ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
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PROJECT TITLE: Controlling *Gibberella fujikuroi,* "Bakanae Disease," in Rice Seed Using Ozone

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OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

The goal of this proposal was to evaluate the potential for ozone to control bakanae, a seed borne disease that erupted in CA rice in 1999. Preliminary data on lab tests indicated that ozone may have potential as a seed treatment prior to seeding to control this fungus. Ozone is triatomic oxygen (O_3), a pungent, unstable bluish gas that is a highly reactive oxidizing agent that readily breaks down organic molecules. It is routinely used as disinfectant in the food industry to control bacterial or fungal growth for fresh fruit and vegetables. The positive attributes of ozone are: that it is highly reactive with organic molecules, thus can be used as relatively low doses; it readily breaks down into O_2 thus not having any environmental concerns with disposal as with chlorine bleach; and has it has been declared generally recognized as safe (GRAS) by a panel of food experts, giving it widespread acceptance in the US; it is an excellent surface biocide. These attributes made it an excellent candidate to test on bakanae as the spores of the causative fungus *Gibberella fujikuroi* are primarily found on the surface of rice seed. The objectives of our research were:

1. Determine the efficacy of ozone treatments in controlling bakanae in rice seed in the laboratory.
2. Determine the efficacy of ozone treatments in controlling bakanae in rice seed in field studies.
3. Design a treatment method for applying ozone in the field at the time of aerial seeding.

SUMMARY OF 2003 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE:

Objective 1

Preliminary data (not shown) indicated that ozone was effective on controlling bakanae fungal spores on presoaked seed, but not on dry seeds. This was likely due to the activated or growing state of the spores under moist conditions making them most vulnerable to eradicate. Under dry conditions, spores are dormant thus protected against effects of biocides. The presoaking of rice seeds prior to seeding thus provided an excellent opportunity to effectively apply ozone.

Experiment 1. Moisture content of field trucks prior to seeding. As moisture content affects mass flow, thus dose of ozone gas through a matrix (presoaked rice), we designed experiments to determine the actual moisture content of presoaked seed in soaking bins and trucks immediately prior to seeding. We sampled three field instances (bins and truck trailers) and one lab instance at different periods after draining so that actual field conditions could be mimicked in our lab and field trials. Each bin or trailer was sampled at three different depths to represent the whole. Seed was weighed immediately after sampling and after 17 hours drying at 103°C. Percent moisture was calculated as (wet weight-dry weight)/dry weight x 100. The data indicates that after a 20-24 hour soak, rice seed has little variation (average decrease 2.5%) over a 24 hour period in trucks and ranged from 16 to 22%. This trend was consistent with results in 350 g bags of seed used for field experiments (Figure 1). Most of the variation was within a trailer as opposed to over time.

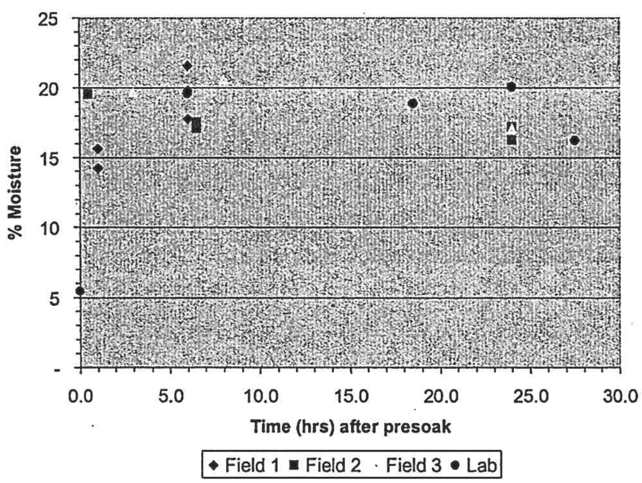

Figure 1. Moisture content of presoaked rice seed in field (trucks) and lab (350 g bags) instances. The datapoint at 0 hours represents moisture content of dry seed (5.5 %).

