Influences from Long-Term Crop Rotation, Soil Tillage, and Fertility on the Severity of Rice Grain Smuts

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Abstract

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False smut (*Ustilagoidea virens*) and kernel smut (*Neovossia horrida*) are diseases of rice (*Oryza sativa*) that reduce both grain yield and quality. Susceptible rice cultivars are in widespread use on production acreage in the United States, and the effects from crop management practices on smut control are poorly understood. We studied the long-term effects of crop rotation, soil tillage, and fertility level on rice smut severity. The highest levels of false smut observed in this study were on cultivars grown in rotation with soybean, on traditionally tilled soils, with high fertilizer treatments. The highest levels of kernel smut were observed in a rice-soybean rotation with winter wheat grown

between summer crops. These rotations are commonly used in rice-growing regions of the southern United States. Using combinations of crop rotation, soil tillage, and fertility rate, several alternative crop-management practices were identified that provided effective control of smuts in susceptible rice cultivars. The most effective method for controlling both false smut and kernel smut was in 3-year rotations of rice, soybean, and corn. Regardless of rotation order or tillage and fertility treatments within the rotations, rotating out of rice for 2 years was the most effective approach for smut control.

False smut (*Ustilagoidea virens* (Cooke) Takah.) and kernel smut (*Neovossia horrida* (Takah.) Padwick & A. Khan, syn. *Tilletia barclayana* (Bref.) Sacc. & P. Syd.) are important fungal diseases of rice (*Oryza sativa* L.) that reduce grain yield and quality (29). Little research has been conducted to date to understand the etiology, epidemiology, and host plant susceptibility to these diseases, and rice-breeding priorities have not included developing smut-resistant cultivars. This has led to the widespread cultivation of susceptible rice cultivars in the United States (27), and a concurrent lack of disease management in rice production, due to the sporadic occurrence of severe smut epidemics. However, recent epidemics have drawn attention to these diseases and the need for their control.

Reports of false smut emerging as a disease of importance for the southern United States began in 1997 (8,23), although the disease was known to exist in the United States since 1906 (22). However, the historical distribution and full impact of the disease over the past century is unknown. In Arkansas, false smut was documented in all rice-producing regions in 1999 (7) and, since that time, multiple outbreaks have occurred, including significant epidemics in 2008 and 2009 (4,15).

Kernel smut has been a chronic disease problem for U.S.-based rice production (6,9,10), and also has significant economic impact. For example, during the winter of 2008–09, the presence of kernel smut halted the export of infested U.S. rough rice in transit to rice mills in Mexico (17). Although the disease has the potential for

significant yield losses (29), phytosanitary and export restrictions are also a concern for seedborne pathogens like *N. horrida*.

False smut and kernel smut present unique challenges for disease management. Currently, all major inbred rice cultivars and hybrids grown in the southern United States are susceptible to false smut, and the inbred cultivars are also susceptible to kernel smut (16,27). Susceptible germplasm combined with a cultivation system (in rotation with soybean (*Glycine max* (L.) Merr.) on tilled soil, with high fertilizer input) that promotes high levels of smut severity (2) has created a situation where smut diseases are not being controlled. Fungicides can be used to control fungal diseases of rice (blast and sheath blight) in the United States. However, midseason fungicide applications to control smut diseases are a financial gamble for rice farmers for the following three reasons. (i) The visual symptoms of disease are not present until after grain fill; therefore, a timely fungicide application must be made without any knowledge of disease incidence in apparently healthy plants. (ii) There is the risk of significant yield loss if no action is taken in rice fields with a history of the diseases. (iii) Fungicides do not offer complete protection from smuts (18,26) and there has been no analysis of cost versus yield protection to show a financial gain from treatments. In order to control grain smuts on susceptible rice cultivars, fungicides are not the optimal method of disease control as they are for foliar diseases.

Previously (2,3), we demonstrated that the severity of smut diseases could be reduced in susceptible rice cultivars without the use of fungicides. By changing crop-management practices to reduced-input systems, high levels of disease control were obtained without yield penalties. Herein, we have expanded upon this work to understand the influences from alternative crop management systems on smut disease severity. Alternatives to the common rice-soybean rotation are used in Arkansas, and are often driven by commodity prices where repeat rice crops or rotations to corn are used, because these options can be more profitable than soybean (28). For long-term sustainability of rice production, additional alternatives are needed to address soil conservation (11,13,19), limited irrigation water (14), greenhouse gas emissions (1,12), and waterway pollution from fertilizer runoff (5). Additional research is needed from an agronomic standpoint to address these issues of sustainable rice production. From a disease-management perspective,

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changes to rice production systems to address these pressing issues can also have positive or negative impacts on disease severity.

