# ANNUAL REPORT COMPREHENSIVE RESEARCH ON RICE January 1, 1995 - December 31, 1995

PROJECT TITLE: Cause and Control of Rice Diseases

STATUS OF PROPOSAL: Continuing

PROJECT LEADER: R.K. Webster

PRINCIPAL UC INVESTIGATORS: T. Miller, N. Cintas, Res. Associates

COOPERATORS: S. Scardaci, M. Brandon

LEVEL OF 1995 FUNDING: 34190.00

#### OVERALL OBJECTIVES AND STATUS OF THE PROJECT:

The primary objective of this project continues to be to gain an understanding of the biology of rice diseases that occur in California and to develop methods for their control.

The relationship of rice residue management methods and the epidemiology of stem rot and aggregate sheath spot is based in their disease cycles that are dependant on overwintering inoculum in the form of sclerotia to initiate disease the following year. Both pathogens, Sclerotium oryzae and Rhizoctonia oryzae sativae are known to overwinter and increase in rice residue. This has been the basis for the beneficial aspects of burning rice residue in regard to disease management.

It is apparent that burning of residue will not be available to all growers in the future. As a result, growers are exploring different methods and schedules for managing the straw after harvest. It is necessary to understand the effects of different methods of dealing with the residue between crops on both rice pathogens and the potentially beneficial organisms, particularly those that affect the overwintering disease propagules.

As open field burning is phased out, the need for prediction of potentially high disease fields will increase due to the provision that growers will be granted permits to burn in fields where disease loss in the future is apparent. Disease severity data for subsequent sample times was collected for use in refining our ability to predict fields where continued burning may be justified.

Continuous year trials for comparing residue management alternatives have been established in Colusa and Butte Counties in cooperation with other UC researchers. Data has been collected for two years from the Colusa trial and for one year from the Butte County trial.

The specific objectives for this year are derived from previous results that we view as promising avenues of continued study in attempts to devise disease management strategies.

# OBJECTIVES FOR 1995 AND EXPERIMENTS TO ACCOMPLISH OBJECTIVES:

- [1] a. Study the occurrence and severity of stem rot ( $\underline{S}$ .  $\underline{oryzae}$ ) and aggregate sheath spot ( $\underline{R}$ .  $\underline{oryzae}$  sativae) under various residue management treatments. Comparisons between the effects of winter flooding, incorporation, burning, soil contact and removal of residue at the Canal Farms site (Colusa County) and the BUCRA, Butte County site were made. We believe that continuous years of data are needed to determine the cumulative effects of these treatments on buildup or decrease of disease inoculum and the levels of disease that occur due to them.
- b. Refine our method to determine soil inoculum levels of R. oryzae sativae. This is needed due to the apparent effects of soil types on the sensitivity of our current method and the differences between the study sites at Colusa and Butte Counties. This study was conducted in the laboratory using various soil samples from the different sites.
- [2] Continue study on the interaction of organisms that have been shown to affect viability of pathogen inoculum and or infection of the rice plant. Emphasis was on the interaction between S. oryzae and R. oryzae sativae to attempt to understand the basis for the "inverse relationship" between stem rot and aggregate sheath spot that has been observed. These studies were conducted in the greenhouse and laboratory this year.
- [3] Continue evaluations of germplasm sources for improved resistance to SR and AGSS. Emphasis this year was on greenhouse studies.
- [4] Continue data collection required for determining ability to predict which fields may justify continued burning. Emphasis this year was on determining when the determination must be made so to allow time to take action in the fall if needed. We are also beginning the analyses to determine the reliability of predicting inoculum levels in spring seed beds from disease levels in a particular field the previous fall.
- [5] Greenhouse screening of environmentally acceptable candidate fungicides for field testing to determine the feasibility of controlling stem rot and aggregate sheath spot in the field with chemical applications.

SUMMARY OF 1995 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE:

Objective 1 a.

Study of the occurrence and severity of stem rot and aggregate sheath spot under various residue management treatments was carried out at the Colusa and Butte County sites.

### Colusa County:

This site is a cooperative effort coordinated by S. Scardaci. Residue treatments under study include: straw burned, straw incorporated in the fall, straw rolled after harvest, straw baled and removed. Winter flooding is tested as a main plot treatment onto each of the above also with a non-flood comparison. Each treatment is established with a separate water system which precludes movement of pathogen inoculum between treatments. This year we completed collection of the second full season of data from this site.

