Reactions of Selected Rice Cultivars
to *Ustilaginoidea virens* in Arkansas

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**ABSTRACT**

False smut, caused by *Ustilaginoidea virens*, was found in Arkansas in 1997 and the disease has now been identified in most counties in which rice is grown. The disease is normally identified by the presence of orange and black sori (= spore balls, pseudomorphs) that appear on the maturing heads or panicles. The disease cycle for this emerging disease of rice is poorly understood and its erratic nature on many different cultivars across locations has hampered the development of effective management strategies for this disease. In 2012, among all of our tests, we conducted a preliminary experiment at one location in Arkansas in which five selected cultivars were planted in replicated plots for the purpose of investigating their reaction to false smut. In this test, we selected a site in which the soil was uniform. We determined the number of infected panicles/m² (= incidence) twice after the first appearance of the sori and before harvest from each of the plots. We also determined the number of sori per panicle (= severity) in order to compare the severity and incidence of sori on panicles for the five cultivars over time. The results of these investigations are similar to results we obtained in 2011 with a larger number of cultivars. The data suggest that there are differences in the reaction among the selected cultivars in response to seedborne and soilborne inoculum of the fungus. Resistance or tolerance may be a useful strategy in understanding and managing this emerging disease of rice in Arkansas.

**INTRODUCTION**

False smut of rice is caused by the fungus *Ustilaginoidea virens*. This pathogen has been in the United States for many years, but was first reported in Arkansas in 1997 (Cartwright and Lee, 2001; Wilson et al., 2005). It has been previously reported that
this disease does not typically affect yield, but quality issues remain important due to production of ustiloxin, a microtubule inhibitor toxic to animals (Koiso et al., 1992; Miyazaki et al. 2009). More recently, the literature suggests that yields can be significantly reduced (Hedge and Anahosur, 2000; Zhou et al., 2003).

Knowledge concerning the disease cycle and epidemiology of *U. virens* is minimal and incomplete (Guo et al., 2012; Lee and Gunnell, 1992). Recent research conducted by Ashizawa et al. (2010), Ditmore and colleagues (2006, 2007), Ikegami (1963), Schroud and TeBeest (2006), TeBeest et al. (2011), and Zhou et al. (2003) suggests that rice plants may be colonized from seedborne and/or soilborne inoculum within a few weeks after emergence. There is growing evidence than flowers can be infected by injection of spores into the boots prior to their emergence (Ashizawa et al., 2011; Tang et al., 2012). Fungicides, applied at heading may only suppress the disease from developing.

Disease resistance is an important tool in managing false smut. It has been widely suggested that the number of sori found on mature panicles or the degree of blanking (= chaffing) varies according to cultivar and therefore may be related to the level of resistance in the cultivar (Cartwright et al., 1999a; Hedge and Anahosur, 2000; Lu et al., 2009). The methods used to evaluate resistance have measured the occurrence of sori in several ways, including number of sori per panicle (Branson et al., 2009; Cartwright et al., 1999b; Hedge and Anahosur, 2000), the maximum number of sori per head (Cartwright et al., 1999a), and the number of sori per pound of harvested grain (Brooks et al., 2009, 2010; Parsons et al., 2004). In addition, Hedge and Anahosur (2000) and Lu et al. (2009) developed disease scoring systems that contain several categories based on the number of sori per panicle. The categories generally describe the disease severity from 0 sori/panicle to a category that includes 10 or more sori /panicle.

Brooks et al. (2009, 2010) reported that the severity of disease on several selected cultivars may also depend on soil fertility and flood water depth. In 2011, TeBeest et al. reported that the occurrence of sori on panicles also differed according to location and cultivar. Many rice cultivars grown in Arkansas were evaluated or rated for resistance to false smut between 2001 and 2009. The rating system was largely based on the number of sori produced per panicle, the number of sori per pound of harvested seeds, and/or on historical observations (Branson et al., 2009; Cartwright et al., 2002; Robinson et al., 2010). Many of the cultivars and breeding lines rated for resistance to false smut during these years were rated as very susceptible, susceptible, or moderately susceptible. However, Bengal, CL121, Jefferson, Kaybonnet, Katy, Koshihikari, M201, M202, Newbonnet, and Saber were rated as moderately resistant to false smut (Cartwright et al., 1999a, 1999b; Cartwright et al., 2000a,b; Cartwright and Lee 2001; Parsons et al., 2004).

