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# EVALUATION OF ASULAM AND 2,4-DB CROP SAFETY AND DOCK CONTROL IN RED CLOVER GROWN FOR SEED

*K.C. Roerig, N.P. Anderson, A.G. Hulting, D.W. Curtis, and C.A. Mallory-Smith*

## Introduction

Dock species (*Rumex* spp.) are persistent perennials in the Polygonaceae family that develop a robust taproot. Dock continues to be a problematic weed in clover grown for seed. Seed cleaners report that dock is frequently found in clover seed lots and is difficult to clean out of harvested seed, thus affecting clover seed quality (Anderson and Hulting, 2015). The difficulty associated with removing dock seed increases the importance of controlling this weed during seed production. However, currently registered herbicides for use in clover seed production provide poor control of dock species.

Asulam (Asulox) is a group 18 (DHP inhibitor) herbicide registered for use in alfalfa grown for seed. Past studies indicate that Asulam can be used to control dock in clover. 2,4-DB (Butyrac 200) is a group 4 herbicide (synthetic auxin) (Shaner, 2014) registered for use in soybeans, peanuts, alfalfa, and seedling birdsfoot trefoil. Both are considered potential candidates for registration in clover grown for seed through the IR-4 process. Fluthiacet was included in this trial because it is registered for broadleaf control in soybeans and may have potential uses in red clover seed production. None of these herbicides is currently registered for use in clover seed production.

## Materials and Methods

This trial was conducted in a commercially grown red clover field in Yamhill County infested with a mixture of curly dock (*Rumex crispus* L.) and broadleaf dock (*Rumex obtusifolius* L.). Due to the difficulty of discerning between these two species, separate evaluation of control was not attempted.

Applications were made January 27 and March 6, 2015. Prior to harvest, dock seed heads were removed and counted, thus giving a quantitative measure of control and preventing contamination in harvested seed, which distorts clover seed yield data. Plots were windrowed into 6-foot swaths on August 11 and threshed with a small plot combine on August 17, 2015. The harvested

seed was cleaned on an air screen cleaner, and clean seed weights were used to determine yield.

## Results and Discussion

Application of 2,4-DB and Asulam provided the best control of dock when applied in early March. There were no significant differences between Asulam and 2,4-DB ( $P = 0.05$ ) for dock control (Table 1). None of the treatments reduced clover seed yield or seed germination (data not shown).

Some leaf cupping was observed following the 2,4-DB application, but in this trial and in previous trials this effect has not resulted in a decrease in clover vigor or yield. 2,4-DB labels caution against the addition of nonionic surfactants in legumes due to increased risk of crop injury. In this trial, no additional injury was noted when a nonionic surfactant was added to 2,4-DB. Additionally, there were no differences in clover injury or yield or in dock control between the 1.0 and 1.5 lb ai/acre rate of 2,4-DB.

In previous studies, Asulam efficacy on dock species was reduced when Asulam was applied too early, while clover injury was unacceptable when applied too late. In this trial, the late January and early March timings seem to be within the optimal window of good dock control and acceptable crop injury. Split application did not improve control of dock species with either Asulam or 2,4-DB.

Fluthiacet safety in red clover was excellent, but it did not provide any dock control. Evaluation of this product will continue because it may control other important broadleaf weeds.

## Conclusions

Results from this study and previous studies indicate that 2,4-DB and Asulam provide good control of dock and good crop safety, thus supporting registration of these products for use in clover grown for seed

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Table 1. Red clover tolerance and dock control in established red clover, 2015, Yamhill County, Oregon.

	Rate (lb ai/a)	Date applied	----- Dock -----		----- Red clover -----	
			Control <sup>1</sup> (%)	Heads/plot <sup>2</sup> (number)	Injury <sup>2</sup> (%)	Seed yield <sup>3</sup> (lb/a)
Untreated	—	—	0	70	0	284
Oxyfluorfen	0.094	Jan. 27	41	56	0	265
+ diuron	1.5	Jan. 27	—	—	—	—
+ paraquat	0.75	Jan. 27	—	—	—	—
Asulam	1.5	Jan. 27	74	25	15	289
+ NIS	0.418	Jan. 27	—	—	—	—
2,4-DB	1.0	Jan. 27	61	32	5	263
2,4-DB	1.5	Jan. 27	38	88	5	313
2,4-DB	0.75	Jan. 27	85	11	5	254
+ 2,4-DB	0.75	March 6	—	—	—	—
Asulam	0.835	Jan. 27	88	3	25	281
+ NIS	0.418	Jan. 27	—	—	—	—
+ asulam	0.835	March 6	—	—	—	—
+ NIS	0.418	March 6	—	—	—	—
Asulam	1.5	March 6	100	1	38	314
+ NIS	0.418	March 6	—	—	—	—
2,4-DB	1.0	March 6	95	9	0	306
2,4-DB	1.5	March 6	94	11	0	286
2,4-DB	1.5	March 6	86	2	0	297
+ NIS	0.418	March 6	—	—	—	—
Fluthiacet	0.00427	March 6	13	83	5	330
+ NIS	0.418	March 6	—	—	—	—
Fluthiacet	0.0064	March 6	31	59	0	351
+ NIS	0.418	March 6	—	—	—	—
LSD $P = 0.05$			32	78	19	104

<sup>1</sup>Visual evaluation June 25, 2015

<sup>2</sup>Visual evaluation July 27, 2015

<sup>3</sup>Harvested August 17, 2015

# TRINEXAPAC-ETHYL TIMING AND RATE EFFECTS ON CRIMSON CLOVER SEED YIELD

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## Introduction

Crimson clover is one of the important forage legume seed crops grown in the Willamette Valley of Oregon. The Willamette Valley produces about 95% of the total U.S. crimson clover seed crop, and the value of production reached \$20 million in 2014.

Crimson clover seed yields have more than doubled since the mid-1970s. Research in red clover seed crops suggests that further improvement in crimson clover seed yield is possible. One factor in yield increases in red clover seed crops has been foliar application of the plant growth regulator (PGR) trinexapac-ethyl (TE), an anti-lodging agent (Øverland and Aamlid, 2007; Anderson et al., 2015; Anderson et al., 2016).

The effects of application of TE or any other PGR to crimson clover seed crops have not been studied and published. Preliminary on-farm trials have shown that TE can increase crimson clover seed yield by 10 to 24% over the untreated control (Anderson et al., unpublished). More information is needed to identify the optimum application rate and stage of crimson clover development for TE application to achieve the best economic use of this product in crimson clover.

The objective of this multiyear study was to evaluate the effects of TE timing and application rate on crimson clover seed crops and to establish recommendations for TE application to crimson clover in the Willamette Valley.

## Materials and Methods

The field trial was established at Hyslop Farm near Corvallis, OR. Crimson clover seed was planted on October 2, 2014 by using a Nordsten drill set at a 6-inch row spacing. The seeding rate was 17 lb/acre. SelectMax (Clethodim) and MCP Amine 4 (MCPA) herbicides were applied at 12 oz/acre and 10 oz/acre, respectively, at BBCH 12 to control weeds in the crop.

The experimental design was a randomized block design with four replications. Both TE rate and timing treatments were evaluated. TE was applied at stem elongation (BBCH 32, late March) and bud emergence (BBCH 50, mid-April). Four TE rates were applied at each of the two timings: 1, 2, 3, and 4 pt/acre.

Application timings and rates were compared to an untreated control.

Soil water content was determined by time domain reflectometry (TDR) in early May. Seed yield components (including numbers of stems, heads, and florets) were determined on samples taken at peak bloom (BBCH 65, mid-May). Canopy characteristics (including above-ground biomass and canopy height) were also measured at peak bloom.

The crimson clover was swathed with a modified John Deere 2280 swather on June 10, 2015 and was combined with a Hege 180 plot combine on June 23, 2015. The seed was cleaned with a M2-B Clipper seed cleaner, and 1,000-seed weight was recorded after counting with an Old Mill Company Model 850-2 seed counter. Seed number was calculated based on seed yield and 1,000-seed weight values obtained from each plot. Analysis of variance (ANOVA) was used to test TE treatment effects, and Fisher's protected least significant difference (FPLSD) test was used to separate treatment means.

## Results and Discussion

The ANOVA revealed that most characteristics of crimson clover seed production were not affected by application of TE PGR (Table 1). These characteristics

Table 1. ANOVA for trinexapac-ethyl treatment effects on crimson clover seed yield and seed yield components.

Characteristics	Treatment significance <sup>1</sup>
Seed yield	ns
Seed weight	**
Seed number	ns
Cleanout	**
Biomass	ns
Stems/ft <sup>2</sup>	ns
Heads/ft <sup>2</sup>	ns
Florets/ft <sup>2</sup>	*
Canopy height	**
Soil water content	*

<sup>1</sup>\* $P \leq 0.05$

\*\* $P \leq 0.001$

ns = not significant

included seed yield, seed number, stem number, above-ground biomass, and head number. Very dry conditions prevailed in the spring of 2015, with only 58% of normal rainfall occurring April through June, and these dry conditions likely influenced the results.

Seed yields were variable and lower than the 10-year average yield of 910 lb/acre for the Willamette Valley as a result of extreme drought and high temperature conditions (Table 2). There was no effect of TE PGR on seed yield at either application timing or for any of the four rates tested. These results were inconsistent with the preliminary on-farm trials in prior years, which showed a seed yield increase with TE.

Seed weight was reduced with all TE treatments (Table 2). Overall, seed weight generally declined with increasing rate of TE and the later application time.

There was no effect of TE on seed number, which was the primary factor responsible for the seed yield increase by TE PGR in red clover in previous studies (Anderson et al., 2015; Anderson et al., 2016).

Canopy height of the crop was reduced with the TE application. Height reductions increased with increasing TE rate at the BBCH 32 timing, but not at the BBCH 50 timing (Table 3). The number of florets increased at the BBCH 32 application timing with 1 to 3 pt/acre rates, but not with 4 pt/acre. Only the 3 pt/acre rate

Table 2. Effect of trinexapac-ethyl timing and rate on seed yield, seed weight, and seed number in crimson clover.<sup>1</sup>

----- Treatment -----				
Timing	Rate	Seed yield	Seed weight	Seed number
	(pt/a)	(lb/a)	(mg/seed)	(seeds/ft <sup>2</sup> )
Untreated control	—	362 a	5.67 a	667 a
BBCH 32	1	346 a	5.38 b	673 a
	2	364 a	5.17 c	733 a
	3	383 a	5.05 cd	792 a
	4	305 a	4.79 de	669 a
BBCH 50	1	278 a	5.11 c	566 a
	2	301 a	4.88 de	643 a
	3	290 a	4.49 f	676 a
	4	278 a	4.38 f	660 a

<sup>1</sup>Means within each column are not significantly different by Fisher's protected LSD values ( $P = 0.05$ ) if followed by the same letter.

Table 3. Trinexapac-ethyl timing and rate effects on seed production characteristics in crimson clover.<sup>1</sup>

----- Treatment -----					
Timing	Rate	Soil water content	Canopy height	Floret number	Cleanout
	(pt/a)	(%)	(cm)	(floret/ft <sup>2</sup> )	(%)
Untreated control		23.0 a	71.1 a	7,309 c	2.23 bc
BBCH 32	1	19.4 b	61.1 bc	9,374 ab	1.86 bc
	2	20.8 ab	58.3 cd	9,200 b	1.74 bc
	3	19.4 b	55.7 d	11,264 a	1.72 c
	4	19.9 b	53.6 d	7,329 c	1.87 bc
BBCH 50	1	23.1 a	65.2 b	7,981 bc	1.87 bc
	2	20.0 b	63.9 b	8,709 bc	2.29 b
	3	21.7 ab	63.8 b	9,776 ab	3.03 a
	4	20.8 ab	62.4 bc	8,663 bc	2.96 a

<sup>1</sup>Means within each column are not significantly different by Fisher's protected LSD values ( $P = 0.05$ ) if followed by the same letter.

significantly increased floret production at the BBCH 50 timing. Cleanout represents the quantity of nonseed material harvested. Cleanout increased with 3 and 4 pt/acre of TE at the BBCH 50 timing.

The reduction in canopy height by TE most likely opened up the canopy, thereby allowing a greater loss of soil water through evaporation (Figure 1). Coupled with the abnormally dry and hot conditions, the reduction in canopy coverage with TE reduced the amount of soil water available for seed filling, likely contributing to the reduction in seed weight.

In summary, severe drought and heat during flowering and seed filling caused low and variable seed yields in crimson clover. Seed yield was not affected by TE application. The trials will be repeated in the 2015–2016 crop year.

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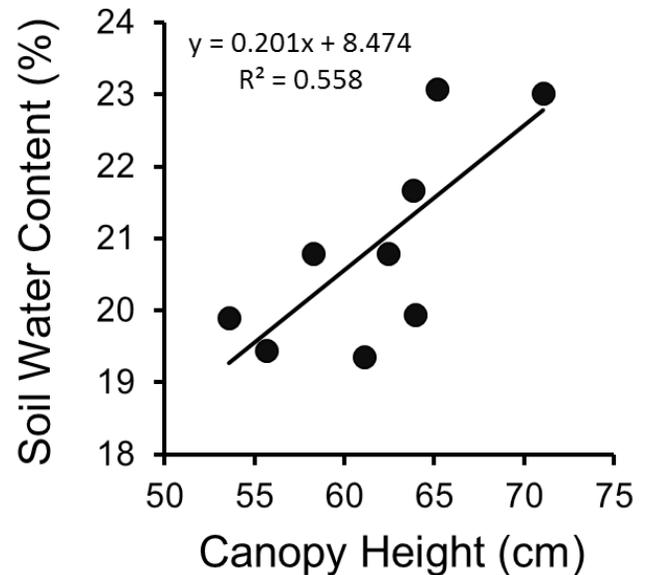


Figure 1. Effect of canopy height reduction by trinexapac-ethyl PGR on soil water content in crimson clover seed production trials.

# SEARCHING FOR GENETIC TOLERANCE TO CHOKE IN ORCHARDGRASS GERMPLASM

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## Introduction

The sexual cycle of the endophyte *Epichloe typhina* can “choke” seed production in certain forage and turf grasses (Kirby, 1961). Orchardgrass (*Dactylis glomerata*) in particular is susceptible to choke in Oregon’s Willamette Valley. Several years ago, seed yield losses were reported at 9% (Pfender and Alderman, 2006); losses likely are much higher now.

Multiple reports have studied the occurrence, spread, yield loss, and possible fungal infection routes of choke in orchardgrass, as well as insect vectors that aid in fertilization of the sexual fungus. What has emerged is a complicated pattern. Incidence of choke has spread throughout the Willamette Valley since 1997. Once choke is detected within a field, it pervades in a quadratic response (Pfender and Alderman, 2006). The fungus is considered weak when outside a plant, and it grows slowly once inside the plant. Its lackadaisical growth changes abruptly upon an unknown flowering signal from the plant; at that time, stroma quickly grow, engulf the seed head, and prevent seed production.

Thus far, fungicides have had little or no effect (Pfender and Alderman, 2003). Although several insect vectors have been studied, none has proven to account for the majority of fertilization of the fungus (Rao et al., 2012). Thus, no efficacious treatment or control for choke has been found.

Tolerance or resistance to fungal pathogens is often found in plants, and variation in these traits is used to breed superior cultivars. Development of tolerant or resistant cultivars is a lengthy process; however, when feasible management options are lacking, this approach is a viable direction. In the case of choke and orchardgrass, tolerance vs. resistance is difficult to define, but it may include gene products that prevent or dilute the plant flowering signal that induces rapid fungus growth, as well as plant structure or natural plant chemicals that limit fungal entry or survival in mature plants. Although the exact cause cannot be efficiently determined and assessed, resistance or tolerance (hereafter deemed tolerance) can be indicated in replicated field trials by a lack or paucity of choke incidence in some varieties over time. The objective of our research has been to assess whether there is

variation for incidence of choke among orchardgrass collections and varieties.

## Materials and Methods

Forty-eight orchardgrass varieties and wild collections (hereafter called entries) were planted in two sites: one near Albany, OR and the other near Corvallis, OR. The accessions represented the wide range of flowering times present in orchardgrass, as well as a number of accessions from Mediterranean origin. Each location was planted with 3 replications of 16 plants for each entry, in a randomized complete block design. Seedlings of each entry were germinated in Logan, UT and transplanted at the two Willamette Valley locations in the summer of 2012. Plots were maintained with fertilizer, pesticide, and herbicide as needed, and data were collected from 2013 through 2015.

For each year of data collection, heading and swathing dates were determined at the Albany site (Table 1). Choke scoring occurred at anthesis and was recorded as the number of plants in each 16-plant plot that had one or more choked panicles. The location of the choked plants was also recorded for nearest neighbor analysis using the Smith and Casler (2004) method. Plots were swathed at both locations based on maturity of seed at the Albany location and were not swathed in one single event. Plant material was discarded 1 week after swathing.

Preliminary analysis for 2014 and 2015 observations was conducted to indicate patterns of infection and identify varieties that are likely tolerant. Correlations between heading dates for each year, and between heading dates and the average number of plants with choke for each variety, were estimated using the Corr procedure of SAS.

## Results and Discussion

From a starting date of January 1, heading dates ranged from 104 to 140 days, with correlation coefficients of 92% between replications. One of our previous studies in the Willamette Valley examined choke incidence without swathing; we found no choke after 3 years of observation, despite the presence of highly infected border plants, insects, and ascospores each year. This previous data suggested that swathing is necessary for

choke infection. Swathing dates in the current study ranged from 180 to 190 days from January 1 and were moderately but negatively correlated with heading dates at  $r = -0.39$  ( $P < 0.05$ ). Further analysis of heading date, swathing date, and choke incidence will be completed after one more season of data collection.

