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Research Organisation Name :	SunRice
Principal Investigator Details :	
Name :	Bronwyn Sigmund
Address :	Yanco Avenue, Leeton, NSW 2705
Telephone contact :	0269530075

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Summary

The Australian rice industry must continue to meet customers' quality expectations, both domestically and internationally. These are controlled largely by Integrated Pest Management and Food Safety.

Due to the phase-out of methyl bromide for all but pre-shipment and quarantine use it has been necessary to develop alternative fumigants for the disinfestation of durable food commodities.

The phase out of Methyl Bromide has impacted the world heavily. The rice industry has been impacted heavily by drought, resulting in a reduced crop which makes each grain of extreme value to the business. Fumigation is highly necessary to ensure SunRice is delivering high quality products to our customers, and as a result relies heavily on successful fumigation. It is crucial that a replacement disinfestation method is found that offers equal or improved effectiveness in controlling pest infestation. The trials undertaken are both extensions on past trials that have given us confidence in their initial studies and new methods that give us hope that we may be able to reduce or replace the use of Methyl Bromide with another method of fumigation.

I Sub-Project Name: Dryacide and Absorba-Cide Trial (2001-2002)

Initial Trial - Slurry application of Dryacide and Absorbacide (Flour beetle and Grain weevil)

Background

Dryacide™ has been the standard product used by Ricegrowers' after emptying paddy storage to control insect pest. The supplier of Dryacide™ has approached Ricegrowers' to trial an alternate product Absorba-Cide™.

Absorba-Cide™ is a disinfectant specifically designed for use in harvesting and other grain handling machinery, food processing, warehousing and grain storage facilities and for the control of insect pests in stored grain. Absorba-Cide™ mode of action is absorption directly into the insects cuticle, ultimately leading to dehydration. Absorba-Cide™ can be purchased at a lower cost to Dryacide™ and supplies can be guaranteed.

Objectives

The aim of this trial is compare the effectiveness of Dryacide™ and Absorba-Cide™, and evaluate and observe the effectiveness of desiccant dusts in general.

Methodology

Materials

12 x Glass Petri dishes (90 mm diameter)
A good cleaning agent
Balance accurate to 0.001g
Weighing paper
Inert dust (to apply 6 gm⁻²)
Dryacide™ dust
Absorba-Cide™ dust
Insect jars (with filter paper lids)
Insect food (whole rice, white rice, rice flour and yeast)

Methods

Pre testing of glassware

Successful preparation of an even test deposit depends on Petri dishes being sufficiently clean to allow water (1.5 mL), pipetted into the dish, to spread evenly and to completely wet its surface. A pre test for glass Petri dishes is therefore advised.

Pipette water (1.5 mL) into the centre of a cleaned glass Petri dish. Rotate and tilt the dish in an attempt to spread the water evenly over the surface. Select only the dishes where the spread of water is even. Soak any dishes that fail this test in a suitable detergent for one hour, scour with a powder cleanser, rinse thoroughly with water then ethanol and dry before retesting.

Application of slurry deposit

Weigh dust (0.054g) on weighing paper and transfer to the centre of four clean glass Petri dish. Add water (0.9mL). Rotate the Petri dish by hand to suspend the dust in the water and to disperse the dust evenly over the base and sides of the dish. A very

fine artist's paintbrush or a gloved fingertip used to assist the dispersion of the deposit. Evaporate the water component of the slurry by placing the dish in an oven (~ 80°C) and rotate the dish approximately each 5 minutes until the water has evaporated. The four remaining clean petri dishes used as controls.

Sitophilus spp- Grain Weevil – Insect selection and survival

Ten *Sitophilus spp.* (*Grain weevils*) were placed in each petri dish for 24 hours. Each petri dish was painted with Fluron™ around the outer edge to prevent insects escaping. Additionally, filter paper 150mm in diameter was placed between the base and lid to aid in the securing of the insects inside the petri dish.

The insect survival was assessed immediately after the set exposure period (24 hours). Insects are assessed as alive, if able to move normally and respond to stimuli, as dead, if unable to do either. Transferred insects to 40 grams of paddy rice white rice and yeast. (The neck of each jar is initially ringed with Fluon suspension). Store jars in a controlled environment. Further mortality counts carried out seven days after the end of the exposure period. At this stage, insects able to move or respond to stimuli are classified as live. Insects are normal or clearly dead after the seven day holding period. Assess mortality against control mortality. Subject control insects to all treatments applied to the test insects except the dust application.

Calculation of survival rates calculated using Abbott's formula for corrected mortalities:

$$= \frac{\text{Proportion dead in TREATMENT} - (\text{Proportion dead in CONTROL})}{1 - (\text{Proportion dead in CONTROL})}$$

Tribolium spp.- Flour beetles- Insect selection and survival

Twenty *Tribolium spp.* (*Flour beetles*) were placed in each petri dish for 24 hours. Insect survival was assessed immediately after the set exposure period (24 hours). Insects were assessed as live, if able to move normally and respond to stimuli, as dead, if unable to do either. Transfer insects to 20 grams of flour and yeast. Store jars in a controlled environment. Further mortality counts were conducted after seven days, after the end of the exposure period. At this stage, insects that were able to move or respond to stimuli are classified as live. Insects are normal or clearly dead after the seven day holding period. Assess mortality against control mortality. Subject control insects to all treatments applied to the test insects except the dust application.

Results

The average mortalities of *Sitophilus spp* as calculated using Abbott's formula for corrected mortalities indicate that after a seven day period 35.7% of the population were dead in the Absorba-cide treatment and 92.8% of the population were deceased in the Dryacide treatment.

Table 1 *Sitophilus spp.* (*Grain weevils*) mortality after 7 days

TREATMENT	AVERAGE MORTALITY
<i>Absorba-cide</i>	35.7%
<i>Dryacide</i>	92.8%

The average mortalities of *Tribolium spp* as calculated using Abbott's formula for corrected mortalities indicate that after a seven day period 0% of the population were deceased in the Absorba-cide treatment and only 5% of the population were dead in the Dryacide treatment.

Table 2 *Tribolium spp.* (Flour Beetles) mortality after 7 seven days

TREATMENT	AVERAGE MORTALITY
<i>Absorba-cide</i>	0%
<i>Dryacide</i>	5.7%

Discussion

Preparation

It was noted that the Absorb-Cide was easier to disperse on the petri dish and that the Absorba-Cide™ dried more rapidly than the Dryacide™.

Particle size- OHS issue if a significant proportion less than 15µm. Dryacide is coated with a silica gel to aid in the moisture absorption therefore potentially aids in the absorption of desiccant dusts.

Treatment effectiveness

The mortality of *Sitophilus spp.* (Grain weevils) after 7 days was calculated using Abbott's formula, which takes into consideration the number of deaths that occurred in the control treatment. After 7 days the Dryacide treatment proved comprehensively, more effective in killing the grain weevils, killing in excess of 90% of the population over the replicated treatments.

The mortality of the *Tribolium spp.* (Flour beetles) after 7 days was also calculated using Abbot's formula. The results indicate low mortalities for both the Absorba-cide and Dryacide treatments, with 0% and 5% mortality being achieved, respectively.

Implications and recommendations

The conclusions that can be drawn from this trial include the difficulty in using a single treatment to control a wide range of pest insects. Absorba-cide™ in this trial has proven to be only moderately effective against *Sitophilus spp* (Grain weevils) and not effective against *Tribolium spp* (Flour beetle). Also the current treatment used to control insects population in the rice industry Dryacide™ has been shown to be highly effective against *Sitophilus spp* (Grain weevils) and but ineffective against *Tribolium spp.* (Flour beetle).

Further trials are required to test the efficacy of both products, although it appears that neither is highly effective in controlling all grain pests of stored rice. Therefore other alternative products should be tested in similar trials.

Appendix

Appendix 1 *Sitophilus sp.* results

	<i>After 24 hrs</i>		<i>After 7 days</i>	
	Alive	Dead	Alive	Dead
Absorba-cide 1	7	3	5	6
Absorba-cide 2	9	1	5	5
Dryacide 1	8	2	0	9
Dryacide 2	8	2	0	10
Control 1	9	1	7	3
Control 2	8	2	7	3

Appendix 2 *Tribolium sp.* results

	<i>After 24 hrs</i>		<i>After 7 days</i>	
	Alive	Dead	Alive	Dead
Absorba-cide 1	20	0	17	3
Absorba-cide 2	20	0	18	2
Dryacide 1	18	2	15	5
Dryacide 2	20	0	18	2
Control 1	17	3	16	4
Control 2	20	0	19	1

Trial Phase 2 – Slurry application of Dryacide and Absorbacide (increased numbers) (Flour beetle and Grain Weevil)

Past Observations

In light of the results obtained in the initial trial, further work is required to confirm or contest results obtained. In this experiment increased numbers will be used as a large population sample and the insects left a long period post their initial exposure to the relevant treatments.

Objectives

The aim of this trial is compare the effectiveness of Dryacide™ and Absorba-Cide™, and evaluate and observe the effectiveness of desiccant dusts in general, over a 14 day period.

Methodology

Materials

12 x Glass Petri dishes (90 mm diameter)

A good cleaning agent

Balance accurate to 0.001g

Weighing paper

Inert dust (to apply 6 gm⁻²)

Dryacide™ dust

Absorba-Cide™ dust

Insect jars (with filter paper lids)

Insect food (whole rice, white rice, rice flour and yeast)

Methods

Pre testing of glassware

Successful preparation of an even test deposit depends on Petri dishes being sufficiently clean to allow water (1.5 ml), pipetted into the dish, to spread evenly and to completely wet its surface. A pre test for glass Petri dishes is therefore advised.

