PART III. Selected Management Practices for Rice Diseases

Section 3. Managing Biological Control Agents

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1. Introduction

Biological control involves a population-levelling process by using either native or introduced living organisms for suppressing the harmful ones either directly or indirectly. It has become useful for controlling insects, mites, weeds, and plant diseases. Broadly, this involves predation, parasitism, or competition. This technology, historically, has the origin with the control of insect pests and weeds and moved for the control of plant diseases in 1920s. Research on this aspect was intensified in the 1980s to become one of the important components of integrated pest management (IPM) strategies. It involves active human management. Biological disease control employs natural enemies of pests or pathogens to eradicate or control their populations. The management principles are built on three basic strategies: importation (sometimes called classical biological control), augmentation, and conservation. Besides this, induction of plant resistance by nonpathogenic or incompatible microorganisms also forms part of the biological control system.

Biological control is meant to suppress plant disease development using another living organism. It may consist of using different methods and approaches to introduce this organism. Deployment of host plant resistance (HPR) was also considered by some as part of this strategy (Cook 1993). However, in the true sense of biological control, mere planting a resistant variety is not commonly included. Garrett (1966) provided a broad definition of biological control to include "any condition under which or practice whereby survival or activity of a pathogen is reduced through the agency of any other living organism (except human intervention), with the result that there is a reduction in the incidence of the disease caused by the pathogen." This is the general definition adapted here. The activity of either naturally occurring or artificially introduced biological control agents (BCAs) may result in suppressing a pathogen, thus enhancing rice crop productivity. BCAs may target more than one pathogen, thus more than one disease.

However, research on biological control of rice diseases is limited and only a few successes can be narrated and patterned for general practice by farmers. Most of the efforts seem to end at identifying, characterizing and testing different kinds of BCAs. No technology of biological control has been developed nor commercialized, but a few BCAs have been actually deployed for practical use for rice disease control. The challenge ahead then was to find ways to make BCAs work in rice fields that could be used by rice farmers. To promote the effort and to assess the microbial resources in rice ecosystems, a collaborative project to explore the potential of biological control of rice diseases was initiated among Asian rice pathologists in the early 1990s. Scientists from various countries conducted collaborative research and met annually to discuss methods of isolation, screening, field performance evaluation, and application. Results from this collaboration further confirmed that the rice ecosystem is rich in microbial resources potential for rice disease management (**BCA Tables 1** and **2**) (T. Mew and A.M. Rosales, International Rice Research Institute, 1992, unpublished). This section mainly devotes to the conclusions drawn from this collaborative research.

BCA Table 1. Diversity and abundance of bacterial biological control agents associated with the rice ecosystems. (Adapted from Mew, 1998).

Genera/species	Sources							
	1	2	3	4	5	6	7	8
Bacillus subtiles	x		x			x	x	x
Bacillus laaterosporus		x						
Bacillus pamilux		x						
Serratia marcescens				x				
Serratia-like		x	x			x		
Erwinia berbicola-like	x							
Pseudomonas spp.	x	x	x			x	x	x
P. aeruginosa		x	x	x				
Burkholderai cepacia								x

Sources: 1= paddy water; 2= healthy plant; 3= sclerotia of Rhizoctonia solani AG1; 4= rhizosphore soil of paddy rice;
5= nonshizosphere soil of paddy rice; 6= sheath blight lesions; 7= blast lesions; 8= rice seeds.

BCA Table 2. Rank-dominance of predominant colony-types on isolation plates from rice seed.

Dominance rank	Identification (FAME_MIS)	Rank	
		Frequency of occurence	Mean abundance
1	*Pantoea stewartii*	1	1
2	*Curtobacterium flaccumfaciens*	1	3
3	*Pseudomonas aeruginosa*	1	4
4	*Burkholderia glumae*	5	2
5	*Enterobacter cloacae*	3	6
6	*Actinomycetes*	5	5
7	*Pseudomonas oryzihabitans*	2	9
	Bacillus pumilus	4	7
8	*Pantoea dispersa*	2	10
9	*Cellulomonas flavigena*	5	8
10	*Xanthomonas spp.*	3	13
11	*Bacillus subtilis*	5	12
	Staphylococcus gallinarum	6	11

* Dominance rank was calculated as the sum of ranks for frequency of occurrence and abundance of each colony-type.

2. Rice ecosystem: source of BCAs

The rice ecosystem is rich in microbes and the rice plant is a natural habitat, not only of harmful but also of beneficial microorganisms. The contribution of free-living microorganisms in enriching soil fertility has been demonstrated in great detail as early as 1980 (Watanabe et al 1980). However, it is not known whether these "free-living" microorganisms either promote rice growth or suppress rice diseases until the 1990s. The types and compositions of these "free-living" microorganisms were not known except those causing harmful effect, i.e., pathogens. In the early 1990s, research was promoted to look at the potential of using naturally occurring microbes in rice ecosystems to provide biological control of important rice diseases where use of HPR has been elusive or not practical. For diseases that cannot be entirely controlled by HPR, complementary measures to neutralize the disease effects with the enemies of the pathogens where microbial diversity is a component, have been considered. The purpose is to obtain knowledge on how microbes in target rice ecosystems associated with deleterious effect on crop production and those that are beneficial by suppressing the pathogens. Knowledge of composition and structure could lead to better management of microbial communities for natural disease control. The initial procedure thus focused on isolating BCAs from rice ecologies and testing their antagonistic effect on selected rice fungal pathogens. The attempt was to find alternative methods of disease management specifically on rice fungal diseases such as sheath blight (see **Part II, Section 1, Chapter 3**) where genetic improvement for resistance is still elusive. The target BCA has been bacteria in irrigated rice. Bacteria were favored because if effective, they are likely to provide a better option than other forms of microbes like fungi in anaerobic conditions created by standing water during rice crop growth.

Using common laboratory culture media, bacteria were isolated from samples of paddy water, rice plants, straw, and seed (Mew and Rosales 1986). Their abundance from various sources, especially from rice seed, is encouraging. Rice seed harbor a large number of bacteria (**BCA Table 3;** Cottyn et al 2009). The type of bacteria based on colony appearance or morph-types varied according to seed sources, seed health status, and whether germinating seed (after 24 hr of water soaking) or pregermination whole seed was used (Chen et al 1999, Cottyn et al 2001, Xie et al 2001, Cottyn et al 2009). The bacterial population included both pathogenic and nonpathogenic forms of different genera (**BCA Table 4).** Among the pathogenic forms, there are known plant pathogens and those classified as opportunistic pathogens, which can cause plant diseases when the host plants are under stress. While some are pathogenic, many of the bacteria, while in large populations, seemed to have no apparent effect on the rice plant in the tests **(BCA Table 5).** Among the nonpathogenic forms, there was a diverse group, some of which possessed antagonistic effect on selected rice fungal pathogens such as *Rhizoctonia solani* AG1-IA, *Fusarium fujikuroi, Magnarporthe oryzae, Sarocladium oryzae,* etc. but others showed both plant growth promotion at the early stage of seedling development and an antagonistic effect against the tested pathogens (Mew et al 1994, Rosales et al 1993, Cottyn et al 2001). The growth promotion property of selected bacterial isolates has also been observed in field experiments under different rates of nitrogen fertilizer **(BCA Figure 1;** Mew et al 2004). However, this trait of the BCA is not consistent and has been difficult to duplicate in subsequent experiments. The cause of such variability remains unclear.

Interestingly, there has been a shift in the broad categories of the bacteria harbored by seed during germination and seedling growth. Of the Gram-positive bacteria, *Bacillus* spp. are common and they were detected more often from pregermination whole seed than from germinated seed and mature plant parts, either senescent leaves or leaves with injury. When seed began to germinate, the number of *Bacillus* species decreases but that

BCA Table 3. Rice seed bacteria with in vitro antagonistic activity towards Rhizoctonia solani and Pyricularia grisea

Identification (FAME_MIS)	Tested	Antagonists
Enterobacteriaceae		
Pantoea stewartii	23	6
Pantoea dispersa	15	5
Enterobacter cloacae	12	1
Pseudomonas		
Pseudomonas aeruginosa	22	4
Pseudomonas oryzihabitans	10	3
Pseudomonas putida	9	9
Other gram-negative rods		
Burkholderia glumae	13	9
Acinetobacter spp.	7	2
Gram-positive cocci		
Staphylococcus simulans	12	3
Micrococcus lylae	3	2
Coryneform bacteria		
Brevibacterium epidermidis	7	1
Microbacterium esteroaromaticum	4	2
Endospore-forming rods		
Bacillus subtilis	11	10
Bacillus sphaericus	8	2
Bacillus cereus	7	2
Paenibacillus lentimorbus	2	2
Actinomycetes with aerial mycelium	18	3

BCA Table 4. Bacteria isolated from rice seeds with antagonistic activity against two tested fungal pathogens in vitro. Source: Cottyn et al (2001).

Isolates	**Antagonist (isolate no.)**	**Suppressing the growth of**	
		***Rhizoctonia solani* AG1** (no.)	***Magnaporthe oyrzae*** (no.)
Pantoea spp. *(B)*	17	11	9
Enterobacter cloacae (B)	3	3	1
Klebsiella mobillis (B)	2	2	1
Nonfluorescent pseudomonads	9	3	7
Pseudomonas putida (B)	2	2	2

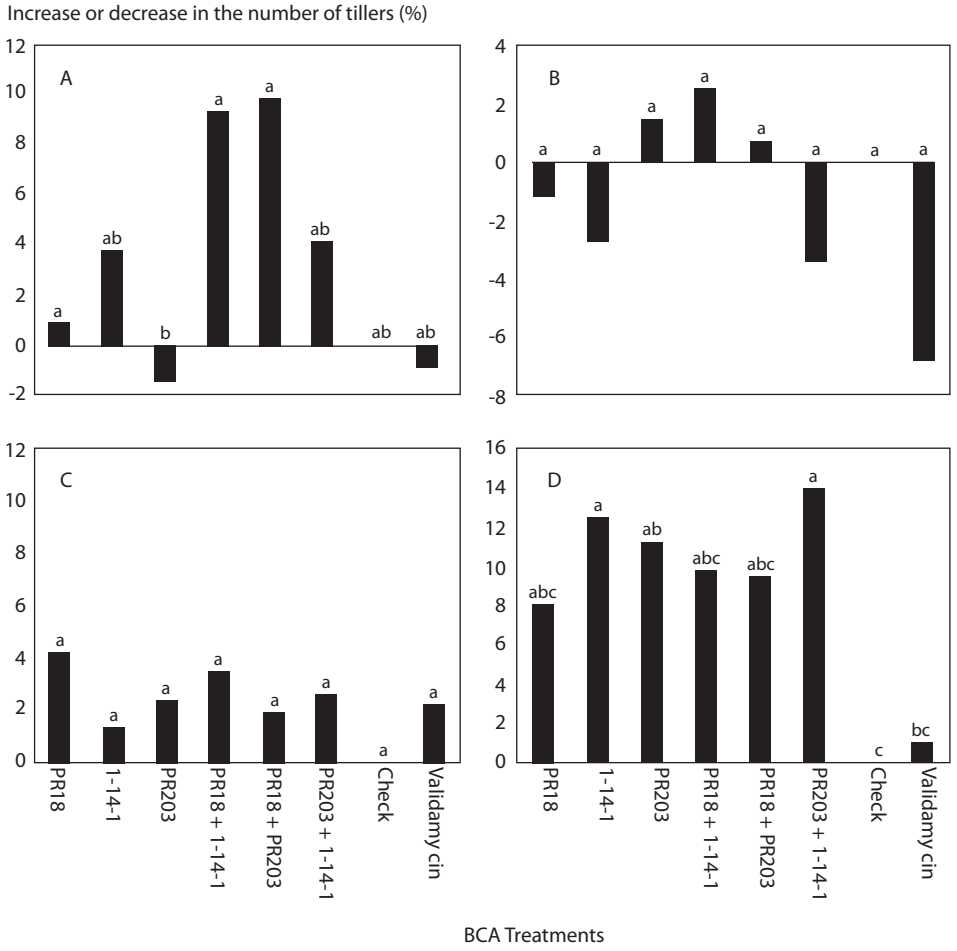
Isolates	Antagonist (isolate no.)	Suppressing the growth of	
		***Rhizoctonia solani* AG1** (no.)	***Magnaporthe oyrzae*** (no.)
Acinetobacter baumannii	3	2	2
Agrobacterium spp.	1	1	0
Bacillus subtitlis	19	17	11
Bacillus cereus	2	2	1
Bacillus coagulans	1	1	1
Paenibacillus spp.	1	0	1
Microbacterium spp.	2	2	0
Brevibacterium spp.	1	1	1
Cellulomonas flavigena	1	1	0
Staphylococcus spp.	2	1	2
Micrococcus spp.	2	1	2
Actinomycetes	3	3	2

Identifications were obtained by FAME-MIS (version 4.15); (B) = Biolog GN MicroPlate system (Microlog version 3.50).
Inhibition of fungal growth was determined by dual-culture test on pigment production medium (PPM) incubated at 28°C and scored for inhibition after 2 to 3 days. Each fungal x bacterial combination was done in three replicates.