In addition to the cultural effects on disease severity, Lu et al. (2009) suggested that there are six pathogenicity groups among the 59 isolates of *U. virens* they tested. Resistance to false smut expressed by three hybrids differed significantly among the isolates tested. They suggested that disease assessments, based on the ability of the isolates to produce sori on the panicles of these three hybrids, could be used to further differentiate the isolates of *U. virens* for virulence to rice. Clearly, the interactions of disease assessments, cultural conditions, cultivar resistance, and the potential role of pathotypes must be understood before effective management tools can be developed.
The overall goal of our research on false smut is to gain a clearer understanding of the disease cycle as it is expressed in Arkansas and of how the disease spreads and develops on some of the current cultivars grown in Arkansas in order to facilitate improving our current management strategies. As part of this overall goal, the specific objective of the work reported here was to evaluate five selected cultivars that had previously shown differences to infection by false smut with two specific sub-objectives: 1) to quantify the number of heads infected by false smut per unit area over time, and 2) to examine the number of sori produced on the panicles of the selected cultivars.

PROCEDURES

Five rice cultivars were selected for these field tests based on the results previously conducted at the Newport Research Station, Newport, Ark., and the Pine Tree Research Station, Colt, Ark., in 2011. Seeds of the five cultivars (Katy, Kaybonnet, Neptune, Taggart, and CL151) were harvested from our research plots in 2011. All seedlots were visibly infested with sori of *U. virens*. Some seeds were also visibly contaminated (blackened) with false smut spores.

Four, 400-gram samples of seeds of each of the five cultivars were prepared and placed individually in envelopes. Treatments (cultivar) were planted in a field with a history of false smut in a randomized complete block design with 4 replications of each treatment. Plots were 5 ft (1.5 m) wide and 100 ft (30.5 m) long and consisted of 7 rows with 7-inch (17.8-cm) spacing between rows. The design of the test (randomization and length of each plot) was intended to minimize differences that might occur within the area with respect to soil fertility (pH, EC, macro- and micro-nutrient levels) which can affect incidence of disease (Brooks et al., 2009, 2010). These data are shown in Table 1. There were no additional seed treatments or inoculations made at any time. Due to the limited space available within a field in which the soil was uniform and the number of times we were able to quantify the disease after first appearance, we can only consider this data as preliminary in nature.

Plots were planted on 18 May 2012 at Pine Tree and seedlings began to emerge on 25 May 2012. Plots were treated with several herbicides, including 0.5 lb/acre Facet and 2 oz/acre Permit on 18 May 2012 and 4 qt/acre Stam and 0.5 oz/acre Permit on 30 May. In addition, 0.8 oz/acre Clincher and 0.25 lb/acre of Facet were applied on 10 July 2012. In addition, plots were treated 150 units of nitrogen (362 lb/acre urea) applied pre flood on 21 June 2012. The plots were put into permanent flood on 22 June 2012. Plots were drained on 1 Oct. 2012 and all plots were harvested on 21 October 2012.

Symptom Development, Disease Incidence and Severity, and Collection of Infected Panicles

In order to determine when signs and/or symptoms of false smut appeared in the tests on the five cultivars, all 20 plots at Pine Tree were examined daily beginning in mid-August, 2012, with the onset of booting of the first cultivar. Data on disease incidence (panicles/m²) were recorded within one week of first appearance of false smut sori and with a final determination on all cultivars in late September to permit
full expression of the disease on all cultivars. Disease surveys of all plots began at booting and the average number of infected panicles/m² was determined for each plot by counting the number of infected panicles/m² by two experienced investigators. Two random counts were made at six locations within each plot in rows exclusive of edge rows. These counts were averaged for each location within each plot.