Pipette water (1.5 ml) into the centre of a cleaned glass Petri dish. Rotate and tilt the dish in an attempt to spread the water evenly over the surface. Select only the dishes where the spread of water is even. Soak any dishes that fail this test in a suitable detergent for one hour, scour with a powder cleanser, rinse thoroughly with water then ethanol and dry before re-testing.

Application of slurry deposit

Weigh dust (0.054g) on weighing paper and transfer to the centre of four clean glass Petri dish. Add water (0.9mL). Rotate the Petri dish by hand to suspend the dust in the water and to disperse the dust evenly over the base and sides of the dish. A very fine artist's paintbrush or a gloved fingertip used to assist the dispersion of the deposit. Evaporate the water component of the slurry by placing the dish in an oven (~ 80°C) and rotate the dish approximately each 5 minutes until the water has evaporated. The four remaining clean petri dishes used as controls.

Sitophilus spp- Grain Weevil – Insect selection and survival

Twenty-five *Sitophilus spp.* (*Grain weevils*) were placed in each petri dish for 24 hours. Each petri dish was painted with Fluron™ around the outer edge to prevent insects escaping. Additionally, filter paper 150mm in diameter was placed between the base and lid to aid in the securing of the insects inside the petri dish.

The insect survival was assessed immediately after the set exposure period (24 hours). Insects are assessed as alive, if able to move normally and respond to stimuli, as dead, if unable to do either. Transferred insects to 40 grams of paddy rice white rice and yeast. (The neck of each jar is initially ringed with Fluron suspension). Store jars in a controlled environment. Further mortality counts carried out seven days after the end of the exposure period. At this stage, insects able to move or respond to stimuli are classified as live. Insects are normal or clearly dead after the seven-day holding period. Assess mortality against control mortality. Subject control insects to all treatments applied to the test insects except the dust application.

Calculation of survival rates calculated using Abbott's formula for corrected mortalities:

$$= \frac{\text{Proportion dead in TREATMENT} - (\text{Proportion dead in CONTROL})}{1 - (\text{Proportion dead in CONTROL})}$$

Tribolium spp.- Flour beetles- Insect selection and survival

Twenty-five *Tribolium spp.* (*Flour beetles*) were placed in each petri dish for 24 hours.

Insect survival was assessed immediately after the set exposure period (24 hours). Insects were assessed as live, if able to move normally and respond to stimuli, as dead, if unable to do either. Transfer insects to 20 grams of flour and yeast. Store jars in a controlled environment. Further mortality counts were conducted after seven days, after the end of the exposure period. At this stage, insects that were able to move or respond to stimuli are classified as live. Insects are normal or clearly dead after the

seven-day holding period. Assess mortality against control mortality. Subject control insects to all treatments applied to the test insects except the dust application.

Results

The average mortality's of *Sitophilus spp.* as calculated using Abbott's formula for corrected mortality's indicate that after a 7 day period 54.8% of the population were dead in the Absorba-cide treatment and 95.2% of the population were deceased in the Dryacide treatment. The number of dead were again counted after at 14 days after the initial exposure, the average mortality's of *Sitophilus spp.* was 70.7% in the Absorba-cide treatment and 97.6% of the population was deceased in the Dryacide treatment (Table 1).

Table 1 *Sitophilus spp.* (Grain weevils) mortality after 7 and 14 days

TREATMENT	MORTALITY after 7 days	MORTALITY after 14 days
<i>Absorba-cide</i>	54.8%	70.7%
<i>Dryacide</i>	95.2%	97.6%

The average mortality's of *Tribolium spp.*, as calculated using Abbott's formula for corrected mortality's indicate that after a seven day period 14.89% of the population were deceased in the Absorba-cide treatment and 31.91% of the population in the Dryacide treatment were deceased (Table 2).

The mortality for the *Tribolium spp.* population exposed to Absorba-cide after 14 days had a mortality of 9.1% and for the Dryacide treatment a mortality of 27.3% was recorded.

Table 2 *Tribolium spp.* (Flour Beetles) mortality after 7 and 14 days

TREATMENT	MORTALITY after 7 days	MORTALITY after 14 days
<i>Absorba-cide</i>	14.89%	9.1%
<i>Dryacide</i>	31.91%	27.3%

Discussion

Preparation

Both treatments dispersed evenly and dried within the 5 minutes in the oven at 80°C.

Treatment effectiveness

After 7 days the effectiveness of the two treatments (Absorbacide and Dryacide) for the two insect pests resembled those of the results obtained in the initial trial conducted, however in both cases the treatments appeared to be more effective, with 'higher average mortalities' being recorded. Dryacide was highly effective against *Sitophilus spp.* killing 95% of the population within seven days. The Absorba-cide was less effective however a modest 54.8% of the population had died within the seven-day period.

Both insecticide treatments proven significantly more effective against *Tribolium spp.* in this trial. After 7 days 14.89 percent of the population treated with Absorba-cide were dead and 31.91% of the population treated with Dryacide were dead. Although mortalities are higher than in Trial 1, the effectiveness of either treatment is not satisfactory for commercial use.

After a 14-day period the survival numbers were again calculated. The *Sitophilus spp.* treated with Dryacide killed 97.6% of the population. The Absorba-cide has proven again to be less effective killing in total 70.7% of the population.

The number of fatalities of *Tribolium spp.* did not increase from the number counted at day 7 to the number counted on day 14 (Appendix 2). However the number of casualties in the control did increase, therefore reducing the overall calculation of population mortalities. The overall and final mortality's of the *Tribolium spp.* was for Absorba-cide and Drya-cide was 9.1% and 27.3%, respectively. The overall effectiveness of both treatments is extremely low and not an effective means of controlling this insect species.

Implications and recommendations

In the repetition of this trial it was hoped to consolidate the results from the previous trial, this has been achieved. The comparison of Absorba-cide and Dryacide shows that the Dryacide treatment is highly effective in the control of *Sitophilus spp.* Absorba-cide was effective (71% kill) however is not considered to a long-term viable alternative to Dryacide, with the increased risk of resistance developing.

Both treatments failed to effectively control *Tribolium spp.* with the number of mortality's less than 30%. Neither treatment could be recommended for use in a commercial situation. Therefore it is recommended that further research be conducted into an alternate methods of testing the efficacy of these treatments against *Tribolium spp.*

Appendix

Appendix 1 Sitpolius sp. results

	After 24 hours		After 7 days		After 14 days	
	Alive	Dead	Alive	Dead	Alive	Dead
<i>Absorba-cide 1</i>	23	2	16	17	4	21
<i>Absorba-cide 2</i>	22	3	11	14	8	17
<i>Dryacide 1</i>	22	3	0	25	0	25
<i>Dryacide 2</i>	23	2	2	23	1	24
<i>Control 1</i>	23	2	21	4	20	5
<i>Control 2</i>	25	0	21	4	21	4

Appendix 2 Tribolium sp. results

	After 24 hours		After 7 days		After 14 days	
	Alive	Dead	Alive	Dead	Alive	Dead
<i>Absorba-cide 1</i>	24	1	22	3	22	3
<i>Absorba-cide 2</i>	24	1	18	7	18	7
<i>Dryacide 1</i>	25	0	20	5	20	5
<i>Dryacide 2</i>	25	0	12	13	12	13
<i>Control 1</i>	25	0	22	3	21	4
<i>Control 2</i>	25	0	25	0	23	2

Trial Phase 3 - Slurry application of Dryacide™ and Absorba-Cide™ on concrete (Flour Beetle)

Past Observations

Due to the less than satisfactory results for the slurry application of diatomaceous earths directly to petri dishes methodology to apply as a slurry on concrete surface was simulated using a similar surface to the interior walls of rice sheds and mills.

Objectives

The aim of this trial is compare the effectiveness of Dryacide™ and Absorba-Cide™, and evaluate and observe the effectiveness of desiccant dusts in general, over a 14 day period, using a slurry application on concrete surface to control Flour Beetle.

Methodology

Materials

6 x Glass Petri dishes (90 mm diameter)

A good cleaning agent

Balance accurate to 0.001g

Weighing paper

Inert dust (to apply 6 gm⁻²)

Dryacide™ dust

Absorba-Cide™ dust

Insect jars (with filter paper lids)

Insect food (whole rice, white rice, rice flour and yeast)

Methods

Concrete preparation

A 1cm layer of concrete spread evenly across 9cm diameter petri dishes and allowed to cure.

Application of slurry deposit

Weigh dust (0.054g) on weighing paper and transfer to the centre of four concrete filled Petri dishes. Add water (0.9mL). Rotate the Petri dish by hand to suspend the dust in the water and to disperse the dust evenly over the base and sides of the dish. A very fine artist's paintbrush or a gloved fingertip used to assist the dispersion of the deposit. Evaporate the water component of the slurry by placing the dish in an oven (~ 80°C) and rotate the dish approximately each 5 minutes until the water has evaporated. The four remaining petri dishes used as controls.

Tribolium spp.- Flour beetles- Insect selection and survival

Twenty-five *Tribolium spp.* (Flour beetles) were placed in each petri dish for 24 hours.

Insect survival was assessed immediately after the set exposure period (24 hours). Insects were assessed as live, if able to move normally and respond to stimuli, as dead, if unable to do either. Transfer insects to 20 grams of flour and yeast. Store jars in a controlled environment. Further mortality counts were conducted after seven days, after the end of the exposure period. At this stage, insects that were able to move or respond to stimuli are classified as live. Insects are normal or clearly dead after the seven-day holding period. Assess mortality against control mortality. Subject control insects to all treatments applied to the test insects except the dust application.