BCA Table 5. Population density[1] of Pseudomonas (including Burkholderia spp), Bacillus and Total Heterotropic Bacteria (THB) on leaf sheaths and blades of IR72 and IR58 at maximum tillering and booting stages of rice growth.

Bacteria	Wet season 1992		Dry season 1993	
	IR72	IR58	IR72	IR58
Pseudomonas spp.				
maximum tillering	8.31	7.54	7.6	4.91
booting stage	8.13	8.46	5.22	7.39
*Bacillus*spp.				
maximum tillering	7.59	8.41	4.32	8.15
booting stage	1.11	0.66	7.63	4.14
THB				
maximum tillering	9.11	9.19	7.74	8.11
booting stage	8.57	8.55	7.9	7.68

[1] x 10-3



BCA Fig. 1. Effects of BCA treatments on the increase or decrease in the number of tillers of rice variety IR72 at various amounts of applied nitrogen fertilizer; A, O, B, 90, C, 120 and D, 180 kg/ha during the 1994 wet season at the International Rice Research Institute. PR18 and PR 203 are Bacillus spp. Strains. The Burkholderia cepacia strain used is 1-14-1. Source: Mew et al (2004).

of Gram-negative bacteria increases (Mew et al 2004, Pamplona et al 2001). Strains of *B. subtilis, B. laterosporus,* and *B. pumilus* are among the most promising BCA of this group (Mew and Rosales 1992).

The components of a particular group of bacteria also vary with space and time. The number of Gram-negative bacteria varies from one site to another and from one season to another. During seed germination, the flora of seed-associated antagonistic bacteria changed. The few well-established BCAs such as *P. putida, Pseudomonas fluorescens*, and *Burkholderia cepacia* were all isolated from rice both in temperate and tropical environments. *Serratia marcescens* and *Erwinia herbicola* were also commonly detected in rice plants (Chen et al 1999; Rosales et al 1993, 1995)**.**

The initial results have shown an abundance of microbial resources in the irrigated rice ecosystems with potential for bio-control of some of the major rice fungal diseases where genetic improvement for resistance is either lacking or not yet pursued (**BCA Table 5**). The next issue then is to determine the approach in terms of disease management

using the microbial antagonists. Can we enhance these naturally occurring BCAs in rice ecosystems in kind (i.e., diversity) and number (i.e., density)? Or, should we rely on the fungicidal approach to control the number and destructive rice diseases for stable rice crop production? The initial success was encouraging but the subsequent experiments with variable results failed to match expectations, raising concerns if microbial biocontrol is indeed a viable option for rice disease management. Deacon (1983) echoed: "The inoculants of biological agents have been ecologically unsuited to the environments where they must operate and, to overcome this, we must design more appropriate screening strategies." As Rovira et al (1992) conceded years ago, "with our knowledge, we still could not always understand and explain the successes or failures of the events of biological control and how to make it work on design and on desire." The future seemed uncertain but it offered an opportunity to reassess what is needed to make it work. In rice, no one could deny that the ecosystems were rich in microorganisms. Potentially useful BCAs could be isolated from various sources. These bacteria are beneficial not only as BCAs but also as plant growth-promoting agents in terms of seed germination and seedling vigor (Mew and Rosales 1986, 1992) (**BCA Tables 6** and **7**). The promise is there and the antagonists of primary interest are ***Bacillus*** and ***Pseudomonas*** spp., which showed broad-spectrum antagonistic activity that may fulfill this promise if we come to understand the relationship between disease development and antagonist occurrence and function. It was more interesting to find that there were many bacterial antagonists isolated from sheath blight lesions toward later stage of development (Lan and Mew 2001, Li et al 2003, Mew et al 2004; **BCA Table 8**). Why was it so? Was there a relationship between failure or restriction of sheath blight development and colonization of BCA in nature? Could there be a varietal difference on lesion development and BCA colonization? Much could be asked but more needed to be done and the clue may simply be pointing toward the future of our research on biological control.

BCA Table 6. Suppression of *Rhizoctonia* sheath blight development by antagonistic bacteria associated with different sources in rice ecosystems. Source: Mew and Rosales (1992).

	Isolates providing protection (%)				
Sources	**High**	**Median High**	**Median low**	**Low**	**None**
Paddy water	0	0	11	33	56
Infected plants (lowland)	0	4	46	21	29
Infected plants (upland)	0	13	13	27	47
Healthy plant (lowland)	2	26	42	15	15
Healthy plant (upland)	0	9	32	41	18
Sclerotia1 from lowland	0	14	38	28	20
Sclerotia1 from upland	0	11	33	26	30
Rhizosphere of healthy plant	2	5	45	39	9
Rhizosphere of infected plant	0	2	28	43	27

High= > 75%; Median high= 50-75%; Median low= 25-50%; low= 1-25%; none= no protection.

BCA Table 7. Occurrence of antagonistic bacterial strains inhibitory to the mycelial growth in vitro of three groups of rice fungal pathogens. Source: Mew and Rosales (1992).

Pathogens	Total (no.)	No. with antagonistic activity	% of total
Seedborne pathogens			
Alternaria padwickii	441	106	24
Fusarium moniliforme	441	66	15
Sarocladium oryzae	441	72	16
Sclerotia-forming pathogens			
Rhizoctonai solani AG1	1856	441	24
Sclerotium oryzae	441	180	41
Sclerotium hydrophium	441	133	30
Gaeumannomyces graminis	441	142	32
Foliar pathogens			
Bipolaris oryzae	441	135	31
Magnaporthe oryzae	441	176	40

BCA Table 8. Isolation of biological control and plant growth promoting agents from the sheath blight lesions on rice plants.

Isolate no.	Origin	Antagonistic activity[a]
4084	sheath blight lesion	22.3
4097	sheath blight lesion	23.6
4103	sheath blight lesion	25.3
4241	sheath blight lesion	21.1
4261	sheath blight lesion	20.2
4274	sheath blight lesion	21.4
4298	sheath blight lesion	24.7
4348	sheath blight lesion	26.3
4349	sheath blight lesion	22.5
4351	sheath blight lesion	21.2
4355	sheath blight lesion	22.6
4358	sheath blight lesion	22.5
4361	sheath blight lesion	20.5
4363	sheath blight lesion	22.7
4364	sheath blight lesion	21.3
4760	sheath blight lesion	25.6
4761	sheath blight lesion	27.4
4766	sheath blight lesion	26.4
CK #1	Rice seedlings upland field	26.7
CK #2	Rice field soil	26.5
CK #3	Rice seed	21.4
CK #4	Rice plant	22.5

[a] Inhibition zone (mm), mean of three replicates.
CK #1 = Pseudomonas fluorescens strain 7-14.
CK #2 = Bacillus subtilis strain B-916.
CK #3 = Burkholderia cepacia strain P-6858.
CK #4 = Pseudomonas putida strain 1821.

3. Consideration of BCA isolation and screening

Despite the presence of numerous BCAs of microbial origin in rice ecosystems, those tested and studied, not to mention used, are very low. In a BCA screening program, the main criteria for selecting a "good BCA" is often based on growth inhibiting ability in a dual culture test: the larger the inhibitory effect, the better the BCA strain. With this method, a limited number of BCA strains can only be screened. The number of BCAs may be unlimited, but the number of BCA mechanisms cannot be indefinite (Cook 1993). Alternatively, the approach may be targeting at screening of strains adaptable to a wide range of crops or soil conditions to which the targeted BCA occurs naturally. Still, the best BCA may be a combination of strains of different traits for crop environment or site. Searching for the right BCA or BCA combination is different from screening chemical fungicides. There is a need to preset the target on how a BCA must work and where it is to be deployed later. Therefore, for effective biological control, we cannot rely on a single "super" strain to work well in all local conditions for all diseases of all rice production environments. BCA strains are more location-specific and they should be targeted to match local rice crop production conditions and cropping systems. Therefore, more strains must be identified and characterized to obtain better option for BCA application, singly or in combination (mixture).

It is easy to find a strain that is antagonistic to the target pathogen causing a disease. The difficulty is in finding a highly effective and competitive strain that performs consistently at the site of deployment. It is even more difficult to understand how the antagonistic strain works ***in vivo*** against the target pathogen during pathogenesis in the plant environment. What is the stage of crop growth and disease development when an antagonistic strain should be deployed so that its function will be fully expressed to suppress the activity of the pathogen? When a known population of a BCA is deployed, it may not immediately function either because of the decline in its initial population after the introduction or because the required secondary metabolites may not have yet produced an adequate amount to be functional. While working with the biological control of wheat take-all caused by ***Gaeumannomyces graminis*** var. ***tritici*** with strains of fluorescent pseudomonads, Weller (1983**)** distinguished two distinct phases for the introduction of antagonist into the host environment. One is the "distribution phase" in which the BCA moves from the inoculum source, the bacterized seed "downward into the rhizosphere with the advancing roots." The next is the "multiplication and/or survival phase," which is characterized by maintenance or an increase in population of the introduced bacteria in the rhizosphere in competition with the indigenous rhizospheric microbiota. In the case of rice sheath blight, it is from the inoculated seed to the above-ground parts of the rice plant, the shoot and new leaves as they emerge. The initial introduction is always high but the population of the introduced antagonist decreases rapidly before its population becomes stable. In rice, one option is to let the introduced bacterial antagonist (has to be a bacillus) to colonize the sheath blight lesions as the lesions begin to develop. As a saprophyte, the antagonist should be competitive there, if not, there is little or no chance for BCAs to function.

Until we launched investigations on the biological control of diseases, there was little understanding on how, when, and in what manner a BCA strain would function after its introduction into the rice-pathogen environment. It was inevitable that we took all these into consideration when designing a testing program of BCA of rice diseases. The significant conclusions drawn are presented below.

3.1. Isolation procedures

For the isolation of a BCA, the dual culture method was used in the beginning (**See isolation procedure #1 below**) to test the inhibition effect and to identify effective bacterial antagonists against the rice fungal pathogens. Isolation was achieved from different sources such as sclerotia, leaf tissue, seed wash, and paddy water on agar plates as the first step (Mew and Rosales 1986). Antagonistic bacteria from each of these sources were then tested for their antagonism against three fungal pathogens, ***F. fujikuroi***, ***R. solani*** AG1-IA, and ***M. oryzae*** and for the ability to suppress diseases caused by these pathogens in the laboratory, greenhouse, and nursery with plants raised in microplots.

With the knowledge gained on the procedure of isolating BCAs, we designed a methodology for using seed germination as an indicator to identify potential isolates for later testing. The method offered a means to identify if seedlots carried naturally occurring BCAs and plant growth-promoting bacteria. The method had been used by scientists participating in a collaborative project in regions working on biological control of rice fungal diseases. The protocol observed is as follows.

3.1.1. Procedure #1. Isolation of BCAs and plant growth-promoting bacteria has the following steps.

- Step 1. Weigh 50-g of seed sample from a seedlot.
- Step 2. Soak the seed in 50 ml sterile distilled water with agitation by shaking for 2 hr in a rotary shaker at 100 rpm for suspending the bacterial cells from ungerminated (pregermination) seed.
- Step 3. Obtain bacteria from germinated seed. Soak 50-g of seed from the same seedlot as in Step 2 and incubate under agitation by shaking for 3 days at 30°C.
- Step 4. To compare the bacterial population and the difference between germinated and ungerminated rice seed from the same seedlots shown in Steps 2 and 3, plate the seed washes by dilution plating on King's medium B. and pigment production medium for bacterial count and isolation. Identify bacteria initially based on colony types and purify on tryptic soy agar for storage at -86°C.
- Step 5. Test isolates for pathogenicity using the injection method on 30 day-old IR24 seedlings.
- Step 6. Test nonpathogenic isolates as potential BCAs using the dual culture (food poisoning technique) method on agar plates.

3.1.2. Procedure #2. The method of testing rice seed carrying naturally occurring growth promotion bacteria uses the sources of the same and different varieties harvested from the same site (locality).

- Step 1. Soak 10 g of IR64 seed in 100 ml of sterile distilled water in a 250-ml flask and incubate for 24 hr at room temperature under agitation by shaking at 100 rpm (SS1).
- Step 2. Decant the seed wash into a sterile 250-ml flask. Use this seed wash to soak another 10 g of IR64 seed surface-sterilized with 70% ethanol for 1 min, rinsed three times with sterile distilled water, and blotted dry on sterile paper towel (SS2). The time and manner of soaking is as in Step 1.
- Step 3. Sow about 200 seed of each of SS1 and SS2 in plastic trays (previously surface-sterilized with 70% ethanol) containing about 3 kg of fine, air-dried and steamed soil collected from a rice field and properly irrigate the trays.
- Step 4. Place the trays in the greenhouse and allow the seed to germinate and the seedlings to grow for 7 to 14 days.

- Step 5. Count germination 7 days after sowing.
- Step 6. At 14 days after sowing, carefully remove 20 to 30 randomly selected seedlings from each tray and wash roots in a running tap to remove the soil. Measure the lengths of shoot and roots separately.
- Step 7. Wrap 20 seedlings (both shoots and roots) in aluminum foil, oven-dry at 50°C for 7 days, and assess the dry weight.

