YEAR 2)

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

Introduction

Forage grass seed crops, including annual ryegrass (*Lolium multiflorum* L.), are a vital part of seed production enterprises in Oregon. Like other cool-season grasses, annual ryegrass produces only 15–33% of its potential seed yield. Lodging of the crop during flowering is one of the major factors limiting seed yield. Making better use of management practices that reduce stem length and decrease lodging is one area that should be further explored to address seed yield losses.

Seed yield is reduced by lodging during anthesis and early seed fill as a result of self-shading in the canopy and reduction in pollination. While trinexapac-ethyl (TE) has been shown to increase yield in perennial ryegrass, its use patterns and potential effects on the seed yield of annual ryegrass are relatively understudied, especially in Oregon. Previous work in the northern hemisphere suggests that seed yield responses of annual ryegrass to TE are generally small (Mellbye et al., 2007; Rijckaert, 2010; Macháč, 2012). However, new studies conducted in New Zealand report seed yield increases of 30–50% when TE is applied (Trethewey et al., 2016).

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

Recent research shows that even greater seed yield increases in annual ryegrass crops are possible when TE applications are strategically timed with spring defoliation. For example, Rolston et al. (2012) reported seed yields of 3,015 lb/acre when 200 g ai TE/ha was applied to annual ryegrass that had been defoliated at BBCH 32–33. This represents a 35% increase over the treatment with the same TE rate applied to annual ryegrass defoliated once at BBCH 30–31 and a 123% increase over the zero TE treatment. This response to TE and later-timed defoliation was related to delayed lodging and better light interception by the standing crop.

Current prices of TE are relatively low, and many annual ryegrass growers are accustomed to grazing fields. If we could better understand how these two lodging reduction strategies can best work together in the Oregon environment, there is strong potential for economic benefit to the grower. The objectives of this work are to define optimum treatment applications of TE across multiple defoliation timings for annual ryegrass seed crops and to determine whether interaction between TE and defoliation will further reduce lodging and increase seed yield. The work presented in this article represents information from year 2 of a 2-year project. Data from year 1 can be found in OSU's *2018 Seed Production Research Report*.

Materials and Methods

A field trial with Oregon 'Gulf' and New Zealand 'Winterstar II' annual ryegrass varieties was established in September 2018 at OSU's Hyslop Research Farm. The experimental design for the trial is a randomized complete block with a split-plot arrangement of treatments and four replications. Plot size is 11 feet x 45 feet. Plots were established with conventional tillage during fall. Spring nitrogen (N) was applied as urea (46-0-0) at 130 lb N/acre. Routine herbicide sprays were applied to manage weeds as needed. Defoliation by grazing was simulated using a flail mower. Main plots were defoliation timings, and subplots were TE rate. Subplots were randomly allocated within defoliation main plots.

Defoliation main plots included the following timings:

- Untreated control (no defoliation)
- Single cutting at BBCH growth stage 31 (appearance of first node)
- Triple cutting: once at BBCH growth stage 31 and twice when regrowth was at BBCH 32–33

TE subplots included the following application rates and timings:

- Untreated control (no PGR)
- Trinexapac-ethyl (Palisade EC): 1.4 pt/acre at BBCH 32
- Trinexapac-ethyl: 2.8 pt/acre at BBCH 32
- Trinexapac-ethyl: 4.2 pt/acre at BBCH 32

Defoliation by flail mowing occurred on March 20, 2019 for the single cutting and on March 20, March 31, and April 18, 2019 for the triple cutting. The TE treatments were applied at the two-node stage (BBCH 32) using a bicycle-type boom sprayer operated at 20 psi delivering 20 gpa with XR Teejet 8003VS nozzles. Above-ground biomass samples were taken from each annual ryegrass plot near crop maturity, and dry weight was determined. The crop height of annual ryegrass was also measured for each treatment at harvest maturity. Lodging ratings were recorded weekly from the start of anthesis until harvest.

Seed was harvested by a small-plot swather and combine, and seed was cleaned to determine 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 above-ground biomass, was also quantified.

Results and Discussion

In year 2, the triple-mow treatment increased seed yield in 'Gulf' (Table 1), and both the single- and triple-mow treatments increased seed yield in 'Winterstar II' (Table 2). For 'Gulf', maximum seed yield was attained with a triple mowing, which resulted in a 45% seed yield increase (Table 1). This contrasts with data from year 1 of the project, when the maximum seed yield with 'Gulf' was obtained with a single mowing. In year 2, there was no advantage or disadvantage to the single-mow over the no-mow treatment with 'Gulf'. For 'Winterstar II', maximum seed yield was also attained with the addition of mowing, with a 23.1% and 31.2% increase with single- and triple-mowing treatments, respectively. Both mowing treatments also increased seed number and decreased lodging (data not shown), fertile tiller length, and spike length in both varieties. There were no effects on biomass for either variety.

Seed yield was also increased by PGR treatments for both varieties (Tables 1 and 2). For 'Gulf", maximum seed yield was attained with the 2.8 pt TE/acre rate applied at BBCH 32 (two-node stage), although there was also a significant increase at 1.4 pt TE/acre. For 'Winterstar II', maximum seed yield was attained with the 1.4 pt TE/acre rate applied at BBCH 32. All PGR treatments increased seed number, while decreasing lodging (data not shown), 1,000-seed weight, tiller length, and spike length in both varieties. There were

Table 1. Interaction of spring mowing and plant growth regulators (PGRs) on seed yield, yield components, and growth characteristics of Oregon 'Gulf' annual ryegrass.[1]

TE treatment	Mowing treatment	Seed yield	Cleanout	Seed weight	Fertile tillers	Tiller length	Biomass	Seed number	Harvest index
(pt/a)		(lb/a)	(%)	(g)	(no./ft^2)	(cm)	(kg/ha)	(no./m^2)	(%)
Untreated	0x	772 ab	2.2	3.345 h	54.6	164.9 g	15,402	25,956 ab	11.8
1.4	0x	1,052 abcd	1.9	3.286 h	73.9	153.3 f	16,939	36,111 abcd	7.6
2.8	0x	1,070 cde	2.1	3.188 g	76.1	145.6 e	18,159	37,728 cde	8.0
4.2	0x	1,268 de	2.2	3.043 ef	78.6	125.3 d	14,077	46,936 de	8.5
Untreated	1x	1,074 bcd	1.6	3.096 fg	86.4	145.3 e	11,909	38,922 bcd	13.7
1.4	1x	920 abc	1.8	2.987 de	96.6	140.5 e	12,258	34,578 abc	10.5
2.8	1x	1,334 def	1.6	2.862 c	94.4	122.8 d	11,446	52,217 def	16.0
4.2	1x	1,402 ef	1.5	2.750 b	99.4	110.1 c	12,396	57,240 ef	10.2
Untreated	3x	732 a	1.4	2.923 cd	103.5	112.6 c	9,512	28,129 a	15.2
1.4	3x	1,653 fg	1.2	2.664 b	117.8	99.9 b	10,120	69,578 fg	16.1
2.8	3x	1,785 gh	1.2	2.531 a	108.9	88.2 a	8,369	79,008 gh	15.2
4.2	3x	1,956 h	1.2	2.546 a	124.4	83.1 a	9,428	86,082 h	18.0
P-value		0.0002	0.5560	0.0202	0.9099	0.0335	0.4562	0.0000	0.6309

[1]Means followed by the same letters are not significantly different at LSD ($P = 0.05$).

no PGR effects on biomass, spikelet number, or HI for either variety.

An interaction of spring mowing and PGR for seed yield, seed number, and lodging was evident in both varieties in year 2. The combined effects of these two management practices further increased seed yield and seed number over individual treatment (spring mowing or PGR) effects. While seed weight was reduced with the combination of spring mowing and PGRs, the increase in seed number still allowed for a greater overall seed yield.

Conclusion

The results of this 2-year project indicate that spring mowing (single or triple) combined with at least 2.8–4.2 pt TE/acre applied at BBCH 32 can increase annual ryegrass seed yield by as much as 151–215% and 72–185% in 'Gulf' and 'Winterstar II', respectively. The combination of these spring management practices should be evaluated on a large scale in growers' fields to further validate what appear to be promising results for achieving higher seed yields in annual ryegrass seed crops in Oregon.

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Table 2. Interaction of spring mowing and plant growth regulators (PGRs) on seed yield, yield components, and growth characteristics of New Zealand 'Winterstar II' annual ryegrass.[1]

TE treatment	Mowing treatment	Seed yield	Cleanout	Seed weight	Fertile tillers	Tiller length	Biomass	Seed number	Harvest index
(pt/a)		(lb/a)	(%)	(g)	(no./ft^2)	(cm)	(kg/ha)	(no./m^2)	(%)
Untreated	0x	1,191 ab	2.1	4.340 de	46.2	158.1	13,251	30,395 ab	14.9
1.4	0x	1,577 cd	1.9	4.342 d	45.0	142.4	12,463	40,828 cd	15.0
2.8	0x	1,119 a	1.8	4.397 de	53.8	133.2	15,005	28,522 a	13.2
4.2	0x	1,526 bcd	1.9	4.196 c	54.3	125.5	13,388	40,875 cd	13.9
Untreated	1x	1,210 ab	1.5	4.455 e	75.4	142.4	13,434	30,404 ab	12.1
1.4	1x	1,535 bcd	1.6	4.354 d	75.6	131.4	12,909	39,510 bc	13.8
2.8	1x	2,007 e	1.4	4.183 c	72.1	116.5	12,200	53,751 e	15.0
4.2	1x	1,948 e	1.4	4.055 ab	89.3	106.7	14,348	53,849 e	13.7
Untreated	3x	1,337 abc	1.5	4.332 d	79.5	111.7	10,043	34,535 abc	15.4
1.4	3x	1,856 de	1.5	4.131 bc	81.8	98.5	10,042	50,401 de	18.9
2.8	3x	2,068 e	1.3	4.058 b	92.5	97.9	10,367	57,261 e	17.0
4.2	3x	1,880 de	1.5	3.960 a	88.5	90.7	8,920	53,261 e	22.0
P-value		0.0040	0.6666	0.0007	0.7789	0.4805	0.7804	0.0023	0.5924

[1]Means followed by the same letters are not significantly different at LSD ($P = 0.05$).

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SPRING-APPLIED NITROGEN AND PLANT GROWTH REGULATOR EFFECTS ON SEED YIELD OF THIRD-YEAR ORCHARDGRASS

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

Introduction

Forage grass seed crops, including orchardgrass (*Dactylis glomerata* L.), are a vital part of seed production enterprises in Oregon. Like other cool-season grasses, orchardgrass produces only a fraction of its potential seed yield. Making better use of nitrogen (N) and plant growth regulators (PGRs) is a way to possibly obtain higher seed yields. In comparison with tall fescue and perennial ryegrass, seed yield response to PGRs in orchardgrass is relatively understudied.

Since lodging is exacerbated in the high-N environments present in grass seed production systems, additional work is needed to determine possible interactions between PGRs and spring-applied N under western Oregon conditions. Recommendations for application rates of N fertilizer in orchardgrass have not been revised and have not appeared in the international seed production literature since PGRs were introduced in this important forage seed crop. In Oregon, OSU fertilizer recommendations (Doerge et al., 2000) for orchardgrass seed crops are more than 15 years old, and new information is needed to evaluate whether N rate recommendations should be adjusted to further increase seed yield in current management environments.

The first- and second-year results of this study indicate that a combination of spring-applied N and PGRs can increase orchardgrass seed yield in western Oregon conditions (Anderson et al., 2018; Anderson et al., 2019). Maximum seed yield was attained with 100 lb N/acre, and there was no additional benefit from higher N rates. Conversely, there was no seed yield increase from applying spring N in the third harvest year. Seed yield was also significantly increased by trinexapac-ethyl (TE) and TE + chlormequat chloride (CCC) PGR treatments (by 55% and 37% in the first year and second year, respectively). An interaction of spring-applied N and PGR for seed yield was evident in this first-year study but not in year 2. One interesting finding from both years was that seed yield was enhanced by the use of PGRs even when no spring N was applied.

The objectives of this 3-year study were to (1) measure the effects of multiple N fertilizer rates in the presence and absence of TE and TE + CCC PGRs and (2) define optimum treatment and timing applications of TE and TE + CCC PGR combinations for third-year orchardgrass seed crops.

Methods

A field trial with 'Persist' orchardgrass was established in October 2015 at OSU's Hyslop Research Farm. Plot size is 11 feet x 38 feet. Fungicide and insecticide treatments were applied to manage pests as needed. Fall N was applied to all plots at a rate of 40 lb N/acre during 2015–2018. The third harvest was taken in 2019. The experimental design for the trial was a randomized complete block with a split-plot arrangement of treatments and three replications. Main plots were spring-applied N rates of:

- 0 lb N/acre
- 100 lb N/acre
- 140 lb N/acre
- 180 lb N/acre

PGR subplots included the following treatments and application rates:

- Untreated control (no PGR)
- 1.5 pt TE/acre applied at BBCH 32 (two nodes)
- 1.5 pt TE/acre applied at BBCH 51 (panicles 10% emerged)
- 0.75 pt TE/acre + 1.34 lb CCC/acre at BBCH 32

Spring N was applied on March 5, 2018 using a tractor-mounted orbit-air spreader system with appropriate amounts of 46-0-0. The PGR treatments were applied at the two-node stage (BBCH 32) and when panicles were 10% emerged (BBCH 51) using a bicycle-type boom sprayer operated at 20 psi delivering 20 GPA with XR Teejet 8003VS nozzles. Above-ground biomass samples were taken from each plot near crop maturity, and dry weight of the standing crop was determined. Total tissue N content was measured from the above-ground biomass samples. Tiller height was measured for each treatment at harvest maturity.

Seed was harvested by a small-plot swather and combine, and seed was cleaned to determine 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 above-ground biomass, was also quantified.

Results and Discussion

Spring-applied N had no effect on seed yield in third-year orchardgrass (Table 1). Unlike the first- and second-year studies, maximum seed yield was attained without N fertilizer, and there was no additional benefit from higher N rates. Nitrogen increased seed weight, total above-ground biomass, and fertile tiller number, but had no effect on percent cleanout, seed number, tiller height, or HI. Total tissue N concentration did not increase when rates above 100 lb N/acre were applied (data not shown).

Seed yield was significantly increased (by 29%) by PGR treatments (Table 2). As in the second year of this study, PGR application timing at the two-node stage (BBCH 32)—both TE and TE + CCC mixture—resulted in significantly increased seed yields and HI compared to TE applied when panicles were 10% emerged (BBCH 51) (Table 2). There was no benefit of TE + CCC PGR combination over TE alone. All PGR treatments increased seed number and decreased tiller height and percent cleanout, but there were mixed effects on seed weight and biomass. There were no PGR effects on fertile tiller number.

An interaction of spring-applied N and PGR for seed yield was not evident in this third-year study. Spring N and PGRs enhanced seed yield independently of one another. In summary, it appears that new recommendations should be developed to optimize seed yields in orchardgrass seed crops grown in the Willamette Valley. Overall results indicate that no more than 100 lb N/acre is needed in the spring and that PGRs should be used and timed at the two-node (BBCH 32) growth stage.

Table 1. Effect of nitrogen (N) on seed yield, yield components, and growth characteristics of third-year orchardgrass.[1]

N treatment	Seed yield	Cleanout	Seed weight	Seed number	Biomass	Fertile tillers	Tiller height	Harvest index
(lb/a)	(lb/a)	(%)	(mg/seed)	(seeds/m^2)	(kg/ha)	(no./ft^2)	(cm)	(%)
0	420	29.9	0.916 a	39,744	4,249 a	22.9 a	78.9	11.8
100	551	17.9	0.959 b	52,905	7,836 ab	36.1 b	86.8	8.3
140	594	19.9	0.940 ab	56,781	7,332 b	35.7 b	90.4	9.4
180	623	18.6	0.947 b	60,048	8,335 c	39.2 b	85.1	8.6
P-value	0.1109	0.0859	0.0238	0.0673	0.000	0.0050	0.2653	0.1765

[1]Means followed by the same letters not significantly different at LSD (*P* = 0.05).

Table 2. Effect of plant growth regulators (PGRs) on seed yield, yield components, and growth characteristics of third-year orchardgrass.[1]

PGR treatment	Seed yield	Cleanout	Seed weight	Seed number	Biomass	Fertile tillers	Tiller height	Harvest index
	(lb/a)	(%)	(mg/seed)	(seeds/m^2)	(kg/ha)	(no./ft^2)	(cm)	(%)
Control (no PGR)	450 a	23.1 b	0.920 a	43,980 a	7,575 b	35.9	110.8 c	6.9 a
Palisade 1.5 pt/a (BBCH 32)	590 c	21.4 a	0.967 c	54,095 b	6,566 ab	32.2	76.6 ab	11.1 b
Palisade 1.5 pt/a (BBCH 51)	546 b	20.9 a	0.933 ab	53,344 b	7,514 b	37.0	85.7 b	8.7 a
Palisade 0.75 pt/a + CCC 1.34 lb ai/a (BBCH 32)	602 c	20.9 a	0.942 b	58,060 b	6,097 a	28.8	68.0 a	11.4 b
P-value	0.0000	0.0186	0.0001	0.0001	0.0333	0.0517	0.0000	0.0001

[1]Means followed by the same letters not significantly different at LSD (*P* = 0.05).

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EFFECTS OF STRAW REMOVAL ON SOIL HEALTH IN TALL FESCUE SEED PRODUCTION (YEAR 1)

E.C. Verhoeven, W.P. Jessie, A.D. Moore, and D.M. Sullivan

Introduction

Soil health describes a soil's ability to maintain productive yields and provide ecosystem services such as reduced nutrient leaching, good water retention, and nutrient cycling. Soil health test packages are available from commercial and university laboratories, but there is no standardized set of measurements for these test packages. Generally, there is consensus 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, or aggregate stability are commonly used to assess the physical condition of a soil, while measurements of pH and macro- and micronutrients are used to assess the chemical condition. Measurements of respiration (often called a CO_2 burst test), organic matter (OM), active carbon (C), soil protein content, and potentially mineralizable nitrogen (N) rates can be used to evaluate the biologic 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 (Nunes et al., 2018; Awale et al., 2017). However, it is important to remember that many measures of soil health are also affected by inherent soil or site properties that cannot be changed, such as clay content, landscape position, or climate. To evaluate soils across soil types and textures, large datasets are likely needed to develop regional scoring calibrations for different texture classes (Fine et al., 2017).

