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EVALUATING THE RESPONSE OF SLUG POPULATIONS AND ACTIVITY TO TILLAGE PRACTICES IN ANNUAL RYEGRASS GROWN FOR SEED

C.S. Sullivan and S.E. Salisbury

Introduction

Slugs remain one of the most damaging economic pests of the grass seed industry in the Willamette Valley. As no-till acreage has increased, it appears that slug pressure has also increased (Dreves et al., 2014). No-till systems, coupled with full-straw-load residue management, provide ideal slug habitat. Slug counts in no-till annual ryegrass fields have been recorded at up to 29 times greater than in conventionally tilled fields (Fisher et al., 1996).

In theory, tillage should reduce slug populations by crushing and burying slugs, disrupting their burrows, and reducing the food source. However, slug populations within conventionally tilled acres may also reach economic-infestation levels, requiring control measures for successful crop establishment. While anecdotal information exists suggesting tillage reduces slug populations, little research has been done under controlled experimental conditions to evaluate this theory. As frustration rises for growers using both conventional tillage and no-till practices, it is apparent that more information is needed on the use of tillage as a slug management tool.

A long-term annual ryegrass seed production trial established in 2005 with varied tillage cycles offered the opportunity to evaluate treatment effects on slug populations. The present study was designed to determine the impact of four tillage treatments on slug emergence and total numbers during the fall of one season.

Methods

The study was conducted at OSU's Hyslop Research Farm near Corvallis, OR, in the fall of 2014. The study plots were part of an existing annual ryegrass cropping systems trial that was established in 2005 (Mellbye et al., 2009; 2011). The original study evaluated six treatments in a continuous annual ryegrass monoculture for seed production; the treatments were different combinations of conventional tillage, conventional planting, and no-till practices. The fall of 2014 was the ninth year of treatments imposed on these plots. An established population of slugs was confirmed in this location.

The experimental design was a randomized complete block with three replications. The following four tillage treatments were studied in 2014:

- Continuous conventional tillage (CT)
- Continuous no-till (NT)
- Alternate-year tillage (NT/CT)
- Third-year tillage (volunteer/NT/CT)

Each treatment plot area measured 25 feet x 60 feet. On all treatments, the crop residue was flail-chopped and left on the plots after harvest. Conventional tillage included plowing to a depth of 8 to 10 inches, disking, and harrowing. Treatments 1 and 3 were conventionally tilled on October 6, 2014, while treatments 2 and 4 were left to volunteer. None of the plots were reseeded in 2014, as there was sufficient seed in the soil for crop regrowth.

Once adequate fall moisture was received (about 1.5 inches), slug blankets were used to monitor slug emergence and numbers. In each plot, three slug blankets (18 inches x 18 inches, designed by Liphatech) were soaked in water and evenly distributed down the length of the center of the plot. In the no-till treatments, the residue was brushed aside to expose the soil surface before placing the blanket. The blankets were secured in place in the afternoon, and the number of slugs per blanket was recorded the following morning. Ten such slug counts were conducted between October 12 and November 28: every three days from October 12 to 24, and then at seven-day intervals from October 24 to November 28. Slugs were removed and blankets rewetted for each count.

For each date, the number of slugs per plot was determined by averaging the three slug blankets counted. Data were analyzed as a two-way factorial analysis of variance using Statistix 9.0 software. Main effects of tillage treatment and slug count timing and their interaction were run at the 95% confidence level ($P < 0.05$). Means within treatments were compared using LSD at the 5% significance level.

Results and Discussion

Tillage treatments, slug count timing, and the interaction between tillage treatments and timing of slug activity

Table 1. Average slug count per sample date in relation to recorded air temperature, soil temperature, and cumulative precipitation at Hyslop Research Farm, fall of 2014.

Sample date	Slug count[1]	Mean air temperature	Mean soil temperature	Cumulative precipitation[2]
		(°F)	(°F)	(inches)
Oct. 12	5.5 d	54.7	62.7	0.03
Oct. 15	13.5 bc	54.7	59.1	0.99
Oct. 18	9.1 cd	62.7	63.9	1.19
Oct. 21	11.3 cd	56.6	60.2	1.92
Oct. 24	12.3 cd	51.5	56.5	3.44
Oct. 31	21.2 b	53.9	58.3	4.94
Nov. 7	35.4 a	47.2	53.8	5.80
Nov. 14	11.5 cd	33.7	40.3	8.28
Nov. 21	30.8 a	50.0	47.2	9.28
Nov. 28	30.0 a	52.1	51.8	10.86

[1] Means followed by the same letter are not significantly different (*P* <0.05).
[2] Cumulative precipitation beginning October 1, 2014.

all produced significant differences (*P* <0.05). Over the course of the sampling period, average slug counts were naturally affected by the weather (Table 1). Slug populations tended to increase with moisture except under freezing conditions. There was a significant interaction between tillage treatment and sample date (*P* = 0.01). Therefore, the effect of tillage on the population and activity of slugs was significant, but varied over the fall (Figure 1).

Differences between tillage treatments were less pronounced early in the season, but measurable differences appeared in late October/early November. The data clearly indicate that alternate-year tillage resulted in both the lowest and most consistent population of slugs over the sampling period (Figure 1). Overall, continuous NT resulted in the earliest slug emergence and greatest total number of slugs. Tillage did delay slug emergence; however, continuous CT had the same average number of slugs as tillage only every third year (Table 2).

The annual ryegrass seed yield averages after six and eight years of this trial were consistently lowest in

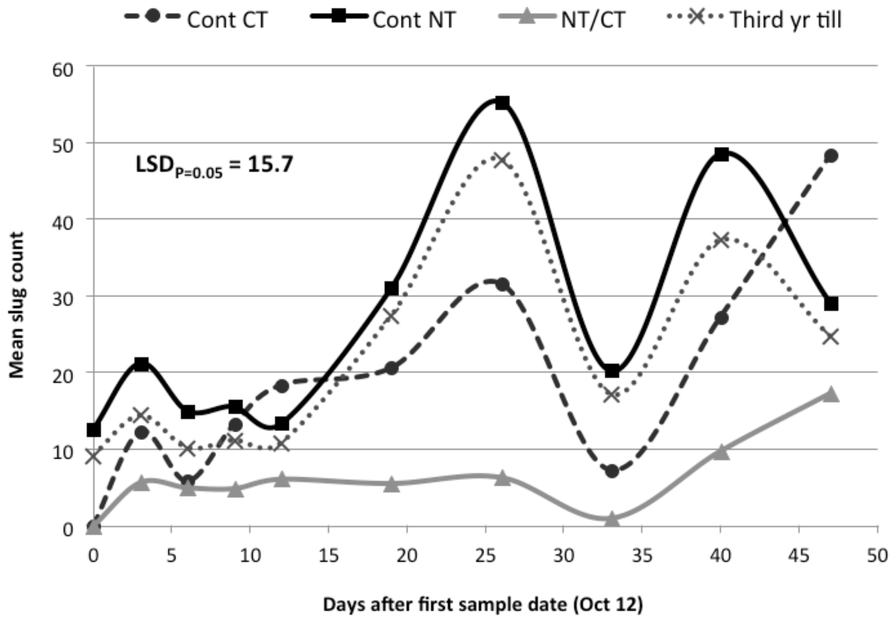

Figure 1. Average slug count per tillage treatment as measured over the course of the sampling period.

the continuous NT treatments (Mellbye et al., 2011; Chastain et al., 2014). The continuous CT, alternate-year tillage, and third-year tillage treatments all produced statistically similar yields after six and eight years. Both Mellbye et al. (2011) and Chastain et al. (2014) noted heavy slug pressure as a likely cause of yield reduction in the NT plots, and slug numbers in the current study were significantly higher in the NT treatments (Table 2).

As shown in Figure 1, the alternate-year tillage treatment resulted in both the lowest population of slugs as well as the most consistent population. The data suggest that some form of disruption (i.e., alternating CT and NT) decreased slug pressure—perhaps by simply altering conditions so that slugs did not become habituated. Similarly, Chastain et al. (2014) found that seed yields in CT treatments were increased when they were cycled with NT or volunteer treatments. In studying annual ryegrass cropping systems over time, it was determined that some frequency of tillage, disturbance of crops residues, and occasional removal of crop residues produced the best seed yields overall (Chastain et al., 2014). These results have been corroborated by growers' observations, who have noted improved slug management and yields when using tillage every other year. With regards to a slug management strategy, alternate-year tillage likely would lead to improved control of slugs with bait applications because of the consistency of slug activity. This scenario both alleviates the risk of "missing" the best opportunity to control slugs and has the lowest level of slug activity to control.

Results from this study indicate several opportunities for follow-up research. The hypothesis that slug activity is more consistent in alternate-year systems, and that slug bait applications therefore are more effective, should be studied with larger scale on-farm slug bait trials. It is important to note that slug migration between plots was highly possible in this study due to the narrow width of the plots. There is also interest from growers in assessing the impacts of shallower tillage on slug populations, as vertical tillage becomes more prominent in the Willamette Valley. Additional trials evaluating not only the efficacy of slug baiting under different tillage strategies, but also the economics of slug bait and land management practices, would be valuable to growers.

Table 2. Average slug count per tillage treatment.

Tillage treatment	Slug count[1]
Continuous tillage	18.5 b
Continuous no-till	26.3 a
Alternate-year tillage	6.3 c
Third-year tillage	21.1 b

[1] Means in a column followed by the same letter are not significantly different ($P < 0.05$).

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CAN KNOWLEDGE OF SPATIAL VARIABILITY IN SLUG POPULATIONS HELP IMPROVE STAND ESTABLISHMENT?

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Introduction

Slugs have been repeatedly identified as one of the most serious pest problems in a wide variety of Willamette Valley crops, including grasses grown for seed. 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.

Materials and Methods

Tests were conducted in the grass seedling establishment phase of three major crop rotations: (1) radish followed by fall seeding of new perennial ryegrass (PR) stands, (2) white clover followed by fall seeding of PR, and (3) established PR taken out of production by conventional tillage and replanted to the same PR variety. Tests were conducted at a total of five sites, two in Polk County and three in Linn County (Table 1). Both no-till and conventional tillage were used in removal of radish and white clover stands. All PR stands were planted by growers.

Weekly counting of slugs, predatory beetles, and earthworms began before crop emergence and continued until stands were well established by mid- to late winter. Slug blankets were placed in grid patterns spaced at approximately one blanket per acre, 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.

Slugs were counted over a period of 19 weeks from early October through early February, although not all sites could be counted every week due to field conditions. Slug counts were made at sites 1, 2, 3, 4, and 5 a total of 8, 13, 14, 12, and 15 times, respectively, ignoring all counts made before the first slugs were present at the soil surface in early fall. Access to fields was more often a problem in the three conventionally tilled fields than in the two no-till fields. Timing of slug counts in this report refers to the number of weeks since the end of September, with week 1 being the period from September 28 to October 4, 2014. Experiments were terminated once crops were well established and final counts of crop stands had been taken.

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 provides more useful information on statistical significance than IDW or Kriging and therefore was chosen for mapping slug populations within fields over time. Slug count data were normalized

Table 1. Test site conditions, fall 2014.

Site no.	County	Previous crop	Seedbed preparation	Planting date	Number of slug counts	Number of times slug bait was applied[1]
1	Polk	Perennial ryegrass, 3-year stand	Conventional tillage	Oct. 9	8	9
2	Polk	Radish for seed	Conventional tillage	Oct. 15	13	4
3	Linn	White clover	No-till	Sept. 24	14	9
4	Linn	White clover, 3-year stand	Conventional tillage	Oct. 6	12	2
5	Linn	Radish for seed	No-till	Sept. 29	15	4

[1] Slug bait applications were made at site 1 on Oct. 27, Oct. 28, Oct. 31, Nov. 4, Nov. 7, Nov. 8, Nov. 24, Dec. 12, and Jan. 20; at site 2 on Oct. 21, Nov. 10, Nov. 16, and Dec. 15; at site 3 on Oct. 2, Oct. 8, Oct. 16, Oct. 30, Dec. 10, Dec. 22, Dec. 30, Jan. 7, and Jan. 22; at site 4 on Oct. 31 and Nov. 24; and at site 5 on Sept. 27, Oct. 31, Nov. 7, and Nov. 25. Total rates applied over the season within treated areas were 54, 60, 48, 20, and 32 lb/acre at the five sites. Most applications were broadcast, but some were limited to areas with the worst slug problems.

by converting zero counts into small positive values between 0 and 1 based on average slug counts and the fraction of plots with non-zero counts at a given site on a given day, followed by log transformation of the revised slug count numbers.

