Comparison of Two Fusarium Head Blight Inoculation Methods in Wheat

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Introduction

Fusarium graminearum Schwabe is the principal causal agent of Fusarium head blight (FHB) in the United States. Infection with this pathogen may result in reduced grain yield and test weight, as well as the contamination of grain with mycotoxins. While fungicide and cultural practices provide some control, the most effective means for preventing this damage is the development of resistant wheat cultivars. Development of host resistance requires the consistent differentiation of resistance levels. This process relies on the use of inoculation methods, as natural infection is inconsistent in most areas. Many inoculation and evaluation methods are used to identify breeding lines with resistance to FHB (2). We compared several methods in 2008, and obtained highly correlated results (1). We evaluated a spray and bag method with a conidial suspension inoculation followed by a period of bagging. If this method is highly correlated with the widely used and accepted inoculated grain spawn and misted nursery for a second year of evaluation this could be a reliable method for multilocation evaluation of FHB resistance. It would not require the establishment of a misted nursery at all sites, saving time and labor.

Objectives

- To determine if FHB resistance ratings from a spray and bag method were highly correlated with data from an inoculated grain spawn misted nursery for a second year of evaluation and over multiple locations.
- To determine if the same breeding lines would be selected or discarded using the two inoculation methods.

Materials and Methods

Plant Material

- Evaluations of FHB resistance were conducted on 120 advanced breeding lines with varying reactions to FHB. Of the 120 lines evaluated in each method, 118 lines were the same across methods.
- Plots were inoculated with the spray and bag method at two locations, Urbana and Brownstown, IL, with two replications in 2010. The first 20 entries also underwent inoculation without a bag, to determine the effect of bagging on FHB symptoms.
- Grain spawn inoculated plots were evaluated at a single location with three replications.
- Field experimental design was a randomized complete block design.

Spray-and-Bag Method - Figure 1

- Spray inoculum was composed of a balance of conidia from ten different isolates of *F. graminearum*.
- Conidia were produced in carboxymethyl - cellulose liquid media and quantified using a hemacytometer.
- Approximately 25 wheat heads per plot were sprayed during mid to late anthesis using a all purpose household sprayer (Model: ACE 11690, ACE Hardware Corporation).
- Heads were sprayed with approximately 6 mL of inoculum at 100,000 conidia mL^{-1}.
- Inoculated heads were marked with a ring made plastic string trimmer line, which provided a rigid, durable, and reusable marker for inoculated heads.
- A 1.1L Whirl-Pak™ bag with six air holes was placed over the heads and secured.
- Bags were removed after 48 hours.
- Heads were rated approximately 21 days after inoculation as follows:
  - Incidence - estimate of percentage of infected heads.
  - Severity - percentage of infected spikelets per head, mean over seven heads.

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Disclaimer

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Table 1. 2010 Spearman correlation coefficients and probability values for disease data on wheat breeding lines using two different inoculation methods to evaluate Fusarium head blight (FHB) resistance in wheat, N=120.

