Spatial Patterns of Ergot and Quantification of Sclerotia in Perennial Ryegrass Seed Fields in Eastern Oregon

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Abstract

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Ergot, caused by *Claviceps purpurea*, is a major disease of perennial ryegrass grown for seed in eastern Oregon. The objective of this research was to quantify and describe the spatial patterns of ergot severity in each of three 50-ha commercial fields of perennial ryegrass grown for seed in 2012 and 2013. In total, 1,433 and 1,405 quadrats were sampled among the three fields in 2012 and 2013, respectively, and the percentage of quadrats with ergot ranged from 59 to 90%. The mean incidence of infected seed heads in each quadrat ranged between 13 and 29%, while mean severity in each quadrat ranged from 0.2 to 0.5 sclerotia per seed head. Significant autocorrelation and clustering were observed in all three fields in both years, as indicated by Moran's *I* and spatial analysis by distance indices of aggregation. The mean number of ergot sclerotia collected from each field after harvest ranged between 4 and 15 sclerotia m^{-2} in 2012 and 18 and 119 sclerotia m^{-2} in 2013. Sclerotia left in perennial fields after harvest are a significant source of inoculum that should be targeted for control. This is the first study to quantify spatial patterns of ergot in perennial ryegrass and provides insights into possible mechanisms that contribute to ergot etiology and epidemiology.

Production of cool-season grass seed in the United States is mostly centered in Oregon State where, in 2013, perennial ryegrass (*Lolium perenne* L.) was grown on approximately 42,700 ha (annual production of over 70,760 metric tons) at an economic value of over U.S. $111 million (Anonymous 2014). In the semiarid Columbia Basin of eastern Oregon, perennial ryegrass is often grown in large (up to 50 ha) fields under center-pivot irrigation. Seed is planted in late August to early September and the grass seed crop is typically windrowed and harvested in July. Field burning is usually not practiced and growers cut and bale grass residues after harvest. Fields are left in production for 2 to 3 years before being rotated to onion, potato, corn, melons, or other crops. Crops are intensively managed for insect pests and diseases.

Ergot, caused by *Claviceps purpurea* (Fr.) Tul., is a major disease of perennial ryegrass grown for seed in the Columbia Basin (Alderman et al. 1993; Dung et al. 2013). Airborne ascospores, the primary inoculum of *C. purpurea*, infect unfertilized or recently fertilized ovaries, ramify through the tissue, and replace seed with sclerotia (Parbery 1996; Scheffer and Tudzynski 2006; Tudzynski and Scheffer 2004). Infected flowers exude a sticky mixture of conidia and plant sap, called honeydew, which serves as secondary inoculum and can make swathing and harvesting grass seed difficult for growers (Butler et al. 2001; Luttrell 1980; Mantle and Shaw 1976; Mantle et al. 1977; Rykard et al. 1984; Vizoso et al. 1984). In addition to yield losses due to seed replacement, repeated cleanings are required to remove ergot sclerotia from highly infested seed lots in order to meet certification standards, resulting in increased seed loss and time and labor costs during seed-cleaning operations. If ergot sclerotia cannot be removed below a threshold level, seed lots can be rejected for certification. Further losses are incurred if seed screenings contaminated with ergot cannot be pelletized to be sold as feed because of toxic ergot alkaloids within sclerotia (Rottinghaus et al. 1993).

Ergot sclerotia are soilborne and overwinter and germinate in the spring to produce ascospores (Alderman 1993; Mitchell and Cooke 1968). The number and potential impact of sclerotia left in perennial ryegrass fields after harvest is not entirely understood but a previous study found between 35 and 39 sclerotia in a total of eight 1-m^2 sample sites located in a first-year perennial ryegrass field after harvest (Alderman et al. 1993). If large numbers of sclerotia are left in perennial ryegrass fields after harvest, they can have a significant impact on ergot epidemics because a single sclerotium can potentially produce thousands of ascospores (Cooke and Mitchell 1967).

Little is known about the epidemiology and spatial patterns of ergot in the large (50-ha), center-pivot-irrigated fields that are typical of the Columbia Basin where perennial ryegrass seed is produced. Spatial patterns of diseased plants and pathogens are the result of numerous interacting factors, including sources of inoculum, pathogen and host biology, the environment, cultural practices, and other conditions (Ristaino and Gumpertz 2000). Whether ergot develops in random, regular, or aggregated spatial patterns (Madden et al. 2007) or varies at different hierarchical scales over time in perennial ryegrass fields is not known. Spatial pattern analyses can be helpful in developing methods of disease sampling and assessment, identifying potential sources of inoculum to target control measures, inferring ecological and dispersal processes contributing to epidemics, and developing more effective management tactics (Gent et al. 2012; Johnson et al. 2006; Madden et al. 2007; Turechek and Madden 1999).

Therefore, the objectives of this study were to (i) quantify and describe the spatial patterns of ergot incidence and severity in large commercial perennial ryegrass fields over time, (ii) determine the degree of aggregation of ergot among individual seed heads of perennial ryegrass, and (iii) quantify the number of ergot sclerotia left in perennial ryegrass fields after harvest.

Materials and Methods

Field information. Three 50-ha commercial perennial ryegrass fields in Umatilla County, OR were included in this study. All three fields were planted in fall 2011 and irrigated using center-pivot irrigation with wheel tracks spaced 50 m apart. Field number 1 was

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planted to 'Pavilion' and situated at approximately 271 m in elevation. Field number 2 was located at 217 m in elevation and planted to 'Top Hat II', while field number 3 was at 263 m om elevation and planted to 'Provocative'. All fields were managed by the same grower and subjected to similar cultural practices, including fungicide applications during anthesis for ergot control. Fungicide applications to control ergot in 2012 included one application of azoxystrobin + propiconazole in late May, one application of azoxystrobin in mid-June, and one application of azoxystrobin + propiconazole in late June. In 2013, the three fields each received applications of azoxystrobin + propiconazole in mid-May, azoxystrobin and propiconazole in early June, and azoxystrobin + propiconazole in mid-June.