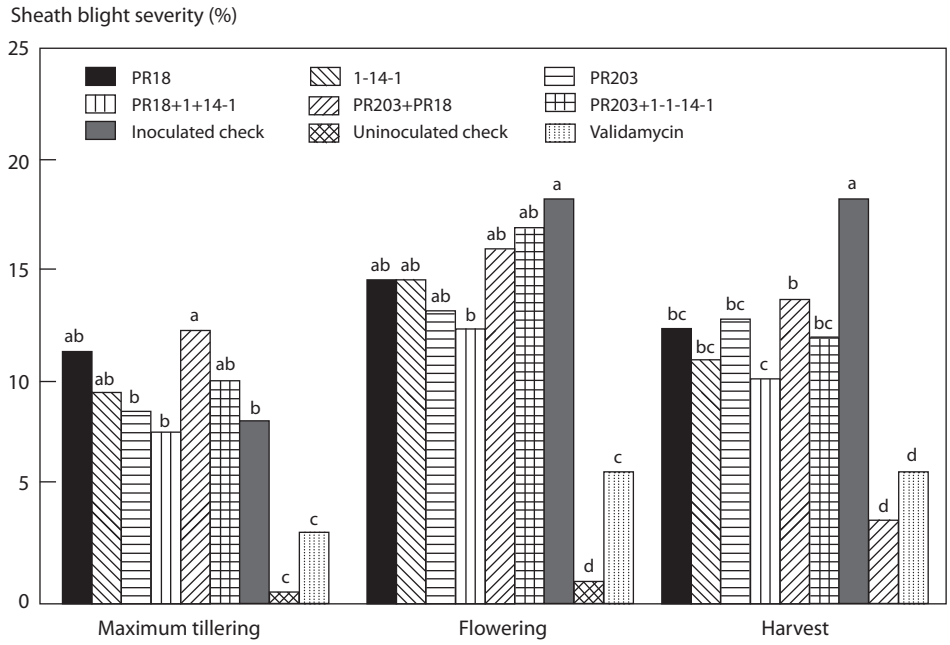
4. Conclusions from IRRI-based collaborative project

In India, experiments on evaluation of ***Pseudomonas fluorescens*** for reduction of rice sheath rot showed promising results (Sakthivel and Gnanamanickam 1986). In greenhouse tests, plants of variety IR20 treated with ***P. fluorescens*** showed a reduction in lesion length of sheath rot by 54%. Sheath rot severity was reduced from 20 to 40% among five rice varieties treated with antagonistic bacteria in three experiments conducted in the field. Additionally, the effect of seed bacterization seemed to enhance plant height, number of tillers, and grain yield from 3 to 160%.

In Korea, several bacteria were isolated from rhizosphere soil, paddy water, sclerotia of ***R. solani*** AG1 I-A, and the leaf sheath of rice plants. They were evaluated for their antagonistic activity against rice pathogens (Choi and Lee 1990**)**. The population of antagonistic bacteria was the highest in rhizosphere soil and paddy water, while the fluorescent bacteria seemed to outnumber the nonfluorescent on healthy rice plants. About 55% of the fluorescent bacteria showed inhibition of mycelial growth of ***R. solani*** in vitro compared to 20% of the nonfluorescent bacteria (Choi and Lee 1990). When rice seed were coated with the antagonistic bacteria, the efficacy of an isolate of ***P. aureofaciens*** in reducing rice sheath blight development during the early-growth stage was greater than those of ***P. putida*** and ***P. fluorescens***. Some antagonistic isolates were effective on rice seedling blight caused by ***Pythium ultimum*** in an artificially infested soil in the nursery. The percentage of seedling blight was significantly lower in response to coating the seed with ***P. aureofaciens***. Isolates of ***P. putida*** and ***P. aureofaciens*** incorporated into soil suppressed bakanae development caused by ***F. fujikuroi***. Seedling blight caused by ***P. ultimum*** and sheath spot and aggregated spot caused by ***R. oryzae*** and ***R. oryzae-sativae***, respectively, were also reduced by either seed treatment or soil incorporation of these three antagonists, but spray application of bacteria was not effective against seedling blight (Choi and Lee 1990).

In similar studies conducted at IRRI in the Philippines since 1986 (Mew and Rosales 1986, Mew et al 1994), rice plants had significantly less sheath blight when grown from seed treated with either fluorescent or nonfluorescent bacteria than plants from untreated seed. Plants from treated seed had smaller lesions than plants from untreated seed in both first and second plantings. Plant imprints from 7-day-old seedlings pressed on KMB agar plates showed that the fluorescent bacteria were on the intact seed of the seedlings as well as on the roots, coleoptiles, and first and second leaves. In irrigated rice, sheath bight usually starts with infections of the sheath near or slightly above the waterline (Kozaka 1975). If after seed bacterization the bacteria only colonize the roots or other portions of the rice plants below the waterline, their efficiency would not be very effective. But the strains of fluorescent bacteria used for seed treatment in our studies were detected on portions of the leaf sheath immediately above the waterline and further on the above-ground plant parts even after 45 days of sowing, indicating a promise of their application in relation to sheath blight control.

The properties of BCA isolates have also been evaluated with field-grown plants. The greater disease reduction came with a mixture of ***Pseudomonas putida*** and ***Bacillus cepacia*** than with individual bacterium when tested in both wet and dry reasons in direct seeded and transplanted IR72 (**BCA Figure 2**)**.** This bacterial combination, which



BCA Fig. 2. Sheath blight severity in rice cultivar IR72 at various BCA treatments in an experiment conducted during the 1994 wet season at the International Rice Research Institute. PR18 and PR 203 are Bacillus spp. strains. The Burkholderia cepacia strain used is 1-14-1. Source: Mew et al (2004).

gave better disease control than treatments with a single bacterium, seemed to exhibit synergism. However, the effect on growth promotion has not been consistent in all the treatments and in subsequent tests despite better biocontrol activity in selected bacterial strain mixture compared with individual bacterial strains (T. W. Mew, International Rice Research Institute, unpublished data). Bacillus spp. produce inhibitory substances (Rosales et al 1995) and phytohormones that induce resistance. P. putida and B. cepacia are known to produce secondary metabolites, 2,4-diacetylchloroglucinol and pyrrolnitrin, respectively (Rosales et al 1995). The presence of a cocktail of metabolites may enhance the suppressive effect on the target pathogen. Hence, in a bacterial strain mixture, compatibility of the BCAs is an important consideration.

During the course of the collaborative research, a distinctive finding has unfolded that among the BCA isolates, many possessed the property of growth promotion, which was measured in the context of seed germination. Seed may serve as an important carrier of BCA to be introduced into the field. Later in the research, the focus was on microflora of rice seed. Rice seed harbor a large number of bacteria, some of which are pathogens but many are not pathogens. Among the nonpathogens, two groups were distinguished: one had no obvious effect on both rice growth and their pathogens while the other showed biological control especially against different rice fungal pathogens and plant growth promotion effect. Based on seed germination and suppression of seedborne ***F. fujikuroi***, the causal pathogen of bakanae (see **Part II, Section 1, Chapter 1**), the antagonistic bacteria were classified into three groups: those that promoted seed germination and enhanced seedling vigor, those that had no effect on seed germination, and those that had a deleterious effect on seed germination (Rosales and Mew 1997). Evidently, these microorganisms formed part of the bacterial community associated with the rice ecosystem.

The finding further confirms that microorganisms with biocontrol potential against rice fungal and bacterial diseases occur naturally in association with the rice plant. It also indicates that a certain population of plant-associated microorganisms may increase in response to disease development and then decline to their lowest population in the ab-

sence of the pathogen. Wheat take-all may be a classical example of this (Kwak and Weller 2013).

However, there has been limited research aimed at commercializing the technology of biological control for rice disease management. One of the most successful cases of these research collaborations is the commercialization of ***Bacillus subtilis*** strain B916 for sheath blight and false smut management conducted by Chinese scientists at Jiangsu Academy of Agricultural Sciences, China. See the case study further below. But first we continue on the methodology and design of the research in relation to technology development.

5. Distribution of rice-associated BCA on the plant

On plant surfaces, competition among microbes for substrates and space may play an important role in regulating the overall qualitative and quantitative nature of different bacteria (Hirano and Upper 1986). It is desirable to determine whether the BCAs are naturally present in or introduced to the rice plant. The populations of ***Pseudomonas***, ***Bacillus,*** and total heterotrophic bacteria (THB) at different plant growth stages were assessed by following a time series of the dilution plating technique. Significant differences of each bacterial group in the populations were noticed. Each bacterial group seemed to follow a definite trend, apparently influenced by crop growth stages. IR58, a short-growth duration, semidwarf, high-yielding variety, appeared to support a higher population density of ***Bacillus*** and THB when the plants were affected severely by sheath blight caused by ***R. solani*** AG1-I A in the field (Mew et al 1994). An increase in pseudomonad population density corresponded to the decrease in population level of ***Bacillus*** as the rice crop approached the booting stage. However, a reverse trend was observed toward the later stages when the rice crop approached maturity and leaves began to show senescence when the bacillus population tended to increase. Similar observation was made on the bacillus population associated with rice blast lesions, which appeared to cause a corresponding decrease in the population of pseudomonad population. This negative interaction may be related to niche competition on plant surfaces such as competition for nutrients or production of secondary metabolites between genera or species. Different kinds of secondary metabolites are reported to be produced by species of pseudomonads from rice plants (**BCA Table 9**; Rosales et al 1995).

Although antibiotics may be important in the rhizosphere, there is little information on their role in microbial interactions in the phyllosphere or in a natural system related to biological control. Regardless of the initial size of BCA applied to the rice plant, it appeared that the temporal distribution was similar at the end of the crop growth or when the crop approached death due to infection, further confirming that there are both a "distribution phase" and "multiplication and or survival phase" of applied BCA (Weller 1988). In addition, the applied population declined rapidly and the spatial distribution of pseudomonad and bacillus BCA on rice plant surface was inversely related to growth stage of the crop even though the THB population remained unchanged. This may confirm an early observation that isolation of pseudomonad BCA was more common in young rice plants or leaves than in aged or senescent ones from which it is easy to isolate bacillus BCA. The rice growth stage had a significant influence on the colonization by a specific type of BCA. In a given condition, our experience showed that bacillus BCA was easily established on rice at the booting stage on lower (old) leaves and the leaf sheath. The age of disease lesions, in the case of sheath blight for example, seemed to have little influence on the kinds of BCA isolation.

BCA Table 9. Production of antifungal metabolites by antagonistic bacteria from different sources associated with the rice system. Modified from Rosales et al (1995).

Strains	Identification	Sources	Rice pathogens inhibited	Metabolites
In-b-6854	*Burkholderia cepacia*	rice seed	*Rhizoctonia solani* AG1	pyrrolnitrin
In-b-6858	*B. cepacia*	rice seed	*Fusarium moniliforme* Sarocladium oryzae *Magnaporthe oryzae* *R. solani* AG1 *F. moniliforme* *S. oryzae* *M. oryzae*	pyrrolnitrin
In-b-1821	*Pseudomonas putida*	rice plants	*R. solani* AG1 *F. moniliforme* *S. oryzae* *Biploris oryzae* *Gaumannomyces graminis*	2,4-diacetyl phloroglucinol
In-b-109	*P. aeruginosa*	soil	*R. solani* AG1 *G. graminis*	phenazines, pyocyanine
In-b-748	*P. aeruginosa*	rice plants	*R. solani* AG1 *M. oryzae* *F. moniliforme* *S. oryzae* *G. graminis*	phenazines, pyocyanine
In-b-7-14	*P. fluorescens*	rice plants	*R. solani* AG1 *F. moniliforme* *M. oryzae*	several unidentified active compounds

6. Performance of BCA application

There are many issues related to performance of biological control of crop diseases. Among these is the inconsistency of BCA performance as pointed out by Rovira et al (1992). Compared to chemical fungicides, BCAs are "affected by factors that influence their survival, proliferation, and spread." One of the issues that seemed to stand out distinctively was the perception of the researchers. BCA was often treated and applied in the same manner as chemical fungicides are used. Thus, biological control has been equated to chemical control. And like chemical control, the expectation is that BCAs should function or be effective as chemicals, i.e., their effect should be immediate after application. In reality, microorganisms intended for use as introduction must be treated in a biological instead of a chemical model (Cook 1993). They are more pathogen-, crop- and site-specific than a chemical fungicide.

The other issue that we have often faced is whether microbial biocontrol agents should be applied as "preventive" or "curative" measures. To make BCAs curative, they need to maintain an 'effective dosage" on the plant surface for a longer duration before

the pathogen arrives. Unfortunately, research has shown that, after introduction, the BCA population rapidly declines to an undetectable level within 24 to 48 hr. The "window" of its efficacy was very small compared to chemicals (Mew et al 1994). A BCA could be curative if it is introduced at the "right time" with an effective dosage in the presence of the pathogen inoculum at a 'reasonable" level. Without any knowledge of the "right time", and how a BCA functions ***in situ,*** it must be applied more frequently, which is impractical and uneconomical. There must be a niche where the BCA can sustain its population in the canopy. Inevitably, some knowledge of the disease epidemic or disease development process, and pathogen biology are also needed as the BCA application needs to fit into the niche to offset or disrupt the process of disease development. This could not be achieved without knowledge of the disease triangular relationship.