Nearest neighbor analysis indicated no pattern of spread in the first and second year after plot establishment, consistent with previous studies and consistent with the mobile ascospores of *E. typhina*. Some entries appeared particularly susceptible to choke, however, with more than 50% of the plants showing one or more choked panicles after 2 years. These “susceptible” entries can be used to test inoculation techniques and to look for plant signals that induce choke growth.

After 2 years, the number of entries at the Albany field with no choke was seven. At the Corvallis field, which had more plant mortality due to deleterious planting conditions, eight entries had no choke after 2 years. Two entries with no choke and little mortality were shared by both sites: the Canadian cultivar ‘AC Killarney’ and the Israeli accession PI578597 (Table 1). Seven other entries had only one plant with choke across all replications in both sites (Table 1).

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Table 1. Entries showing little or no signs of choke after 2 years and their collection origin, heading dates, and swathing dates at the Albany site.

Entry	Origin	Heading date <sup>1</sup>	Swathing date <sup>1</sup>
AC Killarney	Ag. Canada	135	178
PI 578597	Israel	118	185
PI 223250	Afghanistan	117	191
Barlegro	Barenbrug USA	140	189
PI 250928	Iran	124	178
PI 231484	France	121	175
PI 371948	Bulgaria	115	189
PI 538922	Russia	140	183
PI 634258	Albania	123	189

<sup>1</sup>Dates represent the number of days since January 1.

# CONTROL OF *POA ANNUA* AND *POA TRIVIALIS* IN CARBON-SEEDED TALL FESCUE AND PERENNIAL RYEGRASS GROWN FOR SEED

*D.W. Curtis, K.C. Roerig, A.G. Hulting, and C.A. Mallory-Smith*

## Introduction

Diuron applied preemergence to the grass crop and weed species, combined with a 1-inch-wide band of activated carbon over the top of the seeded row, has enabled growers to establish weed-free plantings. Populations of *Poa annua* have now evolved resistance to diuron herbicide, and many herbicides have been evaluated as candidates for diuron replacement. Previous research from Oregon State University has evaluated preemergence herbicides for weed management in carbon-seeded grasses grown for seed (Cole et al., 2003; Curtis et al., 2011, 2012). For example, in 2011, we reported that pyroxasulfone (Zidua), rimsulfuron (Matrix), and indaziflam (Alion) herbicides performed well as replacements for diuron (Curtis et al., 2011).

The potential exists to label a combination of pyroxasulfone premixed with flumioxazin for use in grass seed production. Testing of this premix product has been initiated by the IR-4 project for registration on established grasses grown for seed. Rimsulfuron is also in the IR-4 project for use in carbon seeding. The manufacturer of indaziflam has indicated they would support a label in grasses grown for seed for use with carbon seeding and also in established grass seed stands.

Two studies were conducted at Hyslop Research Farm during the 2014–2015 season to assess the effect

of preemergence herbicides on crop injury and on control of roughstalk bluegrass (*Poa trivialis*) and diuron-resistant annual bluegrass (*Poa annua*) in new plantings of both tall fescue and perennial ryegrass. The studies compared the following preemergence herbicide treatments: (1) indaziflam, (2) pyroxasulfone/flumioxazin, (3) rimsulfuron, (4) rimsulfuron plus pronamide, (5) diuron followed by ethofumesate (standard), and (6) diuron plus pronamide (standard).

## Study 1—Tall Fescue

### Materials and Methods

Plots were 8 feet x 35 feet and were arranged in a randomized complete block design with four replications. Three rows of *Poa trivialis* seed and three rows of diuron-resistant *Poa annua* seed were planted on 12-inch row spacing in the front portion of the plots. Fifteen rows of ‘Rebel XLR’ turf-type tall fescue were planted in the rear portion of the plots on 18-inch row spacing. The tall fescue was planted 0.25 inch deep with a 1-inch-wide band of activated carbon applied over the rows at 300 lb/acre. Planting was completed on September 16, 2014.

Herbicide application and soil data are presented in Table 1. Herbicide treatments were applied on September 17 and November 24 with a compressed-air pressurized boom mounted on a unicycle frame and calibrated to deliver 20 gpa at 20 psi. The planting was

Table 1. Herbicide application and soil data, tall fescue.

	----- Application date -----	
	Sep.17, 2014	Nov. 24, 2014
Crop growth stage	Preemergence	5 tiller
<i>Poa trivialis</i> growth stage	Preemergence	5 tiller
<i>Poa annua</i> growth stage	Preemergence	5 tiller
Air temperature (°F)	69	53
Relative humidity (%)	70	85
Wind (mph, direction)	1, NE	2, SW
Cloud cover (%)	90	60
First irrigation (inches)	Sep. 17 (0.25)	—
Soil temperature at 2 inches (°F)	70	47
pH		5.3
OM (%)		2.82
CEC (meq/100g)		13.3
Texture		Silty clay loam

irrigated with 0.25 inch following the preemergence application. Irrigation was continued through crop emergence. Injury to the tall fescue and percent control of the *Poa* species were evaluated visually on April 6, 2015. The tall fescue was swathed on June 25, harvested with a small plot combine on July 7, and seed was cleaned.

### Results and Discussion

Competition from a background population of diuron-susceptible *Poa annua* reduced yields in the untreated check treatment (Table 2). This *Poa annua* population was controlled in the herbicide-treated plots, resulting in tall fescue yields that were greater than those in the untreated check plots. Diuron-resistant *Poa annua* control was greater than 97% in the herbicide treatment plots, with the exception of the rimsulfuron alone and the diuron followed by ethofumesate treatments (Table 2). *Poa trivialis* was controlled greater than 95%, with the exception of the rimsulfuron alone and the diuron followed by ethofumesate treatments.

Diuron-resistant *Poa annua* and *Poa trivialis* populations were not controlled by preemergence applications of rimsulfuron, but were controlled by preemergence applications of indaziflam, pyroxasulfone/flumioxazin, and pronamide + diuron. The addition of pronamide to rimsulfuron improved control of both species over rimsulfuron alone.

### Study 2—Perennial Ryegrass

#### Materials and Methods

The second study was established in carbon-seeded perennial ryegrass. Plots were 8 feet x 35 feet and were arranged in a randomized complete block design with four replications. Three rows of *Poa trivialis* seed and three rows of diuron-resistant *Poa annua* seed were planted on 12-inch row spacing in the front portion of the plots. Twenty-four rows of ‘APR 2105’ perennial ryegrass were planted in the rear portion of the plots on 12-inch row spacing. The perennial ryegrass was planted 0.25 inch deep with a 1-inch-wide band of activated carbon applied over the rows at 300 lb/acre. Planting was completed on October 8, 2014.

Herbicide application and soil data are presented in Table 3. Herbicide treatments were applied on October 8 and November 24 with a compressed-air pressurized boom mounted on a unicycle frame and calibrated to deliver 20 gpa at 20 psi. Rainfall of 0.23 inch occurred on October 10. Injury to the perennial ryegrass and percent control of planted *Poa* species were evaluated visually on April 6, 2015. The perennial ryegrass was swathed on June 29, harvested with a small plot combine on July 8, and seed was cleaned.

#### Results and Discussion

For most treatments, diuron-resistant *Poa annua* control was greater than 96%; however, the rimsulfuron

Table 2. Control of *Poa* species and crop injury with herbicide treatments in carbon-seeded tall fescue, 2014–2015.

Treatment	Rate (lb ai/a)	----- Control <sup>1</sup> -----		Crop injury <sup>1</sup> (%)	Clean seed yield (lb/a)
		<i>Poa annua</i> ----- (%) -----	<i>Poa trivialis</i> ----- (%) -----		
Untreated check	0	0	0	0	928
Indaziflam	0.02	99	99	19	973
Pyroxasulfone/flumioxazin	0.1	100	95	4	997
Pyroxasulfone/flumioxazin	0.14	100	99	23	1,252
Pyroxasulfone/flumioxazin + pronamide	0.13 —	100 —	100 —	6 —	1,049 —
Rimsulfuron	0.05	13	38	0	1,020
Rimsulfuron	0.06	15	63	0	1,033
Rimsulfuron + pronamide	0.05 + 0.13	75	88	0	1,079
Diuron	2.3	13	63	0	1,015
Diuron followed by ethofumesate	1.0	—	—	—	—
Pronamide + diuron	0.25 + 1	97	98	0	1,080
LSD ( <i>P</i> = 0.05)	—	23	25	5	305
CV	—	26	24	68	20

<sup>1</sup>% control and crop injury evaluated April 6, 2015.

Table 3. Herbicide application and soil data, perennial ryegrass.

	-----Application date -----	
	Oct. 8, 2014	Nov. 24, 2014
Crop growth stage	Preemergence	1 tiller
<i>Poa trivialis</i> growth stage	Preemergence	1 tiller
<i>Poa annua</i> growth stage	Preemergence	1 tiller
Air temperature (°F)	78	53
Relative humidity (%)	55	85
Wind (mph, direction)	3, E	2, SW
Cloud cover (%)	0	60
First rainfall (inches)	Oct. 10 (0.23)	Dec. 1 (0.61)
Soil temperature at 2 inches (°F)	70	47
pH		5.3
OM (%)		2.82
CEC (meq/100g)		13.3
Texture		Silty clay loam

treatments and the diuron followed by ethofumesate treatments did not provide adequate control (Table 4). *Poa trivialis* was controlled greater than 95%, with the exception of the rimsulfuron alone and the diuron followed by ethofumesate treatments.

### Conclusions

Diuron-resistant *Poa annua* and *Poa trivialis* in perennial ryegrass can be controlled by preemergence applications of indaziflam, pyroxasulfone/flumioxazin, and pronamide + diuron. The addition of pronamide to rimsulfuron improved control of the *Poa* species over rimsulfuron alone. Rimsulfuron with the addition of pronamide provided 75% control of the diuron-resistant *Poa annua* and 88% control of the *Poa trivialis* in the tall fescue study. Rimsulfuron plus pronamide controlled 74% of the *Poa annua* and 95% of the *Poa trivialis* in the perennial ryegrass.

These data indicate that indaziflam and pyroxasulfone/flumioxazin have excellent potential for crop establishment of tall fescue and perennial ryegrass utilizing carbon seeding. Rimsulfuron needs the addition of pronamide to provide control of the *Poa* species in these studies. Based on the injury ratings, tall fescue is more sensitive to the preemergence herbicides than perennial ryegrass. These data suggest that the application rate of pyroxasulfone/flumioxazin should be no more than 0.1 lb ai/acre. Further research is needed to determine if lower rates of both indaziflam and pyroxasulfone/flumioxazin could be used while maintaining satisfactory weed control results.

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Table 4. Control of *Poa* species and crop injury with herbicide treatments in carbon-seeded perennial ryegrass, 2014–2015.

Treatment	Rate (lb ai/a)	----- Control <sup>1</sup> -----		Crop injury <sup>1</sup> (%)	Clean seed yield (lb/a)
		<i>Poa annua</i>	<i>Poa trivialis</i>		
		----- (%) -----			
Untreated check	0	0	0	0	1,520
Indaziflam	0.02	96	96	9	1,596
Pyroxasulfone/flumioxazin	0.1	100	100	1	1,492
Pyroxasulfone/flumioxazin	0.14	100	100	19	1,535
Pyroxasulfone/flumioxazin + pronamide	0.13 —	100 —	100 —	9 —	1,727 —
Rimsulfuron	0.05	60	78	0	1,751
Rimsulfuron	0.06	73	85	0	1,686
Rimsulfuron + pronamide	0.05 + 0.13	74	95	0	1,690
Diuron	2.3	76	80	0	1,719
followed by ethofumesate	1.0	—	—	—	—
Pronamide + diuron	0.25 + 1.0	100	100	0	1,642
LSD ( <i>P</i> = 0.05)	—	24	10	2	306
CV	—	21	8	37	13

<sup>1</sup>% control and crop injury evaluated April 6, 2015.

# ENHANCING FERTILIZER EFFICIENCY IN PERENNIAL RYEGRASS SEED CROPS WITH UREASE INHIBITORS

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

## Introduction

Nitrogen (N) is the most important fertilizer used in grass seed production (Hart et al., 2013). Applied N increases seed yield in grass seed crops by increasing the number of seeds produced and by increasing seed weight (Chastain et al., 2014). Nitrogen application increases the profitability of grass seed production enterprises, but the cost of this valuable input has been steadily increasing over time.

The enzyme urease catalyzes the reaction of urea to ammonia, thereby making applied N susceptible to losses through volatilization. Results indicate that the greatest losses occur when there is dry weather for several days following fertilizer application.

The loss of applied N through ammonia volatilization can represent a significant economic cost. Nitrogen use efficiency is reduced by volatilization losses, and seed growers might not be getting maximum benefit from all of the N that they apply. Losses of 5 to 25% of the total N applied have been measured recently in western Oregon wheat and pasture systems (Anderson et al., unpublished report). Seed growers might be able to obtain greater seed yields with the same or less amount of applied N if volatilization were controlled, thereby reducing the cost of production.

Ammonia lost through volatilization is also an environmental pollutant. In the atmosphere, the reaction of ammonia with nitrous oxide (N<sub>2</sub>O) and sulfur dioxide (SO<sub>2</sub>) creates particulate aerosols that scatter light, resulting in haze. This has been a concern in the Columbia Basin, where deposition of inorganic N has been measured from N originating from livestock operations and fertilizer applications on crop fields (Fenn et al., 2007). The potential for reduction in emissions of greenhouse gases such as NO<sub>x</sub> exists with urease inhibitors. The application of urease inhibitors in irrigated pastures in New Zealand reduced NO<sub>x</sub> emissions by up to 12% (Dawar et al., 2011).

A urease inhibitor [N-(n-butyl) thiophosphoric triamide] (NBPT), known by the trade name Agrotain, has been shown to reduce N losses due to volatilization and increase yield in crops such as corn (Hatfield and Parkin, 2014), but little is known about use of this

product in grass seed crops (Hart et al., 2013). Seed yield was increased by 7% with use of a urease inhibitor in perennial ryegrass in New Zealand (Rolston et al., unpublished data). It is not known whether a urease inhibitor could be effective under Oregon conditions.

The objectives of this study were to (1) determine the effect of urease inhibitors on seed yield and seed weight in perennial ryegrass seed crops, (2) measure the effects of urease inhibitors on biomass production, N uptake, and N use efficiency in perennial ryegrass seed crops, and (3) develop recommendations for use of urease inhibitors in grass seed production based on research results and disseminate this information to seed growers and industry practitioners.

## Methods

Large-scale field trials were conducted in first-year perennial ryegrass seed fields at three on-farm sites in 2014–2015 and 2015–2016. The experimental design for the trials was a randomized complete block with three replications at each site. Plot size was approximately 25 feet x 300 feet.

Treatments included two N rates applied as dry urea and representing the range of recommended rates for perennial ryegrass seed crops in western Oregon, with and without the urease inhibitor product Agrotain. A split treatment of dry urea with Agrotain (80 lb N/acre) plus urea ammonium nitrate solution (UAN 32 at 80 lb N/acre) was also included.

Treatments included:

- 120 lb N/acre
- 160 lb N/acre
- 120 lb N/acre + Agrotain
- 160 lb N/acre + Agrotain
- 160 lb N/acre split + Agrotain

Fertilizer applications were made on March 11 and 18 in 2014 and 2015, respectively. UAN 32 fertilizer (80 lb N/acre) was applied to the split treatment plots approximately 1 month after dry fertilizer application.

Weather was monitored to determine timing and amount of rainfall that occurred after the fertilizer was applied. This information is important since the greatest amount

of ammonia is lost from volatilization in the first week following urea applications.

Three above-ground biomass samples were taken from each plot near peak anthesis, and dry weight of the standing crop was determined by drying and subsequent weighing of the harvested material. Seed was harvested with grower combines, and seed yield was determined by use of a weigh wagon. Seed weight was determined by counting two 1,000-seed samples with an electronic seed counter and weighing these samples on a laboratory balance.

## Results and Discussion

An average of 0.19 and 0.17 inch of rainfall was recorded approximately 3 days after fertilizer application in 2014 and 2015, respectively. More than 0.5 inch of rainfall was recorded within 5 days at all sites in both years.

Seed yield was affected by N rate in 2014 (Table 1) but not in 2015 (Table 2). In 2014, the higher N rate (160 lb/acre) increased seed yield by 7% over the lower rate (120 lb/acre) without Agrotain. At the 160 lb N/acre rate, there was no significant difference between a single or split application in either year.

Table 1. Urease inhibitor effects on harvest factors and N tissue concentration in perennial ryegrass seed crops, 2014.<sup>1</sup>

	Yield	Cleanout	Seed weight	Biomass	Tissue N
	(lb/a)	(%)	(mg/seed)	(ton/a)	(%)
Site					
Washington County	1,793	3.9 a	1.707 c	7.1 c	1.95
Yamhill County	1,763	4.7 b	1.541 a	4.9 a	1.92
Marion County	1,863	21.3 c	1.641 b	5.6 b	2.29
Treatment					
120 lb N/a	1,710 a	9.7 a	1.660	5.8	1.71
160 lb N/a	1,831 bc	9.7 a	1.627	5.8	1.99
120 lb N/a + Agrotain	1,806 b	9.5 a	1.625	5.8	2.38
160 lb N/a + Agrotain	1,865 c	10.5 b	1.624	6.0	2.23
Split (160 lb N/a) + Agrotain	1,820 bc	10.5 b	1.613	6.0	1.95

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

Table 2. Urease inhibitor effects on harvest factors and N tissue concentration in perennial ryegrass seed crops, 2015.<sup>1</sup>

	Yield	Cleanout	Seed weight	Biomass	Tissue N
	(lb/a)	(%)	(mg/seed)	(ton/a)	(%)
Site					
Washington County	2,182 b	7.6 b	1.638 a	8.8 c	2.24 b
Yamhill County	1,481 a	4.5 a	1.648 a	8.9 a	1.53 a
Polk County	2,120 b	8.3 c	1.693 b	11.3 b	1.62 a
Treatment					
120 lb N/a	1,978	7.0	1.674	9.5	1.65
160 lb N/a	1,885	6.7	1.650	9.4	1.94
120 lb N/a + Agrotain	1,892	6.9	1.659	9.4	1.83
160 lb N/a + Agrotain	1,926	6.9	1.655	10.2	1.75
Split (160 lb N/a) + Agrotain	1,958	6.4	1.658	10.0	1.82

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

In 2014, the addition of Agrotain increased seed yield at the 120 lb N/acre rate, but had no effect on either the single or split treatment of 160 lb N/acre. There were no seed yield differences between the 120 lb N/acre rate with Agrotain and the 160 lb N/acre treatments. In 2015, Agrotain had no effect on seed yield at either N rate.