Alternatives to standard rice cultivation practices can contribute to long-term sustainability of rice production in the United States but the effects on rice pathogen populations and disease severity are unknown, particularly in no-tillage where crop residue is not burned or incorporated into the soil. Crop rotation has a long history of use in disease management; however, its utility for control of rice smuts was unknown (2). The most common rotation for our region is rice-soybean and, less frequently, the same rotation with winter wheat grown after rice (rice-wheat-soybean). A corn crop can also be included in a rice rotation as a substitute for soybean (rice-corn) or integrated with rice and soybean as a three-phase rotation (rice-soybean-corn or rice-corn-soybean). The use of corn as a rotation crop is of particular interest because it is also a host of *U. virens* (30). Additional alternatives to the rice-soybean rotation are continuous rice and continuous rice with winter wheat (rice-wheat). The primary objective of this work was to determine the effects of tillage management, nitrogen fertilizer rate, and crop rotation on the severities of false smut and kernel smut.

Materials and Methods

Study location. A long-term rice cropping-systems study was established at the University of Arkansas Rice Research and Extension Center (Stuttgart; N 34°27' latitude, W 91°24' longitude) in 2000 (21). The location lies within the Grand Prairie region of eastern Arkansas, where rice is typically grown in rotation with soybean. False smut and kernel smut commonly occur on the experiment station and in production fields of the area (2–4). The experimental plots were known to be naturally infested with false smut and kernel smut prior to 2006, and no fungicides were applied since the inception of the study in 2000.

Crop rotation experiment. As part of the multi-year, multi-cropping-rotation study, the incidence of false smut and kernel smut were recorded each year for 4 years (2006 to 2009). The field experimental layout was a randomized split-strip plot design, where tillage type was the main plot, fertility level was the subplot, and rotation was randomly applied within each of four replicates at the beginning of the study (2,21). Tillage treatments were traditional tillage (TT) and no-tillage (NT). Fertility treatments for rice were “low” (L) (N at 112 kg ha⁻¹ + P₂O₅ at 45 kg ha⁻¹ + K₂O at 67 kg ha⁻¹) and “high” (H) (N at 168 kg ha⁻¹ + P₂O₅ at 67 kg ha⁻¹ + K₂O at 100 kg ha⁻¹) using Agrotain (Agrotain International, L.L.C.) coated urea pellets (46-0-0), triple super phosphate (0-46-0), and muriate of potash (0-0-60). Phosphorus and potassium fertilizers were applied prior to planting and N was applied post emergence at the four- to five-leaf growth stage. The rotation treatments were continuous rice (RR), rice-soybean (RS), rice-corn (RC), rice-wheat (RW), rice-wheat-soybean-wheat (RWSW), rice-soybean-corn (RSC), and rice-corn-soybean (RCS; Table 1).

Four rice cultivars that represent two maturity groups (standard and early) were grown each year. Standard maturity lines (‘Wells’, ‘Cybonnet’, and ‘XL 723’) were spring planted for full-season growth. Wells was grown in all years, whereas Cybonnet (2006) was replaced with hybrid ‘XL 723’ in 2007 through 2009. Early-

maturity lines ‘Spring’ and ‘RU0701124’ were used in rotation with wheat, and were summer planted following the wheat harvest. Spring was grown in all years, and from 2006 through 2008 was entered twice in the experiment because no other early-maturing, locally adapted cultivar was available in sufficient quantity of seed. In 2009, experimental line RU0701124 was included with Spring in the wheat rotations. Seed of inbred cultivars Wells, Cybonnet, and Spring and experimental line RU0701124 were provided by the University of Arkansas. Seed of hybrid XL 723 was provided by RiceTec, Inc. (Alvin, TX). All entries were susceptible to both diseases, with the exception of hybrid XL723 that is moderately resistant to kernel smut (2; *unpublished data*).

Rice was drill-seeded at the rates of 101 kg ha⁻¹ for the inbred cultivars and 39 kg ha⁻¹ for hybrid XL 723 using an Almaco grain drill (Almaco, Nevada, IA) and a 19-cm row spacing (6-by-19-m individual plot size). At the five-leaf growth stage, N fertilizer was applied and a permanent flood 10 cm deep was immediately established and maintained throughout the growing season. Mature rice was harvested with a John Deere 45 combine (Deere & Company, Moline, IL) retrofitted with a Carter weigh bucket (Carter Mfg. Co. Inc., Brookston, IN) at approximately 18% moisture for yield measurements. Grain samples for moisture determinations and disease evaluations were collected from the combine at harvest.

Sampling and disease rating. False smut and kernel smut disease ratings were performed according to Brooks et al. (2). Briefly, false smut sori were recovered by hand sieve from 1- to 1.5-kg grain plot-samples, and disease severity reported as sori number per kilogram of seed harvested. Subsequently, four 50-g subsamples of seed were used to rate kernel smut severity. Each subsample was soaked overnight in 0.27 M KOH to clear the hulls (palea and lemma; 20), and smutted kernels were identified visually over a light box. Kernel smut severity was reported as the number of symptomatic kernels per kilogram of seed harvested. Herein, disease severity is reported as least squares means (LSM) of log-transformed severity values.