Unfortunately, the focus of research on biological control has been more on finding "better and more effective" strains of BCA than understanding the role of BCAs in a disease development process. We have neglected that an effective biocontrol is only a loop in the total system of crop production management. More importantly, to be successful, the aim of biocontrol research should be oriented on technology development rather than an enquiry. Research is needed but technology development is the goal. Therefore, we need to take a systems approach. As such, we should not approach its application based on knowledge or activities of single discipline. There is a need to involve other disciplines such as soil science, crop physiology, microbiology, and microbial genetics.

6.1. Targeting the pathogen and the disease

First of all, the target disease should be defined. In rice, the key to rice disease management is through genetic improvement of the host plant, i.e., disease resistance. However, in resistance breeding, the effort is focused on a few diseases with high epidemic potential. It is unlikely that rice breeders will change their agenda in the near future. More importantly, there are diseases such as sheath blight that have become one of the most limiting biological constraints to high yield and sustainable rice production, yet there is no true genetic resistance identified or proven up to this day. Therefore, alternative approach is needed to address such disease problems during rice crop production in the wet season. In such a situation, biological control may be a potential option exploiting the availability of abundant rice plant-associated BCAs. For practical reasons, the immediate targets are set on fungus diseases, such as sheath blight and seedborne problems. Whether biological control has a niche where its utility can be built into farmers' cultural practices is crucial in the assessment process before major investment in research can be suggested.

The importance of sheath blight is well understood in rice production. Bakanae, a minor disease, is caused by ***F. fujikuroi***, which is seedborne. While biological control of sheath blight is needed, bakanae is a good model system on which to test the principles of biological control using seed as a carrier, i.e., bacterization.

6.2. Tackling performance inconsistency

As mentioned earlier, performance inconsistency is the key issue in using BCAs as a tool in disease management. Because of the variable results obtained in BCA field testing, research was conducted to improve BCA performance in target rice pathosystems. It appears that the manner of microbial introduction that seeks out short-term effects has made BCAs less effective and dependable in disease management. Rice ecosystems are rich in microorganisms. The first consideration must be to explore indigenous BCAs associated with rice plants because of their biological and ecological advantage for sustainability in rice ecosystems. The real potential may well rely on the use of different locally adapted

strains on each crop and in different sites (Cook 1993). It is also important to fully study BCA establishment on rice plant surface and its role in the above-ground disease development.

6.2.1. The hypothesis. There are two approaches to biological control. One is through augmentation, i.e., introduction of BCAs to the host crop where a target pathogen is present. Thus, the aim of augmentation is directly targeting the pathogen in question, whether it is ready to attack or has attacked the host plants, i.e., at pre- or post-infection. The other approach is known as natural biological control—a strategy used more frequently and successfully by entomologists for insect pests. In plant pathology the approach is to modify the environment to favor the growth or action of a BCA either introduced or naturally exiting at the site. A noted example of natural biological control in plant disease is the take-all decline of wheat caused by ***Gaeumannomyces graminis*** var. ***tritici*** (Cook 1993). It has been shown that continuous wheat planting tends to have a high level of natural suppression of take-all. After investigation, the mechanism of suppression is associated with microbes in the rhizosphere. Upon introduction, a BCA is used largely as a "preventive" rather than a "curative" measure (Deacon 1983). It can only be considered "curative" if it is introduced at a time that the pathogen is also present and ready to attack. For this reason, in application of a BCA, either as a "preventive" or "curative" measure, the intervention point in disease development should be identified or understood before its application. In rice diseases, especially for sheath blight, a BCA should be deployed in relation to the disease epidemic process. In practice can we tackle the two approaches, i.e., introduction then enhancement of either the introduced or other naturally occurring BCA for long term and sustainable biological control of rice diseases? This is a different but challenging approach.

To improve BCA performance, two questions arise. The first is how to ensure BCA establishment after its application. Soil amendments either with lime or organic substrates have been applied. Similarly, foliar application of urea by spraying has been proposed. With rice sheath blight, which manifests on the above-ground parts, foliar application of a selective substrate may assist establishment of an applied BCA. However, it needs to be ensured that the amendment has no positive effect on the pathogen and that the applied BCA gets firmly established in the rice crop canopy following the amendment. Nutrients such as urea have been applied to the canopy to support a BCA population established as an epiphyte for some BCA-crop-pathogen systems. Thus, the spread "nutrient" has to be selective. Or, the applied nutrient such as urea may enrich the nontarget microorganisms including the disease pathogen, resulting in a more severe disease outbreak. Taking advantage of the saprophytic nature of BCAs, especially for a bacillus BCA, a disease outcome, such as an initial sheath blight lesion, may provide a "beachhead" for expanding and advancing the population of the applied organism to suppress further development of the lesions. This idea was supported by the fact that large numbers of BCAs have been isolated from sheath blight lesions (Lan and Mew 2002). This has led to the development of the concept of "a support system" to enable the applied BCA to establish a functional initial population on the plant surface in combating the disease pathogen.

If the population of the introduced BCA declines rapidly in the presence of high inoculum of the target pathogen, the applied BCA may not be effective in preventing the attack of the host plant by the pathogen. One way to slow down the activity of the pathogen is to initially treat the host with an effective fungicide spray to "weaken" the pathogen. A half dose of an active fungicide should be adequate. Elad et al (1992) considered this as "a trigger for biological control."

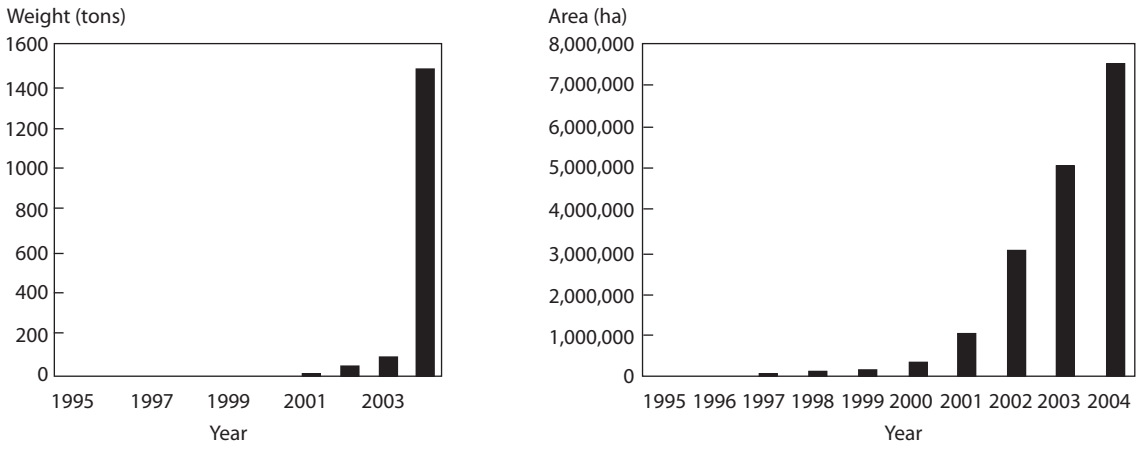
To maintain the efficiency of the applied BCA in the canopy, the initial inoculum of the target pathogen must be weakened or apply the BCA when the initial lesions have

just begun to develop, thus enhancing a "supporting system" on the plant surface. However, this approach is tricky for general disease management using BCAs because once the pathogen hyphae invade the host tissue, as a saprophyte, it is difficult for the BCA to "run after" the rapidly advancing infection. For sheath blight lesion development, invasion is through the runner mycelium and infection cushion. There is a gap. The following case study shows research and technology development on BCA (Mew et al 2004).

Another important aspect is the manner by which a BCA suppresses the disease. If the BCA is functional, it must be able to reduce the disease foci of sheath blight, in number for long-term prospect and in focal point expansion or size of disease foci for a short-term result. Thus, in the case of rice sheath blight, BCA provides a short-term effect by reducing the disease's "focal point expansion" or lesion development and a long-term effect by reducing sclerotium production or the number of disease "foci" in continuous rice cropping. In disease such as blast, the short-term effect includes reducing the "rate of sporulation" or "inoculum efficiency", thus, minimizing the secondary spread of the disease (**BCA Figures 3** and **4**), which in seedborne diseases, minimizing the potential of seedborne inoculum. BCA application after initial disease development may be essential to provide "refuge" or a "support system" to maintain that "effective dosage" on the rice plant surface.

7. Case studies and related research

The initial success in isolating and identifying a large number of bacterial antagonists within the rice ecosystem opened the door for further exploring the potential of biological control of rice diseases. The variable and inconsistent results from testing the bacterial strains from different sites in a coordinated program involving different countries did not immediately demonstrate how BCAs are to be used by the farmers. It was decided to reset the research with an objective to develop a BCA product for use by rice farmers (Mew et al 2004). Sheath blight and bakanae were chosen for the test cases. While the bacterial antagonists were targeted for irrigated rice, their utility in an upland rice cropping system may be limited. Therefore, other approaches for biological control were also tested. The case studies present different attempts made under different rice culture conditions where the rice ecosystem differs. The target disease chosen for the management is also different.



BCA Fig. 3. Area of field expansion use of BCA916 by farmers in Jiang-su China from 1995 to 2004 and in amount of BCA production from 1995 to 2000.

BCA Fig. 4. Product of the formulation of BCA-916 for sheath blight and false smut use, commercialized in Jiang-su, China.

Rice seed naturally carries several BCAs. Various groups of bacteria have been isolated from rice seed (Cottyn et al 2009). The nonpathogenic group that showed antagonism against rice fungal pathogens has further been divided into two subgroups. Subgroup A includes those that promote rice seed germination and seedling vigor along with an antagonistic effect. Subgroup B includes those that show an antagonistic effect exhibiting deleterious effects on rice seed germination (Cottyn et al 2001, Xie et al 2001). The native BCA is part of the internal resource of the rice ecosystems. Biological control with native BCAs is thus regarded as managing the existing biological resource.

7.1. Biological control of sheath blight

The research was formulated to address a series of questions for sheath blight management, which include (1) studying the short- and long-term effects of BCAs in the epidemic process and development of sheath blight, and (2) improving BCA efficacy by adding a dose of a commonly used fungicide (e.g., Jingangmycin in China and Validamycin in Vietnam) for providing an initial trigger for the biological control. For aiding the "distribution" and "multiplication or maintenance" phases of introduced BCAs, there was a need to find ways to "weaken or slow down" the activity of ***R. solani*** AG1 I-A in attacking the rice plant. For this purpose, the antagonistic effect of BCAs with an added dose of fungicide was tested.

To enhance and stabilize the efficacy of BCA, a dose of commonly used fungicide nontoxic to the BCA is added to the BCA suspension. The added fungicide weakens the infectivity of ***R. solani*** AG1 I-A or prevents the development of the infection process before the BCA could reach its functional population. As mentioned earlier, there were two hurdles that the BCA needed to get over after its introduction. One is the distribution phase, which often diluted its initial population to compete with native microflora. The other is

beginning a survival or maintenance phase before multiplication could take place on the plant surface. Addition of a half-dose of Jingangmycin to the BCA strain B916 consistently suppressed sheath blight development in the field at all test sites (**BCA Tables 10** and **11**). The addition of the fungicide into the BCA suspension seemed to reduce the variability of BCA performance (Chen et al 1996). Subsequently, in technology development, we formulated the product of the BCA with half a recommended dose of a commonly used fungicide for sheath blight control and the application was favorably accepted by farmers. To execute the research, a hypothesis of the effect of the BCA on the expansion of the sheath blight focal point was proposed to guide the experiment (**BCA Figure 5**). In testing the long-term effect of the BCA, the fungicide has been eventually replaced with the total BCA formulation.

7.1.1. Limiting focus point expansion. The Subgroup A BCA has been tested in the subsequent studies. In the test, significant reduction in sheath blight was observed at flowering and at harvest regardless of the rates of fertilizer used in the dry-season crop. The reduction in sheath blight by BCA application apparently coincided with lesion expansion. Under field conditions, lesions of sheath blight appeared 5 days after pathogen inoculation (**BCA Figures 6** and **7) b**). At this stage, BCA application establishes an effective contact between the BCA and the fungal mycelium resulting in less lesion development. This suggests that the BCA reduces the inoculum efficiency preventing secondary spread of sheath blight from plant to plant or from leaf to leaf. Thus, the BCA function is to restrict the focal point expansion of the disease in the sheath blight development process (**BCA Figures 8-12**) (Mew and Rosales 1986, Mew et al 2004). Because the antagonistic bacteria used in the studies were saprophytes by nature, it is unlikely that they could enter healthy rice plant tissues in the process of sheath blight development. It is, therefore, also highly unlikely that once the fungal pathogen invaded the host tissues, the saprophytes would reduce the sheath blight severity often measured by lesion length or relative lesion length. This was because of the variable results experienced in most of the experiments initially conducted.

BCA Table 10. Effect of the antagonistic bacterium *Bacillus subtilis* B-916 on incidence of Rhizoctonia sheath blight and rice yield in farmers' fields at three sites in Jiangsu Province, China. Source: Mew et al (2004).