Maintaining soil OM levels is generally considered to be critical to preserve soil health and function. To maintain or increase soil OM, growers must implement practices that decrease OM losses and increase inputs. The main practices that reduce OM losses are practices that reduce soil erosion and reduce the intensity and/or frequency of tillage (Sullivan et al., 2019). Organic matter inputs can come from a variety of sources, such as manure or compost amendments, crop residues, and increased crop biomass from enhanced growth or intercropping. Returning postharvest residues to the field is one method of achieving higher OM inputs to a system.

With the phase-out 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 pressure, and generate immediate farm income from straw sales. The straw is a relatively low-quality organic matter, with negligible amounts of most macro- and micronutrients, but it does contain around 100 lb/acre of potassium (K) and on average around 2,175 lb/acre of carbon (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. Data from Oregon systems are needed to help better inform producers of possible trade-offs associated with long-term straw removal. Data need to be collected in a way that allows us to begin differentiating the effects of management versus soil type on measures of soil health.

The objectives of this study are to:

- Evaluate soil health measurements under bale versus full-straw chop-back management practices in tall fescue seed crops.
- Explore relationships between soil health measures and key soil/site properties (e.g., texture) in tall fescue seed crops

Materials and Methods

This is a 2-year study, with data from 22 fields sampled in year 1 (2019) reported here. In 2020, an additional 20 fields will be sampled, doubling the dataset. Fields sampled in 2019 will not be resampled in 2020.

We identified paired tall fescue seed fields that were 4 years or older throughout the Willamette Valley (one 4-year-old perennial ryegrass pair was included) (Figure 1). Fields with a history of full-straw chop-back ("full-straw") were paired with similar-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.

The fields sampled almost always had more than one soil series. We used Natural Resources Conservation Service (NRCS) soil maps to sample from portions of the field corresponding to dominant soil types and soil types matching the paired field. The most commonly sampled soil series was Woodburn, followed by Dayton and Amity. Other soil series sampled included Quantama, Cornelius-Kinton, Huberly, Aloha, Chehalis, McBee, Nekia, and Jory.

All fields were soil sampled between April 10 and May 2, 2019. In most cases, paired fields were sampled on the same day. Three zigzag transects per field were sampled and analyzed separately. Transects were placed semirandomly in uniform parts of the field and in portions of the field aligned with soil types in the matching paired field. Ten soil cores per transect were taken to an 8-inch depth and mixed to form a composite sample. Penetration resistance and bulk density measurements were conducted in the field. Samples for laboratory analysis were stored at 4°C until laboratory analysis was conducted at OSU's Central Analytical Lab.

The set of analyses outlined in Table 1 follows the framework and protocols outlined by Cornell University in the Comprehensive Assessment of Soil Health (CASH, https://soilhealth.cals.cornell.edu/). In addition to the soil health properties listed in Table 1, soil samples were analyzed for texture (% sand/silt/clay). Soil OM was calculated from total C analysis, but we report only OM.

Methods can vary among labs for biological tests such as respiration and potentially mineralizable N, and changes to the procedure, such as the order of soil wetting, incubation moisture, or temperature, can affect results. It is therefore essential to compare only results analyzed at the same lab and to ask the lab to share their procedure if you are unsure. The full protocol for

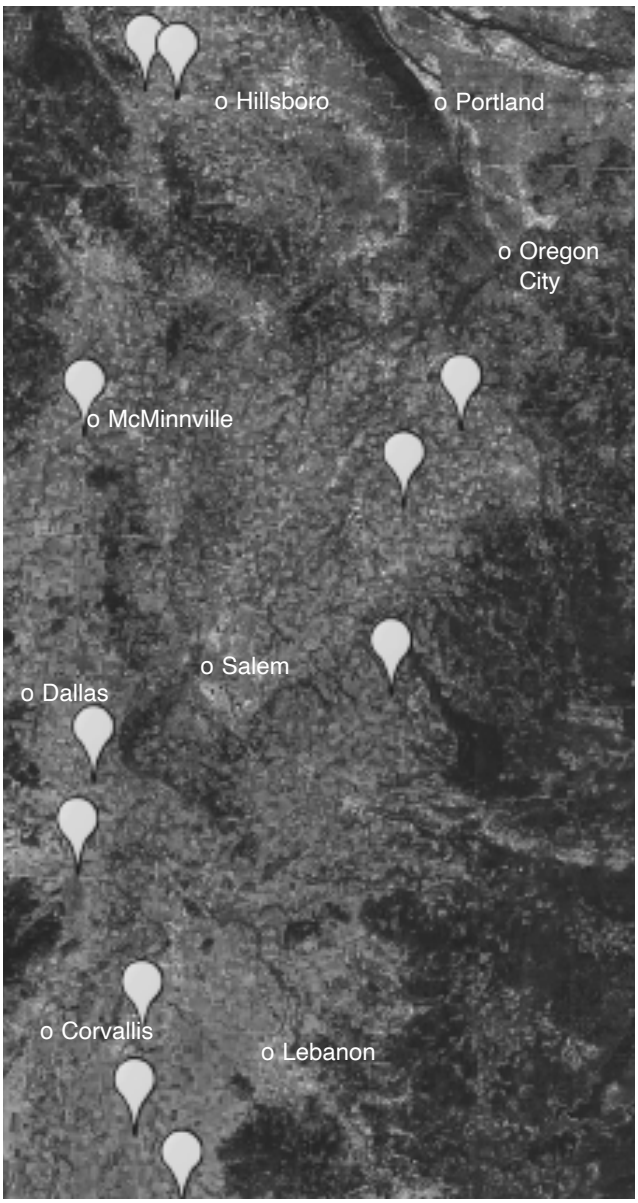

Figure 1. Map of the Willamette Valley showing the general location of the 11 sites (one full-straw and one baled field at each) sampled in 2019.

Table 1. List of chemical, physical, and biologic properties measured to assess soil health.

Chemical/nutrient	Physical	Biologic
pH	Bulk density	24- and 96-hour respiration
Mehlich-3 P, K, Mg, Ca	Penetration resistance	Organic matter % (from total C)
Electrical conductivity (EC)	Wet aggregate stability	Active carbon[1]
Cation exchange capacity		Potentially mineralizable nitrogen

[1]Often referred to as permanganate oxidizable carbon in other texts.

respiration and potentially mineralizable N analysis can be obtained from the Central Analytical Laboratory at OSU (https://cropandsoil.oregonstate.edu/cal). In brief, both analyses were performed at 23°C on air-dried soil, rewetted to 50% water-filled pore space. Respiration was measured by CO_2 accumulation at 24 and 96 hours. Potentially mineralizable N was analyzed using a 28-day aerobic respiration and measuring the accumulation of NO_3^-.

To evaluate the fit of pairing between fields, we compared the mean percent clay and sand between the two fields. Sites with greater than 5% difference in clay or sand content were considered unacceptable pairs and were not included in t-test means comparisons between the management practices. This reduced the number of sites from 11 to 7. To evaluate the relationship between soil health measures and clay or stand age, we performed regression analysis across all sites. We excluded one site from this analysis. This site was located on a Jory/Nekia soil with more than double the OM and total N than that of other sites at both fields.

Results and Discussion

Effects of straw management

Results of four key soil health measures are shown in Figure 2. Among the soil physical properties—bulk density, penetration resistance, and wet aggregate stability—we observed no differences between the management practices. Among soil chemical properties, we found higher K under full-straw management ($P = 0.03$), which is not surprising given that the straw contains high amounts of K (Figure 2). However, these results indicate that in general K removal from baling is not adequately being replaced by potash fertilizer applications. Among the biologic soil properties measured, we observed a trend of higher respiration in the 96-hour measurement in the full-straw fields (Figure 2, $P = 0.073$). 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 but also availability of a food source for microbes, in this case likely C from the straw.

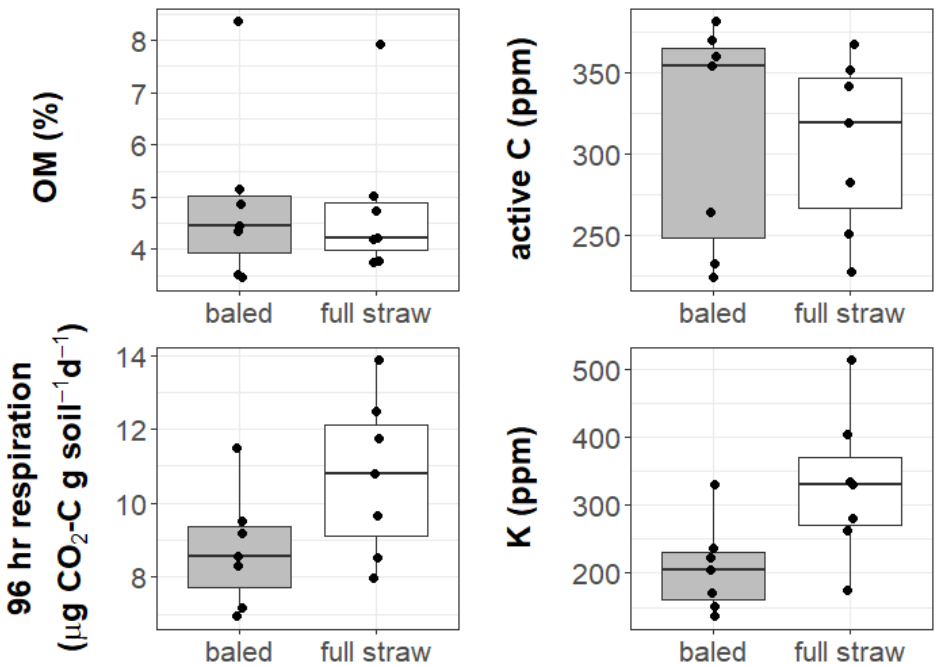

Figure 2. Box plots of key soil health properties in the baled and full-straw fields (n = 7). Each point represents a field and is the average of the three transects. The top of each box represents the 25th percentile, while the lower end of the box represents the 75th percentile (i.e., 25% of observations were above and below the boxed area). Solid bold lines indicate the mean for each management, respectively.

We did not observe any difference in OM or active C between the management practices (Figure 2). We had hypothesized an increase in OM and active C in the full-straw fields. The lack of straw management effect on soil OM could be attributed to the large size of the OM pool in these soils (mean soil OM > 4% for both practices) and/or to more dominant factors that affect OM, such as tillage and below-ground inputs. The soil OM pool is large, and it often takes a long time and significant management changes to detect changes in this pool. Depth stratification may also play a role; straw C in these systems may be more concentrated in the surface layers, but that was not visible when we sampled at 0–8 inches. In 2020, subsamples from the 0- to 3-inch depth will be analyzed for total C, OM, and active C. Active C is a smaller pool of C that is thought

to be more digestible and used relatively quickly by the microbial community; it has been observed to be more responsive to management practices (Awale et al., 2017). However, we found no differences in active C between the management practices.

Effects of soil clay content and stand age

We used regression analysis to examine how soil clay content and stand age affected the measured soil health properties. The older a stand, the more time had passed since it was disturbed by tillage. Stand age ranged from 4 to 14 years, with an average of 7.5 years. Clay content ranged from 15.4 to 47.8%, with an average of 24.7%. Using field averages, we observed that as clay increased, OM, active C, water-stable aggregates, total N (data not shown), and K increased (Figure 3) ($P < 0.05$). Measures of biological activity did not show a correlation with clay content. With respect to stand age, we observed a positive relationship between stand age and OM and total N ($P < 0.05$). Respiration and active C tended to increase with stand age as well ($P < 0.1$) (Figure 4).

Conclusion

In summary, under full-straw management we observed increased K and respiration relative to the baled fields. We did not observe differences in OM or active C between the management practices. Percent OM and total N increased with stand age. This result suggests that C storage in these systems is related to the lack of disturbance (i.e., time since tillage) and is probably also impacted by below-ground root inputs during the life of the stand. For many of the soil health measures examined, we observed a strong effect of soil clay content. It is therefore critical that soil texture be analyzed and taken into account when interpreting soil health measurements. At this time, we strongly caution against using soil analyses to compare fields. Rather, these analyses should be used as relative measures to observe changes over time within a field.

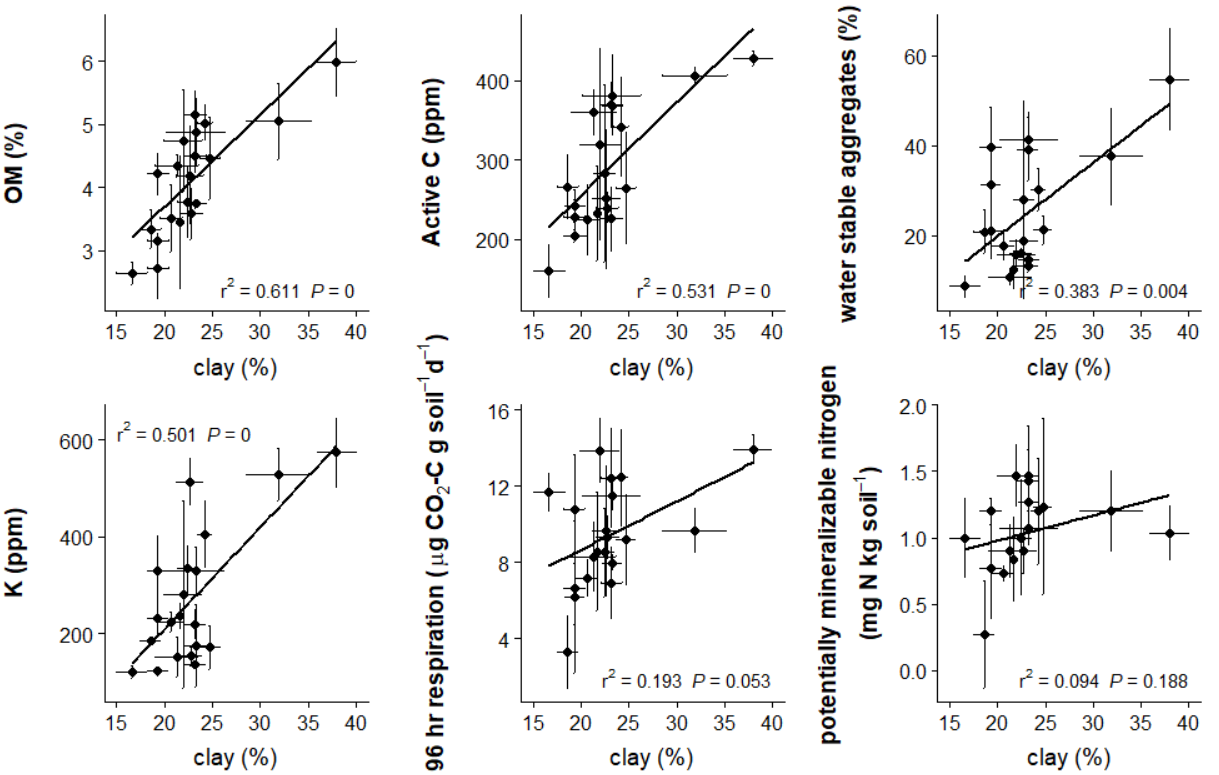

Figure 3. Relationship between soil clay content and select soil health properties across both management practices at ten sites. Each point represents a field and is the average of the three transects.

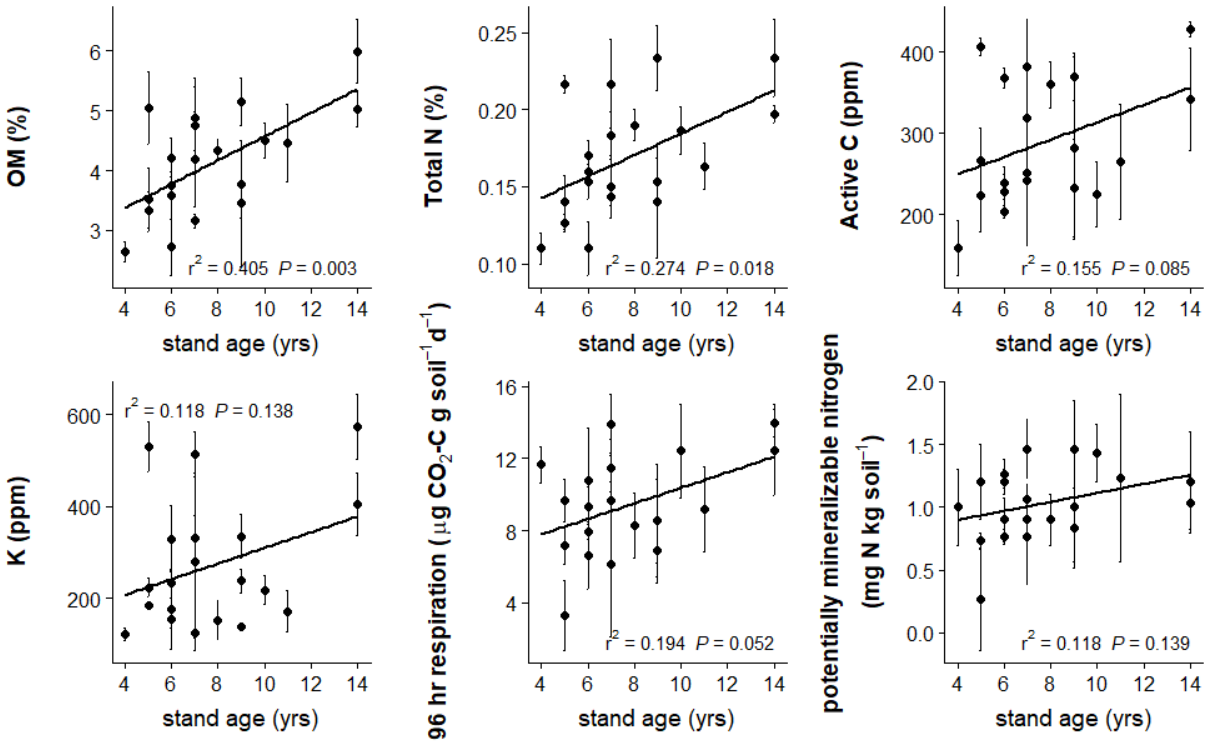

Figure 4. Relationship between stand age and select soil health properties across both management practices at ten fields. Each point represents a field and is the average of the three transects.

