PROJECT REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2002 – December 31, 2002

PROJECT TITLE: Cause and Control of Rice Diseases

PROJECT LEADER: R.K. Webster, Department of Plant Pathology, University of California, Davis, CA 95616

PRINCIPAL UC INVESTIGATORS: R.K. Webster and Laurel Anderson

COOPERATORS: J. Oster

Level of 2002 Funding: $28,897.00

OVERALL OBJECTIVE:

The major goals of this project continue to be to gain an understanding of the biology of the diseases that affect rice in California and to develop methods to minimize their damage to the rice crop. Emphasis is on Stem Rot, Aggregate Sheath Spot, Blast and a new disease, Bakanae. Control methods under study include cultural practices that affect occurrence and severity of diseases and the potential use of fungicides.

OBJECTIVES AND EXPERIMENTS CONDUCTED:

Objective 1:

Continue to monitor the occurrence and spread of the blast disease and to determine the variability of pathogenic races of *Pyricularia grisea* that are occurring in California. Knowledge of the races of *P. grisea* in California is essential to the plant breeding effort to produce improved blast resistance in California cultivars.

We continued to monitor the occurrence, spread and severity of the blast disease both in areas where it is known to have occurred in the past and also in areas where the disease is not known to have occurred previously.

The rice blast disease did not occur in most of the rice producing areas during the 2002 growing season. It was found in only in a few fields (4) on the west side of the Sacramento Valley and in these cases there was very little infection and that was not severe enough to have an economic effect on yield of the fields in which it occurred. In all cases, the disease was not observed until late August or early September, just prior to harvest. In these fields, the occurrence of the disease was sporadic, not generally distributed in the fields and of little consequence except to provide for a continuing overwintering of the pathogen.

Collections of the blast pathogen were made from the fields in which it was found to allow determination of the pathogenic race. All samples tested were of the same race that has been

found in California in previous years. We are confident that there has not yet been a new race evolved and that we are still faced with only one race of the pathogen in California.

Objective 2:

Determine the occurrence, distribution, disease cycle, factors that effect severity, role of soil and residue borne inoculum and impact and control of Bakanae disease in California rice fields.

Occurrence and Distribution of Bakanae:

Bakanae disease was found in California for the first time in 1999 and has since become widespread in the California rice producing areas. We continued to monitor the occurrence and distribution by routine surveys of all rice producing areas of the state. Bakanae was found in all rice producing counties except Fresno during the 2002 season.

Bakanae was observed at various incidence levels in most fields north of Interstate 80. For the most part, the incidence of Bakanae in these individual fields was less than 1% of the total plants established in the field.

However, there were exceptions to these generally low infection levels. In a few cases, the incidence counted exceeded 20% of the established plants. For the most part, these fields with high incidence were planted with seed from a common source.

We are cooperating with Jack Williams to carry out a survey with growers to determine if there were any particular cultural practices or situations that favored the incidence and occurrence of Bakanae in fields where particularly high incidences of Bakanae were observed during the 2002 season. We anticipate that the information may be helpful regarding practices that may be more favorable to Bakanae disease development or some that may be useful in limiting it's occurrence.

Seed as the primary source of inoculum

All present evidence indicates that infested seed is the primary source of inoculum and that the disease organism has become widely spread at various levels throughout the industry. It is also evident that most of our seed growers have shown diligence in selecting fields that showed little or no Bakanae for seed use the following year. We believe that this practice has been useful in limiting the incidence of Bakanae in the majority of fields but the nature of the disease cycle has allowed the disease to continue to spread and be maintained in our seed system. This has resulted in the general distribution of the disease but at the same time has limited the incidence of disease seen in most fields. It remains important for seed producers to continue to provide the cleanest seed possible to prevent fields where Bakanae occurs at high incidences resulting in actual yield loses.

In cases where seed lots have been assayed for percent infestation by the pathogen and then grown for assay of actual occurrence of the disease, the plate assays for percent infestation have consistently shown higher levels of infestation than actual percent disease produced from those seed lots.

We have collected samples of over 200 seed lots (2002 harvest) from several seed growers throughout the industry who intend the seed for the 2003 season. These are being assayed for % infested seed and % Bakanae plants produced from the seed. These studies are not yet complete but thus far the results indicate previous observations i.e: (1) The majority of seed lots contain infested seed and of these the % infested seed varies significantly between lots, (2) The incidence of infested seed is significantly higher than the incidence of Bakanae plants produced from that seed lot.