Nitrogen rate had varied effects on percent cleanout in 2014 and no effect in 2015. Agrotain had no effect on seed weight either year. Above-ground biomass was generally lower in 2014 compared to 2015, but there were no significant differences between any treatments. Nitrogen tissue concentration was 12% higher in 2014 than in 2015, but was not affected by N rate or Agrotain.

Total plant N was higher in 2015 than in 2014 (Figure 1). This may explain why there was no seed yield difference between treatments in 2015. In 2014, when plant N levels were lower, the crop was responsive to N fertilizer application and produced a higher yield when an additional 40 lb N/acre was added. Seed yields plateaued when plant N was about 230 lb/acre. Since plant N was higher than 300 lb/acre for all treatments in 2015, N was not a limiting factor and therefore it is not surprising that there were no differences in seed yield between treatments.

### Conclusions

NBPT-containing urease inhibitors, such as Agrotain, should be considered when conditions are favorable for ammonia volatilization to occur. A seed yield response is most likely to be measured when lower rates of N fertilizer are chosen and when plant N is less than 230 lb/acre. While seed yields might not be increased substantially by the use of urease inhibitors, the cost of N application may be lessened through greater N use efficiency.

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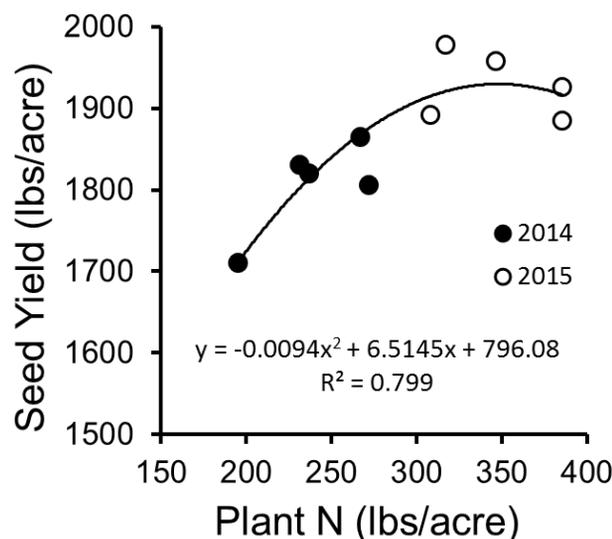


Figure 1. Effect of plant nitrogen on seed yield in perennial ryegrass from six on-farm trials conducted in 2014 and 2015.

# EVALUATION OF A NEWLY FORMULATED SLUG BAIT (FERROXX AQ) FOR CONTROL OF GRAY FIELD SLUGS IN WESTERN OREGON

*C.S. Sullivan*

## Introduction

Slugs remain one of the most damaging economic pests of the grass seed industry in western Oregon. The wet, mild climate of the Willamette Valley is especially conducive to the growth of slug populations, and grass seed fields with heavy amounts of crop residue are usually the crop most damaged by slugs (Dreves et al., 2015). Slug control is a particular challenge in the fall, when field crops are slowly emerging and vulnerable, dropping soil temperatures and increased moisture bring slugs to the soil surface, and baits do not withstand the heavy rains.

Metaldehyde and iron-based baits are commonly used in the Willamette Valley, and companies continue to formulate baits with the goal of making them more attractive to slugs and more rain-fast. Metaldehyde baits are available in multiple formulations (liquid, sand granules, and cereal-based). Their mode of action destroys the mucus-producing system unique to slugs, resulting in fairly rapid death. The cereal-based minipellets with metaldehyde have been shown to provide better coverage and last longer in rainy climates compared to larger baits. However, under wet conditions, baits degrade and slugs reduce feeding, thus potentially ingesting a sublethal dose of metaldehyde (Dreves et al., 2015). Baits also differ in their reapplication windows, and it is important to read the label; for example, Deadline MP has a reapplication interval of 21 days.

There are two types of iron-based baits on the market: iron phosphate and iron chelate baits. Iron-based baits cause slugs to stop feeding, and they usually die underground. Ferroxx (Neudorff North America) is an iron chelate bait that contains the active ingredient sodium ferric EDTA. It has been used for several years in the United States. Neudorff recently released a newly formulated iron bait called Ferroxx AQ, which is meant to hold up better under wet conditions. Both Ferroxx baits have no reapplication restrictions and may be reapplied when needed as the bait is consumed, or every 2 weeks. The objective of this study was to evaluate the newly formulated iron bait for control of slugs in a grass seed field in Oregon.

## Materials and Methods

The study was conducted in a volunteer annual ryegrass field in Linn County during November and December 2015. The field was in its third year of volunteer annual ryegrass production, and no tillage had been completed for 5 years. The plots measured 50 feet x 50 feet and were established in a randomized complete block design that was replicated four times. The trial was located in an area of the field that was known to have high slug pressure.

There were five treatments as outlined in Table 1: (1) untreated control, (2) Ferroxx AQ, (3) Ferroxx, (4) Ferroxx AQ + Ferroxx (Ferroxx 50:50), and (5) Deadline MP. All baits were in minipellet form and

Table 1. Slug bait treatments and corresponding slug days per blanket calculated up to 14 days after bait applications.

Treatment	Composition	Rate	Slug days/blanket <sup>1,2</sup>
Control	NA	0	81.4 a
Ferroxx AQ	3% iron phosphate	15 lb/a	26.6 b
Ferroxx	5% sodium ferric EDTA	15 lb/a	17.7 b
Ferroxx 50:50	see above, 50:50 blend	7.5 + 7.5 lb/a	25.1 b
Deadline MP	4% metaldehyde	15 lb/a	32.7 b

<sup>1</sup>Slug days were calculated by averaging the number of slugs per plot on two consecutive evaluation days, and multiplying the average by the number of days between the evaluations. Slug days between evaluations were summed for the sampling period until 14 days after application.

<sup>2</sup>Means followed by different letters are significantly different ( $P < 0.05$ ).

were applied with a rotary bait spreader at dusk on November 9, 2015. Temperatures were between 50 and 55°F, soil moisture was present, and the wind speed was less than 5 mph.

Slug blankets were used to record slug numbers prior to baiting and at regular intervals after baiting. Three slug blankets (18 inches x 18 inches, designed by Liphatec, Inc.) were soaked in water and randomly placed in each plot. The first slug count (“precount”) was on November 8, one day before the baits were applied. Slug counts were then conducted at 4, 7, 14, and 25 days after the first bait application timing (Table 2). For each date, the number of slugs per plot was determined by averaging the number of slugs counted under the three slug blankets. After each evaluation, the blankets were moved to a new location within the plot.

The Ferroxx treatments were reapplied after 14 days, on November 23. The Deadline MP treatment was to be reapplied after 21 days, but freezing conditions were unfavorable for baiting; therefore, it was decided to delay the reapplication. Due to continued cold weather, precipitation and consultation with Neudorff, the trial was concluded after 25 days, on December 4. The Deadline treatment was not reapplied during the trial.

Data were analyzed by using analysis of variance (Statistix 10), and means were separated by using Fisher’s Protected LSD values ( $P < 0.05$ ). Slug days were calculated by averaging the number of slugs per plot on two consecutive evaluation days (e.g., 4 and 7 days) and multiplying the average by the number of days between the two evaluations (e.g., 4). This calculation was done for each counting interval, and then slug days were summed for a total count across the sampling period. Slug days were compared only until 14 days after application, since the Deadline MP treatment was not reapplied at 21 days.

## Results and Discussion

Approximately 6.25 inches of rain fell during the sampling period of this study. The prebait slug counts revealed high variability in slug numbers across the field (Figure 1). Prebait numbers ranged from an average of 4 to 11 slugs per blanket. Initial numbers were significantly higher in the Ferroxx 50:50 plots than in the Deadline MP or regular Ferroxx plots, and initial numbers were also significantly higher in the Ferroxx AQ than the regular Ferroxx plots (data not shown).

Slug bait applications significantly reduced slug numbers compared to the control plots ( $P < 0.05$ ), both the overall slug days (Table 1) and across the sampling intervals (Figure 1). Results in the control plots demonstrate the effect of weather conditions on slug density; numbers increased as soil moisture increased and temperatures stayed above 40°F. Slug density drastically decreased as temperatures fell toward freezing at 14 days (Figure 1).

The Ferroxx treatments tended to perform better than Deadline MP (Figure 1). All of the Ferroxx treatments had significantly lower slug densities than the Deadline treatment at 25 days after application (data not shown), which is not surprising since Ferroxx baits were reapplied at 14 days and Deadline MP was not. The Ferroxx AQ and Ferroxx 50:50 treatments resulted in the sharpest reduction in slug densities (Figure 1), although the overall slug populations were not the lowest. Overall, the original Ferroxx bait had the lowest slug densities and was able to control slugs the longest (Table 1, Figure 1).

Results of this study indicate that both metaldehyde and iron-based baits can effectively reduce slug densities, as has been found in other bait efficacy studies (Anderson, 2011). The newer, more rainfall-resistant bait formulation of Ferroxx AQ seems to have

Table 2. Weather conditions noted in the field throughout the sampling period.

Sampling interval	Date	Air temperature (°F)	Conditions
Precount	Nov. 8	52	Intermittent rain
4 DAA <sup>1</sup>	Nov. 13	51	Cloudy
7 DAA	Nov. 16	41	Cloudy and windy
14 DAA	Nov. 23	37	Cloudy
25 DAA	Dec. 4	45	Cloudy, windy, rain

<sup>1</sup> Days after application

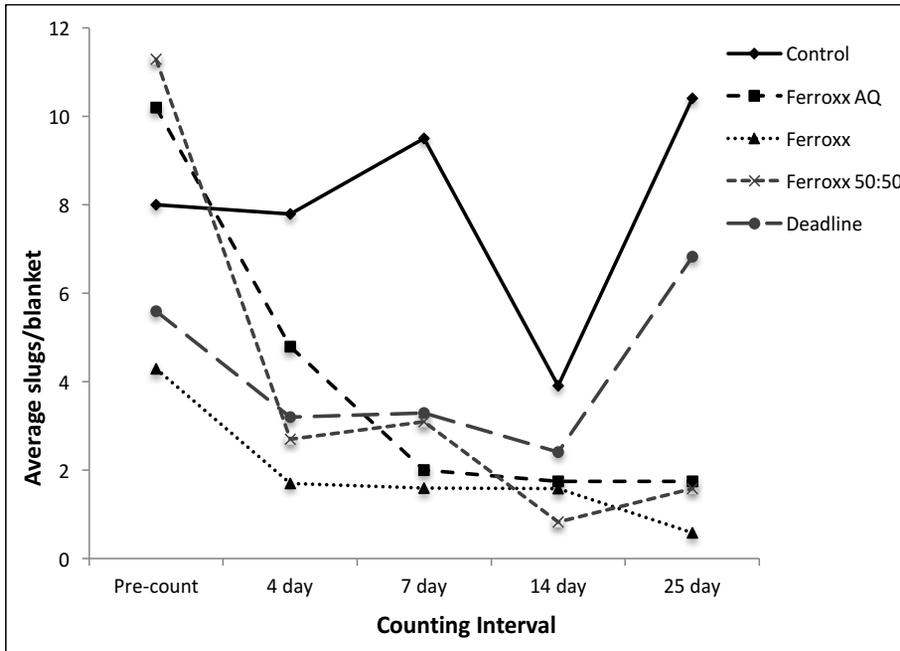


Figure 1. Average slug counts for each treatment across sampling intervals, Linn County, OR.

performed well at reducing slug populations early on, although overall does not seem to have improved the performance of these iron-based baits. Considering that slug populations were so variable across the plots, it would be best to perform additional efficacy studies. We were unable to evaluate the advantage of the narrower reapplication window with the Ferroxx baits as compared to metaldehyde, since Deadline MP was not reapplied at 21 days.

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# SPATIAL VARIABILITY OF SLUG POPULATIONS IN PERENNIAL RYEGRASS STAND ESTABLISHMENT: SECOND-YEAR RESULTS

*G.W. Mueller-Warrant, N.P. Anderson, C.S. Sullivan, G.W. Whittaker, and K.M. Trippe*

## Introduction

Slugs are widely viewed as serious pests of many Willamette Valley crops, including grasses grown for seed, especially during the establishment of new fall plantings. Objectives of this project were to monitor the timing of slug emergence and evaluate the feasibility of identifying areas within fields with highest populations of slugs to help focus control efforts on situations with the greatest risk of crop damage. Fall 2015 was the second year of ongoing research, and this report focuses on results from that year, along with comparisons between 2014 and 2015.

## Materials and Methods

Tests were conducted in the grass seedling establishment phase of four major crop rotations (Table 1): turnip grown for seed followed by fall planting of new perennial ryegrass (PR) stands, (2) red or white clover followed by fall planting of PR, (3) winter wheat follow by fall planting of PR, and (4) green manure cover crops or fallow followed by fall planting of PR.

Traditional small research plots were used at the Hyslop Crop Science Field Lab in Benton County, despite the possibility that slugs could migrate between adjacent treatments. At the Polk and Linn County sites, slug blankets were placed in grid patterns spaced at

approximately 1 acre per blanket, with a minimum of 30 locations per field. Ground chicken mash was applied beneath each water-soaked blanket on one day, and slugs, worms, and beetles were counted the next day. Plywood squares (16 inches x 16 inches) were used to cover the slug blankets to prevent disturbance by wind or water and to help maintain good levels of moisture within the blankets.

Slug baits were applied by growers based on their own experience and on information we provided to them concerning weekly slug counts. In the two Benton County studies, we applied metaldehyde baits, along with several experimental treatments, but only results for currently registered treatments are reported here.

Weekly counting of slugs, predatory beetles, and earthworms began before crop emergence and continued until stands were well established by mid- to late winter. Slugs were counted over a period of 19 weeks from the third week of October through late February, although not all sites were counted every week. Slugs were counted at sites 1, 2, 3, 4, 5A, and 5B a total of 9, 10, 10, 8, 8, and 8 times, respectively, including one or more cases of counts made at the conventionally tilled sites in mid- to late October before any slugs were present at the soil surface (Table 1). Timing of slug counts in this report refers to the number of weeks

Table 1. Test site conditions, fall 2015.

Site no.	County	Previous crop	Seedbed preparation	Planting date	Number of slug counts	Slug bait application dates
1	Polk	Red clover	Conventional tillage	Oct. 21	9	Nov. 10 Nov. 25
2	Linn	White clover	No-till	Oct. 20	10	Oct. 21 Nov. 3 Nov. 25 Dec. 16 Jan. 15
3	Linn	Turnip for seed	No-till	Sep. 7	10	Nov. 12
4	Polk	Wheat	Conventional tillage	Oct. 21	8	Not applied
5A	Benton	Green manure	Conventional tillage	Oct. 16	8	Oct. 22 Dec. 17
5B	Benton	Fallow	Conventional tillage	Oct. 9	8	Oct. 22 Dec. 17

since mid-October, with week 1 being the period from October 18 to October 24, 2015. Experiments were terminated once crops were well established and final counts of crop stands and slug densities had been taken in February. Soil moisture was measured gravimetrically using surface 2-inch-deep soil samples taken each time slugs were counted.

Crop stands were evaluated by counting the number of missing 1-inch-long sections of row in a total of 3,120 inches of row at each plot in a rectangle around the target flag, skipping the center 10 feet x 9 feet because of soil sampling disturbance and crop damage under the plywood squares and slug blankets.

Access to fields (for us to count slugs and for farmers to apply slug bait) was more often a problem in the five conventionally tilled fields than in the one no-till field. In general, growers had fewer problems getting on their fields to apply slug bait in 2015 than in 2014, until the heavy rains in December.

Methods explored to quantify the spatial distribution of slugs and crop damage included inverse distance weighting (IDW) maps, Kriging, Getis-Ord Gi-star hot spot analysis, and both normal and geographically weighted regression. The Gi-star hot spot analysis technique provided more useful information on statistical significance than IDW or Kriging and therefore was chosen for mapping slug populations within fields over time.

### Results and Discussion

Soil moisture content at the conventionally tilled sites ranged from 10 to 15% in late October, delaying crop or weed seedling emergence until early November,

when several light rains finally raised soil moisture above 25%. Growers who planted crops early in the fall reported some instances of spotty perennial ryegrass germination in September followed by seedling death, as soils dried out to below the wilting point.

The unusually hot, dry summer also adversely affected slugs. Comparing fall 2014 (Mueller-Warrant et al., 2015) with fall 2015 (Table 2), slugs first appeared on the soil surface about 3 weeks later in 2015 and at densities of less than half those in the previous year. There was also a dearth of larger individuals in 2015, implying that the slugs that did appear likely did not over-summer as adults, but rather hatched from eggs buried fairly deep in the soil profile. In contrast, in 2014 the slugs that emerged were of varying sizes on most dates, suggesting that multiple “safe havens” existed that year within the soil for adults, juveniles, and eggs.

Predatory beetle populations were highest in the first 2 weeks of counting and declined approximately 10-fold as weather cooled in later fall. Numbers of predatory beetles were lower overall in 2015 (Table 2) than in 2014. Earthworm counts were very low initially and peaked in the third week of November at most sites. Peak earthworm counts were lower than in 2014.