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EFFECTS OF TRINEXAPAC-ETHYL ON KENTUCKY BLUEGRASS IN THE GRANDE RONDE VALLEY OF NORTHEASTERN OREGON (2019)

D.L. Walenta and N.P. Anderson

Introduction

A 3-year study was initiated in the spring of 2018 to evaluate the effects of trinexapac-ethyl (TE, Palisade EC) plant growth regulator (PGR) on seed yield of Kentucky bluegrass (KBG). 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 stem internodes. PGRs are widely utilized in grass seed production around the world to increase seed yield potential via reduced lodging, improved pollination and fertilization, and improved harvestability.

PGR research in 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., 2014, 2015; Silberstein et al., 2002). Results from these studies showed 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 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 in first-, second-, third-, and fourth-year harvest stands. Classes of KBG 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 data presented in this article reflect only the second-year results. The results of the first year were evaluated but delayed until seed cleaning equipment and technical assistance became available in March 2019. The final year of the study will be conducted in 2020.

Materials and Methods

The second year of the study was initiated in spring 2019 in the Grande Ronde Valley of northeastern Oregon by establishing three trials in the same irrigated, commercial KBG seed production fields utilized in study year 1. The experimental design at each site was a randomized complete block with three replications. Plot sizes were 29 feet x 300 feet. Standard crop management practices were provided by cooperator growers, with the exception of the PGR application, which was applied by the researcher using a tractor-mounted R&D research sprayer with a 27-foot boom delivering 16 gal/acre. Crop growth stage, stand planting date, and environmental conditions at application time are provided in Table 1. Trinexapac-ethyl (TE) treatments included an untreated control (no TE) and 0.8, 1.4, and 2.8 pt product/acre.

Above-ground biomass samples were collected (two 1 ft^2 quadrats/plot) in June after anthesis but prior to mature seed development (BBCH 70–80) to determine biomass dry weight/acre, tiller height, panicle density, and ergot infection levels. Twenty-five panicles/plot were collected from windrows after swathing to further evaluate ergot infection levels. Seed was harvested with

Table 1. Crop growth stage and environmental conditions at time of TE application.

	Baron KBG	Skye KBG	Gaelic KBG
Application date	May 4, 2019	May 4, 2019	May 7, 2019
KBG growth stage	1–2 node (BBCH 31–32)	1–2 node (BBCH 31–32)	1–2 node (BBCH 31–32)
Stand planted	Spring 2017	Spring 2016	Spring 2017
Air temperature (°F)	75	81	58
Relative humidity (%)	44	29	56
Cloud cover (%)	5	0	5
Wind velocity (mph)	0–3 from NE	1–3 from NE	0–3 from SE
Soil temperature, surface (°F)	64	85	55
Soil temperature, 1 inch (°F)	64	75	55
Soil temperature, 2 inch (°F)	62	60	53
Soil temperature, 4 inch (°F)	62	57	49

grower-owned equipment, and a Brent weigh wagon was used to measure dirt weight seed yield/plot in the field. Subsamples of seed were collected from each plot and cleaned 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 (International Marketing & Design Model U).

Seed quality samples for each treatment/site were collected by combining 50 g of seed from each replicated treatment at each site to determine seed germination and vigor (three reps x 50 g/rep = 150 g clean seed/treatment/site). A total of 12 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 daily temperature/light cycles of 68°F (16 hours dark) and 86°F (8 hours light) for 2 weeks. Tetrazolium (TZ) testing was performed on two replications of 100 seeds/rep by soaking seed in TZ solution overnight and then counting viable seeds. Accelerated aging tests (AAT) were performed on 4 replications of 50 seeds/rep. These seeds were subjected to stress at 105.8°F for 48 hours before germination, using the same daily temperature/light cycle as was used in the germination test protocol.

Results and Discussion

Seed yield and lodging

'Baron' KBG: Seed yield was not affected by any TE treatments. The 2.8 pt/acre treatment reduced biomass and panicle number by 28% and 38%, respectively (Table 2). Lodging did not occur in any of the treatments. Tiller height was reduced in all treatments, with a notable height reduction of 4–6 inches with the 2.8 pt/acre TE rate. No differences were observed for percent cleanout or seed weight. The difference in purity is attributed to the mechanical seed cleaning process, not to TE application.

'Skye' KBG: Seed yield was increased (32%) across all TE treatments (Table 3). Lodging was reduced at the 1.4 and 2.8 pt/acre TE rates by 48% and 100%, respectively. Tiller height was reduced by 3 inches at the 2.8 pt/acre rate. There were no effects on above-ground biomass, panicle number, seed weight, or purity.

'Gaelic' KBG: Seed yields across all TE treatments were not statistically significant (Table 4). Across all TE treatments, lodging was reduced significantly (63%), but the level of reduction depended on TE rate. The 0.8 and 1.4 pt/acre TE rates did not provide adequate lodging control and resulted in 72% and 40% lodging, respectively. The high TE rate of 2.8 pt/acre resulted in complete lodging control. Tiller height was significantly reduced by all TE treatments, with a notable 10.8-inch height reduction at the 2.8 pt/acre TE rate. There were no effects on percent cleanout, above-ground biomass, panicle number, seed weight, or purity.

Spikelets/panicle and ergot infection

Differences were measured for the number of spikelets/panicle and ergot infection levels between varieties, but no interactions between variety and TE treatment were observed (Table 5). Ergot infection was detected in two of three sites, but frequency and severity levels were low (Table 5). All three varieties began anthesis during the first week of June and ended by mid-June. The flowering stage for 'Gaelic' KBG occurred during

Table 2. Effect of TE on seed yield, yield components, and growth characteristics of second-year harvest 'Baron' Kentucky bluegrass, 2019 (Site 1).[1]

TE treatment	Seed yield	Cleanout	Biomass	Tiller height	Panicle number	1,000-seed weight	Purity	Lodging
(pt/a)	(lb/a)	(%)	(ton/a)	(in)	(no./ft^2)	(g)	(%)	(%)
Control	960	17.3	6.1 a	22.9 a	388 a	0.416	99.0 a	0
0.8	912	16.7	5.3 ab	21.0 b	309 bc	0.418	97.4 b	0
1.4	945	18.3	6.1 a	21.2 b	378 ab	0.412	98.9 a	0
2.8	708	20.7	4.4 b	16.6 c	241 c	0.417	98.7 a	0
LSD (0.05)	NS	NS	0.9	1.6	78	NS	1.0	NS

[1]Values followed by the same letters are not significantly different at LSD (P = 0.05).

Table 3. Effect of TE on seed yield, yield components, and growth characteristics of third-year harvest 'Skye' Kentucky bluegrass, 2019 (Site 2).[1]

TE treatment	Seed yield[2]	Biomass	Tiller height	Panicle number	1,000-seed weight	Purity	Lodging
(pt/a)	(lb/a)	(ton/a)	(in)	(no./ft^2)	(g)	(%)	(%)
Control	512 c	6.8	25.7 a	259	0.418	97.9	100 a
0.8	612 b	6.6	25.7 a	246	0.408	97.5	100 a
1.4	758 a	6.3	26.0 a	232	0.427	96.4	52 b
2.8	666 b	5.8	22.7 b	222	0.421	97.4	0 c
LSD (0.05)	91	NS	1.2	NS	NS	NS	16

[1]Values followed by the same letters are not significantly different at LSD ($P = 0.05$).
[2]Clean seed yield based on 18% percent cleanout for seed subsamples (dirt weights not available).

Table 4. Effect of TE on seed yield, yield components, and growth characteristics of second-year harvest 'Gaelic' Kentucky bluegrass, 2019 (Site 3).[1]

TE treatment	Seed yield	Cleanout	Biomass	Tiller height	Panicle number	1,000-seed weight	Purity	Lodging
(pt/a)	(lb/a)	(%)	(ton/a)	(in)	(no./ft^2)	(g)	(%)	(%)
Control	1,046	46.4	7.3	31.6 a	367	0.406	95.8	99 a
0.8	1,357	36.0	6.4	29.0 b	333	0.417	98.8	72 b
1.4	1,337	40.0	6.3	28.2 b	313	0.393	98.3	40 c
2.8	1,155	40.4	6.4	20.8 c	336	0.411	98.1	0 d
LSD (0.05)	NS	NS	NS	1.4	NS	NS	NS	17

[1]Values followed by the same letters are not significantly different at LSD ($P = 0.05$).

Table 5. Average number of spikelets/panicle and ergot infection frequency/severity by Kentucky bluegrass variety across all trinexapac-ethyl treatments, 2019.[1]

KBG variety	Spikelets/panicle	Ergot-infected panicles	Ergot sclerotia/panicle
	(average no.)	(%)	(average no.)
Baron	128 b	2.2 ab	0.03 ab
Skye	96 c	4.6 a	0.06 a
Gaelic	176 a	0.0 b	0.0 b
LSD (0.05)	14	2.8	0.04
Variety	$P = 0.00$	$P = 0.009$	$P = 0.007$
Treatment	NS	NS	NS
Variety x treatment	NS	NS	NS

[1]Values followed by the same letters are not significantly different at LSD ($P = 0.05$).

a period of very low airborne ergot spore activity following peak spore activity in mid- to late May (data not shown), and ergot infection was not detected. Ergot infection did occur at the 'Baron' and 'Skye' sites, but neither the quantity nor temporal dynamics of airborne ascospores is known since monitoring was not performed at those sites.

Seed quality

Seed quality was not affected by TE at any application rate, and there were no significant interactions between variety and TE treatment (Table 6). Slight differences were observed between varieties for TZ and AAT levels, but differences are not attributed to TE application. Slowed germination in the AAT is attributed to a varietal response to high temperatures and relative humidity.

Table 6. Kentucky bluegrass seed viability and vigor by variety across all trinexapac-ethyl treatments, 2019.

KBG variety	Standard germination test	Tetrazolium test	Accelerated aging test
	(%)	(% viable seed)	(% germination, 2 weeks)
Baron	85	91.0 a	57.4 c
Skye	84	86.2 b	68.6 b
Gaelic	84	92.7 a	81.0 a
LSD (0.05)	NS	2.6	4.6
Variety	NS	$P = 0.0006$	$P = 0.00$
Treatment	NS	NS	NS
Variety x treatment	NS	NS	NS

Conclusion

Overall, results from the second year of the study did not show an interaction ($P = 0.0529$) between variety and TE application rate effect on seed yield. A varietal response to TE application was observed for improved seed yield only in 'Skye', where the 0.8 and 2.8 pt/acre TE rates resulted in statistically similar seed yields, both of which were lower than the 1.4 pt/acre rate. An interaction did occur between variety and TE application rate ($P = 0.00$) for lodging, which indicates that 'Skye' and 'Gaelic' were more susceptible to lodging than 'Baron' at the 0.8 and 1.4 pt/acre TE rates.

Year 1 results (data not shown) were similar to year 2 results, with no observed differences in above-ground biomass. Panicle emergence was delayed approximately 7 days at the 2.8 pt/acre TE rate, with reduced tiller height and lodging at the 1.4 and 2.8 pt/acre TE rates. In first-harvest stands of 'Baron' and 'Gaelic', the 2.8 pt/acre TE rate resulted in fewer panicles/ft^2; however, neither seed weight nor yield was reduced. 'Baron' responded similarly in the second harvest year, with a reduced number of panicles/ft^2. In contrast, no differences were observed for 'Skye' or 'Gaelic'.

Further investigation is needed to examine optimal TE application rates for KBG varieties. This project will be repeated in 2020 to investigate effects of TE on third- and fourth-year seed crop stands in the same fields.

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

R. Qin, N.P. Anderson, D.L. Walenta, and S.B. Lukas

Introduction

Oregon is the leading state in the United States for turfgrass seed production, with a planting area of more than 395,000 acres annually yielding more than 550 million lb of seed valued at $400 million. The main grass seeds crops produced are ryegrass, tall fescue, Kentucky bluegrass (KBG), rough bluegrass, orchardgrass, chewings fescue, red fescue, hard fescue, colonial bentgrass, and creeping bentgrass. Among these grasses, KBG seed is produced on 17,300 acres, yielding 22 million lb of seed. The Columbia Basin region is one of the major KBG seed production zones.

Grass seed crops often suffer from lodging, as their elongating stems cannot support the weight of inflorescences, causing tillers to lodge, or fall to the ground. To mitigate lodging, the use of plant growth regulars (PGRs), which may reduce stem elongation and increase stem thickness, has increased over time in Oregon grass seed production. The practice of reducing lodging with PGRs began as early as the 1980s with paclobutrazol and uniconazole. Currently, Palisade EC (trinexapac-ethyl, or TE) and Apogee (prohexadione calcium) are registered on grass seed crops in Oregon. The active ingredients of these products may inhibit the 3-β hydroxylation of the gibberellin GA20 to GA1. Gibberellins (GAs) are plant hormones that regulate various developmental processes, such as germination, dormancy, stem elongation, flowering, and senescence of leaves and fruits. GA20 is the immediate precursor of GA1, and GA1 has a function of promoting stem elongation.

TE is widely used to control lodging and increase seed yield in perennial ryegrass and tall fescue in coastal Oregon. Application of TE was found to improve seed yield by 45% in perennial ryegrass and by 40% in tall fescue (Chastain et al., 2014b; Chastain et al., 2015). However, the literature on the use of PGRs in KBG seed production is scarce. Moreover, the effects of PGRs on KBG seed production have not been consistent because they increase seed yields of only some cultivars (Butler et al., 2010). Thus, there is a need to conduct more in-depth research on the effect of PGRs on KBG seed yield across different cultivars.

Chastain et al. (2014a) also found that seed yields of perennial ryegrass and tall fescue were affected by interactions of PGR and nitrogen (N) application, and these interactions may also exist in KBG. Acting in opposition to the effect of PGRs, a high-N environment may exacerbate lodging problems in grass seed production systems.

Kentucky bluegrass seed producers in the Columbia Basin have shown great interest in using PGRs, but research-based recommendations are nonexistent. Work is needed to determine possible interactions between PGR and N application for lodging control in KBG seed production in the Columbia Basin region. Therefore, we conducted a field study to evaluate the effect of TE and N applications on KBG growth and seed production. The results of this project aim to provide applied and impactful information for direct dissemination to growers. Meanwhile, this study will fill the current knowledge gap to generate a better understanding of the relationship between cultivars and practices for lodging control and N management.

Materials and Methods

A field study was carried out from September 2017 to July 2018 at the Oregon State University Hermiston Agricultural Research and Extension Center with a soil type of Adkins sandy loam. The experimental design was a randomized complete block with three replications. Individual plots were 5.5 feet x 30 feet, composed of seven rows of KBG on a 6-inch spacing. In the study, a KBG elite, 'Midnight', was planted under center pivot irrigation on September 11. The seed sowing rate was 6 lb/acre pure live seed (PLS).

Before planting, a preplant fertilizer was applied at a rate (lb nutrient/acre) of 16N-45P-112K-22S-4Mg-1Zn-1B. During the crop growing season, urea was split-applied on October 19 at 135 lb N/acre and on March 5 at 125 lb N/acre. Before fertilization, the soil N in the top foot of soil was measured to determine the N application rate.

During the growing season, broadleaf weeds were controlled with an application of Callisto at 4 oz/acre

in mid-October. Powdery mildew was managed with two applications of Tilt at 4 oz/acre in April and May. Escaped broadleaf and grass weeds were removed by hand throughout the growing season.

On May 5, when the KBG was at the early stem elongation stage (BBCH 32), TE was applied at 0, 0.8, 1.4, and 2.4 pt/acre. On the same day, N treatments included two rates at 0 and 50 lb N/acre, applied manually as a top-dress using regular urea. As a result, eight treatments were achieved based on the combination of PGR and N applications.

After treatments were applied, evaluations were conducted in late June to determine lodging score and stem height. At harvest (June 29), the KBG was cut with a swather when the seed was at a high seed moisture content (24–28%). Two weeks later, a small plot combine was used to thresh the seed. Clean seed yield was determined after harvest samples were processed and weights determined.

Results and Discussion

Field measurements indicate that TE application tended to reduce lodging, with the high rate showing a 25% reduction in lodging (Figure 1). However, the application of an additional 50 lb N/acre increased lodging by 11% when compared to treatments without any additional N (Figure 1). KBG tiller height was slightly reduced by applications of TE but was not impacted by the application of an additional 50 lb N/acre (Figure 2). In most cases, KBG seed yields increased 29–74% with the application of an additional 50 lb N/acre. TE application (especially the higher rate) tended to increase seed yields in the N-treated plots (Figure 3).

Overall, no significant differences were found in this experiment because of the large standard deviations among the replicates. It should be noted that seed yield in the study was much lower than that on commercial farms, possibly because strong wind events coincided with KBG harvest (swathing or combine harvest) and may have impacted seed yield due to an unknown amount of seed shatter. Additionally, field management practices might need to be improved.

