Section 1. Fungal diseases

Chapter 1. Seedling diseases

In general, plant pathology, the diseases of germinated seeds and seedlings are usually referred to as ***damping-off*** or ***seedling blights***. These are considered here collectively as ***seedling diseases***. The common features of diseases associated with seed germination retard the emergence of seedlings even with seeds of high germination ability. Damping-off results in patchy appearances in nursery beds and seedling boxes due to death of the germinated seeds and infected seedlings. Emerged seedlings exhibit a water-soaked appearance of the leaves or brown discoloration with shriveling culms.

There are two stages of seedling diseases that may or may not show concomitantly. One relates to preemergence where mortality of germinating seeds is often observed. The other relates to postemergence, also known as seedling blight, which may exhibit various symptoms related to the causal organisms. Seedling blight as "post-damping-off" is the sudden death of the seedlings, often observed on young seedlings that are fully established. The symptoms are yellowing and wilting of the leaves, which leads to the collapse of the seedlings.

The pathogen (or inoculum) either originates from the soil or is carried by the seeds. Traditionally, rice nursery beds are laid out in fields where soils are saturated with water. Very few recorded soilborne fungal pathogens can cause seedling diseases. In recent years, however, as crop intensity has increased with new cultural practices, including cropping systems and mechanization, land use intensity has also increased. Some of the new practices have forced farmers to find new ways of raising seedlings, which have triggered the occurrence of new diseases, especially seedling diseases that have not been previously observed with traditional wet bed nurseries. Two cases illustrate the problem: the wheat-rice system in China and the use of seedling boxes to raise seedlings for mechanical transplanting in Japan (Mew et al 2004). Both systems have attracted new seedling diseases unknown in the past with wet bed nurseries.

Unexpected disease problems, prominent at seedling stage of rice growth, have emerged from changes in cultural techniques in rice-wheat cropping system. In the rice-wheat cropping cycle where in which the two cropping seasons overlap with each other, traditional a wet bed nursery for rice seedlings cannot be laid out in the field before the wheat harvest. Otherwise, seepage from the nursery bed damages the root systems of the standing wheat crop that is ready for harvest in 2 to 3 weeks. To cope, farmers use dry bed nurseries to raise rice seedlings before the wheat harvest, which has resulted in the emergence of many seedling diseases. These diseases include bakanae caused by *Fusarium fujikuroi* (formerly known as *Fusarium moniliforme*), seedling blight caused by *Sclerotia rolfii*, and a few other bacterial seedling diseases (for details about bacterial seedling diseases refer to Chapter 1 of Section 2.

Seedling diseases in seedling boxes in Japan are a good example to illustrate the problem. Japan developed mechanical transplanting technology in the early 1970s (Iwano 2000) that have since been widely adopted by farmers because of its efficiency and accuracy. In parallel, a related technology to produce uniform seedlings for transplanting had also been developed. The seedlings used for mechanical transplanting were raised indoors in plastic seedling boxes under high temperature and high humidity with high seeding rates. Consequently, many new diseases have arisen, with several outbreaks recorded over the years. Bakanae, bacterial seedling blight, and seedling diseases caused by *Rhizopus* spp., *Pythium* spp. and *Trichoderma viride* were common. *Pythium* sp. is also known to attack the young roots of germinated seeds in temperate rice-growing environments although its occurrence is limited to low-temperature conditions. Many of these seedling diseases have not been previously described and studied.

A different scenario of disease occurrence is anticipated to occur from the change in rice cultivation from the traditional method of transplanting to direct seeding and aerobic rice. Some of these diseases have already occurred. The increase in seeding rate and seedlings grown in conditions of high humidity and high temperature leads to another set of problems that farmers or rice growers may not have experienced in the past, but which scientists must anticipate regardless if these diseases are new or previously classified as "minor" diseases.

Under favorable environmental conditions, many pathogens such as *Pyricularia oryzae* and *Bipolaris oryzae* attack the rice plant at all stages of growth, from seedling to maturity. A genuine seedling disease is an infection that is initiated at either the germinating or seedling stages and normally does not occur in older plants. This chapter is restricted only to those diseases.

References

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1. Bakanae (Bak)

Bakanae, caused by *Fusarium fujikuroi* (Sawada) Wollenw (formerly known as *Fusarium moniliforme* J. Sheld.), is one of the oldest reported rice diseases and has been known in Japan since 1828 (Ito and Kimura 1931). Today, it occurs widely in most rice-growing countries. Gupta et al (2015) indicated that it is one of the emerging problems in rice, particularly in basmati-scented rice in India during recent years and becoming more serious threat to sustainable rice production in other parts of the rice-growing world. Recent outbreaks in California, USA (Carter et al 2008) suggest that rice seed, as a carrier of potential inoculum, could introduce the pathogen to—and establish iti—in a new rice production environment. *Fusarium fujikuroi* (former *F. moniliforme*), known to be seedborne (Dodan et al 1994, Hemmi et al 1931, Hino and Furuta 1968, Kim 1981, Manandhar 1999, Sun 1975), and introduced only recently to cause epidemic proportions of the disease in the field where rice has been introduced and grown in California for decades. However, little is known on the epidemiology of an old disease such as bakanae until now.