Similar to 2014, slugs were not uniformly distributed across any of the sites on any single date, and counts varied from a minimum of 0 to a maximum of 34 slugs per blanket. The hot spots for slugs in 2015 tended to be situated at lower elevation locations within each field. This was true both on the nearly level ground of Linn County and on the rolling hills of Polk County. The number of slugs present in spatial hot spots varied from a low of 2.5 per blanket at Site 4, to medium values of 7.3 and 8.3 per blanket at Sites 3 and 1, and to a high

Table 2. Test site results, fall 2015.

Site no.	Average weekly slug count, entire fall season	Highest weekly average slug counts		Slug counts from period most closely related to crop loss		Average counts of other organisms	
		Week <sup>1</sup>	Average number	Weeks included <sup>1</sup>	Average number	Predatory beetles (weeks 1–6)	Earthworms (weeks 1–9)
1	0.8	5	1.4	—	—	0.26	0.4
2	4.0	5	6.4	1–6, 8, 9	4.0	0.22	0.7
3	1.3	4	3.7	3–6	1.5	0.21	10.2
4	0.2	4	0.4	—	—	0.27	2.5
5A	0.8	2	1.5	—	—	0.04	0.7
5B	4.7	9	7.7	2–6, 9	4.7	0.44	3.0

<sup>1</sup>Week 1 of fall establishment season is defined as October 18 to October 24, 2015.

of 19 per blanket at Site 2. Modest numbers of slugs appeared at nearly all locations (other than the hot spots) within each field site.

There were fewer differences over time in 2015 than was the case in 2014. In other words, the higher slug counts tended to occur in the same plots on all dates rather than fluctuating across space as the season progressed. Statistically significant spatial hot spots for slugs remained generally stable in the fall of 2015, in contrast to 2014, when there was a mix of stable hot spots and locations with fluctuating counts—high in some weeks and near zero in other weeks. This may be a result of the limited set of conditions in which slugs were able to survive the summer drought, likely mainly as deeply buried eggs.

Bait applications typically reduced slug counts by approximately two-fold (e.g., six slugs per blanket before treatment and three slugs after treatment). Bait performance was poorer than in 2014, when five-fold reductions were common.

Cutworms were found in the third week of November 2015 at several sites, especially no-till PR into white clover (Site 2). However, their numbers were low and our ability to find them was very limited due to the substantial canopy of surviving clover plants.

There are many ways to analyze and display slug count and crop stand data, and not all results can be presented in this report. We tested multiple relationships between crop stand gaps and slug counts at each site and have shown the best models for each site in Figures 1–4. In general, sites with the highest numbers of slugs had the widest ranges in crop stand loss, while sites with fewest slugs had the least variation in crop stands.

At Site 2, stand loss (missing PR seedlings relative to perfect stands) increased from 20% in plots with no slugs to around 60% in plots with averages of 15 slugs/blanket (Figure 1). However, plots of stand loss versus slug counts revealed the presence of three outliers with severe stand loss but low numbers of slugs (Figure 1). The worst two cases were near one of the spots where cutworms had been detected, suggesting that it was reasonable to assume that the most severe crop damage was from cutworms rather than slugs. The regression in Figure 1 omitted the three points with greatest injury, although relationships between slug counts and stand loss remained significant even when the instances of probable cutworm damage were included in the logistic regressions.

At Site 5B, stand loss increased from 10% in plots with less than two slugs/blanket to around 60% in plots with averages of nine slugs/blanket (Figure 2).

Stands at the other four sites were less affected by slugs, mainly because slug counts were much lower (Table 2). At Site 3, prolonged exposure of PR seed after planting to extremely dry conditions resulted in stand loss of nearly 50%, even in plots averaging less than one slug/blanket, with an additional 20% stand loss at maximum slug populations of three or four slugs/blanket (Figure 3). Site 4 (following winter wheat)

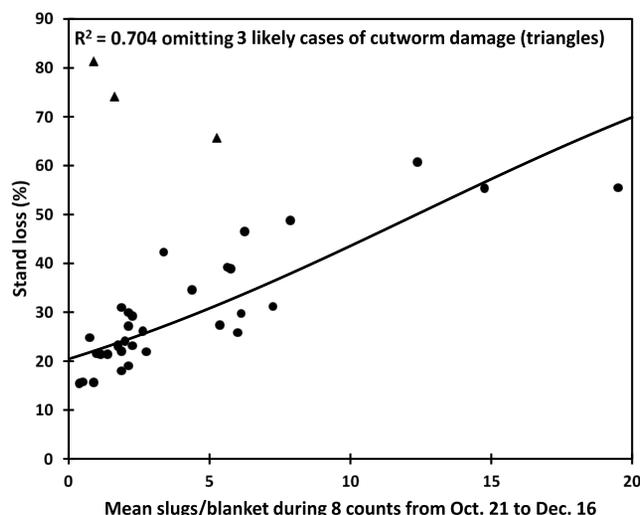


Figure 1. Stand loss from slugs at Site 2.

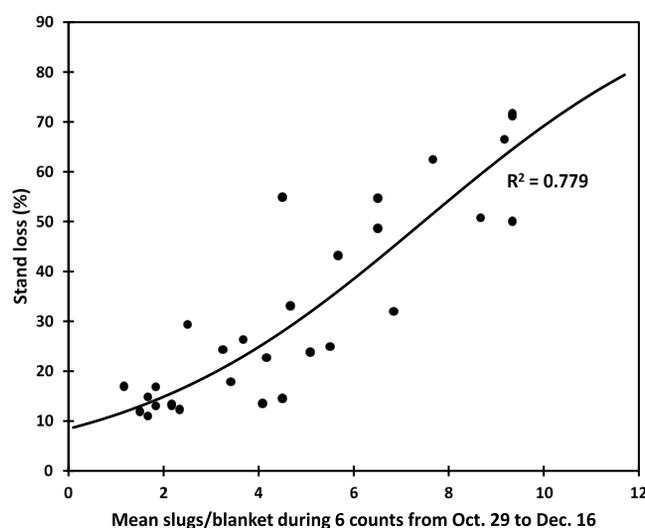


Figure 2. Stand loss from slugs at Site 5B.

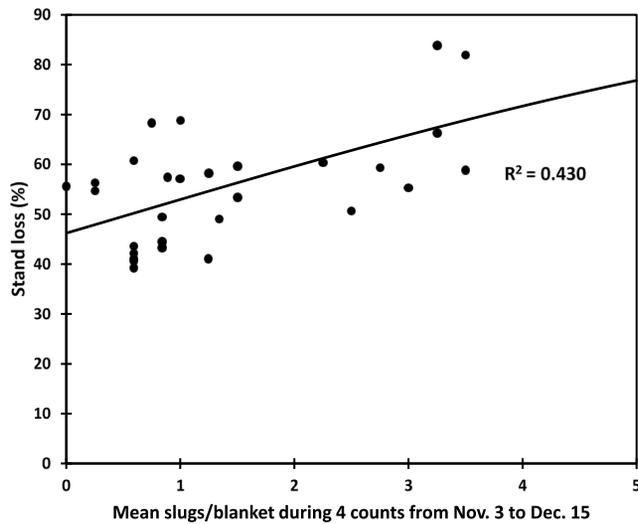


Figure 3. Stand loss from slugs at Site 3.

had excellent PR stands, very low numbers of slugs, and no significant relationship between stand loss and slug counts (data not shown). Site 5A (following green manure cover crop) had mediocre PR stands, moderate numbers of slugs, and only 17% greater stand loss as slug counts increased from zero to four (Figure 4). PR stands at Site 1 were apparently damaged by both cutworms and herbicide injury, and there was no detectable relationship between slug counts and stand loss (data not shown). Stand loss averages of 73% at Site 1 actually understated the problem, as at least half of the ryegrass plants counted were weedy annual ryegrass rather than true PR. In many cases, it was difficult to locate the position of the crop rows due to the low numbers of PR seedlings and the greater relative abundance of annual ryegrass.

The critical period for crop damage caused by slugs (from emergence to appearance of the first tiller) began later in the fall of 2015 than in 2014 because both PR germination and slug emergence were delayed by the prolonged dry weather.

### Conclusions

These findings have several important implications for management of slugs by grass seed growers. First, the absence of cold spots means that entire fields ought to be treated at least once during the peak emergence of slugs after fall rains begin; there were no truly safe locations free of all slug danger. Second, the presence of a few stable hot spots at each of the sites means that some areas will need repeated applications of slug bait. One way to identify those areas is to mark locations where high numbers of slugs have already been found. Third, the stable correlation between lower

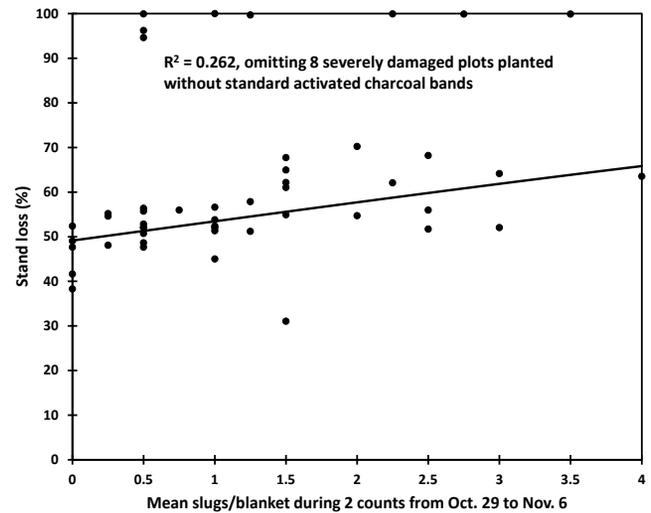


Figure 4. Stand loss from slugs at Site 5A.

elevations and higher slug counts within fields suggests that it may be possible to predict where slug numbers will be highest within fields, at least when tillage and moisture stress combine to limit slug survival to the most favorable positions within the soil profile. Fourth, no-till establishment of PR into existing clover crops can succeed if growers are diligent in their scouting for slugs and other pests such as cutworms and are willing to apply slug bait multiple times over the fall (i.e., whenever more slugs appear at the soil surface ready to eat crop plants). Fifth, the economic threshold for damage to PR seedlings remains a very low number, likely somewhere from two to four slugs/blanket for measurements made during active slug baiting. The threshold would presumably be even lower if no slug bait was ever applied.

We have not yet been able to identify all of the specific factors that would be needed to develop a good predictive model for slug emergence and density. The tendency of lower elevation positions within fields to have higher slug counts failed to correlate with surface soil moisture content throughout the fall, although it remains possible that spatial variation in soil moisture at depths below the tillage zone was important to slug survival over the summer.

Gradients in slug counts from the outer edge to the inside of tilled areas at Benton County Site 5B indicated a need for use of wider borders and larger plots in future research. Further analysis of results from 2015 should refine the experimental treatments worth repeating down to a very small number, allowing use of much larger plot sizes in future experiments.

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# EVALUATION OF NEW FUNGICIDE CHEMISTRIES AND APPLICATION STRATEGIES TO REDUCE ERGOT IN GRASS SEED PRODUCTION SYSTEMS

N. Kaur, S.C. Alderman, D.L. Walenta, K.E. Frost, J.K.S. Dung, and P.B. Hamm

## Introduction

Ergot, caused by the fungus *Claviceps purpurea*, infects the unfertilized flowers of grasses and grains and transforms seed into fungal structures called sclerotia. Sclerotia overwinter and germinate in the spring to produce fruiting bodies called capitula, which in turn release millions of airborne ascospores. Ergot can be difficult to control, considering the inoculum load of airborne ascospores that is present during the flowering stage of grass seed crops grown for seed production in Oregon and Washington. Another major challenge in ergot control is the extremely large number of sclerotia that can be left in perennial grass seed fields after harvest; one study found between 16,000 and 480,000 sclerotia/acre that were deposited in perennial ryegrass fields after harvest (Dung et al., 2015).

Only two fungicides are labeled for ergot control in Kentucky bluegrass grown for seed in the Pacific Northwest: azoxystrobin (FRAC 11) and propiconazole (FRAC 3). These active ingredients are applied either separately or as one of two commercial products that combine both active ingredients in varying amounts. These products are protective rather than systemic and must be applied during flowering.

Growers make multiple fungicide applications in an effort to prevent and control the disease, spending \$14 to \$35/acre/application. Correctly timing fungicide application with flowering and knowing when to make multiple applications during flowering are the two most difficult challenges documented by Kentucky bluegrass seed growers in a recently conducted postharvest ergot survey. Taking into account the repeated applications of similar fungicides for powdery mildew and rust control in grass seed crops, there also is a potential for

fungicide resistance development in fungal grass seed pathogens.

A need exists for new active ingredients or application strategies, due to the limited fungicide options that are available and the inadequate control they often provide. Moreover, the rotation of fungicide chemistries or use of fungicides with multiple modes of action could delay the development of fungicide resistance in ergot and other diseases affecting grass seed crops. In addition to applying fungicides during anthesis, when flowers are susceptible to infection, it may be possible to apply fungicides to sclerotia in the field during the fall and/or as they begin to germinate in the spring before they release spores (Dung et al., 2012). This approach could reduce the amount of primary inoculum available in the spring to cause ergot infection and, in the long term, break the ergot disease cycle that occurs in perennial fields.

The first objective of this study was to evaluate the efficacy of newer fungicide active ingredients to protect flowers against ergot infection. The second objective of this research was to assess the efficacy of soil-applied fungicides to reduce sclerotia germination.

## Materials and Methods

### Evaluation of new fungicides to protect flowers from ergot infection during anthesis

Plots of perennial ryegrass cultivar ‘Derby Extreme’ were established at the Oregon State University Hermiston Agricultural Research and Extension Center (OSU-HAREC) in September 2014. Four replicated plots (20 feet x 3.5 feet) with 10-foot buffer zones were arranged in a randomized complete block design. Five fungicide treatments (Table 1) and a nontreated water

Table 1. Fungicide treatments, trade name, and application rate used during anthesis.

Chemical	Product <sup>1</sup>	Rate	FRAC group
Pyraclostrobin + fluxapyroxad	Priaxor	6 oz/a	7 + 11
Benzovindiflupyr	Solatenol	4 oz/a	7
Penthiopyrad	Fontelis	24 oz/a	7
Fluopyram + prothioconazole	Propulse	14 oz/a	3 + 7
Azoxystrobin + propiconazole	Quilt Xcel	14 oz/a	3 + 11

<sup>1</sup>Registered trade name

control were used. Three applications were made at weekly intervals, beginning at Feekes stage 10.51 (first appearance of stigmas/anthers). Applications were made on May 27, June 3, and June 10, 2015 using a CO<sub>2</sub> backpack sprayer at 30 PSI in a volume of 60 gal/acre. Several foliar treatments evaluated in this study are not registered for use in grasses grown for seed.

When honeydew appeared, the number of seed heads showing symptoms of infection was determined out of 40 heads collected randomly from each plot. At harvest, 40 seed heads were randomly collected from each plot, and disease incidence and disease severity were calculated, based on the number of seed heads containing ergot sclerotia and the number of sclerotia present in each seed head, respectively. Data were analyzed using ANOVA, and multiple comparisons were made using Tukey's LSD test.

#### Use of soil-applied fungicides to reduce sclerotia germination

Plots of Kentucky bluegrass cultivar 'Midnight' (four replicates per treatment) were established at OSU-HAREC in September 2014. The field was divided into plots 3.3 feet long and spaced 3.3 feet apart, with seven rows per plot. Each plot was infested in October 2014 with 100 sclerotia collected from perennial ryegrass. Treatments consisting of fall, spring, and fall + spring applications of 12 fungicides (Table 2) and a nontreated water control were applied with a CO<sub>2</sub> backpack sprayer at labeled rates in a volume of 400 gal/acre. Fall treatments were applied on October 21, 2014; spring treatments were applied on April 3, 2015. None of the fungicides is registered for soil application.

Treatment efficacy was determined by counting the number of ergot fruiting bodies (capitula) in May and June 2015. Counts were converted to area under capitula production curves (AUCPC). The mean and maximum number of capitula observed for each treatment during the course of the experiment was also calculated. Data were analyzed using analysis of variance (ANOVA), and multiple comparisons were made using Tukey's LSD test.

### **Results and Discussion**

#### Evaluation of new fungicides to protect flowers from ergot infection during anthesis

Applications of Propulse (fluopyram + prothioconazole), Quilt Xcel (azoxystrobin + propiconazole), and Priaxor (pyraclostrobin + fluxapyroxad) during anthesis significantly reduced ergot honeydew (Figure 1) ( $P = 0.002$ ). Disease severity

was significantly reduced in all fungicide treatments compared to the nontreated water control ( $P = 0.0079$ ), indicating that several of these new fungicides were just as effective as the grass seed industry standard (Quilt Xcel) at reducing ergot honeydew during the season and sclerotia after harvest. Additionally, these new fungicides contain succinate dehydrogenase inhibitors and represent a different FRAC group (FRAC 7) that could potentially be used in a fungicide rotation program for ergot management.

#### Use of soil-applied fungicides to reduce sclerotia germination

Significant reductions in sclerotia germination were not observed compared to the water-treated control plots (Figure 2). Despite the lack of statistical differences, fall applications of Propulse (fluopyram + prothioconazole) reduced AUCPC values by 75%, while spring and fall + spring applications resulted in 40% and 48% reductions, respectively. Fall and fall + spring application of Abound (azoxystrobin) resulted in reduced AUCPC values.

Propulse (fluopyram + prothioconazole; FRAC 3+7), Abound (azoxystrobin; FRAC 11), and Priaxor (pyraclostrobin + fluxapyroxad; FRAC 7 + 11) were the most promising fungicides identified against ergot in multiple field trials conducted between 2012 and 2015 (unpublished data). Soil applications of two fungicides in particular (azoxystrobin and fluopyram + prothioconazole) have reduced sclerotia germination by up to 75% in these trials. We will continue to screen these fungicides in field conditions to generate data that can be used to enter new fungicides/application methods into the IR-4 program. Results are considered preliminary and should not be considered as product endorsement or recommendation for commercial use.