Calculation of survival rates calculated using Abbott's formula for corrected mortalities:

$$= \frac{\text{Proportion dead in TREATMENT} - (\text{Proportion dead in CONTROL})}{1 - (\text{Proportion dead in CONTROL})}$$

Results

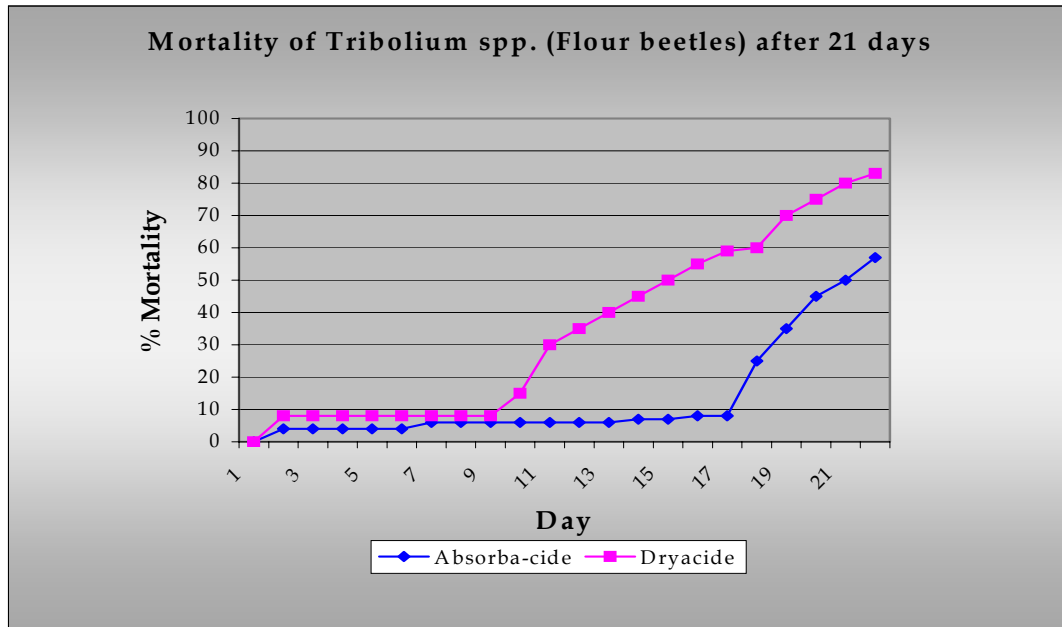


Figure 1- Mortality of *Tribolium spp* (Flour beetles) after 21 days

The average mortality's of *Tribolium spp.* as calculated using Abbott's formula for corrected mortality's indicate that after a 21 day period 57% of the population exposed to Absorba-cide died and 83% exposed to Dryacide died after the same time period. A rapid increase in the numbers of observed mortalities was seen for the Dryacide treatment day 10 and a similar rapid increase was observed for Absorba-cide after 18 days.

Discussion

Preparation

The application for the dusts to concrete was made difficult due to the abrasive nature of the surface and its ability to absorb water. The limited quality of water available to disperse the dust made the even application almost impossible.

Both treatments when dispersed dried within the 5 minutes in the oven at 80°C.

Treatment effectiveness

The results of this trial demonstrate that the application to concrete did not improve the efficacy of either Dryacide or Absorba-cide, resulting in a less than satisfactory kill of insects after 21 days. Comparatively the application for slurry mixtures directly to clean petri dishes proved more effective over a short time frame.

Implications and recommendations

In the repetition of this trial on a concrete surface was hoped to improve the overall efficacy of the trailed compounds. As with previous work the efficacy of the products proved to be less than satisfactory with only 57% and 83% of populations being controlled by the application of Absorba-cide and Dryacide, respectively.

Therefore it is recommended that further research be conducted into an alternate method of testing the efficacy of these treatments against *Tribolium spp.*

Trial Phase 4 - Dust application of Dryacide and Absorbacide (Grain Weevils)

Past Observations

Due to the less than satisfactory results for the slurry application of diatomaceous earths directly to petri dishes alternate bioassay methods have been sought. In light of discussion and attending the NWP Conference in Melbourne the application of the diatomaceous earths as a dust has been demonstrated to deliver improved efficacy.

Objectives

The aim of this trial is compare the effectiveness of Dryacide™ and Absorba-Cide™, and evaluate and observe the effectiveness of desiccant dusts in general, over a 14 day period, using a dust application directly to the glass surface of the petri dish for the control of Grain Weevils.

Methodology

Materials

12 x Glass Petri dishes (90 mm diameter)

Parafilm

Balance accurate to 0.001g

Weighing paper

Inert dust (to apply 6 gm⁻²)

Dryacide™ dust

Absorba-Cide™ dust

Insect jars (with filter paper lids)

Insect food (whole rice, white rice, rice flour and yeast)

Methods

Pre testing of glassware

Successful preparation of an even test deposit depends on Petri dishes being sufficiently clean to allow water (1.5 mL), pipetted into the dish, to spread evenly and to completely wet its surface. A pre test for glass Petri dishes is therefore advised.

Pipette water (1.5 mL) into the centre of a cleaned glass Petri dish. Rotate and tilt the dish in an attempt to spread the water evenly over the surface. Select only the dishes where the spread of water is even. Soak any dishes that fail this test in a suitable detergent for one hour, scour with a powder cleanser, rinse thoroughly with water then ethanol and dry before re-testing.

Application of dust deposit

Weigh dust (0.014g) on weighing paper and transfer to either a top or bottom of the petri dish. Place another top onto a top and a bottom onto a bottom then join with parafilm strips. Rotate and tap the deposit to distribute it between the two dishes then hold the joined dishes high and give a few quick sharp shakes. This creates static electricity and sticks the dust particles to the surfaces. Examine the dishes to see if the deposit is distributed evenly between the two dishes. If the deposit is unequal, a little tap and another shake corrects the distribution. Remove parafilm and separate

dishes.the centre of four clean glass Petri dish. The four remaining clean petri dishes used as controls.

Sitophilus spp- Grain Weevil – Insect selection and survival

Twenty *Sitophilus spp.* (Grain weevils) were placed in each petri dish for 24 hours. Each petri dish was painted with Fluron™ around the outer edge to prevent insects escaping. Additionally, filter paper 150mm in diameter was placed between the base and lid to aid in the securing of the insects inside the petri dish.

The insect survival was assessed immediately after the set exposure period (24 hours). Insects are assessed as alive, if able to move normally and respond to stimuli, as dead, if unable to do either. Transferred insects to 40 grams of paddy rice white rice and yeast. (The neck of each jar is initially ringed with Fluron suspension). Store jars in a controlled environment. Further mortality counts carried out seven days after the end of the exposure period. At this stage, insects able to move or respond to stimuli are classified as live. Insects are normal or clearly dead after the seven-day holding period. Assess mortality against control mortality. Subject control insects to all treatments applied to the test insects except the dust application.

Calculation of survival rates calculated using Abbott’s formula for corrected mortalities:

$$= \frac{\text{Proportion dead in TREATMENT} - (\text{Proportion dead in CONTROL})}{1 - (\text{Proportion dead in CONTROL})}$$

Results

The average mortality’s of *Sitophilus spp.* as calculated using Abbott’s formula for corrected mortality’s indicate that after a 5 day period all insects had expired (Figure 1).

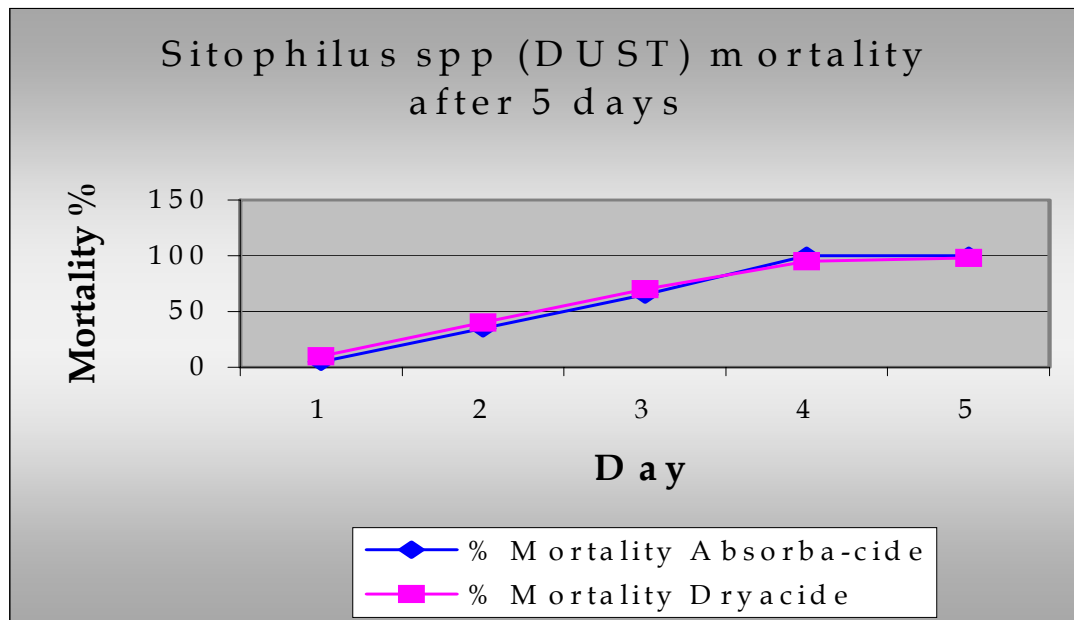


Figure 1 *Sitophilus spp.* (Grain weevils) mortality after 5 days
The efficacy of Absorba-cide was slightly high, with 100% mortality recorded after 4 days.