In the early decades of the last century, bakanae was extensively studied in Japan. Prior to the discovery of seed-treatment fungicides, it was considered a major problem in rice production. Since fungicides have been discovered and used extensively for seed treatment, the importance of bakanae as a constraint in rice production had diminished. However, in recent years in Japan and other countries, the disease has re-emerged as a troublesome disease in seedlings grown in seed boxes for mechanical transplanting or in dry bed nurseries. This is partly due to the neglect on the seedborne nature of the fungal pathogen. It remains a problem in other countries such as Nepal (Desjardines et al 2000).

1.2. Symptoms

The unique symptom of Bak is the abnormal elongation of the shoot growth after seed germination (**Bak Figure 1**). The term "bakanae", adopted from Japanese, stands for "crazy growth or foolish seedlings". Infected seedlings grow faster and several inches taller than normal seedlings in a seedbed or later in the field. The "bakanae" seedlings are thin and yellowish green, thus, weak. In a hill, the affected tillers stand out very conspicuously and occurrence is scattered in the main field while severely infected seedlings die before transplanting in the nursery.

Bak Fig. 1. Symptoms of bakanae disease in rice.

When the crop reaches maturity, the flag leaves of infected tillers are pale green. The infected plant may also produce adventitious roots at the lower nodes of the culm and its leaves dry up one by one from below and die in a few weeks before reaching maturity. Infected plants that develop to maturity may have only a very few tillers that produce sterile or fully developed panicles bearing infected grains. Severely infected grains develop a reddish discoloration due to the presence of conidia of the pathogen. More often, the whole grain in the panicle is discolored.

Several types of symptoms of Bak have been reported in the literature (Yamanaka and Honkura 1978), which appear to be more related to infection caused by different inoculum density carried by seeds rather than by different types of isolates as postulated by some earlier scientists (Sun and Snyder 1981). Besides infection of germinated seeds, which expresses its symptom in the growth of the seedlings, no other vegetative parts above the ground are sites for infection. However, sooty growth of the mycelium on the leaf sheath surrounding the culm is occasionally observed in the field. This is epiphytic growth of the fungus, which produces ascospores that infect the panicles (Sun 1975, Yu and Sun 1976).

A number of *Fusarium* spp. have been isolated from rice seeds (**Bak Table 1**) and initially named fungal species, but *F. moniliforme* is now known to comprise a number of distinct species collectively termed as the *Gibberella fujikuroi* species complex. It has been questioned whether these different *Fusarium* spp. are all involved in any of the symptoms described above (Desjardins et al 2000; Mew and Gonzales 2002). This question may be addressed by future research, but the general features shared by the species include both soilborne and seedborne inocula that can cause seedling blight exhibiting stunting and chlorosis of the seedling. The bakanae tillers have always been associated with seedborne inoculum at a high density. The severity of the disease is also related to inoculum density and not to the types of isolates or species.

Bak Table 1. *Fusarium* species isolated from Nepalese rice samples (Desjardins et al 2000).

Fungal species[b]	Samples infected with the indicated species (no.)[a, c]			Strains identified from each species (no.)	
	Group I	Group II	Group III	Group I	Group II
Total no. of samples	18	8	22	67	100
G. fujikuroi species complex	15	8	11	36	24
G. fujikuroi MP-A	0	0	2	0	0
G. fujifuroi MP-C	6	0	0	7	0
G. fujifuroi MP-D	5	7	6	6	13
F. fujikuroi, nonfertile	13	7	4	23	11
G. zeae	3	7	7	14	34
F. graminearum, nonfertile	1	1	3	1	1
F. semitectum	4	7	5	13	36
F. equiseti	1	3	2	1	3
F. chlamydosporium	1	1	0	1	1
F. oxysporium	0	0	2	0	0
F. avenaceum	1	0	0	1	0
F. acuminatum	0	1	0	0	1
F. anguioides	0	0	1	0	0
None (clean sample)	2	0	8		

[a] Group 1 comprised 18 samples of the Bak-susceptible, improved variety Khumal-4 from the Kathmandu valley and the following regions: Kavre district (four samples), Dolakha district (six samples), and Lalitpur district (eight samples). Group II comprised eight samples of bakanae -resistant varieties from the Kathmandu valley and the following adjoining regions: two samples each from improved varieties chainung-242 and Taichung-176 from Bhaktapur district, one sample of local variety Sankharika from Lalitpur district, and one sample each of improved varieties IR51672 and Masuli from Kavre district. Group III comprised 22 samples of local varieties from Lamjung district, whose Bak susceptibility and resistance is unknown.

[b] Surface-disinfected seeds with jusks (50 seed from each sample), were placed on a selective medium. For all samples except 14 Lumjung samples (Group III) that showed no infection, an additional 50 to 75 seeds were tested. For 29 of the 38 infected seed samples, every Fusarium colony was identified to species. For the nine most highly infected seed samples, colonies were placed into groups by morphological criteria, and representative colonies were identified to species.

[c] Numbers in columns sums exceed the total numbers of samples due to infection of some samples with multiple Fusarium sp.