Role of Soil and Residue as a Source of Inoculum:

We have demonstrated that seedlings can become infected from inoculum in the soil and residue from fields with a history of Bakanae in greenhouse and laboratory experiments by planting known "clean seed" in soil from fields where Bakanae occurred and by placing infested residue in sterile soil and planting clean seed.

To determine the amount of infection that may occur in the field from soil and/or residue we established experiments in Colusa County in a field that had a relatively high amount of Bakanae the previous year. Aluminum rings were placed in the top and lower half of the field after the seedbed was prepared and covered with plastic to exclude grower seed. After the fields were flooded and planted the covers were removed and a known amount of seed planted into each ring. Seed with a known level of infestation (28% infestation on Fusarium selective Media) was planted after soaking in water only and seed which showed 17% infestation after soaking in 5% Clorox served as the other treatment. This treatment was included in an attempt to assure non infested seed to determine if infection occurred from the pathogen in the soil or residue remaining from the previous crop but we were unable to attain Bakanae clean seed for this comparison. The levels of Bakanae infected plants resulting are shown in Table 1 for both 30 days after planting and just prior to harvest of the field.

The results indicate that the seed with 28% infestation soaked in water only resulted in less than 1% Bakanae plants 30 days after planting while there were 2% Bakanae plants at the end of the season. For this seed there were less than .5% Bakanae plants in the intake end of the field compared to 2% at the drain end of the field. The seed with 17% infestation at planting showed less than .1% Bakanae at 30 days from planting and less than .25% prior to harvest. There was a small increase in Bakanae plants in both seed sources between the counts observed 30 day from planting and those observed at harvest time. This suggests that either there were delayed infections from the infested seed or possibly there were infections from soil or residue during the season. The fact that there were a higher number of Bakanae plants in the rings placed at the drain end of the field may suggest that due to water movement during the previous season from the top to the bottom of the field that there was an increase in carry over inoculum at the drain end, however the results do not provide for a firm conclusion that infection from soil or residue occurred since Bakanae plants occurred from both seed sources. The results do confirm that there is a significantly less amount of Bakanae developed in the field than the percent of infested seed determined by the Fusarium Selective Media Assay. Also, soaking seed in 5% Clorox reduces the amount of infested seed transmission of the disease to seedlings in the field but that this concentration does not completely eliminate the disease resulting from infested seed.

Table 1. Incidence of Bakanae disease resulting form water only and NaOCl soaked seed planted in a field with a history of Bakanae.

Treatment	Replication – Observation Dates					
	June 28			September 12		
	I	II	III	I	II	III
	Water Intake end of Field					
Water Soak	0	1	0	2	2	0
NaOCl Soak	0	0	0	0	0	0
	Drain end of Field					
Water Soak	0	0	1	2	4	2
NaOCl soak	1	0	0	2	1	0

Rings placed in the field and covered prior to planting of field
200 seed per ring planted after treatment. Seed for the water soak treatment was from an infected field in 2001. It assayed as 28% infested on Fusarium selective media. Seed for the NaOCl soak treatment was assayed at 17.0% infested on FSM.
NaOCl soak was 5% Clorox (Household bleach) Clorox contains 5.2% NaOCl.

Attempts in grower's fields to determine the possibility of infection from residue or soil, effects of water depth during seedling establishment and plant to plant spread of the disease are hampered by the uncertainties of presence of the pathogen in the soil, carry over of infested residue, possible movement of inoculum in the water source and difficulties of maintaining precise water depths and in precise observations of infected plants. To circumvent these difficulties we established a series of basins at a research site at Davis. Ten foot square basins were established by removing approximately 16 inches of soil and lining the basins with plastic. The soil was replaced into the basins and a water system utilizing gated irrigation pipe to supply water from Lake Berryessa known to be free of the Bakanae pathogen was set up. This allowed us to establish experiments with soil known to be free of the pathogen, easily observe possible plant to plant spread of the disease and to maintain precise water depths throughout the duration of the experiments. Comparisons of the effect of NaOCl soaking of infested seed were also carried out at the site.

Residue known to be infested with *Gibberella fujikuroi* was added to basins prior to flooding and planting. One thousand seed which was soaked for 24 hours in 5% bleach was planted in each basin for this test. Plants were observed until heading. No Bakanae developed in these basins indicating that at least in this test, infested residue did not serve as an effective source of inoculum.