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Table 2. Fungicide treatments, trade name, application rate, FRAC group, and timing of application in soil-applied fungicide trial.

Chemical	Product <sup>1</sup>	Rate	FRAC group	Timing
Nontreated control	NA	NA	NA	Fall + Spring
Pyraclostrobin + fluxapyroxad	Priaxor	6 oz/a	7 + 11	Spring
Cyproconazole	Alto	5.5 oz/a	3	Spring
Propiconazole	Tilt	8 oz/a	3	Spring
Prothioconazole	Proline	5.7 oz/a	3	Spring
Fluopyram	Luna	5.5 oz/a	7	Spring
Benzovindiflupyr	Solatenol	4 oz /a	7	Spring
Penthiopyrad	Fontelis	24 oz/a	7	Spring
Penthiopyrad	Fontelis	24 oz/a	7	Fall
Fluopyram + prothioconazole	Propulse	14 oz/a	3 + 7	Fall
Fluopyram + prothioconazole	Propulse	14 oz/a	3 + 7	Spring
Fluopyram + prothioconazole	Propulse	14 oz/a	3 + 7	Fall + Spring
Azoxystrobin + propiconazole	Quilt Xcel	14 oz/a	3 + 11	Fall
Azoxystrobin + propiconazole	Quilt Xcel	14 oz/a	3 + 11	Spring
Azoxystrobin + propiconazole	Quilt Xcel	14 oz/a	3 + 11	Fall + Spring
Pyraclostrobin	Headline	12 oz/a	11	Fall
Pyraclostrobin	Headline	12 oz/a	11	Spring
Pyraclostrobin	Headline	12 oz/a	11	Fall + Spring
Picoxystrobin	Aproach	18 oz/a	11	Fall
Picoxystrobin	Aproach	18 oz/a	11	Spring
Picoxystrobin	Aproach	18 oz/a	11	Fall + Spring
Azoxystrobin	Abound	15.5 oz/a	11	Fall
Azoxystrobin	Abound	15.5 oz/a	11	Spring
Azoxystrobin	Abound	15.5 oz/a	11	Fall + Spring

<sup>1</sup>Registered trade name

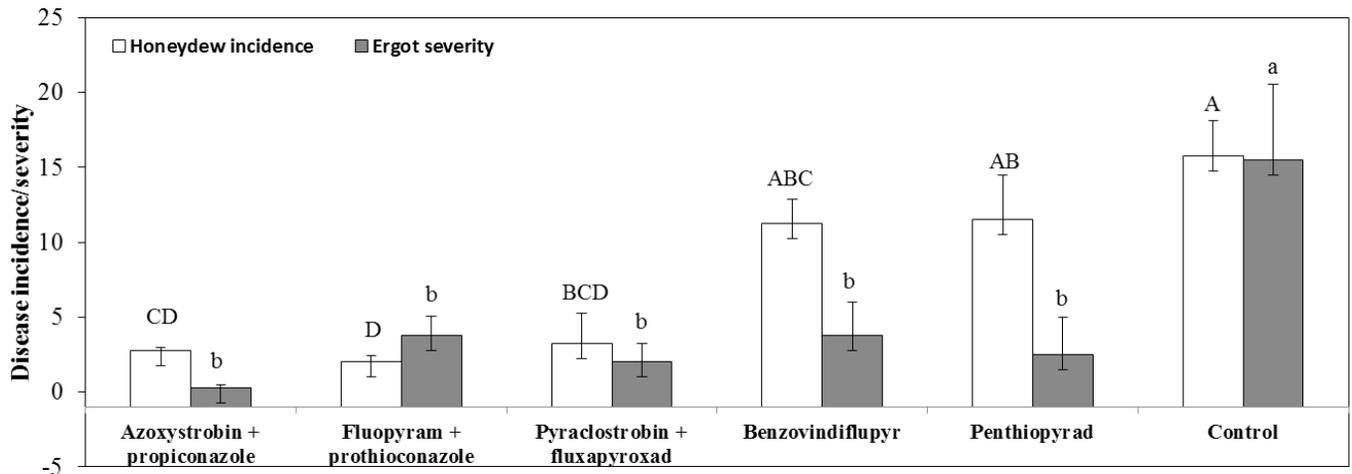


Figure 1. Effect of fungicides on the number of perennial ryegrass seed heads with honeydew (white bars) and disease severity based on the number of sclerotia in seed heads (gray bars). Treatments with the same letters are not significantly different from each other.

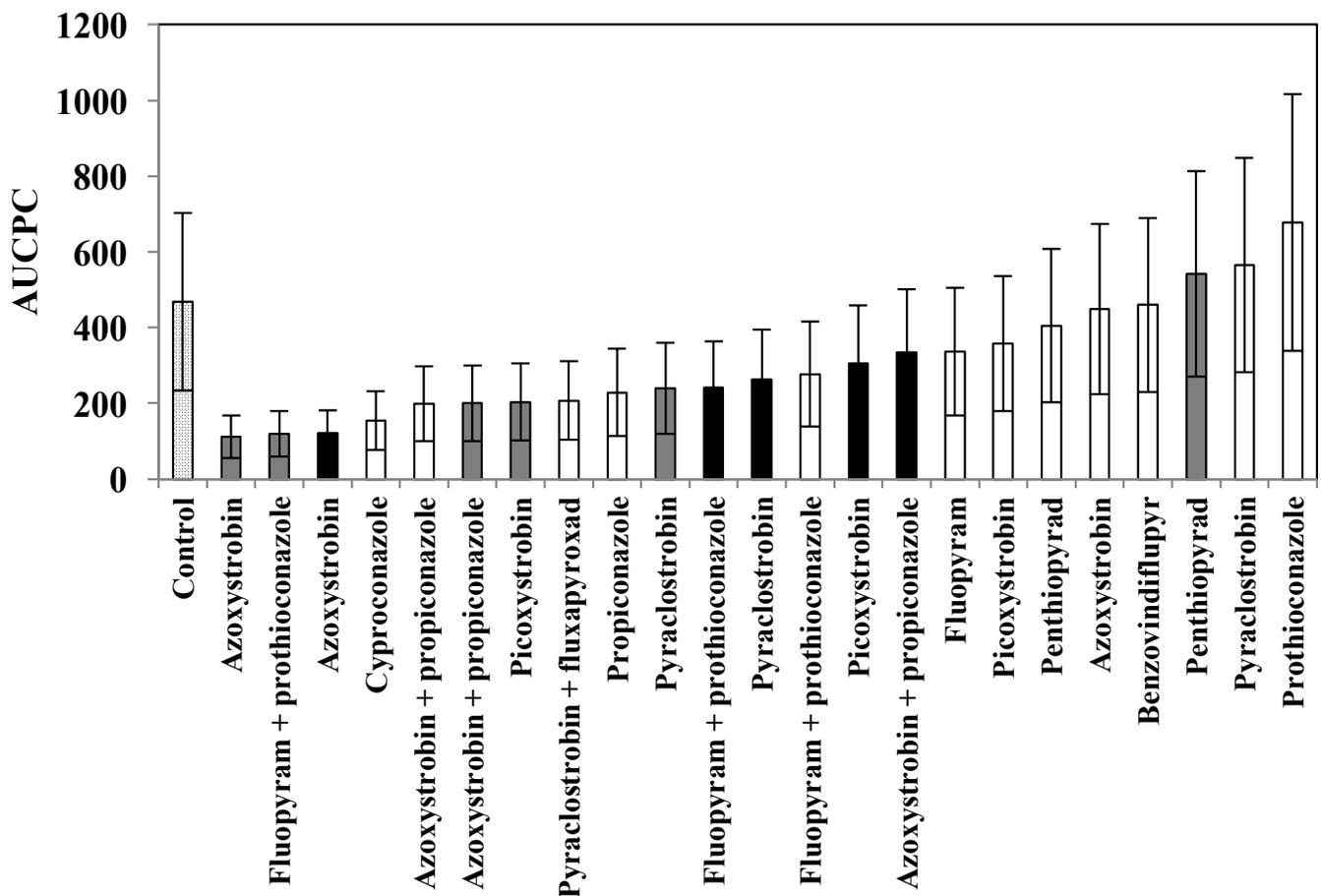


Figure 2. Mean area under capitula production curve (AUCPC) values in experimental plots infested with ergot sclerotia from perennial ryegrass in October 2014 and treated with soil-applied fungicides in fall 2014 (gray bars), spring 2015 (white bars), or in both fall 2014 and spring 2015 (black bars). The bar with dotted diamond pattern represents the water-treated control.

# ERGOT ESCAPE POTENTIAL OF COMMERCIAL CULTIVARS OF PERENNIAL RYEGRASS

*N. Kaur, J.K.S. Dung, S.C. Alderman, D.L. Walenta, K.E. Frost, and P.B. Hamm*

## Introduction

Ergot, caused by the fungal pathogen *Claviceps purpurea*, is an important disease of cool-season grass seed crops. It results in yield reduction and hinders seed certification efforts. Toxic alkaloids contained in contaminated bales may impact livestock health. This disease is a persistent problem in many perennial ryegrass (PRG) and Kentucky bluegrass (KBG) growing regions in the Pacific Northwest (Alderman et al., 1996; Alderman et al., 1998). The fungus has a very wide host range in North America, including important grains grown for human and livestock consumption, as well as forage, turf, and weedy grasses (Alderman et al., 2004).

The pathogen infects the unfertilized flowers of grasses and grains and transforms seed into fungal structures called sclerotia. Sclerotia overwinter and germinate in the spring to produce fruiting bodies called capitula, which in turn release millions of airborne ascospores. In addition to ascospores, asexual spores called conidia are produced in large numbers and mix with plant sap exuded from infected ovaries to form a substance referred to as honeydew. Honeydew can serve as secondary inoculum if splash- or insect-dispersed to uninfected flowers during the growing season.

Ascospore release typically coincides with grass flowering (anthesis), which is the only period of host susceptibility. However, Kentucky bluegrass fields for seed production in eastern Oregon have been able to escape the disease in some years because most of the ascospores are released prior to flowering, or because the cultivar may flower before or after peak ascospore release (Menzies and Turkington, 2015).

Limited information exists on the disease escape mechanism in commercial PRG and KBG cultivars grown for seed. In this study, we sought to evaluate PRG cultivars for their potential to escape infection by ergot. We hypothesized that cultivars that flower before or after peak ergot spore production, or those with shortened periods of anthesis, would escape infection and have less disease than do those cultivars that flower when ergot spores are present at high levels.

## Materials and Methods

This study was conducted at the Hermiston Agricultural Experiment and Research Center, Hermiston, OR, for 2 consecutive years (April to July of 2014 and 2015). Twelve PRG cultivars ('Applaud II', 'IG2', 'Integra II', 'PST-2M20', 'Silver Dollar', 'Quickstart II', 'Top Hat 2', 'Derby Extreme', 'Esquire', 'Fiesta 4', 'SR 4600', and 'Karma') were evaluated. Cultivars were selected based on turfgrass breeders' recommendations of cultivars that differed in their initiation of anthesis, length of anthesis, and possible ergot resistance level.

Plots (30 feet x 4 feet) were arranged as a randomized complete block design with four replicates. For the 2014 study, experimental plots were established in September 2013, followed by artificial infestation in October 2013. For the 2015 trial, cultivars were planted in September 2014 and infested in October 2014. Plots were infested with 200 sclerotia collected from the seed cleaning facilities that sorted PRG lots harvested in 2013 and 2014, respectively.

A Burkard 7-day recording volumetric spore sampler was used to determine the timing of ascospore release in the PRG plots. The spore sampler was placed in the middle of the plots from April to June in both 2014 and 2015, with the air intake orifice located approximately 2 feet above the soil surface. Spore trap tapes were collected weekly, and each tape was cut into daily segments and stained. The number of *C. purpurea* ascospores was determined microscopically for each hour and then totaled to establish daily counts.

Crop phenology was assessed weekly between May and June in both years to determine the timing and duration of anthesis for each PRG cultivar. Crop phenology was measured using the Feekes scale, whereby the appearance of stigmas and/or anthers is considered the beginning of flowering (stage 10.51) (Cook and Veseth, 1991). Flowering was considered to be completed when at least 90% of the plot reached Feekes stage 11.1 (ripening). The timing and duration of anthesis for each cultivar was recorded to determine when the cultivar is susceptible to infection or has the potential to escape infection.

Once honeydew production was initiated, disease incidence was recorded for 2 weeks, based on the percentage of infected flowers out of 40 flower spikes collected randomly from each plot. Upon maturity, disease incidence was calculated based on the number of seed heads containing ergot sclerotia out of 100 randomly collected seed heads. Disease severity was calculated based on the number of sclerotia present in each infected seed head. Seed heads were swathed and harvested to determine seed yield and the number of sclerotia per 100-gram sample in each treatment. Based on statistical analysis, the 2 years' data could be pooled and statistically analyzed using ANOVA, with multiple comparisons made using Tukey's test.

### Results and Discussion

Combined analysis indicated that there was a significant difference in the time of anthesis initiation (start date), anthesis termination (stop date), and anthesis duration among the cultivars evaluated in both years (Table 1). The cultivar 'Quickstart II' initiated flowering earlier in the season in both years than did other cultivars, and its length of anthesis period was significantly extended (Table 1). On average, the flowering period of 'Quickstart II' began on the 127<sup>th</sup> day of the year (May 7), which coincided with peak ascospore production (1,410 spores). As a consequence, the highest disease incidence was observed in 'Quickstart II' (Table 1). Conversely, the two varieties 'PST-2M20' and 'Derby Extreme' exhibited significantly less disease incidence ( $P < 0.0001$ ), based on the frequency of honeydew production (Table 1). Flowering in 'Derby Extreme' began later in the season (late May), was significantly shorter in duration, and coincided with the least ascospore production (528 spores), likely resulting in disease escape.

A significant negative correlation existed between ergot infection and anthesis initiation date ( $P = 0.0021$ ;  $r = -0.3$ ) (Figure 1), thereby indicating the potential for disease escape in cultivars that tend to initiate flowering later in the season. Other host plant resistance mechanisms (host fertilization status, postpollination stigmatic constriction, etc.) may have played roles in the observed disease incidence, yet were outside the scope of this study. We will continue to evaluate these cultivars in 2016 to gather more data under both field and greenhouse conditions.

### Conclusions

It is important to consider the difference between escape and resistance throughout the breeding process

(i.e., when selecting new grass cultivars; see Figure 2). Identification of anthesis traits that enable disease escape may aid plant breeders in the development of cultivars that avoid ergot infection. A parallel study evaluating disease escape potential in commercial KBG cultivars was conducted in 2015 at the Central Oregon Agricultural Research Center in Madras, OR. This study will be repeated in 2016. Additional KBG plots were planted in La Grande, OR for evaluation in 2016. Further studies are needed to understand the other mechanisms of host plant resistance not explored in the current study.

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### Acknowledgments

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Table 1. Day of the year corresponding to anthesis timing, total spores during anthesis, ergot incidence, and ergot severity for 12 perennial ryegrass cultivars evaluated during 2014–2015.<sup>1</sup>

Cultivar	Anthesis initiation <sup>2</sup> (date)	Anthesis termination <sup>2</sup> (date)	Anthesis duration (days)	Total spores during anthesis	Incidence <sup>3</sup>	Severity <sup>3</sup>
Applaud II	131.8 cd	163.1 cd	31.2 abc	972.7 ab	34.3 bcd	4.2
1G2	137.1 abc	166.5 c	29.3 abc	873.0 ab	15.6 ef	2.6
Integra II	132.9 bcd	168.3 bc	35.3 a	1,116.7 ab	20.4 def	4.5
PST-2M20	138.1 abc	174.0 a	35.9 a	805.6 ab	5.4 f	4.5
Silver Dollar	135.1 abcd	164.6 cd	29.5 abc	914.8 ab	41.7 ab	9.9
Quickstart II	126.5 d	160.7 d	34.2 ab	1,410.2 a	65.0 a	7.5
Top Hat 2	140.9 ab	163.7 cd	22.7 c	584.1 b	51.1 ab	2.1
Derby Extreme	142.4 a	172.3 ab	29.9 abc	528.4 b	5.4 f	1.9
Esquire	140.8 abc	168.7 abc	27.8 abc	637.8 b	34.2 bcde	6.3
Fiesta 4	137.6 abc	166.9 bc	29.2 abc	600.2 b	34.3 bcd	4.1
SR 4600	140.7 abc	163.6 cd	22.8 c	571.1 b	47.5 ab	5.6
Karma	141.9 ab	167.5 bc	22.7 c	537.4 b	25.3 cde	2.8
F-value ( <i>P</i> -value)	5.92 (<0.0001)	5.21 (<0.0001)	8.90 (<0.0001)	2.66 (0.0035)	17.74 (<0.0001)	NS <sup>4</sup>

<sup>1</sup>Means followed by the same letters are not statistically different using Tukey’s comparison.

<sup>2</sup>Dates are based on perpetual calendar days or day of the year (e.g., 126 = May 6; 174 = June 23).

<sup>3</sup>Disease incidence (number of infected seed heads) and severity (number of sclerotia) were determined from a random sample of 100 seed heads collected from each plot at harvest.

