Project No. RP-2

ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 1988 - December 31, 1988

PROJECT TITLE: Cause and Control of Rice Diseases

PROJECT LEADER AND PRINCIPAL UC INVESTIGATORS:

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LEVEL OF 1988 FUNDING: $41,322

OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

Continuing objectives are to understand the etiology and epidemiology of rice diseases that occur in California. Knowledge gained is used to devise methods for control of the diseases. We have emphasized study on interactions of various culture practices which contribute to disease occurrence and severity with a long range goal of determining alterations that minimize disease affects on yield.

Management of rice residue has received particular emphasis. The tradition of burning residue, although known to be beneficial in controlling diseases of rice, is under continued scrutiny. In addition, rice economics and increased emphasis on sustaining productivity has forced many growera to re-evaluate culture systems. There is renewed interest in green manure crops, incorporation of rice residue and effects of fallow seasons between rice crops. A major effort this year has been to establish sites to study the cumulative effects of these alternatives in culture systems on severity of rice diseases. In addition, detailed studies to determine the feasibility of enhancing the biodegradation of rice residue and potential for biocontrol of rice diseases have been initiated.

Additional studies to identify safer seed treatments for control of seedling disease, control for crown rot and screening for disease resistance were carried out. Many aspects of these studies require consecutive years of evaluation in the field to determine cumulative effects.

Specific Objectives for 1988:

(1) Continue studies on potential enhancement of biodegradation of rice residue and overwintering propagules of the rice pathogens, S. oryzae, R. oryzae,-sativae and R. oryzae.

(2) Re-evaluate and test rice-seed treatments to replace Kocide and Captan.

(3) Complete development of screening techniques to identify resistance to kernel smut.

(4) Test soil (seed-bed) applications of fungicides to control crown rot (Pythium).

(5) Continue establishment and monitoring of trials for management of rice residue and diseases.

Experiments were carried out in 1988 in University of California laboratories, greenhouses, the Rice Research facility and in growers' fields.

SUMMARY OF 1988 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE:

Objective 1: The potential management of rice residue with saprophytic organisms requires accomplishment of several sub-objectives: (a) establish methods and sites for monitoring the microflora on rice, (b) isolation, quantification and identification of rice microflora, (c) determination of appropriate methods to compare residue degradation capacities of the organisms from a and b, and (d) determine potential of biocontrol of rice pathogen inoculum.

Three widely separated fields of rice (cv. M202) in Butte County were selected as microflora survey sites. These fields were chosen because the same rice cultivar could be monitored under different locales, management, and disease levels. It was hoped that the different sites would thus indicate the diversity of the microflora on rice in Butte County.

Different disease levels and complexes were present at each site. Site 1 was over-fertilized and had a very high level of aggregate sheath spot (R. oryzae-sativae). It had a very low level of stem rot (S. oryzae) and sheath blight (R.. oryzae). Site 2 featured moderate and approximately equal amounts of stem rot and aggregate sheath spot with no sheath blight detected. Site 3 had a low level of stem rot and very low levels of the other two diseases.

Random samples of rice plant portions (above water and submerged) were collected each week until harvest and assessed in the lab for resident microflora. Of several methods attempted, we settled on a combination dilution plate and tissue incubation procedure. Approximately 1 gm (dry weight) subsamples of collected plant parts were washed twice for 30 minutes on a reciprocating shaker, the two washing fluids combined and serially diluted to 10^{-5}. Aliquots (.1 ml) of 10-,

10^{-2}, and 10^{-3} dilutions (for fungi) and 10^{-4}, 10^{-5} dilutions (for bacteria) were spread plated on Difco PDA and Difco CMA (fungi) or Difco nutrient agar (NA) (bacteria). PDA and CMA were amended with 30 ug/ml chlortetracycline and streptomycin to control bacteria and NA was amended with 50 ug ml cycloheximide to control fungi. Dilution plates were incubated at room temperature (19-24 C) and inspected at 3-7 days depending on growth. Each morphologically distinct fungal or bacterial colony was assigned a number, respective CFU's (colony-forming units) tabulated, and a representative colony isolated in pure culture for later identification and study.

Washed tissue from the above procedure was directly plated on PDA and CMA then incubated at 10 C and room temperature for 3-14 days (duplicate plates). Predominant fungi growing from the tissue were ranked and a representative of each isolated in pure culture.

