Section 2. Bacterial diseases

Chapter 4. Grain diseases

Introduction

Rice grains can infected through three routes, boot leaf sheath infection spreading to florets, direct infection of the florets after ear emergence or the developing grains, and during storage. This chapter deals with grain rot and bacterial palea browning. These cause concern when raising seedlings in nursery boxes and reducing the commercial value of the grains, respectively.

Obviously, grain infection, when transmitting via seed an infectious agent, is bound to be reflected in the emerging seedlings one way or another. Such a relationship exists with the grain rot disease, the pathogen of which also causes "Seedling rot" in nursery boxes (**see Chapter 1 in this Section**).

Other bacteria, such as *X. oryzae pv. oryxicola* and *B. plantarii*, can also infect grain but rice is not a primary target. In this online resource, these bacteria are not listed and discussed as bacterial pathogens of grain diseases. The grain rot pathogen, *Burkholderia glumae* and another species of the same genus, *B. gladioli*, are known to infect grains independent of each other, causing a disease syndrome known as "bacterial panicle blight" in the U.S

1. Grain rot (GnR)

As early as 1955, bacterial GnR was observed in Kyushu, Japan (Goto and Ohata 1956). Subsequently, it has been reported in Taiwan (Chien et al 1963), Korea in 1995 (Cha 1993, Song and Hyung Moo 1999), and mainland China in 2007 (Luo et al 2007). In the southern United States, bacterial panicle blight similar to grain rot had been reported in the early 1990s (Nandakumar et al 2009). Initially, the symptoms were thought to be caused by an abiotic stress related to high temperature, water, or toxic chemicals. The malady was later confirmed to be of bacterial origin caused by *B. glumae* and *B. gladioli* and identical to grain rot reported in Japan (Shahjahan et al 2000). It is then possible that other disease problems exist that may be attributed to nonbiological factors. In the United States, the disease is called bacterial panicle blight but in other countries the disease is known as grain rot.

In Japan, GnR was initially regarded as a minor problem. However, since the 1970s, the incidence and severity continued to intensify in southern and central Japan (Uematsu et al 1976a,b), which coincided with the time when the conventional field nursery had been replaced by the nursery box for raising seedlings. In mid-1970s, the first incidence of *B. glumae* infecting seedlings in a nursery box was observed, showing that the bacterial pathogen attacks rice both at the seedling and panicle development stages (Uematsu et al 1976a,b). The importance of *B. glumae* as a rice bacterial pathogen has since been recognized. *B. glumae* has since been manifested as grain rot of mature plants at the heading stage in the field and as seedling rot in nursery boxes. However, seedling rot seldom occurs in field nursery. While examining the bacterial species associated with grain or sheath rot in a large number of seed samples from 22 different countries, Zeigler and Alvarez (1990) noted that *Pseudomonas glumae* (syn. *B. glumae*) among the four frequently encountered bacterial seed pathogens with a worldwide distribution.

1.1. Symptoms

The disease manifests itself into distinct types of symptoms on rice plants (Sayler et al 2006; Uematsu et al 1976a,b). At the seedling stage in a nursery box, it is seedling rot while on grains in fields, it is grain rot. On leaf blades, especially flag leaves, the disease appears as small tan lesions measuring 1 to 5 mm with brown margins. On the leaf sheath, the lesion may be several centimeters long with a tan center and reddish brown margins on individual flag leaf sheaths of infected tillers. Infection occurs on spikelets causing kernel abortion before the kernels are completely filled. Infected florets may show dark gray or brown bases, but the rachis of the panicles remains green. GnR is usually realized when the infected seeds are sown for germination in nursery boxes. Panicles of infected tillers remain erect instead of bending due to loss of grain weight.

The infection of seedlings results in seedling rot, likely derived from seedborne inoculum. Seed infestation or infection increases during presowing and water soaking or when seedlots carry the bacterial inoculum. Preemergence "damping off" causes GnR where the seeds fail to germinate and also forms a source of inoculum for other germinated seeds of disease foci in nursery boxes. The symptoms on seedlings caused by inoculum from rotting seeds usually develop into brown lesions on the coleoptile (Uematsu et al 1976a,b), which can cause seedling mortality. Lesions on infected and developed seedlings show brown, water-soaked soft rot of the leaf sheath accompanied by wilting or soft rot of the leaves (**GnR Figure 1**).