Discussion

Preparation

The distribution of both treatments (Dryacide and Absorbacide) proved difficult to distribute evenly across the two petri dishes, however with persistence even distribution was achieved.

Treatment effectiveness

Sitophilus spp (Grain Weevil)

After 4 and 5 days respectively the Absorba-cide and Dryacide treatments achieved 100% mortality. Compared to efficacy of slurry applications a mortality rate of 97% was observed for Dryacide applications and 71% for Absorba-cide after a 14 day period. This increased efficacy of the diatomaceous earths applied as dust applications demonstrates the abrasive nature of these compounds. Although Allen (2000) suggests that the efficacy of slurry applications is superior to dust, in a number of replicated experiments.

Implications and recommendations

The use of dust application of the treatments of Dryacide and Absorbacide for the control of *Sitophilus spp* (Flour Beetle) proved equally as efficient with 100% of both populations succumbing to the treatment in less than five days. Also in comparison to the mortality rate of using slurry application (Exp. 1), the dust application appears to be significantly more effective, with complete mortality in slurry not occurring after 14 days. However the practical application of either Dryacide or Absorbacide as a dust could pose potential Occupational, Health and Safety issues.

Trial Phase 5 - Dust application of Dryacide and Absorbacide, continuous exposure. (Flour Beetle)

Past Observations

Due to slower than anticipated mortality of *Tribolium spp* (Flour Beetle) it was thought that the length of exposure should be increased in order to replicate the potential exposure in a commercial situation and to determine if anyway resistance to the treatments may have developed.

Objectives

The aim of this trial is to compare the effectiveness of Dryacide™ and Absorba-Cide™, and evaluate and observe the effectiveness of desiccant dusts in general, over a 14 day period, with constant exposure to the treatments within the petri dish.

Methodology

Materials

6 x Glass Petri dishes (90 mm diameter)

Parafilm

Balance accurate to 0.001g

Weighing paper

Inert dust (to apply 6 gm⁻²)

Dryacide™ dust

Absorba-Cide™ dust

Methods

Pre testing of glassware

Successful preparation of an even test deposit depends on Petri dishes being sufficiently clean to allow water (1.5 mL), pipetted into the dish, to spread evenly and to completely wet its surface. A pre test for glass Petri dishes is therefore advised.

Pipette water (1.5 mL) into the centre of a cleaned glass Petri dish. Rotate and tilt the dish in an attempt to spread the water evenly over the surface. Select only the dishes where the spread of water is even. Soak any dishes that fail this test in a suitable detergent for one hour, scour with a powder cleanser, rinse thoroughly with water then ethanol and dry before re-testing.

Application of dust deposit

Weigh dust (0.014g) on weighing paper and transfer to either a top or bottom of the petri dish. Place another top onto a top and a bottom onto a bottom than join with parafilm strips. Rotate and tap the deposit to distribute it between the two dishes then hold the joined dishes high and give a few quick sharp shakes. This creates static electricity and sticks the dust particles to the surfaces. Examine the dishes to see if the deposit is distributed evenly between the two dishes. If the deposit is unequal, a little tap and another shake corrects the distribution. Remove parafilm and separate dishes. The four remaining clean petri dishes used as controls.

Tribolium spp.- Flour beetles- Insect selection and survival

Twenty *Tribolium spp.* (*Flour beetles*) were placed in each petri dishes. Insect survival was assessed immediately after the set exposure period (24 hours). Insects were assessed as live, if able to move normally and respond to stimuli, as dead, if unable to do either. Further mortality counts were conducted every until complete mortality was achieved.

Results

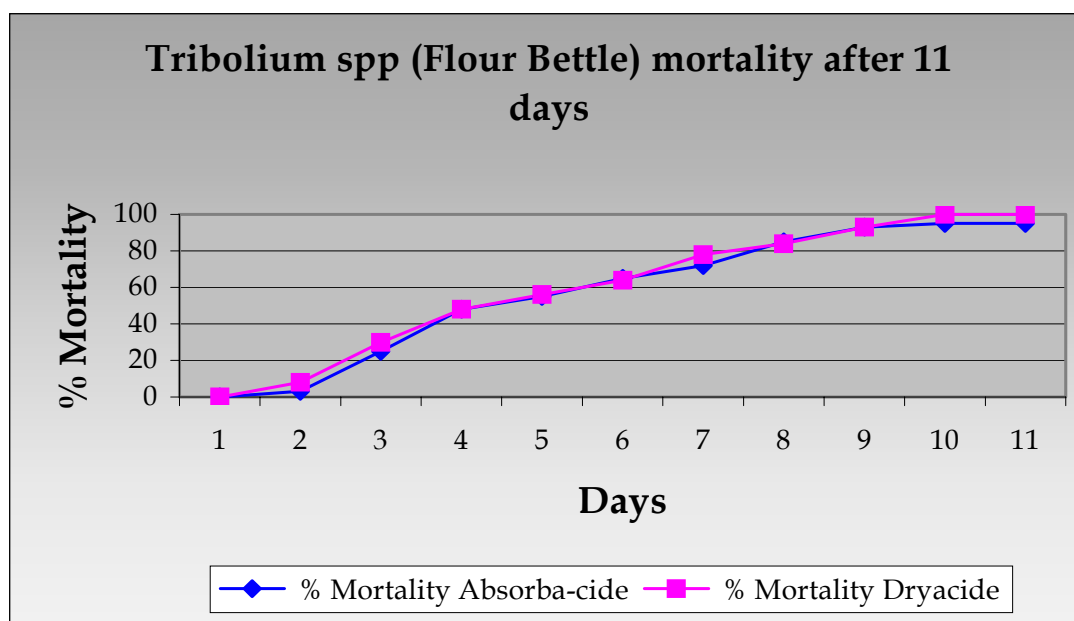


Figure 1 *Tribolium spp.* (*Flour Beetles*) mortality after 11 days

After 10 days 100% mortality was achieved in the Dryacide and treatment and 95% of the population exposed to Absorba-Cide died after 10 days (Figure 1). Both treatments showed similar rates of mortality over the observation period, however the Absorba-Cide treatment failed to kill all insects.

Discussion

Preparation

Both treatments proved difficult to distribute evenly across the two petri dishes, however with persistence even distribution was achieved.

Treatment effectiveness

Tribolium spp (Flour Beetle)

This trial demonstrated that with continuous exposure to either Dryacide or Absorbacide that above 95% mortality can be achieved in Flour Beetles. The Dryacide treatment proved completely effective after 10 days, with 100% mortality. These results confirming earlier work that shows that Dryacide is more effective than Absorbacide. Also this trial demonstrates that the constant exposure of the insect population to these treatments in the dust form is more effective and a limited 24-hour exposure. Therefore highlighting the need to increase the time between the application of the treatment and in-loading grain.

Implications and recommendations

This trial demonstrates the increased exposure time equates to increased mortality. One hundred percentage can be achieved after 10 days using the Dryacide treatment. Again demonstrating need for increased time between application of structural treatment and in loading of grain.

This experiment in conjunction with all other trials in this series demonstrates the inferior efficacy of the structural treatment Absorbacide.

Trial Phase 6 - Slurry application of Dryacide and Absorbacide (continuous exposure) (Lesser Grain Borer)

Past Observations

Rhyzopertha dominica (Lesser grain borer) until recently has been a minor insect pest of the rice industry, therefore little recent research has been conducted into the effectiveness of current chemical and no chemical treatments in controlling these insect pests.

Other bulk handlers of grain have also expressed problems in controlling *Rhyzopertha dominica* due to resistance issues.

Objectives

Due to limited knowledge about the effectiveness of Dryacide on *Rhyzopertha dominica* (Lesser Grain Borer) a trial was conducted to gauge it's effectiveness as a structural treatment controlling these insects.

Methodology

Materials

4 x Glass Petri dishes (90 mm diameter)

Parafilm

Balance accurate to 0.001g

Weighing paper

Inert dust (to apply 6 gm⁻²)

Dryacide™ dust

Methods

Pre testing of glassware

Successful preparation of an even test deposit depends on Petri dishes being sufficiently clean to allow water (1.5 mL), pipetted into the dish, to spread evenly and to completely wet its surface. A pre test for glass Petri dishes is therefore advised.

Pipette water (1.5 mL) into the centre of a cleaned glass Petri dish. Rotate and tilt the dish in an attempt to spread the water evenly over the surface. Select only the dishes where the spread of water is even. Soak any dishes that fail this test in a suitable detergent for one hour, scour with a powder cleanser, rinse thoroughly with water then ethanol and dry before re-testing.

Application of dust deposit

Weigh dust (0.014g) on weighing paper and transfer to either a top or bottom of the petri dish. Place another top onto a top and a bottom onto a bottom then join with parafilm strips. Rotate and tap the deposit to distribute it between the two dishes then hold the joined dishes high and give a few quick sharp shakes. This creates static electricity and sticks the dust particles to the surfaces. Examine the dishes to see if the deposit is distributed evenly between the two dishes. If the deposit is unequal, a little tap and another shake corrects the distribution. Remove parafilm and separate dishes. The four remaining clean petri dishes used as controls.

Rhyzopertha dominica.- Lesser Grain Borer- Insect selection and survival

Twenty *Rhyzopertha dominica*. (Lesser Grain Borers) were placed in each petri dishes.

Insect survival was assessed immediately after the set exposure period (24 hours). Insects were assessed as live, if able to move normally and respond to stimuli, as dead, if unable to do either. Further mortality counts were conducted every until complete mortality was achieved.