Data analyses. Analyses of all dependent variables were performed using the GLIMMIX procedure in SAS (version 9.2). False smut and kernel smut were lognormally distributed. This probability distribution was specified in the model after the variables were coded by adding +1 to each value. Yield followed a Gaussian (normal) distribution. Tillage, fertility, rotation, and their interactions were considered fixed effects in the statistical models. The replicated block was random as well as the cultivar within the rotation. Cultivar was a random effect because we were inferring the range of all rice cultivars and we were not implicitly focused on the means results of the specific cultivars included in this study. The data were originally assessed on a year-to-year basis (*not shown*). The fixed effects showed similar trends in all years of the study. The full experiment over all years was analyzed by incorporating the random time effect (years) upon the repeated measurement of each experimental unit (cultivar plot within each rotation). A Toeplitz covariance structure was modeled for each cultivar within rotation across years. This approach allowed for more sampling and power to interpret the rotation and the variability of the random cultivar within rotation. The Kenward-Roger degrees of free-

Table 1. Summary of crop rotations and rice cultivars evaluated for false smut and kernel smut severity by year

Year	Rice cropping systems ^x							Rice cultivars			
	1	2/3 ^y	4/5 ^y	6	7/8 ^y	9 ^z	10 ^z	V1	V2	V3	V4
2006	RR	RS	RC	...	...	...	...	Wells	Cybonnet	...	...
2007	RR	RS	RC	...	...	...	...	Wells	XL 723	...	...
2008	RR	RS	RC	RW	RWSW	RSC	RCS	Wells	XL 723	Spring	Spring
2009	RR	RS	RC	RW	RWSW	...	...	Wells	XL 723	Spring	RU0701124

^x Crop rotation abbreviations indicate the rotation-years sampled for false smut and kernel smut. RR = continuous rice, RS = rice-soybean, RC = rice-corn, RSC = rice-soybean-corn, RCS = rice-corn-soybean, RW = rice-wheat, and RWSW = rice-wheat-soybean-wheat.

^y Cropping systems 2/3, 4/5, and 7/8 were paired so that rice was grown every year for each rotation (i.e., when system 2 was planted in rice, system 3 was planted with soybean, but both are identical rice-soybean rotations).

^z Cropping systems 9 and 10 were three-phase rotations where rice was grown once every 3 years. Rice occurred only once (2008) in these rotations during the 4 years of this study. These systems were not paired and differ because rice follows corn in system 9 but follows soybean in system 10.

dom method was used in all variable models. Best linear unbiased predictors and predicted standard errors (SEs) of cultivar were calculated using the SOLUTIONS option. Estimated means, SEs, and differences of means in the fixed effects were calculated using the LSMEANS option. Specific comparisons of fixed effects were calculated via ESTIMATE statements.

Results

False smut and kernel smut disease severities were evaluated from 2006 through 2009 under a variety of crop-management systems. The statistical approach used detected high SEs which were attributed to the inherent variability of each disease, natural infestation of the research plots, environmental variability over the 4-year research period, and the frequency that individual treatment combinations were present over the course of the study. The latter was most important for the presentation of the results that follow, because the frequency in which treatment combinations were measured resulted in unbalanced treatment groups and unequal variances. Therefore, the three-phase rotations that appeared only once in the study had the highest SEs, followed by the wheat rotations (2 years), and the lowest SEs occurred in the rotations (RR, RS, and RC) that were present in all years (Tables 1 and 2). Because unequal variances were present, the letter groupings presented in the tables do not reflect all significant comparisons. To make the presentation of the data the least cumbersome, the additional significant comparisons (treatment combinations with relatively small SEs) are noted in the text and appropriate tables with *P* values.

The statistical analysis of false smut results for the study shows that the main effects of tillage, fertility, and a rotation–tillage interaction significantly affected disease severity (Table 3). Although there were differences in disease severity between crop rotations (Table 2), these differences were not significant at *P* < 0.05 and were due to a high level of variance within each rotation (Table 2, SE). The high SEs resulted from the inherent variability of a naturally infected field experiment conducted over 4 years (see covariance parameter estimates below). The only near-significant comparisons for false smut between rotations occurred when a corn crop was added to the RS rotation (RSC and RCS), which resulted in *P* values of 0.0550 and 0.0523, respectively (Table 4). The rota-

tion–tillage interaction provided more informative comparisons between the paired effects of rotation and tillage (Table 5).