The results of this preliminary study indicate that both TE and seasonal N applications are important tools for KBG growth and production. The additional N application during the stem elongation stage might be beneficial for seed production, although the positive

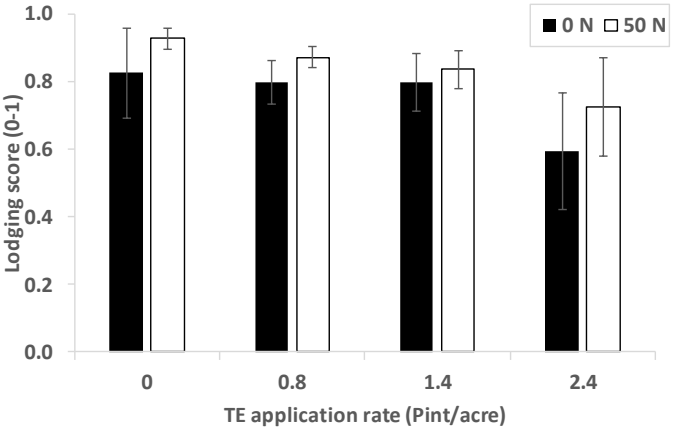

Figure 1. The effect of plant growth regulator and nitrogen applications on the lodging score of Kentucky bluegrass. Lodging scores range from 0 to 1, with 0 representing no lodging and 1 representing 100% lodging.

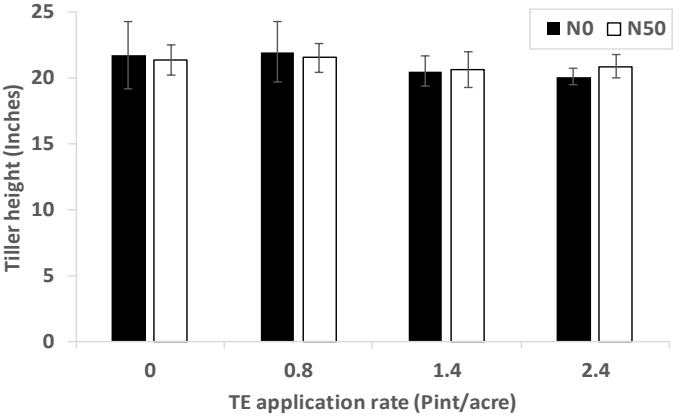

Figure 2. The effect of plant growth regulator and nitrogen applications on tiller height of Kentucky bluegrass.

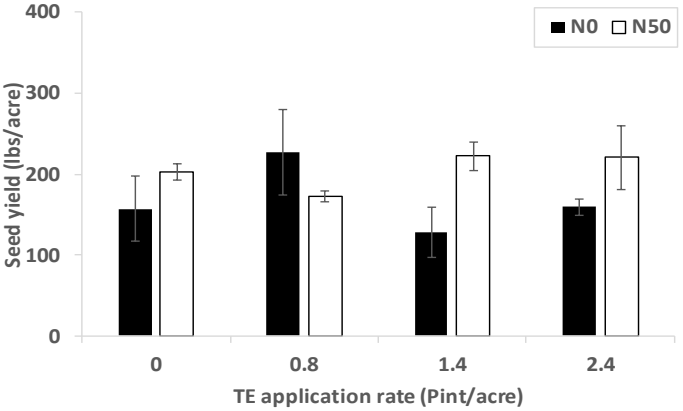

Figure 3. The effect of plant growth regulator and nitrogen applications on seed yield of Kentucky bluegrass.

effect might be weakened by the increased potential for lodging. In order to make conclusive recommendations, more data is needed to further evaluate the interactive effect of PGR and N applications on plant growth and seed production in KBG.

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Projects are funded by the OSU Agricultural Research Foundation and the Washington Turfgrass Seed Commission. Cory Zita, Dan Childs, and Tim Weinke of the OSU Hermiston Agricultural Research and Extension Center provided technical support for field management. Nikkole Duitsman, Avery Treadwell, and Schae Borrego contributed to the field measurements and data collection.

EFFECTS OF TRINEXAPAC-ETHYL ON KENTUCKY BLUEGRASS IN THE COLUMBIA BASIN OF OREGON

R. Qin, N.P. Anderson, D.L. Walenta, and S. Fei

Introduction

Oregon is one of the few states in the United States with significant Kentucky bluegrass (KBG) seed production, with more than 17,300 acres in production. The Columbia Basin of eastern Oregon is one of the state's major KBG seed production areas. In this area, KBG seed production occurs primarily on coarse-textured soils such as loamy fine sand and fine sandy loam.

KBG is susceptible to lodging because of a lack of stem strength and the prevalence of windy conditions during the reproductive stage. Lodging can result in significant yield loss and harvesting difficulties for growers. The plant growth regulator (PGR) trinexapac-ethyl (TE) is widely used to reduce lodging and increase seed yield in perennial ryegrass and tall fescue in western Oregon. Application of TE was found to increase seed yield by 45% in perennial ryegrass and by 40% in tall fescue (Chastain et al., 2014; Chastain et al., 2015). However, the literature on the use of PGRs in KBG seed production is limited. In one published study, the effects of PGRs on KBG seed production were inconsistent, with only certain cultivars showing increased seed yields (Butler et al., 2010). Therefore, there is a need for more in-depth research on the effect of PGRs on KBG seed yield across different KBG cultivars.

To fill the knowledge gap, a study was conducted to measure the effect of TE at various application rates on three KBG cultivars that differ in growth habit. By evaluating plant growth, lodging, flowering, and crop yield, the objective of this project was to identify the most effective TE application rates for KBG seed production. The overall goal is to improve our understanding of the relationship between cultivars, management practices, and utilization of PGRs.

Materials and Methods

A field trial was conducted from September 2018 to July 2019 at the Oregon State University Hermiston Agricultural Research and Extension Center on an Adkins sandy loam soil. Three KBG cultivars ('Mercury', 'Bluecoat', and 'Midnight') were selected to represent classes that differ in growth habit and seed yield potential. For example, 'Mercury' and 'Bluecoat' have taller tiller height and higher yield potential than 'Midnight'. The cultivars were planted on August 30 under a center pivot system. The sowing rate for 'Mercury' and 'Midnight' was 6 lb/acre, with the rate for 'Bluecoat' increased by 20% (7.2 lb/acre) to adjust for seed age difference. Each cultivar was planted with a cone planter into a block measuring 24 feet x 135 feet (four drill passes/block) oriented side by side.

Preplant fertilizer was applied and incorporated into the trial site at a rate (lb nutrient/acre) of 14N-40P-100K-20S-4Mg-1Zn-1B-73Cl. During the growing season, the trial site was top-dressed with regular urea on October 30 and April 3, delivering 145 and 150 lb N/acre, respectively. Nitrogen application rates were based on the N content in the top foot of soil.

Broadleaf weed and volunteer potatoes were controlled with the application of 4 oz/acre Callisto herbicide in mid-October. In April, the fungicide Tilt was sprayed at a rate of 4 oz/acre to control powdery mildew disease. Grass weeds (annual ryegrass and occasionally volunteer wheat) and mallow were hand weeded throughout the growing season.

In mid-April, within each KBG cultivar block, each drill pass was divided into five 25-foot-long plots with a 2.5-foot border on each end. As a result, each cultivar block was a randomized complete block experimental design with five treatments and four replicates. TE was applied with a backpack sprayer on April 22, when plants were at the early stem elongation stage (BBCH 32). TE rates were 0, 0.9, 1.9, 2.8, and 3.8 pint product/acre.

Following treatment, lodging score, stem height, and percent flowering were measured regularly throughout the growing season. Lodging was evaluated using a customized chart with a scoring system ranging from 1 to 9, where 1 represents the most serious lodging and 9 represents no lodging.

A small plot swather was used to windrow each cultivar at a high seed moisture content (24–28%) on July 2. Threshing was done with a small-plot combine in mid-July. The dirty and clean seed yields were determined for each treatment. The data were analyzed as a one-way ANOVA using Sigmaplot 13. When the F-test was significant, the means were separated using the Bonferroni test at the 5% level.

Results and Discussion

Lodging

Results from the study indicate that the application of TE significantly reduced the severity of lodging, with the higher application rates resulting in less lodging (Figure 1). A significant effect of TE for lodging control was observed during the period of June 4–June 21 for 'Bluecoat', from May 22 to June 21 for 'Mercury', and on June 4 for 'Midnight' ($P < 0.05$).

However, it appears that TE lost effectiveness at harvest time regardless of the application rate. This may be attributed to two factors. First, strong wind events may have resulted in high levels of lodging for the TE-treated plants. Second, the effect of TE may last for only a certain length of time, after which the crop may be able to resume normal growth, as indicated by stem heights measured in this study. One solution to the short-lived effect of TE might be a split application of TE at the two-node stage (BBCH 32) and at flag leaf emergence (BBCH 37–39). Research related to prolonging the effect of TE is needed.

Among the three cultivars, TE was more effective at controlling lodging in 'Mercury' and 'Bluecoat' and less effective for 'Midnight' (Figure 1). The lodging response difference may be attributed to the overall plant height growth potential of each cultivar. In this study, 'Midnight' exhibited a shorter plant height than did 'Bluecoat' and 'Mercury'.

Stem height

Our data indicate a significant reduction in stem height with increased TE application rate (Figure 2). Similar to the lodging score, the TE effect on stem height reduction diminished with time (Figure 2). For example, in 'Mercury', the significant differences in stem height reduction between TE application rates occurred in both May and June and disappeared at harvest time. For 'Bluecoat' and 'Midnight', the significant differences in stem height disappeared beginning June 21. Field observations indicate that flowering was delayed for approximately 1 week in all KBG cultivars at TE application rates ≥ 2.8 pt/acre (data not shown).

At harvest, uncleaned seeds and clean seed yield were determined for each treatment. It should be noted that during the cleaning procedure an unknown number of clean seeds are removed. The highest KBG clean seed yields for 'Bluecoat' and 'Mercury' were obtained with TE application rates of 1.9–2.8 pt/acre ($P < 0.05$) (Figure 3). Compared to other cultivars, the seed yield response to TE application in 'Midnight' was limited. The average internode length of 'Midnight' is much shorter than that of the other two cultivars, and the effect of TE in reducing internode elongation was limited. Among the three cultivars, the lowest seed production was measured in 'Midnight'.

It should be noted that seed yield in the study was much lower than that on commercial farms, possibly because strong wind events coincided with KBG harvest (swathing or combine harvest) and may have impacted seed yield due to an unknown amount of seed shatter. Additionally, field management practices might need to be improved.

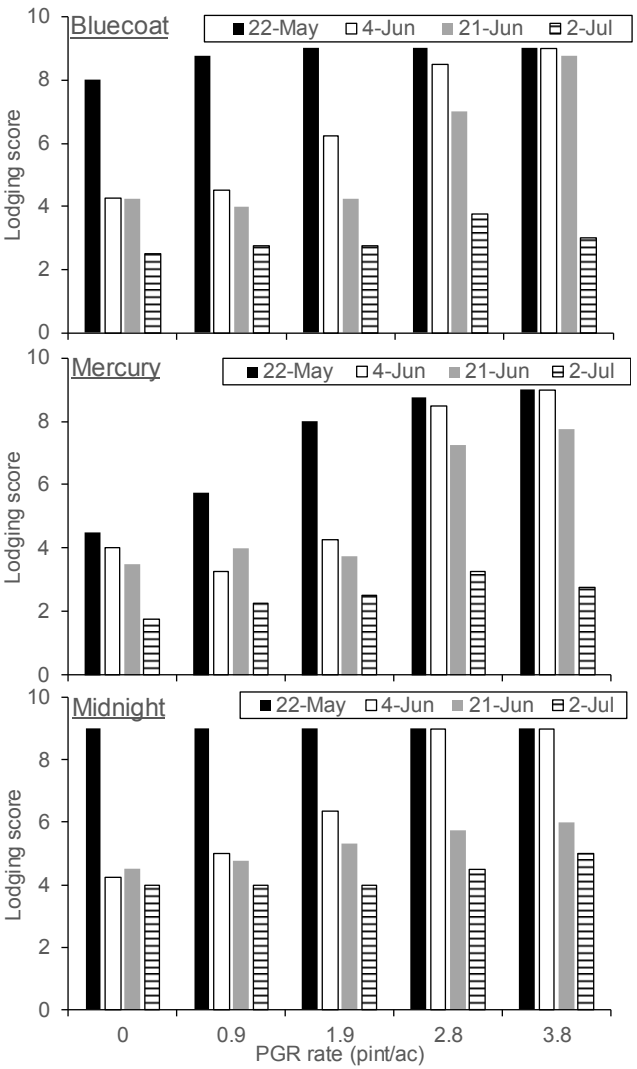

Figure 1. The effect of TE applications on lodging in Kentucky bluegrass. Lodging scores range from 1 to 9, with 1 representing the most serious lodging and 9 representing no lodging.

Results from the first-year field study suggest that the TE effect may vary with different cultivars. This finding is consistent with previous research demonstrating differential cultivar response to TE (Butler et al., 2010). The application of TE had a significant effect on lodging and stem height reduction on cultivars with greater plant height and yield potential. An additional year of data is needed in order to confirm the effect of TE and to refine TE application recommendations for KBG seed producers.

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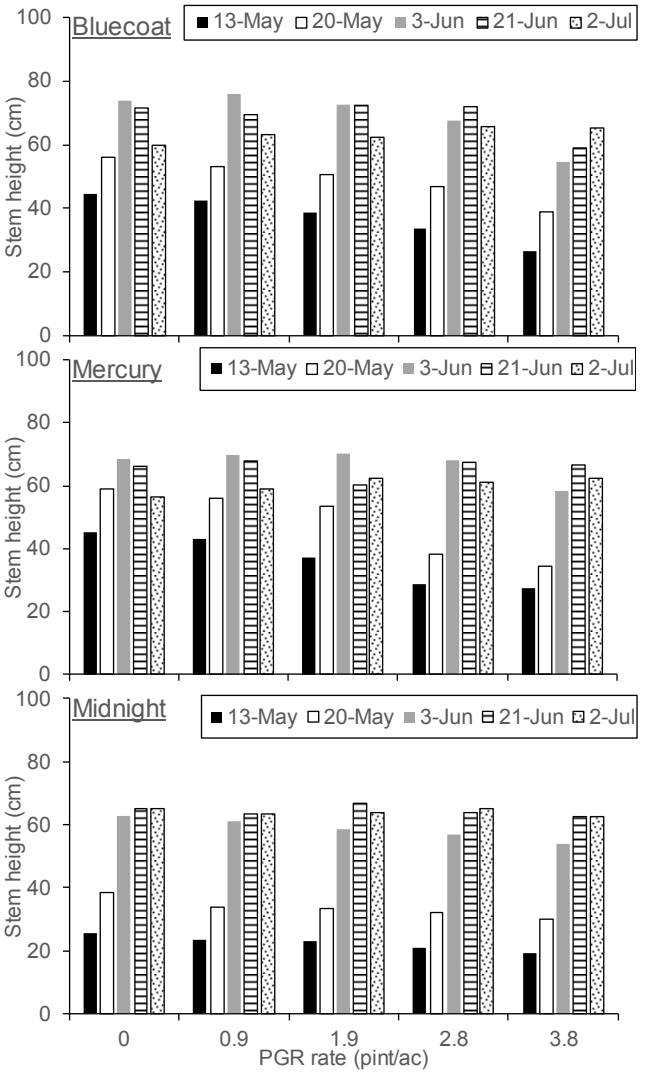

Figure 2. The effect of TE applications on stem height of Kentucky bluegrass.

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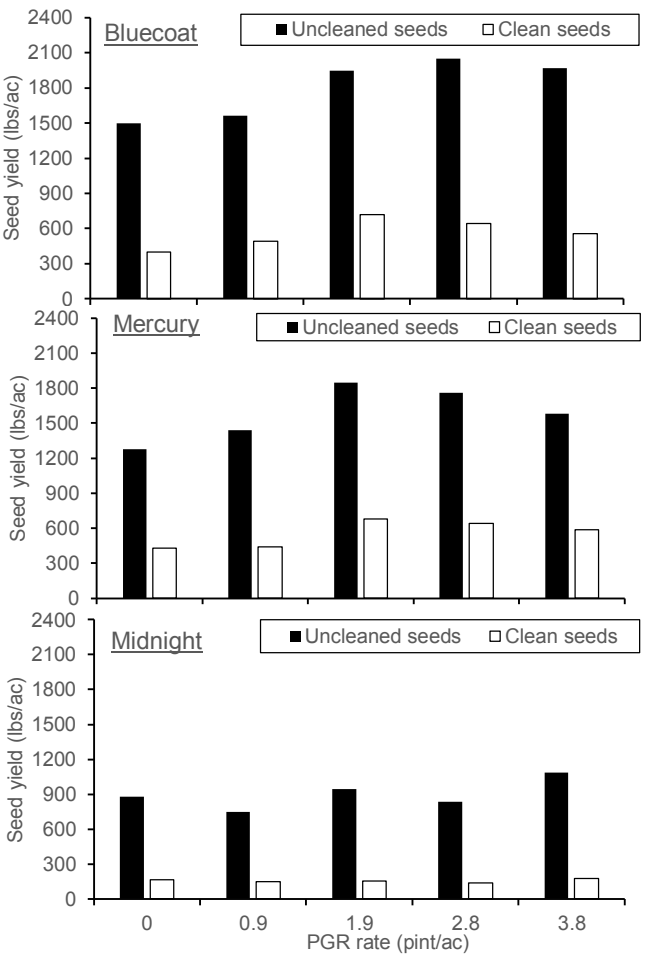

Figure 3. The effect of TE applications on seed yields of Kentucky bluegrass.

EXAMINING THE NITROGEN FERTILIZER NEEDS OF DRY FIELD PEAS IN THE WILLAMETTE VALLEY

E.C. Verhoeven, W.P. Jessie, and C.S Sullivan

Introduction

Dry field peas grown for seed are a valuable rotation crop that has expanded in acreage in the Willamette Valley in recent years. Although field peas have been produced extensively in the Pacific Northwest, they are a new crop for many Willamette Valley growers, and optimal management practices are still emerging. A fertilizer guide for field peas grown in western Oregon does not recommend application of any nitrogen (N) if nodulation is effective (Gardner et al., 2000). Effective nodulation occurs when there are sufficient and active populations of *Rhizobia* sp. bacteria, either from native populations or from successful inoculation. Despite the ability of peas to fix N through this association with Rhizobia bacteria, in-season N fertilizer application rates average around 60 lb N/acre in the Willamette Valley.