1.3. Causal organism

Ou (1985) provided a historical account of the causal fungus summarized as follows. Hori (1898) first described the causal organism of Bak as *Fusarium heterosporium* Nees Fujikuro and found the teleomorph. Sawada (1917) named the fungus as *Lisea fujikuroi*, which was later placed under the genus *Gibberella* as *G. fujikuroi* (Sawada) Ito (Ito and Kimura 1931) with *Fusarium moniliforme* Sheld as its anamorph.

The name of the anamorph had been changed a few times in recent years with the progress on taxonomy of *Fusarium* species (Seifert et al 2003). The progress in taxonomy also led some to question whether Bak is caused by not one but several *Fusarium* spp. (Desjardins et al 2000). The confusion is perhaps partly due to the uncertainty of the taxonomic status of *F. moniliforme*. Are different organisms responsible for bakanae disease or has the name of the real causal fungus just been frequently changed? A simple answer to this question is that the causal organism is unlikely to be different from what was originally known as the causal agent of the disease. To understand this, it may be necessary to indicate that, in classical plant pathology, the mycological and pathological functions of a fungus were often treated inseparable as "two-in-one". From the beginning, scientists worked on the science of both mycology and plant pathology. A mycologist may be more interested in taxonomy while a pathologist focuses on function of the fungus and of the host plant. As science advances, it is inevitable that the two had to separate. We need to realize that, for the mycologist-taxonomist, the question is "what the organism is" while for the plant pathologist, it is "what the organism does" relative to the disease it causes. However, the plant pathologist must possess some understanding of the taxonomical status of the organism while working on it as a pathogen of the host. Some of the latest developments on the taxonomy of the fungus that causes rice Bak disease are briefly described next.

There is little to argue that rice Bak is caused by Gibberella *fujikuroi* (Sawada) Wollenweb. var. *fujikuroi* (anamorph: *Fusarium moniliforme* J. Sheld.). *F. moniliforme,* the conidial stage of the fungus, occurs on a wide variety of host plants besides rice, including maize, sorghum, etc., causing seedling blights, stem rots, and fruit decay (Kuhlman 1982). Although bearing the same "name," the question is whether the organisms from different crops are similar in pathogenicity, or whether they belong to the rice taxon that causes Bak. Equally important is to ask whether the fungus bearing the same name from different regions is the rice *F. moniliforme* described in the early years or the *Fusarium* under section *Liseola*. The answer to this question is not only important scientifically (a mycologist's concern), but also carries practical implications in plant quarantine regulations on seed movement and trading since the fungus causing Bak of rice is seedborne, which plays an important role in disease development (a plant pathologist's concern). In early studies, the causal organism of Bak was listed in the *Fusarium* section *Liseola,* which composes of species pathogenic to a wide range of economically important crops such as maize, rice, sorghum, millet, sugarcane, pineapple, and mango (Amoah et al 1995, Leslie 1991). The actual number of species in this section is not known, but mating studies had shown it consisted of six reproductively isolated groups, labeled as A-F (Hsieh et al 1977) that were later modified by other studies (Kerényi et al 1999; **Bak Table 2**). Early studies indicated that all rice isolates appeared to belong to group C, and mostly from Southeast Asia (Hsieh et al 1977, Kuhlman 1982).

Three mating populations of the *G. fujikuroi* complex have been associated with Bak of rice. Although at one time, Group C was assumed to have a strong specificity for rice, it had also been reported in maize and sorghum (Leslie 1991). Mating population A (MATA) (anamorph: *Fusarium verticillioides*) and mating population D (MATD) (anamorph: *Fusarium proliferatum*) have been isolated from rice from Asia, while MATD alone has been isolated

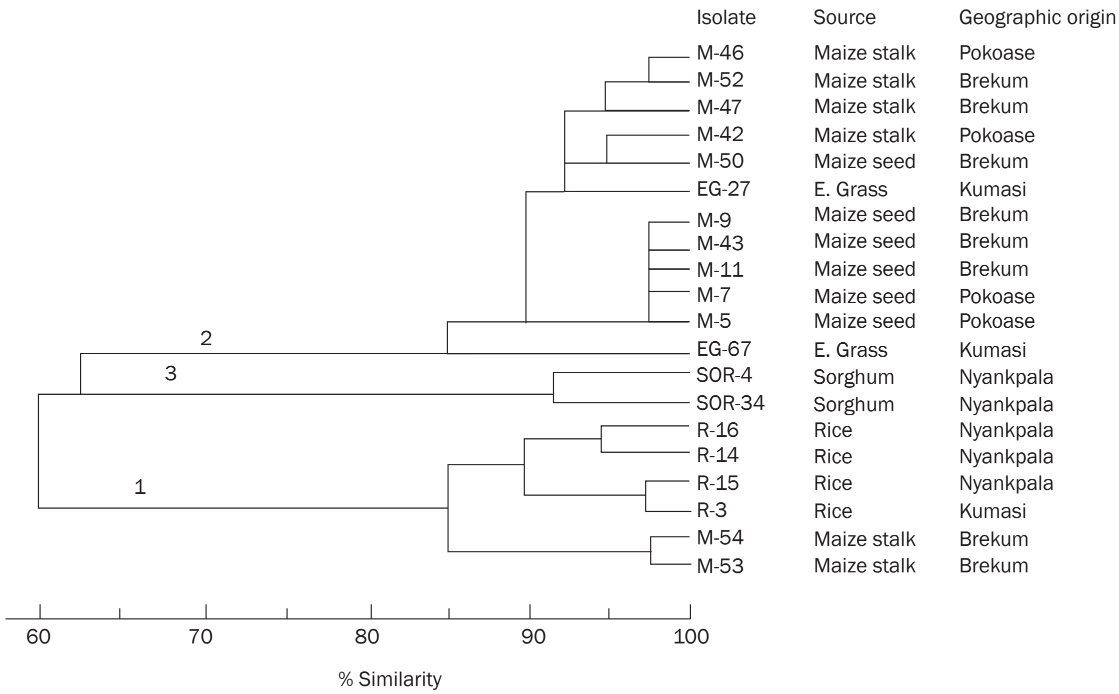
Bak Table 2. Correspondence between previous mating type terminology and revised mating type terminology for the Gibberella fujikuroi spp. complex (Kerenyi et al 1999).