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.

Slug baits were applied by growers based on their own experience and on information we provided to them concerning weekly slug counts.

Results and discussion

Maximum soil moisture content (>40%) was reached by early December at all sites.

Results are summarized in Table 2. Predatory beetle populations were highest in the first month of counting, and declined to near 0 when weather cooled in November. Earthworm counts remained high at most sites through early December, although there were large differences among plots at any site and also among the five sites.

Slugs were never uniformly distributed across any of the sites on any date, and counts varied from a minimum of 0 to a maximum of 86 slugs per blanket. Slug counts (both raw and log-transformed data) were significantly affected by date and by plot location within each site. Bait applications typically reduced slug counts by approximately 5-fold (e.g., 25 slugs per blanket before treatment and 5 slugs after treatment).

There were 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 at each site in Figures 1–5.

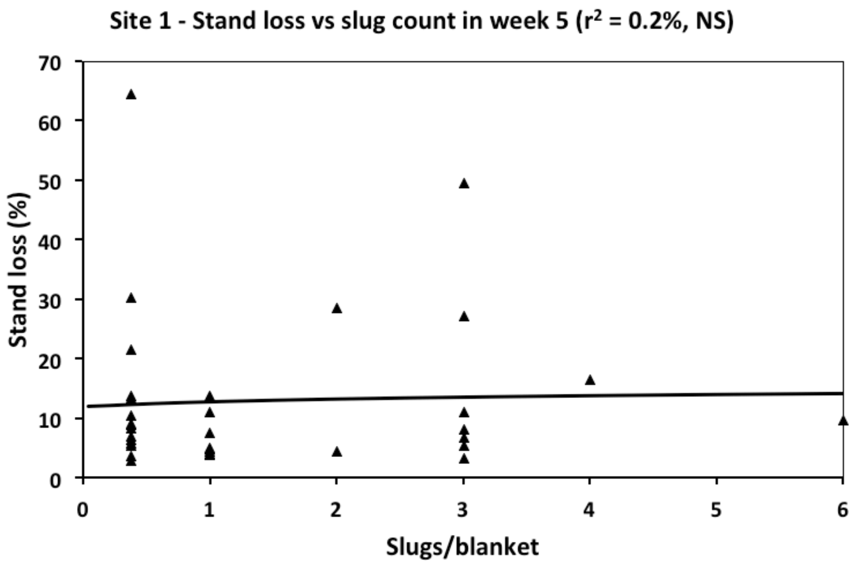
At site 1 (conventional tillage into a three-year stand of PR), crop stand loss was generally low and showed no sign of any relationship with slug counts (Figure 1). Problems accessing the field limited us to a total of eight counts over the entire period, seven fewer than at the most easily accessed field. Serious feeding by cutworms was seen in some areas of the field in mid-fall, prompting the grower to apply insecticide on November 26 to save his crop. We were unable to obtain detailed information on the distribution of cutworms, but it seems probable that most of the small patches of missing crop in the field were caused by cutworms rather than by slugs.

At site 2 (conventional tillage into radish), crop stand loss was best explained by a combination of slug counts at weeks 3, 4, and 6 (Figure 2), with an r^2 value of 51.5%. This particular site had the greatest amount of stand damage seen at any of the sites, and many patches in the field suffered close to 100% stand loss. These patches were irregularly shaped, but were often 10 to 20 feet in one direction by 50 to 100 feet perpendicularly. Slug counts with the strongest relationships to stand loss were those taken from mid-October through early November, corresponding with emergence and early growth of PR seedlings. There was a dense stand of volunteer radish at this site, likely

Table 2. Test site results, fall 2014.

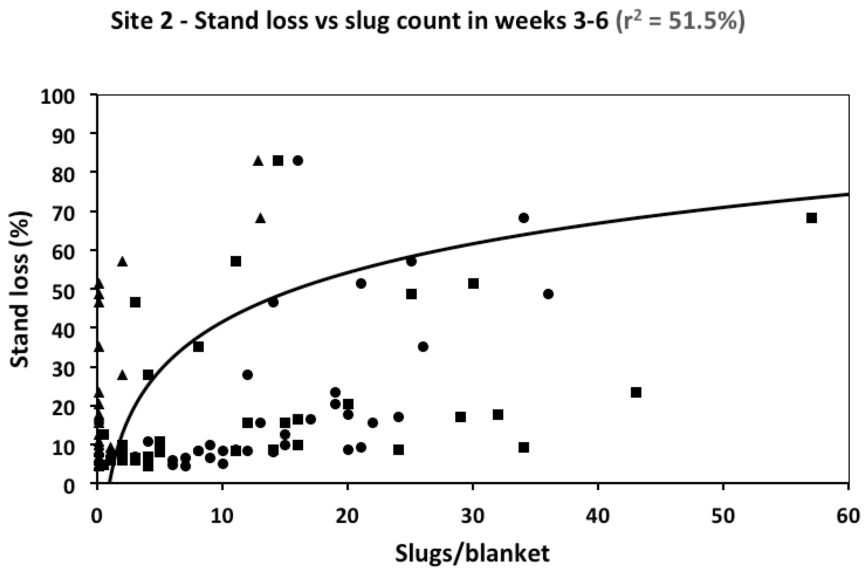
Site no.	Average weekly slug count, entire season	Highest weekly --- average slug counts --- Week[1]	Average number	Slug counts from period most closely related to crop loss Weeks included	Average number	Average counts of other organisms Predatory beetles (weeks 3–6)	Earthworms (weeks 4–11)
1	1.4	6	2.8	5	1.4	0.8	8.1
2	7.2	9	21.1	3, 4, 6	9.4	1.5	0.6
3	4.1	3	9.8	3, 4, 6–11	4.6	0.1	4.2
4	3.9	5	7.9	4, 5	4.3	3.1	14.6
5	1.3	5	2.3	4	1.0	0.1	18.3

[1] Week 1 of fall establishment season is defined as Sept. 28 to Oct. 4, 2014.



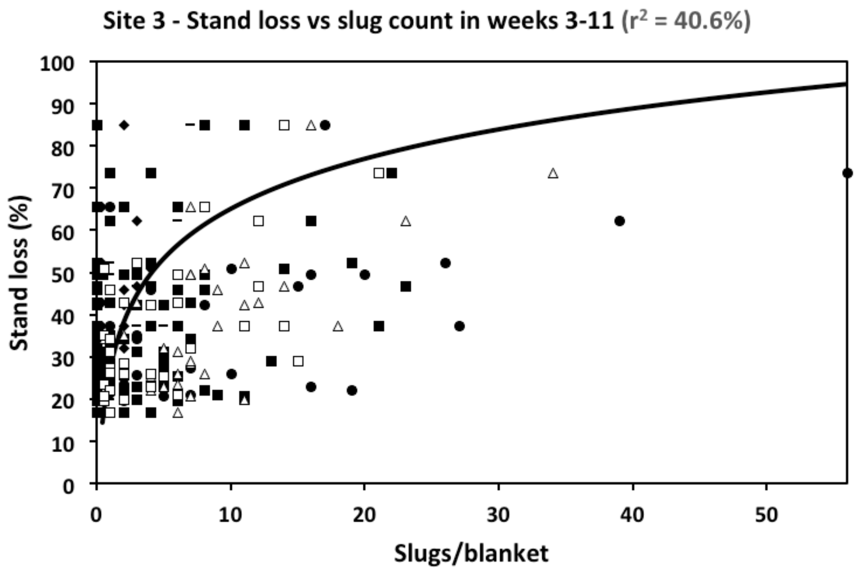
Site 1 - Stand loss vs slug count in week 5 (r^2 = 0.2%, NS)

Figure 1. Stand loss from slugs at site 1.



Site 2 - Stand loss vs slug count in weeks 3-6 (r^2 = 51.5%)

Figure 2. Stand loss from slugs at site 2.



Site 3 - Stand loss vs slug count in weeks 3-11 (r^2 = 40.6%)

Figure 3. Stand loss from slugs at site 3.

maintaining relatively good moisture conditions on the soil surface throughout the daytime hours in mid-fall, thereby maximizing slug activity. This was the only site at which new slug egg masses were found on the soil surface during the fall.

Site 3 was a no-till planting into a dense stand of white clover. It suffered from highly variable crop emergence, probably related to poor soil moisture at planting time and erratic seed/soil contact. Germination was spread out over many weeks, and the dense stand of white clover suppressed development of PR seedlings for several months before it was finally killed by herbicides. Crop stand loss at site 3 was best explained by slug counts over the period of time from week 3 through 11 (Figure 3), with an r^2 value of 40.6%. The combined result of slow emergence and heavy competition apparently left seedling PR vulnerable to slug feeding/damage over a prolonged period of time. The firm soil, however, allowed the grower good access to the field to repeatedly apply slug baits, and moderately good crop stands eventually developed over much of the field.

Stand loss at site 4 (conventional tillage into white clover) and site 5 (no-till into radish) was much less serious than at sites 2 and 3. Crop emergence was relatively uniform and prompt at sites 4 and 5, with no competition from weeds at site 4 and only temporary competition at site 5 until herbicides were applied to control the volunteer radish. Crop stand loss was most strongly related to slug counts in weeks 4 and 5 at site 4 (Figure 4) and to those in week 4 at site 5 (Figure 5), with r^2 values of 39.1 and 36.0%, respectively. Weeks 4 and 5 represented the point at which soils had received

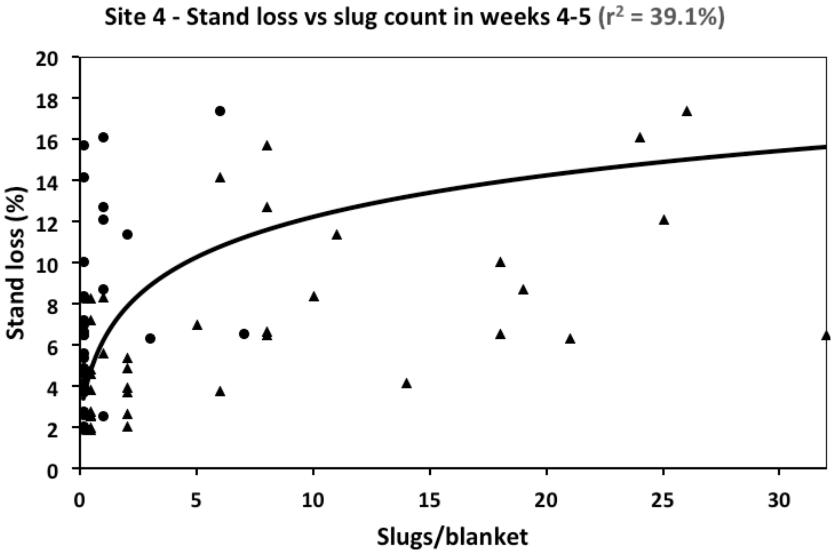


Figure 4. Stand loss from slugs at site 4.

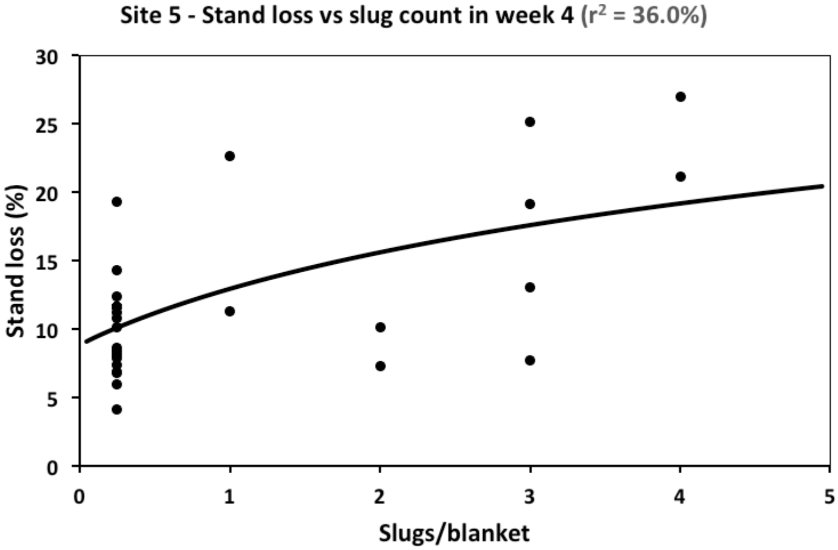


Figure 5. Stand loss from slugs at site 5.

enough rain to become good environments for slugs to safely forage for food while the weather was still warm enough for the slugs to be active. Soil moisture at site 4 climbed from 14% on October 10 to 37% by October 31. Growers at both sites 4 and 5 maintained relatively good control over slugs with timely application of slug baits. Although slug counts at site 4 included five cases of more than 20 slugs per blanket, PR stand damage was less than 18% even in the worst plots, in contrast to multiple plots with more than 80% stand loss at sites 2 and 3.