			Spray and Bag							Grain Spawn and Mist Irrig.				
			Urbana		Brownstown		Locations Combined							
			Severity	FHB index	Incidence	Severity	FHB index	Incidence	Severity	FHB index	Incidence†	Severity‡	FHB index§	FDK#	ISK index*
Spray and Bag	Urbana	Incidence	0.19 0.0352	0.48 <.0001	0.14 N.S.¶	0.23 0.0130	0.23 0.0106	0.92 <.0001	0.26 0.0041	0.45 <.0001	0.23 0.0124	0.09 N.S.	0.15 N.S.	0.14 N.S.	0.20 0.0320
		Severity		0.92 <.0001	- 0.10 N.S.	0.25 0.0063	0.24 0.0095	0.14 N.S.	0.75 <.0001	0.69 <.0001	0.17 N.S.	0.38 <.0001	0.37 <.0001	0.25 0.0074	0.33 0.0002
		FHB index			- 0.06 N.S.	0.32 0.0004	0.31 0.0006	0.41 <.0001	0.75 <.0001	0.77 <.0001	0.27 0.0035	0.39 <.0001	0.41 <.0001	0.28 0.0020	0.40 <.0001
	Brownstown	Incidence			0.23 0.0101	0.31 0.0006		0.43 <.0001	0.12 <.0001	0.19 0.0348	0.00 N.S.	0.07 N.S.	0.03 N.S.	0.07 N.S.	0.10 N.S.
		Severity				0.99 <.0001		0.30 0.0009	0.81 <.0001	0.81 <.0001	0.42 <.0001	0.44 <.0001	0.48 <.0001	0.24 0.0079	0.44 <.0001
		FHB index						0.33 0.0002	0.80 <.0001	0.81 <.0001	0.41 <.0001	.44 <.0001	0.47 <.0001	0.24 0.0079	0.43 <.0001
	Locations Combined	Incidence							0.29 0.0014	0.49 <.0001	0.22 0.0166	0.13 N.S.	0.17 N.S.	0.16 N.S.	0.22 0.0153
		Severity								0.96 <.0001	0.38 <.0001	.53 <.0001	0.55 <.0001	0.25 0.0065	0.49 <.0001
		FHB index									0.4 <.0001	0.51 <.0001	0.54 <.0001	0.26 0.0048	0.50 <.0001
Grain Spawn and Mist Irrig.		Incidence										0.57 <.0001	0.76 <.0001	0.37 <.0001	0.80 <.0001
		Severity											0.95 <.0001	0.47 <.0001	0.82 <.0001
		FHB index												0.49 <.0001	0.91 <.0001
		Kernel Qualtiy													0.75 <.0001

†Incidence = the percentage of heads with symptoms ‡Severity = the percentage of spikelets in a head with symptoms
§FHB index = (incidence × severity)/100 #FDK =Fusarium damaged kernels percentage
*ISK index = [(0.3 × incidence) + (0.3 × severity) + (0.4 × kernel quality)]
¶N.S. = Not Significant at α = 0.05

Table 2. Number of wheat breeding lines distributed between the specified proportions for mean Fusarium head blight (FHB) index for plots using two different inoculation methods to evaluate FHB resistance in wheat, 2010.

Spray ang Bag		Grain Spawn Method					
		Top 10%	Top 11-30%	Middle 40%	Bottom 11-30%	Bottom 10%
Brownstown	Bottom 10%	0	2	2	5	**2**
	Bottom 11-30%	0	3	9	**6**	6
	Middle 40%	4	8	**22**	9	4
	Top 11-30%	4	**9**	8	3	0
	Top 10%	**3**	2	6	1	0
Urbana	Bottom 10%	0	1	5	2	**3**
	Bottom 11-30%	0	3	9	**8**	4
	Middle 40%	5	6	**22**	10	4
	Top 11-30%	4	**10**	7	3	0
	Top 10%	**2**	4	4	1	1
Locations Combined	Bottom 10%	0	2	1	4	**4**
	Bottom 11-30%	0	1	13	**6**	4
	Middle 40%	4	11	**17**	12	3
	Top 11-30%	3	**7**	12	2	0
	Top 10%	**4**	3	4	0	1

Figure 1. Spray and Bag Method


Figure 2. Grain Spawn and Mist Irrigation

Table 3. Means and standard deviations (SD) for incidence, severity, Fusarium head blight (FHB) index, Fusarium damaged kernels (FDK) percentage, and ISK index for two different inoculation methods to evaluate FHB resistance in wheat. N=120

	Spray and Bag			Grain Spawn
	Urbana	Brownstown	2 loc. Mean	
Trait	x̄ ± SD	x̄ ± SD	x̄ ± SD	x̄ ± SD
Incidence, %	87.4 ± 10.5	97.6 ± 3.6	92.5 ± 7.8	82.3 ± 11.5
Severity, %	57.0 ± 10.2	59.0 ± 13.5	58.0 ± 12	32.7 ± 12.3
FHB index†, 0-100	50.2 ± 11.1	57.7 ± 13.2	54 ± 12.2	29.0 ± 11.9
FDK, %	-	-	-	31.4 ± 8.1
ISK index‡, 0-100	-	-	-	48.3 ± 7.2