### Butte County:

This site is a cooperative effort with other UC researchers and is coordinated by Marlin Brandon. The treatments being compared are identical to those at the Colusa County trial. The experiment is designed with separate water systems for each replicate of the individual treatments. The individual plot sizes are smaller due to limitation of the total size of the test site. This year we completed collection of the first full season of data from this site.

Soil samples were collected from finished seedbeds from each of the replicated treatments at three sites per basin at both the Colusa and Butte County sites. The samples were ground with a soil grinder and subsamples from each were processed by removing sclerotia by floatation and soil sieving. Sclerotia of both S. oryzae and R. oryzae sativae recovered from samples were counted and plated on agar to determine their identity and viability. Viability information is needed to determine differential survival of sclerotia from one season to the next due to various residue treatments.

Plant samples were collected from each of the sites approximately where the soil samples were collected. These were rated for stem rot severity (1=healthy - 5 = severe disease). Aggregate sheath spot incidence was also determined for each sample. The values obtained from the subsamples from each of the replicate basins per treatment were averaged for that treatment and the data analyzed by standard statistical methods.

## Butte County Site:

Stem Rot ( $\underline{S}$ .  $\underline{oryzae}$ ): Total sclerotia recovered, viable sclerotia (inoculum level) and percent viable sclerotia obtained in each of the various residue treatments for  $\underline{S}$ .  $\underline{oryzae}$  (stem rot) are shown in Table 1.

Table 1

Total Number of Sclerotia, Percent Viability and Viable Sclerotia\gram seedbed Soil of S. oryzae at Butte County,1995

	Treatment	Number Scler/gr	Percent Viable	Viable gram\soil	Stem rot Severity
	Straw Burned, winter-flooded	4.61 abc	31 a	1.43 bc	2.64 ab
	Straw burned, non-flooded	4.24 bc	29 a	1.24 c	2.57 ab
*	Straw incorporated winter-flooded	, 6.06 ab	35 a	2.13 a	2.81 ab
	Straw incorporated non-flooded	, 5.30 abc	28 a	1.43 bc	2.36 b
	Straw rolled, winter-flooded	6.13 a	32 a	1.89 ab	2.60 ab
	Straw rolled, non-flooded	3.95 c	32 a	1.24 c	2.33 b
	Straw baled, winter-flooded	4.46 abc	30 a	1.42 bc	2.94 a
	Straw baled, non-flooded	5.34 abc	29 a	1.54 abo	2.43 b

<sup>\*</sup> values with same letter are not significantly different \*\* significant differences based on Duncan's mean separation \*\*\* stem rot severity based on 1=healthy - 5 severe disease.

The first years results show that there is a difference in total number of sclerotia and percent viability of the sclerotia between the residue treatments. Even so the viable sclerotia per gram soil resulting in the finished seedbeds is 1.2 or higher in all of the treatments. Previous study has shown that inoculum levels above .6 scl\grm result in no significant differences in disease severity.

The experimental design is a main plot, flooding versus non-flooding with burning, incorporating, rolled and baled as subplots. The effect of the main and subplot treatments on various parameters measured at the site are summarized in Table 2.

Table 2

Affect of flooding and various residue treatments on viability of <a href="S.oryzae">S.oryzae</a> inoculum, disease severity and yield at Butte Site, 1995

Treatment	% viable	via.\scle gr.soil	Disease Severity	Yield * dry\wt.
Winter Flood	.32	1.71	2.74	6361 lb\ac
Burned	.31 a	1.42 bc	2.64 ab	
Incorp.	.35 a	2.13 a	2.81 ab	
Rolled	.32 a	1.89 ab	2.60 ab	
Baled	.30	1.42 bc	2.94 a	
No Winter Flood	.295	1.36	2.40	6867 lb\ac
Burned	.29 a	1.24 c	2.51 ab	
Incorp.	.28 a	1.42 bc	2.36 b	
Rolled	.32 a	1.24 c	2.33 b	
Baled	.29 a	1.54 abc	2.43 b	
Subplot Yield	Burn	Incorp.	Rolled	Baled
Flooded	6510	6559	6417	5960
No Flood	6937	6937	6839	6753
Mean	6723	6748	6628	6356

<sup>\*</sup> The yield data given is for the large plot harvests at the experimental site. We believe that there are factors in addition to disease that are affecting yields in the various treatments. As the experiment progresses it should be possible to determine what factor or factors these differences are due to.