Knowing that the critical period (as defined by when small crop plants are most easily destroyed by slugs) was usually only the first month after planting reduced our concern about the stability of slug hot spots over the entire period from early fall through midwinter. However, situations such as delayed crop emergence or excessive competition from established plants or volunteer seedlings of previous stands could extend the period of vulnerability past the first month. In that case, the stability of slug populations would become more important.

Figures 6–10 show the statistical significance of clustering by slugs into hot (and occasionally cold) spots over multiple periods of time. The center bulls-eye of each ring in Figures 6–10 shows significance for weeks 11 to 19 of slug counts, the next larger ring shows significance for weeks 7 to 10, and the third ring shows significance for the first 6 weeks. In Figures 7–10, the outermost (largest) ring of each circle shows clustering significance of slugs at the period of time most strongly linked to crop stand loss. In many cases, there was at least some similarity in location of hot spots over time. It was relatively uncommon, however, for hot spots to remain at the same locations over the entire four-month period.

Most slugs seen at the five sites hatched over a month-long period from early October through early November. Factors causing juvenile slugs to appear at the soil surface in variable and sometimes damaging numbers over this period of time are uncertain, but likely include the depth at which eggs were laid in the spring, the degree of mortality from predatory beetles, and local spatial variability in soil moisture and temperature regimes.

Once slugs hatched and began feeding on crop seedlings and other vegetation, variations in uniformity of slug bait application rates and patterns of earthworm feeding on slug baits probably induced further spatial variability in slug populations by allowing some juveniles to survive and develop while others were killed. Some stand damage due to cutworms also occurred, although growers at the two sites most impacted by cutworms quickly brought them under control by timely insecticide applications. Cutworms were most serious at site 1, where spatial variation in stand loss was unrelated to slug counts on any date or range of dates.

Several results stand out from this research regarding general recommendations for applying slug control baits to new grass plantings. First, the most critical period for applying slug baits is when soils first become thoroughly wet and eggs hatch out into hungry neophytes. In the fall of 2014, this point in time was preceded by approximately four weeks of intermittent, light rainfall that germinated PR seedlings while maintaining a generally dry soil surface. Heavier rains soon after planting likely would shorten the time period in which seedlings are safe from slugs, probably leading to greater crop damage. Second, no-till has distinct advantages over conventional tillage in allowing access to fields when needed. In conventional tillage systems, access is limited to times when rainfall patterns allow equipment to drive on the fields. Third, multiple applications of slug bait were required at all sites, particularly when crop germination was extended over a lengthy period or early applications were somewhat ineffective. Fourth, growers limited some of their slug baiting efforts to spot treatments rather than whole-field applications, but with mixed results. Treating only those parts of a field firm enough to drive on would be an invitation to slugs to damage crops in the rest of the field. In contrast, treating areas with known slug populations and leaving other areas untreated worked reasonably well as long as all areas of the field were accessible for monitoring.

Analysis is continuing in an attempt to identify reasons for the patchiness of slug populations and crop damage. Until a predictive model can be developed, the best that growers can do is to initially treat entire fields and then continue treating areas with noticeable damage in order to maintain adequate stands of newly planted crops such as PR. Shifting locations of slug hot spots in the early weeks of the fall growing season suggest that hatching and emergence are delayed or promoted by factors such as soil moisture, soil texture, depth of tillage, composition of pre-existing vegetation, and prevalence of predators. Further research into these factors will be crucial for developing predictive models useful in designing variable rate and hot spot application programs for slug baits.

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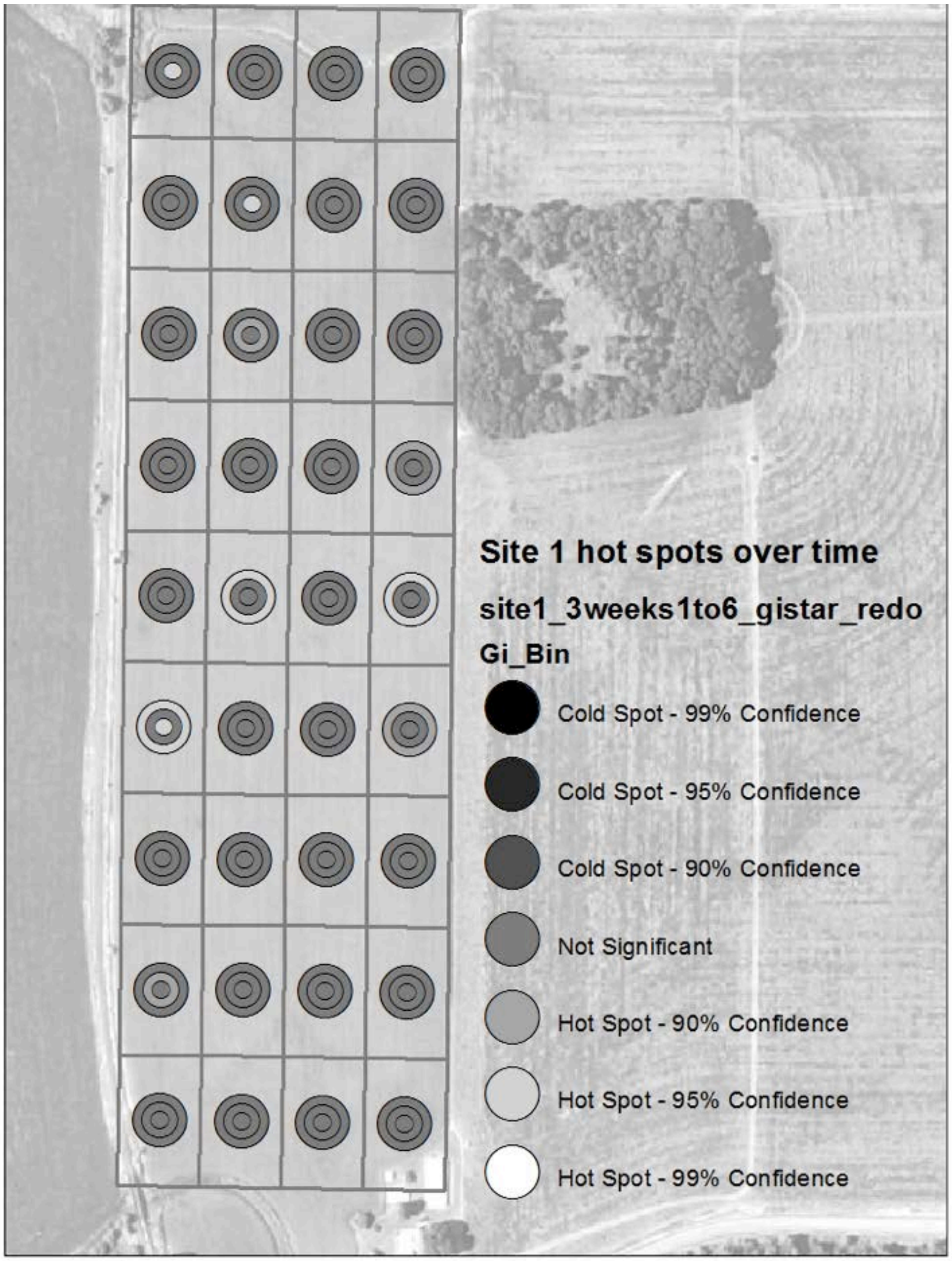

Figure 6. Significance of slug hot spots over time at site 1.

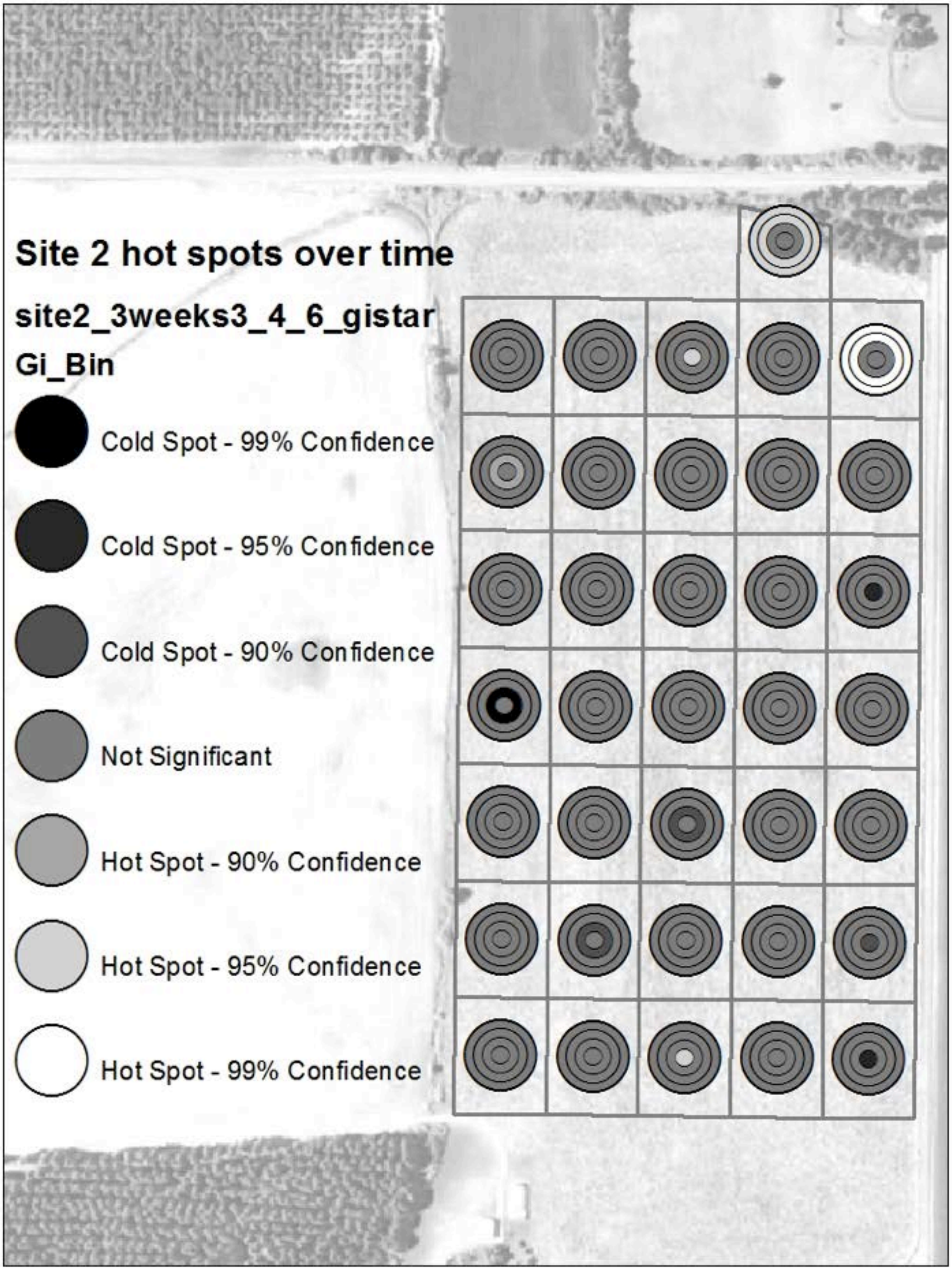

Figure 7. Significance of slug hot spots over time at site 2.