GnR Fig. 1. Symptoms of early infection by the pathogen of rice grain rot showing water soaked discoloration on the lower part of the grain. Photo by Dr. Donald Groth, Lousiana State University, AgCenter

On grain rot, the panicles are shrunken and pale green, becoming dirty yellow to brown and dry. The symptoms may appear on the glumes and kernels. However, in Japan, panicle rachises and branches do not show symptoms. The hulls of infected grains on young panicles rapidly fade in color from the base, turning greenish white at first and then pale pink to yellowish brown. They soon wither and dry up. The infected grains are either sterile or carry brown rice with dark-brown discoloration at the base. A brown margin between the infected and healthy parts of the grain is a diagnostic feature of the disease (**GnR Figure 2**). The bacterium may cause mild rot of the flag leaf sheath of the leaf sheath collar.

GnR Fig. 2. The characteristic two-tone discoloration of rice grain rot. Photo by Dr. Donald Groth, Lousiana State University, AgCenter

1.2. Causal organism

GnR is caused by *Burkholderia glumae* (formerly *Pseudomonas glumae* Kurita et Tabei 1967). The bacterium is a gram-negative, nonspore-forming rod, measuring 0.5-1.0 x 1.5-3.0 µm, and with round ends. The cells occur singly, rarely in pairs, and are mobile by

means of one or several polar flagella. It forms white colonies on nutrient agar. A fluorescent pigment is also produced on potato agar (Urakami et al 1994). Granules of poly-β-hydroxybutyrate are accumulated in the cells. The bacterium grows well at 30°C and at 37°C but not at 42°C. The optimum pH for bacterial growth is between pH 5.0 and 7.5, but not at a pH value below 3.0 or above 9.5. Strains of *B. glumae* produce crystals of calcium oxalate on agar medium containing calcium chloride. The genetic diversity of *B. glumae* isolates is relatively low as revealed by 16S-23S rDNA ITS sequence analysis (Sayler et al 2006).

1.3. Host range

Natural infection of the bacterial pathogen has been reported on rice. When artificially inoculated with the pathogen, several plant species were weakly infected but their role in the life cycle of the bacterium remains insignificant.

1.4. Disease cycle

B. glumae is seedborne and epiphytic on rice plants. The bacterium invades the glumes through the stomata located outside the lemmas colonizing the space between the cells in the outer epidermis and spongy parenchyma of the lemma. From the infected seeds, the bacterium may grow epiphytically at a low level until panicle emergence when its population increases rapidly on the grain. The bacterium multiplies rapidly on the surface of emerging panicles, and infect flowers just after emergence (Goto 1992). The disease is favored by high night temperature (28°C) and high humidity.

1.4.1. *Toxoflavin, a phytotoxin produced by* B. glumae. On various media, *B. glumae* produces bright pigments identified as the phytotoxins toxoflavin (1,6-dimethylpyrimidio[5,4-e]-1,2,4-triazine-5,7(1***H***,6***H***)-dione), a 7-azapteridine antibiotic, and fervenulin in culture. Both toxins produce chlorosis and reduce the growth of leaves and roots (Sato et al 1989; Liyama et al 1994,1995). Toxoflavin-induced wilt symptoms in other field crops such as tomato, sesame, and perilla indicate the general broad spectrum of this toxin (Jeong et al 2003). Toxoflavin induces a wilt symptom in the field on crop species other than rice. This symptom on other crop species is similar to that caused by *Ralstonia solanacearum*. Phytotoxins have been reported in many pseudomonad bacteria but none has been implicated in pathogenicity. Toxoflavin is one of the few shown to be involved in pathogenicity and its requirement for quorum sensing for pathogenicity is the first report for plant pathogenic bacteria.

1.4.2. Detection of* B. glumae *from rice seed. *B. glumae* is seedborne and can be detected from rice seeds. It can also be selectively isolated from rice seed using a selective medium described by Tsushima et al (1986) where *B. glumae* produces circular, convex colonies with smooth margins that are reddish brown or opalescent with a purple or red-purple center. However, this technique may not be accurate for plant quarantine purposes.