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From a stem rot disease standpoint, the effects of the various treatments this year are most interesting regarding the differences in percent viability of sclerotia in the treatments and the total viable sclerotia that resulted in each treatment.

The experiment is designed to determine the cumulative affect of the various treatments on the production and survival of overwintering inoculum of <u>S. oryzae</u>. The beginning inoculum levels were beyond the limit of linear disease increase as affected by inoculum level and thus it was not expected to see differences in amount of stem rot disease between the treatments this year. This site provides an ideal opportunity to measure effects of the treatments on stem rot epidemiology over successive years since each treatment will be repeated in the same basins.

Aggregate Sheath Spot ( $\underline{R}$ .  $\underline{\text{oryzae}}$   $\underline{\text{sativae}}$ ) Similar observations on aggregate sheath spot disease were made at the Butte site. The sclerotial counts and incidence of aggregate sheath spot observed during the first year (1995) are summarized in Table 3.

Table 3

Aggregate Sheath Spot Incidence Under Various Residue
Management Treatments at the Butte County Site 1995

Treatment	% Tillers infected with AGGS	Viable Sclerotia per gram soil
Straw Burned, winter flooded	56.8 a	.071 a
Straw Burned, not flooded	78.2 ab	.114 bc
Straw Incorporated, winter flo	ooded 53.3 a	.116 c
Straw Incorporated, not-flood	ed 84.2 bc	.121 c
Straw Rolled, winter flooded	59.7 ab	.076 a
Straw Rolled, not flooded	84.6 bc	.064 a
Straw baled, winter flooded	56.1 a	.086 ab
Straw baled, not flooded	76.5 ab	.081 a

<sup>\*</sup> disease incidence determined as percent infected tillers

Viable sclerotia per gram soil from the seed bed of the aggregate sheath spot pathogen are considerably lower than those for the stem rot pathogen. This is typical of the group of pathogens to which it belongs and comparisons between the two pathogens on numbers of propagules per gram soil are not valid.

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Aggregate Sheath Spot incidence in flooded main plots was 56.5 and 80.9 in the non-flooded mainplots. This first years data from Butte County on Aggregate Sheath Spot provides an ideal base to determine the cumulative affects of various residue management treatments on inoculum and disease incidence under continuous years of the treatments.

## Colusa County Site:

The 1995 season was the second year for data collection at the Colusa County site. Total number of  $\underline{S}$ .  $\underline{\text{oryzae}}$  sclerotia, percent viable, viable sclerotia per gram soil and stem rot disease severity observed in 1995 are summarized in Table 4.

Table 4

Total Number of <u>S</u>. <u>oryzae</u> sclerotia, percent viable, viable sclerotia per gram soil and stem rot disease severity at Colusa County study site, 1995.

Treatment	Number Sclerotia	Percent Viable Viable gram\soil	Stem Rot ** Severity
Straw Burned winter-flooded	2.95 d *	21.0 c .62 c	3.06 c
Straw Burned non-flooded	3.68 bcd	17.1 c .63 c	3.49 ab
Straw incorp winter-flooded	3.25 cd	24.3 c .79 c	2.92 c
Straw incorp	4.55 bc	33.1 b 1.51 b	3.86 a
Straw rolled winter-flooded	4.51 bc	32.5 b 1.47 b	3.21 bc
Straw rolled non-flooded	6.17 a	36.4 a 2.25 a	3.70 a
Straw baled winter-flooded	3.79 bcd	27.4 bc 1.04 bc	3.51 ab
Straw baled non-flooded	4.56 b	29.6 b 1.35 b	3.78 a

<sup>\*</sup> values with same letter are not significantly different based on Duncan's mean separation

<sup>\*\*</sup> stem rot severity based on 1=healthy - 5=severe disease

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There are significant differences between total sclerotia and survival of sclerotia in the treatments being compared however even in the burn treatments the inoculum levels are .62 and .63. Both of these inoculum levels are above those reported where correlations between inoculum level and disease severity are known to continue to be linearly related.

Main plot affects, winter flooding vs non winter flooding, are summarized in Table 5.

Table 5

Affect of winter flooding and various residue treatments on viability of <u>S</u>. <u>oryzae</u> inoculum, stem rot disease severity and yield at the Colusa County Site, 1995.