After harvest, rice residue was collected from the sites at 2-week intervals and processed as above.

Sclerotia of S. oryzae placed in the fields in August were recovered after 8 weeks and plated on respective media to detect any colonizing microflora.

At this point, no analysis of the population data has been performed nor has adequate identification of isolates (especially bacteria) been accomplished. Some general trends are evident, however.

Before harvest, predominant fungi detected on dilution plates from upper rice plant parts include: Cladosporum spp. (3), Sporobolomyces sp., Epicoccum nigrum (2 forms), Colletotrichium sp., Acremonium sp., Alternaria spp. (2), Nigrospora sp., an unidentified buff, dry yeast, a white slimy yeast, and three sterile white mycelial fungi. Plated tissue yielded Cladosporium, Sporobolomyces, Epicoccum, Alternaria, Nigrospora, R. oryzae-sativae, R. oryzae, and S. oryzae.

Submerged rice plant parts yielded Colletotrichum sp. (not the same as above), Cladosporium spp., Acremonium spp. (3), and Gliomastix sp. Several unidentified yeast and sterile mycelial forms were again consistently isolated. Plated submerged tissue yielded Fusarium sp., Cladosporium, Absidia, Phycomyces ?, Sclerotium hydrophilum, R. oryzae-sativae, R. oryzae, and S. oryzae. Water samples had a similar pattern as the dilution plates from submerged tissue washings.

After harvest, population levels changed drastically but in general the same group of fungi was recovered. The buff-colored yeast and Sporobolomyces, were reduced to minor components of the microflora. Additional fungi, including Aureobsidium (3 forms), Penicillium (3 spp.), and Fusarium (2 spp.) have consistently been detected since harvest.

Bacterial populations were generally more dynamic than fungi under our conditions. Again, population structure differs between the upper rice plant and submerged portions with consistent respective groups until harvest. After harvest, population levels dropped considerably, especially on stubble collected from burned areas. This was not unexpected and we have noted subsequent increases on straw collected after the first fall rains.

As a result of the above work, we have approximately 80 different fungi and 60 different bacteria (including 6 Actinomycetes) isolated in pure culture for later research. These organisms represent those consistently isolated over time even if present at low population levels.

Our initial interest in rice microflora is for potential straw degrading or biocontrol organisms. We also wish to understand this group because some members may have the potential to interfere with introductions of useful microorganisms at some later date. We will continue to monitor the microflora of rice stubble during the winter and spring and collect the dominant components.

Several interesting observations were noted when sclerotia of S. oryzae were recovered from the field and plated on various media. Colonizing fungi consistently isolated from the sclerotia included Chaetomium, Penicillium, and an unidentified Basidiomycete. None of these were isolated in other efforts involving rice plants or stubble. Sclerotia colonized by Chaetomium and the Basidiomycete were often dead but this was not true in the case of Penicillium. All three fungi were antagonistic to S. oryzae in culture.

Several bacteria were also consistently isolated from the sclerotia with one species - a hyaline, fast spreading, thin colony type, gram-positive rod - being very inhibitory to sclerotial germination. Surface sterilized sclerotia from the same sample germinated normally. All of these observations are very preliminary and controlled experiments with these isolated organisms need to be conducted before further steps are taken.

Methods for analysis of field collected straw over time were reviewed during this period. An acid detergent digestion method to analyze crude fiber appears to offer the most promise. Information obtained with this method includes hemicellulose, cellulose, lignin, and silica content of the straw. These compounds make up 80% of the dry weight of rice straw and the first three are the components attacked by degradation microbes. We are in the process of analyzing all microbes obtained thus far by this method of analysis to determine which are most efficient as residue decomposers. We are continuing collections of straw samples every 2 weeks for additional study of the microflora to determine if major changes in microflora occur during the overwintering period.

We are also screening the microbial isolates we have collected for straw degradation ability for antagonism to S. oryzae, R. oryzae-sativae, and R. oryzae. Organisms showing promise during initial screening will be further tested in the greenhouse for ability to control the pathogens.

We are proceeding with data analysis and microbe evaluations for both straw degradation and bioactivity against the pathogens.

Hopefully, the time course of decay can be usefully correlated with the population data from microflora monitoring. Collection and analysis of overwintering sclerotia of S. oryzae, R. oryzae-sativae, and R. oryzae (if any) will continue in order to identify, isolate, and study any additional antagonistic colonizers.