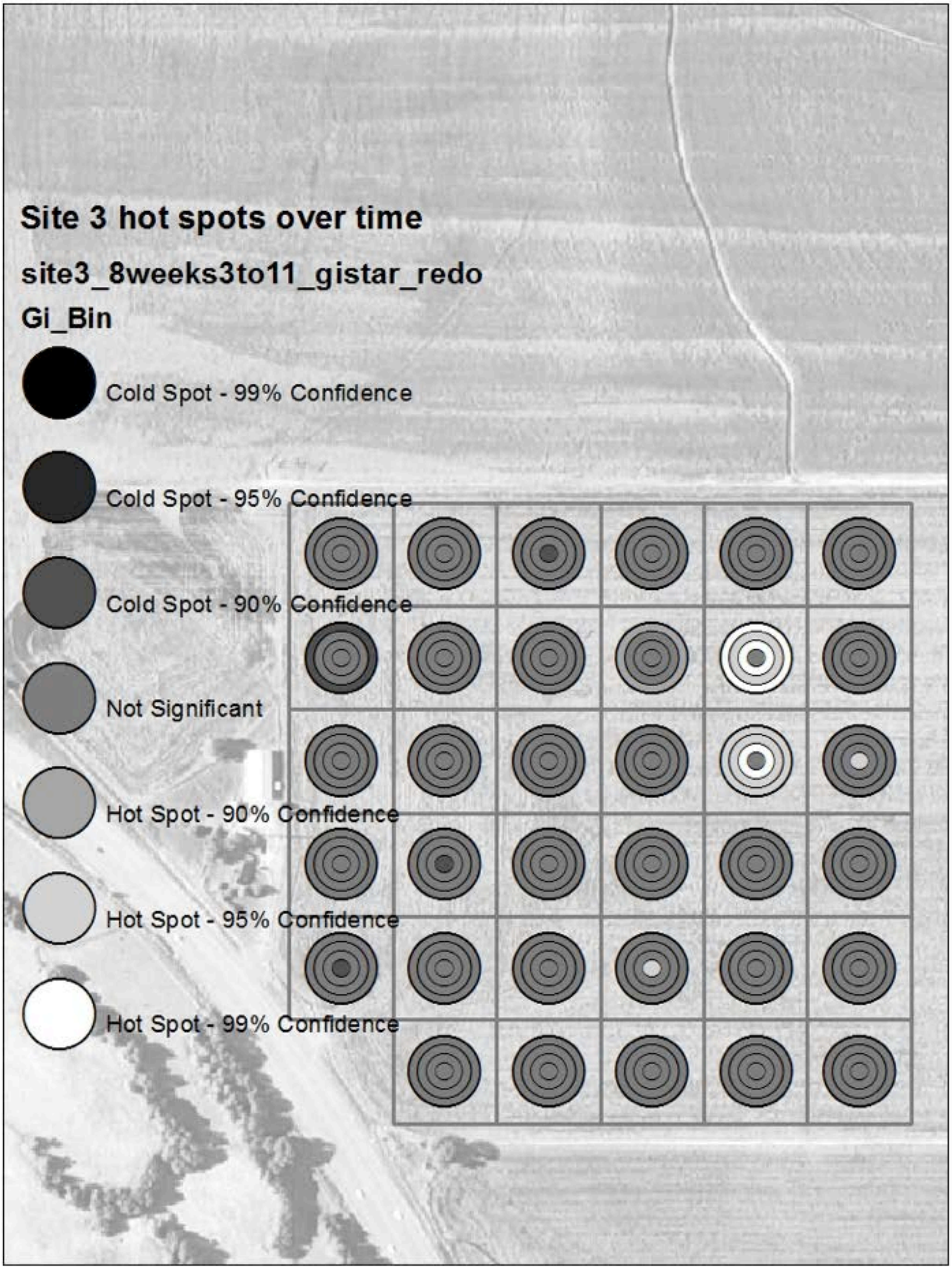

Figure 8. Significance of slug hot spots over time at site 3.

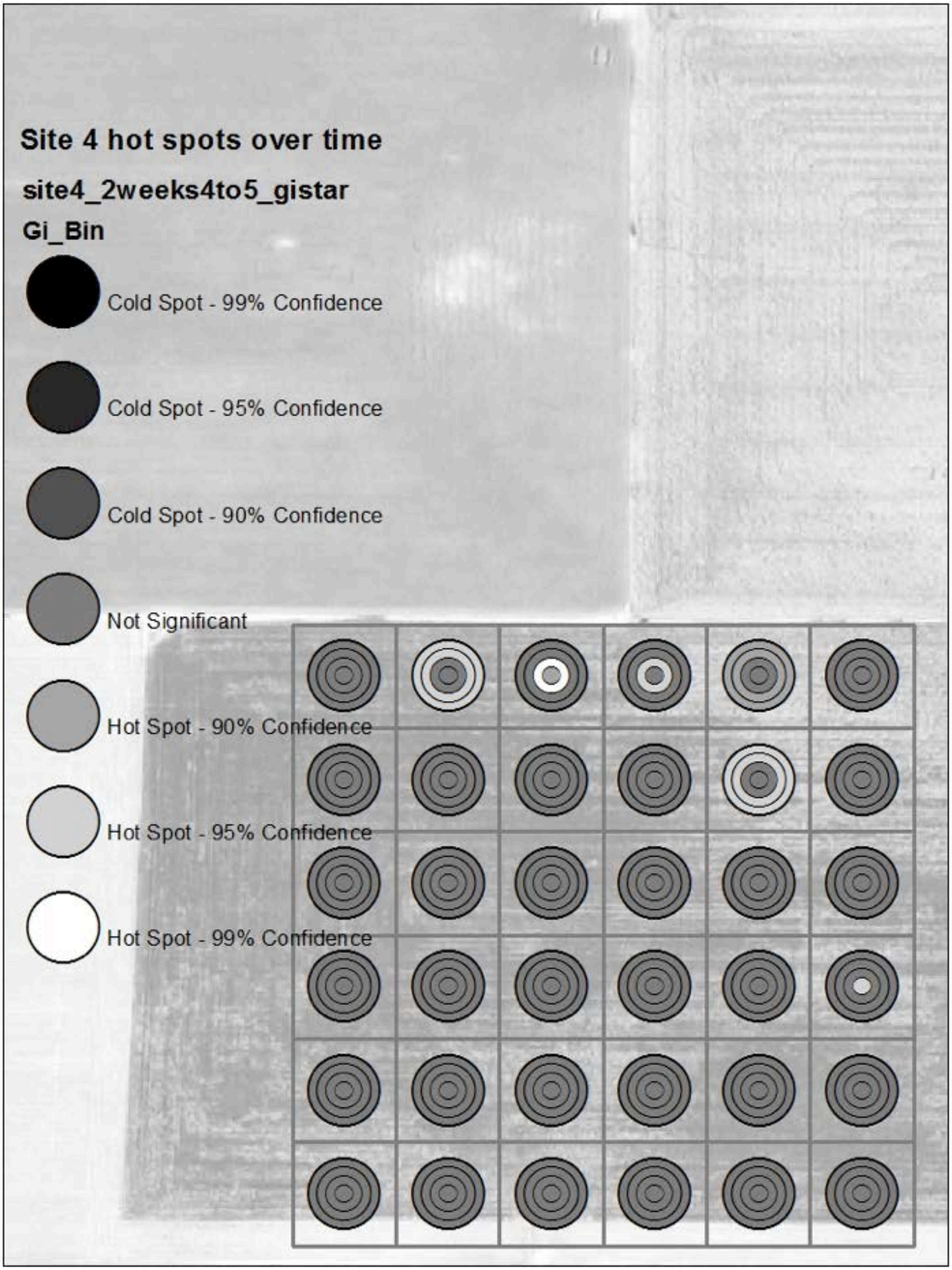

Figure 9. Significance of slug hot spots over time at site 4.

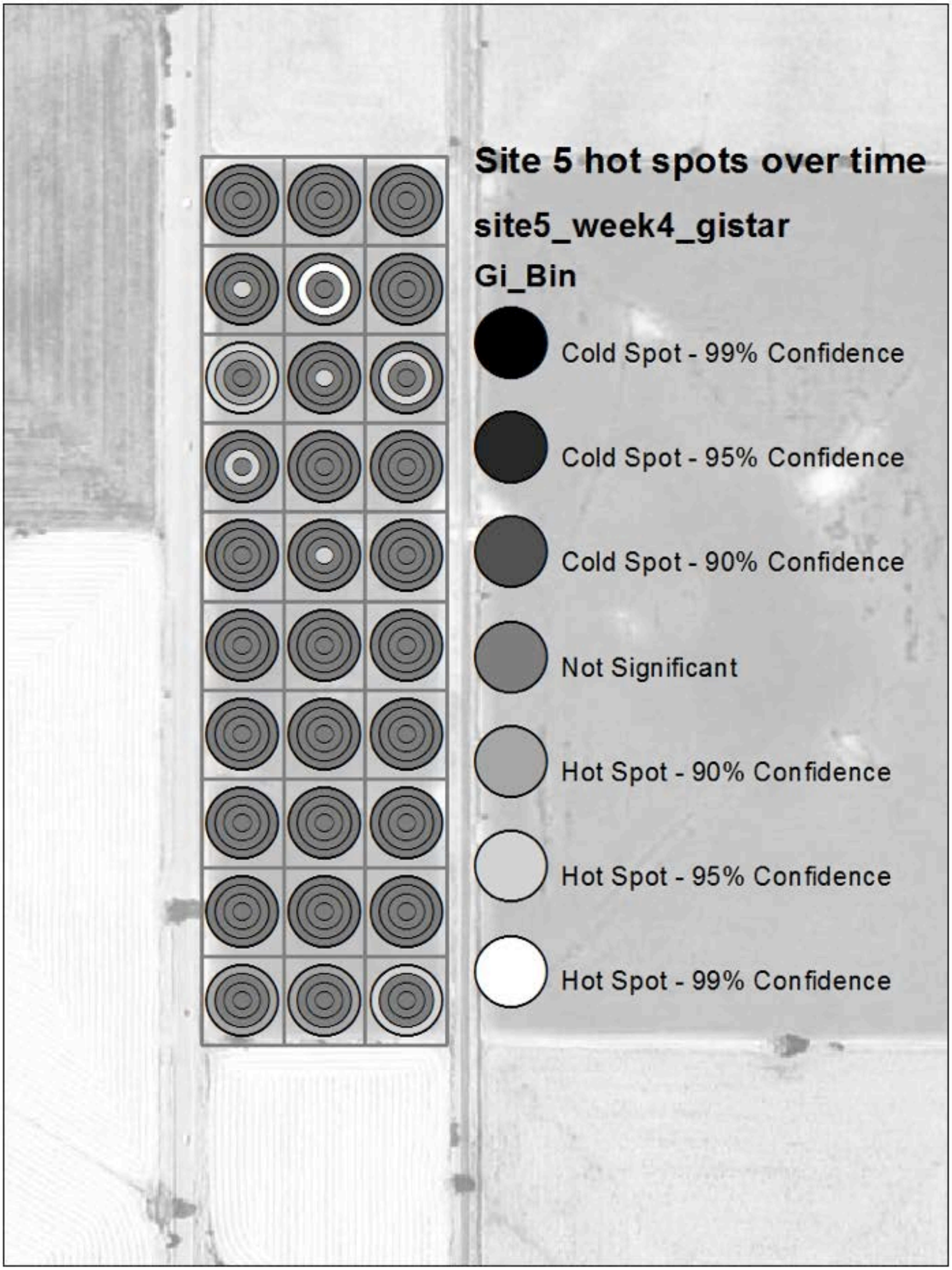

Figure 10. Significance of slug hot spots over time at site 5.

ENHANCING FERTILIZER EFFICIENCY IN WESTERN OREGON GRASS 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. However, the cost of this input has been steadily increasing over time, so enhancing fertilizer N efficiency is important.

The enzyme urease catalyzes the reaction of urea to ammonia, thereby leaving applied N susceptible to losses through volatilization. The loss of applied N through ammonia volatilization not only has an environmental cost, but can also be a significant economic cost. Nitrogen use efficiency is reduced by volatilization losses; thus, seed growers may not receive the maximum benefit from all of the N that they apply. Losses of 5–25% of the total N applied have recently been measured in western Oregon wheat and pasture systems (Anderson, et al., unpublished report). Results indicate that the greatest losses occur when there is dry weather for several days following top-dress N fertilizer application. Controlling volatilization may allow seed growers to obtain greater seed yields with equal or lower N rates, thereby reducing the cost of production.

A urease inhibitor [N-(n-butyl) thiophosphoric triamide] (NBPT), known by the trade name Agrotain Ultra®, has shown considerable efficacy in reducing N losses due to volatilization and in increasing 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 NBPT-containing urease inhibitor in perennial ryegrass in New Zealand (Rolston et al., unpublished report). However, it is not known whether NBPT urease inhibitors are effective under Oregon conditions. While seed yields may not increase substantially with the application of NBPT urease inhibitors, it is anticipated that the cost of N application will be reduced through greater N use efficiency, thus increasing profitability.

The potential for reduction in emissions of greenhouse gases such as nitrous oxide also exists with use of urease inhibitors. The application of urease inhibitors in irrigated pastures in New Zealand reduced nitrous oxide emissions by up to 12% (Dawar et al., 2011).