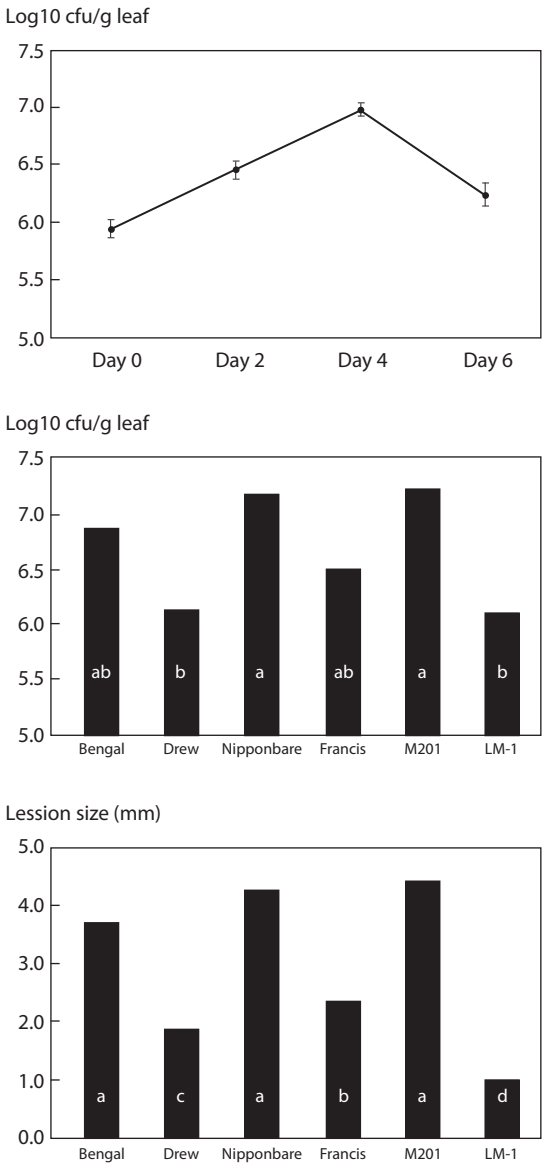
As it is a seedborne disease, GnR was listed as a plant quarantine disease in China in 2007. Attempts have been made to devise sensitive methods of detection using molecular tools. A real-time PCR method was developed to detect and identify the bacterium from rice seed using specific PCR primers based on the sequences of internal transcribed spacer (ITS) region of the 16S-23S rDNA of representative isolates from Japan and Arkansas, USA (Sayler et al 2006). Yuan et al (2009) developed a real-time PCR and a general PCR diagnostic method for its detection in seedlots. A 319-bp fragment specific to *B. glumae* produced in both these methods confirms the presence and identity of the pathogen.

1.5. Control measures

Options to control GnR are limited to using clean seed and resistant varieties.

1.5.1. Seed health approach using clean seed. For seedborne rice diseases, such as those reported here, seed health management may be one of the most preferred control practices. Healthy seed must be used for planting. Seeds from fields known to harbor grain rot must be avoided. Infected seeds can easily be recognized because these are generally lighter in weight although research on this may not be adequate. For field bulk seed planting, seeds may be suspended in salt water to sort out the best quality seeds for planting (Goto 1992). This is an old method but remains one of the most effective means to manage seedborne rice diseases. The various approaches to seed health are described in detail in **Section 2 of Part III**, Selected Management Practices.

1.5.2. Varietal resistance. If resistance sources are available, deployment of resistant varieties is another option in managing grain rot. However, there is meager research on host plant resistance on grain rot. The long grain rice varieties, mostly indica types, are more tolerant to grain rot or panicle blight than medium to short grain types (Sayler et al 2006). There is no data to support any genetic basis on the variable responses of these two types of rice to the pathogen. Nevertheless, based on bacterial multiplication in leaf sheath at the 4th day after inoculation by injection, and based on lesion size on seedlings after spray inoculation, variations among tested varieties were evident (**GnR Figure 3**; Sayler et al 2006). There is still a lot to understand on the genetics of resistance to grain rot and in developing grain rot-resistant rice varieties. There is also the need to elucidate the role of seedling infection and bacterial multiplication and movement as epiphyte on the plant till panicle initiation or boot leaf stage to get an opportunity of infecting the florets for grain rot development.



GnR Fig. 3. Evaluation of five rice cultivars and one breeding line for resistance to *Burkholderia glumae*. (A) Bacterial growth over time in the rice cultivar Bengal as measured by dilution plating. (B) Bacterial growth in different rice cultivars 4 days after inoculation ($P = 0.004$). (C) Bacterial lesion size in different rice cultivars 2 weeks after spray inoculation. Cultivars with statistically significant difference in disease severity (lesion size) were designated with different letters ($P < 0.0001$) Source: Sayler et al (2006).

1.5.3. Chemical control. There are several chemicals known to be effective in controlling GnR, especially for seedlings raised using seed boxes. However, proper guidance is necessary for their use.