Studies from regions where field peas are more commonly grown (i.e., Canadian prairies, Dakotas, and Montana) have generally shown that in-season N fertilization is not necessary if field peas are successfully inoculated and starting N (soil + starter fertilizer) provides 10–15 lb total available N/acre (Jones and Olson-Rutz, 2018). Research from North Dakota has shown that 60–80% of the N found in a field pea is derived from N_2 fixation, with the remainder being derived from soil N sources (Franzen, 1998). Furthermore, it has been shown that high levels of available soil N (more than 50 lb/acre) reduce nodulation because the legume crop preferentially uses soil N and reduces utilization of "free N" from fixation.

Nearly all research conducted on dry field peas has been done in climates that are very different from the Willamette Valley, such as semiarid environments where soils carry over N from the previous crop. In the Willamette Valley, it is assumed that most NO_3-N present in the fall is leached and lost over winter.

A trial was conducted at three locations (Benton, Linn, and Polk counties) in 2016 to investigate the effect of in-season N rates on field pea yield and yield components (Sullivan, 2019). In that study, there was no effect of in-season N rate (0–120 lb/acre) on seed yield or yield components. Following harvest, higher residual soil N was observed at the higher N rate treatments (80 and 120 lb/acre), indicating that N was likely in excess of plant demand.

Results from a repeated study (2019) are presented in this report. Combined with the 2016 data, this work provides on-farm yield data across two growing seasons in the Willamette Valley. This work will help growers decide whether they can eliminate or reduce in-season N rates to reduce input costs and maximize the N_2 fixing abilities of field peas. In addition, nutrient accumulation data for macro- and micronutrients beyond N were also collected and will help guide growers in developing a pea fertility program.

Objectives:

- To demonstrate the effect of no in-season N fertilization on field pea growth and seed yield.
- To measure the effect of in-season N fertilizer rate on root nodulation, seed yield, and seed yield components.
- To develop recommendations for in-season N fertilizer use in field pea production based on research results and to disseminate this information to growers.

Table 1. Trial activities and dates completed at three field pea sites, 2019.

Activity	Linn	Benton	Marion
Preplant soil sample	Mar. 18	Mar. 25	Mar. 16
Fields planted	Mar. 21	Mar. 26	Mar.17
Flagged	Apr. 10	Apr. 11	Mar. 20
Grower fields fertilized	Apr. 17	Apr. 20	Apr.19
OSU trials fertilized	Apr. 23	Apr. 25	Apr. 24
Nodulation sampling	May 28	May 28	May 27
Swathed[1]	N/A	N/A	Jul. 13
Combined	Aug. 14	Aug. 12	Jul. 27
Postharvest soil sample	Aug. 19	Aug. 13	N/A[2]

[1]Linn and Benton county sites were direct combined.
[2]Plot flagging was removed at harvest, and postharvest soil samples could not be obtained.

Materials and Methods

Three trials were established in the spring of 2019 on growers' fields in Linn, Benton, and Marion counties (Table 1). Field peas were planted with grower equipment on March 17 (Marion), March 21 (Linn), and March 26 (Benton). The experiment was a randomized complete block design with four replications. Plots at the Linn and Marion sites were 0.13 acre, while Benton plots were 0.09 acre. Plot width was determined by grower harvest equipment. Preplant soil samples (0–6 inches) were taken at each site prior to planting and starter fertilizer application. Seed was not inoculated at any site. See Table 1 for field activity dates.

Soil samples were taken prior to the start of the trial to determine the starting nutrient concentrations in all fields. Starting nitrate (NO_3^--N) ranged from 11 to 31 lb NO_3^--N/acre. Phosphorus (P) and potassium (K) levels varied quite widely among the sites (36.4–156.8 ppm P and 146–263 ppm K) but in all cases were sufficient for crop growth. Soil pH at the sites ranged from 5.6 to 6.4. A starter fertilizer was applied preplant or at planting at all sites (grower field and trial area). Application of starter N did not exceed 20 lb N/acre. In-season grower N application to the surrounding field was 60 lb N/acre.

At the four-leaf stage (V4), four fertilizer treatments (urea, 46-0-0) were applied at the following rates: 0, 40, 80, and 120 lb N/acre. Initial soil N levels were not used to adjust fertilizer rates; the same rate was applied at each site. Fertilizer was applied using an air-inducted Orbit Air spreader at the Benton site and using a manual spin spreader at the Marion and Linn sites.

Roots from each plot were sampled approximately 10 weeks after planting, and root nodulation was assessed visually according to the *Nodulation and Nitrogen Fixation Field Assessment Guide* published by the Saskatchewan Ministry of Agriculture (Risula, 2016). In this protocol, nodulation and N fixation potential of a legume plant are scored based on: (1) plant growth and vigor, (2) nodule color and abundance, and (3) nodule position. A score is then calculated corresponding to a rating of effective nodulation, less effective nodulation, or poor nodulation.

At harvest, two 1 ft^2 plant biomass samples were taken per plot. Biomass samples were separated into stems and pods, dried at 140°F, and analyzed separately. The stems were analyzed for total biomass and percent N concentration. Pods were processed to measure the number of pods/ft^2, peas/pod, and peas/ft^2. Harvest was performed with grower equipment and a weigh wagon. Seed yield was calculated based on the seed weights from individual plots. Postharvest soil samples (0–6 inches) were taken within 5 days of harvest from each plot to determine residual soil N.

In addition to yield and yield components, two 1 ft^2 biomass samples were taken from the 80 lb N/acre treatment on five dates during the growing season, roughly corresponding to the following: two-node stage (V2), eight-node stage (V8), onset of flowering (R1), green seed fill (R4), and harvest. In each case, whole plant biomass was weighed, ground, and analyzed for nutrient concentrations (N, P, K, Mg, S, B). Total nutrient quantities at each sampling time were calculated by multiplying the nutrient concentration by the biomass and extrapolating to a lb/acre basis. At harvest, total above-ground biomass was sampled from all treatments, partitioned into plant biomass and peas, and analyzed for the nutrient concentration as above.

Results and Discussion

At all sites and N rates, we observed effective nodulation, meaning the plants were green and vigorous and active pink nodules were present. At all sites, we observed a tendency of higher nodulation scores in the 0 N plots, but there was no significant difference in nodulation score among treatments. In the 2016 study, less effective nodulation was observed in the 80 and 120 lb N/acre treatments. An overall rating of less effective nodulation reflects a combination of lower number of pink or active nodules, reduced crown nodulation, and reduced vigor (Risula, 2016). This result follows the expectation that less nodulation will occur when N is readily available from fertilizer. It is possible that we did not see this effect in 2019 because of the timing of our N applications. In the 2019 growing season, N fertilizer was applied in late April, immediately after a period of high rainfall. Following application, there was a period of no rainfall that lasted nearly 3 weeks. This timing may have resulted in some ammonia volatilization and delayed fertilizer incorporation, thereby minimizing and delaying the impact of the fertilization treatments.

There were no differences in seed yield or plant samples taken at harvest (Table 2). A trend of increasing N content and N uptake was observed with increasing N rate, but this result was not significant. Among the seed yield components (pods/ft^2, peas/pod, and peas/ft^2), we observed no differences between the treatments. Similarly, in the 2016 trial, no differences in seed yield components were observed (data not

shown). Relatively high residual postharvest soil N was observed among all treatments at the Linn and Benton sites, ranging from 39 to 51 lb/acre. A trend of increasing soil N with N fertilizer rate was observed at the Linn site, while no pattern was observed at the Benton site. In the 2016 trial, higher residual soil N was observed in the 80 and 120 lb N/acre fertilizer treatments relative to 0 N, indicating that N was in excess and was not completely taken up by the pea crop (data not shown).

Pea plant nutrient uptake was correlated to biomass growth. At the Marion site, nutrient accumulation was most rapid through the end of May for N, K, Mg, and S (Figure 1). The other sites were direct combined, and plants matured in the field for approximately a month longer. At these sites, plants seemed to accumulate nutrients most rapidly in July. This difference is likely a combination of variety difference, drought stress (which seemed to be more severe at the Marion site), and a longer growth period at the Benton and Linn sites.

Table 2. Field pea biomass, stem and pod characteristics, nodule ratings, yield, and postharvest soil sample results, 2019.[1]

	------ Postharvest plant biomass ------								
N rate	Total N concentration	Biomass	N uptake	Nodulation rating	Pod/ft²	Peas/pod	Peas/ft²	Yield	Postharvest soil N
	(%)	(lb/a)	(lb/a)		(no.)	(no.)	(no.)	(lb/a)	(lb/a)
0	1.13	5,600	61	Effective	65	4.1	270	3,351	40
40	1.18	5,762	65	Effective	69	4.1	281	3,330	39
80	1.21	6,421	76	Effective	75	4.7	334	3,265	51
120	1.23	5,932	71	Effective	70	4.6	312	3,355	49

[1]Results are averaged across three locations in the Willamette Valley (Marion, Linn, and Benton counties) under four N fertilization rate treatments.

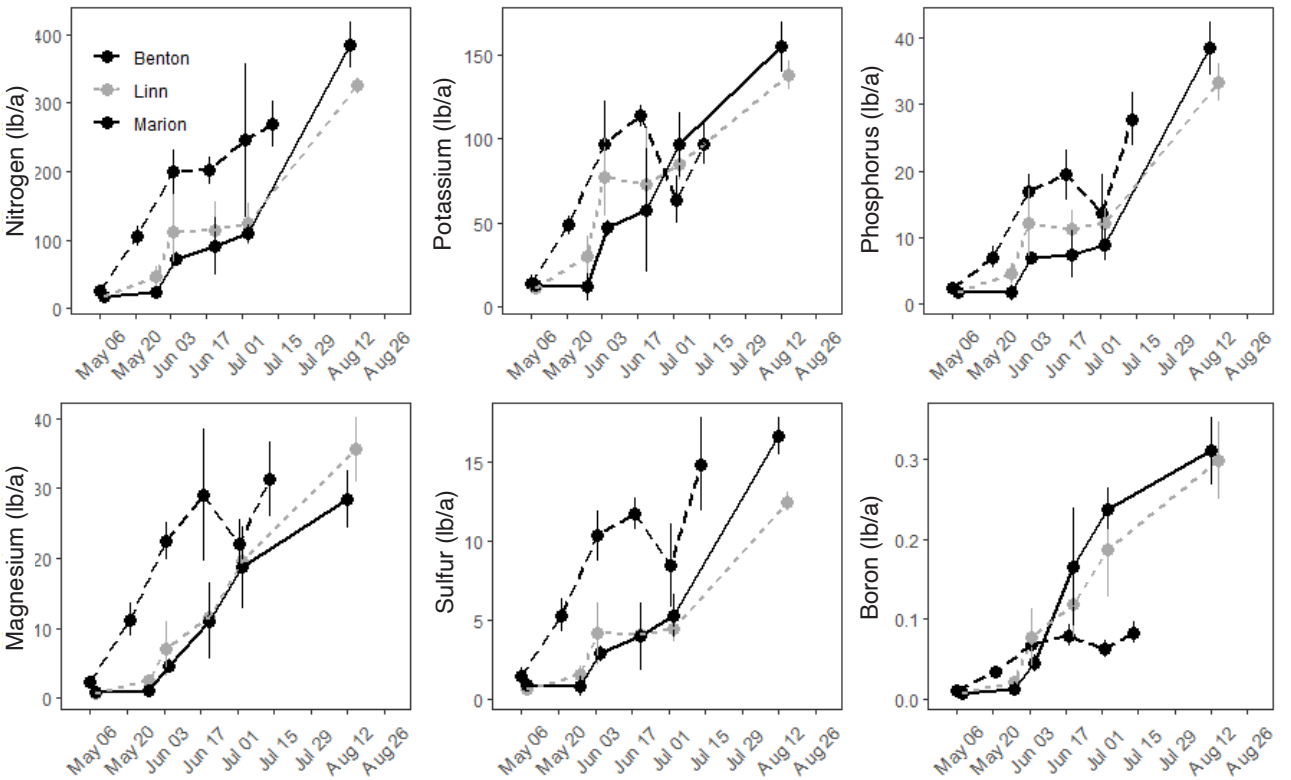

Figure 1. N, K, P, Mg, S, and B uptake throughout the growing season in the 80 lb N/acre treatment (n = 4). Nutrient uptake was calculated by multiplying plant biomass by nutrient concentration.

Table 3. Total nutrient uptake in postharvest plant biomass and peas.[1]

Portion of plant	N rate	N	P	Mg	K	Ca	S	B
				(lb/a)				
Plant biomass	0	61.4 (19.1)	3.9 (1.5)	19.3 (3.4)	54.9 (18.6)	144.3 (35.9)	3.8 (1.2)	0.1 (0.1)
Plant biomass	40	65.2 (18.4)	4.1 (1.1)	19.9 (7.0)	58.6 (18.9)	155.8 (54.3)	3.9 (1.5)	0.2 (0.1)
Plant biomass	80	76.2 (16.1)	4.7 (1.6)	23.7 (4.9)	68.0 (17.5)	177.6 (53.2)	4.4 (1.1)	0.2 (0.1)
Plant biomass	120	71.1 (18.7)	4.1 (1.3)	21.6 (6.7)	59.8 (19.2)	149.3 (46.7)	4.1 (1.6)	0.2 (0.1)
Peas	0	207.9 (71.9)	24.5 (7.6)	6.5 (2.1)	52.5 (19.2)	5.5 (2.2)	8.6 (1.9)	0.04 (0.03)
Peas	40	200.8 (65.3)	24.6 (7.7)	6.4 (2.0)	51.3 (16.4)	5.4 (2.4)	9.0 (2.9)	0.04 (0.02)
Peas	80	250.5 (61.4)	28.4 (6.0)	7.7 (1.8)	61.1 (16.5)	6.2 (2.2)	10.5 (2.3)	0.05 (0.02)
Peas	120	200.4 (50.4)	24.8 (8.7)	6.2 (1.7)	50.4 (17.9)	5.1 (1.8)	8.4 (2.1)	0.04 (0.02)

[1]Results were averaged across all three sites with the standard deviation in (). Biomass nutrients were left on the field, while pea nutrients were removed with harvest.

Plant biomass from all treatments was analyzed for nutrient concentration at harvest (Table 3). If fertilizer application practices are designed to maintain soil fertility, the average nutrient load removed with the peas can be used to guide total fertilization rates. One exception is N; because peas fix N, a large portion of N will come from the atmosphere. On average, peas removed 26 lb P/acre, 7 lb Mg/acre, 54 lb K/acre, 9 lb S/acre, and 0.04 lb B/acre (Table 3).

As in the first year of this trial (2016), seed yields were not increased by N fertilizer rate (Table 4). These data confirm previous findings demonstrating that in-season N fertilizer is not needed to obtain maximum seed yield in field pea crops grown in the Willamette Valley. Given the variability we observed in starting soil NO_3^- concentrations, a starter fertilizer with some N is still recommended but should not exceed 20 lb N/a.

Table 4. Yield results of three field pea trials in Benton, Linn, and Marion counties under different N rates as compared to grower field average, 2019.[1]

	Yield		
Treatment	Benton	Linn	Marion
(lb N/a)		(lb/a)	
0	2,564	4,374	3,115
40	2,781	4,252	2,957
80	2,575	4,406	2,815
120	2,675	4,483	2,908
Trial average	2,649	4,379	2,949
Grower field average	3,348	4,700	2,213
Grower fertilizer rate	60 lb N/a	60 lb N/a	60 lb N/a

[1]Grower fertilizer rates included. No differences between treatments within a field or between treatments summarized across sites were observed.

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THE EFFICACY OF FOUR SPECIES OF SLUG-KILLING NEMATODES ON THE GRAY FIELD SLUG

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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. Current control measures focus heavily on the use of molluscicidal baits, but growers report considerable variation in the efficacy of the most widely used active ingredients, i.e., metaldehyde, iron phosphate, and sodium ferric EDTA (McDonnell and Anderson, 2018). Hence, there is an urgent need to identify and develop alternative control practices for producers in the region.

Biological control offers a compelling option: the use of a pest's natural biological enemies to combat it in the field. Nematode worms in the genus *Phasmarhabditis* are important natural enemies of slugs. In fact, in Europe, a species called *Phasmarhabditis hermaphrodita* is currently used as a commercially available biological control agent labeled under the trade name Nemaslug to successfully manage slug pests in a wide range of crops. It is currently illegal to use Nemaslug in Oregon because the nematode has not been found in the United States. However, over the past 2 years we have surveyed a diverse range of crops for these nematodes and have discovered four species of *Phasmarhabditis* in various locations throughout the Willamette Valley. These discoveries potentially open up Oregon for the use of *Phasmarhabditis* as biological control agents. There is now an urgent need to investigate the infectivity of all four nematodes so the species most lethal to key slug pests, such as the gray field slug (*D. reticulatum*) can be identified for future research and testing as a biological control agent.

Methods

Slug and nematode specimens

Gray field slugs were hand collected in April 2019 from a field of perennial ryegrass grown for seed in Tangent, Linn County, OR, 48 hours before trials were initiated. Specimens were placed into plastic containers (35.9 cm x 20 cm x 12.4 cm), with 30 slugs per container, and were maintained in a growth chamber at a temperature of 18°C and a 12-hour photoperiod. The containers were kept moist with a single paper towel saturated with deionized water. Several slices of organic carrot were placed in each container as food. The paper towels and carrot were replaced three times weekly. Nematodes used in this study were isolated from slugs collected throughout Oregon. DNA identification of these nematodes is ongoing; consequently, they are referred to simply as *Phasmarhabditis* A, B, C, and D for the purposes of this report.