Species*	G. fujikuroi mating population	Mating type designation	
		Previous studies	This study
Fusarium moniliforme	A	matA+	MATA-2
(Fusarium verticillioides)		matA-	MATA-1
(Gibberella moniliforme)			
Fusarium subglutinans	B	matB+	MATB-2
(Fusarium sacchari)		matB-	MATB-1
Fusarium fujikuroi	C	matC+	MATC-1
		matC-	MATC-2
Fusarium prolifertum	D	matD+	MATD-2
		matD-	MATD-1
Fusarium subglutinans	E	matE+	MATE-2
(Gibberella subglutinans)		matE-	MATE-1
Fusarium thapsinum,	F	matF+	MATE-1
Gibberella thapsina		matF-	MATE-2
Fusarium nygamai,	G	matG+	MATG-1
Gibberella nygamai		matG-	MATG-2

*Anamorph name (Fusarium species), followed by teleomorph name (Gibberella species) if other than a mating population of G. fujikuroi. The names in parentheses are synonyms.

from rice from Africa, Australia, and the United States. It appears that all rice isolates from Ghana belong to the D population (Amoah et al 1996). The only previously reported rice isolate from group D was from Sierra Leone in West Africa (Kuhlman 1982), raising the question on whether the C population actually occurs in Ghana and other parts of Africa or whether the rice in this region has been infected from the D group isolates originating from staple cereal crops of the region, such as maize and sorghum. Three mating populations of the *G. fujikuroi* complex have been associated with Bak of rice (Desjardins et al 1997). Mating population C (anamorph: ***F. fujikuroi***) (Hsieh et al 1977) and mating populations D (anamorph: *F. proliferatum* [synonym, *F. moniliforme*]) have also been isolated from rice from Asia. Population D has been isolated from rice grown in nearby fields of maize in Africa (Amoah et al 1996; Desjardins et al 1997, 2000). It is further suggested that cross infection of the maize population may be pathogenic to other host plants including rice.

Analysis of random amplified polymorphic DNA (RAPD) fingerprints of isolates of *Fusarium moniliforme* from rice, maize, and sorghum placed the isolates into three discrete groups that appeared to be generally related to the hosts from which they were originally obtained (Amoah et al 1995; **Bak Figure 2**). The groupings had been further confirmed by restriction fragment length polymorphism (RFLP) analysis, providing additional evidence that these groups constituted discrete populations. This information also confirmed results published earlier by others (Hsieh et al 1977, Kuhlman 1982, Leslie 1991). Pathogenicity test on rice seedlings of isolates from different hosts indicated that only the rice isolates produce the Bak tillers. The isolates can also be characterized into vegetative compatibility group (VCG), and within the same VCG, through variations in virulence and gibberelic acid production. There is no evidence to show that isolates pathogenic to other crop plants



Bak Fig. 2. Relatedness dendogram of *F. monitiforme* isolates from different hosts and tissues based on RAPD polymorphisms. Groups are labelled on the branches. Source: Amoah et al (1195).

can also produce gibberellic acid (Sunder and Satyavir 1998). Some rice isolates behave like isolates from other hosts causing stunting of seedlings, which is related to fusaric acid, a general toxin produced by many necrotrophs. Thus, fusaric acid and stunting of seedling may also come from different *Fusarium* spp. and are nonhost specific.

RAPD analysis suggested considerable genetic variation among isolates of *F. moniliforme* isolated from seeds of different crop species including rice, maize, sorghum, etc. from the same country (Kini et al 2002). In general, isolates from the same crop grouped together in the cluster analysis, indicating that variation was less among isolates from a seed sample of the same species compared with the variation among isolates of different crop species, confirming the results of Amoah et al (1995). Because geographical variation of the isolates is low, the difference in variation is mostly related to crop specificity. Cropping seasons of different hosts overlap in most countries in the developing world, which may lead to the spread of inoculum from one crop species to another subsequently raised crop species. The importance of the source of inoculum in spreading the disease in the field from one host crop to another may not be as important as the source of inoculum originating from the seed.