•		-		
Treatment	% viable	via.\scle gr. soil	Disease severity	Yield dry\wt
Winter Flood	26.3	.98	3.17	8980
Burned	21.0 c	.62 c	3.06 c	
Incorp.	24.3 c	.79 c	2.92 c	
Rolled	32.5 b	1.47 b	3.21 bc	
Baled	27.4 bc	1.04 bc	3.51 ab	
No Winter Flo	od 29.1	1.43	3.70	7980
Burned	17.1 c	.63 C	3.49 ab	
Incorp.	33.1 b	1.51 b	3.89 a	*
Rolled	36.4 a	2.25 a	3.70 a	
Baled	29.6 b	1.35 b	3.78 a	- 5
Subplot	Treatments	Yield		
Inc Rol	Burn Incorporate Roll Bale		ields not signi ifferent in sub	

A comparison of the data obtained for stem rot inoculum levels and disease severity for 1994 and 1995 is given in Table 6.

Table 6

S. oryzae inoculum levels and Stem Rot Severity at the Colusa County Study site for the 1994 and 1995 Rice seasons

Treatment	viable sclerotia per gram soil	Stem Rot Severity
	1994 1995	1994 1995
Winter Flood		
Burn	1.0 .62	2.54 3.06
Incorp.	1.5 .79	2.55 2.92
Roll	1.5 1.47	2.60 3.21
Bale	1.4 1.04	2.75 3.51
Not Flooded		
Burn	1.1 .63	3.10 3.49
Incorp	1.4 1.51	2.78 3.86
Roll	2.4 2.25	2.73 3.70
Bale	1.2 1.35	3.32 3.78

Inoculum levels of  $\underline{S}$ .  $\underline{\text{oryzae}}$  in all treatments tested are relatively high (above .6 scl\grm) and thus correlations between inoculum level and disease severity do not occur due to saturation of the disease system.

Stem Rot disease severity levels in all treatments are higher in 1995 than they were in 1994. This is typical of the overall 1995 season and probably due to the many aspects of the effects of the particularly wet winter and difficulties experienced with field preparation, planting schedules etc. It is also unknown what the effects of the wet winter of 94 and 95 were on the mainplot treatments of flooding and non-flooding. We expect that the continuous years of study at both sites will reveal the cumulative effects of the treatments under study.

Incidence of aggregate sheath spot disease that occurred in the various residue treatments at the Colusa site was also determined and the data for 1994 and 1995 are compared in Table 7.

Table 7
Aggregate Sheath Spot Incidence at the Colusa County Study Site for the 1994 and 1995 Rice Seasons

Treatment	Percent Ti	llers Infected 1995	
Winter Flood	.067	1.20	
Burn	.06	.92	
Incorp.	.10	2.36	
Roll	.05	.46	
Bale	.06	1.07	, •
Not Flooded	.052	1.26	
Burn	.05	1.16	
Incorp.	.07	1.73	
Roll	.05	.08	
Bale	.04	2.08	

There was an increase in aggregate sheath spot incidence in the 1995 season over that observed for the 1994 season in all treatments tested at the Colusa County site. This increase was greatest in the incorporated treatments and the roll or soil contact treatments.

## Objective 1 b.

Determination of quantitative inoculum levels of the AGSS pathogen Rhizoctonia oryzae sativae are complicated due to variation in sclerotial size, shape, similarity to other soil borne fungal propagules and buoyancy when utilizing floatation from soil. We continued efforts to refine our method this year. Studies comparing floatation solutions prepared with different densities to floatation on water only, recovery of only floating sclerotia vs total sclerotial recovery (those that sink) and effects of surface sterilization on viability of recovered sclerotia revealed that a method where only those sclerotia that float from soil in water is as reliable as any other tested for determining relative levels of sclerotial inoculum of R. oryzae sativae. We have concluded that a water floatation method similar to that used for S. oryzae when

accompanied by culturing to assure proper identification of propagules is effective in measuring changes in AGSS inoculum levels due to the residue management treatments being tested. The full details of these studies on methodology will be presented elsewhere.

Objective 2.

Continued study on the interaction of organisms that effect occurrence and severity of the stem rot and aggregate sheath spot diseases emphasized the potential relationship between the various residue management treatments being studied at the Colusa and Butte County sites and their effects on the populations of various bacterial species that occur naturally in rice fields. We have collected samples from the various treatments, isolated the bacteria that occur and are presently identifying them to determine if the bacterial flora differ between treatments and also if they are changing due to the treatments.