The rotation by tillage interaction was the result of differing effects from tillage treatments on false smut severity among rotations (Table 5). For example, when data were pooled across fertility treatments, the effect of tillage on the two rotations containing a wheat phase (RW and RWSW) was opposite from those that did not contain wheat. RW/NT and RWSW/NT treatment combinations had higher levels of false smut than the tilled plots in the same rotations. This difference was 30% in the RW rotation (RW/NT > RW/TT; LSM diff. = 0.6242, SE = 0.3353, *P* = 0.0646), and 52% in the RWSW rotation (RWSW/NT > RWSW/TT; LSM diff. = 1.2571, SE = 0.2524, *P* < 0.0001). In the remaining two-phase rotation–tillage treatment combinations, NT resulted in lower levels of false smut than TT (RR/NT < RR/TT, *P* = 0.0181; RS/NT < RS/TT, *P* < 0.0001; RC/NT < RC/TT, *P* < 0.0001). The reduction in false smut severity from NT among these rotations was 21% in RR, 50% in RS, and 51% in RC. No significant differences were observed in the three-phase rotation by tillage treatment combinations (RSC/TT-RSC/NT and RCS/TT-RCS/NT). The three-phase rotations had numerically less false smut in the NT treatment but this difference was not significant due to the comparatively high SEs relative to the low levels of disease (Table 5). Although the tillage effect in wheat rotations was opposite from the corresponding summer crop rotations, greater differences were observed in rotations containing a soybean phase (RS/TT > RS/NT = 50% and RWSW/NT > RWSW/TT = 52%) than when a winter wheat crop was added to an RR rotation (RR/TT > RR/NT = 21% and RW/NT > RW/TT = 30%).

Table 3. Analysis of variance summary of *P* values for false smut and kernel smut for the fixed effects of rotation, tillage, fertility, and their interactions

Source of variation	<i>P</i> values	
	False smut	Kernel smut
Rotation	0.3498	0.0180
Tillage	0.0002	0.2782
Fertility	<0.0001	0.0141
Tillage × fertility	0.5084	0.4066
Rotation × tillage	<0.0001	0.9888
Rotation × fertility	0.8189	0.9580
Rotation × tillage × fertility	0.3709	0.8782

Table 2. Rice false smut and kernel smut severity under different crop rotations^w

Rotation ^x	LSM ^y	SE ^y	LSM grouping ^z
False smut			
RS	2.8104	0.6350	A
RC	2.1986	0.6349	A
RR	1.9929	0.6349	A
RW	1.8001	0.8029	A
RWSW	1.7830	0.7928	A
RSC	0.5823	0.8106	A
RCS	0.5522	0.8106	A
Kernel smut			
RWSW	4.6494	0.6581	A
RW	3.8843	0.6712	AB
RS	2.9669	0.5059	AB
RC	2.9070	0.5058	AB
RR	2.7165	0.5058	AB
RCS	1.0812	0.7027	B
RSC	0.8387	0.7027	B

^w Disease severity = the number of infected kernels per kilogram of harvested seed. Disease severity values for each rotation are inclusive of both fertility and tillage treatments.

^x RR = continuous rice, RS = rice-soybean, RC = rice-corn, RSC = rice-soybean-corn, RCS = rice-corn-soybean, RW = rice-wheat, and RWSW = rice-wheat-soybean-wheat.

^y Least squares mean (LSM) of log-transformed disease severity values and standard error of the mean (SE).

^z Conservative letter groupings of differences of LSMs were derived from log-transformed data for the multiple comparisons and use of the Tukey-Kramer *P* value adjustment. Within a treatment group, values followed by the same letter are not significantly different at $\alpha = 0.05$.

Table 4. Comparisons of rice false smut and kernel smut severity between specific crop rotations^x

Rotation comparison ^y	Difference ^z	SE ^z	<i>P</i> value
False smut			
RR, RR vs. RW	0.1928	1.0236	0.8544
RS, RS vs. RWSW	1.0274	1.0157	0.3363
RS vs. RC	0.6118	0.8980	0.5124
RC vs. RSC	1.6163	1.0297	0.1467
RC vs. RCS	1.6464	1.0297	0.1400
RS vs. RSC	2.2281	1.0297	0.0550
RS vs. RCS	2.2583	1.0297	0.0523
Kernel smut			
RR vs. RW	-1.1678	0.8305	0.1863
RS vs. RWSW	-1.6826	0.8199	0.0649
RS vs. RC	0.05986	0.7036	0.9340
RC vs. RSC	2.0684	0.8562	0.0307
RC vs. RCS	1.8259	0.8562	0.0521
RS vs. RSC	2.1282	0.8562	0.0269
RS vs. RCS	1.8857	0.8562	0.0458

^x Disease severity = the number of infected kernels per kilogram of harvested seed. Disease severity values for each rotation are inclusive of both fertility and tillage treatments.

^y RR = continuous rice, RS = rice-soybean, RC = rice-corn, RSC = rice-soybean-corn, RCS = rice-corn-soybean, RW = rice-wheat, and RWSW = rice-wheat-soybean-wheat.

^z Difference = Difference in least squares means within individual comparisons as calculated via ESTIMATE statement. SE = standard error of the difference of means.