Preharvest field sampling and disease assessment. Fields were sampled to quantify ergot incidence and severity prior to harvest approximately 1 week before windrowing. Field 1 was sampled on 11 and 12 July 2012 and on 4 July 2013; field 2 was sampled on 6 July 2012 and 2 July 2013; and field 3 was sampled on 18 July 2012 and 7 July 2013. Wheel tracks from the center-pivot irrigation system located 50, 150, 250, and 350 m from the center of the fields were used as circular transects (Fig. 1). Sampled areas consisted of 1-m^2 quadrats located 2 m outside of the wheel tracks and spaced approximately 10 m apart. The location of each quadrat was recorded using a Garmin Foretrex 401 (Garmin International, Inc., Olathe, KS) handheld global positioning system (GPS). In total, 492, 487, and 454 quadrats were sampled in fields 1, 2, and 3, respectively, during 2012. In 2013 476, 471, and 458 quadrats were sampled in fields 1, 2, and 3, respectively. Ten seed heads were arbitrarily collected from different plants in each quadrat and evaluated in the laboratory for ergot infection. Honeydew can be difficult to accurately quantify; therefore, ergot incidence was estimated as the percentage of seed heads (spikes) with sclerotia out of the 10 seed heads collected from each quadrat. The number of sclerotia on each of the 10 seed heads was recorded and the total number of sclerotia was used to determine ergot severity in each quadrat. Ergot incidence and severity were calculated among plants within quadrats and among quadrats within fields. Pearson's correlations between ergot incidence and severity for quadrats within fields were determined for each field in 2012 and 2013 using PROC CORR in SAS (ver. 9.4; SAS Institute, Cary, NC).

Deviations from randomness for the disease incidence data were determined by calculating the index of dispersion (D) using the formula $s_y^2/[\bar{y}(1-\bar{y})/n]$, where s_y^2 is the unbiased estimate of the variance, $\bar{y}$ is the mean proportion of diseased seed heads, and n is the number of seed heads sampled from each quadrat (Fisher 1925; Madden et al. 2007). A D value >1 or <1 was indicative of an aggregated or uniform distribution of ergot-infected plants, respectively, while a D value close to 1 indicated a random distribution of ergot-infected plants. Variance-to-mean ratios (σ^2/μ) of ergot severity were calculated to measure the degree of aggregation of ergot sclerotia among plants within quadrats and among quadrats in each field (Madden et al. 2007). A variance-to-mean ratio >1 indicated aggregation of ergot severity and a variance-to-mean ratio <1 indicated a uniform distribution of ergot severity. A variance-to-mean ratio close to 1 suggested a random distribution of ergot severity.

Spatial analyses. Fits of binomial and β-binomial distributions to the disease incidence data were determined using PROC NLMIXED and the goodness-of-fit (GOF) macro (available online at http://www.oardc.ohio-state.edu/aps-statsworkshop/downloads/downloads.htm from L. V. Madden at The Ohio State University) in SAS (Madden and Hughes 1994; Madden et al. 2007). Fits of Poisson and negative binomial distributions to the disease severity data were assessed using PROC COUNTREG in SAS (Madden et al. 2007; White and Bennetts 1996). Adjacent categories with small expected frequencies (<10 for ergot severity data) were pooled and GOF was determined with χ^2 tests using PROC FREQ in SAS. Model fit was compared using the Akaike Information Criterion (AIC) (Akaike 1974).

Global spatial autocorrelation of infected seed heads and ergot severity among quadrats in each field was measured using Moran's Index (Moran's I) (Moran 1950). Moran's I was calculated using the VARIOGRAM procedure in SAS to determine whether disease incidence and severity exhibited significant spatial autocorrelation

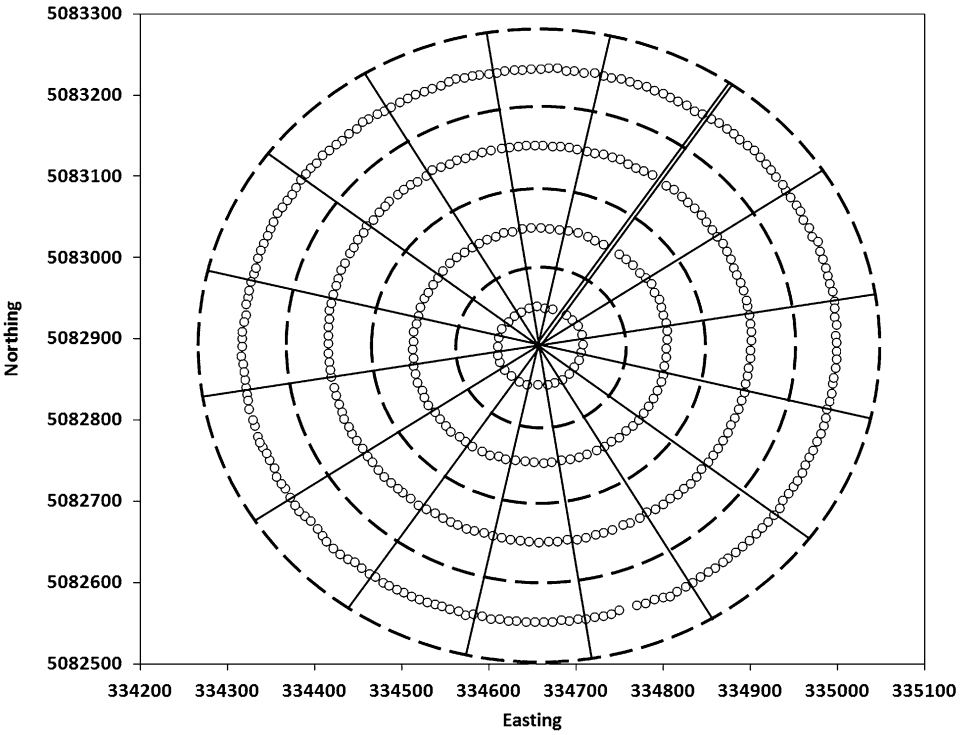

Fig. 1. Example of the sampling pattern used in this study. Small open circles represent sampled quadrats which were approximately 1 m^2 in size (not to scale) and followed alternating wheel tracks spaced at 50, 150, 250, and 350 m away from the center of the 50-ha field. Quadrats were located approximately 2 m away from the wheel track and spaced 10 m apart. Dotted lines represent nonsampled wheel tracks. Solid and dotted lines represent the divisions used for spatial analysis by distance association analysis. The double solid line represents the access road to the center-pivot system. Distances between lines are approximate.