Objective 2: The need to re-evaluate chemicals being used for seed treatment control of seedling disease was accentuated this past season. Unfavorable weather conditions and the fact that some growers planted untreated seed resulted in many stand-establishment problems, some replanting and some less than optimum stand densities. In addition regulatory agencies are signaling that this will be the last year Captan will be available and several seed plants have indicated they will no longer use Kocide.

Field tests to identify replacement fungicides were conducted during this season. Apron, Anchor, Vitavax, Maneb and Gus-F-44 were tested at two sites with Captan as a standard. Captan, Apron, and Anchor provided near equal control and were all significantly better than untreated controls at both sites. Maneb, Vitavax and Gus-F-44 were about equal in effect but only slightly better than the untreated control. Additional work on rates and formulation of these compounds is needed to determine if any are adequate replacements for the currently used chemicals.

Objective 3: Studies to develop satisfactory screening techniques for differences in resistance to kernel smut were completed in the greenhouse. Comparison of two inoculation techniques on 8 cultivars showed that the best results are obtained when sporidial suspensions are injected into the early boots stage of growth and the plants maintained under conditions of high humidity. With this method we obtained over 50% smutted kernels in panicles of long grain cultivars and 30-35% in medium and short grain cultivars. No significant differences in resistance were noted between cultivars of similar grain type. The results indicate new sources of germplasm can be adequately screened for potential resistance to kernel smut.

Objective 4: Researchers in Louisiana have reported increased stand establishment and yields by soil (seed-bed) applications of the watermold-specific fungicide, Ridomil. They suggest this practice may control Pythium crown rot of rice and contribute to control of seedling disease. We conducted two trials to test the possibility that ridomil may control early dying (crown rot) and also contribute to seedling disease control in California. Ridomil 2E was applied to seedbeds at 1 pint a.i./acre just prior to flooding in two trials with 4 replicates/trial. Stand counts, percent crown rot and yield was not significantly different between treated and untreated plots at either site. We will not pursue this approach to disease control further.

Objective 5: Effects of alternate cropping systems (residue incorporation, green manure crops, fallow seasons) on sustainability of rice production and severity of rice diseases is being studied at several locations. This year we identified or established sites where long-term effects of the above can be compared to the standard system being used by most growers. Initial disease levels and inoculum levels for comparing with changes over time have been established. We are comparing the following systems:

(a) Rice following rice - straw chopped on ground following harvest/ incorporated into soil (fall or spring).

(b) Fallow following rice - straw unburned, unchopped/incorporated early summer/irrigated.

(c) Rice following fallow-field where straw was incorporated.

(d) Rice following rice-straw burned/conventional.

(e) Straw incorporated/winter vetch crop.

(f) Straw burned/winter vetch crop. Cumulative effects of the above are being determined. Results of 2 or 3 years are needed to interpret which provides the best means to minimize rice diseases.

Publications or Reports:

Webster, R. K. Report to the California Rice Research Board. Project RP-2. Cause and control of Rice Disease, p. In Annual Report of Comprehensive Rice Research. 1987. University of California and U. S. Dept. of Agriculture.

Rutger, J. N., R. A. Figoni, R. K. Webster, J. J. Oster, and K. S. McKenzie. 1987. Registration of early maturing, marker gene and stem rot resistant germplasm lines of rice. Crop Science 27:1319-1320.

Concise General Summary of Years Results:

Microflora that occur on rice were isolated and are being tested for ability to biologically degrade rice straw. Several candidate microbes for biological control of rice pathogens have been isolated, partially identified and are being screened for activity against S. oryzae, R. oryzae-sativae and R. oryzae. Field sites with varying reside management schemes, with or without a green manure crop have been established for comparing microflora populations and their efficacy as residue decomposers and/or biocontrol organisms.

Additional fungicides effective to control rice seedling disease have been identified. Trials this year will emphasize rate and formulation to maximize efficacy.

A reliable method for evaluating differences between cultivars and rice lines for resistance to rice kernel smut was developed.

Attempts to control crown rot of rice by fungicide applications to soil were not successful.

Field study sites to compare the affects of residue incorporation, green manure crops and fallow seasons in various combination on rice disease severity were established and initial data collected. Continued study is needed to determine cumulative effects.