It is logical to expect changes in populations of organisms other than pathogens when large changes such as the manor of residue management are being implemented into the rice culture system. The possibility that such changes may be exploited to the benefit of disease management should not be ignored. Results thus far are incomplete and will be reported at the appropriate time.

Objective 3.

Evaluation of collections of wild <u>Oryzae</u> species in an attempt to identify improved sources of resistance to both stem rot and aggregate sheath spot were completed. Accessions studied varied greatly in plant type, flowering date, maturity time, seed size and sterility. None tested were considered as useful for future breeding efforts by standard breeding techniques. Although new technologies may allow transfer of only desirable characters we did not identify such.

This year we began screening of collections of  $\underline{O}$ . Sativae from the National Germplasm Collection concentrating on collections from countries with a similar climate as that of California. Evaluations of over 550 entries in this collection revealed only 6 that were as resistant to stem rot as is M-202 and none were more resistant. The selections evaluated varied considerably more in reaction to Aggregate Sheath Spot but again were not considered promising as sources for resistance to AGGS. We have obtained additional selections from the collection and will continue evaluating them.

Objective 4.

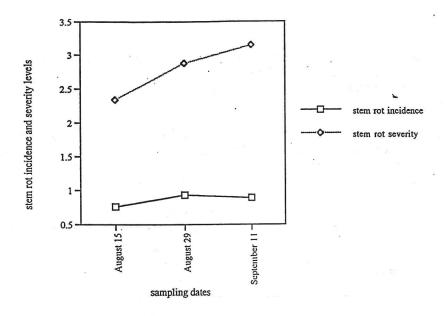
The safe-harbor provision in the legislation restricting burning of rice residue accentuates the need for a reliable means to determine when disease can be expected to be limiting in a

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particular field and allow timely decisions regarding exceptions for burning. We believe that continuous years data, particularly on correlations between disease severity in the fall and carry over inoculum level in the field the following spring and resultant disease from that carry over inoculum provide the best basis for this prediction.

To allow adequate time for decision makers to determine who may be eligible for exceptions to burn, it is also necessary to determine how early in the growing season disease severity ratings may be made that correlate to the final amount of disease experienced in a particular field. Results obtained last year indicated that Aggregate Sheath Spot incidence (percent infection) continues to increase during the month prior to harvest. Stem Rot incidence changes very little but severity continues to increase since this disease causes most of it's damage during the time that the pathogen is continuing to penetrate and rot the culmes of infected rice tillers. The increase of stem rot severity over time versus stem rot incidence is shown if Figure 1 for three sampling dates ranging from August 15 to September 11 for 1995.

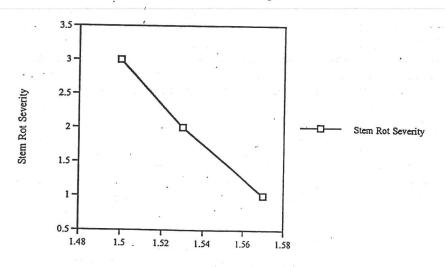
Stem rot incidence and severity levels over time in a Colusa county rice straw management trial



These results are similar to those obtained last year for the stem rot disease and they indicate that stem rot severity ratings are more reliable than stem rot incidence data in measuring potential losses due to stem rot.

Figure 2 shows the relationship between stem rot severity ratings of individual tillers rated severity category 1 (healthy) to category 3 (moderately diseased) diseased and the reduction in weight of panicles for the different disease categories.

## Stem rot severity versus panicle weight



Panicle Weight (g)

As seen there, even though tillers showing these moderate levels of stem rot severity produce tillers and grain there is an over all reduction in the grain weight produced on the infected tillers.

Representative data for reduction in grain weight as stem rot severity increases on individual tillers from healthy (non-infected) through severe, category 5 (culm entirely penetrated, small to no tiller and fewer to no grain) are given below.

Mature Panicle Weights (Grams) at Five Levels of Stem Rot Severity from Three fields in Butte County

Field	Stem 1	Rot Dis	ease Sever 3	ity Cate 4	gory 5
1	1.95	1.77	1.75	1.73	1.59
2	1.63	1.41	1.37	1.29	1.15

Disease Severity Ratings: 1 = Healthy - 5 = Severe Values are averages of six random samples per field with at least 70 tillers per sample (total 350 +)

These results indicate that predictions of fields in which a significant amount of stem rot may be expected to occur the following year are possible based on disease severity ratings from that field prior to harvest the previous year.

Objective 5.