Experiment 2. Effect of ozone on bakanae spores on rice seed. An experiment was designed to test three doses of ozone (75,000, 150,000 and 300,000 ppm) and three times after presoaking seed (1, 8 and 17 hrs) on bakanae spore count to confirm preliminary results and to narrow down the number of treatments for field trials. Spores were counted at 24 and 72 hours after treatment (Table 1). All treatments showed a significant reduction in spores relative to untreated control, confirming preliminary data. Although numerically, the longer times after presoaking had lower 24 hr spore counts, these differences were not significant (see standard error). Similarly, dose response within a time treatment were not significantly different. As seen with other treatments such as bleach, spore counts after 72 hours were significantly higher if seed was kept moist. Germination was not significantly affected by ozone dose. Greenhouse evaluations were not pursued as they are not effective in the spring (when tests were done) due to poor fungal growth under cool conditions.

Table 1. Effect of time after presoaking and ozone dose on spore counts from bakanae infected rice seed

Time after presoak (hrs)	Ozone Trt (ppm)	Moisture content (%)	Germination (%)	24 hr count (spores/ml)	72 hour count (spores/ml)
1.0	Untreated control	26.4	97.0	2296	105,920
1.0	75,000	26.4	95.0	114	80,080
1.0	150,000	26.4	96.0	40	95,520
1.0	300,000	26.4	96.0	42	97,200
9.0	75,000	24.1	95.0	176	68,480
9.0	150,000	24.1	95.0	54	88,160
9.0	300,000	24.1	93.0	208	>240,000
18.0	75,000	21.3	94.0	16	>48.000
18.0	150,000	21.3	92.0	0	>32,000
18.0	300,000	21.3	95.0	8	>60,000
Standard error			1.26	71.3	10,608

Objective 2

Based on experiments 1 and 2, it was decided that three ozone doses, 75,000, 150,000 and 300,000 ppm ozone should be tested in the field with approximately 20% moisture content of the seed to reflect field moisture levels immediately prior to seeding or in soaking bins after draining. The three treatments were included in replicated trials in a Random Complete Block Design (RCBD) in four locations in Colusa, Glenn, Yuba and Sutter counties with infested and uninfested controls and 15 other bleach and fungicide treatments. For each treatment, 350 g of presoaked seed (24 hours soak and 2 hour drain) was treated with ozone. The seed for all four replicates was treated in bulk so that all replicates received exactly the same dose for a given location. Seed was planted within 24 hours of treatment with the rest of the trial. Data were taken on for stand, seedling vigor, number of bakanae seedlings/ft^2, number of dead heads, tillers/ft^2 and yield. Disease was expressed as percent bakanae and percent dead heads at seedling and mature stages, respectively. Although bakanae infested seed (>20%) was used for the trials, 2003 was a poor year for development of bakane on rice in CA overall. This can be seen by the comparing the infested (+) and uninfested (-) controls. Seedling bakanae scores varied by 0.33 plants/ft2 or just over 1%. As a result only limited conclusions can be made on the efficacy of ozone on bakanae control in the field. Although significant F tests were found across locations for all traits but yield, the LSD_{01} showed that all ozone treatments were not statistically different than controls for all traits measured. Ozone treatments had a significant reduction in seedling vigor at the Lauppe site, but this was not observed at the other sites (data not shown). Numerically, ozone treatments were not as efficacious as bleach treatments in all sites with the 75,000 ppm treatment showing less control than higher ozone doses based on seedling and mature (dead heads) bakanae evaluations. Further field evaluations are necessary to determine if ozone can be an effective treatment to control bakanae in rice in the field.

Table 2. Efficacy of seed treatments on bakanae control, 2003, mean of four locations.