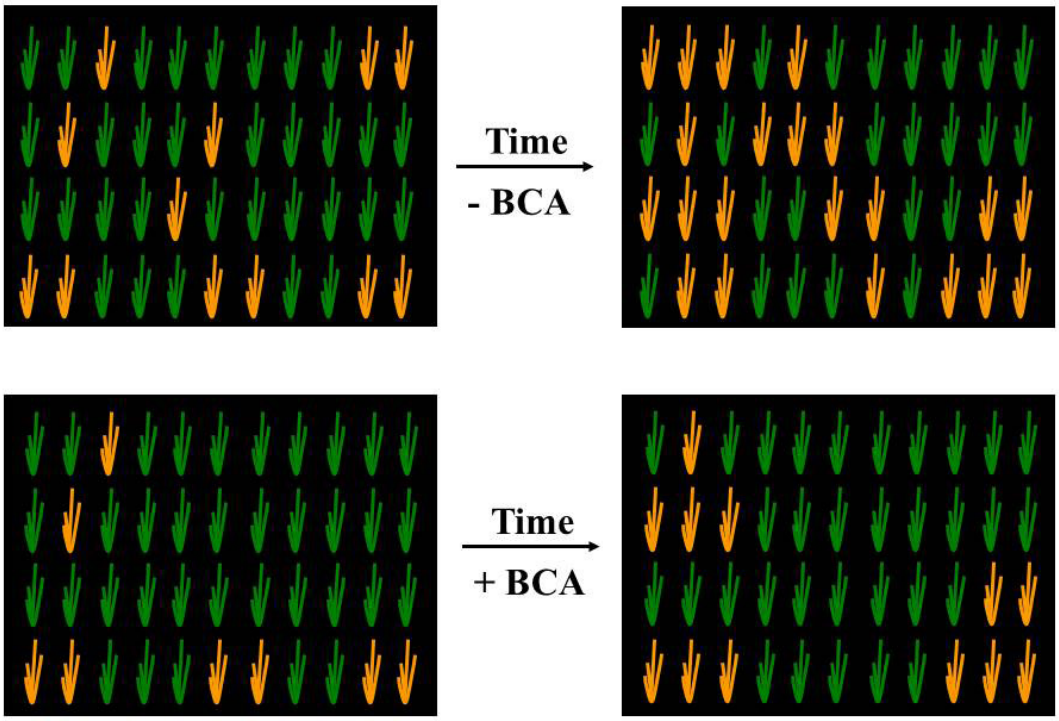
Year and treatment	Dosage (L/ha)	Disease incidence (%) and yield (t/ha)					
		Juroung		Jiangyan		Wujiang	
1996							
B-916	3.75-4.50	9.9b	8.7a	10.5b	9.2a	18.8b	9.2a
Jingangmycin	3.75-4.5	11.7b	8.5a	9.6b	9.3a	12.4c	9.4a
Check		29.4a	7.6b	42.8a	7.2b	48.8a	7.6b
1997							
B-916 + Jingangmycin	2.25 + 2.25	8.7b	8.5b	2.1b	9.2a	8.06b	9.1a
Jingangmycin 4.5		11.0b	8.4b	2.4b	9.3a	4.61b	9.3a
Check		38.5a	7.0a	17.2a	7.9b	33.84a	7.6b

Each disease incidence is a mean of 10 replicates, and for yield a mean of six replicates.
Means in a column within the same year having the same letter are not significantly different each other (P>0.01) by Duncan's multiple range test.

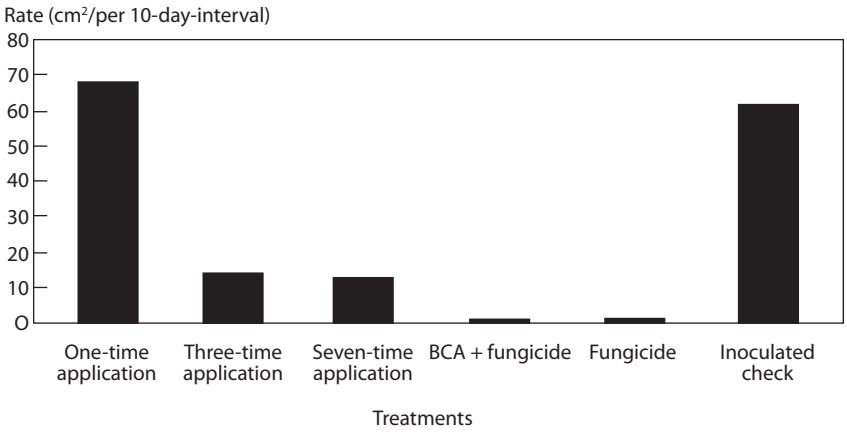
BCA Table 11. Performance of antagonistic bacterium Bacillus subtilis B-916 on incidences of Rhizoctonia sheath blight in farmers' fields at three sites in Jiangsu province, China.

Year	Treatment	Dosage (L/ha)	Disease Incidence (%)* Jurong	Jiangyan	Wujiang
	B - 916	3.75 – 4.5	9.90 b**	10.50 b	18.80 b
1996	Jingangmycin	3.75 – 4.5	11.70 b	9.60 b	12.40 c
	Check		29.40 a	42.80 a	48.80 a
	B - 916	4.5	8.70 b	-	-
	B – 916 + Jingangmycin	2.25 + 2.25		2.10 b	8.06 b
1997	Jingangmycin	4.5	11.00 b	2.40 b	4.61 b
	Check		38.50 a	17.20 a	33.84 a

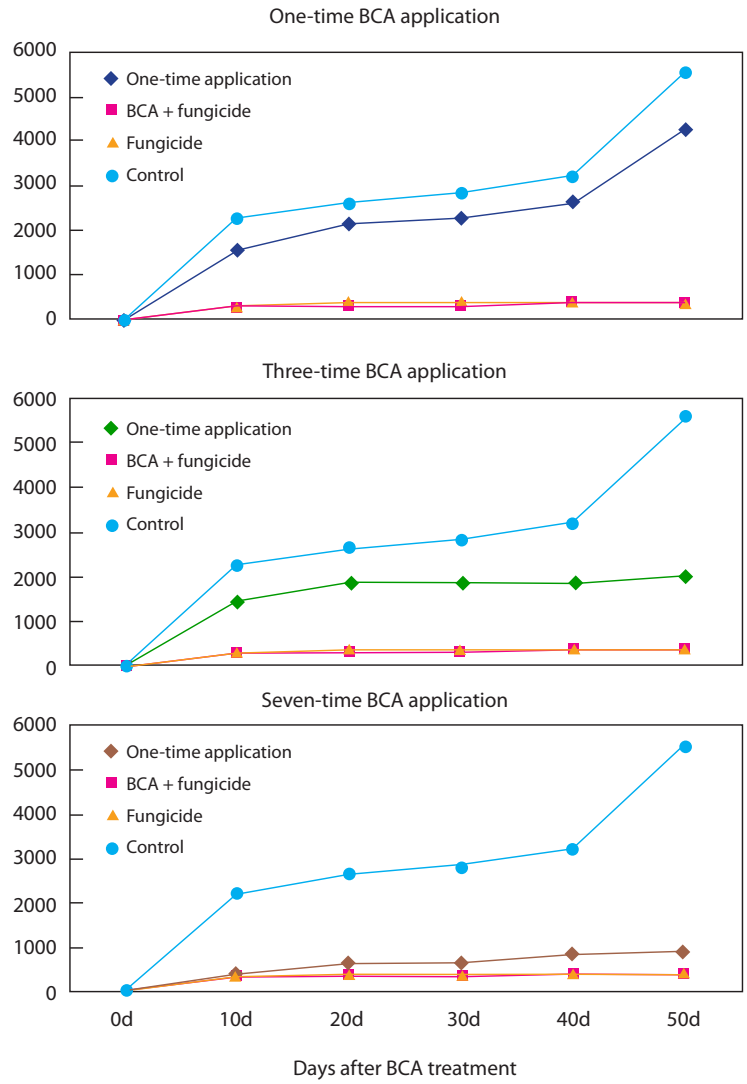
* Each disease incidence data is a mean of 10 replications.
** Means in a column within the same year having the same letter are not significantly different from each other (P<0.01) by DMRT.



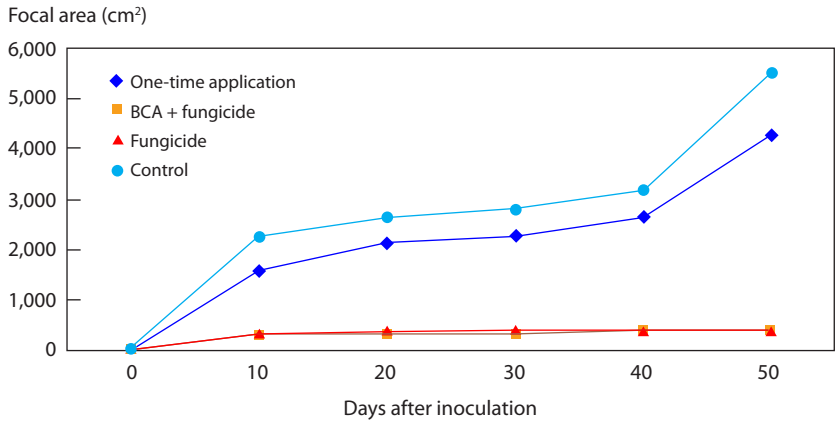
BCA Fig. 5. A working hypothesis to illustrate the efficacy of the BCA on focal-point expansion (+ BCA) of sheath blight development as compared to the case of no BCA application (- BCA).



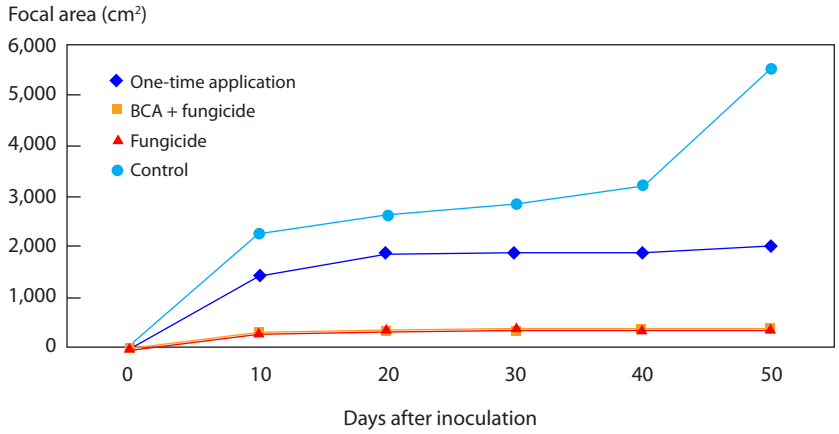
BCA Fig. 6. Rate of focal area spread on BCA-treated plots vs. control. 2002 dry season.



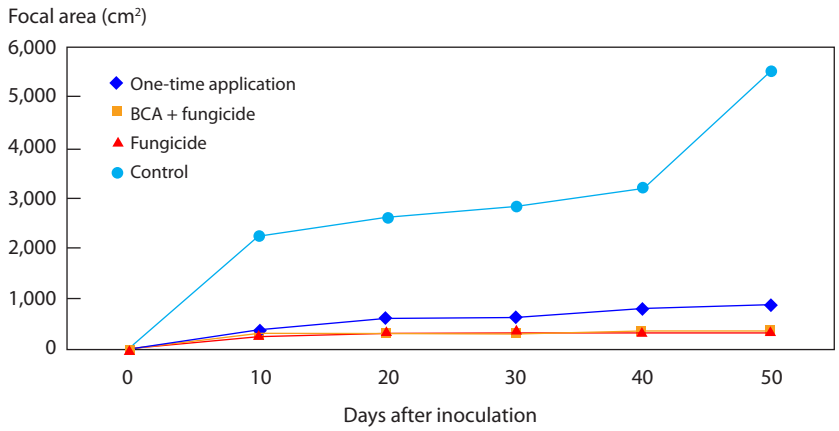
BCA Fig. 7. Focal expansion of sheath blight of rice as affected By duration of BCA application period. 2002 dry season.