The potential of economic and environmental benefits for Oregon's grass seed industry makes the timely investigation of urease inhibitors for reduction of N losses a priority. The objective of this study was to determine the effect of NBPT-containing urease inhibitors on seed yield and seed yield components, biomass production, and N uptake in perennial ryegrass seed crops.

Materials and Methods

Trials were conducted in first-year perennial ryegrass seed fields at three on-farm sites in 2013–2014 in Marion, Yamhill, and Washington counties. The experimental design for the trials was a randomized

Table 1. Nitrogen application rates, source, timing, and urease inhibitor materials used in three first-year stands of perennial ryegrass grown for seed production.

Treatment	N application rate	Source	Timing	Urease inhibitor
	(lb/acre)			
1	120	40-0-0-6	March 11	—
2	160	40-0-0-6	March 11	—
3	120	40-0-0-6	March 11	Agrotain®
4	160	40-0-0-6	March 11	Agrotain
5	160 split	40-0-0-6 + UAN	March 11 + April 14	Agrotain (on 40-0-0-6 only)
6	120	40-0-0-6	March 11	N-Veil®[1]

[1] N-veil is a NBPT product similar to Agrotain Ultra. It was evaluated only at the Marion County trial site.

complete block with three replications at each site. Plot size was approximately 25 feet x 300–355 feet. Soil samples were taken prior to spring fertilizer application to measure pH and percent organic matter. Soil pH values ranged from 5.4 to 5.9, and organic matter ranged from 3.5 to 4.2%.

Fertilizer treatments included the following (Table 1):

- Two N rates applied as 40-0-0-6, representing the range of recommended rates for perennial ryegrass seed crops in Oregon (120–160 lb N/acre), with and without Agrotain Ultra
- A split application of 160 lb N/acre, with 50% applied as 40-0-0-6 with Agrotain Ultra and 50% applied as liquid UAN
- At the Marion County site only, an N application of 120 lb N/a, applied as 40-0-0-6 with N-Veil®, another NBPT-containing urease inhibitor

All dry fertilizer was applied as a single application on March 11, and the liquid UAN was applied on April 14. Agrotain Ultra and N-Veil were each applied at a rate of 3 qt/ton of N fertilizer.

Above-ground biomass samples were taken at peak anthesis, and dry weight of the standing crop was determined by drying and subsequent weighing of the harvested material. Total C and N in plant tissue samples were determined by using a LECO CNS analyzer. Seed was harvested with grower combines, and seed yield was determined with 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.

Data were analyzed by using analysis of variance, and means were separated by using Fisher's Protected LSD values ($P = 0.05$).

Results and Discussion

N fertilizer rates and NBPT-containing urease inhibitors influenced seed yields differently among the three sites. At the Marion and Washington county sites, the 160 lb N/acre rate produced higher seed yields than the 120 lb N/acre rate with both single and split applications, while no difference in seed yield was evident among N rates at the Yamhill County site (Table 2).

There was a significant seed yield response when NBPT was used at one of the three trial sites. In Washington County, seed yield was increased by 15.4 and 10.1% with Agrotain Ultra at 120 lb N/acre and 160 lb N/a, respectively. The split application totaling 160 lb N/acre with Agrotain Ultra produced a 6.3% seed yield increase over the 160 lb N/acre without Agrotain Ultra. No significant seed yield response to NBPT urease inhibitor treatments, in either a single or split application, were observed at the Marion or Yamhill county sites.

There were no significant differences in seed weight, above-ground biomass, or total tissue C and N among N rate or urease inhibitor treatments at any of the sites (Table 2). Nonetheless, tissue N content tended to be elevated where NBPT was used. The lack of differences in seed weight at the trial sites indicate that increased seed number per unit area, rather than changes in seed weight, were responsible for the seed yield increases observed as a result of increasing N rate or use of NBPT.

No rain was measured in the Willamette Valley region for three days following N fertilizer application; however, approximately 0.45 inch of rainfall was measured during the four- to seven-day period following fertilizer application. Previous studies conducted in the Willamette Valley and elsewhere indicate that the amount of ammonia loss to volatilization following top-dress N applications is drastically reduced when a rain event occurs within seven days following N fertilizer application.

The relatively dry conditions that occurred during the study period likely resulted in some N loss to ammonia volatilization, resulting in a positive seed yield response when Agrotain Ultra was used at the Washington County site. Since there was no seed yield response when a higher N rate was used at the Yamhill County site, it is evident that the lower N rate sufficiently supplied the crop. Therefore, it is not surprising that there was no response to the addition of NBPT.

This is the first report on effects of NBPT-containing urease inhibitors on grass seed production in western Oregon. The results to date suggest that these particular urease inhibitors appear to have potential for improving N fertilizer use efficiency in grass seed crops produced under western Oregon conditions. Additional studies will be conducted in 2015, and updates will be provided when data is available.

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Table 2. Harvest characteristics and tissue analysis of three first-year perennial ryegrass fields on Marion, Yamhill, and Washington county sites under different N rates with and without the use of a NBPT-containing urease inhibitor, 2014–2015.

Marion County site

Treatment	Yield[1]	Cleanout	Seed weight	Biomass	C	N
	(lb/a)	(%)	(mg/seed)	(lb/a)	(%)	(%)
120 lb N/a 40-0-0-6	1,762 a	20.7	1.693	11,131	43.5	1.95
160 lb N/a 40-0-0-6	1,956 c	21.3	1.649	11,268	43.4	2.42
120 lb N/a + Agrotain	1,797 ab	20.2	1.603	10,515	43.9	2.48
160 lb N/a + Agrotain	1,898 bc	22.6	1.629	10,805	43.9	2.63
Split (160 lb N/a +Agrotain)	1,880 bc	21.6	1.629	11,879	43.4	1.97
120 lb N/a + N-Veil	1,848 abc	22.4	1.614	—	—	—

Yamhill County site

Treatment	Yield	Cleanout[1]	Seed weight	Biomass	C	N
	(lb/a)	(%)	(mg/seed)	(lb/a)	(%)	(%)
120 lb N/a 40-0-0-6	1,767	4.5 a	1.607	9,385	42.6	1.80
160 lb N/a 40-0-0-6	1,779	4.1 a	1.512	8,809	44.1	1.64
120 lb N/a + Agrotain	1,772	4.6 a	1.557	9,892	43.0	2.52
160 lb N/a + Agrotain	1,763	4.7 a	1.542	11,077	43.2	1.87
Split (160 lb N/a +Agrotain)	1,715	5.7 b	1.490	9,964	43.1	1.77

Washington County site

Treatment	Yield[1]	Cleanout	Seed weight	Biomass	C	N
	(lb/a)	(%)	(mg/seed)	(lb/a)	(%)	(%)
120 lb N/a 40-0-0-6	1,591 a	3.90	1.677	14,323	42.9	1.38
160 lb N/a 40-0-0-6	1,744 b	3.70	1.721	14,361	43.1	1.91
120 lb N/a + Agrotain	1,836 bc	3.60	1.716	14,253	43.1	2.13
160 lb N/a + Agrotain	1,920 c	4.10	1.701	14,032	43.3	2.19
Split (160 lb N/a+Agrotain)	1,853 c	4.40	1.720	14,083	42.9	2.12

[1] Means followed by the same letter are not significantly different from each other at LSD (0.05).

OCCURRENCE AND TRENDS OF WEED SEED AND ERGOT CONTAMINANTS IN OREGON-GROWN *POA PRATENSIS* AND *POA TRIVIALIS* SEED LOTS

S.C. Alderman, S.G. Elias, and A.G. Hulting

Introduction

In 2013, we published a study in Seed Technology (vol. 35, pages 237–250) on assessing the frequency of occurrence of various weed species and ergot (sclerotia of *Claviceps purpurea*) in Kentucky bluegrass (*Poa pratensis*) and rough bluegrass (*Poa trivialis*) grown in Oregon during 1986–2012. Following are highlights of the findings of that study. A reprint of the original paper can be viewed at the OSU Seed Crops website (http://cropandsoil.oregonstate.edu/group/seed-crops).

Materials and Methods

Data for the frequency of occurrence of several weed species in Kentucky bluegrass (KBG) during 1986–1995 were obtained and compiled from a summary of weed seed occurrence in certified seed sample purity test records at the OSU Seed Lab (Dade, 1996). Data for 2002–2012 were obtained from the OSU Seed Lab purity testing records for certified seed lots of KBG and rough bluegrass (RBG).

Results

Kentucky bluegrass

Within the 21 years of the study, 155 different weed contaminants were identified in KBG, including 113 identified to species, 39 to genus only, and 3 to family only. The most common weed contaminants, occurring in 20 or more years of the study period, were foxtail (*Alopecurus* spp.), windgrass (*Apera spica-venti* (L.) P. Beauv.), common lamb's-quarters (*Chenopodium album* L.), wild carrot (*Daucus carota* L. subsp. *carota*), downy brome (*Bromus tectorum* L.), henbit (*Lamium amplexicaule* L.), witchgrass (*Panicum capillare* L.), annual bluegrass (*Poa annua* L.), and rattail fescue (*Vulpia myuros* (L.) C. C. Gmel.) (Table 1).

A trend of decreasing percentage of contaminated lots was identified by regression analysis for the following species: windgrass (r^2=0.56, F <0.001), downy brome (*Bromus tectorum* L.) (r^2=0.73, F <0.001), shepherd's-purse (*Capsella bursa-pastoris* (L.) Medik.) (r^2=0.38, F=0.003), henbit (r^2=0.32, F=0.007), poplar hybrids (*Populus* spp.) (r^2=0.64, F=0.003), Lemmon's alkaligrass (*Puccinellia lemmonii* (Vasey) Scribn.) (r^2=0.69, F <0.001), curly dock (*Rumex crispus* L.) (r^2=0.44, F <0.001), common groundsel (*Senecio vulgaris* L.) (r^2=0.35, F=0.005), and rattail fescue (r^2=0.23, F=0.03). No contaminants were found to have a trend of increasing percentage of contaminated lots over time.

Rough bluegrass

Within the 11 years included in the RBG survey, 40 weed contaminants were detected, including 26 identified to species and 14 to genus. The most common contaminants, occurring annually, were windgrass, KBG, and rattail fescue.

A significant trend of declining percentage of lots containing KBG was found (R^2=0.72, F=0.001). This is the only contaminant in RBG with a significant increasing or decreasing trend with respect to time.

Ergot

Between 2002 and 2012, the percentage of lots with ergot ranged from 22 to 61% for KBG and 0 to 10% for RBG (Figure 1). Among the three production areas in Oregon (Columbia Basin, central Oregon, and Grand Ronde Valley), the percentage of lots with ergot varied among years and areas (Figure 2). As few as 18% of lots from central Oregon had ergot in 2002, and as many as 82% of lots from the Grand Ronde Valley had ergot in 2006. In most years, a higher percentage of samples with ergot were found in the Grande Ronde Valley than in the Columbia Basin or central Oregon.

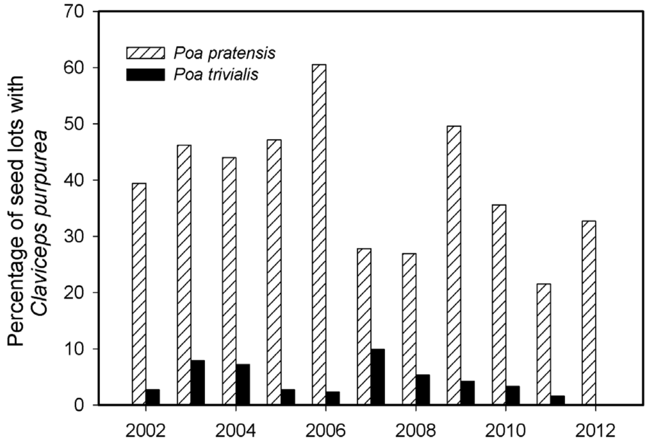

Figure 1. Percentage of certified seed lots of *Poa pratensis* and *Poa trivialis* contaminated with *Claviceps purpurea* (sclerotia) during 2002–2012.

Table 1. Frequency of occurrence of the most common weed species found in *Poa pratensis* L. (Kentucky bluegrass) seed lots grown in Oregon and the range of percentage of seed lots contaminated each year. Data summarized for years 1986–1995 and 2002–2012 for taxa occurring in 10 or more years out of 21.