Does plant to plant spread of Bakanae occur?

To determine if plant to plant spread occurred, similar basins were established and rows of similar bleach soaked seed were planted. In each of rows, 10 known infested seed (46% by assay) were planted and marked with a stake to allow observations of the same plants throughout the season. Additional attempts to create disease foci were made by planting inoculated seed at 1

foot intervals in other basins. Bakanae disease did occur in the sites where known infested seed was planted as the season progressed but only in the disease foci marked where the infested seed was planted. Though the disease was present in the intentional disease foci, no diseased plants were observed outside the disease foci. This suggests that at least in these experiments (clean soil and clean water) that there was no plant to plant spread of the disease. This conclusion is consistent with field observations where Bakanae plants are observed quite evenly dispersed throughout fields and seldom occurring in clusters.

Affects of water depth on occurrence of Bakanae

Greenhouse experiments to determine the affects of different water depths during the seedling stage on the incidence of infection of seedlings suggested that infection by the Bakanae causal fungus (*Gibberella fujikuroi*) maybe encouraged by draining the water from paddys during stand establishment and weed control applications. To test this possibility under field conditions we established eight basins as described above with four treatments each treatment repeated twice. Four thousand seed was planted in each basin. Water was maintained at either 4 inches or just saturated at one half inch deep or less. The results and treatments are shown in Table 2.

Table 2. Effect of Water Depth and NaOCl preplant soak on Bakanae Incidence

Treatment	Water Depth	Bakanae Incidence
Bleach 5%	4-6 inches	2
Bleach 5 %	0-1 inches	3
Water only soak	4-6 inches	87 (2.1%)
Water only soak	0-1 inches	84

4000 seed per basin; values are means per basin of Bakanae plants 30 days after planting
Seed FSM assay showed 11% infested; mean of 87 Bakanae plants represents approximately 2.0% of the 4000 seeds planted per basin
Soil and water free of *G. fujikuroi*

The results in Table 2 suggest that water depth does not affect Bakanae incidence, as essentially the same amount of disease occurred at both water depths tested. These data also show that while the 5% bleach soak of infested seed significantly reduces disease incidence, it is not completely effective in eliminating the occurrence of disease when infested seed is planted although less than .01 % of the bleach soaked seed planted resulted in Bakanae plants.

Longevity of *G. fujikuroi* in soil

Studies on the longevity of the bakanae pathogen in soil and residue are being continued. Thus far it is apparent that the pathogen survives in both soil and residue long enough to carry over between seasons, particularly in situations where rice is grown for successive years. This information is needed to understand the role of over-wintered inoculum in soil and residue, as a

possible source of disease in situations where clean seed in planted and to allow determination of the possibility of eliminating the pathogen from a field by rotation from rice.

Objective 3:

Determine the variability and population structure of *G. fujikuroi* as it is occurring in California. Although this objective is not a part of the research funded under the proposal for this project, information on the nature and variability of the pathogen population is needed for understanding the disease cycle and in formulating approaches for control of the disease. These studies include the occurrence and role of the perfect stage (teleomorph) or ascospores in regard to survival and as seed borne inoculum and variability between isolates in their ability to produce giberellin, the basis for the elongated symptoms that characterize the disease.

We have continued to collect isolates of the bakanae pathogen from throughout the areas where the disease is occurring for use in studies on pathogen variability. Studies thus far utilizing NIT mutants for vegetative compatibility analyses are being continued. Thus far studies indicate that there are at least three vegetative compatibility groups in the California population. We anticipate that with study of additional isolates that additional groups will be identified. The significance of information regarding these groups relates not only to the variability of the population but also to the implications regarding the extent of the occurrence of the perfect stage and production of ascospores by the pathogen in fields. It is quite probable that when understood more fully, that the occurrence and role of ascospores will provide valuable insights into efforts to control the disease through clean seed or possible other approaches.