Several biological control agents (BCAs) have been discovered that are effective against the bacterial pathogen (Sumida and Takaya 2002). A general procedure for raising seedlings in nursery boxes is to presoak the seeds overnight before sowing. No research has shown whether BCAs can be formulated for large-scale seed-soaking and commercialized. However, there appears to be a good opportunity to formulate BCAs that can be used in conjunction with seed-soaking to suppress seedborne inocula. For details, see Section 3 on *Managing microbial antagonists for rice disease control* in Part III, Selected rice disease management practices.

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2. Bacterial palea browning (BpB)

BpB of rice was first reported in Japan (Azegami et al 1983). More recently, it has been found in China (Xie 2001) and Korea (Kim et al 1989). The proportion of discoloration of the palea (bract-like organs in the spikelet) sometimes amounts to about 30% of the total number of grains on a panicle. There were reports in some localities that disease incidence could be as high as 32% with 1,000-seed weight reduction. The disease also adversely affects grain quality. Yellow pigmented bacteria have been frequently isolated from affected grains. Similar bacteria have been isolated from the other parts of rice plants in various localities in Japan and also from apparently healthy paleas. Some of these bacteria were pathogenic to the glume when artificially sprayed onto rice plants at the flowering stage. Isolates of the bacteria exhibit similar morphological and bacteriological properties, suggesting its closeness to *Erwinia* sp.

2.1. Symptoms

BpB symptoms usually first appear at early flowering stage. Initially, light brown, water-soaked lesions occur on the lemma or palea, which later turns dark brown. The discoloration occurs most frequently on the palea, thus the name of the disease. Infected panicles have more immature grains and lighter grains at harvest. Infected grains become brown after milling.

2.2. Causal organism

The bacterium responsible for BpB has been identified as *Erwinia herbicola* (Lohnis) Dye. Although the biochemical properties such as acid production from melibiose, cellobiose, and glycerol but not from dextrin, indole production, negative reaction on nitrate reduction, and absence of phenyalanine deaminase are similar to those of *E. ananas* Serrano, the bacterium is now named *Pantoea ananas* Serrano. The bacterium is gram-negative, rod-shaped, facultatively anaerobic, fermentative, mobile with peritrichous flagella, and produces a yellow water-soluble pigment (Azad et al 2000, Coutinho and Venter 2009).

2.3. Host range

Natural infections of *P. ananatis* causing disease occur in a wide range of economically important agricultural crops and forest trees of both monocotyledonous and dicotyledonous plants. The natural hosts include pineapple, corn, onion, sudangrass, sorghum, Eucalyptus, tomato, and melon and upon, artificial inoculation, sugarcane, oats, and cotton also get infected (Azad et al 2000, Coutinho and Venter 2009). Because of this wide pathogenic

ability, *P. ananatis* is regarded as an emerging pathogen (Coutinho and Venter 2009). Unlike a majority of plant pathogenic bacteria, unconventionally, it is capable of infecting humans (Maayer et al 2010).

2.4. Disease cycle

P. ananatis survives in nature on different hosts as an epiphyte, a saprophyte and a pathogen (Cota et al 2010). High epiphytic populations of *E. herbicola* (syn. *P. ananatis*) are very common on rice. During harvest time in temperate environments, the presence of the bacterium as an epiphyte may be involved in ice nucleation and can cause cold damage to rice panicles toward harvest (Kim et al 1989). Using *E. ananas* (syn. P. ananatis), transformed with bioluminescence genes, the sites of multiplication of the bacterium on the inoculated rice plant was traced (Hasegawa et al 2003). Post-flowering, the bacterium has been located on the anthers and on dead tissues of leaf tips, lower leaf sheaths, and leaf blades. In spikelets exhibiting disease symptoms on the palea, the pathogen has been detected on the stigmas, basal parts of ovaries, and lodicules indicating that the multiplication of the bacterium in these internal tissues of the spikelets is associated with the appearance of browning on the paleas.The disease occurs when heading coincides with periods of rain and high temperatures in the range of 30-35°C. Disease incidence increases in fields with high levels of nitrogen fertilization, particularly at heading.

2.5. Control measures

Since the causal bacterium is seedborne, the best management option is the use of clean and healthy seeds. Coinoculation of rice plants with a nonpathogenic ***P. ananatis*** and one of its lytic bacteriophage suppressed BpB (Azegami 2013), suggesting the possibility of developing an eco-friendly biocontrol strategy for disease management.

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