among sampled quadrats in the field. Moran's I values close to 0 indicate a random, independent spatial pattern and lack of autocorrelation, while values close to −1 or 1 indicate negative or positive spatial autocorrelation, respectively.

Ergot severity data were subjected to the spatial analysis by distance indices (SADIE) to quantify and describe the spatial patterns of ergot severity among quadrats in each field (Dallot et al. 2003; Franke et al. 2009; Hay and Pethybridge 2005; Kamdem et al. 2012; Navas-Cortés et al. 2008; Perry 1995; Turechek and Madden 1999). The index of aggregation (I_a) was calculated in SADIE to quantify the aggregation of ergot (Perry 1995). I_a values >1 represent clustering and values <1 represent a regular spatial pattern. The estimated probability that an I_a value greater than or equal to the observed value would occur due to chance (P_a) was also calculated. Above-average clusters or below-average gaps were identified using the SADIE ν, where $\nu_i > 1.5$ indicates above-average clustering, $\nu_i < -1.5$ values indicate below-average gaps, and ν_i values between −1.5 and 1.5 indicate a random spatial distribution. Spatial interpolation of ν_i and ν_i values between sampled quadrats was performed using the kriging method and visualized as red-blue contour plots (Perry et al. 1999) using Surfer (ver.12.5.905; Golden Software Inc., Golden, CO). The overall spatial association between clustering indices in 2012 and 2013 was measured using the index X (Perry and Dixon 2002; Winder et al. 2001). Because GPS-referenced quadrats were not located at the exact same coordinates each year, fields were divided into 64 sections (Fig. 1) and the total number of sclerotia for all quadrats was determined within each section. Index X was calculated using sclerotia totals from each of the 64 sections and the significance of X was determined using a randomization test after adjusting for small-scale spatial autocorrelation (Dutilleul 1993).

Postharvest quantification of ergot sclerotia. The number of sclerotia remaining in each field was determined in both years by vacuum sweeping plots after harvest and residue management operations were completed. Postharvest residue management consisted of the baling and removal of hay after harvest by the grower. A commercial vacuum sweeper (Chastain et al. 1997) was used to collect ergot sclerotia from each of the three fields. The vacuum sweeper was pulled behind a tractor at approximately 1.6 km h^{-1}. Samples were collected from 24 vacuumed plots per field. Vacuumed plots were centered on quadrats that were sampled for ergot incidence and severity before harvest. Vacuumed plots were selected among quadrats using a stratified random sampling method. Briefly, a quadrat was randomly selected from each transect using a random number generator. Subsequent vacuum plots were selected based on the number of quadrats in each concentric transect so that vacuumed plots were equally spaced within each transect. In total, 3, 5, 7, and 9 plots were sampled from each 50-, 150-, 250-, and 350-m circular transect, respectively. Vacuumed plots in 2012 were not vacuumed in 2013 to avoid biasing the results in 2013. The first nine plots vacuumed in field 2 in 2012 were 2 m wide and 10 m long in size. Subsequent vacuum plots were reduced to 2 m wide and 5 m long in order to decrease the total amount of material collected to less than 0.25 m^3. Large fractions of hay and small fractions of soil were removed from each sample using a Clipper Eclipse 324 seed cleaner (A.T. Ferrell Company, Inc., Bluffton, IN). A number 14 round-hole screen was used for the top and middle screens and a 6-by-34 wire-mesh screen was used for the bottom screen. The fraction between the middle and bottom screen was kept for further processing. Seed and sclerotia were separated from the remaining soil and plant residues using an air screen machine, indent cylinder, air column separator, and hand screens, as previously described (Alderman et al. 1993). A stereomicroscope was used to identify and count sclerotia. The number of sclerotia per square meter was calculated for each vacuum plot and divided by the total mass of the sample to account for differences in sample mass before correlation analysis. Pearson's correlations between postharvest sclerotia per square meter and ergot severity were calculated using PROC CORR in SAS.

Results

Preharvest field sampling and disease assessment. In total, 1,433 and 1,405 quadrats were examined in the three perennial ryegrass fields in 2012 and 2013, respectively (Table 1). The percentage of quadrats per field containing at least one seed head with ergot ranged from 59 to 90% in 2012 and 63 to 86% in 2013. The

Table 2. Aggregation of ergot incidence among quadrats and aggregation of ergot severity among plants within quadrats and among quadrats within fields in three perennial ryegrass seed production fields[a]

Field (cultivar)	Year	D[c]	Variance-to-mean ratio[b]	
			Among plants within quadrats	Among quadrats within fields
1 (Pavilion)	2012	1.56	1.61	2.69
	2013	2.45	1.91	5.35
2 (Top Hat II)	2012	1.97	1.91	4.25
	2013	2.71	2.06	6.03
3 (Provocative)	2012	1.87	2.05	4.04
	2013	2.45	2.03	6.54

[a] Ergot incidence was determined from 10 seed heads collected from each quadrat. Ergot severity was determined based on the mean number of ergot sclerotia in 10 seed heads collected from each quadrat.