All comparisons of tillage within rotation for false smut, where additional observations were made, are listed in Table 5. Most notable are the RS/TT and RC/TT treatment combinations that had the highest levels of false smut. The lowest levels of false smut were observed in the three-phase rotations, where disease severities were reduced by 80% (RSC/TT) or more (in RCS/TT, RSC/NT, and RCS/NT treatment combinations) compared with RS/TT (RSC/TT < RS/TT; LSM diff. = 2.9866, SE = 1.0422, $P = 0.0157$). These data also show that incorporating wheat into an RS/TT treatment combination reduced the severity of false smut by 69% (RWSW/TT < RS/TT; LSM diff. = 2.5815, SE = 1.0273, $P = 0.0304$). However, this effect was lost with the NT treatment because false smut severities were similar in RWSW/NT and RS/NT. Finally, inclusion of winter wheat in continuous rice cropping had no effect on false smut severity regardless of rotation–tillage treatment comparisons (RW[TT or NT] versus RR[TT or NT]).

The fertility treatment effect was consistent across all other treatments (no significant interactions, Table 3); therefore, a study-wide value for the reduction in false smut severity attributable to reduced fertility was a relevant estimate. The low-fertility treatment reduced the severity of false smut by 20% compared with the high-fertility treatment across all rotation and tillage treatment

combinations. Comparisons of fertility treatments within rotation are presented in Table 6. The fertility treatment was significant within all two-phase rotations. The reduction in false smut severity from the low fertility treatment was 27% in RW, 19% in RC, 18% in RS, and 15% in RR (P values = 0.0361, 0.0011, <0.0001, and 0.0190, respectively). The remaining rotations (RSC, RCS, and RWSW) followed the same trend of reduced levels of false smut in the low-fertility treatment compared with the high-fertility treatment but these differences were not significant (Table 6).

The greatest level of false smut in the high fertility–rotation treatment combinations occurred in the RS rotation (Table 6, RS/H). The lowest levels were in the RSC and RCS rotations, where both fertility treatments were significantly lower than the RS/H treatment combination. Three-way comparisons of rotation–tillage–fertility show that the RS/TT/H treatment combination had the highest levels of false smut (LSM = 4.1275) for the entire study while RSC/NT/L and RCS/NT/L had the lowest (LSMs = 0.3973 and 0.3466, respectively), which represented a 91% average reduction in false smut.

Table 5. Comparison of tillage by rotation treatment effects on disease severity within each crop rotation for rice false smut and kernel smut^a

Rotation ^y	Tillage ^w	LSM ^x	SE ^x	Group ^y
False smut ^z				
RS	TT	3.7360	0.6410	A
RC	TT	2.9516	0.6410	AB
RWSW	NT	2.4116	0.8028	ABC
RR	TT	2.2202	0.6410	ABC
RW	NT	2.1122	0.8034	ABC
RS	NT	1.8849	0.6411	ABC
RR	NT	1.7656	0.6410	ABC
RW	TT	1.4880	0.8367	ABC
RC	NT	1.4457	0.6410	BC
RWSW	TT	1.1545	0.8028	BC
RSC	TT	0.7494	0.8243	BC
RCS	TT	0.7145	0.8243	BC
RSC	NT	0.4152	0.8243	C
RCS	NT	0.3899	0.8243	C
Kernel smut				
RWSW	TT	4.8074	0.6740	A
RWSW	NT	4.4915	0.6740	AB
RW	TT	4.0345	0.7191	ABC
RW	NT	3.7341	0.6744	ABC
RS	TT	3.0785	0.5174	ABC
RC	TT	3.0331	0.5174	ABC
RS	NT	2.8553	0.5176	BCD
RR	TT	2.8109	0.5174	BCD
RC	NT	2.7809	0.5174	BCD
RR	NT	2.6221	0.5174	CD
RCS	NT	1.0854	0.7229	DE
RCS	TT	1.0769	0.7229	DE
RSC	TT	0.9751	0.7229	DE
RSC	NT	0.7023	0.7229	E

^a Tillage by rotation values include both fertility treatments. Disease severity = the number of infected kernels per kilogram of harvested seed.

^y RR = continuous rice, RS = rice-soybean, RC = rice-corn, RSC = rice-soybean-corn, RCS = rice-corn-soybean, RW = rice-wheat, and RWSW = rice-wheat-soybean-wheat.

^w TT = traditional soil tillage and NT = no-tillage.

^x Least squares mean (LSM) of log-transformed disease severity values and standard error of the mean (SE).

^y LSM grouping. Conservative letter groupings of differences of LSM were derived from log-transformed data for the multiple comparisons and use of the Tukey-Kramer P value adjustment. Within a treatment group, values followed by the same letter are not significantly different at $\alpha = 0.05$.

^z Additional significant comparisons for false smut: RS-TT, RS-NT: LSM diff. = 1.8512, SE = 0.1764, P value < 0.0001; RC-TT, RC-NT: LSM diff. = 1.5059, SE = 0.1758, P value < 0.0001; RWSW-NT, RWSW-TT: LSM diff. = 1.2571, SE = 0.2524, P value < 0.0001; and RR-TT, RR-NT: LSM diff. = 0.4546, SE = 0.1758, P value = 0.0181.