We have found the perfect stage in a few California fields and were able to reproduce it in co-inoculated rice in basins at our Davis site during the 2002 season. An interesting aside to the observation of the perfect stage and ascospores in growers fields is that this would require both mating types of the fungus to be present for this to occur. Thus far we have not found the perfect stage in a high percentage of fields that have Bakanae, but when it has been found there was a higher percentage of Bakanae infected plants than observed in most fields. This observation raises the question of the relative importance of conidia or ascospores as the primary source of overwintering inoculum on infested seed. It also could help explain the observation that there has been a higher % of infested seed as determine by Fusarium Selective Media (FSM) than % Bakanae has been observed in fields and experiments planted with that seed. In either case, it is apparent that the original introduction of the Bakanae pathogen into the California rice system included both mating types of *G. fujikuroi.* Wheather the introduction was in the form of ascospores, conidia or both is not as important as a need to know if both or only one of the spore forms is playing a more significant role in survival on seed or in soil than the other. This could be particularly relevant as it could affect attempts to develop methods to treat or eliminate infestation from seed, particularly if one or the other form is more difficult to eliminate by treatments being tested.

PUBLICATIONS OR REPORTS:

Webster, R.K. Report to the California Rice Research Board: Project RP-2. Cause and Control of Rice Diseases, 8 pp. In Annual Report of Comprehensive Research. 2001. University of California and the U.S. Department of Agriculture.

Anderson, L.L. and R.K. Webster, 2002. Bakanae Disease of Rice in California – An Old Pathogen in a New Venue. Proceedings 29th Rice Technical Working Group. Pp. 88-89.

Choi, Jung-Sup, Daniel A. Sumner, Robert K. Webster and Christopher A. Greer, 2002. Economic Consequences of a New Exotic Pest: The Introduction of Rice Blast Disease in California. IN: Exotic Pests and Diseases: Biology, Economics and Public Policy for Biosecurity. Iowa State Press.

CONCISE GENERAL SUMMARY OF CURENT YEAR'S RESULTS

Rice Blast disease did not occur in most of California's rice fields in the 2002 season and was of little or no consequence to yield in the few fields where it was observed. New races were not detected and it is concluded that there is still only one race of the pathogen in California.

Efforts to further define and understand the disease cycle of the Bakanae disease were continued by emphasizing studies on occurrence, distribution, factors that affect incidence and severity, plant to plant spread, role of soil and residue as possible sources of inoculum, seed as the primary source of inoculum and efficacy of NaOCl as a seed soak to eliminate the pathogen from seed.

Distribution and Incidence: Bakanae disease was observed in all counties of the rice producing area except Fresno. It was observed at varying levels of incidence in most fields north of Interstate 80 but at usually less than 1% of total plants established in any given field. There were exceptions where incidence levels were considerably higher, exceeding 20% in a few fields.

Seed as the Primary source of Inoculum: All present evidence indicates that infested seed is the primary source of inoculum and also the main means of spreading the disease from field to field. When seed lots have been assayed for presence of the pathogen with Fusarium Selective Media and subsequent grow outs, the percentage of pathogen infested seed has always been higher than the percentage of plants that develop Bakanae from that seed lot.

Role of Soil and Residue as Possible Inoculum Sources: Experiments established in grower fields to determine if plants could be infected from the pathogen in the soil or in residue were inconclusive. In more controlled tests in basins with soil known to be free of the pathogen and maintained with known pathogen free water there was no development of Bakanae when infested residue was added to the soil. In similar experiments, when Bakanae infection foci were established and clean seed planted next to them, there was no plant to plant spread of infection observed.

Experiments comparing the effect of water depth on development of Bakanae revealed that there was no significant difference in incidence between depths of 0-1 and 4-6 inches of constant water flood.

In all tests conducted this year, soaking seed with 5% Bleach (5 parts household bleach, 5.2 % NaOCl, to 95 parts water) incidence of Bakanae was highly significantly reduced but not completely eliminated.

Studies to determine the survival time of G. fujikouri in soil and residue are being continued. Thus far it is apparent that the pathogen survives at least long enough to carry over between seasons, particularly in situations where rice is grown for consecutive years in the same field. Fortunately, results thus far indicate that inoculum form soil and or residue plays a minor role in the overall disease cycle of Bakanae.

Studies on the variability and population structure of *G. fujikouri* in California, have thus far shown that there are multiple vegetative compatibility groups and that both mating types required for the production of the perfect stage (teleomorph) are present. We have found the perfect stage and ascospores in several fields and have been able to reproduce it in experiments utilizing co-inoculation with compatible strains. We are continuing attempts to determine the role and significance of the perfect stage and the ascospores in the disease cycle of Bakanae and also if there are significant implications regarding attempts to develop control measures.