[b] Variance-to-mean ratios for ergot severity were estimated using the formula σ^2/μ. A variance-to-mean ratio > 1 indicates aggregation of ergot severity and a variance-to-mean ratio < 1 indicates a uniform distribution of ergot severity. A variance-to-mean ratio close to 1 suggests a random distribution of ergot severity. Quadrats were 1 m^2 in size and spaced approximately 10 m apart. Quadrats were located 2 m outside of every other pivot wheel track. In total, 492, 487, and 454 quadrats were sampled in fields 1, 2, and 3, respectively, during 2012. In 2013, 476, 471, and 458 quadrats were sampled in fields 1, 2, and 3, respectively.

[c] Index of dispersion (D) was calculated using the formula $s_y^2/(\bar{y}(1-\bar{y})/n)$, where s_y^2 is the unbiased estimate of the variance, $\bar{y}$ is the mean proportion of diseased seed heads, and n is the number of seed heads sampled from each quadrat. A D value > 1 indicates aggregation of ergot-infected plants and a D value < 1 indicates a uniform distribution of ergot-infected plants. A D value close to 1 suggests a random distribution of ergot-infected plants.

Table 1. Incidence and severity of ergot, and correlations between ergot incidence and severity, in quadrats surveyed in three fields of perennial ryegrass in the first (2012) and second (2013) years of production

Field (cultivar)	Year	Number of quadrats[a]	Quadrats with ergot (%)[b]	Infected plants/quadrat (%)[c]	Mean severity[d]	r[e]
1 (Pavilion)	2012	492	84	20.5 (± 15.9)	0.29 (± 0.28)	0.89
	2013	476	63	15.1 (± 17.7)	0.23 (± 0.35)	0.92
2 (Top Hat II)	2012	487	59	12.7 (± 14.7)	0.18 (± 0.28)	0.88
	2013	471	79	26.3 (± 22.9)	0.45 (± 0.52)	0.87
3 (Provocative)	2012	454	90	25.3 (± 18.8)	0.41 (± 0.41)	0.87
	2013	458	86	29.2 (± 22.6)	0.49 (± 0.56)	0.86

[a] Quadrats were 1 m^2 in size and spaced approximately 10 m apart. Quadrats were located 2 m outside of every other pivot wheel track.

[b] Percentage of quadrats with at least one infected seed head.

[c] Incidence of infected heads determined from 10 seed heads collected from each quadrat. Values in parentheses indicate standard deviations of the means.

[d] Mean number of ergot bodies per seed head. In total, 10 seed heads were collected from each quadrat. Values in parentheses indicate standard deviations of the means.

[e] All r values significant at $P < 0.0001$.

percentage of quadrats with ergot increased from 2012 to 2013 in field 2 but decreased in the other two fields. Mean ergot incidence (the mean percentage of seed heads bearing at least one sclerotium within each quadrat) ranged between 13 and 25% in 2012 and 15 and 29% in 2013 (Table 1). Mean severity, or the mean number of ergot bodies in each quadrat, ranged from 0.2 to 0.4 sclerotia/seed head in 2012 and 0.2 to 0.5 in 2013. The mean number of infected seed heads and ergot severity increased from 2012 to 2013 in fields 2 and 3. Ergot incidence and severity in quadrats were significantly and positively correlated in all three fields in both years (Table 1).

D values ranged from 1.56 to 2.71 and were significant at $P < 0.0001$ using the χ^2 test, indicating a deviation from randomness. Variance-to-mean ratios of ergot severity suggested that ergot sclerotia were clustered on infected seed heads within each quadrat, with values of 1.61 to 2.05 in 2012 and 1.91 to 2.06 in 2013 (Table 2). Ergot severity was also clustered among quadrats, as indicated by variance-to-mean ratio values between 2.59 and 4.25 in 2012 and 5.35 and 6.54 in 2013 (Table 2).

Spatial analyses. Frequency distributions of ergot incidence and severity were skewed right (Fig. 2). The β-binomial distribution fit the disease incidence data collected from all fields in both years ($P < 0.05$), with the exception of field 3 in 2012 (Table 3). The binomial distribution did not fit any of the data sets well based on χ^2 analysis. The negative binomial distribution fit the disease severity data collected from field 1 ($P = 0.13$) and field 2 ($P = 0.84$) in 2012, indicating significant aggregation of ergot severity among quadrats (Table 3). In 2013, the negative binomial fit the disease severity data for field 2 ($P = 0.95$) and field 3 ($P = 0.86$). However, the negative binomial distribution did not fit field 3 in 2012 ($P = 0.0005$) or field 1 in 2013. Ergot severity was poorly modeled by the Poisson distribution, which did not fit any of the ergot severity datasets ($P < 0.0001$) based on χ^2 GOF tests.

Moran's *I* indicated significant ($P < 0.0001$) positive global spatial autocorrelation of ergot severity in all three fields in both 2012 and 2013. Although observed Moran's *I* values were close to 0 and ranged between 0.0519 and 0.0708 in 2012 and 0.0303 and 0.0638 in 2013,