Table 6. Comparison of fertility by rotation treatment effects on disease severity within each crop rotation for rice false smut and kernel smut^a

Rotation ^u	Fertility ^v	LSM ^w	SE ^w	Group ^x
False smut ^y				
RS	H	3.0809	0.6389	A
RS	L	2.5400	0.6389	AB
RC	H	2.4294	0.6388	AB
RR	H	2.1582	0.6388	AB
RW	H	2.0795	0.8138	AB
RC	L	1.9678	0.6388	AB
RWSW	H	1.9656	0.8011	AB
RR	L	1.8276	0.6388	AB
RWSW	L	1.6005	0.8011	AB
RW	L	1.5208	0.8138	AB
RCS	H	0.6891	0.8226	B
RSC	H	0.6458	0.8226	B
RSC	L	0.5189	0.8226	B
RCS	L	0.4152	0.8226	B
Kernel smut ^z				
RWSW	H	4.8758	0.6712	A
RWSW	L	4.4231	0.6712	AB
RW	H	4.1122	0.6879	ABC
RW	L	3.6564	0.6879	ABC
RC	H	3.2325	0.5137	ABC
RS	H	3.2130	0.5137	ABC
RR	H	3.0006	0.5137	BCD
RS	L	2.7208	0.5139	BCDE
RC	L	2.5815	0.5137	CDE
RR	L	2.4324	0.5137	CDEF
RCS	H	1.3020	0.7203	DEF
RSC	H	0.9988	0.7203	EF
RCS	L	0.8603	0.7203	EF
RSC	L	0.6785	0.7203	F

^a Fertility by rotation values include both tillage treatments. Disease severity = the number of infected kernels per kilogram of harvested seed.

^u RR = continuous rice, RS = rice-soybean, RC = rice-corn, RSC = rice-soybean-corn, RCS = rice-corn-soybean, RW = rice-wheat, and RWSW = rice-wheat-soybean-wheat.

^v H = high fertility rate and L = low fertility rate.

^w Least squares mean (LSM) of log-transformed disease severity values and standard error of the mean (SE).

^x LSM grouping. Conservative letter groupings of differences of LSM were derived from log-transformed data for the multiple comparisons and use of the Tukey-Kramer P value adjustment. Within a treatment group, values followed by the same letter are not significantly different at $\alpha = 0.05$.

^y Additional significant comparisons for false smut: RS-H, RS-L: LSM diff. = 0.5409, SE = 0.1411, P value < 0.0001; RC-H, RC-L: LSM diff. = 0.4616, SE = 0.1403, P value = 0.0011; RR-H, RR-L: LSM diff. = 0.3306, SE = 0.1403, P value = 0.0190; and RW-H, RW-L: LSM diff. = 0.5587, SE = 0.2656, P value = 0.0361.

^z Additional significant comparisons for kernel smut: RC-H, RC-L: LSM diff. = 0.6510, SE = 0.1792, P value = 0.0029; RS-H, RS-L: LSM diff. = 0.4922, SE = 0.1799, P value = 0.0165; and RR-H, RR-L: LSM diff. = 0.5681, SE = 0.1792, P value = 0.0072.

Covariance parameter estimates for false smut show that 30% of the total experimental variance was due to year, 44% due to cultivar within rotation, and 26% due to experimental error. The annual variation in disease severity was a useful measure because all research plots were naturally infected with false smut. Plots of false smut severity values by year show consistent trends for rotations evaluated in all 4 years (RR, RS, and RC; Fig. 1A). Years 2006 and 2009 had the highest levels of false smut, whereas 2007 and 2008 were the lowest years tested. The low levels of false smut in 2008 were attributed to early planting and harvest, because severe out-

breaks of false smut were recorded on late planted rice that year (*data not shown*).

Kernel smut severity was also evaluated using the same study, time frame, and experimental treatments as above. The statistical analysis shows that the main effects of fertility and rotation were significant and there were no interactions (Table 3). Tillage was not detected as a significant main effect for kernel smut (Table 3); however, there was a consistent trend of lower disease severity in the NT treatment combinations when compared with the TT combinations (Table 5). Fertility effects for kernel smut were con-

Fig. 1. Log-transformed smut severity values (number of symptomatic kernels per kilogram of harvested seed; Y-axes) plotted by each year of the study (X-axes) for continuous rice (RR), rice-soybean (RS), and rice-corn (RC) rotations. **A**, False smut and **B**, kernel smut plots show the range of values obtained within rotation by year.

sistent across all rotation and tillage treatment combinations, and the study-wide value for the reduction in kernel smut due to low fertility was 16% ($H > L$; LSM diff. = 0.4831, SE = 0.1298, $P = 0.0141$). Comparisons of fertility treatments within rotation are presented in Table 6. Fertility comparisons were significant within RR ($H/L = 19\%$; $P = 0.0072$), RS ($H/L = 15\%$; $P = 0.0165$), and RC ($H/L = 20\%$; $P = 0.0029$) rotations. The remaining rotations followed the same trend of reduced levels of kernel smut in the low-fertility treatment but these differences were not significant (Table 6). Therefore, variability in the magnitude of the fertility effect and corresponding SEs resulted in some significant comparisons, although the trend was consistent over the entire study.