Results

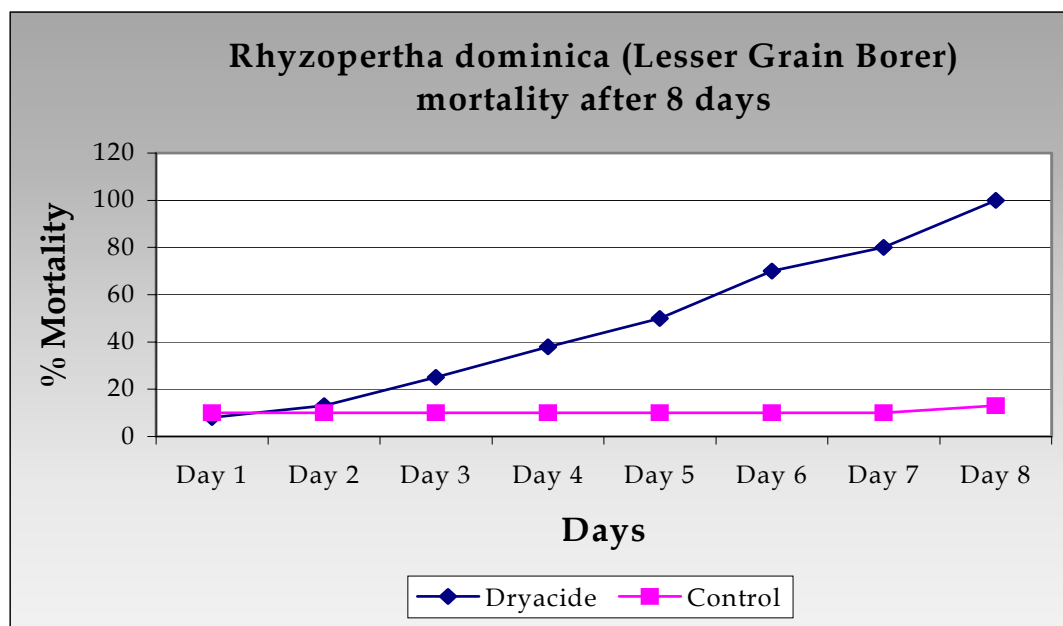


Figure 1 *Rhyzopertha dominica* mortality after 8 days

One hundred percent mortality was achieved after 8 days exposure to the Dryacide treatment. In that time the control treatment 13% of the population died.

Discussion

Treatment effectiveness

The Dryacide treatment effectively controlled 100% of the *Rhyzopertha dominica* population. This demonstrates that Dryacide is effective in controlling Lesser Grain Borer and potentially the rice industry does not yet have the same level of resistance report to this product in other grains industries in the region.

Collectively in this series of experiments it has been shown that an increased time between application and in-loading of grain is necessary to gain control of all insect problems in *Rhyzopertha dominica*.

Implications and recommendations

From this trial it can be seen that the Rice Industry currently can successfully use Dryacide to control *Rhyzopertha dominica*. In this trial 100% control of the population was achieved in 8 days of constant exposure this is potentially a shorter exposure period than required for other major insect problems such as Flour Beetle and Grain Weevil.

Project Intellectual Property

N/A

References

N/A

Acknowledgements

Mrs. Tania Bauer
The Paddy Projects Team

II Sub-Project Name: Impact of storage at -20°C on rice properties (1999-2000)

Background

Losses resulting from insect infestations are widespread and involve more than loss of quality. Insect infestation also causes a reduction of nutrients in the grain. Controlling of insects with insecticides/pesticides rather than using preventive methods incurs great cost. In addition, infestations result in dissatisfied customers and related market problems that develop a poor reputation in marketing channels.

Rice markets are becoming increasingly discerning so there is a need to develop alternative means to avoid use of post-harvest chemicals. The most prevalent means for disinfecting insects from stored grain relies on chemical fumigants such as phosphine and methyl bromide. However, the range of fumigants currently in use is decreasing as these are removed from-permitted lists due to health considerations. In addition, there is increasing consumer resistance to the use of pesticides in general due to perceived problems related to health and wellbeing.

One alternative to pesticides for disinfection of stored grain is the use of inert dusts. However, there are problems with these, including their overall effectiveness, effects on flow properties and bulk density of grain, and subsequent removal of the inert dust for sale and further processing of the grain. This is likely to be a particular problem for white rice. The use of low temperature storage avoids the problem of the use of pesticides and consequently the grain would be free of chemical residues. This would enable disinfested rice to be produced, which could be fairly and accurately described as residue free or any similar terms for domestic and export marketing.

No previous study has been done to examine the effect of low temperature on rice properties. The present study was planned to avoid use of chemicals during storage and assessing the effect of low temperature storage on quality characteristics of rice.

Objective

To determine the impact of storage at -20°C on rice properties

Methodology

One tonne bulkers of paddy, brown and white rice along with 1kg retail packs of white rice were stored at -20°C for a period of 5 weeks. Over the course of storage, temperature was measured continuously.

After five weeks had elapsed, samples of each product were taken and evaluated for milling yield, cooking behaviour and RVA pasting properties. Also measured were in vitro starch digestibility and crystallinity by X-ray diffraction. These results were compared with those from control samples stored under ambient conditions.

Results

It was found that storage at -20°C had very little impact on most of the rice properties examined.

Implications and recommendations

Low temperature storage at -20°C does not affect most of the rice properties such as milling yields, quality parameters, bulk density, water absorption, cooking time, pH and soluble solids significantly. However, during the trial it took three weeks to establish -20°C in 1 tonne bulkers. It is evident from the study that low temperature technique is very useful and gives us further indication that control of insects can be tried by exposing them at low temperature without affecting quality of grains.

This technique will avoid the problems associated with pesticides and consequently grain will be free of chemical residues. It will enable disinfested rice to be produced which could fairly and accurately be described as residue free or any similar term for both domestic and export marketing.

Construction of facilities capable of storing all rice harvested and processed in a year would be a major cost and something that would require planning and assessing over the coming years. Additionally, the extended periods of time taken to reach -20°C in bulker bags, suggests this method was not economically viable.

Project Intellectual Property

No IP

References

N/A

Acknowledgements

Ms Robyn Delves - Ricegrowers' Co-operative Limited, Leeton
Prof Michael Wootton - University of New South Wales, Sydney

III Sub-Project Name: Fumigation with ethyl formate and carbonyl sulphide (1999-2000)

Background

Due to the phase-out of methyl bromide for all but pre-shipment and quarantine use it has been necessary to develop alternative fumigants for the disinfestation of durable food commodities. Carbonyl sulphide (COS) and ethyl formate are two alternatives under development at the Stored Grain Research Laboratory (SGRL) at CSIRO Entomology. Both these materials have been through a research path aimed at defining the condition for their use and their efficacy against some insect pests of stored products.

Neither carbonyl sulphide nor ethyl formate has been systematically tested on rice and rice products. The research undertaken is the first stage of assessing the applicability of the alternative fumigants to these products, hopefully opening a pathway for effective fumigation using a material other than methyl bromide.

Objective

The work undertaken was designed to investigate the following three major areas:-

1. Sorption of COS and ethyl formate on the commodity;
2. Airing-off of fumigant-rate and completeness; and
3. Detrimental effects on product quality.

Methodology

Metal tins (~20L) were filled with 5kg of rice and sealed with silicone. Pressure tests were performed on each tin and a leak detector fitted. Fumigations were carried out at 10°C and 20°C.

After fumigation, samples were split and analysed by SunRice and SGRL.

Results

Milled white and brown rice

The results obtained from SGRL laboratory exposures of rice products indicate that there are unlikely to be any problems obtaining conditions that will control insects in milled rice (white or brown) at 25°C with either ethyl formate or carbonyl sulphide. In this domain (i.e. milled product 25°C), and on the basis of results obtained, operational requirements, in terms of application and ventilation, should be similar to those developed for cereal grains.

Paddy and rice flour

Paddy and rice flour were more sorptive of both fumigants than expected. This means that the concentrations reached after application was lower than expected and the level of insect control would be much more doubtful.

Implications and recommendations

Milled white and brown rice

The interpretation of the 10°C results is more equivocal as it is outside the domain of most previous insect toxicology studies. On the observed results it is likely that carbonyl sulphide will be effective on milled rice (brown and white) at this temperature but studies elsewhere have shown that a longer exposure period than

those tested may be necessary. There are very few studies on the toxicity of ethyl formate at low temperatures, those that exist suggest its toxicity is not greatly affected but very considerable work is needed before a definitive answer can be given for all species likely to be encountered.

A next step in proving the fumigants and progressing towards a future registration would be small-scale field application on milled product under ambient conditions, assuming problems are not revealed during quality assessment.

Paddy and rice flour

Higher dose rates are likely to remedy this but are more likely to have an adverse impact on quality. Further exposures and quality testing would be needed to ensure this does not occur before field trials could be attempted.

Since this trial carbonyl sulphide has failed as a potential replacement for MeBr. Ethyl formate has remained a possibility and further studies have continued.

Project Intellectual Property

No IP

References

Annis, P. and Reuss, R., 2001. Fumigation of rice products with the potential methyl bromide replacements, ethyl formate and carbonyl sulphide. A contracted report to Ricegrowers' Co-operative Limited prepared by CSIRO, Entomology.

Acknowledgements

Mr Rainer Reuss – CSIRO

Mr Peter Annis – CSIRO

Mrs Robyn Delves - SunRice

IV Sub-Project Name: Treatment of rice products with carbon dioxide applied to shipping containers as dry ice for the purpose of insect control (2000-2001)

Background

The trial was designed to re-investigate the use of shipping containers as fumigation enclosures, primarily for carbon dioxide treatment but also for other gaseous treatments. The current investigation was designed as an investigation into the possibility of using this type of fumigation in a routine large-scale industrial operation to replace methyl bromide fumigation for final disinfestation of rice products.