<sup>4</sup>NS = not significant

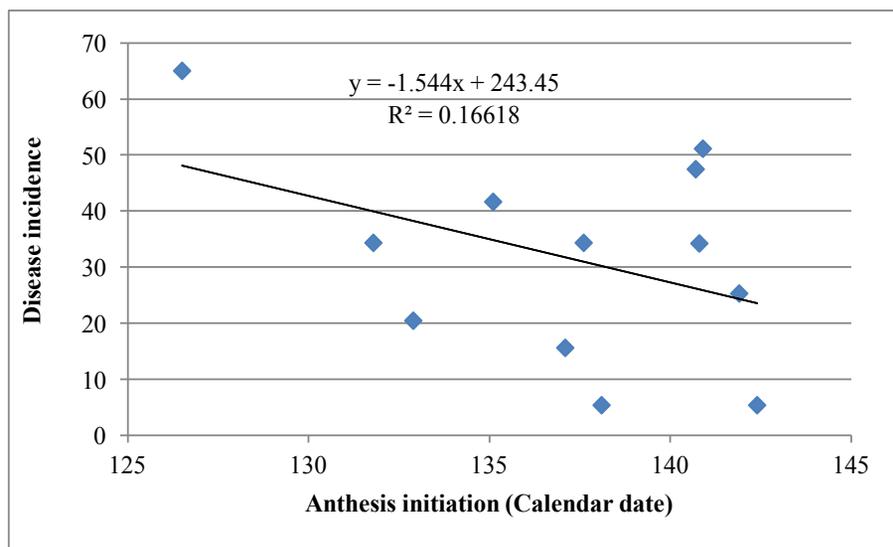


Figure 1. Correlation between observed ergot incidence and anthesis initiation date ( $r = -0.3$ ;  $P = 0.0021$ ) in 12 perennial ryegrass cultivars evaluated during 2014 and 2015.

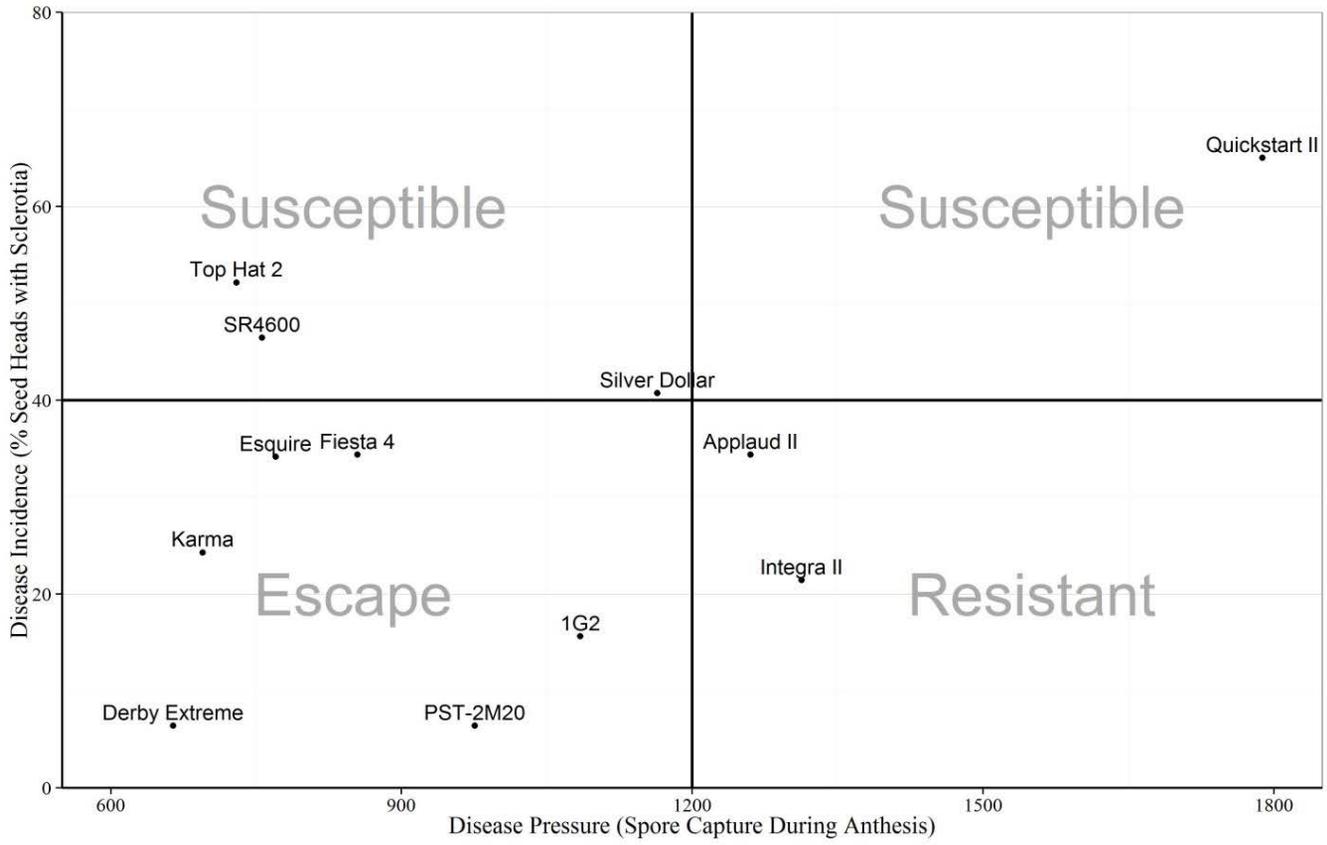


Figure 2. Response of commercial perennial ryegrass cultivars (disease incidence) to disease pressure (number of ascospores present during anthesis), indicating potential disease escape, susceptibility, or resistance.

# DEVELOPMENT OF A DNA-BASED PROTOCOL TO DETECT AIRBORNE ERGOT SPORES IN COOL-SEASON GRASS SEED FIELDS

*J.K.S. Dung, J.C. Scott, S.C. Alderman, N. Kaur, D.L. Walenta, K.E. Frost, and P.B. Hamm*

## Introduction

Ergot, caused by the fungal pathogen *Claviceps purpurea*, is a floral disease of grasses and a persistent problem in irrigated grass seed production (Alderman et al., 1996; Alderman et al., 1998; Alderman et al., 2015). The pathogen infects the unfertilized flowers of grasses and grains and transforms seed into fungal structures called sclerotia. Sclerotia overwinter and germinate in the spring to produce fruiting bodies called capitula, which in turn release millions of airborne ascospores.

One commonly used method to detect airborne ergot spores (ascospores) utilizes Burkard volumetric 7-day recording spore traps, in which air samples are continuously collected on a sticky tape surface (Figure 1). The tape is then examined under a microscope for the presence of spores. Although spore trapping is effective in providing quantitative data on airborne spore numbers, processing and microscopic examination of the tapes is time consuming (up to 8 hours for a 7-day tape). Another issue encountered during the microscopic examination of spore tape samples is that sections of the tape can be uncountable because of overlapping layers of pollen or high densities of soil particulates that can occur on dry, windy days. Counting can also be difficult if large numbers of spores have been trapped. Finally, the potential for misidentification exists if other fungal spores with similar morphology are present, especially if the technician is not properly trained in the identification of *C. purpurea* spores.

DNA-based spore detection methods are more specific and sensitive than traditional microscopic identification. DNA-based methods, mostly based on variations of polymerase chain reaction (PCR), have been used to detect airborne spores of other plant pathogenic fungi. PCR utilizes short, specific fragments of DNA (called primers) that bond to a precise region of DNA associated only with the target fungus. When primers are combined with enzymes called DNA polymerases under the appropriate conditions, sufficient copies of the DNA sequence of interest are synthesized to allow confirmation of its presence in the sample. In quantitative PCR (qPCR), a fluorescent dye is used to quantify the amount of synthesized DNA. When

compared to a standard curve of known DNA quantities, qPCR can quantify the number of DNA copies that are present in a sample.

Quantitative PCR allows for the sensitive, specific, and reproducible detection and quantification of target DNA sequences. In addition, less time and labor are typically involved, providing faster results to support an IPM program with less expense, once a protocol is developed and technicians are trained. Quantitative PCR has been used for many plant pathogenic fungi, including *Claviceps* species such as *C. africana*, *C. sorghi*, and *C. sorghicola* (Tooley et al., 2010); however, a similar protocol does not yet exist for *C. purpurea*. The objective of this research project was to develop and validate a qPCR protocol for the detection of airborne ergot spores in cool-season grass seed crops, which would provide a more accurate and less time-consuming process to quantify the number of *C. purpurea* spores found in spore traps.

## Materials and Methods

Sclerotia of *C. purpurea* were collected from infected perennial ryegrass (*Lolium perenne*) in Oregon and infected Kentucky bluegrass (*Poa pratensis*) seed lots grown in Oregon and Washington between 2010 and 2014. Sclerotia from rye (*Secale cereale*) and smooth brome (*Bromus inermis*) were collected from

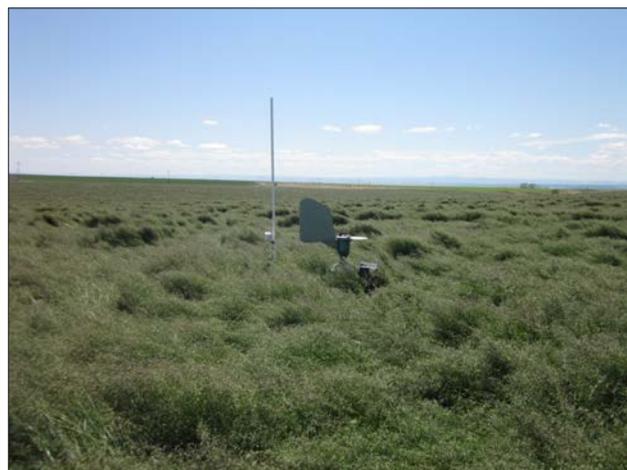


Figure 1. A Burkard volumetric 7-day recording spore trap and weather data logger deployed in a commercial Kentucky bluegrass seed production field (Benton County, WA).

field borders in Oregon and Washington, respectively. Freeze-dried tissue of *C. purpurea* from other states and countries and freeze-dried tissue from other *Claviceps* species (*C. africana*, *C. fusiformis*, *C. paspali*, and *C. pusilla*) were obtained. Isolates were obtained in pure culture by plating surface-sterilized sclerotia on potato dextrose agar. Pure culture isolates were maintained in pure culture until DNA extraction. Genomic DNA was obtained using a phenol-chloroform extraction followed by a sodium acetate-ethanol precipitation. Additional DNA extracts from *C. cynodontis* and *C. maximensis* were also obtained. The genomic DNA extracts obtained from the pure culture isolates were used to develop, test, and optimize the qPCR assay prior to validation with spore trap tape samples from the field.

Spore trap tape samples (ascospores) were obtained from commercial fields of perennial ryegrass (Umatilla County, OR) and Kentucky bluegrass (Jefferson County, OR and Union County, OR) in 2014 and 2015. Additional tape samples were collected from artificially infested experimental Kentucky bluegrass plots located at the Central Oregon Agricultural Research Center (Madras, OR) and perennial ryegrass plots at the Hermiston Agricultural Research and Extension Center (Hermiston, OR).

A qPCR assay was developed to amplify a species-specific 96 base pair region of the *C. purpurea* genome. A standard curve ranging from 10 nanograms to 1 picogram was achieved using 10-fold serial dilutions of genomic DNA. A second standard curve was obtained

using DNA extracts from known spore amounts (4, 40, 400, 4,000, and 40,000 spores) collected from pure cultures. Quantitative PCR reactions were performed in triplicate or duplicate, and melt curve analysis was used to distinguish potential nonspecific PCR products. A no-template water sample was included as a negative control in all PCR experiments. A cycle threshold (Ct) value < 40 was interpreted as a positive detection if the melt curve matched that of *C. purpurea*.

Comparisons between traditional and molecular approaches to quantifying spore numbers were accomplished by cutting each tape sample in half lengthwise and using one half for microscopic quantification of spores and the other half for DNA extraction and qPCR. Samples used for qPCR were selected to represent a range of spore counts observed using microscopic methods (0 to 1,054 spores/half tape). Quantitative PCR reactions were performed as described above, and each sample was subjected to four technical replicates. All reactions were repeated once (eight total reactions/sample).

### Results and Discussion

Species specificity of the primers was confirmed against 41 *C. purpurea* isolates collected from six hosts, including perennial ryegrass (17 isolates), Kentucky bluegrass (19 isolates), barley (1 isolate), rye (2 isolates), smooth brome (1 isolate), and cordgrass (1 isolate). The species-specific primers did not amplify DNA from closely related isolates of *C. africana* (2 isolates), *C. cynodontis* (1 isolate), *C. fusiformis*

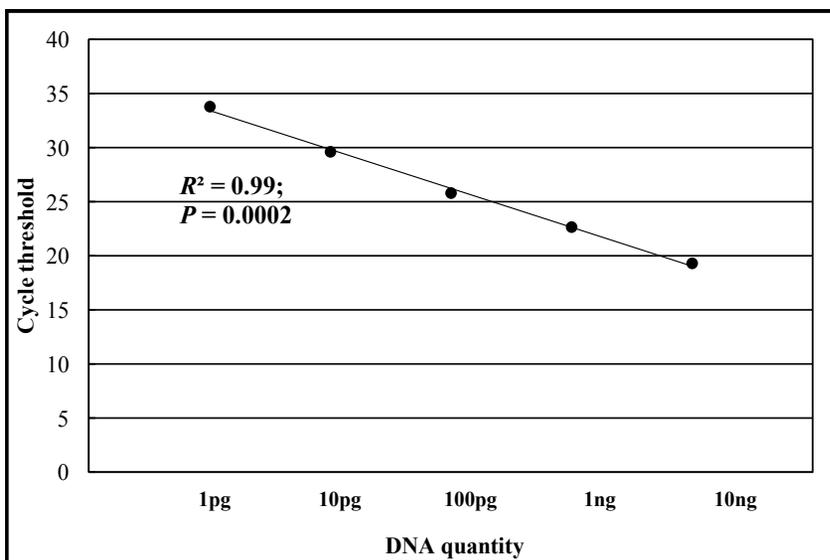


Figure 2. Standard curve of cycle threshold values calculated from serial dilutions of DNA from *Claviceps purpurea*.

(3 isolates), *C. maximensis* (1 isolate), *C. paspali* (2 isolates), or *C. pusilla* (2 isolates). In addition, the assay was highly sensitive and could detect as little as 1 picogram (one trillionth of a gram) of *C. purpurea* DNA and as few as four spores.

The qPCR reactions were highly efficient (97.6%), suggesting a high degree of specificity. Melt curve analysis confirmed that the qPCR reactions were highly specific and generated a single product (data not shown). Significant relationships were observed between Ct values and DNA quantity ( $R^2 = 0.99$ ;  $P = 0.0002$ ) and between Ct values and the number of spores ( $R^2 = 0.99$ ;  $P = 0.0004$ ) used for standard curves (Figures 2 and 3). These results indicated that the assay was applicable for samples containing a wide range of DNA (1 picogram to 10 nanograms) or spores (4 to 40,000 spores).

Microscopic examination of spore trap tape samples detected ergot spores in 23 out of 26 tape samples collected from perennial ryegrass fields and 6 out of 8 tape samples collected from Kentucky bluegrass fields. Quantitative PCR of tape samples detected ergot spores in 23 out of 26 tape samples collected from perennial ryegrass fields and all 8 tape samples collected from Kentucky bluegrass fields. Quantitative PCR detected spores on 2 KBG tape samples and 2 PRG tape samples from which spores were not observed using microscopic methods. This result could be due to the higher sensitivity of qPCR compared to traditional methods; difficulty in counting spores on tapes with large amounts of pollen, sand, or other debris; or unequal distribution of spores among the tape halves.

There were also five PRG tape samples in which spores were observed using microscopic methods but were not detected using qPCR. The reasons for these false negative results are not known, but it may have been due to excessive amounts of non-target DNA (e.g., pollen, other fungi), PCR inhibitors (e.g., humic acid, polysaccharides), or unequal distribution of spores among the tape halves. Quantitative PCR was repeated using a 1:10 dilution of these five samples, resulting in a positive detection in three of the samples, thus indicating that PCR inhibitors likely were present in these three samples. Inhibitors may be present in tape samples with excessive amounts of soil or other natural materials that are subsequently carried over into the DNA extraction. Regardless, a significant correlation for Ct values and the number of spores from spore trap tapes was observed ( $r = -0.68$ ;  $P < 0.0001$ ) (Figure 4).

### Conclusions

Microscopic methods used to detect and quantify ergot spores captured by spore traps are usually not rapid enough to allow for the detection and reporting of results in a timely manner, preventing growers from using this information in the current season or in an IPM system. A fast and reliable detection protocol for the presence of airborne *C. purpurea* spores will enable grass seed growers to make better-informed decisions regarding fungicide applications. When used in conjunction with predictive models, a qPCR detection protocol for airborne *C. purpurea* spores could help growers decide if, and when, to spray protective fungicides (Dung et al., 2013).

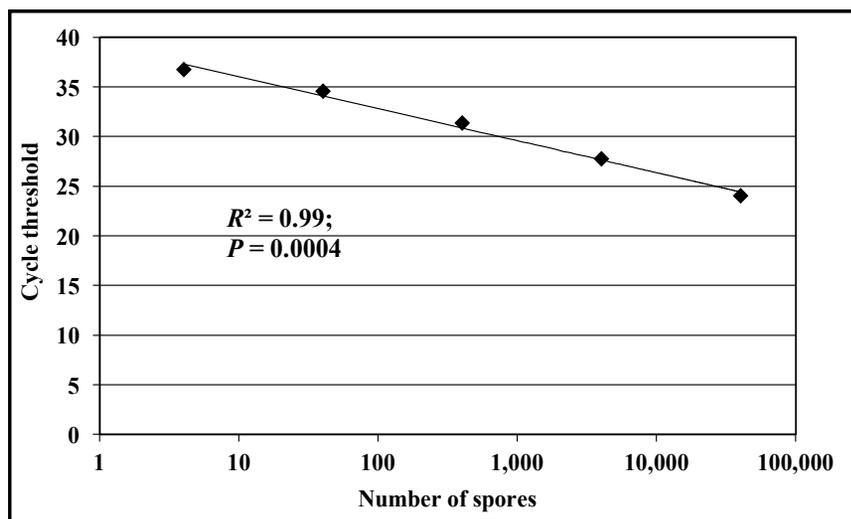


Figure 3. Standard curve of cycle threshold values calculated from serial dilutions of spores from *Claviceps purpurea*.