Collection of Mature Infected Panicles

After all cultivars reached maturity and after the data on the incidence of false smut were collected, 15 to 20 infected panicles were collected at random from the center rows within each plot. The panicles were pooled as a collection for each plot (treatment) and placed in paper bags, then placed in boxes before transport to the laboratory. In the laboratory, the number of sori/panicle was determined by counting the number of typical sori on 10 randomly selected panicles from each individual bag (plot). Thus, we collected 4 replications of 10 infected panicles from each treatment.

Statistical Analyses

The design for this experiment was a randomized complete block design with four replications of each treatment. An analysis of variance of the means of each treatment was conducted using PROC GLM of SAS v. 9.2 (SAS Institute, Inc., Cary, N.C.) and a least significant difference test (LSD) of the means was used to separate differences at P = 0.05 for the dependent variables (infected panicles/m² and across the two sample times and sori/panicle).

RESULTS

Symptom Development

As expected, visible symptoms of infection did not appear on any of the cultivars used in the study at any time until 31 August 2012, when young sori were still encased within a membrane and a few orange sori were found developing on a few panicles already at the flowering stage. The infected plants were distributed throughout the test although there were differences between treatments. There were no indications of disease aggregation at this time.

Reaction of Selected Rice Cultivars

Grown in Arkansas to Infection by *U. virens*

The average number of panicles visibly infected by false smut/m² was collected twice before harvest, once on 6 September 2012 and again on 24 September, 6 and 24 days after the first appearance of sori, respectively. Disease levels were assessed as described above by counting the number of visibly infected heads at multiple locations within each plot and these data were then averaged for each plot. A panicle was considered infected if it had a clearly identifiable sorus.
Analysis of variance shows that there were very significant differences between the cultivars for the number of infected panicles/m² for the data collected for both collection dates (Table 2). In Table 3, the mean numbers of infected panicles/plot show a wide and statistically significant range of incidence of infection by false smut at Pine Tree. On 6 Sept., the incidences of infection ranged from 0.75 panicles/m² on Katy to more than 16 infected panicles/m², in some plots, on CL151. On 6 Sept., there were no significant differences in disease incidences between Katy, Kaybonnet, and Taggart, and no differences between Taggart and Neptune. In comparison, the incidence of infection was very significantly different for CL151 and all other cultivars.

The results of the survey conducted on 24 Sept. showed that there had been a general increase in the number of infected panicles/m² for all cultivars when compared to the levels found 18 days earlier, on 6 Sept. For the data collected on 24 Sept., there were no significant differences between Katy, Kaybonnet, Taggart, and Neptune; although the levels of infection were visibly higher on both Taggart and Neptune than on either Katy or Kaybonnet. This was probably due to variances between replications and samples among the data for Taggart and Neptune. In sharp contrast to these cultivars there were, on average, more than 18 infected panicles/m² on CL151, in contrast to only 1.33 to 7.33 infected panicles/m² on the other four cultivars.

The data in Table 3 also suggest that there might have been a significant increase in the number of infected panicles/m² for four of the cultivars on the second sampling date when the data is compared to the data from the first sampling date. However, statistical evaluation of the data between sampling times for all five cultivars revealed that there was no significant increase in disease over time (Table 2). Examination of the data revealed significant overlapping of the incidence data for the different sampling stations within each plot over time for all cultivars. Taken together, the data suggest that there was a rapid increase in disease incidence between the time of its first appearance in the plots and 6 days later and an insignificant increase in disease between 6 and 24 days after the first appearance of the disease in this test. We also found no evidence of interplot interferences due to dispersal of spores after examining the data according to position in the field plots. Similarly, adjacent field tests in which 12 susceptible cultivars (including these five cultivars) had been planted did not appear to have been affected by the close proximity (3 m) to this test.