Determination of potential control of stem rot and aggregate sheath spot with fungicides was carried out. Greenhouse tests indicated that there are compounds that deserve testing in the field and these trials are planned for this year. Candidate compounds to be tested are: ICIA5504 a wetable granule formulation that reduced incidence and severity of both SR and AGSS in greenhouse trials. Researchers from other rice producing states have also observed effectiveness of the compound in controlling sheath blight and to a lesser extent, stem rot.

Benomyl has been reported to control sheath blight and stem rot in other states. Our tests with Benomyl showed no control of stem rot in both greenhouse and field tests. It did however reduce the incidence of AGSS by about 40% when two applications were applied. We will compare the effectiveness of both Benomyl and ICIA5504 to Duter (previously shown to control stem rot but failed to obtain registration) in this years studies.

## PUBLICATIONS OR REPORTS

Webster, Robert K. Report to the California Rice Research Board: Project RP-2. Cause and Control of Rice Diseases, 14 pp. In: Annual Report of Comprehensive Research. 1994. University of California and the U. S. Dept. of Agriculture.

Cintas, N.A., R.K. Webster, and T. Miller, 1995. Interactions of <u>Sclerotium oryzae</u> and <u>Rhizoctonia oryzae</u> sativae on Rice. Phytopathology 85: 1038.

Cartwright, R.d., R.K. Webster and C.W. Wick, 1995. Mycoparasitism of <u>Sclerotium oryzae</u> and other fungi by an unusual <u>Ascochyta</u> sp. from California rice fields. Mycologia

## CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS

The establishment of continuous year trials to monitor the effects of various residue management treatments on rice disease epidemiology is essential in determining the optimum rice disease management system as open field burning is curtailed. The second year of data was obtained from the Colusa site and the first full year of data from the Butte site. Results thus far can be summarized as follows:

Stem Rot Disease.

Results at the Butte site showed differences in viable sclerotia in the seedbeds of treatments tested. In all treatments the viable sclerotia in the seedbed were above 1.2 or higher per gram soil and stem rot severity relatively high. Previous study has shown inoculum levels above .6 viable sclerotia per gram result in no significant differences in disease severity. Thus continued study to determine if any of the treatments result in a reduction of the initial levels of inoculum will be critical.

In comparing the main plot effects at the Butte site during the first year the mean viable sclerotia per gram seedbed soil was lowest in the non-flooded mainplots and the mean yield was highest. The reverse was observed at the Colusa site where mean viable sclerotia per gram seedbed soil was lowest in the winter-flooded mainplots and yield was significantly higher for the second year in a row. The initial inoculum levels at the entire Butte site were relatively high and there was an unusual amount of rain during the past winter which may have influenced the results comparing the winter-flooded and non-flooded mainplots.

There were significant differences in viable sclerotia surviving between treatments at the Colusa site with the burn treatments being the lowest after two years. These inoculum levels are just above .6 where correlations between inoculum level and stem rot disease are known to be linearly correlated. This accentuates the value of the continuous years study to determine the cumulative effects of the treatments being compared.

We observed plant conditions at both trial sites that appeared to be abnormal and probably related to nutritional factors, possible plant toxicities or both. Thus the differences in yield between some of the treatments cannot be attributed to disease alone.

Aggregate Sheath Spot:

The detectable inoculum levels and disease incidence of aggregate sheath spot at the Butte site were significantly higher for the first year than in either of the two years at the Colusa site. There was an increase in AGSS at the Colusa site in 1995 over that in 1994 with the largest increase occurring in the incorporation treatments. The differences in the levels of disease at the two sites provides an ideal situation to study the cumulative effects of the treatments on this disease.

Evaluation of over 550 collections of  $\underline{O}$ . sativae from the National Germplasm Collection revealed only 6 that were as resistant to stem rot as M-201 and none of these were more resistant. None of the 6 were considered promising as sources of

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resistance to aggregate sheath spot. Evaluations of additional collections will be continued.

Preliminary tests to determine candidate fungicides for extensive field trials in 1996 were completed and three compounds were selected for these tests.

Attempts to predict which fields may be considered eligible for the safe-harbor provision of the reduction in burning legislation were continued. Results show that evaluations made within a month of harvest give reliable estimates of the effect of stem rot on the present crop. Correlations between disease severity of the present crop and expected carry inoculum levels in the next years seedbed were also strong. This approach is very labor intensive and dependant on the validity of the sampling techniques used. This effort is being continued.