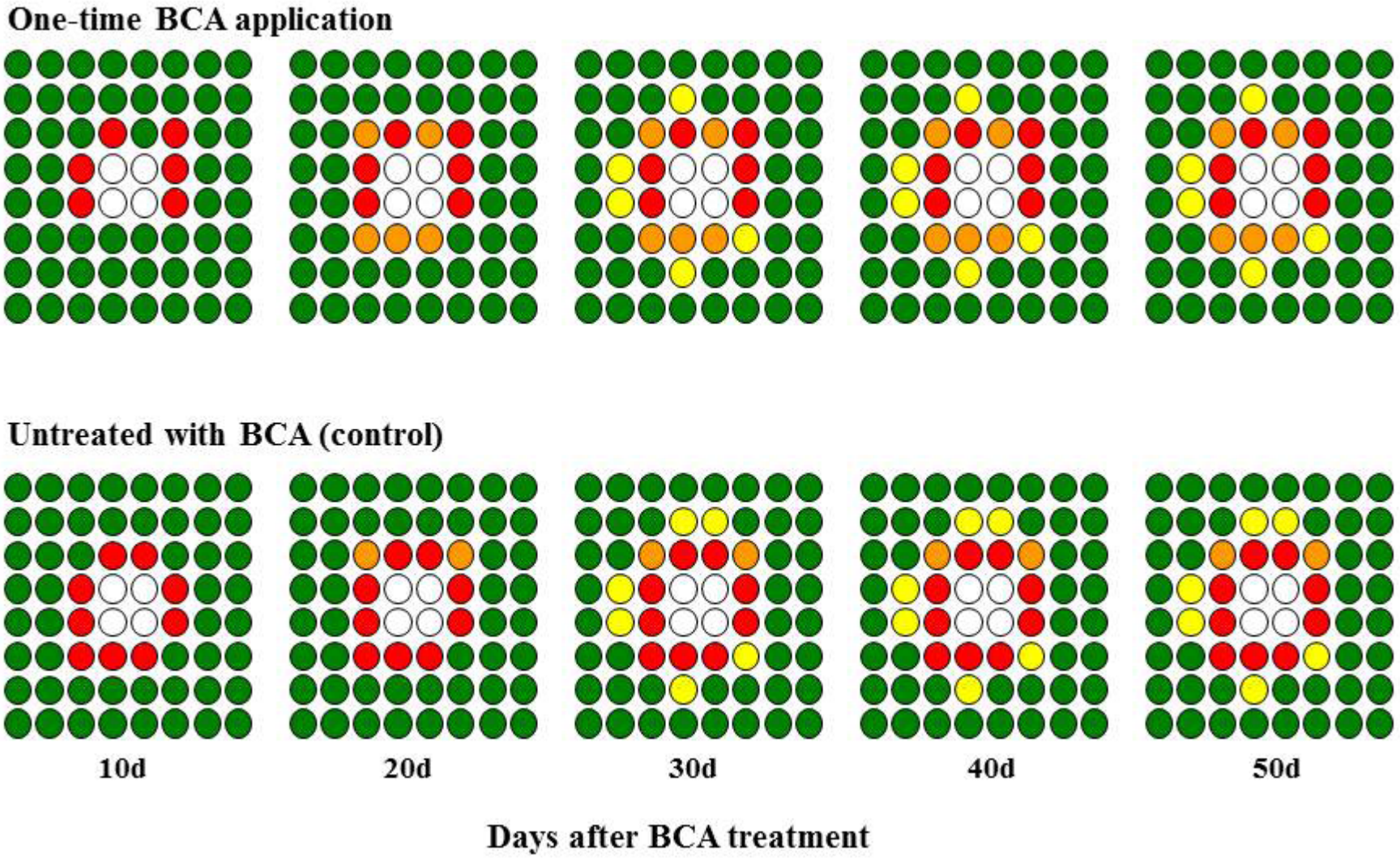
BCA Fig. 8. Sheath blight focal area expansion after one BCA application.



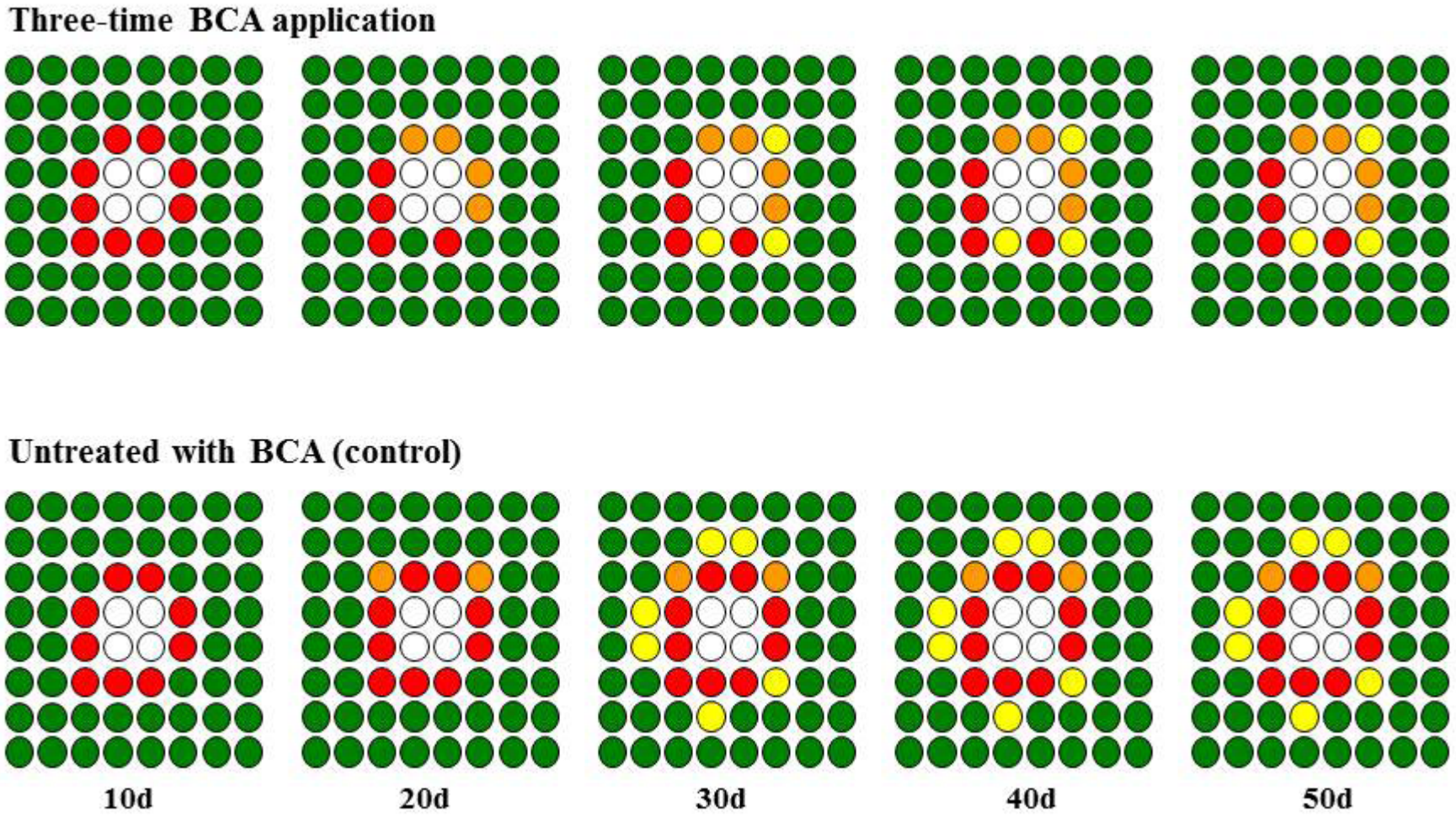
BCA Fig. 9. Sheath blight focal area expansion after three BCA applications.



BCA Fig. 10. Sheath blight focal area expansion after seven BCA applications.



BCA Fig. 11.Focal expansion of sheath blight with one BCA application vs. control.

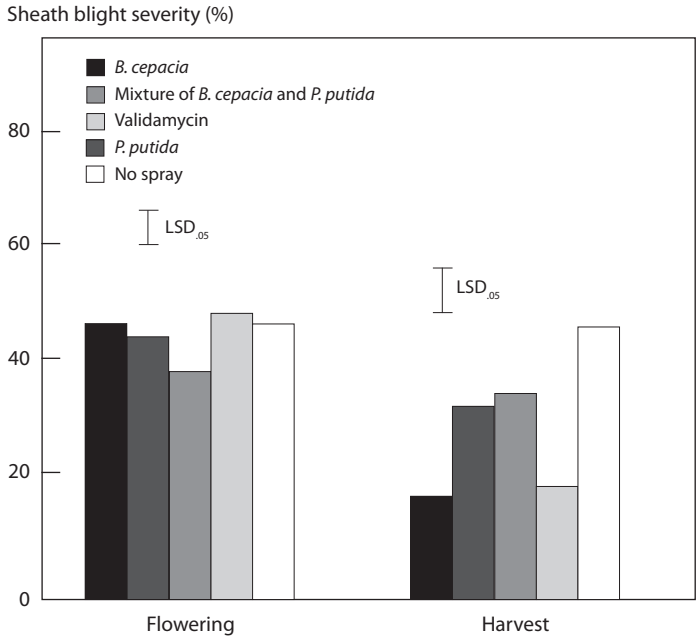


BCA Fig. 12. Focal expansion of sheath blight with three BCA applications vs. control.

This hypothesis is further supported by an experiment conducted in the 1997 wet season at the IRRI Farm. In that experiment, sheath blight incidence differed among plots with and without BCA introduction initially. As crop growth advanced, sheath blight continued to develop rapidly. Disease assessment and visual observation did not reveal significant difference between the treatments. When a strong typhoon passed across the area during the experiment, all crops lodged a month before harvest. However, at harvest, sheath blight between treatments was visibly different in the field plots. The difference appeared to be related to secondary infection that took place during the time when the plants were lodged (**BCA Figure 13**). This observation suggests that BCAs are more effective in preventing secondary instead of primary infection and the effect considerably prolongs in the field. BCAs may also restrict the formation of sclerotia, and, if formed, are likely to be colonized by BCAs. Thus, the infectivity of the sclerotia gets affected preventing secondary infection after the rice plants have lodged in field plots where the BCA was applied compared to the check plots.

In a continuation of this interesting observation, to further verify the BCA effect on focal point expansion, BCA application was targeted at a time shortly after the initial sheath blight lesions were observed in the field. Under such situations, the results indicated that in the context of sheath blight epidemic process, BCAs are more effective in reducing inoculum potential for new infections, i.e., secondary spread of the focal point expansion rather than reducing sheath blight severity after the pathogen established in the rice plant tissues.

7.1.2. Reduction of sheath blight foci. *R. solani* AG1 I-A may be a soilborne pathogen but the disease it causes, sheath blight, is a foliar disease. The pathogen survives in paddy soil as sclerotia, in plant debris as mycelium, and on weed hosts as an active par-



BCA Fig. 13. Effects of biological control agent (BCA) treatments on sheath blight severity (%) in direct seeded rice cultivar IR72 at flowering and harvest in an experiment conducted during the 1997 wet season at the International Rice Research Institute (Mew et al., 2004).

asite. The BCA is indigenous to the rice ecosystem. Introducing more as an augmentation at a higher population density, either singly or in a mixture, needs to be competitive to be established in the plant surface environment. In reality, if the initial inoculum of ***R. solani*** AG1 I-A is abundant with high inoculum potential, the infection process is fast under the favorable environment and, if the fungal pathogen has already entered into the host plant tissue, it is difficult for the introduced BCA to be functional (Lan and Mew 2001). Augmentation is often used to off-set this deficit in research during the critical period of disease development when the host is vulnerable to pathogen attack. For practical disease management, this is not feasible economically. Alternatively, efforts should be made for the long-term effect of the BCA.

To determine the long-term effect of BCAs on sheath blight, research was conducted in farmers' fields with direct seeded rice in central Thailand from July 1995 to May 1999 (Nilpanit et al 2001). A sampling unit of 121 to 123 per field was assessed on the initial sheath blight development. Disease incidence and severity were systematically recorded in these sampling units throughout the rice crop season for 12 crop cycles in five fields with different predetermined treatments including control plots.

In Field 1, the BCA was applied for 7 consecutive crop seasons in the beginning and, thereafter, the field was left with no additional BCA applications. In all crop seasons, 231 sampling units were taken randomly and the number of disease foci was then assessed.

In Field 2, the rice crop was grown from the bacterized seed from the 1st through 6th crops. Thereafter, the rice crop was grown from normal seed. The disease foci were assessed based on 121 sampling units in all the crops.

In Field 3, the experiment was started in September 1996. The bacterized seed were used for raising the crop in the first 4 crop seasons and the disease foci were assessed based on the 121 sampling units taken randomly from the field, similar to Field 2, in all the crops.

Field 4, located adjacent to Field 1, was included as a check since January 1997. It was later discovered that this field was at the low-end of Field 1, and the paddy water from Field 1 overflowed into this field. So, there was a need to include an additional check (Field 5). Sampling also consisted of 121 units for this treatment.

Field 5 was included in June 1998.

The cultural practice and crop management of all five fields were done by farmers using their usual practices. This long-term test revealed that the number of sheath blight foci decreased in the fields where the BCA was applied in each cropping season over three to five seasons (**BCA Table 12**) (Nilpanit et al 2001, Mew et al 2004). Apparently, the BCA lowered the inoculum potential, reducing the disease foci over time instead of merely achieving immediate disease control with a one-time application in a single crop season. The success of immediate disease control with a one-time BCA application in a single crop season was probably more by the chance application when the disease began to take off, rather than a designed effect of the BCA. BCA introduction in this continuous rice-cropping system revealed the importance of the BCA in sustaining crop production and disease management. The inconsistent results seemed to be related to finding out the perfect timing for BCA introduction, which was not always attainable under field conditions. Initial pathogen inoculum density and disease occurrence under natural field conditions are variable and influenced BCA efficiency. If the density of pathogen inoculum is too high, it is unlikely that the BCA would be able to suppress the rapid infection caused by the pathogen, especially under conditions favoring disease development. Therefore, in biological control, the role of BCAs through augmentation should be targeted for long-term instead of for short-term effects.