The data in Table 1 shows that there were significant differences between treatments (= cultivars) in the average number of sori/infected panicle at \( P = 0.05 \). In Table 2, the data clearly indicates a significant difference between CL151 and the other four cultivars tested. In addition, Katy and Kaybonnet were the only cultivars with an average of fewer than 2 sori/panicle and these two cultivars were the only two cultivars that satisfied the requirements for a disease rating of 1 according to the Lu et al. (2009) scale. All three of the other cultivars were rated as a 2 although CL151 nearly had a rating of 3 according to this scale. We did observe some panicles with more than 9 sori/head on this cultivar. Despite our precautions in site selection, there may have been replication effects originating from overwintering inoculum or soil characteristics that may have affected the incidence of false smut and the number of sori/panicle (Table 2).
DISCUSSION

To our knowledge, this is the first report on the development of the disease on the basis of the time required for false smut sori to appear and reach a maximum incidence levels on cultivars grown in Arkansas. The disease progress curve for false smut in this test was very steep and different from the disease progress curves we have noted for rice blast or anthracnose diseases of grain sorghum and northern jointvetch and *Alternaria* on *Anoda cristata*, other diseases that have significant secondary dispersal and infection cycles (Li and TeBeest, 2009; Long et al., 2001; Moore et al., 2010; Yang and TeBeest, 1993). The cultivars used in this study were previously described as susceptible or moderately susceptible and all of the cultivars grown developed signs of infection. However, it was visibly and statistically evident that there were widely different levels of incidence and severity of false smut among the cultivars at Pine Tree in 2012. In general, Katy and Kaybonnet appeared to be the more ‘resistant’ or ‘tolerant’ of the five cultivars tested under the conditions in which they were grown at Pine Tree in 2012.

In the absence of visible symptoms of infection prior to heading, many of the previous studies described above have estimated the relative resistance of cultivars on the basis of the development of visible signs of infection on the panicles or the number of infected heads/unit area. Others have measured the number of sori found in harvested grain. In this study, we collected data on two dependent variables: the number of infected panicles/m\(^2\) in individual field plots and the number of infected panicles from each of 5 cultivars planted, which were previously described as either susceptible or moderately susceptible to *U. virens*. Analysis of variance indicated that there were significant differences in the incidence of false smut between the treatments (cultivars) when measured within 6 days and 24 days after the initial appearance or emergence of the smut sori. The two data sets, gathered 18 days apart from the same general areas of the same plots, are presented as separate events. Statistical evaluation of the incidence data suggests that there were no significant increases in disease incidence for any cultivar after the initial data taken 6 Sept. It is relatively clear from these preliminary data that additional work on the epidemiology of false smut is necessary. Studies to define the rate of increase in the emergence of sori over time in relation to the emergence of panicles from the boots and relative to the levels of resistance within cultivars, would be beneficial. In addition, since the incidences of infection for these five cultivars were much higher in 2011 than in 2012 when measured with the same techniques, it raises questions concerning the role and source of primary inoculum, cultural conditions, and environmental effects on the final incidence of this disease.

Hedge and Anahosur (2000) and Lu et al. (2009) attempted to describe susceptibility of different cultivars on the basis of the number of spore balls/panicle. In 2011, TeBeest et al. used a similar assessment tool and a larger number of cultivars and found that there were statistically significant differences in the number of false smut sori on panicles for the cultivars used in that study. The data in this report also show statistically significant differences in both the severity (= sori/panicle) and in the incidence (= number infected panicles/m\(^2\)) of false smut on the five cultivars tested in 2011 at Newport, a location consistently more conducive to false smut development.
SIGNIFICANCE OF FINDINGS

False smut is an emerging and increasingly significant pathogen of rice in Arkansas. Although first reported in a single field in White County, Ark., in 1997, it is now considered to be widespread within the state. Disease resistance is a mainstay of managing many plant diseases. Finding germplasm that demonstrates resistance or tolerance to false smut across the different soil and environmental conditions in the state may be crucial toward successful and integrated management practices. Based on the preliminary data in this test and on the evidence already in the literature for false smut and other diseases, we have begun to develop the methodologies necessary to identify and evaluate germplasms across locations and fields with reasonable assurances of success. In that sense, the results of work conducted in 2011 and 2012 continues to warrant further investigation if they remain inconsistent over time and location.