†FHB index = (incidence × severity)/100

‡ISK index = [(0.3 × incidence) + (0.3 × severity) + (0.4 × FDK)]

Table 4. Best linear unbiased predictors (BLUPs) for disease measurements for Fusarium head blight resistance in plots with and without a bag placed over inoculated wheat heads. N=20

		Incidence, %	Severity, %	FHB index, (0-100)
No Bag	Brownstown	46.6	29.8	21.3
	Urbana	39.4	32	7
	2 Location Mean	43	30.9	14.2
Bag	Brownstown	97.4	60.7	65.4
	Urbana	90.3	62.8	51.1
	2 Location Mean	93.8	61.7	58.2
No Bag vs. Bag		(-)50.8****	(-)30.8	(-) 44.0*
Brownstown vs. Urbana		7.18***	(-)1.6	16.0****

* significant at 0.1
** significant at 0.05
***significant at 0.01
**** significant at <.0001

Literature Cited

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Materials and Methods

Grain Spawn and Mist Irrigation Method – Figure 2

- Grain spawn composed of corn kernels infected with 10 different isolates of *F. graminearum*.
- Grain spawn was disseminated with a broadcast spreader two and four weeks before anthesis at a total rate of 287 kg ha^{-1}.
- The nursery was mist irrigated three times per 24 hours during anthesis and for two weeks after the last wheat genotype flowered.
- Azoxystrobin (Quadris®; Syngenta Crop Protection, Greensboro, NC), was sprayed at a rate of 0.04 Kg a.i. ha^{-1} at Feekes growth stage 8.0 – 9.0 to control foliar diseases.
- Lambda-cyhalothrin (Warrior®; Syngenta Crop Protection, Greensboro, NC) insecticide was applied at a rate of 0.006 Kg a.i. ha^{-1} in the fall and spring to control aphids transmitting *Barley yellow dwarf virus*.
- FHB was evaluated approximately one month after heading date as follows:
  - Incidence – a whole plot visual estimate of percentage of infected heads.
  - Severity – a mean of the number of infected spikelets per head based on seven heads.
- A visual estimate of Fusarium damaged kernels (FDK) percentage was determined after harvest by comparison to known standards.

Statistical Analysis

- Data were analyzed using SAS v9.2 (3) with a significance threshold of α=.05.
- Spearman rank-order correlation coefficients were calculated using PROC CORR of SAS.
- PROC MIXED of SAS was used to analyze the effect of bagging with the ESTIMATE option to calculate best linear unbiased predictors. These data were analyzed as a split plot, with the variety (N=20) as the whole plot, and the bagging treatment as the subplot. Variety was considered as a random effect.

Results

- Extremely high incidences were observed for the spray and bag method, especially in Brownstown, IL (X=97.6%) (Table 3), likely overwhelming genetic resistance. This resulted in little distinction between resistant and susceptible lines for Type I resistance.
- Severity (r=0.53) and FHB index (r=0.54) were significantly (P <.0001) correlated between the two location means for the spray and bag and the grain spawn method (Table 1).
- Correlations between locations were weak, 0.14 for incidence and 0.31 for FHB index.
- The number of lines shared between methods for specified proportions was similar for the two locations compared to the grain spawn method (Table 2).
- Only 38 lines were in complete agreement between methods for the specified proportions. (Table 2)
- Placing a bag on inoculated heads significantly increased disease incidence and FHB index (Table 4).
- Plots without a bag correlated poorly with the grain spawn method (data not shown).

Conclusions

- High disease levels in spray and bag plots likely overwhelmed genetic resistance, making resistant lines appear susceptible. Also the high disease levels likely contributed to the weak correlations between methods. Of concern, there was poor reproducibility between locations of the spray and bag.
- Reducing the inoculum concentration would likely reduce overall disease levels and improve correlations between methods and locations.
- Not all the lines identified resistant in one method would have been selected using the other method..
- Our significantly correlated results for two years indicate that data collected using this method could be used in conjunction with a grain spawn nursery to obtain multiple locations of FHB data on wheat breeding lines, but further refinement of the technique is needed.