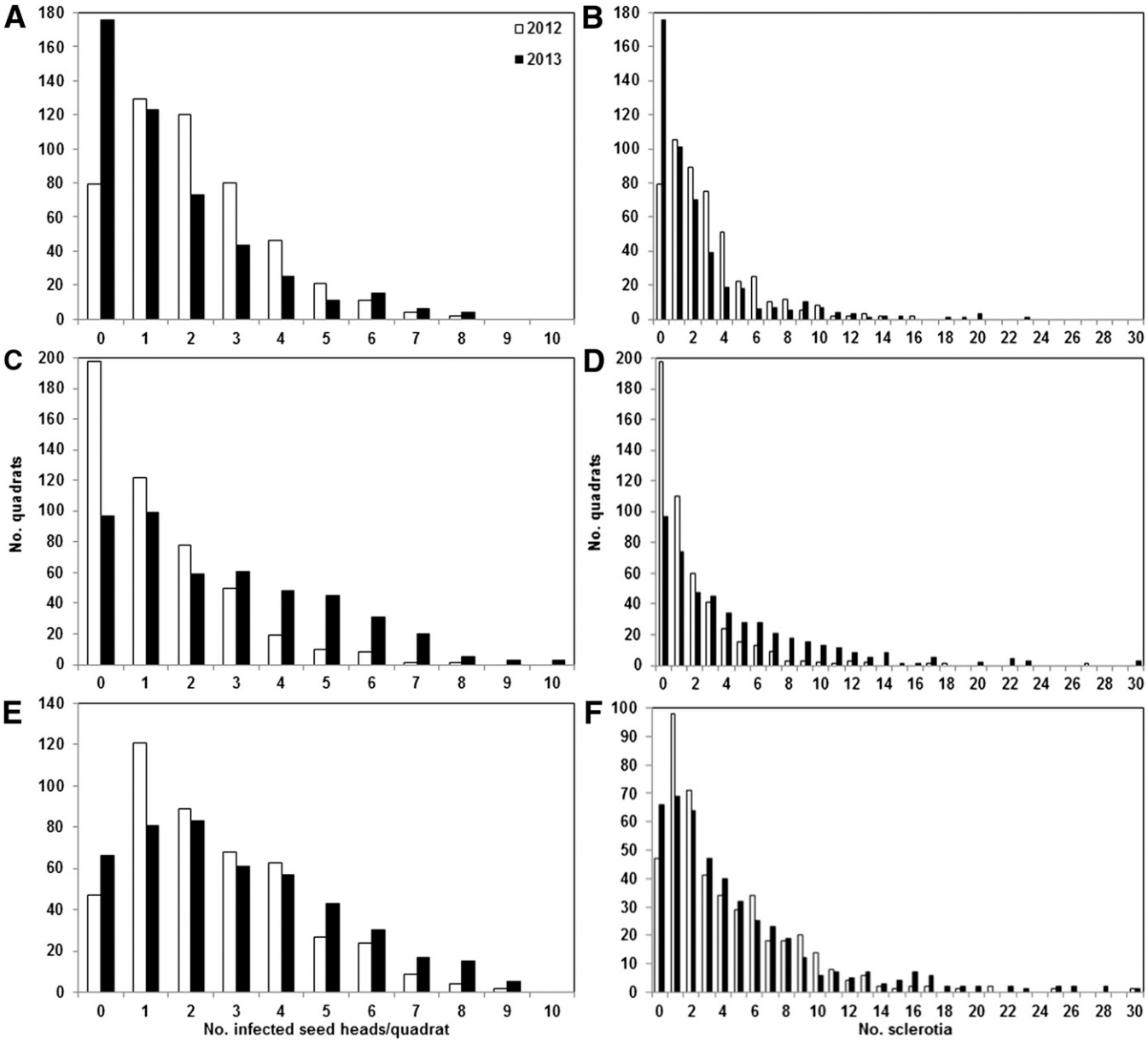

Fig. 2. Frequency distributions of ergot incidence and severity in three fields of perennial ryegrass grown for seed. **A,** Ergot incidence and **B,** ergot severity in field 1 ('Pavilion'); **C,** ergot incidence and **D,** ergot severity in field 2 ('Top Hat II'); and **E,** ergot incidence and **F,** ergot severity in field 3 ('Provocative'). In total, 492, 487, and 454 quadrats were sampled in fields 1, 2, and 3, respectively, during 2012 (white bars). In 2013, 476, 471, and 458 quadrats were sampled in fields 1, 2, and 3, respectively (black bars). Incidence of infected heads was determined from 10 seed heads collected from each quadrat. Severity was determined based on the mean number of ergot sclerotia in 10 seed heads collected from each quadrat.

the observed values were significantly different from the predicted values in all fields in both years (Table 4). SADIE of aggregation and clustering indicated significant clustering of ergot severity among quadrats in all three ryegrass fields for both years (Table 5). SADIE were lower in field 1 and field 3 in 2013 compared with 2012 but increased in field 2 from 2012 to 2013. Significant patches and gaps of high ($\nu_i > 1.5$) and low ($\nu_i < -1.5$) ergot severity were observed for all three fields in both years using red-blue plots (Fig. 3). Significant associations between clustering indices in 2012 and 2013 were not observed in any fields (Table 5).

Postharvest quantification of ergot sclerotia. The mean number of sclerotia remaining in fields after harvest in 2012 was 4.4, 15.3, and 3.8 sclerotia m^{-2} in fields 1, 2, and 3, respectively (Table 6). In fields 1 and 3, 75% of plots had ≤5 sclerotia m^{-2}. High sclerotia levels were found in field 2, where >10 sclerotia m^{-2} were recovered from 50% of the sampled plots and one plot contributed over 75 sclerotia m^{-2}. A greater number of sclerotia were collected after harvest in the three fields in 2013, with fields 1, 2, and 3 containing a mean of 18.4, 119.4, and 66.4 sclerotia m^{-2}, respectively (Table 6). Significant correlations between ergot severity assessments made before harvest and the number of ergot sclerotia collected after harvest with the vacuum sweeper were only observed in 2012 in field 2 ($P = 0.02$, $r = 0.49$) and field 3 ($P < 0.01$, $r = 0.54$), but not in field 1 ($P = 0.06$; r = 0.40). Significant correlations were not observed in field 1 ($P = 0.67$; r = 0.09), field 2 ($P = 0.66$; r = 0.09), or field 3 ($P = 0.94$; r = –0.02) in 2013.