Weed species	Common name	Frequency[1]	Range[2]
			(%)
Alopecurus spp.	Foxtail	20	0–7.1
Amaranthus spp.	Pigweed	11	0–4.0
Amaranthus retroflexus L.	Redroot pigweed	10	0–.2
Amsinckia spp.	Fiddleneck	11	0–0.7
Anthemis cotula L.	Dogfennel	16	0–3.9
Apera spica-venti (L.) P. Beauv.	Windgrass	21	0.6–5.1
Brassica spp.	—	10	0–0.7
Bromus tectorum L.	Downy brome	21	0.3–8.2
Capsella bursa-pastoris (L.) Medik.	Shepherd's-purse	12	0–3.2
Chenopodium album L.	Common lamb's-quarters	20	0–4.4
Chorispora tenella (Pall.) DC.	Blue mustard	16	0–2.6
Daucus carota L. subsp. *carota*	Wild carrot	21	0.9–3.1
Echinochloa crus-galli (L.) P. Beauv.	Barnyardgrass	11	0–3.8
Elymus repens (L.) Gould	Quackgrass	10	0–0.4
Festuca spp.	Fescue	11	1.0–5.5
Festuca arundinacea Schreb.	Tall fescue	11	0–1.5
Galium spp.	Bedstraw	14	0–0.9
Lamium amplexicaule L.	Henbit	20	0–8.6
Lolium spp.	Ryegrass	11	0–7.0
Malva neglecta Wallr.	Common mallow	19	0–2.8
Matricaria discoidea DC.	Pineappleweed	14	0–2.0
Panicum capillare L.	Witchgrass	21	1.3–6.1
Poa annua L.	Annual bluegrass	20	0–11.5
Poa bulbosa L.	Bulbous bluegrass	16	0–2.0
Poa compressa L.	Canada bluegrass	10	0–2.4
Poa secunda J. Presl.	Big bluegrass	10	0–1.4
Poa trivialis L.	Rough bluegrass	11	0–11.1
Poaceae	Poaceae	13	0–1.0
Polygonum aviculare L.	Prostrate knotweed	15	0–1.5
Puccinellia spp.	Alkaligrass	17	0–7.6
Puccinellia lemmonii (Vasey) Scribn.	Lemmon's alkaligrass	14	0–10.1
Rumex crispus L.	Curly dock	19	02–3.1
Salsola spp.	Russian thistle	13	0–1.5
Senecio vulgaris L.	Common groundsel	15	0–3.6
Setaria italica (L.) P. Beauv. subsp. *viridis* (L.) Thell.	Green foxtail	15	0–1.3
Sisymbrium altissimum L.	Tumble mustard	12	0–1.4
Solanum villosum Mill.	Hairy nightshade	16	0–1.4
Stellaria media (L.) Vill.	Common chickweed	10	0–4.7
Taraxacum officinale F. H. Wigg. aggr.	Dandelion	15	0 -0.9
Thlaspi arvense L.	Field pennycress	12	0–4.0
Triticum spp.	Wheat	11	0–4.3
Vulpia myuros (L.) C. C. Gmel.	Rattail fescue	21	2.1–20.9

[1] F = Frequency of occurrence (years out of 21)

[2] Range = Percentage of seed lots contaminated within a year

Discussion

Results from this study indicated a large diversity of weed seed contaminants in KBG and RBG seed lots. Most contaminant species occurred at a low level and in few years. This indicates that seed growers are, for the most part, utilizing effective weed management practices for seed production and that seed cleaners are effectively removing most of the contaminants prior to sampling and testing.

Weeds listed as problematic in KBG and RBG seed production fields in central Oregon included downy brome, rattail fescue, common groundsel, prickly lettuce (milk thistle) (*Lactuca serriola* L.), common mallow (*Malva neglecta* Wallr.), stinking chamomile (*A. cotula* L.), pinweed (*Erodium cicutatrium* (L.) L. Hér.), curly dock, and quackgrass (*Elymus repens* (L.) Gould) (Butler et al., 2002a, 2002b). Not surprisingly, these species were some of the common contaminants in seed samples.

Weed contaminants such as windgrass, wild carrot, downy brome, witchgrass (*Panicum capillare* L.), and rattail fescue, which occurred annually in KBG, indicate difficulty in control within the field as well as in separating out the weed seeds during seed cleaning operations. Limited effective chemical control options for species such as wild carrot and rattail fescue result in production of large quantities of seed, which are harvested with the KBG crop. Physical properties (such as size, shape, and/or density) of these species' seed are also similar to those of KBG seed, making it difficult to separate the seed from KBG seed during seed cleaning. In addition, these weed species are common in the grass seed production areas of Oregon east of the Cascade Range (Colquhoun et al., 2001).

In RBG, the most significant weed contaminants were KBG and rattail fescue. During 2002 to 2005, 20 to 27% of samples contained KBG, but the percentage dropped to 8 to 12% during 2007 to 2012, indicating improved management of KBG.

The sources or mechanisms of weed seed contamination in seed lot samples were not determined and are beyond the scope of this study. We hypothesize that the sources of most contaminants were weed populations persisting in individual fields, but we cannot exclude the possibility of contaminant sources outside the production fields, including wind-borne seed or inadvertent introduction of contaminants during transport, storage, or conditioning of seed lots. Additional studies would be needed to determine the source of specific weed contaminants.

Ergot occurred at a much higher frequency in KBG than in RBG. It is likely that RBG is not as susceptible as KBG to ergot. In most years, *C. purpurea* was

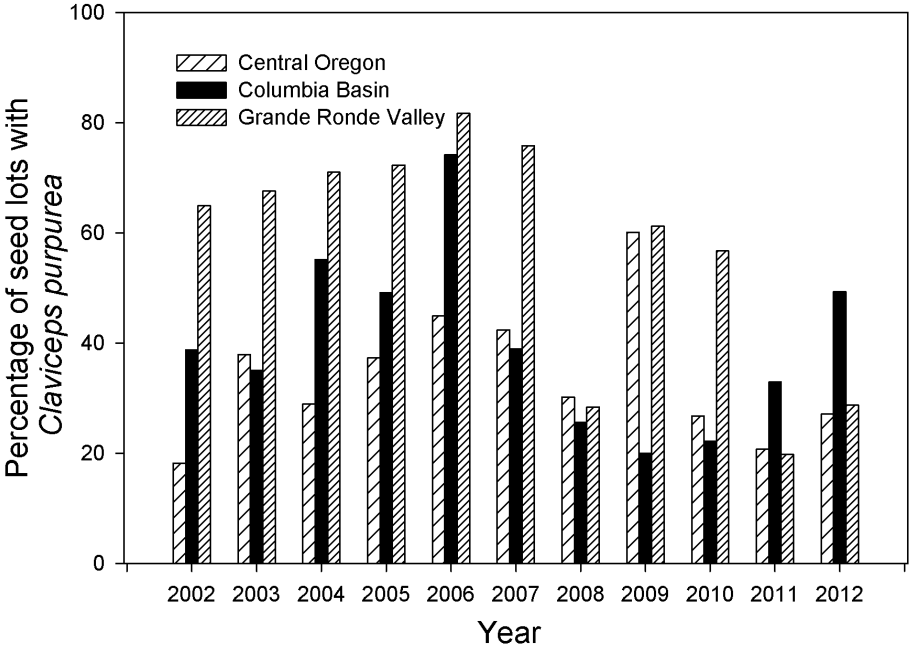

Figure 2. Percentage of certified seed lots of *Poa pratensis* grown in central Oregon, the Columbia Basin, and the Grand Ronde Valley contaminated with *Claviceps purpurea* (sclerotia) during 2002–2012.

most prevalent in the Grand Ronde Valley; however, it is not clear whether this is due to environmental conditions or to the bluegrass cultivars grown in that region. Most cultivars are unique to each production area. In addition, the cultivars grown within each area vary from year to year as new cultivars are introduced and replace existing cultivars. Although most cultivars are susceptible to ergot, some have a greater level of resistance than others. Some of the yearly variation in ergot can be accounted for by environmental conditions, which can affect the timing of pathogen spore release. Current management of ergot relies to a large extent on fungicide use (Pscheidt and Ocamb, 2013), which provides only partial control.

This study suggests that robust weed management practices, coupled with awareness on the part of seed cleaning facilities, results in the production of *Poa* spp. crops with minimal weed seed contaminants. Ergot likely will continue to be problematic in bluegrass spp. seed production and as a seed contaminant in cleaned seed.

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DIVERSITY AND ERGOT INCIDENCE AMONG INSECT POPULATIONS IN KENTUCKY BLUEGRASS AND PERENNIAL RYEGRASS SEED FIELDS IN THE COLUMBIA BASIN

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Introduction

Ergot, caused by the fungus *Claviceps purpurea*, is an important floral disease of perennial ryegrass (PRG, *Lolium perenne*) and Kentucky bluegrass (KBG, *Poa pratensis*) seed crops in the Pacific Northwest (Alderman, 1991). The fungus colonizes unfertilized ovaries, resulting in production of elongated black sclerotia that replace the seed. During infection, asexual spores (conidia) mix with plant sap and exude from florets in what is referred to as the "honeydew" stage of infection.

Ergot honeydew can serve as a food resource for insects such as flies, beetles, wasps, and piercing and sucking insects. When these insects land or feed on the flowers, they may facilitate the insect-mediated secondary dispersal of *C. purpurea*. Based on microscopic examinations, Butler et al. (2001) reported that 75% of flies and 100% of moths collected from KBG fields in central Oregon carried ergot conidia. However, there is limited information on the association of ergot disease and insect abundance in KBG and PRG fields in northeastern Oregon. Furthermore, ergot conidia can be morphologically similar to other fungal spores. Therefore, molecular techniques could aid in identifying and distinguishing *C. purpurea* conidia from other fungal spores in or on insects.

The need to understand the role of insects in ergot dispersal was prompted by growers' concerns and inadequate information about insect vectors. A previous study by Rondon indicated that the majority of insect species in PRG and KBG fields were beneficial ground beetles, rove beetles, and other predatory insects, based on data collected using pitfall traps, sweep nets, and sod samples (Rondon, 2009). The information obtained from the 2008 study was used by Rondon's lab to refine monitoring, trapping, and identification methods in 2009. This effort provided a foundation for understanding the insect population structure in grass seed crops and is the basis for our investigation in 2014 to obtain a better understanding of the potential role that insects may play in ergot dispersal.

The objectives of our study were to: (1) estimate insect abundance in PRG and KBG research trials planted at the Hermiston Agricultural Research and Extension Center (HAREC) in 2009 and in both commercial fields and research trials in 2014; (2) determine whether ergot conidia are associated with insects and whether a correlation exists between insect abundance and ergot incidence; and (3) develop a high-fidelity polymerase chain reaction (HF-PCR) protocol to confirm the presence of *C. purpurea* spores on insects.

Materials and Methods

Survey of insect abundance—2009

Arthropod diversity was monitored in one KBG trial (cv. 'PST 102-68') and one PRG trial (cv. 'Metropolitan') located at HAREC during April and June 2009. Each field was 0.15 acre in size and split into four equal sections to represent replicated plots. Samples from 6 pitfall traps, 10 sweeps with a standard 15-inch sweep net, and 6 sod samples (1 foot in diameter by 4 inches deep) were taken weekly in each section of each field. Insects were collected, sorted, and counted from all samples. Species identifications were made by using identification keys and by comparing sampled insects with voucher specimens held at the Irrigated Agricultural Entomology Program Laboratory at HAREC and at the Oregon State Arthropod Collection at Oregon State University in Corvallis, OR.

Survey of insect abundance and ergot incidence—2014

Insect abundance was monitored from May to June 2014 in four commercial KBG fields (cvs. 'Midnight', 'Arrowhead', 'Brooklawn', and 'Bonaire') and three commercial PRG fields (cvs. 'Pavilion', 'Presidio', and 'TopHat2') located in the Columbia Basin of Washington and Oregon, respectively, and in experimental field plots (0.1 acre) situated in a PRG variety trial located at HAREC. Each commercial field was approximately 125 acres and divided into four equal quadrants representing four replicates. Sampling was conducted weekly in each quadrant using universal black light traps, delta traps, yellow sticky cards, and sweep nets. Ergot incidence in the commercial fields was calculated at crop harvest, based on the number of infected seed heads containing sclerotia out of 100 seed heads collected from each quadrant of field sampled. Correlations between ergot incidence and insect abundance were calculated.