Objective

- To explore the gas holding of "normal" containers used by Ricegrowers' for shipping;
- To investigate the modifications required to dry-ice applications needed to match carbon dioxide treatment requirements to the actual shipping container leakage;
- To evaluate the gas-tightness of shipping containers for fumigation with other gases

Methodology

Containers and commodity

Four containers that were part of a normal consignment of containers received at Ricegrowers' Deniliquin Mill were used in the experiment. All four containers had plywood floors, were of various ages, and on visual inspection were in fair to very poor condition (Table 2). Each container was loaded with 9-10 tonnes of white rice placed on ten wooden palettes.

Temperature and relative humidity (rh) measurement

Table 2 -Containers used for carbon dioxide treatment

Number	Condition	Age of container	Bag type	Data Logger	Dry ice added
1	Fair	7 month	Bulk	-	75kg
2	Fair	9 month	Bulk	-	75kg
3	Poor	>10 month	20kg	-	110kg
4	Very Poor	12 month	20kg	-	95kg

Application of dry ice to rice products

Dry ice used was in the form of 5 kg blocks. For each container, eight blocks of dry ice (40 kg) were insulated with paper and cardboard to slow the release of CO₂ gas and placed on top of the bags inside the containers. Immediately following addition of dry ice containers were sealed. Containers were treated on 4 April, 2001 and remained sealed until the 14 April, 2001, ten days in total.

Carbon dioxide measurements

CO₂ concentrations were measured with a Gow-Mac Instruments portable GC-TCD (Gas chromatograph with thermal conductivity detector) fitted with a sample pump. Samples were drawn through 1/4 inch nylon sample lines that had been passed through the door seals of the containers. Measurements were carried out several times a day over the 10-day exposure period.

Results

Table 3 -Results from the 10-day CO₂ container fumigation.

Number	Average Temp (°C)	Ave CO ₂ (%)	CO ₂ (%)	Insect Control
1	9.5	35	10	X
2	19.9	30	14	X
3	-	26	12	X
4	19.4	45	10	X

Implications and recommendations

In-transit CO₂ treatments cannot be seen as a reliable method for end point disinfestation of rice unless an acceptable and reliable process of container selection for gas-tightness can be devised. The logistics are such that this selection has to take place prior to the empty containers being shipped to up-country rice mills.

Shipping containers are unlikely to be gas tight after a short period of time due to the nature of their handling.

Project Intellectual Property

No IP

References

Annis, P. and Reuss, R., 2001. A Treatment of rice products in shipping containers with carbon dioxide applied as dry ice for the control of insects. A contracted report to Ricegrowers' Co-operative Limited prepared by CSIRO, Entomology.

Acknowledgements

Mr Daryl Hill - SunRice

V Sub-Project Name: A study of Phosphine sorption by paddy rice (2001-2002)

Background

Paddy rice, due to its structure and chemical properties, is highly sorptive of phosphine. In order to determine effective dosage rates, fumigation design and employee safety, the sorption profile of paddy rice was determined.

The current label rate in Australia is 1.5g of phosphine per m³. Maintaining a concentration of 100ppm in a fumigation vessel for a minimum of 14 days is expected to result in a successful fumigation.

Objective

This study aimed to determine sorption profile (% gas loss per day) of both pure gas and self-generating pellet phosphine formulations in medium grain paddy.

Methodology

1. A small-scale experiment was undertaken to quantify the loss of phosphine gas through sorption onto paddy
2. On a larger scale, experiments were undertaken to observe the interaction between sorption of phosphine by paddy and generation of phosphine from aluminium phosphide

Gaseous phosphine

Fumigations were conducted in glass desiccators of 2.5-2.7L. The desiccator lids had glass stoppers fitted with a septum.

Two paddy samples were split to determine implications of fill rate and temperature on end result.

Table 1 –Samples treated with phosphine

Sample	ID	Fill Rate (%)	Temperature (°C)	Dosage Rate (g/m ³)
1	A	40	15	1.5
2	B	90	15	1.5
3	C	40	25	1.5
4	D	90	25	1.5

Aluminium Phosphide

Large-scale laboratory fumigation was conducted in 63.5L drum with an internal diameter of 380mm and a height of 570mm. Each was fitted with a septum fitting above the bottom rim and centrally in the removable lid. Lids were sealed with gas tight seals and pressure tested successfully.

Drums were filled to 90% capacity with paddy rice and a weighing tray containing aluminium phosphide tablets placed on the surface.

Phosphine standards

Gas analysis was conducted by the generation of gas using aluminium phosphide tablets and aqueous sulphuric acid. Determination of gas was performed using a Gow Mac gas density balance on a Tracor gas chromatograph fitted with a Poropak Q80-100 column.

Phosphine concentrations during the experiments were measured and determined by comparison with prepared phosphine standards.

Results

Gaseous phosphine

Temperature and fill rate both affected the loss of phosphine from the headspace. The rate of phosphine loss from the headspace was higher with the higher fill rate and the higher temperature.

This suggests that a vessel filled to 90% with paddy rice at a rate of 1.5g/m^3 and at 25°C , would require topping up to a calculated 500ppm approximately every 6 days to maintain a concentration above 100ppm. If the temperature were reduced to 15°C , the time between topping-up would be extended to approximately 10 days.

Figure 1: Gaye Weller of CSIRO Entomology performing the small-scale fumigations using gas chromatograph to determine phosphine concentration in glass desiccators



Aluminium phosphide

The recommended label dose for phosphine fumigation to target tolerant species such as *Sitophilus oryzae* in the temperature range $15\text{-}20^\circ\text{C}$ is for a minimum exposure period of 14 days maintaining a concentration greater than 100 ppm.

The application rate of 3.0g/m^3 maintained a concentration of phosphine in the headspace above 200ppm for 13 days. However, the application rate of 1.5g/m^3 maintained a concentration above 200 ppm for only six days and was less than 35ppm after 12 days. This would necessitate a top-up with gaseous phosphine to meet the requirements for a successful fumigation.

These results are similar to the small-scale results.

Implications and recommendations

The results from these studies agree with previous findings that paddy rice is highly sorptive of phosphine gas and that the rate at which it is sorbed is dependent on temperature, fill rate and whether the paddy has been fumigated with phosphine previously.

This study suggested that an actual application method is available to ensure the effective fumigation of bulk paddy. An application of phosphine blankets at 1.5g/m^3 will maintain the concentration of phosphine above 100ppm for eight days, after which a top up with gaseous phosphine will be required to maintain the concentration above 100ppm for the required 14-day exposure period. If the label rate is increased to 3.0g/m^3 , an initial application of aluminium phosphide preparation would ensure that the 14 days above 100ppm was achieved.

Project Intellectual Property

Weller, G., 2002. A Study of Phosphine Sorption by Paddy Rice. A contracted report to Ricegrowers' Co-operative Limited prepared by CSIRO, Entomology.

References

N/A

Acknowledgements

Ms Gaye Weller – CSIRO Entomology

Mr Daryl Hill - SunRice

VI Sub-Project Name: Phosphine Fumigation of Paddy Rice, Shed 10, Emery(2003-2004)

Background

SunRice has erected two sealed storages on both Deniliquin and Emery sites. These storages are both 12 sided and of 20,000T capacity.

Due to the limitations of harvesting rice between February and June and using this over a 12-18 month period, quality of grain can be difficult to maintain. The intention of such storages is to hold grain for extended periods under fumigation to ensure quality is maintained and improved through limiting or ceasing insect damage and associated quality reduction such as stackburn.

This trial was conducted by Dr Joanne Holloway and Kathryn Smith of NSW Agriculture, Wagga Wagga, NSW.



Figure 1: 12-sided shed at Emery Site

Methodology

The Roundhouse at Emery, a 12-sided shed of 20,000 t capacity, was surveyed prior to filling with last season's paddy rice. Evidence of rice weevil (*Sitophilus oryzae*) and lesser grain borer (*Rhyzopertha dominica*) were found in some old grain that had not yet been cleared away. SunRice followed their normal procedure of cleaning out and spraying the shed with a surface protectant treatment prior to inloading the paddy rice.

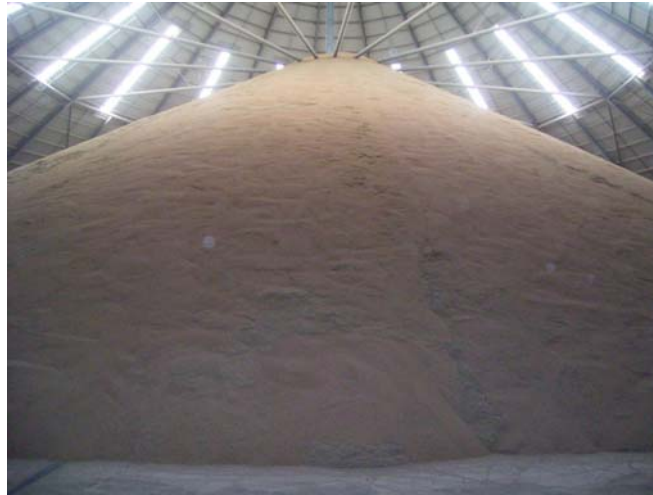


Figure 2: Shed 10 – Emery filled with 7000T of paddy rice

A bulk of 7,000 t was inloaded with each load of paddy rice sieved, to check for any natural infestation of insects, but none were found. A phosphine concentration of 100 ppm for at least 20 days was recommended to ensure a successful fumigation. William Shore, from GasApps Australia Pty Ltd, was in charge of calculating and filling the shed with the required amount of phosphine. To allow for sorption of the gas into the rice and any losses due to small leakages within the shed, approximately double the required concentration of phosphine was pumped into the shed.