Given that infestations of seed and soil with viable spores can lead to infections, we need to understand the roles that inoculum, cultivar genetics, and soil fertility may have on the general significance of false smut in Arkansas. The possibility that pathotypes of this fungus may already exist in Arkansas, although as yet not described, must not be overlooked. Lastly, the epidemiology of this disease is poorly understood. Further work on the disease progress curves across cultivars and locations in relation to flowering could provide useful information regarding management of this disease with fungicides or disease resistance.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of the Arkansas Rice Research and Promotion Board and the Director of the University of Arkansas Agriculture Experiment Station. We are grateful for the seeds of the test cultivars provided by R. Cartwright and K.A.K. Moldenhauer and the Arkansas Rice Foundation Seed program. The authors are indebted to J. Velie, Systems Analyst, University of Arkansas Agricultural Statistics Laboratory, for his assistance with the statistical analyses and Jody Hedge for his vigilance and dedication to maintaining our research plots at the Pine Tree and Newport Research Stations.

LITERATURE CITED


Table 1. Results of analysis of soil samples collected from the field plots at the Pine Tree Research Station, Colt, Ark., in 2012.

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample†</th>
<th>pH</th>
<th>EC</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
<th>Na</th>
<th>Fe</th>
<th>Mn</th>
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</thead>
<tbody>
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<td>6.9</td>
<td>107</td>
<td>66.1</td>
<td>123</td>
<td>1566</td>
<td>245</td>
<td>18.6</td>
<td>46.5</td>
<td>560</td>
<td>111</td>
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<td></td>
<td>2</td>
<td>6.5</td>
<td>114</td>
<td>79.5</td>
<td>138</td>
<td>1565</td>
<td>230</td>
<td>28.0</td>
<td>47.7</td>
<td>587</td>
<td>97</td>
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<td>3</td>
<td>7.0</td>
<td>122</td>
<td>53.3</td>
<td>111</td>
<td>1965</td>
<td>289</td>
<td>21.7</td>
<td>47.6</td>
<td>536</td>
<td>439</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.6</td>
<td>105</td>
<td>70.9</td>
<td>121</td>
<td>1648</td>
<td>265</td>
<td>16.1</td>
<td>43.1</td>
<td>594</td>
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<td>128</td>
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<td>1708</td>
<td>284</td>
<td>22.1</td>
<td>53.9</td>
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<td>6</td>
<td>6.8</td>
<td>120</td>
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<td>270</td>
<td>20.1</td>
<td>43.1</td>
<td>590</td>
<td>127</td>
</tr>
</tbody>
</table>

† All data based upon six replicated samples collected on 6 June 2012 and analyzed by the Agricultural Diagnostic Laboratory, University of Arkansas, Fayetteville. Values for EC and pH (1:2 soil/water ratio), Mehlich-3 (1/10 ratio) Analysis by Spectros Arcos inductively coupled plasma mass spectrometry.
Table 2. Analysis of variance of the number of infected panicles counted per square meter and the number of sori found per panicle for five selected cultivars grown in four replications at the Pine Tree Research Station, Colt, Ark., in 2012.