BCA Table 12. Effect of a long-term trial on BCAs on rice sheath blight caused by Rhizoctonai solani AG 1, Central Thailand.

Field no.	Crop										
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th crop
	1995	1996	1996	1996	1997	1997	1997	1998	1998	1998	1999
1	73.6	48.5	84.4	36.4	22.5	13	9.5	11.7	10.8	18.2	1.7
2				43	47.9	20.7	5	5.6	4.1	18.2	0.8
3				29.8	11.6	10.7	8.3	2.5	6.6	9.9	0.8
Control #1					100	71.1	41.3	1.7	8.3	5	3.3
Control #2									54	73.3	24.8

This was a farmer-managed experiment in their fields. BCA treatment was done by researchers, both planting and crop management was based on their own practices.

Note: The figures denote for disease incidence based on means of 100 sampling points per field.

Field of Control # 1 was at the low-lying of Field #1 and was found later the water was down-flow from Field # 1 to field of Control #1, thus a second Control was included.

7.2. Biological control of seedborne pathogens

Fusarium fujikuroi (*F. moniliforme*) causes the rice disease bakanae, which is known to be seedborne. The syndrome of "bakanae" comprises the well-known seedling elongation, foot rot, seedling rot, grain sterility and grain discoloration (Ou 1985). The fungus is both seedborne and soilborne. Generally, the seedborne inoculum initiates the primary infection in the nursery bed or main field under direct seeding. Since chemical seed treatment became popular in the 1950s, bakanae had become less important as a rice disease until the 1980s when open-field nursery beds were replaced by seedling boxes to raise the seedlings for machine transplanting in Japan and later in other countries. This also happened when wet nursery beds were replaced by dry nursery beds. Bakanae has reappeared as a common seedling disease in seedling boxes. Its recent occurrence in California, although claimed to be new introduction (Carter et al 2008) of the pathogen, may also be related to some cultural changes in rice production. Chemical seed treatment has been extensively applied since the switch from nursery beds but resistance of the fungal pathogen to the fungicides has been reported (Ogawa 1988). There is a need to look for an alternative approach for bakanae management in long-term rice crop production.

Many tested BCAs (113 of of 441 isolates) were effective against ***F. fujikuroi*** (Rosales and Mew 1997). The use of BCA to bacterize naturally infected rice seed reduced bakanae incidence both in seedling boxes and in nursery beds (**BCA Figure 14).** In a nursery bed experiment with IR42 seed soaked in BCA suspensions, bakanae incidence and disease control ranged from 1.0 to 7.0% and 72.0 to 96.0%, respectively **(BCA Table 13**). From the 3-year field trials, 10 BCA strains reduced bakanae incidence (Rosales et al 1986). Among the 10 strains, 5 consistently reduced bakanae incidence while the other 5 exhibited variable effects among trials. The specificity of BCA suppression against different pathogenic isolates of ***F. fujikuroi*** from various locations in the Philippines has also been observed. The seed bacterization seemed to suppress the pathogen inoculum carried naturally by seed during germination (**BCA Table 14**). In this case, no fungicide was needed to weaken the pathogen activity because BCA is functional before infection is initiated by the fungal pathogen.

7.3. Rice straw decomposition

In rice-based cropping systems, "rice after rice" is only one type of rice-based crop pattern. Others, especially those under the rainfed lowland or dryland rice system in which a dryland crop is often planted after rice. In both systems, sheath blight is observed. ***R.***

BCA Fig. 14. Seedlings grown from bacteria-treated seed were more healthy and had less bakanae infection than did untreated seedlings. Source: Rosales and Mew (1997).

BCA Table 13. Influence of rice seed bacterization of IR42 grown in farmer's field on intensity of natural bakanae infestation, Laguna, Philippines. Source: Rosales et al (1996).

Treatments[1]	Disease control (%)[3]	Disease control (%)[3]		Disease intensity (%)[4]
		C1	C2	
Bacterial strains				
In-b-150-F	21.0 c	58	70.4	2.2 c
In-b-590-NF	20.0 c	60	71.8	2.4 c
In-b-17-NF	19.0 c	62	73.2	2.3 c
In-b-24-F	18.0 c	64	74.6	2.2 c
Benomyl	17.0 c	66	76.1	1.0 c
Control 1	49.5 b			4.5 a
Control 2	71.0 a			13.5 a

[1]Seed source for bacterial strains, benomyl treated and Control 1 were from IRRI. Seedlings of Control 1 were raised by the Dapog method in the greenhouse.

Seedlings for Control 2 were from farmers' seeds and raised in nursery bed in farmers' fields.

[2]Means of three replicates, figures followed by the common letter are not significantly different (P=0.05).

[3] C1 is the percent disease control based on Control 1 while C2 on Control 2.

[4] Disease intensity was estimated based on bakanae of the entire field.

BCA Table 14. Bakanae incidence (%) in relation to isolates of Fusarium and isolates of antagonistic bacteria applied as seed treatment in the greenhouse. Source: Rosales and Mew 1997).

Bacterial isolates	Fusarium isolates				
	F-2 (Cavite)	F-13 (Leyte)	F-27 (Nueva Ecija)	F-22 (Laguna)	F-19 (Bicol)
In-b-521	1.7a*	4.0a	12.7ab	5.0ab	2.7a
In-b-442	3.0a	4.7a	8.3a	3.7a	3.0a
In-b-527	4.7a	6.3a	8.7a	1.3a	2.0a
In-b-715	18.0b	18.0abc	29.7c	16.7bcd	18.0b
In-b-33	24.3bc	32.0cde	64.0fg	51.3fgh	50.0d
In-b-520	25.3bc	25.0bcde	36.7cd	19.3cd	28.0bc
In-b-714	33.7cde	19.7abcd	24.3bc	10.0abc	23.3b
In-b-178	31.7cde	35.3de	60.7efg	52.3h	49.3de
In-b-837	27.0bcd	14.7ab	30.0c	26.7de	22.7b
Check	30.7cd	31.7cde	58.7ef	40.0fgh	26.7be

* Means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

solani AG1 1-A attacks a wide range of host plants, which include rice, maize, sorghum, and legumes (cowpea, mungbean) and are also involved in rice-based cropping systems in tropical Asia. The saprophytic survival of ***R. solani*** AG 1 1-A in soil, paddy or dryland, relates to crop residues left in the field after harvest. Rice straw and stubble are an important reservoir of ***R. solani*** AG1 1-A that survives during the off season **(**Kobayashi et al 1997). Conventionally, these crop residues may be burned shortly after rice harvest. In a rice-based cropping system, especially in rainfed lowland rice, the field is usually left fallow for a brief period before a dryland crop is established in January or February of the second year. If sheath blight pathogen or some other sclerotia-producing rice disease pathogens occur during the rice crop, the infected straw and stubble then become a reservoir of the surviving pathogens for other crops or the rice crop to be planted later. Traditionally, rice farmers often turn over the stubble immediately after rice harvest when the field is still wet. In the tropical environments, this enhances rapid decomposition of the crop residues, which exhausts the nutrient supply in the field. As rice production intensifies and labor becomes more scarce, this is now done during land preparation for planting of the following crop.

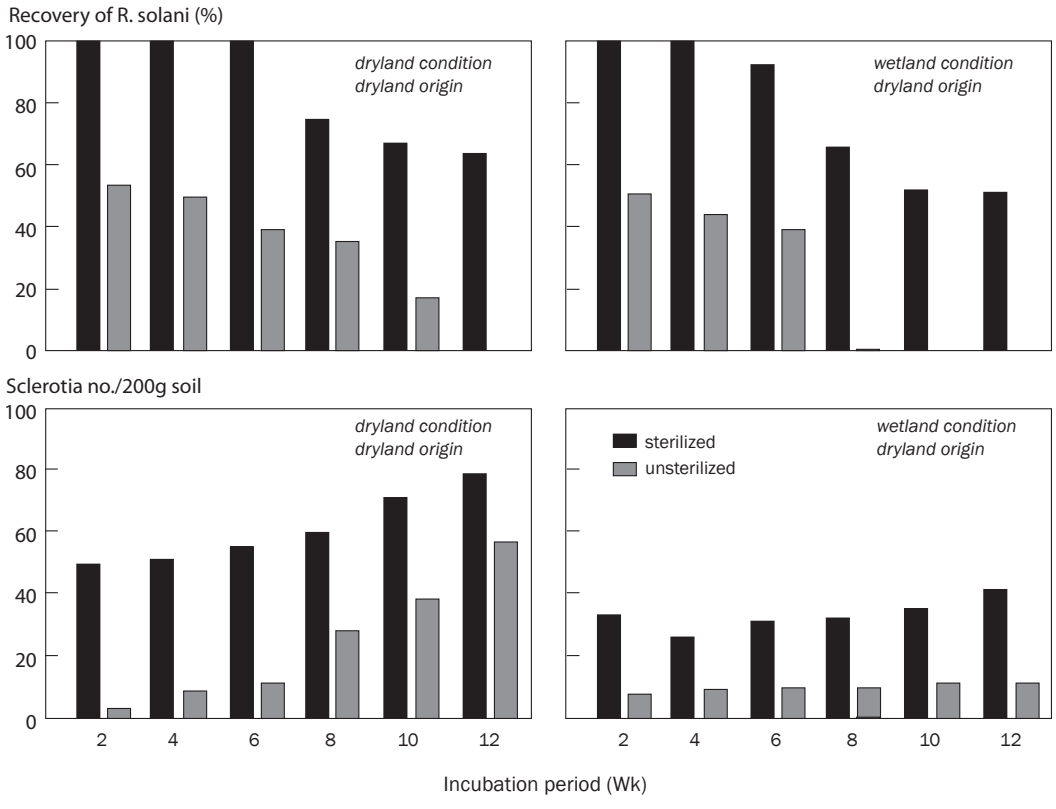
In the rice–legume pattern, we have tested an approach to decompose rice straw by introducing ***Trichoderma*** spp. in the system since the 1980s, to exhaust the nutrient supply for the surviving fungal pathogens.

Trichoderma harzianum has been isolated from rice straw buried in soil from a dryland rice field. Its presence was surveyed in different types of rice cultures. ***T. harzianum*** is usually not detected from soil of irrigated fields or soil of rainfed rice field with standing water but has been isolated in rainfed rice field when rice was harvested and the field left fallow with no standing water. In dryland rice field, it might be isolated throughout the crop growth period. ***T. harzianum*** is found in rainfed soil when the field is planted to a dryland crop after rice. Occasionally, it may be isolated from soil of rainfed field when the field is left fallow. The frequency of isolation was higher in soil planted with a dryland crop than in soil planted only with rice, but highest in the dryland field that had never been flooded.

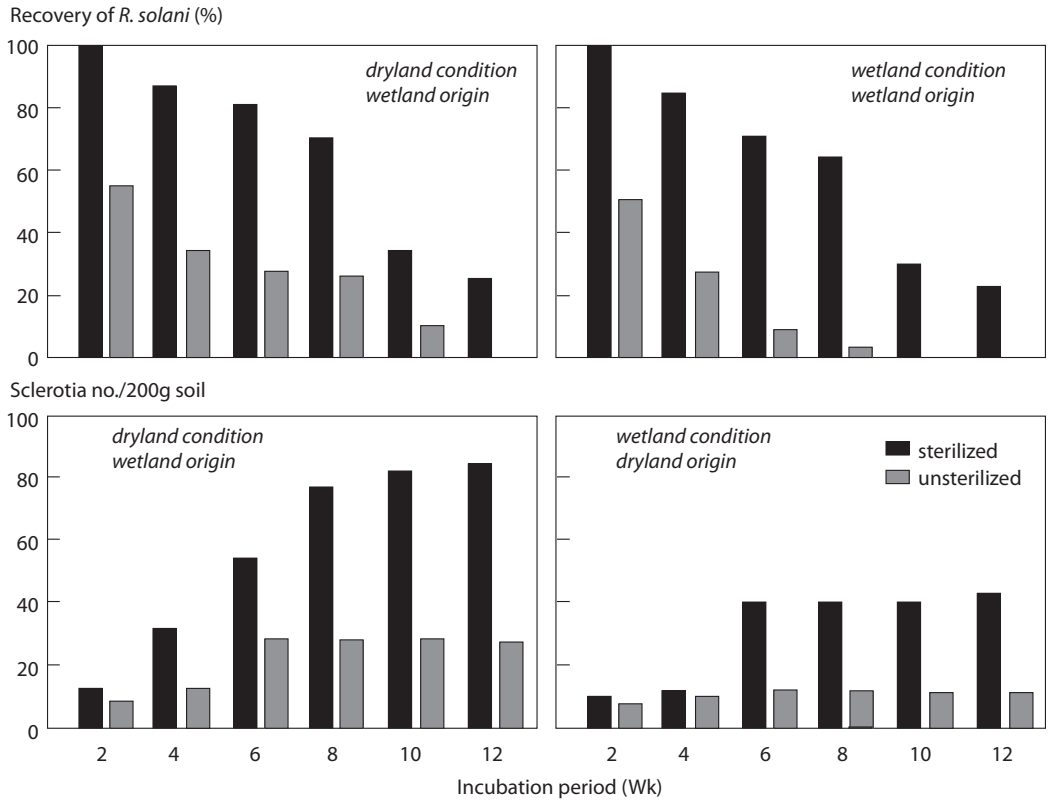
The saprophytic survival of ***R. solani*** AG1 1-A in rice straw is affected by unsterilized soil from the rice field. Recovery of the fungus in rice straw samples buried in soil declined with time. The difference between soil from dryland and irrigated rice fields indicated that

survival of ***R. solani*** AG1 1-A is better in dryland than in flooded conditions with unsterilized. In sterilized conditions, although the percentage of recovery of the fungus was lower in irrigated soil than in dryland soil, the pathogen was recovered at the end of the experiments, i.e., 12 weeks after incubation (**BCA Figures 15** and **16**). The survival of the pathogen in flooded conditions appears to be related not only to anaerobic conditions but also to other microbial activity. From 2 to 4 weeks after incubations, sclerotia production in dryland conditions was higher in sterilized dryland soil than in sterilized irrigated soil; at 6 weeks and thereafter, there was no difference in sclerotia production between the two soils. The trend in flooded conditions was similar for unsterilized soil, but the number of sclerotia was lower. ***T. harzuanum*** colonized the straw pieces buried in dryland rice soil but not those in irrigated soil. The scloratia produced in such conditions were also colonized by the ***Trichoderma.***