Infectivity trials

The infectivity trial arenas consisted of circular 8-oz plastic containers with 25 g of autoclaved topsoil and perforated lids. The soil was moistened by adding 10 ml of deionized water and mixing thoroughly. We used two nematode treatment rates: 20,000 (low rate) and 40,000 nematodes (high rate) for each species of nematode. Nematodes were placed in 5 ml of deionized water and then pipetted across the soil surface. A nematode-free container was used as a negative control. Six adult gray field slugs were then placed on the soil in the center of the arenas. A total of five replicates were used per nematode treatment and ten replicates for the control. Arenas were maintained in a growth chamber at a temperature of 18°C and a 12-hour photoperiod. A slice of organic carrot was placed in each container for 24 hours on day 8 as a source of slug food. The number of dead slugs was recorded daily for 2 weeks. A slug was deemed to have died when it did not respond to being poked by a blunt needle and/or when its tissue had liquefied. In addition, a dead slug typically would be covered with thousands of nematodes and thus would be visually obvious.

Statistical analysis

It was not possible to normalize the data, so nonparametric statistics were used for analysis. Differences between nematode treatments and controls were investigated using the Kruskal-Wallis test. Posthoc analysis was completed using Dunn's test incorporating the Bonferroni correction for multiple comparisons. Levels of significance corresponding to $P < 0.05$, $P < 0.01$, and $P < 0.001$ were used. All statistical analyses were carried out using IBM SPSS version 24.

Results and Discussion

The analysis showed that there was a significant difference ($P < 0.001$) in median percentage slug mortality between the different nematode treatments and control on days 3 to 14 (Table 1, page 34). No

slug mortality occurred on days 1 and 2. The high rates for both *Phasmarhabditis* A (16% mortality, $P < 0.05$) and *Phasmarhabditis* B (50% mortality, $P < 0.001$) were the first nematode treatments to cause significantly more slug mortality than the controls (0%), with mortality beginning on day 3. The low rates of *Phasmarhabditis* A (100%) and *Phasmarhabditis* B (66%) caused significantly ($P < 0.01$) more mortality versus the control on days 7 and 4, respectively. For *Phasmarhabditis* C, the high (100%, $P < 0.01$) and low (100%, $P < 0.05$) rates resulted in significantly higher *D. reticulatum* mortality compared to the control on day 7 (8%) and 9 (16%) respectively. At no point during this infection trial did *Phasmarhabditis* D cause significantly greater slug mortality compared to the other treatments and control (Table 1).

Complete mortality in all replicates occurred after 5 days, 6 days, 6 days, 8 days, 9 days, and 10 days for *Phasmarhabditis* B high, *Phasmarhabditis* B low, *Phasmarhabditis* A high, *Phasmarhabditis* A low, *Phasmarhabditis* C high, and *Phasmarhabditis* C low, respectively. Complete mortality in all replicates did not occur in the *Phasmarhabditis* D treatments (Table 1).

The high rate of *Phasmarhabditis* B was the only nematode treatment that caused a significantly higher percentage slug mortality when compared to other nematode treatments, and this occurred on days 3, 4, and 5 (Table 1). On day 3, this treatment was significantly ($P < 0.05$) more lethal to *D. reticulatum* than were the *Phasmarhabditis* C low rate, the *Phasmarhabditis* C high rate, and the *Phasmarhabditis* A low rate. It also caused significantly ($P < 0.05$) more slug mortality than the *Phasmarhabditis* C low rate on days 4 and 5 and significantly more mortality than the *Phasmarhabditis* D low rate on day 5.

Conclusion

As pest slugs, including *D. reticulatum,* continue to cause serious damage to crops throughout Oregon, there is a growing need to develop new management tools to help mitigate the losses caused by these pests. This need is particularly pressing given that slug populations are able to proliferate in cropping systems due to several factors, including prohibition of residue burning as a postharvest management option, an increase in no-till and conservation tillage acres, and grower dissatisfaction with the level of control with existing slug baits (McDonnell and Anderson, 2018). Biological control has often been identified as one such tool, and in recent years nematodes in the genus *Phasmarhabditis* have been cited repeatedly as having important potential (Tandingan De Ley et al., 2017). Although past research on *Phasmarhabditis* has focused almost exclusively on *P. hermaphrodita,* primarily due to commercial availability in Europe under the trade name Nemaslug (Rae et al., 2007), other species within the genus have been largely overlooked despite having clear biological control potential. Our data demonstrates that the four species of *Phasmarhabditis* we have discovered in Oregon are also lethal to the key pest, *D. reticulatum,* in laboratory infection trials. However, the extent of mortality and time to cause complete mortality varied greatly among nematode species.

Phasmarhabditis B was the most lethal of the four species investigated here, causing complete slug mortality in all replicates after 5 days with the high inoculation rate and killing significantly more slugs than the controls after just 4 days at both rates. This nematode species should be a prime candidate for additional biological control testing. *Phasmarhabditis* D caused more than 75% slug mortality after 14 days at both rates but was the least virulent of the four species to *D. reticulatum*. In fact, at no point during this infection assay was slug mortality significantly greater with this species of *Phasmarhabditis* compared to the controls, and hence it appears to have the lowest potential for further biological control testing.

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Table 1. Mean (+SE) percentage gray field slug mortality recorded daily for the low and high rate treatments of four *Phasmarhabditis* species and controls.[1]

	A High	A Low	B High	B Low	C High	C Low	D High	D Low	Control
Day 3	19.4 ± 3.4[a]	3.2 ± 3.2[b]	43.2 ± 4.2[b,c,d,e]	9.6 ± 3.9	3.2 ± 3.2[d]	3.2 ± 3.2[e]	6.4 ± 3.9	6.4 ± 3.9	0[a,c]
Day 4	36.2 ± 8.1	9.6 ± 3.9	89.8 ± 4.2[f, h]	63.0 ± 11.1[g]	13.0 ± 6.1	6.4 ± 3.9[h]	9.8 ± 6.6	13.2 ± 8.1	1.6 ± 1.6[f,g]
Day 5	73.0 ± 8.5[i]	36.2 ± 9.8	100.0 ± 0[j,k,l]	89.8 ± 4.2[m]	23.0 ± 6.6	6.4 ± 3.9[k]	13.0 ± 6.1	13.2 ± 8.1[l]	1.6 ± 1.6[i,j,m]
Day 6	100.0 ± 0[n]	66.4 ± 15.0	100.0 ± 0[o]	100.0 ± 0[p]	63.0 ± 6.1	29.6 ± 6.4	19.6 ± 6.2	19.6 ± 6.2	6.4 ± 2.6[n,o,p]
Day 7	100.0 ± 0[q]	93.2 ± 4.2[r]	100.0 ± 0[s]	100.0 ± 0[t]	93.2 ± 4.2[u]	59.6 ± 3.9	29.6 ± 3.4	22.8 ± 4.2	9.7 ± 3.6[q,r,s,t,u]
Day 8	100.0 ± 0[v]	100.0 ± 0[w]	100.0 ± 0[x]	100.0 ± 0[y]	93.2 ± 4.2[z]	79.6 ± 6.4	33.0 ± 5.4	26.2 ± 6.8	11.3 ± 3.5[v,w,x,y,z]
Day 9	100.0 ± 0[A]	100.0 ± 0[B]	100.0 ± 0[C]	100.0 ± 0[D]	100.0 ± 0[E]	93.2 ± 4.2[F]	39.8 ± 4.2	36.2 ± 8.1	12.9 ± 3.3[A,B,C,D,E,F]
Day 10	100.0 ± 0[G]	100.0 ± 0[H]	100.0 ± 0[I]	100.0 ± 0[J]	100.0 ± 0[K]	100.0 ± 0[L]	43.0 ± 6.6	46.4 ± 11.1	14.5 ± 2.9[G,H,I,J,K,L]
Day 11	100.0 ± 0[M]	100.0 ± 0[N]	100.0 ± 0[O]	100.0 ± 0[P]	100.0 ± 0[Q]	100.0 ± 0[R]	49.8 ± 13.0	59.8 ± 17.3	17.8 ± 3.0[M,N,O,P,Q,R]
Day 12	100.0 ± 0[S]	100.0 ± 0[T]	100.0 ± 0[U]	100.0 ± 0[V]	100.0 ± 0[W]	100.0 ± 0[X]	59.8 ± 13.6	66.6 ± 14.0	17.8 ± 3.0[S,T,U,V,W,X]
Day 13	100.0 ± 0[Y]	100.0 ± 0[Z]	100.0 ± 0[$]	100.0 ± 0[€]	100.0 ± 0[&]	100.0 ± 0[¥]	76.6 ± 14.6	73.2 ± 11.3	26.2 ± 6.2[Y,Z,$, €,&,¥]
Day 14	100.0 ± 0[£]	100.0 ± 0™	100.0 ± 0[©]	100.0 ± 0[®]	100.0 ± 0[@]	100.0 ± 0[§]	79.8 ± 13.4	83.2 ± 10.6	31.3 ± 8.8[£,™,©,®,@,§]

[1] No slug mortality was recorded on days 1 and 2, and consequently these days were omitted from the table. On specific days, values with the same superscript letter indicate significant differences between the treatments/controls. Pairwise posthoc tests with significance values adjusted by the Bonferroni correction for multiple tests for all time points.

Day 3: H=30.64, N=50, df=8, P=0.000. **a**: Ph High v Control, test statistic=23.70, P<0.05; **b**: Pp High v Ph Low, test statistic=27.90, P<0.05; **c**: Pp High v Control, test statistic=32.30, P<0.001; **d**: Pp High v Pc High, test statistic=27.90, P<0.05; **e**: Pp High v Pc Low, test statistic=27.90, P<0.05

Day 4: H=34.19, N=50, df=8, P=0.000. **f**: Pp High v Control, test statistic=34.10, P<0.001; **g**: Pp Low v Control, test statistic=29.40, P<0.01; **h**: Pp High v Pc Low, test statistic=29.30, P<0.05

Day 5: H=39.94, N=50, df=8, P=0.000; **i**: Ph High v Control, test statistic=28.30, P<0.01; **j**: Pp High v Control, test statistic=35.70, P<0.001; **k**: Pp High v Pc Low, test statistic=31.80, P<0.05; **l**: Pp High v Pu Low, test statistic=28.80, P<0.05; **m**: Pp Low v Control, test statistic=32.10, P<0.01

Day 6: H=42.75, N=50, df=8, P=0.000; **n:** Ph High v Control, test statistic=34.20, P<0.001; **o**: Pp High v Control, test statistic=34.20, P<0.001; **p**: Pp Low v Control, test statistic=34.20, P<0.001

Day 7: H=45.41, N=50, df=8, P=0.000; **q:** Ph High v Control, test statistic=33.00, P<0.01; **r**: Ph Low v Control, test statistic=28.00, P<0.01; **s**: Pp High v Control, test statistic=33.00, P<0.01; **t**: Pp Low v Control, test statistic=33.00, P<0.01; **u**: Pc High v Control, test statistic=28.00, P<0.01

Day 8: H=45.41, N=50, df=8, P=0.000; **v:** Ph High v Control, test statistic=31.40, P<0.01; **w**: Ph Low v Control, test statistic=31.40, P<0.01; **x**: Pp High v Control, test statistic=31.40, P<0.01; **y**: Pp Low v Control, test statistic=31.40, P<0.01; **z**: Pc High v Control, test statistic=25.80, P<0.05

Day 9: H=46.15, N=50, df=8, P=0.000; **A:** Ph High v Control, test statistic=30.30, P<0.01; **B**: Ph Low v Control, test statistic=30.30, P<0.01; **C**: Pp High v Control, test statistic=30.30, P<0.01; **D:** Pp Low v Control, test statistic=30.30, P<0.01; **E**: Pc High v Control, test statistic=30.30, P<0.01; **F**: Pc Low v Control, test statistic=24.30, P<0.05

Day 10: H=47.60, N=50, df=8, P=0.000; **G:** Ph High v Control, test statistic=29.35, P<0.01; **H**: Ph Low v Control, test statistic=29.35, P<0.01; **I**: Pp High v Control, test statistic=29.35, P<0.01; **J:** Pp Low v Control, test statistic=29.35, P<0.01; **K**: Pc High v Control, test statistic=29.35, P<0.01; **L**: Pc Low v Control, test statistic=29.35, P<0.01

Day 11: H=41.50, N=50, df=8, P=0.000; **M:** Ph High v Control, test statistic=27.55, P<0.01; **N**: Ph Low v Control, test statistic=27.55, P<0.01; **O**: Pp High v Control, test statistic=27.55, P<0.01; **P:** Pp Low v Control, test statistic=27.55, P<0.01; **Q**: Pc High v Control, test statistic=27.55, P<0.01; **R**: Pc Low v Control, test statistic=27.55, P<0.01

Day 12: H=42.68, N=50, df=8, P=0.000; **S:** Ph High v Control, test statistic=28.20, P<0.01; **T**: Ph Low v Control, test statistic=28.20, P<0.01; **U**: Pp High v Control, test statistic=28.20, P<0.01; **V:** Pp Low v Control, test statistic=28.20, P<0.01; **W**: Pc High v Control, test statistic=28.20, P<0.01; **X**: Pc Low v Control, test statistic=28.20, P<0.01

Day 13: H=39.53, N=50, df=8, P=0.000; **Y:** Ph High v Control, test statistic=26.70, P<0.01; **Z**: Ph Low v Control, test statistic=26.70, P<0.01; **$**: Pp High v Control, test statistic=26.70, P<0.01; **€:** Pp Low v Control, test statistic=26.70, P<0.01; **&**: Pc High v Control, test statistic=26.70, P<0.01; ¥: Pc Low v Control, test statistic=26.70, P<0.01

Day 14: H=34.64, N=50, df=8, P=0.000; £**:** Ph High v Control, test statistic=24.00, P<0.01; **™**: Ph Low v Control, test statistic=24.00, P<0.01; ©: Pp High v Control, test statistic=24.00, P<0.01; ®**:** Pp Low v Control, test statistic=24.00, P<0.01; **@**: Pc High v Control, test statistic=24.00, P<0.01; §: Pc Low v Control, test statistic=24.00, P<0.01

DEVELOPING ERGOT DISEASE DETECTION TECHNOLOGY FOR ENHANCED IPM IN GRASS SEED CROPS

Q. Cheng, D. Walenta, K. Frost, and J. Dung

Introduction

Ergot is an important seed replacement disease of perennial ryegrass and Kentucky bluegrass (KBG) seed crops grown in irrigated production regions of the lower Columbia Basin of Washington and Oregon. The ergot fungus (*Claviceps purpurea*) infects unfertilized flowers of grasses and transforms ovaries into dormant resting structures (sclerotia), which overwinter and produce spores the following season. The disease reduces yield and can be difficult to manage with fungicides alone (Cheng et al., 2019). Additional losses are incurred during seed cleaning to remove ergot.

Since 2015, our research team has provided regional Ergot Alerts to stakeholders during the grass seed production season (Walenta et al., 2016). The weekly Ergot Alerts provide growers and crop consultants in the Columbia Basin, Grande Ronde Valley, and central Oregon with disease risk potential based on spore counts and a predictive model. Recently, ergot has become a disease of concern in irrigated production regions of the upper Columbia Basin. The disease has also been reported in dryland production regions. As reports of ergot expand across the Pacific Northwest, a need exists for expanding disease monitoring and modeling efforts. However, the number and geographic range of Ergot Alert sites that can be monitored are limited due to the cost of equipment and the time required to travel to each site for sample collection.

Materials and Methods

Instead of Burkard spore samplers, which are relatively expensive (more than $5,000) to purchase and maintain, we deployed rotating-arm samplers, which were relatively inexpensive (less than $100) and easy to build and maintain. Validation of the rotating-arm samplers was performed at the Central Oregon Agricultural Research and Extension Center (COAREC, Madras, OR) in a 5-acre, second-year KBG seed field with a history of ergot. Three plots were established in the 5-acre field for the validation. In each of the plots, one Burkard spore sampler was placed alongside three rotating-arm samplers to collect ergot spores simultaneously. The three rotating-arm samplers in each plot were set at different heights (2, 3, and 4 feet) to test the effect of sampling height on spore collection efficiency. Different sampling periods (1, 2, 3, 4, and 7 days) were tested and compared with the standard Burkard spore sampler. Samples were processed following the standard phenol-chloroform DNA extraction procedure and quantified by quantitative PCR (qPCR) (Dung et al., 2018). Precipitation data were collected to determine the performance of the rotating-arm samplers under wet conditions. The collection efficiencies of traps with different heights and sampling periods were compared using analysis of variance. Results from Burkard samplers were provided through the Ergot Alert Blog (http://blogs.oregonstate.edu/coarecplantpathology/) as in previous years.

Results and Discussion

During the first year of validation, 238 sampling events were performed, and ergot spores were detected on 211 (88.7%) of the sampling days. Detection events from the rotating-arm samplers were consistent with results from the Burkard spore sampler for 91.6% of the data points. False negatives, which were defined as days on which ergot spores were not detected by an individual sampler but were detected by at least one other sampler in the array during the same sampling period, were greatest in the 4-foot rotating-arm sampler and Burkard spore sampler (seven false negatives each), followed by the 2-foot rotating-arm sampler (four false negatives) and the 3-foot rotating-arm sampler (two false negatives) (Table 1).

Results from rotating-arm samplers set at collection heights of 2, 3, and 4 feet were compared with those from the standard Burkard spore sampler, which collects at a height of 2 feet. There were no statistical differences among collection heights and the Burkard sampler ($P = 0.15$) (Table 2), indicating that rotating-arm samplers performed equally at different collection heights and were comparable to the standard Burkard spore sampler. As the season progressed, operation of the rotating-arm samplers at 2-foot heights was compromised by the canopy, so it will be recommended that rotating-arm samplers be placed just above the expected canopy height at anthesis.

Different sampling periods (1, 2, 3, 4, and 7 days) were tested and compared with the standard Burkard spore sampler. There was a significant difference among sampling periods ($P < 0.0001$) (Table 3). Overall,

Table 1. Number of days on which ergot spores were detected or not detected using different rotating-arm sampling periods and heights and compared with a Burkard spore sampler (collects at a height of 2 feet).

