The Role of Rice Stink Bug in the Transmission of Bacterial Panicle Blight in Rice

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ABSTRACT

Bacterial Panicle Blight (BPB) is a seed- and residue-borne disease caused by the pathogens *Burkholderia glumae* and *B. gladioli*. In recent years, the incidence and severity of BPB appears to be correlated with the increases in abundance of insect pests of rice in Arkansas, notably rice stink bug (*Oebalus pugnax* (F.); RSB). The objective of this study was to investigate the extent to which the BPB pathogens were found associated with RSB in different locations throughout the state. Our analyses show that RSB populations are capable of harboring the BPB pathogens at a very low frequency. The presence of the pathogen in the gut but not on the exterior suggests that the pathogen may be a gut symbiont of BPB; bacteria in the genus *Burkholderia* are known symbionts in the gut of other hemipteran insects. While a pathogen detection rate of ~3% in the RSB may seem like a very small proportion (i.e., low vector potential of RSB), the high abundance of RSB across rice agroecosystems (typically in the order of millions of insects in any given location or year) means that this can still translate into high absolute numbers of RSB that carry the pathogen. To truly understand the risk of vectoring of BPB by RSB, the efficiency of transmission of this disease by RSB (i.e., vectorial capacity) needs to be evaluated.

INTRODUCTION

Bacterial Panicle Blight (BPB) is a seed- and residue-borne disease caused by the pathogens *Burkholderia glumae* and *B. gladioli*. In some years, BPB can be the most significant disease of rice in Arkansas and other rice-growing regions of the southern U.S., and is capable of reducing yields by 10% to 20% and affecting milling quality.
The pathogen overwinters in infected seed and crop residue in the soil and is capable of re-establishing populations on rice seedlings, and subsequently going on to impact yield and grain quality by infesting the panicles as the plant matures. The pathogen is transferred between plants in the field through the action of wind and rain, and this movement is localized. Agents of longer distance dispersal of the pathogen are poorly understood.

In recent years, the incidence and severity of BPB appears to be correlated with the increases in abundance of insect pests of rice in Arkansas, notably rice stink bug \textit{(Oebalus pugnax) (F.)}. This correlation could be equally explained by two possibilities: (a) environmental conditions that are favorable for the incidence of BPB are also the same ones that are suitable for rice insects, or (b) rice insect pests are an important vector of BPB either through incidental or vectored movement, and transmission of the bacterial pathogen between rice plants (such disease-vectoring is known in other stink bug species). If the former explanation is true, then management of BPB and rice insect pests can continue to be treated as independent pest pressures in rice production. However if the latter is true, then the management of both the bacterial disease and the impacts of rice insect pests may be contingent on the effectiveness of the management against insects. Understanding the role of insect pests in the transmission of BPB is therefore important in the context of facilitating effective management of both bacterial and insect pressures on rice production.

The objective of this study was to investigate the extent to which the BPB pathogen was found associated with RSB in different locations throughout the state.

**PROCEDURES**

**Field Sampling**

Rice stink bugs were collected using standard sweepnet-based sampling techniques from 27 grower fields (and associated field margins) across ten counties of Arkansas (Arkansas, Lonoke, Prairie, Chicot, Ashley, Desha, Mississippi, Clay, Jackson, and Poinsett counties), and from fields at the University of Arkansas System Division of Agriculture Rice Research and Extension Center (RREC) near Stuttgart, Ark., with a known presence of the BPB-causing pathogens (Table 1). The majority of the sampling was conducted when rice plants were at the milk to soft dough stages of panicle development, as this is the preferred feeding stage for RSB and therefore when disease transmission by RSB is most probable (Table 1). Field-collected insects were grouped by sampling location to avoid cross contamination of samples, and stored live in a cooler with ice for transport to the laboratory.

**Laboratory Methods**

The field-collected insects were held alive in a refrigerator in the laboratory prior to examining them for the presence of the BPB pathogen on their exterior surface and in their gut. In all, 565 individual RSB were examined for the presence of the BPB pathogen.
pathogen (Table 1). Two methods were used to detect the BPB pathogen. This included traditional diagnostics using a selective growth medium (CCNT) to culture and detect the BPB pathogens (Kawaradani et al., 2000), and a polymerase chain reaction (PCR)-based molecular diagnostic method that used *Burkholderia*-specific markers to detect the presence of the BPB pathogens (Maeda et al., 2006). Standard axenic protocols were followed in the handling of all samples, and included oven sterilization of all glassware at 130 °C for at least 20 minutes between each dissection and flame sterilization of all handling and dissection instruments to avoid cross-contamination.