Detection of fungal spores

Insects collected during 2014 were sorted, counted, and stored at −20ºC until preliminary microscopic examination for the presence of ergot spores. Subsamples of 30 insect specimens from each field were used in microscopic studies. Insects were dissected, and fluid from the insect digestive tract was examined under a compound microscope for the presence of ergot conidia. Since this technique is time consuming and could lead to inaccurate identification of fungal spores, a HF-PCR protocol was developed to detect the presence of ergot spores. Whole insect samples were subjected to genomic DNA extraction using a modified CTAB method as described by Zhang et al. (1998). Genomic DNA was used to confirm the presence of ergot spores in insect extracts using the HF-PCR protocol. Ergot specific forward (5'-GCTCTAGACTGCTTTCTGGCAGACC-3') and reverse (5'-CGTCTAGAGGTACCCATACCGGCA-3') β-tubulin primers (Tooley et al., 2001) were used to amplify the target gene. PCR products were visualized on a 1% agarose gel. The subset of positive PCR products were cloned, sequenced, and compared with sequences in GenBank to confirm the identification of fungal isolates belonging to *C. purpurea*.

Table 1. Species composition and average number of insects collected in perennial ryegrass (PRG) and Kentucky bluegrass (KBG) fields between April and June 2009.

Order Common name Genus/species	PRG	KBG
	Average number	
Coleoptera		
Ground beetles		
Bembidion spp.	2.3	29
Amara quenseli	10	2.5
Amara conflata	0.7	1
Rove beetles		
Philonthus fuscipennis	179	0
Hymenoptera		
Parasitoid wasps		
Ichneumonid spp.	1	0
Bracon spp.	22	0
Diptera		
Lesser house fly		
Fannia canicularis	71	0

Results and Discussion

Survey of insect abundance—2009

The 2009 study provided a better understanding of the biodiversity of insects in grass seed crops. Ground beetles (*Bembidion* spp. and *Amara* spp.) were found in both KBG and PRG (Table 1), while rove beetles (*Philonthus fuscipennis*) and parasitoid wasps (ichneumonids and braconids) occurred only in the PRG field. Similarly, the lesser house fly (*Fannia canicularis*) occurred only in the PRG field. The difference in insect populations between these two crops could be due to differences in crop management practices, habitat suitability, and/or food availability.

Survey of insect abundance and ergot incidence—2014

Dipteran insects were the largest proportion of the insect community in both KBG and PRG seed fields in 2014, comprising 60% of the insects collected (Figure 1). An abundance of dipteran insects was also observed in PRG fields during the 2009 insect survey (Table 1). A significant and positive correlation existed between insect abundance and ergot incidence in PRG fields surveyed in 2014 (Figure 2). However, correlations could not be established in KBG fields because ergot incidence was negligible (data not shown). The reason for the low occurrence of ergot in KBG fields is not known at this time, but spore trap data indicate that very few ascospores were present in these fields (unpublished data).

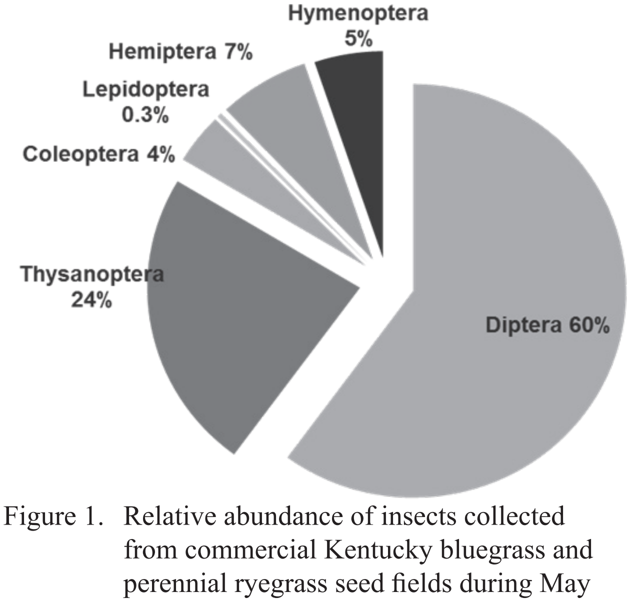

Figure 1. Relative abundance of insects collected from commercial Kentucky bluegrass and perennial ryegrass seed fields during May and June 2014. (No differences in the population structure between the two crops were found; therefore, data were pooled.)

Detection of fungal spores

Initial microscopic examination revealed the presence of ergot conidia in at least 50% of the insect gut subsamples; however, taking into account the similarity between ergot conidia morphology and that of other fungi, we relied on molecular detection for further confirmation. The HF-PCR protocol amplified a 527 base pair product from *C. purpurea*. Using HF-PCR, up to 35% of dipterans (muscid flies) and 27% of moths (noctuid moths) tested positive for ergot, indicating accuracy and sensitivity of the method used. These results were consistent with the findings of Butler et al. (2001), in which moths and flies were most commonly contaminated with ergot spores. Understanding the importance and the mechanism of insect-mediated ergot dispersal may aid in developing new strategies to manage this disease.

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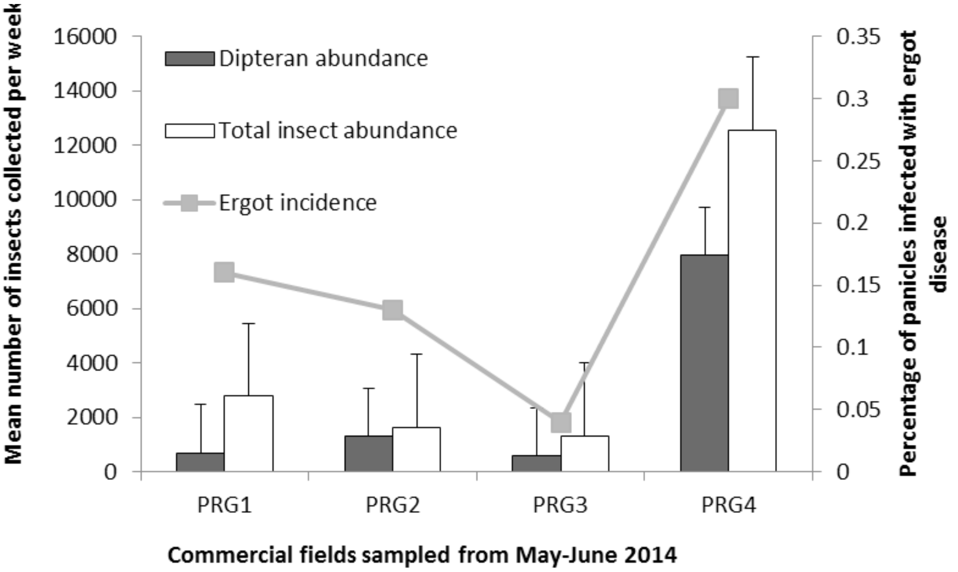

Figure 2. A significant positive correlation ($r = 0.9$, $P < 0.05$) existed between total insect abundance and ergot incidence, as well as between dipteran abundance and ergot incidence ($r = 0.9$, $P < 0.05$) in four commercial perennial ryegrass (PRG) fields during May and June 2014.

IRRIGATION AND TRINEXAPAC-ETHYL EFFECTS ON SEED YIELD IN FIRST- AND SECOND-YEAR RED CLOVER STANDS

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

Introduction

Red clover is an important forage legume, with annual worldwide seed production second only to that of alfalfa (*Medicago sativa* L.) (Boller et al., 2010). In Oregon, red clover is harvested for seed on approximately 15,000 to 21,000 acres annually, making it the state's most important forage legume seed crop. In this region, red clover is commonly grown for two years in mostly dryland environments; however, irrigation is becoming increasingly available. There is continued interest among red clover seed producers in evaluating agronomic management practices that increase seed yield.

Oliva et al. (1994) showed that irrigation strategically timed to coincide with flowering increased seed yield over that of non-irrigated red clover. It is unclear how the combination of irrigation and use of a plant growth regulator (PGR) might affect seed yield and yield components in red clover.

Plant growth regulator use in seed crop production aims to increase the number of seeds produced per unit. Harvest index may be increased by producing a higher seed yield and by reducing the amount of vegetative biomass produced in relation to seed produced. Previous work with PGRs in legume seed production has centered on gibberellin biosynthesis inhibitors such as paclobutrazol and uniconazole (Silberstein et al., 1996). More recently, trinexapac-ethyl (Palisade EC®) was evaluated on red clover seed crops in Oregon, New Zealand, and Norway. In Norway, red clover seed yield was increased when trinexapac-ethyl (TE) was applied at BBCH 32[1] (Øverland and Aamlid, 2007). Anderson et al. (2015) reported a 9 to 16% seed yield increase across New Zealand and Oregon environments when 1.7 and 3.4 pt/acre was applied at the same timing.

The objective of this study is to quantify the impact of irrigation and its potential interaction with PGR use in first- and second-year stands of red clover seed crops grown under western Oregon conditions.

Methods

Two plantings (2011 and 2012) of red clover seed crops were established in the fall at Hyslop Research Farm near Corvallis, OR, and each was followed over a two-year period to examine the effects of irrigation and TE PGR. The experimental design was a randomized complete block with a split-plot arrangement of treatments and four replications. Plant growth regulator treatment subplots (11 feet x 50 feet) were randomly located within irrigated and non-irrigated main plots. The TE PGR treatments were made on the subplots at two application timings and at three rates of TE. Control plots were not treated (Table 1).

The red clover seed crop was flailed in mid-May (prior to bud emergence), and residue was removed from the field. Once regrowth occurred, approximately 4 inches of irrigation water was applied to main plots over a two-day period using a custom-designed Pierce AcreMaster linear system equipped with minimum-drift Nelson sprinklers. This single irrigation was strategically timed to coincide with first flowering (BBCH 60). Trinexapac-ethyl was applied at rates listed in Table 1 to subplots at stem elongation (BBCH 32) and bud emergence (BBCH 50). Seed was harvested with a small-plot swather (modified JD 2280) and threshed with a Hege 180 small-plot combine. Harvested seed was processed through a M2-B Clipper cleaner, and clean seed yield was determined.

Table 1. Trinexapac-ethyl (TE) application timings and rates used in two first- and second-year stands of red clover grown for seed production.

Application timing (BBCH scale)	TE application rate
	(pt/a)
Untreated control	0
BBCH 32 (stem elongation)	2
	3
	4
BBCH 50 (bud emergence)	2
	3
	4

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[1] BBCH refers to the Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie system of crop development staging.

Plots were sampled at peak bloom (BBCH 65) to determine the number of inflorescences, florets per inflorescence, primary stems, and above-ground biomass. Harvest index was determined for each plot based on harvested seed yield and above-ground biomass. Seed weight was measured by counting two 1,000-seed samples from harvested, cleaned seed material and determining the weight. Seed number was calculated based on seed yield and 1,000-seed weight values obtained from each plot.

Analysis of variance was conducted for each year and stand age. Irrigation and PGR means were separated by Fisher's protected LSD values at the 5% level of significance.

Results and Discussion

There was no interaction of TE and irrigation for seed yield in first- or second-year stands across all three years of the study. Nevertheless, irrigation consistently increased seed yield across both stand ages and years. Red clover seed yield was increased by application of TE at BBCH 32 only in second-year stands.

A single irrigation application consistently increased seed yield of red clover by an average of 13.1% across first- and second-year stands from 2012 to 2014 (Table 2). Irrigation consistently increased seed weight across all years and stand ages by an average of 5% (data not shown). Seed number was increased by an average of nearly 10% with irrigation in first- and second-year stands in 2013, but no significant differences in seed number attributable to irrigation were noted in the first-year stand in 2012 or the second-year stand in 2014. Together, the increases in seed weight and seed number contributed to the seed yield increases as a result of the single irrigation. Irrigation did not have an effect on above-ground biomass, stem number, inflorescences or harvest index, but had varied effects on florets.

Seed yield was not affected by application of TE at any rate or timing in first-year stands, regardless of year (Table 3). However, TE applied at BBCH 32 produced seed yield increases of 15 to 19% in second-year red clover seed stands. Seed number was increased by TE application at BBCH 32 but not at BBCH 50 (data not shown). This increase in seed number likely resulted from increased inflorescences with TE application. Seed weight was reduced by TE regardless of application timing or rate, but the decline in seed weight was more pronounced when TE was applied at BBCH 50.

Table 2. Effect of irrigation on red clover seed yield in first- and second-year stands. A single irrigation (4 inches) was applied at BBCH 55.[1]

		Stand 1		Stand 2		
Irrigation	Water applied	1st year	2nd year	1st year	2nd year	Yield increase
	(in)	(lb/a)				(%)
None	0	787 a	678 a	505 a	667 a	0
Single	4	867 b	746 b	624 b	723 b	13.1

[1]Means within columns followed by the same letter are not significantly different by Fisher's LSD values (*P* = 0.05).