Quantification of ergot inoculum during the season may also enable growers to predict if seed lots may require additional cleaning after harvest, allowing them to plan their postharvest operations accordingly.

This assay provides a means for detecting and monitoring airborne *C. purpurea* spores in the field and in experimental plots. It was highly specific, was useful over a wide range of spore densities, and could be performed in a matter of hours instead of days. The protocol could be useful not only for ergot detection in cool-season grasses, but also for important grain crops (e.g., barley, rye) and wild or weedy grass hosts of ergot.

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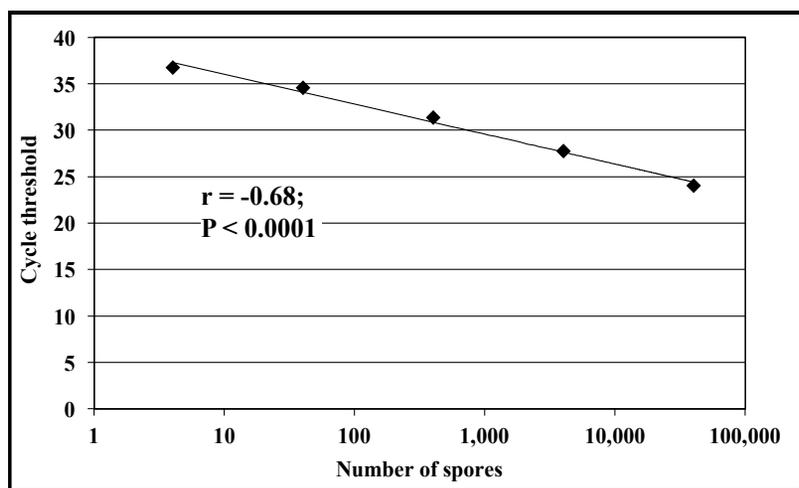


Figure 4. Correlation between cycle threshold values and log<sub>10</sub>-transformed counts of *Claviceps purpurea* spores obtained using traditional microscopic methods.

# USING INFORMATION TECHNOLOGY TO ADVANCE INTEGRATED ERGOT DISEASE MANAGEMENT IN PERENNIAL GRASS SEED CROPPING SYSTEMS

*D.L. Walenta, N. Kaur, S.C. Alderman, K.E. Frost, P.B. Hamm, and J.K.S. Dung*

## Introduction

Ergot is an important fungal disease of perennial ryegrass and Kentucky bluegrass in the Pacific Northwest (PNW). The ergot fungus can infect only unfertilized flowers of grasses, so timing of fungicide application(s) at the beginning and/or during the early stages of the flowering period are critical for successful ergot control in grass seed production fields. Fungicides can prevent infection but cannot cure the disease once the fungus has germinated and colonized the ovary of the flower. Growers are challenged with applying fungicides at the optimum time due to weather conditions, overall workload, and the variation in crop maturity and time of flowering for different cultivars. Multiple fungicide applications are often required to protect flowers during extended flowering stages.

In years when very few or no ergot spores are detected, growers could reduce the number of fungicide applications or even avoid fungicide application completely, thus saving approximately \$14 to \$35/acre/application. These treatment costs are based on 2014–2015 custom application rate estimates (application + product cost) for products registered for ergot control in grasses grown for seed. Please refer to the *2015 PNW Disease Management Handbook* (<http://pnwhandbooks.org/plantdisease>) for details.

Growers and consultants can more effectively manage disease problems with readily accessible information regarding potential for disease development. Information and communication technology continues to evolve, giving university Extension personnel the opportunity to utilize new platforms for the delivery

of time-sensitive information and deployment of new decision-aid tools. The Ergot Team’s objective for the *Ergot Alert Newsletter* was to develop an electronic alert system that would provide the PNW grass seed industry with timely and region-specific information regarding ergot spore production and crop development progress, in addition to providing ergot management recommendations for Kentucky bluegrass and perennial ryegrass seed crops.

## Materials and Methods

In 2015, seven Burkard spore traps were deployed in three grass seed production regions east of the Cascade Mountain Range: Columbia Basin (Oregon and Washington), Grande Ronde Valley (northeast Oregon), and central Oregon (Table 1). Spore traps were used to collect continuous air samples from mid-April through late June in each area. Personnel collected the spore trap samples and performed trap maintenance on a weekly basis. Weather data were collected with data loggers within the field and/or obtained from AgriMet and AgWeatherNet weather stations located near the spore traps.

The weekly samples were examined at the USDA-ARS National Forage Seed Production Research Center in Corvallis, OR, using microscopic methods to detect and quantify ergot spores collected from each trap. Weekly spore trap counts were available within approximately 7–10 days.

The spore trap results were compared to weekly crop development observations made at each trap location to determine potential risk of ergot infection based on local inoculum pressure. Observations and results were compiled weekly/biweekly.

Table 1. Burkard spore trap monitoring site descriptions.

Site	County	Grass species	Cultivar	Planting date
PRG-1	Umatilla, OR	Perennial ryegrass	Multiple (cultivar trial)	Aug. 29, 2014
PRG-2	Umatilla, OR	Perennial ryegrass	Pavilion	Sep. 20, 2013
KBG-1	Benton, WA	Kentucky bluegrass	Arrowhead	Sep. 2, 2014
KBG-2	Benton, WA	Kentucky bluegrass	Arrowhead	Sep. 3, 2014
KBG-3	Union, OR	Kentucky bluegrass	Wildhorse	May 5, 2010
KBG-4	Union, OR	Kentucky bluegrass	Baron	April/May 2014
KBG-5	Jefferson, OR	Kentucky bluegrass	Multiple (cultivar trial)	Aug. 11, 2014

*Ergot Alert Newsletters* were distributed on a weekly/biweekly basis between May 13, 2015 and July 2, 2015. Information contained in the newsletters was intended to assist growers in field monitoring efforts to determine optimum timing for fungicide application(s), if needed. Due to differences in ergot spore production and rate of crop development, newsletters were tailored to each region. The regional newsletters were e-mailed directly to stakeholder distribution lists for each of the three production regions.

In total, the newsletters were sent to 61 recipients in central Oregon, 310 recipients in the Columbia Basin, and 65 recipients in the Grande Ronde Valley of northeastern Oregon. Newsletters were also posted online at OSU-HAREC, OSU-COARC, and OSU Extension-Union County websites.

An OSU Institutional Review Board-certified survey tool was developed to assess impact and perceived importance of the *Ergot Alert Newsletter*. The survey tool consisted of two components, including: (1) a preharvest survey tool to determine current ergot management practices and informational needs for improved ergot management, and (2) a postharvest survey tool to determine whether the newsletter improved stakeholder knowledge about ergot disease and management practices. The survey tool was designed as a paper-based survey, and an electronic version (Qualtrics) was made available online and via e-mail. The paper-based preharvest survey was deployed in May 2015 at the annual Hermiston Grass Seed Field day, followed by launch of the online version on May 27, 2015. The survey closed on September 4, 2015. The online postharvest survey was launched on September 4, 2015 and closed January 14, 2016.

## Results and Discussion

### Preharvest survey

The preharvest survey was completed by 22 respondents representing mostly crop consultants/field scouts (55%) and growers (45%), with a portion of the group also involved in seed cleaning (18%). Most respondents were from the Columbia Basin (50%) and central Oregon (40%).

Ergot was viewed as a moderately to highly important disease (3.6 on a scale of 1 to 5) for grass seed crops, with many comments indicating that significant yield loss can occur before harvest and that ergot makes harvest difficult and causes additional seed loss during cleaning operations. Others mentioned concerns for seed quality and disposal of ergot-infected pellets made from screening materials. Many respondents who indicated that ergot was not important were from the northern Columbia Basin of Washington, where ergot is not as much of a problem in dryland production systems.

Current ergot management practices in grass seed cropping systems rely on fungicide application (95% of respondents) to grass seed crops (Figure 1). Other common practices include crop rotation (57%), control of weed/volunteer grasses in rotational crops (48%), and propane flaming (43%). These results indicate that many growers are already implementing various cultural control practices in an effort to reduce ergot inoculum sources.

Fungicide use for ergot control ranged from one to four applications (median two applications) per growing season. The survey did not collect data related to fungicide use for other disease management needs. Information most often used by growers/consultants to

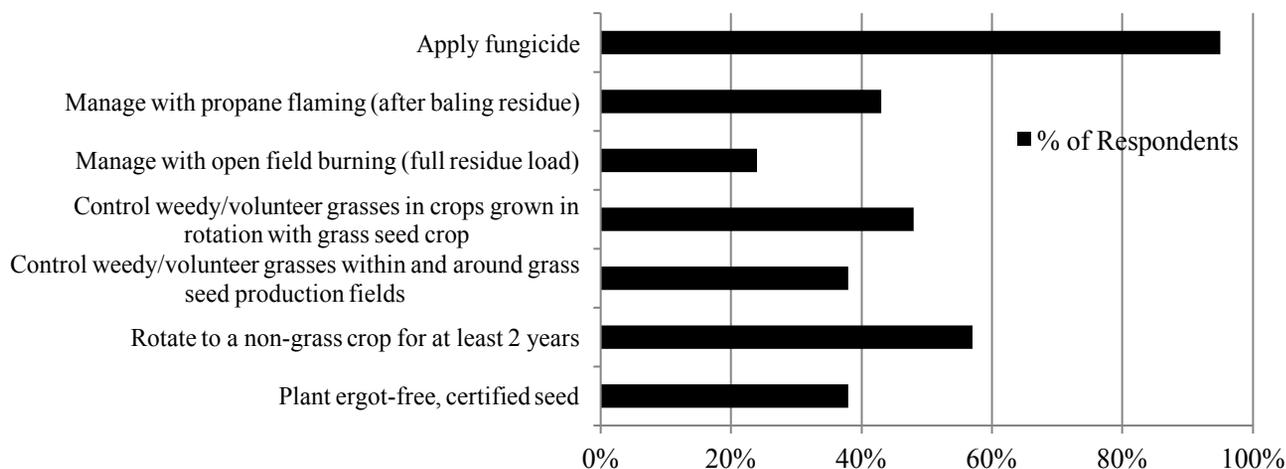


Figure 1. Tactics currently used to manage the fungal disease ergot, 2015 preharvest survey.

make fungicide application decisions focused on the history of ergot presence in production fields (92%), observations when scouting fields (62%), and time of flowering initiation (62%). Fewer than half of the respondents utilized information related to length of the flowering period, stand age, or proximity to other seed production fields with previous/current ergot infection.

Fungicide application programs for ergot control were initiated by 60% of respondents at early crop growth stages, ranging from stem elongation (Feekes 5) to the boot stage (Feekes 10), which are well in advance of the susceptible flowering stage (Feekes 10.5). These responses suggest that opportunities still exist to help some growers optimize timing of early fungicide applications for ergot control.

All respondents indicated it would be helpful to receive notifications about ergot spore presence in their region at certain times during the growing season, particularly before and/or during flowering. An overwhelming 95% of respondents expressed interest in receiving the *Ergot Alert Newsletter*. The majority of respondents (86%) indicated willingness to adopt new IPM management practices by basing future fungicide application decisions on prediction models and/or knowledge of ergot spore presence/absence in the air.

#### Postharvest Survey

The postharvest survey was completed by 21 respondents representing primarily growers (62%) and crop consultants/field scouts (33%), with a portion of the group also involved in seed cleaning (19%). Most respondents were from the Columbia Basin (48%) and central Oregon (33%). Ergot was considered to be moderately difficult to manage in 2015 (2.4 on a scale of 1–5). Pre- and postharvest yield losses for both species were mostly in the 1 to 10% range; however,

some respondents reported pre- and postharvest yield losses up to 11 to 25% in Kentucky bluegrass and up to 26 to 50% in perennial ryegrass (Figure 2). The difficulties associated with the fungal disease ergot were many, but timing fungicide application with flowering was considered to be most difficult (86% of respondents), followed by making multiple applications during flowering (Figure 3).

Overall, the *Ergot Alert Newsletter* was rated moderately to highly useful (3.6 on a scale of 1–5), and 90% of respondents reported an improvement in their knowledge of ergot disease. Many respondents indicated they would use multiple tactics in the future to manage ergot, including:

- Use the *Ergot Alert Newsletter* information and field scouting to determine start of flowering in each species and variety.
- Use different fungicide modes of action and improved application timing.
- Continue to use multiple tactics, such as nutrient management, fungicide application, and harvest aids.
- Follow recommendations provided by agronomists and other research professionals.
- Improve weed management around field perimeters.
- Improve flowering stage monitoring and timing of fungicide application.
- Produce varieties with short flowering stages.

The *Ergot Alert Newsletter* also helped 52% of respondents make fungicide application decisions in 2015, resulting in better ergot control (20% of respondents) and reduced fungicide applications (13% of respondents). Comments regarding newsletter usefulness indicated that it was difficult to determine whether or not ergot control was improved due to field-to-field variability in ergot infection and/or the lack of

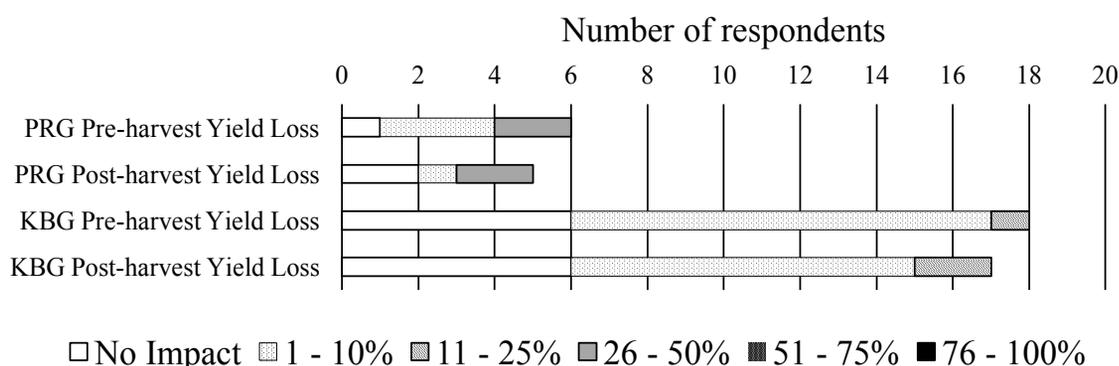


Figure 2. Yield losses of perennial ryegrass (PRG) and Kentucky bluegrass (KBG) in the field (preharvest) and after seed cleaning operations (postharvest), as reported by growers, fieldmen, and seed cleaners, 2015 postharvest survey.

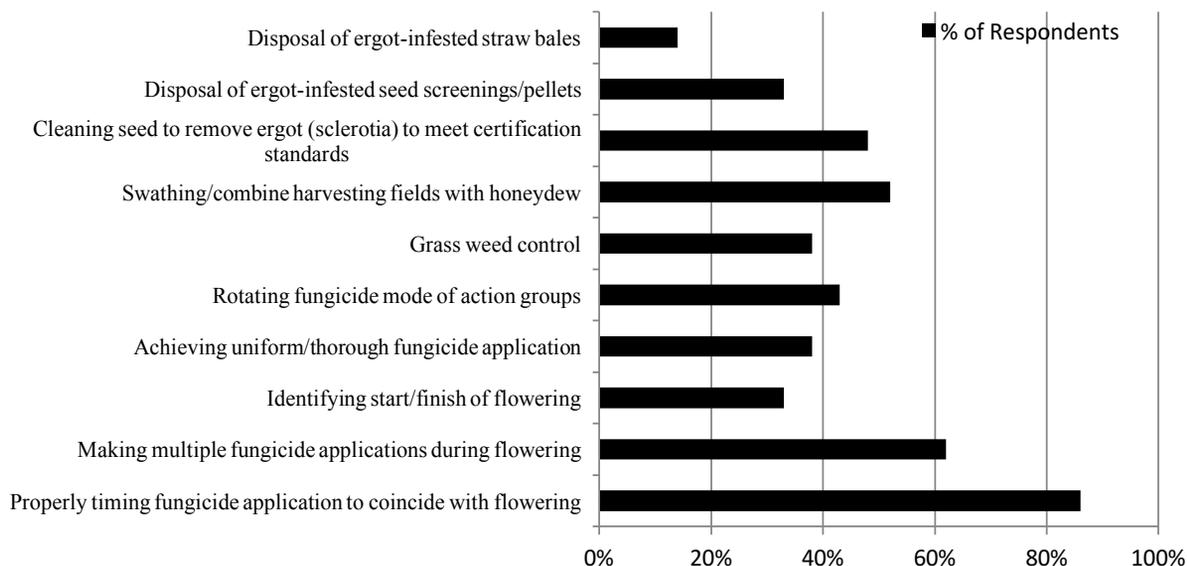


Figure 3. Difficulties associated with the fungal disease ergot, 2015 postharvest survey.

ergot pressure. However, some respondents indicated the newsletter did confirm proper application timing when fungicides were used.

In the future, 86% of respondents were willing to base fungicide application decisions on predictive models and knowledge of airborne ergot spore activity, but very few respondents were willing to use either as a stand-alone decision-making tool. Overall, 100% of respondents would use the tools to help make fungicide application decisions.

Newsletter distribution prior to critical stages of crop development was preferred by most respondents (71%). However, 19% of respondents preferred weekly distribution.

### Conclusions

Improvements to the newsletter in 2016 and beyond will be incorporated as the technology is developed and adapted into the regional alert system. The DNA-based ergot spore detection protocol and predictive models for perennial ryegrass and Kentucky bluegrass are under development and will need validation prior to full deployment for ergot management (Dung et al., 2013). The new tools will facilitate monitoring in more locations and more frequent/timely notifications. Currently, the alert system is limited to seven monitoring locations due to the time- and labor-intensive process of mechanical spore trapping and visual quantification of ergot spores using microscopic methods. Ultimately, the Ergot Team's goal is to provide frequent ergot alert notifications that are compatible

across various information/communication technology platforms (including smartphone technology) to aid field monitoring efforts and achieve optimum timing of fungicide application(s).