Overall comparisons of crop rotation effects on kernel smut severity, pooled over tillage and fertility treatments, were possible due to the consistent main effects and no interactions (Tables 2 and 4). Crop rotations exhibited distinct effects that were categorized by the crop components of the rotations. For example, both rotations containing a wheat phase (RW and RWSW) had the highest levels of kernel smut, and no other rotation-treatment combinations resulted in kernel smut severities that exceeded the wheat rotation values (Tables 5 and 6). Significant differences were observed between the RWSW and either of the three-phase rotations (RWSW-RCS; LSM diff. = 3.5683, SE = 0.9541, $P = 0.0021$ and RWSW-RSC; LSM diff. = 3.8108, SE = 0.9541, $P = 0.0013$), and the comparison of RWSW to the corresponding spring crop rotation RS was nearly significant ($P = 0.0649$; Table 4). In addition, no differences in kernel smut severities were observed when corn was substituted for soybean in two-phase rotations (RS versus RC), whereas the three-phase rotations significantly reduced kernel smut severity when compared with either of the corresponding two-phase rotations (RSC and RCS versus RS or RC; Table 4).

Covariance parameter estimates for kernel smut show that 55% of the total experimental variance was due to year, 18% due to cultivar within rotation, and 25% due to experimental error. The annual variation in disease severity was an interesting measure because all research plots were naturally infected with kernel smut. Plots of kernel smut severity values by year show consistent trends for rotations evaluated in all 4 years (RR, RS, and RC; Fig. 1B). Years 2006, 2007, and 2009 were consistent and had the highest levels of kernel smut, whereas 2008 was the lowest year tested.

Discussion

Previously (2), we reported an analysis of smut severity in RR and RS rotations. In that report, we focused our attention on continuous rice as an alternative to the standard RS rotation that is used on the majority of rice production acreage in the southern United States. An analysis of the effect of continuous rice cropping on smut diseases was done, because there are circumstances where RS rotations are not used. For example, continuous rice is desirable when market prices make it attractive for farmers to repeat rice crops (28), or where poorly draining soil types on precision-leveled ground make soybean an unproductive rotation component for rice farmers. In these situations, understanding the effects of crop rotation on disease can predict future problems, such as expected increases or decreases in diseases due to alterations to rotation patterns. Or, if disease control is a primary concern, specific rotations can be chosen for field-level disease management for susceptible rice cultivars.

The present study expands upon our previous research to examine additional crop rotation effects on smut severity. Corn is an example of an alternate crop that was not commonly grown in rotation with rice in the Mississippi Delta region of Arkansas but has gained acreage due to interest in biofuels and corresponding market prices in certain years. A high-value year for corn will not likely entice rice farmers to switch to an RC rotation but more likely include corn into an established RS rotation. In this case, a three-phase rotation is created but the crop immediately preceding rice cannot be predicted, and rice may follow soybean or corn (RCS or RSC) in rotation. Wheat is another crop that is not common in the rice-producing regions of Arkansas but is used as a

winter crop particularly when dry fall weather permits ground preparation and planting and when wheat prices are acceptable. Winter wheat poses challenges for rice farmers due to the late harvest of the crop (usually in June), which is well after the normal rice-planting period for the region (usually late April to mid-May). This system (RW) requires short-duration cultivars due to the late planting, which changes both the germplasm and the environmental conditions that are relevant to disease severity. Due to limited availability of short-duration rice cultivars, soybean generally follows wheat in rotation (RWSW) due to a greater selection of cultivars and the ability to plant soybean onto the wheat beds without soil tillage.

Previous reports (2,25,29) have shown that fertility is the most important agronomic factor influencing smut diseases, where high nitrogen fertilizer input positively affects smut severity. The present work is in agreement with these findings and, in our previous work (2), we suggested that the differences in disease severity from tillage and rotation treatments were caused by buffering N availability (NT and RR) or nitrogen enrichment of the soil (RS). Therefore, the effect from rotation was closely linked to the rotation's effect on N accumulation or availability. The same phenomenon holds true for the present work, where field history has a greater role in N accumulation and availability than fertilizer applied during the rice cultivation year.

RS, RC, and RR ranked as the three highest rotations in terms of false smut severity. False smut severity was comparable in RS, RC, and RR within tillage groups and, in all three rotations, false smut was significantly higher with TT than NT. The high levels of false smut recorded in RS-TT may be attributed to nitrogen fixation by the soybean crop and in RC-TT by residual nitrogen in the soil from high N fertilizer levels used on corn. Soil organic matter accumulation buffers N availability to rice plants (2,21,24) and may be responsible for the reduction in false smut severity due to NT, but we did not collect soil samples and, thus, cannot confirm these hypotheses.