Despite the complexity of the taxonomic status of the fungus, the results of RAPD analysis conducted by different laboratories confirmed the distinct host association of the fungal pathogen with seed as well as the plant. The next question that warrants an answer is whether regional distinction among the isolates collected from the same host plant, rice for instance, exists or not. This information will show whether rice germplasm, when moved as seeds from one continent to another, may also unintentionally introduce the fungal pathogen, establishing the pathogen in different regions or countries within Asia, and between continents, such as from Asia to Africa. Such information will be useful in setting plant quarantine regulations on a particular pathogen of the same taxonomic status from the same or different crops. The Bak pathogen can serve as a case study in pathogen movement through seed or other host materials.

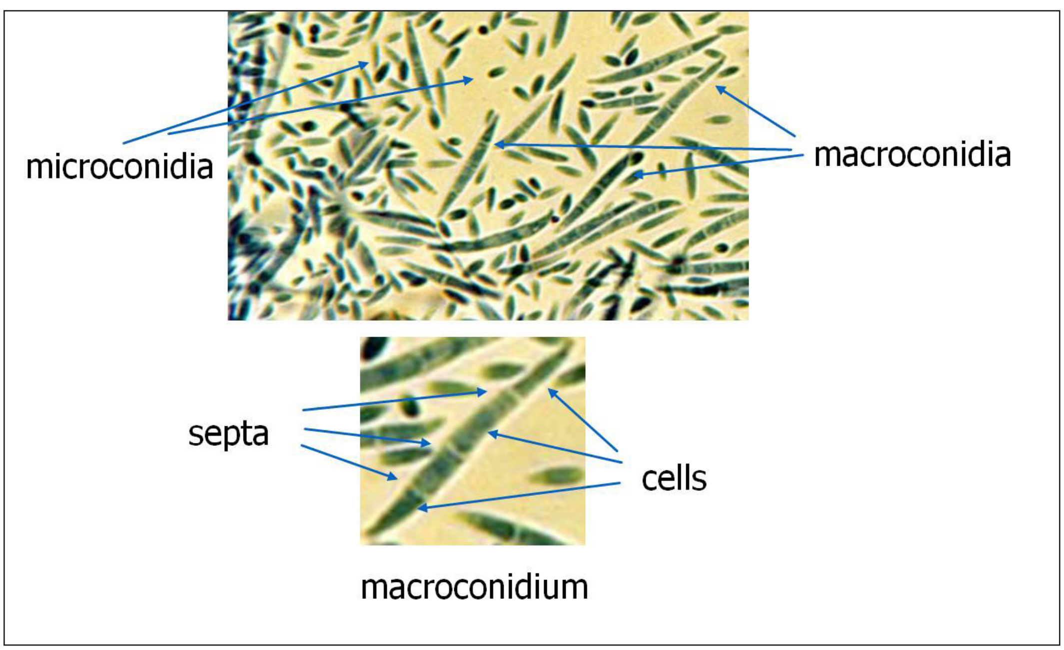
The Bak pathogen produces dark blue perithecia, globose to conical in shape measuring 22-300 x 250-300 µm (**Bak Figure 3**). The asci are ellipsoid to clavate and contain four to eight monostichous to distichous ascospores.

Ascospores are hyaline, elliptical, with one to sometimes three septa,10-20 x 4-7 µm. Microconidia are hyaline, oval to clavate, single-celled, measuring 5-12 x 1.5-2.5 um, and are formed in chains or false heads from monophialides. Macroconidia are somewhat sickle-shaped to almost straight, with a foot-shaped basal cell, thin walls, and usually three to five (but less than seven) septa, and measure 25-60 x 2.5-4 µm. The fungus does not produce chlamydospores. Dark blue sclerotia are sometimes present. *F. fujikuroi* Nirenberg (teleomorph: *Gibberella fujikuroi* Sawada) is a haploid, heterothallic ascomycete fungus.

The fungus is well-known for its production of plant growth substances, the gibberellin. This discovery led to the findings of plant growth regulators (Kurosawa 1926, Yabuta and Hayashi 1939). It should be noted too that the fungus produces a phytotoxin, fusaric acid (Yabuta et al 1934), a nonspecific plant toxin, and at least three mycotoxins, namely, beauvericin, moniliformin, and fumonisin, which affect both human and animal health (Desjardins et al 2000) **(Bak Table 3**). Gibberellin is responsible for the elongation of seedlings, while fusaric acid is thought to be responsible for the stunting and/or root rot of seedling. There is no information whether or not mycotoxins can affect the growth of the host plant. The production of both gibberellin and fusaric acid varies with the strains of the fungal pathogen and nutritional conditions for growth.

1.4. Host range

The causative fungus of rice Bak disease appears to have a wide host range. However, there is a host preference among isolates from different host plants even though they were isolated from different crop species and all are designated as *F. moniliforme*. The actual taxonomical status of the species is discussed in the section on the causal organism above. Besides rice, maize, sorghum, millet, sugarcane, pineapple, and mango have also been reported (Amoah et al 1995, Leslie 1991). More recently, *Echinochloa* spp., such as *E.*



Bak Fig. 3. The micro-and macroconidia of *Fusarium moniliforme*.

Bak Table 3. Fumonisin levels in rice fractions from field samples (Abbas et al 1998).