Figure 3: (from L to R) Steve Hussey (SunRice), Darren Poole (SunRice) and William Shore (GasApps) sealing door and setting up phosphine introduction equipment

Due to a leak in the shed there was a drop in phosphine concentration during the fumigation period, and the shed had to be re-gassed 15 days into the fumigation.

Phosphine Monitoring

Prior to the inloading of paddy rice, 12 lines for monitoring phosphine fumigation were set up by NSW Ag technical staff and another 22 were set-up inside the bulk by SunRice. A further 16 original monitoring valves were located at regular intervals in the wall of the shed for monitoring gas concentrations around the perimeter. For the first 24 hours after gas filling was completed, NSW Agriculture staff monitored phosphine concentrations through the NSW Ag tubes every 2 hours. In addition, 6 hourly concentration readings were also taken from the 16 perimeter monitoring points. Thereafter, staff from SunRice monitored concentrations once a day from their 38 monitoring points (22 through the bulk and 16 around the edge of the shed).



Figure 4: William Shore (GasApps Australia) and Darren Poole (SunRice) measuring gas concentrations

Insect Bioassays



Figure 5: Dr Jo Holloway and Kathryn Smith of NSW Agriculture inserting bioassays into grain bulk

One weak and one strong resistant strain of lesser grain borer, *Rhyzopertha dominica* (SR Rd; WR Rd), and a strong resistant strain of rice weevil, *Sitophilus oryzae* (SR So), were set up to provide 21 cultures (6 experimental and 1 control for each strain) containing all stages (eggs, larvae, pupae and adults) of the insects. For each culture, either 100 adult *R. dominica* or 50 adult *S. oryzae* were placed in 100 g of moisturised grain and maintained at 25 °C and 60 % rh.

Prior to the fumigation, the experimental cultures were transferred to insect-proof assay probes and positioned in the bulk. A further series of 8 assay probes were placed around the circumference of the bulk.

In addition to the assay probes, 2 temperature dataloggers were also placed within the paddy rice bulk. These were located next to the SR Rd at 6 m (location 4) and the WR Rd also a 6 m but on the opposite side of the bulk (location 14). These were also retrieved after the shed was vented and all information downloaded. Unfortunately, due to battery failure, no information could be retrieved from these loggers and, consequently, no temperature data were obtained for this trial.

Results:

Phosphine concentrations

Concentrations of phosphine within the bulk reached between 200-250 ppm 2-3 days after gas filling (Fig. 1). Phosphine concentrations then steadily declined until day 15, when concentrations at all monitoring points dropped below 100 ppm. This was when a leak was discovered by SunRice staff and repaired. At this time gas concentrations within the bulk were between 70-85 ppm. The shed was then topped up with approximately 100 ppm of phosphine in order for the bulk to achieve the recommended concentration of at least 100 ppm for 20 days. Phosphine concentrations again declined and were between 70-100 ppm when the shed was vented 24 days after the fumigation commenced. While the phosphine penetrated throughout the bulk, concentrations were generally lower at the base.

A “successful” fumigation is a cumulative CT product (concentration x time) of 100 g.h/m³. This was achieved prior to the shed being vented at all except 2 of the monitoring points. Both of these points were located at the base and close to the centre of the bulk. Of the other monitoring points, the cumulative CT product of 100 g.h/m³ was not reached until day 22 of the fumigation at 5 locations.

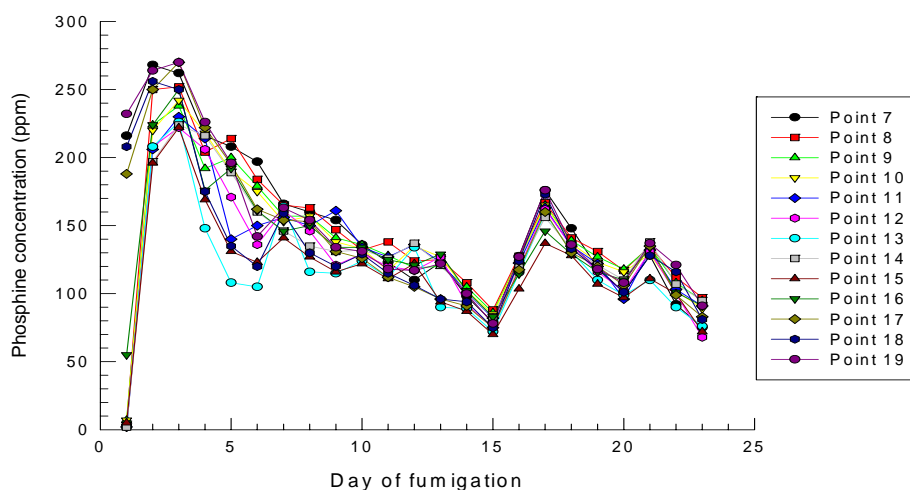


Figure 6: Phosphine concentrations at 13 monitoring points located throughout a 7,000 t bulk of paddy rice during a trial fumigation at the Roundhouse, Emery

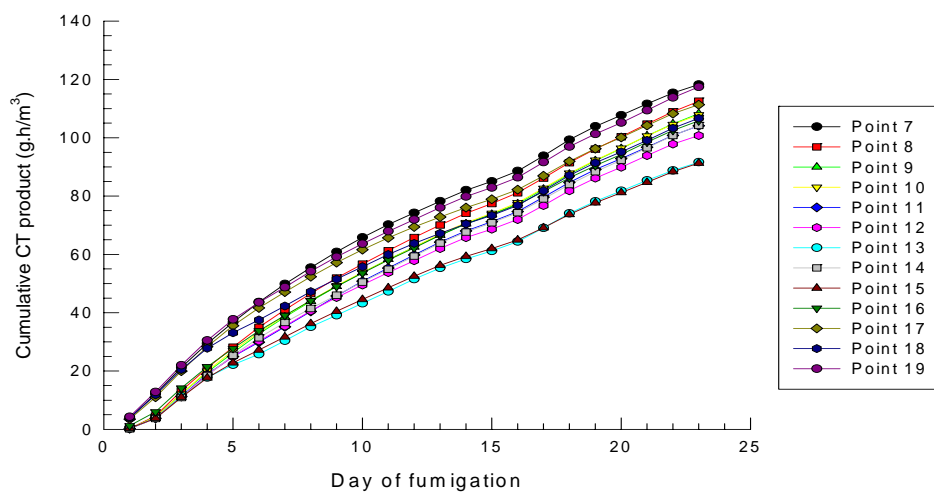


Figure 7: Cumulative CT product of phosphine at 13 monitoring points located throughout a 7,000 t bulk of paddy rice during a trial fumigation at the Roundhouse, Emery

Insect Bioassays

Once retrieved, the probes were taken back to the laboratory, the grain sieved and adult insects counted. No live insects of any of the three strains were found at this stage. The grain was then incubated for 8 weeks to see if any eggs or pupae survived the fumigation and developed into adults. No rice weevil, *S. oryzae*, emerged from these cultures and, therefore, the fumigation was successful in controlling this species. However, the lesser grain borer, *R. dominica*, was not completely controlled, with adults emerging from the grain cultures in both the weak and strong resistant strains (Table 1). Perhaps surprisingly, eggs and/or pupae survived in 4 of the 6 probes containing the weak resistant strain, compared with only 1 of the 6 probes with the strong resistant strain. This anomaly may be explained by a number of reasons, including mislabelling the strains, but was probably due to the lack of robustness of the strong resistant strain. Despite this, the fact that some *R. dominica*, both strong and weak resistant, survived indicates that the concentration of phosphine was not held at a high enough concentration for a long enough period in order to kill all life stages, and that the fumigation failed to control this species.

Implications and recommendations:

While some locations within the paddy rice bulk achieved the recommended phosphine concentration of 100 ppm for 20 days, a number of points failed to attain this dose. Consequently, eggs and/or pupae of resistant *R. dominica* strains survived within the bioassay probes and the fumigation can be deemed to have failed.

There are three possible reasons for this failure. The first, and most likely, is structural leakage. During this trial extreme temperature changes were experienced. Temperatures altered daily from an average minimum of 6.1°C to an average maximum of 43.9°C. These changes commonly were experienced over a 24 hour period which most likely caused the shed to expand and contract, creating breaks in the seal. The shed was seal tested twice immediately prior to this trial and both results were successful.

Another reason for the decline in phosphine concentration could have been sorption in the paddy rice.

Finally, aeration within the shed may have been insufficient to produce a uniform concentration of phosphine throughout the bulk.

In conclusion, the fumigation failed because the phosphine concentration was not held high enough for a long enough period of time. The primary reason for this appears to be leaks within the shed. It is unknown to what extent sorption by paddy rice and aeration may have contributed to the failure, if at all, as the leakage would probably have masked their effects.

Note: This trial is expected to be repeated in March/April 2006 pending availability of paddy rice.

Project Intellectual Property No IP

References

Report: Paddy Rice Fumigation, The Roundhouse, Emery; February 2004 – NSW Agriculture Wagga Wagga 11 June 2004

Acknowledgements

Dr Barry Wallbank - NSW Agriculture

Dr Joanne Holloway Research Entomologist - NSW Agriculture

Kathryn Smith - NSW Agriculture

William Shore – GasApps Australia Pty Ltd

Ms Bronwyn Sigmund - SunRice

Mr Darren Poole – SunRice Finley

Mr Rodney Jones – SunRice Coleambally

Mr Steve Hussey – SunRice Deniliquin

Mr Bill Volleberg – SunRice Coleambally

Mr David Hutchinson – SunRice Coleambally

VII Sub-Project Name: Treatment of rice products with ethyl formate (2004)

Background

Due to the phase out of methyl bromide under the auspices of the Montreal Protocol, alternative treatments are being sought for many commodities that have traditionally been fumigated with that gas. Ethyl formate is a fast acting fumigant which can kill all stages of many insects within hours, which makes it an attractive alternative to methyl bromide.