In contrast, the three-phase rotations had the lowest smut severities regardless of fertility or tillage treatments. Although tillage and fertility within RSC and RCS followed the same trends as expected ($H > L$, $TT > NT$) for both diseases, these differences were not significant. This shows that fertility effects and N availability due to tillage were similar to those in RS and RC rotations. It also shows that high N fertility rates can be used in three-phase rice rotations without the risk of significant smut damage. For instance, on traditionally tilled soil, adding a year of corn into a soybean rotation will reduce both false smut and kernel smut (RS/TT versus RSC/TT or RCS/TT) even with high N input. Based upon this evidence, we hypothesize that rotating out of rice for 2 years may be reducing the amount of viable smut inoculum in the soil, because there is no reason to suspect limited N availability in the three-phase rotations. However, the RSC and RCS rotations occurred only once (2008) over the course of the 4-year study. False smut severity was least in 2008 but comparable with 2007 and 2009, and kernel smut severity in 2008 was noticeably lower than all other years tested (Fig. 1). Therefore, the apparent reduction of disease severity in the three-phase rotations may have been influenced by the overall lower values recorded in 2008.

The effects from corn in rotation with rice are especially interesting for false smut because corn is an alternative host for *U. virens*. In rotations where corn was present, the alternative host did not appear to have any impact on disease severity in subsequent rice crops. Although the degree of susceptibility to *U. virens* in the corn hybrids used is unknown, very little false smut was observed in corn tassels over the course of the study (S. Brooks's field observations). *U. virens* is a minor disease of corn (30), and our data from rice plots immediately following corn in rotation (RC or RSC) show that an alternative host in rotation with rice had no detectable influence on false smut.

Incorporating wheat into continuous rice (RW) or an RS rotation (RWSW) had interesting impacts on false smut severity. False smut severity was consistent in the NT system for RR, RS, and the

corresponding wheat-containing rotations RW and RWSW, whereas false smut severity was not consistent among these rotations in TT; most notably, the 69% reduction of false smut in RWSW/TT compared with RS/TT. Moreover, TT reduced false smut in both rotations containing a wheat phase relative to NT (TT < NT; RW $P < 0.0646$ and RWSW $P < 0.0001$). At this time, we have no explanation for the interaction of wheat and tillage on false smut severity.

Adding a wheat phase to rice rotations had a significant effect on kernel smut severity. In this case, the analysis was simpler because there were no significant interactions and disease severity was consistent across tillage treatments. It appears that the high kernel smut severities were the result of susceptibility in rice entry RU0701124 (on average, 238 smutted kernels per kilogram of seed). As stated above, rice grown in rotation with wheat requires short-duration rice cultivars that are grown later in the cropping season. The combination of susceptibility (in the short-season Spring also) and, possibly, late-season conditions conducive to the disease produced high kernel smut values for the wheat rotations. Therefore, the high levels of kernel smut in wheat rotations may have been the result of germplasm selection and change in environment.

There were several consistent trends within our results that are noteworthy. As mentioned above, the three-phase rotations present the best management strategy for both diseases, where the highest levels of disease control were achieved without confounding factors such as tillage or fertility. In addition, the three-phase rotations had remarkably consistent results for both diseases and both fertility and tillage treatments. Similarly, the RR system also produced consistent results throughout the study. Although disease severity was higher in RR than the three-phase rotations, severity was constant across other treatments. In general, the NT treatment also produced consistent results between rotation and fertility treatment combinations. Finally, the kernel smut disease severity was consistent, where tillage had little effect and fertility effects were similar across rotations. This contrasts with false smut, where disease severity was more erratic within and between rotation and tillage treatments. This was evident in the covariance parameter estimates, where variance due to cultivar was high for false smut (44%) and lower for kernel smut (18%). In contrast, there was greater variance due to time for kernel smut (55%) than for false smut (30%).

This report concludes our research on smut disease control in susceptible rice cultivars using crop-management approaches. We have analyzed a number of agronomic factors that influence smut severity for three reasons: (i) to provide information on disease control for current economically important rice cultivars, because cultivars that occupy the majority of production acreage in the southern United States are all susceptible to grain smuts; (ii) to predict the positive or negative affects of alternate cropping systems on disease severity; and (iii) to use the most important factors for smut disease severity (rotation and fertility) to develop nurseries that maximize disease severity for resistance screening. The final reason is addressed in another report on smut resistance in rice. In this present work and our previous reports (2,3), we identified a number of conditions where smut diseases were controlled in rice cultivation without the use of fungicides or losses in yield. Incorporating NT will require a significant departure from standard practices in the southern United States. However, moderating the use of N fertilizer and three-phase rotations provide the highest levels of smut control and require little additional effort from rice producers. Finally, our results demonstrate the importance of field history influences on smut disease severity. Crop-management decisions are often based upon current-year conditions and, although current production practices are important, the previous rotation crops and tillage practices also have a significant influence on smut severity.

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