Table 3. TE rate and stand age effects on red clover seed yield with TE applied at BBCH 32.[1]

	Stand 1		Stand 2		Yield increase	
TE rate	1st year	2nd year	1st year	2nd year	1st year	2nd year
(pt/a)	(lb/a)				(%)	
0	818 a	698 a	621 a	639 a	0.0	0.0
2	860 a	818 b	618 a	717 b	2.7	14.8
3	852 a	826 b	630 a	741 b	3.0	17.2
4	844 a	833 b	614 a	754 b	-1.4	18.7

[1]Means within columns followed by the same letter are not significantly different by Fisher's LSD values (*P* = 0.05).

Seed yield was likely increased by TE applied at BBCH 32 in second-year stands because the losses in seed weight were more than offset by increased seed numbers. The lack of seed yield response to TE in first-year stands might have been the result of the TE-induced increases in seed number not being able to offset the reductions in seed weight. When TE was applied early (BBCH 32), the harvest index generally increased with increasing application rate. When TE was applied at BBCH 50, the harvest index decreased as the TE rate was increased. Application of TE did not affect above-ground biomass or stem number.

Conclusion

Irrigation and TE can independently increase seed yield in red clover seed crops, but there was no interaction between the two. A first- or second-year stand likely will benefit from a single irrigation; however, data from this study indicate that, under Oregon conditions, TE likely will increase seed yield only when applied at BBCH 32 in second-year stands.

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POTENTIAL FOR MANAGEMENT OF THE CLOVER ROOT BORER PEST IN RED CLOVER SEED PRODUCTION FIELDS USING INSECT PATHOGENIC FUNGI

A.S. Lestari and S. Rao

Introduction

The clover root borer (CRB, also known as clover crown borer) is a major pest of red clover seed production in the Willamette Valley. It was inadvertently introduced into the U.S. more than 100 years ago (Rockwood, 1926). The larvae and adults feed internally within roots. As a result, nutrient and water transport within the plant are disrupted, and infested plants turn brown, wilt, and die (Rockwood, 1926). Koehler et al. (1961) reported that about 40% of first-harvest-year plants are infested by the end of the first season, and by the second harvest year almost all plants are infested. Hence, a third year of seed production is generally not economical. Thus, the impact of the clover root borer is considerable, and effective management is critical for economical red clover production.

CRB is a challenge to control due to its predominantly subterranean life cycle. Adults are present above ground briefly in spring when they emerge, mate, and then migrate to new plants in the same or in different fields (Rockwood, 1926). Subsequent development occurs below ground. Females lay eggs in niches in clover roots, and the emerging larvae burrow within roots, feed on root tissue, develop slowly over the summer, and pupate inside the clover root. Adults emerge by late summer but remain below ground. The pest has one generation per year.

No pesticide is effective in suppressing CRB larvae and adults within roots. In a field trial conducted in 2011, four insecticides labeled for red clover seed crops were evaluated, but none caused significant mortality compared with the controls (Rao et al., 2012). Foliar applications of organochlorine insecticides such as aldrin, dieldrin, heptachlor, and lindane, which had long residual action, were effective against adults present above ground in spring (Gyrisco and Marshall, 1950). However, the persistence of these compounds in soil and groundwater, along with their low water solubility, which resulted in their accumulation in fatty tissue of non-target organisms, including humans and wildlife, led to a ban on their use in the 1970s. Currently no pest suppression strategy exists for CRB.

Insect pathogenic fungi, known as entomopathogenic fungi, have potential for management of subterranean pests (Brownbridge et al., 2006; Pilz et al., 2007). Rockwood (1926) reported one fungus, *Beauveria globulifera*, in association with CRB, but no further information is available on associations of this or other entomopathogenic fungi with CRB. The objectives of this study were to: (1) determine the presence of naturally occurring entomopathogenic fungi in red clover seed fields, and (2) assess virulence of naturally occurring and commercial entomopathogenic fungi against CRB.

Material and Methods

For all studies, CRB adults were collected from red clover seed production fields in the Willamette Valley. Roots of infested plants were transported to a laboratory, where they were cut longitudinally and placed in Berlese funnels overnight for collection of adults.

Objective 1. Determine the presence of naturally occurring entomopathogenic fungi in red clover seed fields

In 2013, during a preliminary survey, five field-collected CRB adults were observed to be infected with a fungus. The fungus was transferred to fungal media, Potato Dextrose Agar (PDA) for isolation and growth for subsequent identification. For further detection of entomopathogenic fungi, the soil around roots from individual plants was collected, placed in separate petri dishes and baited with five waxworms (*Galleria mellonella*). Waxworms were used as surrogate hosts instead of CRB due to their higher sensivity and larger suface area in baiting entomopathogenic fungi from soil. After two weeks, infected waxworms were removed, rinsed with water, and incubated in separate dishes on wet filter paper. Fungi from the infected waxworms were transferred to PDA media for fungal development and identification as described above. Confirmation of the fungal identities using molecular techniques is in progress.

In 2014, four red clover seed production fields were surveyed for further detection of naturally occurring entomopathogenic fungi. At three randomly selected locations in each field, a 4 meter x 4 meter grid was placed on the ground. Twenty-five plants from each grid were randomly dug up, bagged, and transported

Table 1. Entomopathogenic fungi evaluated for pathogenicity against CRB.

Species	Host or source	Type
Isaria fumosorosea (FE 9901)	White fly, Natural Industries	Commercial
Metarhizium anisopliae (F52)	Taenure granular bioinsecticide	Commercial
Beauveria bassiana A	Infected CRB	Field isolated
Beauveria bassiana B	Soil, Galleria baiting, red clover field	Field isolated
Isaria fumosorosea A	Soil, Galleria baiting, red clover field	Field isolated
Isaria fumosorosea B	Soil, Galleria baiting, red clover field	Field isolated
Control	Tween 80 (0.03% solution)	—

to the lab. The soil baiting method previously desribed was used for detection of entomopathogenic fungi. Soil was collected from around the roots of 10 randomly selected red clover plants at each location. Each of the 10 soil samples was placed in a petri dish and exposed to 5 waxworms. After two weeks, waxworms that died were removed and processed as previously described for fungal development. Identification of the fungi is in progress.

Objective 2. Assess the virulence of entomopathogenic fungi against CRB

Fungi evaluated in this study included the naturally occurring entomopathogenic fungi collected from red clover seed production fields (Objective 1) and two commercially available products, *Metarhizium anisopliae* and *Isaria fumosorosea* (Table 1). Field-collected adult beetles, 10 per petri dish, were dipped in fungal spore solutions (10^8 spores/ml) for five to seven seconds and then maintained in an incubator (22°C ± 1°C; 70–75% relative humidity). Spore solutions were dissolved in sterile water with the addition of Tween 80 (0.03%), which served as a surfactant for lowering the water tension for thorough mixing of the spores. CRB adults dipped in Tween 80 (0.03% solution) were used as controls. Parts of red clover roots were added to each dish for beetles to feed on. The experiment was set up as a randomized block design with six replications. Daily observations were made for two weeks on numbers of CRB adults that became infected with entomopathogenic fungi.

Results and Discussion

Naturally occurring entomopathogenic fungi in red clover fields

A single fungus was isolated from five field-collected CRB adults in 2013. Based on morphological characters, the fungus was identified as *Beauveria bassiana*. Of the 20 waxworms used as soil baits in 2013, 8 (40%) died due to fungal infection. Based on the morphology of the spores, spore-producing structures, and fungal colony characteristics, the fungi isolated from these waxworms were identified as belonging to two strains of entomopathogenic fungi, *Beauveria bassiana* (62.5%) and *Isaria fumosorosea* (37.5 %) (Table 2). In all, 13 entomopathogenic fungi were isolated from the red clover field surveyed in 2013 (Table 2). In 2014, out of the 600 waxworms used as baits, 147 (24.5%) died as a result of entomopathogenic fungal infection. The infection rate ranged from 15.3 to 28.7% across the four fields. Isolation and identification of the fungi are in progress.

Table 2. Naturally occurring entomopathogenic fungi isolated from the red clover field surveyed in 2013.

Species	Source	# isolates
Beauveria bassiana A	Soil	4
Beauveria bassiana B	5 dead CRB and soil	6
Isaria fumosorosea A	Soil	2
Isaria fumosorosea B	Soil	1

Virulence of entomopathogenic fungi against CRB

Fungal spores were observed on the mouth, thorax, and anal regions of CRB adults exposed to entomopathogenic fungi (Figure 1). All six fungal treatments caused significantly greater CRB mortality compared with the controls (Figure 2). Within seven days of exposure to fungal treatments, more than 50% mortality of CRB adults was observed, and by two weeks mortality increased to 70% or more (Figure 2).

Overall, the study documented the presence of two naturally occurring entomopathogenic fungi, *B. bassiana* and *I. fumosorosea*, in red clover seed production fields in western Oregon. Additionally, the study documented that field-isolated entomopathogenic fungi had similar levels of virulence compared to commercial products. Thus, *Beauveria bassiana*, *Isaria fumosorosea,* and *Metarhizium anisopliae* have potential as biological control agents for CRB. Further research is needed to determine the extent to which the naturally occurring entomopathogenic fungi are present in Willamette Valley red clover seed production fields, and to evaluate the efficacy of strains of the fungi used in the current study in suppressing CRB populations in the field.

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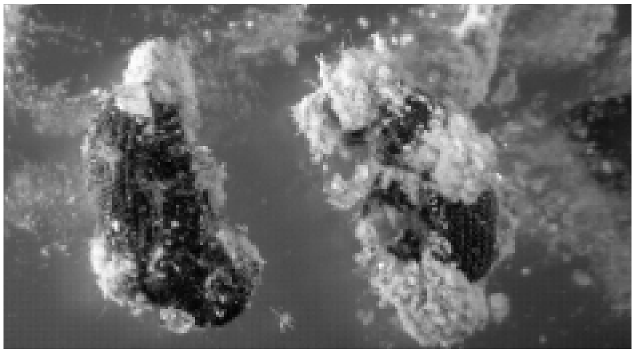

Figure 1. Fungal spores emerging from various body parts of CRB adults after exposure to an isolate of entomopathogenic fungus.

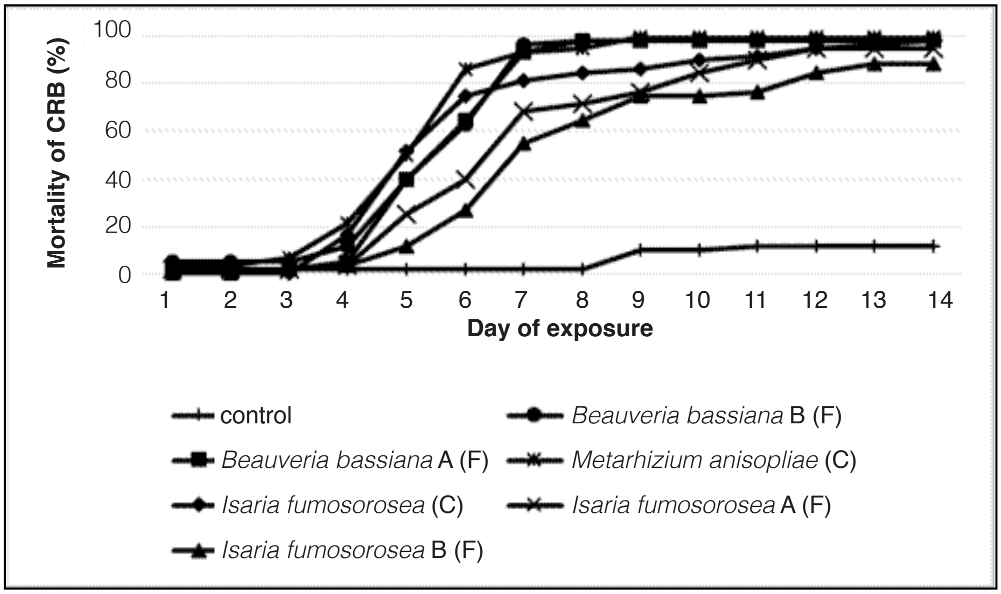

Figure 2. Mortality (%) of CRB adults exposed to spores of field isolates and commercial strains of entomopathogenic fungi. C = Commercial product; F = Field collected.

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