Rice fraction	Lab. No.[a]	Assay methods	Furmonisin levels (ug/g)[b]			Total (ug/g)
			FB1	FB2	FB3	
Unpolished rice	1	HPLC	4.7	1	0.6	6.3
	2	HPLC	2.4	0.7	1.3	4.4
	3	CD-ELISA[c]				3.5
Hulls	1	HPLC	13	2.6	1.2	16.8
	2	HPLC	8.4	1.3	1.6	11.3
	3	CD-ELISA				14.5
Brown rice	1	HPLC	0.9	ND	ND	0.9
	2	HPLC	0.6	ND	ND	0.6
	3	CD-ELISA				1.2
Bran	1	HPLC	3.1	0.6	ND	3.7
	2	HPLC	1.7	0.5	0.5	2.7
	3	CD-ELISA				3.4
White rice	1	HPLC	ND	ND	ND	ND
	2	HPLC	ND	ND	ND	ND
	3	CD-ELISA				ND

[a] Laboratory 1 included Southern Weed Science Laboratory and the University of Minnesota; Laboratory 2 was NVSL, USDA-APHIS; Laboratory 3 was Neogen Corp.

[b] HPLC = high performance liquid chromtography; CD-ELISA = competitive direct enzyme-linked immunosorbent assay. CD-ELISA data are given for total fumonisin because of cross-reactivity among fumonisins.

[c] ND = no detectable fumonisin at 0.5 ug/g when determined by HPLC or at 1.0 ug/g when determined by CD-ELISA.

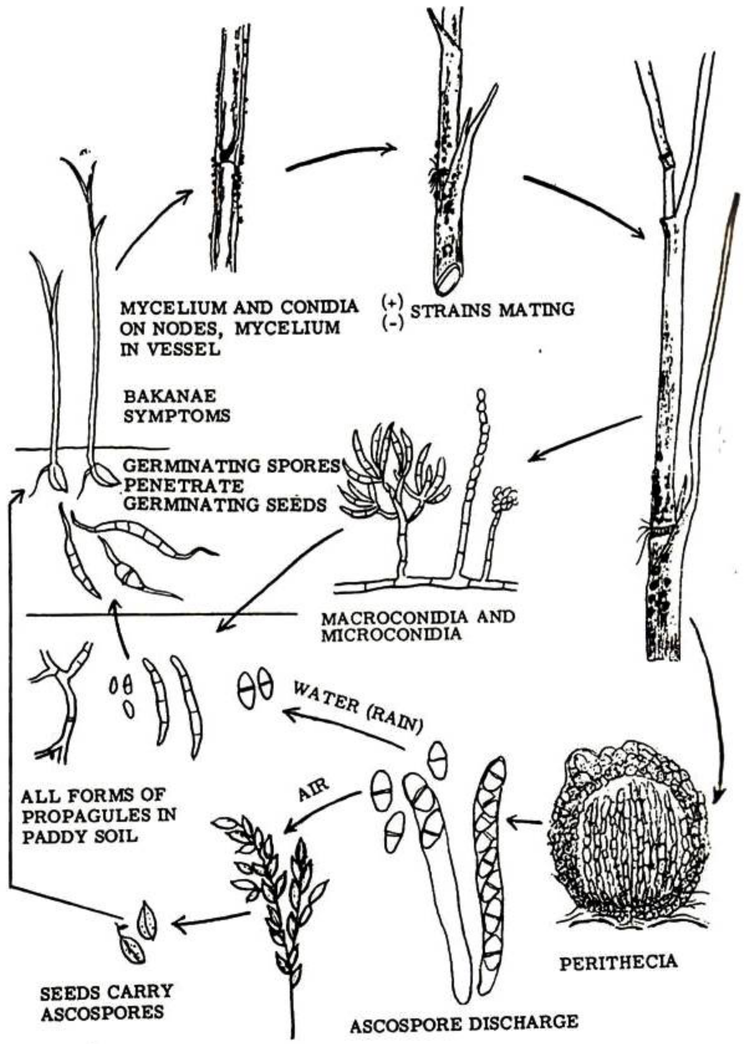
oryzoides and *E. crus-gallis,* are reported, while *E. phyllopogon, Leptochloa* spp., *Scirpus* spp., and *Typha* spp. are also speculated to be potential hosts of the fungus (Carter et al 2008).

1.5. Disease cycle

Bak is a monocyclic disease where the primary source of inoculum is vital to the development of the disease in the field. Although infection may come from ascospores discharged in the air during flowering time (Yu and Sun 1976), the pathogen is dispersed primarily with infected or infested seeds (Sasaki 1987).

1.5.1. Primary inoculum. It is quite certain that Bak can be initiated through both seedborne and soilborne inocula. The seeds are infected at the flowering stage **(Bak Figure 4).** Seedborne inoculum is important for two reasons; it provides inoculum directly to rice crop grown from these seeds and it can repeatedly introduce the fungus to distant regions to establish a new source of inoculum. Once the fungus is established in an area, there are other potential sources of inoculum besides the seed. From a recent rice Bak epidemic in California, it appears that weeds grown around field levees are also a potential source of inoculum (Carter et al 2008).