Discussion

Spatial patterns of ergot epidemics can be influenced by the timing, duration, and amount of primary and secondary inoculum production,

Table 3. Goodness-of-fit (GOF) statistics of ergot incidence data to binomial and β-binomial distributions and ergot severity data to Poisson and negative binomial distributions[a]

	Field 1 (Pavilion)		Field 2 (Top Hat II)		Field 3 (Provocative)	
Criteria[b]	2012	2013	2012	2013	2012	2013
Binomial						
AIC	1,823	1,865	1,653	2,357	1,894	2,273
χ^2 (*P* value)	81.6 (<0.0001)	2541 (<0.0001)	217.1 (<0.0001)	754.3 (<0.0001)	204.5 (<0.0001)	520.1 (<0.0001)
β-Binomial						
AIC	1,765	1,603	1,508	1,959	1,776	1,972
χ^2 (*P* value)	2.14 (0.91)	8.13 (0.23)	3.92 (0.56)	12.53 (0.13)	18.27 (0.01)	4.44 (0.81)
Poisson						
AIC	2,416	2,720	2,297	3,620	2,887	3,555
χ^2 (*P* value)	219.8 (<0.0001)	611.8 (<0.0001)	324.9 (<0.0001)	823.8 (<0.0001)	467.2 (<0.0001)	569.6 (<0.0001)
Negative binomial						
AIC	2,141	1,895	1,763	2,451	2,284	2,247
χ^2 (*P* value)	11.3 (0.13)	20.3 (0.0024)	2.1 (0.84)	3.9 (0.95)	29.8 (0.0005)	3.9 (0.86)

[a] In total, 492, 487, and 454 quadrats (*N*) were sampled in fields 1 ('Pavilion'), 2 ('Top Hat II'), and 3 ('Provocative'), respectively, during 2012. In 2013, 476, 471, and 458 quadrats (*N*) were sampled in fields 1, 2, and 3, respectively. In total, 10 plants were sampled from each quadrat.

[b] Distribution fit criteria. Fits of binomial and β-binomial distributions to the disease incidence data were determined using PROC NLMIXED and the goodness-of-fit (GOF) macro in SAS. Fits of Poisson and negative binomial distributions to the disease severity data were assessed using PROC COUNTREG in SAS. Model fit was compared using the Akaike Information Criterion (AIC) (Akaike 1974). Lower AIC values indicate a better fit. GOF was determined with χ^2 tests using PROC FREQ in SAS, where $P > 0.05$ indicates an acceptable fit.

Table 4. Global spatial autocorrelation of ergot severity in three fields of perennial ryegrass grown for seed in the first (2012) and second (2013) year of production[a]

Field (cultivar)	Year	Observed[b]	Expected[b]	*Z*[b]
1 (Pavilion)	2012	0.0708	–0.00204	16.22
	2013	0.0303	–0.00211	7.03
2 (Top Hat II)	2012	0.0557	–0.00206	12.60
	2013	0.0638	–0.00213	14.01
3 (Provocative)	2012	0.0519	–0.00221	11.33
	2013	0.0360	–0.00219	7.90

[a] Quadrats were 1 m^2 in size and spaced approximately 10 m apart. Quadrats were located 2 m outside of every other pivot wheel track. In total, 492, 487, and 454 quadrats were sampled in fields 1, 2, and 3, respectively, during 2012. In 2013, 476, 471, and 458 quadrats were sampled in fields 1, 2, and 3, respectively. Incidence of infected heads determined from 10 seed heads collected from each quadrat. Severity was determined based on the mean number of ergot sclerotia in 10 seed heads collected from each quadrat.

[b] Observed and expected Moran's *I* values and corresponding *Z* values. Positive *Z* values indicate positive autocorrelation. All values were significant at $P < 0.0001$.

Table 5. Aggregation analysis using spatial analysis by distance indices (SADIE) of aggregation (I_a), patch clusters (v_i), gap clusters (v_j), and association (X) of ergot severity in three perennial ryegrass seed fields during the first (2012) and second (2013) year of production[a]

Field (cultivar)	Year	I_a (*P* value)	v_j (*P* value)	v_i (*P* value)	X (*P* value)
1 (Pavilion)	2012	3.041 ($P = 0.0002$)	–2.959 ($P < 0.0001$)	2.885 ($P < 0.0001$)	0.6226 ($P = 0.0767$)
	2013	1.505 ($P = 0.0082$)	–1.415 ($P = 0.0199$)	1.469 ($P = 0.0112$)	…
2 (Top Hat II)	2012	3.219 ($P = 0.0002$)	–3.269 ($P = 0.0020$)	2.862 ($P < 0.0001$)	0.8030 ($P = 0.0548$)
	2013	3.809 ($P = 0.0002$)	–3.825 ($P < 0.0001$)	3.648 ($P < 0.0001$)	…
3 (Provocative)	2012	2.906 ($P = 0.0002$)	–2.853 ($P < 0.0001$)	2.761 ($P < 0.0001$)	0.7360 ($P = 0.0714$)
	2013	2.225 ($P = 0.0002$)	–2.010 ($P = 0.0002$)	2.083 ($P < 0.0001$)	…

[a] The spatial analysis of distance indices (SADIE) method calculates an index of aggregation (I_a). Values of $I_a > 1$ represent clustering and values < 1 represent a regular spatial pattern. Above-average patch clusters or below-average gap clusters of ergot were determined using the SADIE ν, where $\nu_i > 1.5$ indicates above-average clustering, $\nu_i < -1.5$ values indicate below-average gaps, and ν_i values between –1.5 and 1.5 indicate a random spatial distribution. The SADIE X is a measure of the overall spatial association between clustering indices in 2012 and 2013.

the timing and duration of host anthesis, environmental and cultural conditions in the field, and other factors. Although the percentage of quadrats with ergot ranged from 59 to 90% in the three fields included in this study, ergot incidence exhibited a deviation from nonrandomness, as indicated by significant *D* values. Ergot severity exhibited significant autocorrelation and clustering among quadrats in all three fields in both years and positive variance-to-mean ratios, indicating that ergot was not randomly or uniformly distributed among sampled quadrats. However, significant spatial associations of ergot severity in 2012 and 2013 were not observed in any of the three fields. The temporal changes in spatial patterns of ergot severity between 2012 and 2013 may have been caused by the widespread dispersal of ascospores over large areas in the field or the movement of ergot sclerotia during seed harvest or hay baling, resulting in a redistribution of primary inoculum sources. Other factors that could have contributed to the lack of association observed between the 2 years include the relative role of secondary spread via honeydew, rotation of neighboring fields out of perennial ryegrass and the