Within one week of collection, the external surfaces (dorsal and ventral) of each insect were pressed onto the CCNT medium with a sterile pair of forceps. The CCNT plates were sealed with Parafilm immediately after the plating process, and incubated for 48 h at 39 °C. The presence of BPB in a plate is indicated by the presence of distinct yellow coloration that is the result of a reaction between the medium and toxins produced by the BPB pathogen (Kawaradani et al., 2000).

The exterior of each insect was then surface sterilized using a 2% sodium hypochlorite solution for one minute, and the insect was dissected in a sterile glass dissection Petri dish. The gut of the RSB has been characterized previously (Hamner, 1936), and contains four distinct gut sections and an elaborate salivary gland. The gut was examined for the presence of the BPB pathogens using either the CCNT medium or PCR-based analyses. The gut was aseptically removed, and each of the four gut sections from each insect was separately plated on the CCNT medium; alternatively, the entire gut was stored in a sterile tube at -20 °C for PCR-based analysis. The salivary gland is part of the first gut section, and wherever possible it was plated separately on the CCNT medium. The CCNT plates with the gut tissues were incubated, as described for the insect’s external surface, to document the presence of the BPB pathogens.

Positive detection of the pathogen using molecular methods were done by examining the presence of DNA fragments of a specific size (597, 529, and 479 bp DNA fragments corresponding to the *gyrB* nucleotide sequences of *B. plantarii*, *B. glumae*, and *B. gladioli*, respectively) using gel electrophoresis (Maeda et al., 2006).

Positive controls using a pure culture of *B. glumae* were run for each batch of CCNT plates and PCR samples.

**RESULTS AND DISCUSSION**

None of the CCNT plates of the external surfaces of RSB tested positive for the presence of BPB. However of the 324 gut samples examined by the CCNT method, 9 tested positive for the presence of the BPB pathogens (Table 1). This represents ~3% of the processed samples. Among the positive samples, there was no clear association of a given gut section with the presence of the pathogen. All samples that tested positive were from fields with a known BPB history, suggesting that when the disease is present in the plant, RSB may be capable of acquiring it while feeding on an infected plant.

None of the PCR samples tested positive for the presence of the BPB pathogen. There are three plausible explanations for this: (a) the pathogen is not present in association with the RSB gut, (b) some aspect of our handling of the gut tissue is causing
the pathogen to die prior to amplification by the PCR process, or (c) the amount of pathogen DNA in the insect tissue is below the detection threshold of the PCR-based method. Among these (b) and (c) are the most likely explanations, given our ability to detect the pathogen using the CCNT method. These aspects are topics of ongoing collaborations among the authors.

Our analyses show that RSB populations are capable of harboring the BPB pathogen(s) at a very low frequency. The presence of the pathogen in the gut but not on the exterior suggests that the pathogen may be a gut symbiont of RSB; bacteria in the genus *Burkholderia* are known symbionts in the gut of the other hemipteran insects (Kikuchi et al., 2008). Additional work, specifically tailoring of the PCR-based method for detection of the BPB pathogen in association with insect vs. plant tissues and at the soil-plant interface, is needed to better understand the transmission of this disease.

**SIGNIFICANCE OF FINDINGS**

While a pathogen detection rate of ~3% in the RSB may seem like a very small proportion (i.e., low vector potential of RSB), the high abundance of RSB across rice agroecosystems (typically in the order of millions of insects in any given location or year) means that this can still translate into tens of thousands of RSB that carry the pathogen in a given field. To truly understand the risk of vectoring of BPB by RSB, the efficiency of transmission of this disease by RSB (i.e., vectorial capacity) needs to be evaluated. In addition, other closely related insects in the order Hemiptera need to be examined, and their vector potential and vectorial capacity in the context of BPB transmission needs to be evaluated.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


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**Table 1. Summary of origin and numbers of rice stink bug (RSB) dissected and examined using either a selective growth medium or polymerase chain reaction-based methods for the presence of the bacterial panicle blight (BPB)-causing pathogens.**

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>Sample month</th>
<th>CCNT</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throughout the state</td>
<td>July</td>
<td>158</td>
<td>0</td>
</tr>
<tr>
<td>Throughout the state</td>
<td>October</td>
<td>34</td>
<td>89</td>
</tr>
<tr>
<td>From planting date trials at the RREC</td>
<td>August (March)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>August (April)</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>August (May)</td>
<td>12(1)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>September (May)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>October (May)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>RREC, inoculated plots&lt;sup&gt;c&lt;/sup&gt;</td>
<td>August</td>
<td>48(4)</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>24(4)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

<sup>a</sup> Planting date given in parentheses. Sampling was restricted to rice plants with panicles in the milk to soft dough stage, as this is the stage preferred by the rice stink bug.

<sup>b</sup> Numbers in parentheses indicate the number of insects that tested positive for the presence of BPB pathogens.

<sup>c</sup> Data from the plots at the RREC that had plants that were seed and surface inoculated with the BPB pathogen.