1.5.2. California case study. The recent rice Bak epidemic in California provides a good example of how a seedborne inoculum can be introduced and established in a new



Bak Fig. 4. Disease cycle of rice bakanae caused by *Fusarium moniliforme* (*Gibberella fujikuroi*).

area, and eventually can initiate an epidemic. Because of its relatively "clean" environment (semiarid with low humidity, single rice crop) that is unsuitable for survival of many rice pathogens and establishment of diseases, the outbreak of Bak in California would serve as a good model in understanding the structure and behavior of the pathogen population of an introduced seedborne pathogen striving in its new environment. This incidence may not be found in humid and sub-humid tropics, which are conducive and suitable to the introduction and establishment of many rice fungal or bacterial pathogens. Thus, the research of Carter et al (2008) is worth citing as *F. fujikuroi* is still new in California, and the Bak disease epidemic provides an understanding on pathogen population structure and its establishment into new site.

Although the first report of Bak disease in California was in1999, *F. fujikuroi* was rapidly dispersed and could be observed throughout most of the rice-growing regions through 2002, indicating that epidemic potential coupled with a high sexual recombination and high level of genetic diversity of the pathogen renders it readily adaptable to its new environment and that subsequent planting of seeds harvested from infected plants or fields aids in its further spread. However, Carter et al (2008) assert that "the genetic and genotypic diversity introduced was low with only a few unique genotypes persisting to create an effective population bottleneck." They also observed that "isolates of ***F. fujikuroi*** that infect water grass belong to the same population as do those that infect rice," enabling the pathogen to assume the inoculum spread rapidly in the field with "field reservoir" to provide inoculum for rice planted in the following years. Other findings included:

- Both mating types and six unique amplified fragment length polymorphism haplotypes corresponding to six vegetative compatibility groups were detected.
- The two most frequently isolated haplotypes encompassed 94% of the collected isolates of 172 between 2000 and 2003 when Bak was first observed in 1999 on rice in California, suggesting that clonal reproduction dominates.
- Coefficients of similarity between the unique haplotypes ranged from 0.94 to 0.98, indicating that there is very little genotypic variation in the *F. fujikuroi* population in California.
- The low level of introduced genotypic diversity, in conjunction with the asexual reproductive strategy of this population, will slow the evolutionary process, including adaptation to the California environment.
- The isolate of *F. fujikuroi* from rice is demonstrated to have pathogenicity on water grass species.

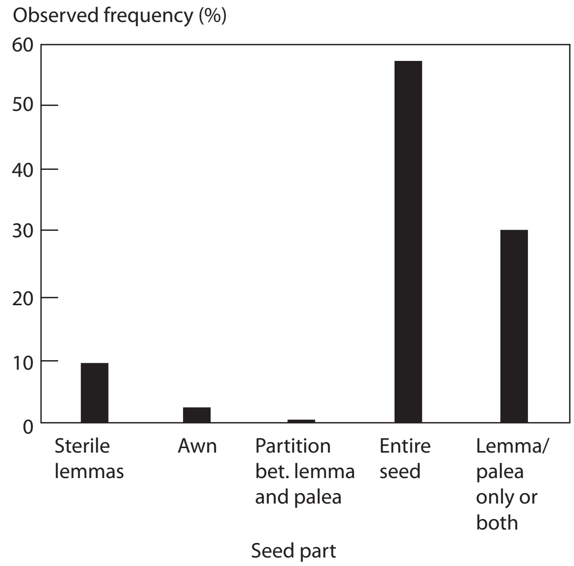
Methods used in this study included:

- Vegetative compatibility groups (VCGs) were used to characterize fungal populations, particularly for species in the genus *Fusarium*.
  - A low level of VCG diversity is an indicator of asexual (clonal) reproduction.
  - Greater VCG diversity suggests that sexual recombination may be important in the life cycle of the fungus.
  - In India and China, indigenous populations of F. fujikuroi isolated from rice all had high levels of VCG diversity, suggesting that sexual recombination occurs with regularity in these field populations.
- Molecular markers were used to elucidate population structure. Amplified fragment length polymorphisms (AFLP) are reproducible for detecting genetic variation in a population and have been used to examine the diversity present in population of *Fusarium* spp.

Other reports indicate that the fungus may survive in soil and infected crop residues (Sun 1975, Sunder and Satyavir 1998). Infected crop residue from the previous season may serve as a source of inoculum (Sun 1975). Many weed species such as *Erchinochloa oryzoides* and *E. crus-galli* in the paddy fields are reported to be hosts of the Bak pathogen (Carter et al 2008). These infected weeds, whether early or late in the crop season, provide a reservoir, hence, a potential source of inoculum during current or future rice cropping in single or double rice-cropping areas. However, there is lack of field survey data from actual research to show that these weed species are potential sources of inoculum.

The seedborne nature of the Bak fungus has been well established since the early days (Hemmi et al 1931) and in succeeding years (Dodan et al 1994, Hino and Furuta 1968, Kim 1981, Manandhar1999, Sasaki 1987, Sun 1975). The fungus can be isolated even from seeds that appear to be healthy, if they are collected from fields showing Bak disease.

When germinated, such seeds give rise to seedlings with Bak symptoms, while the reddish-colored seeds produce stunted seedlings due to fusaric acid. Bak seedlings and stunting growth of rice seedlings may be differentiated by the degree of infection of the seed. The long-term detection frequency and infection levels of rice seed lots are convincing (Mew and Gonzales 2002; **Bak Figure 5**).