Treatment	Stand (plants /ft2)	Seedling vigor	Bakanae seedlings /ft2	Bakanae (%)	Dead heads/ ft2	Tillers / ft2	Dead heads (%)	Yield (g/plot)
Uninfested	25.1	4.0	0.02	0.08	0.02	51.50	0.03	8,252
Infested	26.6	4.3	0.35	1.31	0.18	60.25	0.31	7,995
.5% bleach + .25% Surfonic	25.3	4.2	0.07	0.26	0.01	51.31	0.03	8,372
1% bleach	26.5	4.1	0.04	0.16	0.01	56.75	0.02	7,999
2% bleach	24.1	4.1	0.02	0.08	0.01	53.00	0.03	8,237
3% bleach	25.1	4.0	0.05	0.20	0.02	53.50	0.04	8,149
5% bleach (2hr soak)	26.3	4.2	0.04	0.15	0.01	62.88	0.02	8,281
5% WECO	24.6	4.0	0.04	0.17	0.02	52.38	0.04	8,030
10% WECO	22.1	3.6	0.02	0.11	0.01	51.00	0.02	8,214
5% MC588	25.9	4.0	0.04	0.14	0.01	58.50	0.01	7,712
10% MC588	16.8	3.1	0.01	0.05	0.00	43.00	0.00	8,366
15% MC588	5.9	1.9	0.01	0.18	0.01	37.44	0.03	7,853
Ozone 75,000 ppm	26.9	3.8	0.13	0.48	0.07	65.00	0.11	7,706
Ozone 150,000 ppm	24.1	3.6	0.08	0.35	0.04	51.13	0.08	7,825
Ozone 300,000 ppm	26.5	3.7	0.06	0.24	0.04	57.06	0.07	8,303
.25% phosphorous acid	26.8	4.2	0.02	0.08	0.01	53.50	0.01	8,225
.5% phosphorous acid	27.0	4.2	0.04	0.13	0.00	56.00	0.01	8,153
1.25oz Nusan (30%ai)	26.4	4.1	0.06	0.23	0.02	60.50	0.03	8,341
Protégé 100FS	27.9	4.3	0.12	0.42	0.04	59.13	0.06	8,087
Protégé + Maxim	26.4	4.3	0.08	0.29	0.03	52.75	0.06	8,119
Mean	24.3	3.9	0.06	0.25	0.03	54.3	0.05	8,111
Treatment LSD01	2.60	0.22	0.05		0.03	10.43		NS

Objective 3.

The 3rd objective was to design a treatment method for applying ozone in the field at the time of aerial seeding. The goal was to retrofit a 4 x 4 seed bin with a lid and manifolds to deliver ozone through the presoaked seed and measure the ozone concentration at various locations within the bin to determine if a uniform dose for ozone could be delivered in a gaseous form. Plans and calculations were made to execute this objective. The moisture content for our preliminary data for this proposal was approximately 13% (data not shown). Experiment 1 (Objective 1) indicated that the moisture content of presoaked seed in field scale was about 20% (Figure 1). As mass flow of ozone decreases with increasing moisture content, the amount of ozone applied (but not the effective dose) had to be increased approximately to double on a lab scale when treating seed for field evaluations. Initial calculations for the cost of ozone to control bakanae in rice were based on amortization (7 years) of the capital cost of an ozone generator and operating costs. Based on preliminary data, this would be approximately \$0.005/lb of seed or \$1.00 an acre based on 200 lb/acre seeding rate. This cost is reduced to \$0.001/lb or \$0.20/acre after paying for the generator in 7 years. Correspondingly, the capacity of ozone generator exceeded the portable generator available to us through Ozone Consulting, Davis, CA to deliver ozone through a 4 ft by 4 ft bin. A generator able to deliver 104 scfm would be necessary. As the cost of a larger generator is not significant over 7 years (\$98,000 vs 70,000) the cost per lb of seed does not change significantly ($0.0047 vs 0.0055/lb). Although a generator of this capacity is commercially available, we were not able to locate one available for our experiments in Northern California. As a result Objective 3 was not met. The cost of bleach is significantly less, especially if disposal fees are waived as indicated by OSHA (Chris Greer, personal communication). Consequently, ozone is noncompetitive compared to bleach treatments which have shown good efficacy (see Table 2).

The conclusions of this research are that further evaluations are necessary to determine if ozone can be an effective treatment to control bakanae in rice in the field, and that although ozone may be effective at controlling bakanae in rice, its application is cost prohibitive compared to other seed treatments due to capital costs.

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