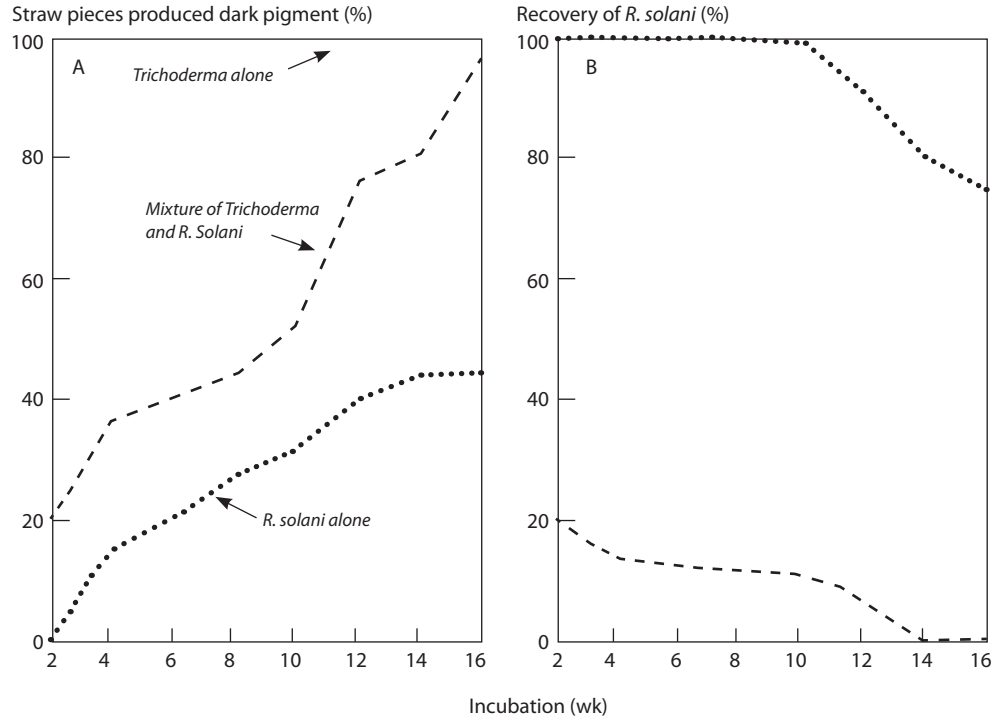
Rice straw infested with ***T. harzianum*** alone turned dark in color 2 weeks after incubation, indicating decomposition. **BCA Figure 17** shows straw decomposition adapted from Mew and Rosales (1985). Only 40% of the straw samples inoculated with ***R. solani*** AG1 1-A became dark-colored after 16 weeks of incubation. The microbial decomposition of rice straw greatly affected the survival of the sheath blight pathogen. In the presence of ***T. harzianum***, recovery of the sheath blight pathogen was reduced to 20% at 2 weeks after incubation. Rice straw weight losses due to colonization by either or both of the organisms were not significantly different as shown in **BCA Figure 18** adapted from Mew and Rosales (1985). ***T. harzianum*** appeared to be highly competitive: its cellulolysis adequacy index was higher than that of ***R. solani*** AG1 1-A whether the substrate used was filter paper or rice straw. Application of ***Trichoderma*** for crop residue decomposition is one way



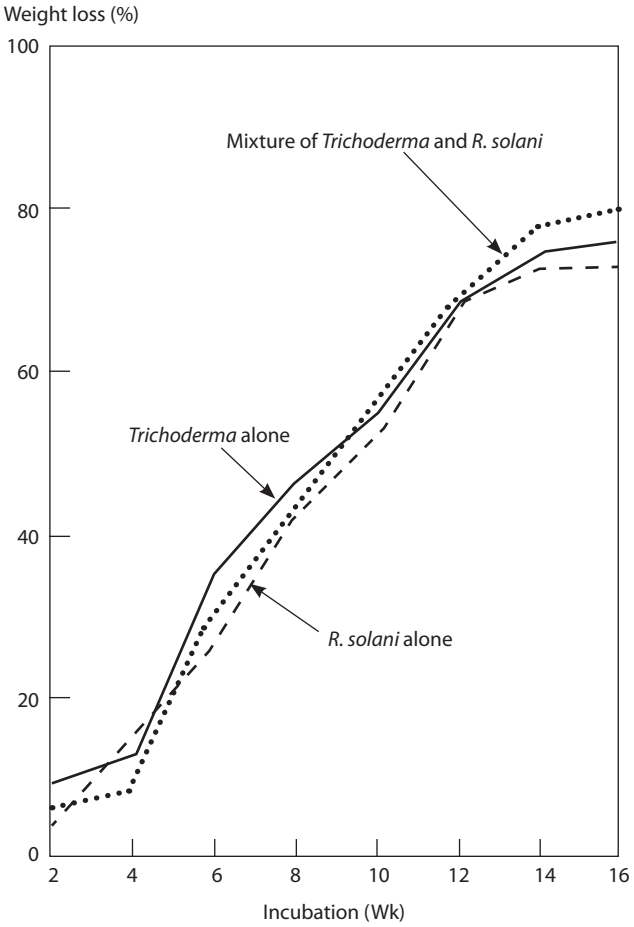
BCA Fig. 15. Survival and sclerotia production of *Rhizoctonia solani* in infected rice straw samples in sterilized and unsterilized soil from dryland rice field maintained as either dryland or wetland conditions. Source: Mew and Rosales (1985).



BCA Fig. 16. Survival and sclerotia production of *Rhizoctonia solani* in infected rice straw samples in sterilized and unsterilized soil from wetland rice field maintained as either dryland or wetland conditions. Source: Mew and Rosales (1985).



BCA Fig. 17. Effect of *Trichoderma harzianum* on rice straw decomposition (A) and on saprophytic survival (B) of the sheath blight pathogen. Source: Mew and Rosales (1985).



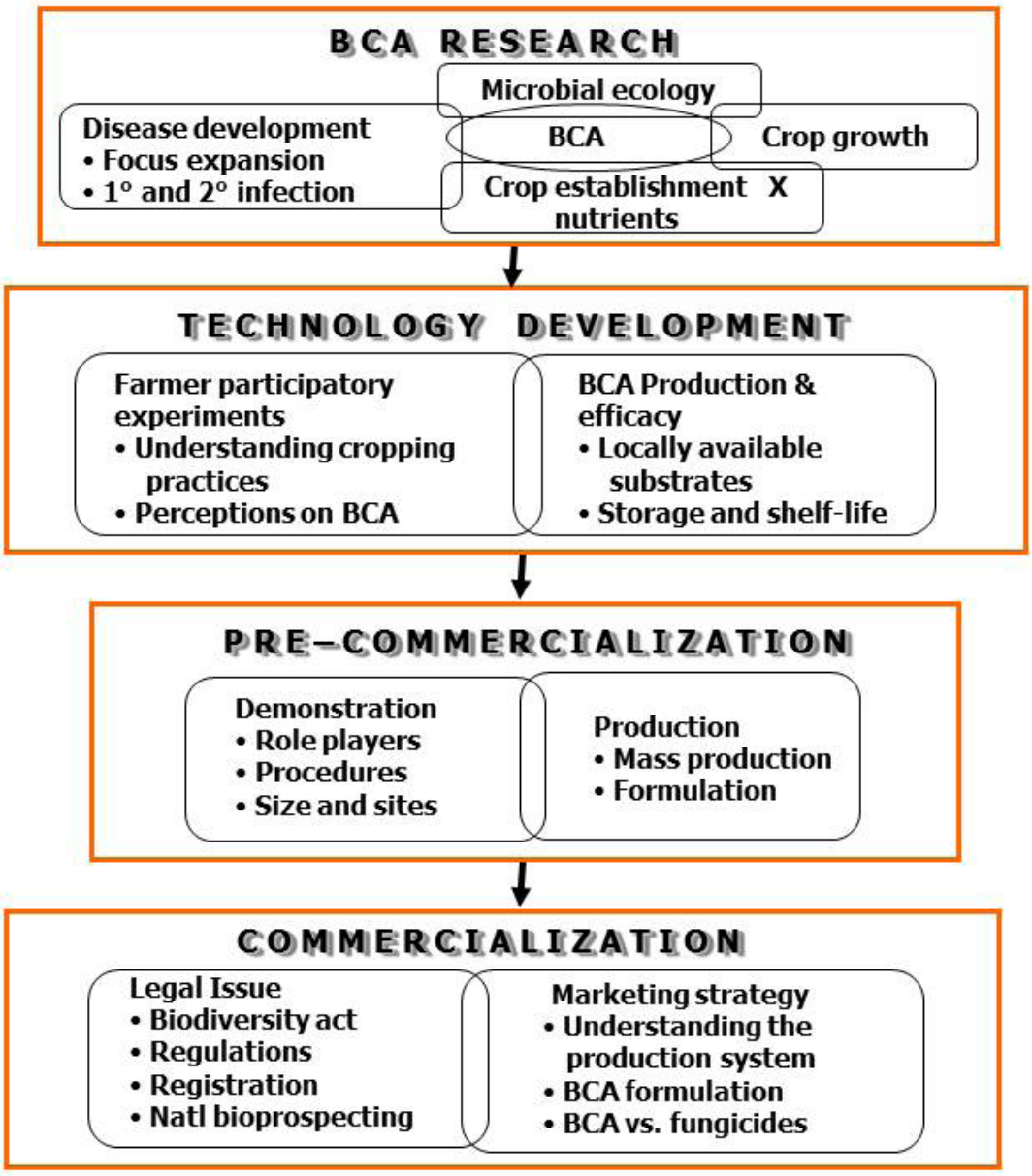
BCA Fig. 18. The weight loss (wet) of rice straw colonized by *Trichoderma harzianum* and *Rhizoctonia solani* at different durations compared with that of rice straw without *T. harzianum* and *R. Solani*. *Source:* Mew and Rosales (1985).

to minimize the sheath blight problem in a rice-based cropping system, while practical application of fungal BCA to suppress ***R. solani*** AG1 1-A is effective and feasible only when it is used to decompose the crop residue that serves as its food.

8. Technology development for BCA production

It would be difficult to market a product for disease management if its effect is not predictable or its field performance varies. To solve this problem, it seems advisable that from the start of the research, the objective should be set on technology development (**BCA Figure 19**). There is a need for different BCA for different disease-crop systems, and the approach must aim at finding the niches of the systems where the pathogens could be suppressed.

For effective preparation of bacteria-based BCAs, adding a dose of a commonly used fungicide in the formulation would assist in inactivating the pathogen while "buying" time for the BCA to recover from the "introduction shock." To scale up BCA technology, two issues are of major concern in its development: "shelf-life" and "storage." We propose a decentralized BCA production system based on a model of "demand and supply" technology development systems, which seems more effective in developing countries on a community basis. In this model, it is important for all parties, the researchers, county agricultural and crop protection specialists, and farmers to work closely together. Proper sites identi-



BCA Fig. 19. A framework of research on biological control redesigned based on the experience gained on the study of BCAs for rice disease management.

fied for large-scale testing with farmers participatory approach is important for success in technology evaluation and in up-scaling. Mass production of BCA products by researchers and delivery to farmers at the right time for field application is equally important. The data from such large-scale testing can be used for production registration. This approach, especially in the formulation, should be applicable to BCA for sheath blight, bakanae, false smut and many other rice diseases. For details, the readers are referred to Mew et al (2004).

In terms of using *T. harzianum* for rice straw decomposition, again, all concerned parties should work closely in a community approach. Potentially, the rice straw compost with *T. harzianum* may be also used for other vegetable production.

In short, there are opportunities to develop the technology for up-scaling, once we have a way to ensure that the technology works positively and predictably.

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