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# EFFECTS OF APPLIED NITROGEN ON YELLOW MUSTARD SEED PRODUCTION IN THE WILLAMETTE VALLEY

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## Introduction

Given recent legislative actions prohibiting production of *Brassica* spp. oilseed crops in much of the Willamette Valley, field crop producers are continuing their search for a productive crop to include in rotation with grass seed crops. Yellow mustard (*Sinapis alba* L.) is a promising, low-input, multipurpose crop that, although a member of the Brassica family, is taxonomically classified in the *Sinapis* genus and is therefore not restricted in the Willamette Valley. Yellow mustard is a distant relative of *Brassica* species vegetable seed crops, but will not hybridize with them (Vaughn, 1997; Brown et al., 2005).

Agriculturalists recognize the versatility that yellow mustard can offer as a green manure, condiment mustard (Vaughn, 1997), and oilseed crop (Brown et al., 2005), as well as its utility as a biopesticide (Beckie et al., 2008). Stemming from this newly realized potential comes the need for recommendations specific to yellow mustard production under Willamette Valley conditions, especially regarding nitrogen (N) fertilizer application rates. No agronomic guide highlighting recommended yellow mustard production practices for the high-rainfall environment of western Oregon is available. Additionally, no data addressing the effect of N fertilizer on yellow mustard plant physiology or the components of seed yield are available in the scientific literature. The objectives of this study were to quantify the effects of applied N on yellow mustard dry matter partitioning, seed yield, components of seed yield, and oil production characteristics.

## Materials and Methods

Field trials were conducted in 2013 and 2014 at the OSU Hyslop Research Farm near Corvallis, OR, utilizing a randomized complete block design and four replications. The cultivar 'IdaGold' was utilized in this study because it is resistant to lodging, seed shatter, and a number of insect pests and diseases. A preplant application of K-Mag (0-0-22-11-22) and a postplant foliar application of Solubor were made to correct nutrient deficiencies identified in preplant soil analysis (sampled at 0–8 inches in depth). A preplant herbicide trifluralin (Treflan) was applied and incorporated each year to control weeds; however, no additional pesticides were utilized. Following seedbed preparation, mustard

seed was planted at 18 lb/acre with a double-disk drill set in 6-inch rows on March 11, 2013 and March 13, 2014.

A tractor-mounted orbit-air fertilizer spreader was used to apply dry granular urea (46-0-0) at five rates: 0, 50, 100, 150, and 200 lb N/acre. Nitrogen application dates coincided with appearance of the first few true leaves (BBCH stages 11–13) on April 4, 2013 and April 18, 2014. Plant growth, development, and lodging rates were tracked weekly throughout the growing season. Vegetative measurements were conducted at the stem elongation (BBCH 30) and inflorescence emergence (BBCH 50) growth stages. At each stage, two adjacent 1-square-foot quadrats of yellow mustard vegetation were randomly cut and removed from each plot.

Immediately following collection, samples were weighed and plant number determined. Ten plants were then randomly selected from each observation and measured for height as well as total one-sided leaf area (measured with a LI-3100 leaf area meter), from which the leaf area index (LAI) was calculated. All biomass was returned to the individual observation sample bags, dried in an air-forced chamber at 158°F for 48 hours, and weighed.

The final biomass collection occurred when approximately 70% of siliques, commonly referred to as seed pods, were ripe (BBCH 87), on July 9, 2013 and July 7, 2014. Two adjacent 1-square-foot quadrat samples per plot were removed, weighed, dried, and then reweighed. Crop growth rate (CGR) was calculated as the change in plant dry weight over time. Ten random and representative plants were subsampled from each quadrat observation, and their plant height was quantified. These plants were also utilized to determine the components of seed yield (raceme/plant, siliques/plant, seeds/silique, and seed weight per raceme type).

Shortly after the final biomass sampling date, plots were swathed and left to dry for 7 to 12 days. The center swath (6 feet x 50 feet) from each plot was harvested and bagged with a plot combine on July 23, 2013 and July 28, 2014. The seed was cleaned and used to ascertain seed yield, seed weight, seed number/square foot, harvest index (ratio of seed yield to above-ground

biomass), and nitrogen use efficiency (NUE) (ratio of seed yield to available N supply). The cleaned seed was further subsampled and utilized for measuring seed oil and protein concentrations via nuclear magnetic resonance spectroscopy. Oil yield was calculated as the product of seed yield and fractional seed oil concentration.

Analysis of variance (ANOVA) was conducted for data on a plot means basis for each year. Vegetative measurements were analyzed for each growth stage. Treatment means were separated by Fisher’s protected LSD values at the 5% level of significance. Regression analysis was used to determine the relationship between seed yield components and seed yield.

## Results and Discussion

### Vegetative measurements

Applied N affected plant height at all developmental stages in 2013 and 2014, with the greatest increase in plant height occurring between stem elongation (BBCH 30) and inflorescence emergence (BBCH 50). Plants attained nearly full mature height during flowering when produced under dry conditions in 2013, but continued to grow under the wet conditions in 2014. Plant height is one of the main factors contributing to lodging, which is a potential problem for many crops produced in western Oregon. Lodging in yellow

mustard was present only with the two highest N rates (150 and 200 lb N/acre) in 2013, and appeared to a lesser extent in 2014 (data not shown). In both years, lodging was not severe, and adverse impacts on seed yield or increased harvest difficulty were not observed.

There was a positive relationship in both years between above-ground biomass and applied N for all developmental stages. Plants in the control plots grew at all measurement timings; however, the control plants consistently accumulated the least biomass when compared to plants in the treatments receiving applied N. Overall, applied N stimulated above-ground biomass accumulation increases ranging from 16% to 169% in 2013 and from 27% to 150% in 2014.

Leaf area index (LAI) is an indicator of potential photosynthetic capacity of a crop canopy. Increased LAI at stem elongation and inflorescence emergence was observed with application of N in 2013; however, only rates greater than 100 lb N/acre produced LAI values greater than the control in 2014 (Table 1). Crop growth rate (CGR) was also influenced in both years by applied N, especially at rates greater than 150 lb N/acre in 2013 and greater than 100 lb N/acre in 2014 (Table 1). The CGR in 2013 was somewhat greater than in 2014, possibly resulting from more favorable growing conditions in 2013, as well as a short-term boron toxicity experienced early in 2014.

Table 1. Nitrogen fertilizer effects on yellow mustard leaf area index (LAI), crop growth rate (CGR), nitrogen use efficiency (NUE), and harvest index (HI), 2013 and 2014.<sup>1</sup>

Year	Nitrogen (lb/a)	----- Leaf area index -----		Crop growth rate (lb/a/day)	Nitrogen use efficiency <sup>2</sup>	Harvest index <sup>3</sup>
		BBCH 30	BBCH 50			
2013	0	1.4 b	1.8 c	132 b	79.6 a	0.15 a
	50	3.1 a	3.0 b	142 ab	23.2 b	0.15 a
	100	3.3 a	2.9 b	157 ab	13.4 c	0.12 a
	150	3.6 a	4.9 a	188 a	10.3 c	0.12 a
	200	3.7 a	5.1 a	190 a	10.6 c	0.15 a
2014	0	0.8 c	1.3 d	86 c	66.2 a	0.18 a
	50	1.3 bc	2.2 cd	110 bc	19.7 b	0.18 a
	100	1.7 ab	3.3 ab	156 ab	13.9 c	0.16 a
	150	2.1 a	4.3 b	181 a	10.6 c	0.16 a
	200	2.4 a	6.9 a	175 a	8.3 c	0.17 a

<sup>1</sup>Means within columns and years followed by the same letter are not significantly different by Fisher’s protected LSD values ( $P = 0.05$ ).

<sup>2</sup>Nitrogen use efficiency = ratio of seed yield to available N supply

<sup>3</sup>Harvest index = ratio of seed yield to above-ground biomass

### Seed yield

Although precipitation during the 2 years was markedly different, seed yield responses to applied N were similar. A positive linear relationship between the rate of applied N and seed yield was found in both years (Figure 1). Average seed yields were 300 to 500 lb/acre lower in 2014 than 2013, with the exception of the 100 and 150 lb N/acre rate treatments (Table 2). Nevertheless, all applied N rates significantly increased seed yield over the control in both years. The 50 lb N/acre rate increased seed yield by 14% in 2013 and by 32% in 2014. Applications of 200 lb N/acre resulted in 68% and 86% greater seed yield than the control in 2013 and 2014, respectively.

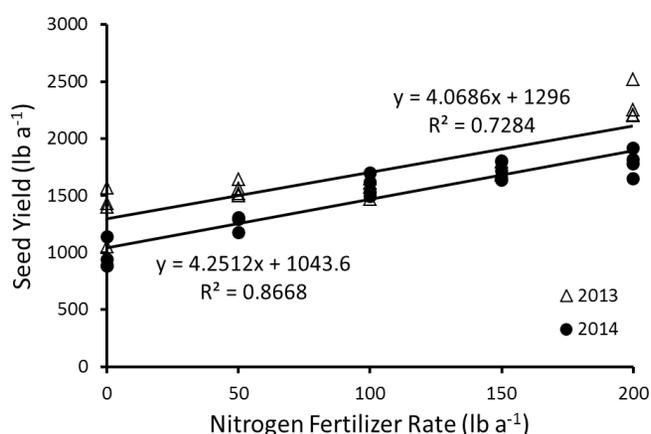


Figure 1. Applied nitrogen effects on yellow mustard seed yield, 2013 and 2014.

Table 2. Nitrogen fertilizer effects on yellow mustard seed yield, seed weight, and seed number, 2013 and 2014.<sup>1</sup>

Year	Nitrogen (lb/a)	Seed yield (lb/a)	Seed weight (mg)	Seed number (seeds/ft <sup>2</sup> )
2013	0	1,363 d	6.92 bc	2,060 c
	50	1,555 c	6.83 c	2,370 b
	100	1,576 bc	6.84 bc	2,400 b
	150	1,725 b	7.04 ab	2,550 b
	200	2,296 a	7.19 a	3,330 a
2014	0	961 d	5.94 c	1,680 c
	50	1,269 c	5.98 c	2,210 b
	100	1,586 b	5.99 c	2,760 a
	150	1,739 a	6.20 b	2,920 a
	200	1,789 a	6.48 a	2,880 a

<sup>1</sup>Means within columns and years followed by the same letter are not significantly different by Fisher's protected LSD values ( $P = 0.05$ ).

### Harvest index and NUE

Harvest index (HI) provides a measure of how applied N might influence partitioning to seed in relation to total above-ground biomass production. Applied N did not affect HI in yellow mustard in either year (Table 1). Values for HI in yellow mustard in this study ranged from 0.12 to 0.18, which were less than those reported for other Brassica family members (Ferguson et al., 2016).

Nitrogen use efficiency (NUE) in yellow mustard was reduced by applications of N in both years (Table 1). The inverse relationship of NUE and N application rates results from changes in N uptake during plant growth and development. The average NUE of yellow mustard in this study, 27.5 and 10.8 in 2013 and 2014, respectively, is within the range of NUE values obtained for oilseed rape (12.0 to 27.0) (Wang et al., 2014).

### Components of seed yield

Applied N affected all seed yield components in both years, with the exceptions of seed weight on primary (1°) branches in 2013 and seeds/silique on 1° branches in 2014. The number of racemes/plant was increased by applied N. The number of 1° branch racemes was increased with 200 lb N/acre in 2013 and with rates greater than 100 lb N/acre in 2014.

The numbers of siliques on the main stem and 1° branch racemes were increased with applied N in both years. Similar results were found in oilseed rape and winter canola (Wang et al., 2014). Siliques borne on the main

stem and 1° branch racemes accounted for roughly 99% of the total silique production in both years. Main stem siliques accounted for 61% and 65% of the total siliques/plant in 2013 and 2014, respectively. Total production of siliques by the plant was greater in 2013 than in 2014 and is likely a contributor to seed yield differences observed between years.

Applied N increased the number of main stem seeds/silique in both years and 1° branch raceme seeds/silique in 2013, but not in 2014. These results are in contrast with reported effects of applied N in winter canola, where the number of seeds/silique was not affected by N application (Ferguson et al., 2016).

Seed number/square foot was increased with all rates of applied N in both years (Table 2). The greatest number of seeds/square foot was observed with 200 lb N/acre in 2013 and with rates greater than 100 lb N/acre in 2014. All N rates resulted in significantly more seeds/plant in 2013 when compared to the control.

Seed weight was increased by applied N with the 200 lb N/acre rate in 2013 and by rates greater than 150 lb N/acre in 2014 (Table 2). The weight of seed produced on the main stem in both years, as well as seed produced on 1° branch racemes in 2014, was incrementally increased with the rate of applied N (Figure 2). However, N had no influence on the weight of seed produced on 1° branch racemes in 2013. Applied N either reduced or had no effect on seed weight produced on main stem and 1° branch racemes in winter canola (Ferguson et al., 2016).

Seed yield was most strongly affected by seeds/square foot rather than by seed weight because variation in seed weight across applied N rates was much less than that observed for seeds/square foot. There was a strong linear relationship between seeds/square foot and seed yield (Figure 3), similar to that reported by Ferguson et al. (2016) in winter canola. Since seeds/plant was the most important characteristic in determining seed yield responses to applied N, the increased number of seeds most likely was a result of N-induced increases in siliques on main stem and 1° branch racemes.

#### Seed oil and protein content and oil yield

Seed protein content is a major consideration in many oilseed crops, yet is of less concern in yellow mustard production due to limited nutritional applications. Contrary to Kovács et al. (2006), who found that seed protein content in yellow mustard was positively

influenced by increasing N fertilizer, seed protein content in this study did not vary from 24%, regardless of N rate (Table 3).

Generally, N fertilizer causes reductions in seed oil content; however, applied N inconsistently affected the oil content in yellow mustard seed in both 2013 and 2014. In this study, yellow mustard seeds averaged 27.3% and 27.5% oil in 2013 and 2014, respectively.

Applied N influenced yellow mustard oil yield, with all N rates increasing oil yield over the control in both years (Table 3). Overall, oil yields were greater in 2013 than in 2014, as a result of more favorable growing environment in 2013. Greatest oil yields were noted with the 200 lb N/acre rate in 2013 and rates greater

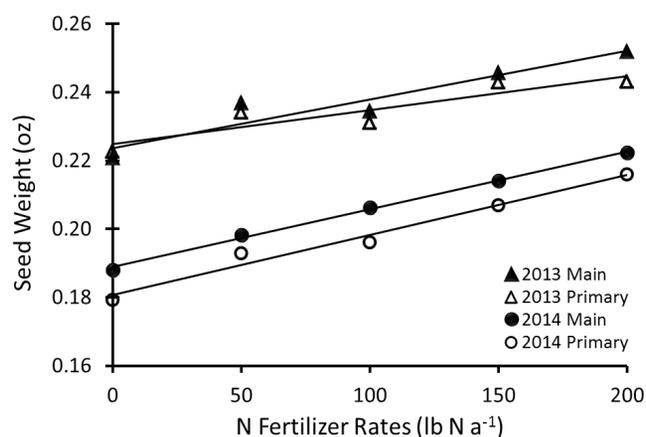


Figure 2. Applied nitrogen effects on yellow mustard main stem and primary seed weight, 2013 and 2014.

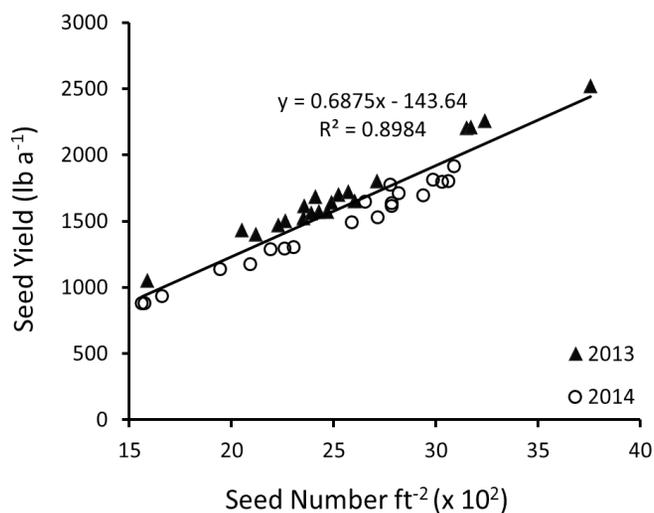


Figure 3. Relationship of yellow mustard seed yield and seed number/square foot, 2013 and 2014.

than 150 lb N/acre in 2014. Yellow mustard oil yields ranged from 271 lb to 604 lb oil/acre.

**Conclusions**

This study was initiated to provide the background information needed to establish N fertilizer recommendations for Willamette Valley produced yellow mustard. Applications of 150 to 200 lb N/acre supported the greatest seed and oil yields over the 2-year trial period and are recommended as the optimal N fertilizer application rate for ‘IdaGold’ yellow mustard cultivated under nonirrigated Willamette Valley growing conditions.

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Table 3. Applied nitrogen effects on seed protein, oil content, and oil yield in yellow mustard, 2013 and 2014.<sup>1</sup>

Year	Nitrogen (lb/a)	Seed protein content (%)	Seed oil content (%)	Oil yield (lb/a)
2013	0	24.2 a	26.3 b	359 c
	50	23.9 a	28.4 a	442 b
	100	24.0 a	28.4 a	448 b
	150	24.2 a	27.0 b	466 b
	200	24.4 a	26.3 b	604 a
2014	0	24.3 a	28.1 ab	271 d
	50	24.2 a	28.7 a	364 c
	100	24.4 a	27.6 b	437 b
	150	24.6 a	26.8 c	466 a
	200	24.9 a	26.4 c	472 a

<sup>1</sup>Means within columns and years followed by the same letter are not significantly different by Fisher’s protected LSD values ( $P = 0.05$ ).

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