Sampling period	Sampling height	Detected	Not detected	False negatives[1]
1 day	Rotating-arm (2 ft)	2	1	0
	Rotating-arm (3 ft)	2	1	0
	Rotating-arm (4 ft)	0	0	2
	Burkard (2 ft)	1	1	1
2 days	Rotating-arm (2 ft)	6	0	0
	Rotating-arm (3 ft)	6	0	0
	Rotating-arm (4 ft)	6	0	0
	Burkard (2 ft)	6	0	0
3 days	Rotating-arm (2 ft)	26	1	3
	Rotating-arm (3 ft)	28	1	1
	Rotating-arm (4 ft)	25	1	3
	Burkard (2 ft)	23	1	6
4 days	Rotating-arm (2 ft)	14	0	1
	Rotating-arm (3 ft)	14	0	1
	Rotating-arm (4 ft)	13	0	2
	Burkard (2 ft)	15	0	0
7 days	Rotating-arm (2 ft)	6	0	0
	Rotating-arm (3 ft)	6	0	0
	Rotating-arm (4 ft)	6	0	0
	Burkard (2 ft)	6	0	0

[1]False negatives are defined as days on which ergot spores were not detected by an individual sampler but were detected by at least one other sampler in the array during the same sampling period.

ergot detection was greatest in samples collected every 7 days, but the 2-day sampling period was not different from the 7-day sampling period (Table 3).

Precipitation events were recorded, and data for sampling periods with precipitation events were analyzed separately in order to validate the performance of spore samplers in wet conditions. Overall, rotating-arm spore samplers performed as well as Burkard spore samplers under wet conditions regardless of sampling height ($P = 0.32$) (data not shown). Samples collected for 7 days performed best during precipitation events but were not different than samples collected after 2 days (data not shown); these results corresponded to those from samples collected under all weather conditions.

Table 2. Effect of different rotating-arm sampling heights (2, 3, and 4 feet) on ergot spore collection and comparison to a Burkard spore sampler.

Sampling height	Mean cycle threshold value[1]
Rotating-arm (4 ft) ($n = 58$)	30.33
Rotating-arm (3 ft) ($n = 60$)	28.24
Rotating-arm (2 ft) ($n = 60$)	28.23
Burkard (2 ft) ($n = 60$)	28.16
P-value	0.15 NS[2]

[1]A smaller cycle threshold value indicates that more spores were collected.
[2]NS = not significant at 0.05 level.

Table 3. Effects of different rotating-arm sampling periods (1, 2, 3, 4, and 7 days) on ergot spore collection.

Sampling period	Mean cycle threshold value[1]
(number of samples)	
1 day (n = 11)	35.25 a
3 day (n = 119)	29.28 b
4 day (n = 60)	28.16 bc
2 day (n = 24)	25.73 cd
7 day (n = 24)	25.29 d
P-value	< 0.0001

[1]A smaller cycle threshold value indicates that more spores were collected. Treatments followed by the same letters are not significantly different by Tukey's test.

Conclusion

Overall, 91.6% of the 238 data points agreed between rotating-arm spore samplers and the standard Burkard spore samplers. Sampling height was not a significant factor affecting sampling efficiency, but the rotating-arm samplers should be placed above the crop canopy. A minimum sampling period of at least 2 days would be recommended. A rotating-arm spore sampler was also deployed in the Grande Ronde Valley as a preliminary test of the new system in a commercial KBG seed production field. This research suggests that rotating-arm spore samplers can perform as well as standard Burkard spore samplers for monitoring airborne ergot ascospores. These results will be further validated in the second year.

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Acknowledgments

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CROP TOLERANCE AND RATTAIL FESCUE CONTROL WITH DRY/LIQUID HERBICIDE FORMULATIONS IN DRYLAND CREEPING RED FESCUE SEED CROPS IN THE GRANDE RONDE VALLEY OF NORTHEASTERN OREGON

D.L. Walenta

Introduction

Winter annual grass weeds such as downy brome (*Bromus tectorum*) and rattail fescue (*Vulpia myuros*) are persistent problems in cool-season turfgrass seed production systems in the Grande Ronde Valley of northeastern Oregon. Research efforts continue in the region in order to identify potential herbicide products that provide improved grass weed control in fine fescue and Kentucky bluegrass seed crops (Walenta, 2017a, 2017b).

Herbicides currently registered for grass weed control in fine fescue seed crops are primarily soil-active products applied preemergence to weeds. Adequate control of winter annual grass weed species with preemergent herbicides is often difficult to achieve, especially in dryland systems, due to: (1) the lack of adequate rainfall in early fall to activate the herbicides for optimal winter annual grass weed control and (2) the presence of crop residue/ash on the field surface (following residue management with baling + propane-flaming), which interferes with the applied herbicide reaching the soil surface. A new approach to preemergence herbicide application was proposed by industry—drop-spreading granular herbicide formulations followed by mechanical disturbance (e.g., harrowing) to move the granules through the residue/ash to the soil surface, where weed control can take place.

A study was conducted during the fall of 2018 and spring of 2019 to evaluate crop tolerance and rattail fescue (RF) control with selected dry and liquid herbicide formulations in established dryland creeping red fescue (CRF) grown for seed. Note: flumioxazin + pyroxasulfone (Fierce EZ) and pendimethalin (Prowl H_2O) are registered for use in Oregon fine fescue seed production. The active ingredients triallate, trifluralin, ethalfluralin, and pyroxasulfone (applied as Zidua) are not registered for use in Oregon fine fescue seed production. Product evaluations are for experimental purposes only; therefore, mention of products used in this trial should not be considered a recommendation for commercial use.

Materials and Methods

The experiment was located in an established commercial field of 'Fenway' CRF in Union County. The field was seeded during spring of 2016, and the second seed crop was harvested in the summer of 2018. After baling the crop residue, the field was propane flamed in late September and was not harrowed afterwards. Environmental conditions at the time of herbicide application are summarized in Table 1. Site of action descriptions for each active ingredient are listed in Table 2.

Plots were 8 feet x 25 feet and arranged in a randomized complete block design with four replications. All liquid

Table 1. Environmental conditions at time of herbicide application.

Application timing	Oct. 18, 2018, preemergence (PRE)	Mar. 30, 2019, late post (LPOST)
CRF growth stage	Regrowth just starting	1½ to 2½ leaf, 1–2 inch height
Rattail fescue growth stage	Not emerged	Not emerged
Air temperature (°F)	64	51
Relative humidity (%)	40	56
Cloud cover	Cloudy	Clear and sunny
Wind velocity (mph)	Calm	0–4 from N
Soil temperature, surface (°F)	62	74
Soil temperature, 1 inch (°F)	62	68
Soil temperature, 2 inch (°F)	58	64
Soil temperature, 4 inch (°F)	52	53

Table 2. Site of action descriptions for herbicides included in the 2018–2019 trial.

Group #	Description[1]
3	Inhibits microtubule assembly (cell division in roots and shoots); swelling of root tips
8	Lipid synthesis inhibitor but not an ACCase inhibitor
14	Inhibits protoporphyrinogen oxidase (PPO); loss of chlorophyll; leaky cell membranes
15	Inhibits synthesis of very long chain fatty acids (VLCFA); affects seedling emergence

[1]Descriptions from the Weed Science Society of America.

herbicide treatments were applied with a hand-held CO_2 sprayer delivering 21 gpa at 35 psi. To minimize drift potential, TeeJet air-induction, extended-range (AIXR) 11002 nozzle tips were used for all applications. Granular formulations were applied with a Gandy drop spreader calibrated for each product by making two 3.5-foot-wide passes per plot. The following day the entire plot was spike tooth harrowed twice in direction with the plots (not across the reps).

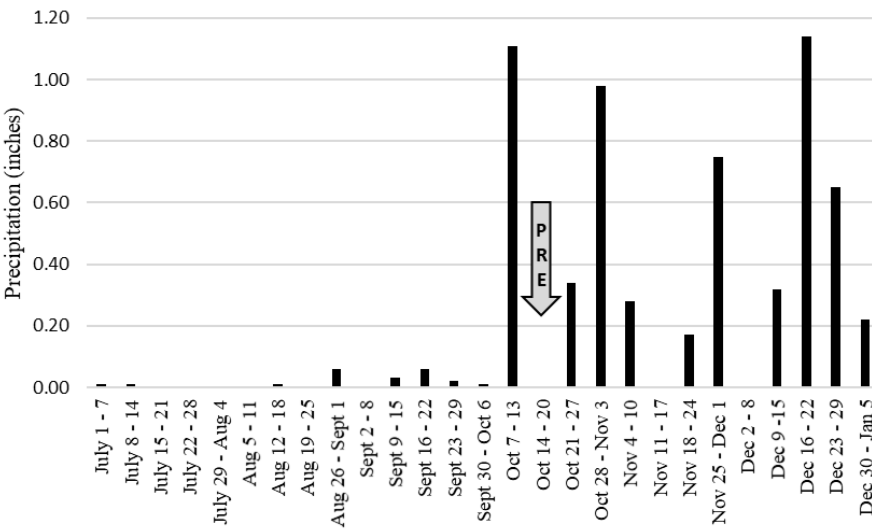

Figure 1. Weekly precipitation amounts at Imbler AgriMet station, fall 2018.

Visual evaluations of crop injury were collected only in the spring of 2019 (March 30, April 1, and May 3) due to the lack of adequate CRF regrowth in the fall of 2018. Visual evaluations of weed control were not possible due to the low RF infestation level. However, RF plant density/plot was collected on June 21 by counting all plants in each plot. The trial site was mowed in late June to comply with crop-destruct requirements for investigating potential candidate nonregistered herbicides. Seed yield was not determined in this study.

Results and Discussion

CRF regrowth and RF emergence were significantly delayed in the fall of 2018 due to persistent dry conditions that lasted until October 2 (Figure 1). Preemergence (PRE) herbicide treatments were applied October 18 to CRF at the start of regrowth, when RF had not yet emerged. Weather and soil conditions remained dry for 8 days after PRE treatment application until late October/early November, when rainfall events delivered 1.38 inches of rainfall over a 2-week period. The delay in receiving adequate rainfall to incorporate and activate herbicide treatments may have reduced grass weed control potential.

Postemergence (POST) herbicide treatments were scheduled to be applied in early November, but weather conditions delayed POST applications until late March, when snow cover finally left the site. At this time, CRF regrowth was only 1.5–2.5 inches in height, RF had not yet emerged, and no crop injury symptoms were observed (data not shown).

Visual crop evaluations were challenging to complete due to the CRF stand being weakened by poor and variable fall regrowth throughout the trial site. Under these conditions, visual crop injury evaluations taken in mid-April indicated significant injury to CRF in all treatments regardless of formulation type (Table 3). By late May, crop injury symptoms were diminished in all PRE liquid herbicide treatments (5, 6, 7, and 8). However, significant crop injury was still evident in the granular herbicide treatments (2, 3, and 4). The late POST application of flumioxazin + pyroxasulfone (treatment 9) resulted in significant crop injury early in the growing season; although diminished, crop

injury was still unacceptable by late May (15%). The RF infestation level was low across the trial site and resulted in no significant differences between treatments for RF control. RF plant counts were highest in the untreated check at 0.05 plants/ft^2 (10 plants/plot).

In summary, the stressed condition of the CRF stand may have increased crop susceptibility to herbicide injury. Thus, results from this trial indicate that further investigation under more vigorous crop health and/or irrigated conditions is warranted to better understand levels of crop tolerance.

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Acknowledgments

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Table 3. Crop injury and rattail fescue control with dry/liquid herbicide formulations in dryland creeping red fescue seed production in the Grande Ronde Valley of northeastern Oregon, 2019.

					----- Crop injury[1] -----		
Treatment	Group	Active ingredient	Rate	Timing	Apr. 13	May 30	Rattail fescue
			(product/a)		---------- (%) ----------		(no./plot)
1		Check			6 c	0 c	10
2	8 + 3	Triallate + trifluralin	12.5 lb[2]	PRE	92 a	90 a	0
3	8	Triallate	15.0 lb[2]	PRE	91 a	85 a	0
4	3	Ethalfluralin	7.5 lb[2]	PRE	93 a	91 a	0
5	3	Ethalfluralin	2.0 pt	PRE	24 bc	0 c	7
6	8	EPTC	3.5 pt	PRE	71 a	4 bc	0
7	3/15	Pendimethalin/ pyroxasulfone	5.0 pt 1.5 oz	PRE LPOST	31 bc	4 bc	0
8	3/3	Pendimethalin/ ethalfluralin	5.0 pt 2.0 pt	PRE LPOST	19 bc	1 c	< 1
9	14 + 15	Flumioxazin + pyroxasulfone	3.0 oz	LPOST	36 b	15 b	< 1
LSD (0.05)					29.9	12.4	NS

[1]Numbers followed by the same letters are not significantly different by Tukey's HSD All-Pairwise Comparisons Test.

[2]Granular formulation

SEED YIELD PERFORMANCE AND FLOWERING INITIATION OF TWELVE RED CLOVER VARIETIES (YEAR 1)

N.P. Anderson, T.G. Chastain, C.J. Garbacik, 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 a valuable rotation crop 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 2018 was $11.5 million, with approximately 20,000 acres harvested (Anderson, 2019). Consistent seed movement and decent 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 objectives of this 2-year study were 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 between varieties. Results from the first year of this study are presented in this report.

Materials and Methods

The field trial was planted on September 27, 2018 at OSU's Hyslop Research Farm. 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 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'

Preplant fertilizer included nitrogen (N), sulfur (S), and boron (B) at 25 lb N/acre, 21 lb S/acre, and 2 lb B/acre. Fertilizer was broadcast and incorporated before planting. All red clover seed was inoculated with N-Dure true clover inoculant and planted at a rate of 7.5 lb/acre with 6-inch row spacing using a conventional drill. Due to extended dry weather in the fall, 0.5 inch of irrigation was applied with an overhead linear system on September 15 and September 17 to ensure uniform stand emergence. Routine herbicide, molluscicide, and insecticide treatments were applied to manage pests as needed. Spring N was applied to all plots at a rate of 20 lb N/acre. An additional 4 inches of irrigation was applied on May 7 due to abnormally dry conditions. All plots were flailed to a height of 2–3 inches on May 22 and in the reverse direction on May 24. When regrowth reached the two-node growth stage (BBCH 32), trinexapac-ethyl plant growth regulator (Palisade EC) was applied at a rate of 2 pt/acre. A final irrigation of 4 inches of water was applied on June 21. 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 above-ground biomass, was also quantified.

Results and Discussion

In this first-year trial, seed yields from 'Medium Red' and 'Kenland' were 499 and 356 lb/acre, respectively (Table 1). Four varieties, including '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'. 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 larger 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. There were no differences in above-ground biomass between varieties. Both 'Relish' and 'Secretariat' had a higher inflorescence number compared to the controls. Inflorescences from all varieties contained floret numbers that were equal to or less than both controls. The HI for 'Medium Red' was significantly higher than for 'Kenland'. All other varieties had a lower HI compared to 'Medium Red' except for 'Secretariat' and 'Dynamite', which were statistically the same.

Flowering initiation varied between varieties (Table 2). 'Vulcano', 'FS3662', and 'Secretariat' began flowering earlier than all other varieties, but differences were less apparent by the second week of bloom. There were some differences in percent flowering near the end of bloom; however, only one variety, 'FS3662' reached full bloom earlier than all other varieties. There is no obvious trend that would indicate a relationship between flowering initiation and seed yield.

This is the first year of a 2-year trial. Results for year 2 of the trial will be reported in the 2020 OSU seed production research report.

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Acknowledgments

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Table 1. First-year seed yield, yield components, and growth characteristics of 12 red clover varieties.[1]

Variety	Seed company	Seed yield	Cleanout	Seed weight	Seed number	Biomass	Inflorescences	Florets/ inflorescence	Harvest index
		(lb/a)	(%)	(mg/seed)	(no./m^2)	(kg/ha)	(no./ft^2)	(no.)	(%)
Medium Red	—	499 ef	11.2 c	1.741 ef	31.8 d	5,939	46 a	115 cde	9.5 cd
Kenland	—	356 bc	7.3 b	1.688 cdef	23.3 b	6,288	49 ab	118 de	6.8 ab
Blaze	Mountain View	344 abc	3.5 a	1.715 def	22.3 ab	6,014	50 ab	113 bcde	6.8 ab
Vulcano	Gentos	314 ab	4.0 a	1.609 abc	21.5 ab	5,619	39 a	116 de	6.6 ab
Freedom! MR	Barenbrug USA	386 cd	5.4 ab	2.150 g	19.8 a	6,710	55 abcd	105 ab	6.6 ab
Redomon	Van Dyke Seed	481 e	6.6 ab	1.620 abcd	33.0 de	7,100	50 ab	123 e	7.8 bc
CISCO	Van Dyke Seed	353 bc	4.6 ab	1.579 ab	24.5 bc	7,268	47 a	120 e	5.5 a
Relish	PGG Wrightson	295 a	14.7 d	1.636 bcd	19.8 a	6,055	71 cd	106 abc	6.1 ab
FS3662	PGG Wrightson	302 a	12.0 cd	1.526 a	22.0 ab	4,982	69 bcd	102 a	6.9 ab
Secretariat	PGG Wrightson	542 fg	7.4 b	1.644 bcde	36.3 e	6,705	73 d	114 cde	9.5 cd
Dynamite	Grassland Oregon	568 g	10.6 c	1.772 f	35.5 e	5,626	52 abc	109 abcd	11.5 d
DLFPS-102/3	DLF Pickseed	411 d	11.5 c	1.660 bcde	27.5 c	5,987	56 abcd	119 e	7.8 bc
P-value		0.0000	0.0000	0.0000	0.0000	0.3679	0.0287	0.0007	0.0002

[1]Numbers followed by the same letter are not significantly different at LSD (0.05).

Table 2. Percent bloom from flowering initiation to full bloom in 12 red clover varieties.[1]

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	—	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	

[1]Numbers followed by the same letter are not significantly different at LSD (0.05).