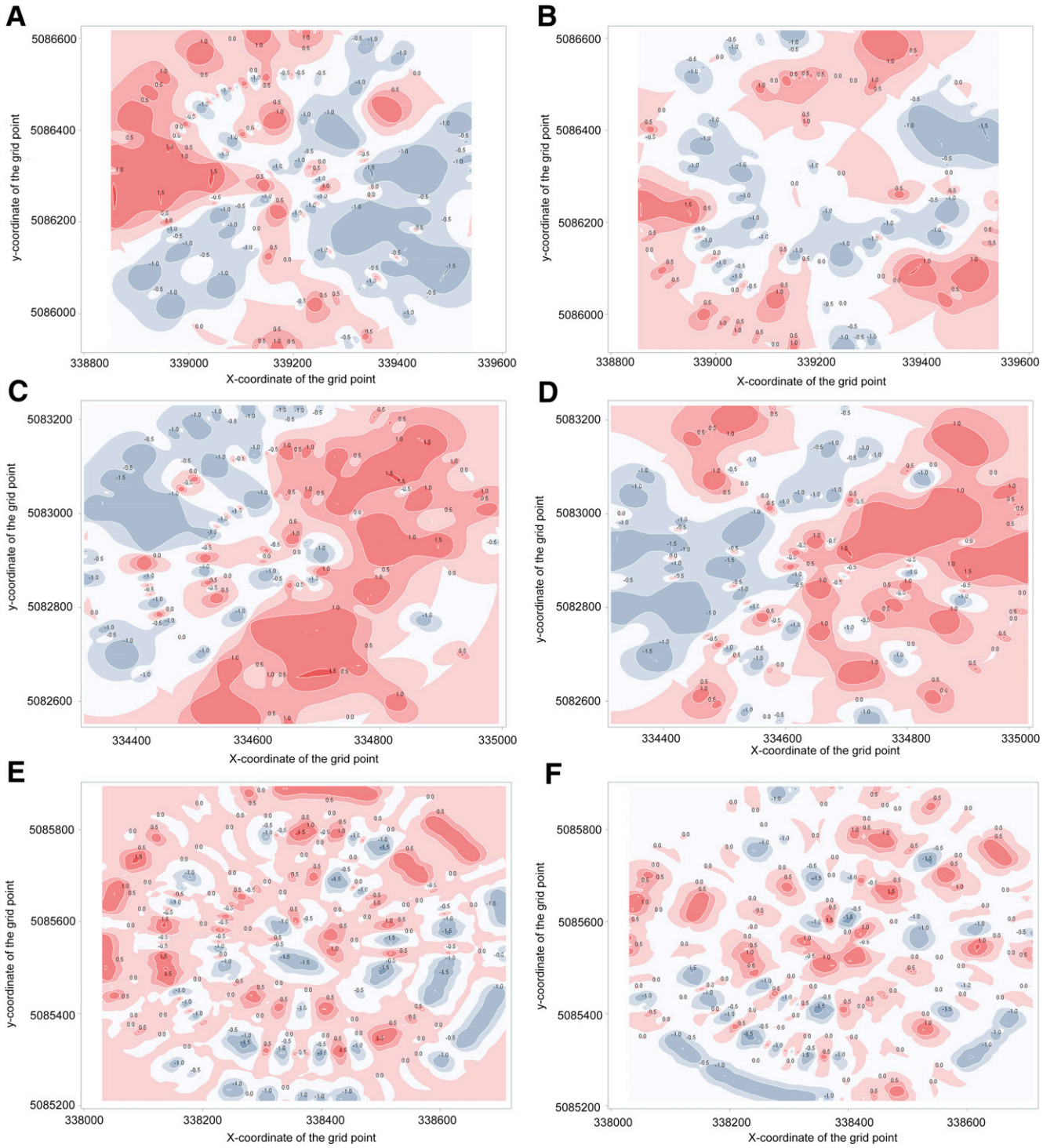

Fig. 3. Red-blue plots of spatial analysis by distance clustering indices (SADIE) of ergot severity in 2012 (left panels) and 2013 (right panels) for **A** and **B,** perennial ryegrass field 1 (top); **C** and **D,** field 2 (middle); and **E** and **F,** field 3 (bottom). Above-average patches or below-average gaps were determined using the SADIE ν, where $\nu_i > 1.5$ indicates above-average patches (red), $\nu_i < -1.5$ indicate below-average gaps (blue), and v_i values between –1.5 and 1.5 indicate a random spatial distribution (white). Closed circles show sampled quadrats with above- and below-average counts (red and blue, respectively) and open circles show sampled quadrats with average counts.

removal of potential sources of primary or secondary inoculum, differences in environmental conditions, or the impacts of cultural practices such as irrigation.

Clustering of sclerotia was also observed on infected seed heads based on variance-to-mean ratios between 1.6 and 2.1 over both years. When only infected seed heads were considered, the mean number of sclerotia per seed head of 1.4 to 1.7 and 1 to 13 sclerotia was observed on individual seed heads. These results are in agreement with previous studies of ergot in Kentucky bluegrass, which found that sclerotia occurred in clusters on infected seed heads instead of being randomly distributed (Alderman and Barker 2003; Wu et al. 2009). Large numbers of ergot sclerotia on individual seed heads could be caused by concentrated sources of sclerotia and ascospores in the field, multiple or prolonged exposures to ascospores during anthesis, secondary spread within and among plants via honeydew, or a combination of factors. In addition, weedy hosts and contaminated insects can contribute to the spread of honeydew and affect the epidemiology of ergot in other hosts (Butler et al. 2001; Hardy 1988; Mantle et al. 1977) but their importance in perennial ryegrass seed crops is not well understood.