Bak Fig. 5. Observed frequency of *Fusarium moniliforme* occurence on seed. Source: Mew and Gonzales (2000).

The importance of airborne ascospores in contaminated grains from the heading stage to maturity has also been proposed (Yu and Sun 1976). In moderately diseased rice fields, 100% of the seeds yielded the fungus causing Bak on agar plates, 30% of which produced Bak seedlings when planted. The ascospores are discharged into the air at night or during rain. When humidity is low, ascospores oozes out from the ostioles.

Inoculum in soil. Inoculum may not survive long in soil; however, if seeds infested with a high density of inoculum are sown in soil, the seedlings may show a variety of symptoms ranging from seedling blight, dwarf seedlings, and yellowing. Bak seedlings may be produced only when the soil inoculum density is moderate. Different forms of propagules, such as macroconidia, microconidia, and ascospores, may be found in infested soil and their presence in the soil are most likely due to the infected rice crop residue, or drip, especially conidia and ascospores, into the soil through rainwater from infected plants (Sun 1975).

1.6. Control measures

The use of resistant cultivars is a practical, cost-efficient, and environment friendly strategy for plant disease management. However, there are few studies on host resistance to the Bak pathogen despite the fact that many rice varieties are reported to be resistant or susceptible in the field. In actual rice production, there are no varieties known to be specifically developed for resistance to Bak. In practical plant breeding, it is also not known if any laboratory would devote a major effort in resistance breeding for Bak.

Despite this deficiency, as the fungus is seedborne, seed treatment and planting of high-quality seed harvested from fields free of Bak disease may be the best cultural option to minimize the disease in the field. Perhaps, most importantly, is the need to emphasize seed health management.

Proper disposal of infected crop residues, both from rice crop and weed plants would be another important cultural practice for minimizing disease incidence (Carter et al 2008).

As a potential target for biological control, Bak has been used as a case study. It has been proven that seed-bacterization with biological control agents with *Pseudomonas* sp. and ***Bacillus*** sp. is effective. For details, refer to *Biological control* in Rosales et al (1986).

Chemical seed treatment has been widely observed in preparing seedlings from seedboxes or dry-land seedbeds. In Japan, Bak was effectively controlled with application of benomyl until fungicide resistance was observed (Ogawa 1988). So, chemical seed treatment becomes less effective when a single fungicide has been widely and intensively used.

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2. Feeder root necrosis and root rot (FrT)

A complex of factors, such as water regimes, environmental conditions, and nutrient levels reflecting on physiological status of the plants besides insect damage, is associated with the occurrence of root rots. In newly emerged seedlings, leaf yellowing and loss of seedling vigor due to poor nutrient absorption are common in the foliage while brown to black root discoloration leading to tissue necrosis and decay can be seen.

FrT is caused by *Pythium* spp. Rice root rot is a result of the interaction between rice weevil and Pythium spp., and the symptoms are characterized by browning and necrosis of the roots. While feeder root necrosis shows no apparent symptoms, it is caused by or associated with *Pythium* spp. Both feeder root necrosis and root rot are reported in temperate environments. Recently in tropical Asian fields where aerobic rice is promoted, root rot in rice showing necrotic symptoms have been reported, and *Pythium* spp. have been isolated from the infected roots (C. M. Vera Cruz, pers. comm.). Whether this is similar or different from feeder root necrosis or root rot reported in temperate environments is not clear.

Many *Pythium* spp. have been isolated from rice roots of tropical rice, most of which seem to be pathogenic to rice under experimental conditions (Schneider et al 1988, Schneider 1992). As to how important the isolates may be in various rice production cultural practices such as alternate wetting and drying of the rice field during crop growth and promotion of aerobic rice remains to be seen. So far, there is little information available on the occurrence and importance of these diseases.

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3. Seedling blight (SdB)

Several seed and soilborne fungal pathogens, such as Cochiobolus, Curvularia, Fusarium, Rhizoctonia, and Sclerotium, have been implicated with the seedling blight syndrome in rice as these agents cause rapid and conspicuous discoloration, wilting, and drying of tissues in the infected plants, collectively referred to as blight syndrome. (**SdB Figure 1**) Incidence of seedling blight affects the crop stand by lowering crop productivity. Some of these pathogens also affect mature plants and therefore are treated separately in this online resource. Here, only blight caused by *Sclerotium rolsfi* (Sacc.) is covered. As the name

SdB Fig. 1. Symptoms of seedling blight. Source: Koji Azegami, Chief Researcher, National Institute of Vegetable and Tea Science, Tsukuba, Ibaraki, Japan.

indicates, the fungus produces sclerotia that survive in soil overwintering and serve as primary inoculum for the disease.

SdB was first described by Reinking (1918) in the Philippines. Subsequently, it was reported in the USA (Tisdale 1921), Malaysia (Thompson 1928), and in many tropical countries. The disease is caused by *Sclerotium rolfsii* under dry-land conditions or in dry-land seedbed nurseries. Although it is has been observed, this disease has not been well studied in rice. Because the pathogen has a wide host range (Aycock 1966), several reports on this disease on other crops are available.

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