<table>
<thead>
<tr>
<th>Date/variable†</th>
<th>Source</th>
<th>DF</th>
<th>Sums of squares</th>
<th>Mean square</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 Sept.</td>
<td>No. panicles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rep</td>
<td>3</td>
<td>51.6625</td>
<td>17.220</td>
<td>4.23</td>
<td>0.0296</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>4</td>
<td>666.5500</td>
<td>166.637</td>
<td>40.89</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Error (MS)</td>
<td></td>
<td>48.9000</td>
<td>4.075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 Sept.</td>
<td>No. panicles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rep</td>
<td>3</td>
<td>107.517</td>
<td>35.838</td>
<td>1.89</td>
<td>0.189</td>
</tr>
<tr>
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<td>Treatment</td>
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<td>744.436</td>
<td>186.109</td>
<td>9.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Error (MS)</td>
<td></td>
<td>12</td>
<td>227.997</td>
<td>18.999</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infected Panicles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rep * Treatment</td>
<td>12</td>
<td>136.897</td>
<td>11.408</td>
<td>0.93</td>
<td>0.544</td>
</tr>
<tr>
<td></td>
<td>Time</td>
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<td>49.506</td>
<td>49.506</td>
<td>4.03</td>
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<tr>
<td></td>
<td>Treatment* time</td>
<td>4</td>
<td>25.935</td>
<td>6.484</td>
<td>0.53</td>
<td>0.717</td>
</tr>
<tr>
<td></td>
<td>Error: MS (error)</td>
<td>15</td>
<td>184.038</td>
<td>12.484</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sori/panicle</td>
<td>Rep</td>
<td>3</td>
<td>2.962</td>
<td>0.987</td>
<td>3.18</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>4</td>
<td>8.068</td>
<td>2.017</td>
<td>6.49</td>
<td>0.005</td>
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<td></td>
<td>Error (MS)</td>
<td></td>
<td>12</td>
<td>3.728</td>
<td>0.310</td>
<td></td>
</tr>
</tbody>
</table>

† Variables = values for the dependent variable, no. of panicles/m², are given as the average number of infected panicles/m² found in replicated plots of five cultivars collected at two different times after first appearance. The dependent variable, sori/panicle, was based on the number of sori counted per panicle collected from 10 infected panicles from each replication of each cultivar (= treatment). Analysis of variances evaluations were performed using a general linear models (GLM) procedure in SAS.

Table 3. The mean number of infected panicles counted per square meter and the number of sori found on panicles of selected cultivars planted in field plots grown at the Pine Tree Research Station, Colt, Ark., in 2012.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Infected panicles†</th>
<th>Sori Rating class‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Katy</td>
<td>0.75 A       1.52 A</td>
<td>1.65 A 1</td>
</tr>
<tr>
<td>Kaybonnet</td>
<td>1.00 A       1.33 A</td>
<td>1.25 A 1</td>
</tr>
<tr>
<td>Taggart</td>
<td>1.62 AB      6.42 A</td>
<td>2.00 AB 2</td>
</tr>
<tr>
<td>Neptune</td>
<td>4.25 B       7.33 A</td>
<td>2.70 AB 2</td>
</tr>
<tr>
<td>Clearfield 151</td>
<td>16.00 C      18.12 C</td>
<td>2.95 B 2</td>
</tr>
</tbody>
</table>

† Means followed by the same letter within a column are not significantly different according to Fisher’s protected least significant difference test at $P = 0.05$. Data are the averages of multiple samples per plot and 4 replications per treatment. The number of sori per panicle is based on 10 randomly selected infected panicles per plot and 4 replications of each treatment.

‡ Disease ratings as reported by Lu et al. (2009). Disease rating classes were assigned based on the average of the number of spore balls/10 panicles per replication/cultivar. Class 0 = 0 sori/panicle; class 1, one sorus/panicle; class 2, two sori/panicle; class 3, three sorus/panicle; class 4, six to nine sori/panicle; and class 5, greater than ten sori/panicle.
Fig. 1. Signs of infection of rice by *Ustilaginoidea virens* found on panicles of one of the more susceptible cultivars in our field plots at Pine Tree in 2012. The nine sori are beginning to turn from their initial orange color to dark green before they become blackened at maturity.