The clustered spatial patterns observed in the surveyed fields suggest that simple random sampling of ergot should be avoided in large, center-pivot-irrigated commercial fields because patches and gaps of disease severity can exist. Additionally, sampling only a portion of a field may not provide an accurate or precise estimate of ergot severity. Systematic, predetermined, and uniform sampling approaches would likely require a sufficient number of sample units in order to obtain an accurate estimate of ergot severity, especially in fields where ergot is highly aggregated but at a low incidence. A comparison of the accuracy and efficiency of sampling methods for ergot, including systematic, stratified, sequential, and adaptive cluster sampling, is needed (Madden and Hughes 1999; Madden et al. 2007; Thompson 1990)

In the Columbia Basin of eastern Oregon, perennial ryegrass seed fields often remain in production for at least 2 to 3 years and grower observations suggest that ergot increases in each successive year of production. We documented an increase in ergot severity from 2012 to 2013 in two of three fields but a reduction in ergot in the third field. The reason for the reduction of ergot in field 1 from 2012 to 2013 is not known, especially because more ascospores were captured in 2013 compared with 2012 (data not shown). In addition, environmental conditions were likely conducive to ergot in 2013 because other fields in the area exhibited the disease. Differences among cultivars, environmental conditions, microclimates, or other factors may have played a role.

Large numbers of sclerotia were collected from all three fields after harvest and postharvest residue management, and approximately 4 to 17 times more sclerotia were collected in 2013 compared with 2012. The annual increases in ergot incidence and severity observed in some fields in this study may primarily be a result of increasing levels of sclerotia left in perennial fields after harvest. Although sclerotia of *C. purpurea* are generally thought to only survive for a year under field conditions (Coley-Smith and Cooke 1971; Cunfer and Seckinger 1977), it is also possible that a polyetic build-up of sclerotia can occur if even a small percentage of sclerotia can remain viable for longer than previously thought. The large numbers of ergot sclerotia observed after harvest in 2012 may have served as local sources of inoculum in 2013 and contributed to the different spatial patterns that were observed in 2013.

Sclerotia left in perennial fields after harvest are a potentially major source of inoculum that should be targeted for control. Open field burning, which can reduce ergot sclerotia viability, is not widely practiced in eastern Oregon due to environmental and health concerns (Hardison 1980). Although tillage of fields previously cropped to perennial ryegrass can bury sclerotia and reduce the potential for germination and ascospore release, it is unlikely that all sclerotia are buried to a sufficient depth to completely prevent the germination of all sclerotia in a field. In addition, the presence of highly erodible soils in the area precludes certain cultural management practices such as burying sclerotia through deep tillage and, given the high likelihood of soil erosion in the area, the use of a moldboard plow is rare (Bretag and Merriman 1981; Pscheidt and Ocamb 2013). As such, sclerotia left in perennial fields after harvest can be difficult to control because management options like crop rotation and deep tillage are not available. Research is currently underway to reduce or prevent germination of ergot sclerotia in perennial grass seed production fields using soil-applied fungicides as a strategy to help manage this disease (Dung et al. 2013).

Established perennial ryegrass fields that are infested with ergot sclerotia may act as potential green bridges for the disease, serving as sources of inoculum for newly planted fields that, in turn, become infested with sclerotia that can produce primary inoculum the following season. This may be especially true in the Columbia Basin, where the edges of neighboring fields may only be separated by access roads less than 5 m wide. In 2012, the greatest disease severity in all three first-year perennial ryegrass fields tended to occur near neighboring ryegrass fields that were in their second or third year of production, and especially older perennial ryegrass fields located upwind (data not shown). Although the distance of *C. purpurea* ascospore dispersal is not known, ascospores of the white mold fungus *Sclerotinia sclerotiorum* are thought to travel up to 4 km, though most are probably deposited within a distance of 100 m from the original source (Cubeta et al. 1997; Kohli et al. 1995). Preliminary experiments at the Hermiston Agricultural Research and Extension Center suggest that ergot ascospores can travel a minimum distance of at least 60 m from a source of sclerotia (personal observations).

Perennial ryegrass seed producers in the Columbia Basin consider ergot to be the most important disease affecting perennial ryegrass seed production in eastern Oregon. Fungicides typically provide adequate control of powdery mildew (*Blumeria graminis*) (Lewis et al. 1996) and stem rust (*Puccinia graminis*) (Pfender 2006), two other major diseases of perennial ryegrass in the region, but protective fungicide applications during anthesis have not provided satisfactory control of ergot. In this study, ergot was observed in all three perennial ryegrass fields surveyed in 2012 and 2013, despite grower efforts to protect flowers with multiple fungicide applications during anthesis. More effective integrated disease management strategies to control ergot in perennial ryegrass seed production in the Columbia Basin of Oregon are needed.

Table 6. Number of ergot sclerotia collected after postharvest residue management operations from three perennial ryegrass seed fields during the first (2012) and second (2013) year of production[a]

	2012				2013			
Field (cultivar)	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
1 (Pavilion)	4.4	4.0	0.6	15.3	18.4	19.7	0.0	64.4
2 (Top Hat II)	15.3	16.8	1.4	75.6	119.4	88.5	27.4	289.3
3 (Provocative)	3.8	2.2	0.6	8.5	66.4	34.2	4.1	142.1

[a] Postharvest residue was baled and removed by the grower-cooperator. Ergot sclerotia were collected from 24 vacuum plots in each field in each year using a commercial-sized vacuum sweeper. The first nine vacuum plots in field 2 in 2012 were 20 m^2 in size. The remaining vacuum plots were reduced in size to 10 m^2 in order to reduce the overall amount of material sampled. Mean = sclerotia per square meter, SD = standard deviation, Min = minimum, and Max = maximum.

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