EVALUATION OF BIFENTHRIN RESISTANCE IN FIELD-COLLECTED CLOVER SEED WEEVILS

N. Kaur, W.P. Jessie, C.S. Sullivan, D.L. Walenta, E.C. Verhoeven, and N.P. Anderson

Introduction

Clover seed weevil (CSW), *Tychius picirostris* Fabricius (Coleoptera: Curculionidae) is one of the key insect pests in white clover seed production cropping systems and requires control in western Oregon. Besides white clover, CSW is also known to attack other clover species, including alsike, arrowleaf, and Ladino clovers (Anderson, 2019). The small, gray weevil (about 0.1 inch in length) has a characteristic long snout and brushes of gray and white hair (Reeher et al., 1950). CSW has a potential to cause significant yield loss, as larvae feed on developing clover seeds for a prolonged period during the growing season. The adult weevils create feeding punctures to feed at the base of the calyx on the florets of clover heads. In contrast, egg punctures are created midway up the calyx.

CSW has two generations per year. First-generation adults tend to migrate from noncrop hosts to white clover in spring and early summer. The first clover flowers can appear as early as April, depending on stand age and management, and may attract adults as they emerge from overwintering sites. Adults mate and lay eggs inside developing pods as early as 1 week after locating flowers. Eggs hatch, and larvae begin to feed on developing seeds inside the pods. Each larva can destroy up to four seeds before reaching the fourth and final instar. Prior to pupation, larvae exit the pod and fall to the soil surface. Within 3 weeks, second-generation adults begin to emerge. It is not advised to control second-generation adults because they neither harm seeds nor lay eggs; the insects can lay eggs only in partially developed seeds (Reeher et al., 1950). At this later stage in the growing season, when second-generation adults appear, seeds are hardened enough to prevent egg laying.

Since 2010, an increase in the number of CSW adults has occurred during field scouting efforts in commercial white clover fields in the Willamette Valley. Such increased CSW populations are speculated to be associated with the recent increase in the acreage of white clover seed production in Oregon (Extension estimates for Oregon legume seed crop acreage, 2010). The close proximity of clover seed production fields without the presence of any noncrop hosts to disrupt dispersal is considered a contributing factor for CSW population growth.

The economic threshold level to treat with insecticide is when an average of two or more weevils are encountered per straight-line sweep (made at 90° in the field). Straight-line sweeps are made by walking in a straight line and sweep sampling with each step (10–15 steps).

Both bifenthrin and chlorpyrifos are recommended for chemical management of CSW in white clover seed fields (Anderson, 2019). However, the use of chlorpyrifos is incompatible with the recommended application timing. Early-season (prebloom) applications of chlorpyrifos do not reduce in-field CSW populations during clover bloom. In recent years, several cases of failed CSW control with bifenthrin application have been reported, but confirmation of bifenthrin resistance has not been investigated nor documented. The objective of this laboratory study was to generate preliminary data and documentation needed to characterize bifenthrin resistance levels among CSW populations collected in commercial white clover seed production fields in the Willamette Valley.

Materials and Methods

In 2019, CSW adults were collected from three field sites in Linn County located more than 3 miles (5 km) away from each other. Adult CSW were kept in separate large, ventilated chambers for 24 hours prior to conducting bifenthrin dose-response assays. Adult weevils were collected three times from each field during May–June, and assays were conducted within 24–48 hours of capture. Adult CSW were exposed to various bifenthrin rates by treating the inside surface of a glass vial with formulated insecticide (Brigade). The treated vials were then placed on a vial roller to dry and to ensure uniform product distribution on the vial's interior surface. For each collection event per site, three vials were prepared for each of six treatment rates: 0.75, 1, 3.9, 6.4, 8, and 12 oz/acre. A fourth vial treated only with water was included for each collection site and time (n = 9). Ten field-collected CSW adults were then placed in each vial, and the vial was closed with a cotton stopper. Vials were inspected for mortality at 12, 24, and 36 hours.

Results and Discussion

For each location and collection time, rates as low as 1 oz/acre killed more than 50% of the population, and the maximum labeled rate (6.4 oz/acre) resulted in good control (> 90% mortality) (Figure 1). The lower label rate (3.9 oz/acre) resulted in approximately 85% mortality. Mortality in untreated vials remained < 10% across all populations. Therefore, bifenthrin-resistant CSW adults were not detected in the populations tested in this study.

Additional work is needed to further clarify the extent to which resistance may be developing in the Willamette Valley, including using molecular tools to assay target-site mutations that may confer resistance. Based on these preliminary results, it may also be advisable to develop additional hypotheses to address the poor CSW control observed in the field. Optimization of agronomic practices, including improved application timings to coincide with economic thresholds, management of clover canopy height and density to promote effective spray coverage, and crop mowing to remove early-season inflorescences, need to be tested and implemented. Currently, the industry does not have access to decision-making tools that will help predict CSW life stages during the growing season. The development of phenology/predictive models would help growers more effectively deploy CSW management tactics.

Observations from fields across Linn County revealed that the earliest application timings (typically in May) always required subsequent applications in late June or early July, as weevil numbers rebounded quickly after the first application. The mobility and abundance of clover seed weevils allows rapid recolonization of fields following early applications, particularly as the crop continues to produce new florets throughout early summer.

One important consideration for insecticide timings is the progression of flowering in white clover. High numbers in the early spring may seem alarming, but applications at this stage (and likely through May) to protect clover florets may not be warranted, as early florets will likely drop/shatter long before swathing and combining operations. It is recommended that the bulk of the florets that should be protected occur at around 20% brown-down (Reeher et al., 1950). It is during this time that the first seed pods form in the heads. The percentage of brown heads can be measured by selecting small areas in several representative sections of the field and counting the brown heads and the heads in full bloom. Applications at this time both maximize the percentage of florets being protected and reduce the likelihood of needing subsequent applications.

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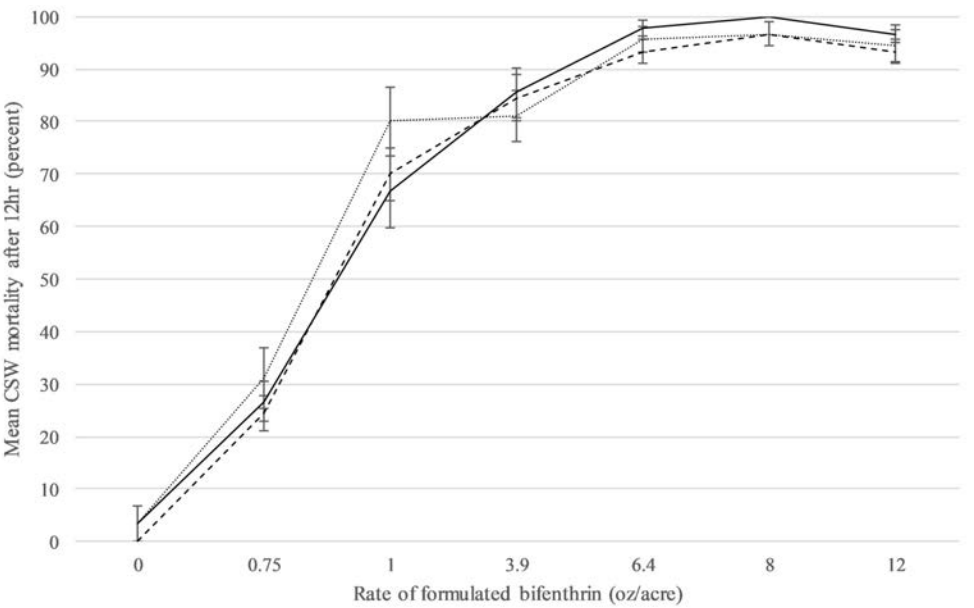

Figure 1. Dose-response curves for three field clover seed weevil populations collected in Linn County. Laboratory test with different rates of bifenthrin.

MONITORING FOR THE RED CLOVER CASEBEARER MOTH IN EASTERN OREGON RED CLOVER SEED PRODUCTION REGIONS

D.L. Walenta, K.C. Tanner, and N.P. Anderson

Introduction

The red clover casebearer moth (RCCB or *Coleophora deauratella*) was first detected in the Grande Ronde Valley of northeastern Oregon in June 2018, which caused great concern since RCCB was only recently discovered (2012) in the Willamette Valley, the primary growing region for clover seed in the United States (Anderson et al., 2014). Initial monitoring efforts in 2018 in the Grande Ronde Valley detected RCCB adult moths at high population levels in 2-year-old stands of red clover (*Trifolium pretense* L.) seed production fields. These observations indicated that the frequency and severity of seed head damage was very low, at 2% of collected seed heads. Adult moth and larvae specimens collected in 2018 were confirmed as *C. deauratella* by entomology personnel located at the Agriculture and Agri-Food Research Center in Beaverlodge, Alberta, Canada.

Based on these preliminary findings, eastern Oregon clover seed growers and industry representatives prioritized a need to increase pheromone-based monitoring efforts to further delineate RCCB distribution, population dynamics during the growing season, and potential impact on seed yield in red clover seed production east of the Cascade Mountain Range. The objectives of this study were to:

- Determine RCCB distribution, abundance, and population dynamics during the growing season in red clover seed production areas in Union, Baker, and Malheur counties in eastern Oregon.
- Evaluate red clover seed head damage/yield loss potential due to RCCB larvae infestation.
- Utilize monitoring data toward development of future phenology models and control strategies for RCCB.

Materials and Methods

On June 11, 2019, sex-pheromone-baited traps (Evenden et al., 2010) were placed in six commercial red clover (*Trifolium pretense* L.) seed production fields in an effort to detect the presence or absence of RCCB male moths. Fields utilized for RCCB monitoring were located in Union (two), Baker (one), and Malheur (three) counties, and all sites were second-year stands of red clover except for one site in Malheur County. At the time of trap installation, red clover growth stage at all monitoring sites was early bloom except for Malheur-2, which started blooming in mid-May, and Malheur-3 (first-year field), which started blooming in early July.

One green UniTrap was placed in each field at least 100 feet from the field edge and at crop canopy height. A gray septa baited with the RCCB male moth pheromone was placed in each trap and replaced one time after the first 30 days had elapsed. An insecticide vapor strip was placed in the bottom of each trap to euthanize captured moths; it was also replaced after the first 30 days had elapsed. Traps were monitored weekly for 8–10 weeks depending on stand age and production area. Monitoring efforts ended in late July to mid-August for all sites except for the 1-year-old stand in Malheur County (Malheur-3), where monitoring continued until September 3, 2019.

Weekly monitoring activities included: (1) collecting moth specimens from each trap for identification and quantification and (2) evaluating red clover seed heads for larvae presence and/or damage. Moths collected from each trap were identified utilizing a stereoscope and counted. All specimens were placed in the freezer until identification confirmation could be completed. Destructive clover seed head samples (25 heads/site) were randomly collected from the mid- to upper crop canopy on a weekly basis. The pink/red heads were processed by removing each individual floret and examining florets for the presence of eggs, larval feeding damage, and/or larvae.

To estimate potential impact on seed yield, 100 mature red clover seed heads were collected from each site prior to commercial harvest. Mature heads were processed and evaluated using the same protocol as that used for weekly destructive seed head evaluation. Soil surface residue and soil samples (three 1 ft^2 quadrats) were collected from the Union-1 monitoring site to determine whether RCCB pupae could be detected in postharvest crop residue after seed harvest.

Results and Discussion

Adult RCCB moths were captured in every field monitored, resulting in a total of 6,789 moths captured during the 2019 growing season. Union County RCCB adult populations were extremely high and represented

98% of the total captured RCCB. Interestingly, the Union-1 monitoring site was the only site that presented a distinct peak in RCCB adult activity from late June to mid-July (Figure 1). In all other monitoring sites, fewer than 100 RCCB adults/site were captured. At the time of trap installation on June 11, adult RCCB populations were already active in all 2-year stands of red clover, as indicated by the high numbers captured during the first week of trap deployment (collected June 17). In contrast, adults were not captured until July 8 in the first-year stand in Malheur County (Table 1, Malheur-3).

A total of 1,200 red clover seed heads were collected during the growing season to evaluate for the presence of eggs, larvae, and/or floret damage. Eggs were not detected on any of the destructive head samples. Overall, RCCB larvae detection was very low across all monitoring sites and resulted in totals of 23, 0, and 17 larvae collected in Union, Baker, and Malheur County, respectively, for the entire growing season (Table 1). Such low larvae detection levels represent 0.03 larvae/head. Larvae detection occurred earlier in the growing season in Union County when compared to Malheur County (Table 1). Floret damage from RCCB larvae feeding activity was very low, with only 0.2 damaged florets/head and only 239 damaged florets (Table 1) detected within an estimated 144,000 total florets examined during this study (based on an average of 120 florets/head).

At maturity, 705 red clover seed heads were evaluated for RCCB larvae damage, and results also indicate low infestation levels (Table 2). Only 3% of heads exhibited larval feeding damage, and only 80 damaged florets were detected in an estimated 80,400 florets examined, which equates to only 0.113 damaged florets/head. No RCCB pupae were detected in postharvest soil surface residue samples. Three species of weevils (*Sitona cylindricollis*, *Hypera nigrirostris*, and *Tychius picirostris*) were also found during the course of this study using destructive head sampling and sweep net techniques, but detection levels were low.

Conclusion

The presence of RCCB was detected in each of the red clover seed production areas of eastern Oregon. Notably, after 2 years of monitoring in commercial seed production fields, the RCCB appears to be very well established in the Grande Ronde Valley (Union County), given extremely high moth capture rates both

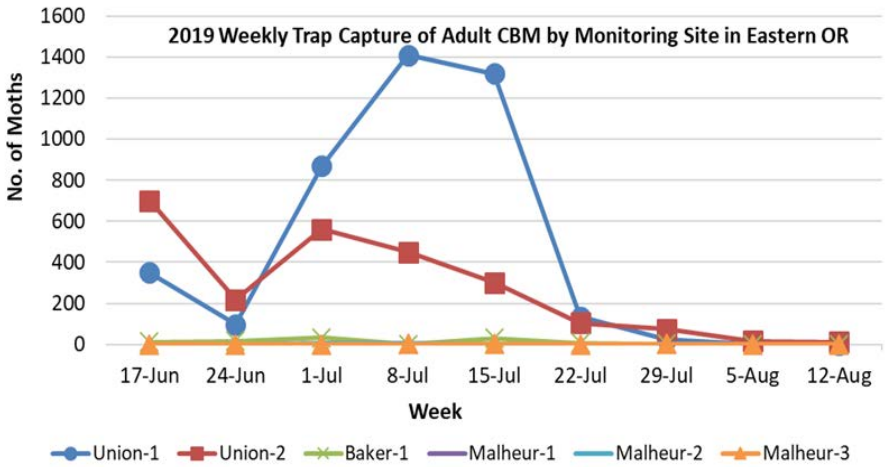

Figure 1. Weekly pheromone trap results for male red clover casebearer moths (RCCB) for each commercial red clover seed production field monitoring site in eastern Oregon. Lines for Malheur-1 and Malheur-2 are obscured by the Malheur-3 line.

Table 1. RCCB larvae and damaged florets from weekly destructive sampling of 25 red clover seed heads collected from each pheromone trap monitoring site in eastern Oregon, 2019.

Site	Field I.D.	Stand age	RCCB larvae/damaged florets								
		(yrs)	Jun. 17	Jun. 24	Jul. 1	Jul. 8	Jul. 15	Jul. 22	Jul. 29	Aug. 5	Total
Union-1	CR-1	2	0	0	0	1/66	0/11	0/12	0	0	1/89
Union-2	BD-4	2	17/8	3/13	2/8	0/2	0	0	0	0	22/31
Baker-1	JH-2	2	0	0	0	0	0	0	0	nd	0
Malheur-1	South-mow	2	0	0	0	1/0	4/9	2/30	1/10	0/1	9/50
Malheur-2	Middle	2	0/1	0	0	0	2/30	3/24	1/6	nd	6/61
Malheur-3	North	1	nd	nd	0	0	0	0	0	2/8	2/8
Total			17/9	3/13	3/8	2/68	6/50	5/66	2/16	2/9	40/239

years. The RCCB is present in Baker and Malheur counties; however, additional monitoring is needed over multiple seasons to better understand population density in those production areas.

Overall, the results of this study indicate that RCCB larvae populations are very low, levels of seed head damage and frequency are very low, and potential impact on seed yield is less than 1% loss. These results are similar to observations made during preliminary monitoring efforts in the Grande Ronde Valley in 2018. High early trap counts suggest RCCB moth flights begin earlier in the growing season, so pheromone monitoring should begin 4–6 weeks earlier. The discrepancy between moth capture rates and low damage levels in eastern Oregon, especially in Union County, suggests that an unidentified biological control agent may be limiting damage to the seed crop. This possibility warrants further investigation, as efforts continue to develop pest management strategies for RCCB.

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Table 2. Mature red clover seed head assessment for RCCB larvae damage from each pheromone trap monitoring site in eastern Oregon, 2019.

Site	Field I.D.	Mature heads collected	Collection date	Visible damage			Damaged florets	Larvae in heads
				Eggs		Larvae		
		(no.)		------ (no. heads with) ------			---------- (no.) ----------	
Union-1	CR-1 (2 yr)	100	Aug. 13	0	8	0	54	0
Union-2	BD-4 (2 yr)	100	Aug. 13	0	6	0	10	0
Baker-1	JH-2 (2 yr)	100	Aug. 6	0	1	0	1	0
Malheur-1	South-mow (2 yr)	100	Aug. 19	0	3	0	4	0
Malheur-2	Middle (2 yr)	105	Jul. 26	0	6	0	10	0
Malheur-3	North (1 yr)	100	Sep. 3	0	1	0	1	0
Malheur-4[1]	By North (2 yr)	100	Sep. 3	0	0	0	0	0
Total		705		0	25	0	80	0

[1]Malheur-4 was not monitored during the season with a pheromone trap; however, mature heads were collected and evaluated due to its proximity to Malheur-3.

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