Ethyl formate can be highly sorptive and the concentration of gaseous ethyl formate may fall rapidly when placed in contact with a commodity, to levels that are not lethal to insects (Reuss and Annis, 2003). If this fumigant can be distributed through a commodity quickly and a lethal concentration maintained for sufficient time, it promises to be a fast and effective fumigant. One method to ensure distribution is to apply the liquid ethyl formate to small units such as bags of finished product, as is currently done in the dried fruit industry.

Objective

To determine whether ethyl formate is a suitable replacement for methyl bromide.

Methodology

In this preliminary study an application rate of 100 mg/kg ethyl formate was assessed for in-bag disinfestation of processed rice. This included bioassays on insects representative of those that may be found infesting rice and points to some of the occupational health and safety issues that may arise from this application method.

Results

Permeability

Films from the 2kg packages (polythene) and the 750g “Clever rice” packages (nylon) were assessed for permeability. The figure shows clearly that the nylon film (750g packaging) is not permeable to ethyl formate. Although there was a small loss of ethyl formate from the injection side, there was not a corresponding increase in the concentration of ethyl formate in the other chamber. Indicating that ethyl formate did not move across the film. In contrast the film used in the 2kg packages was permeable to ethyl formate. The concentration of ethyl formate observed in the injection chamber fell, corresponding to an increase in the opposing chamber. Within 3 days the concentration of ethyl formate in each chamber was the same. This level of permeability may pose an occupational risk if in bag fumigation is pursued. However, it also indicates that external application of ethyl formate as a standard fumigation may be viable.

Bioassays

In the bioassay trials of mixed age cultures of *S. oryzae* exposed to a dose of 100 mg/kg for 7 days, 100% mortality of adults and larvae was observed, 100% mortality of eggs was observed in the 750g packages and reduced mortality for eggs in the 2 kg packages (97.9%) and 500 g packages(57.1%). There were insufficient pupae to assess the mortality.

Increasing the applied dose from 100mg/kg to 200 mg/kg is likely to improve the mortality observed in both the 500g and 2kg packages. Where there were survivors, those insects were exposed to the fumigant as either pupae or eggs. The likelihood of having either of these stages present and viable post processing/packaging should be assessed prior to a decision to increase the dose applied. It is known that *S. oryzae* pupae and eggs are vulnerable to physical damage and are easily killed during the movement of grain which may apply equally to percussive forces that occur on the rice processing line.

Implications and recommendations

In-bag fumigation of packaged white rice may offer an alternative to methyl bromide fumigation post packaging. In these experiments we found that an application of 100mg/kg ethyl formate in 750 g nylon packages would control all life stages of *S. oryzae* and *E. cautella*. The same dose applied to 2 kg polythene packages controlled all adults and larvae, but only resulted in 98% mortality of *S.oryzae* eggs and *E. cautella* pupae. When applied to the smaller 500 g polythene packages, the survival of *S. oryzae* eggs and *E. cautella* pupae was much higher (57% and 67% respectively). Increasing the applied dose to 200 mg/kg may control the surviving life stages in the 2 kg packages; however it is unlikely to control all life stages in the 500 g packages due to the rapid gas loss from these small packages. The 750g nylon bags were highly suited to this treatment as they retained the gas, however the time for the breakdown of the ethyl formate (i.e. a withholding period) needs to be further considered. Whether the applied dose needs to be increased to control all insects that are likely to be in packages of white rice, is dependent on whether eggs and/or pupae of insect pests survive the packaging process. A study of whether percussion forces that occur on the rice processing lines are sufficient to kill eggs and pupae of pest species may negate the need to increase the does required.

Increasing the application rate of ethyl formate from 100 to 200 mg/kg would increase the residual ethyl formate in the packages, but would not double the residues as might be expected. Increasing the applied dose would also increase the concentration of ethyl formate in the vicinity of the packaging line and in storage area and would need to be further assessed for the possibility of exceeding the flammability limit and exceeding the safe working concentration of this gas.

On most packaging lines at SunRice an injection of liquid ethyl formate could be done during the filling process. On some of the packaging lines, suction plates are used to pick up and place the bags into boxes This packaging procedure would be incompatible with the use of ethyl formate in this manner because the vacuum created would forcibly withdraw the gas from the bags before fumigation was complete. Ideally bags fumigated by injection would not be pin wheeled as the holes created in this process are an obvious point for gas loss and possible reinfestation. However, as these studies have shown, fumigation in bag of larger bags that are pin wheeled may still achieve a fumigation profile that will control most insect pests.

Quality assessment was not performed and would need to be done in any subsequent trials of this nature.

The development of the new product “VaporMate™” by BOC gases, a non-flammable mixture of ethyl formate and carbon dioxide (Ryan and Bishop, 2003),

may reduce the risk of exceeding the flammability limit during application and may be suitable for fumigation of packaged product under tarpaulins. Studies would need to be undertaken to determine whether this formulation could penetrate into a bulk and whether pulsed or continuous application over time would produce the best insect control.

Trials in this application to consumer packs is being explored early-mid 2005 and thus far is the most promising replacement for methyl bromide studied this far.

Project Intellectual Property

No IP

References

Weller, GL., 2004. A Study of the suitability of ethyl formate for fumigation of white rice in commercial packaging. A contracted report to Ricegrowers' Co-operative Limited prepared by CSIRO, Entomology.

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Ms Gaye Weller – CSIRO

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VIII Sub-Project Name: Assessment of a lowered dosage of methyl bromide for finished product fumigation (2005)

Background

In its evaluation of an application for a critical use exemption for methyl bromide as a fumigant of consumer packs of rice in Australia, the Methyl Bromide Technical Options Committee (MBTOC) accepted that there was currently no viable alternative treatment but reduced the quantity of methyl bromide available for this use by half (from 12.3 to 6.15 tonnes). In responding to the request, they noted that the dosage rate nominated (48 g m⁻³ for 24 hours) was unusually high for non-quarantine pests and suggested that the dose used by SunRice be reduced to a rate of 24 g m⁻³ for 24 hours in the absence of any particular circumstances that might justify a higher rate. The Australian label rate for general fumigation of cereal products with methyl bromide, to control stored product pests, is 24 – 32 g m⁻³ for 24 hours at atmospheric pressure and temperatures above 15°C (Appendix 1). This is higher than the rate recommended by the European and Mediterranean Plant Protection Organisation (EPPO) for phytosanitary treatment of rice (15 g m⁻³ for 24 hours at temperatures between 10 and 20°C (Appendix 2), but lower than that recommended for quarantine treatments by the Australian Quarantine and Inspection Service (40 g m⁻³ for 24 hours at 15°C and atmospheric pressure)

Objective

To assess a lowered dosage of methyl bromide for finished product fumigation.

Methodology

Two stacks of the same size, commodity and construction were fumigated on site inside a large warehouse at the SunRice facility at Leeton. Each stack consisted of 4 rows of pallets, 3 pallets high and 14 pallets deep.

Each stack was monitored for gas concentration at 6 locations. Gas sampling lines (1/4 inch and 1/8th inch nylon tubing) were run from locations in the stack to outside the 6m exclusion area set up around each stack in accordance with the Australian Fumigation Standard.

Five locations were the same in each stack; 3 sampling lines were inserted between the pallets in the top layer of pallets and 2 between pallets in the bottom layer of pallets. In stack 1, the 6th sample line was inserted into a box containing the rice packages in the centre of the bottom layer of pallets. In stack 2, the 6th sample line was inserted into a bag of rice inside a box in the centre of the bottom layer of pallets.

Results

The developmental stages of each species that survived the fumigation were judged by the time of emergence of adults. The only adult insects observed emerging from bio-assays were *Plodia interpunctella*. Given the time to emergence post fumigation, these were most probably eggs at the time of the fumigation. Bell (1976) reported that a concentration X time (CT product) of 63ghm⁻³ of methyl bromide is required to kill the eggs of Pyralid moths (including *Ephestia cautella*; *Ephestia khuniella* and *Plodia interpunctella*) at 15°C. The concentration observed inside the packages of rice (Line 6, Fumigation 1; Figure1) equates to a CT product of approximately 72ghm⁻³, however the concentration for some of the time is below the minimum effective

concentration for methyl bromide for pyralid moths (2gm^{-3} at 15°C and 3.5gm^{-3} at 25°C ; Bell and Mills, 1983) and should not be included in the calculation of the CT product. The effective CT product would therefore fall below the 63ghm^{-3} . In other sections of the stack, the CT product was measured at higher concentrations, sufficient to kill eggs of pyralid moths however eggs were found to survive at a number of locations throughout the stack.

Implications and recommendations

In this trial, only one line was inserted into bags of rice to observe the concentration of gas within the rice (where any insect infestation would be). To confirm that the concentration of gas inside the packages of rice are this low, a similar trial should be undertaken where multiple lines are inserted into bags of rice so that the low concentration observed in this trial can be confirmed.

This trial is being repeated in April 2005 ensuring monitoring of gas concentrations and placing of bioassays occurs inside packs.

Project Intellectual Property

No IP

References

Weller, GL. and van Graver, JE, 2004. Assessment of reduced application of methyl bromide for the control of insect pests of final product (consumer packages of rice). A contracted report to Ricegrowers' Co-operative Limited prepared by CSIRO, Entomology.

Acknowledgements

Ms Gaye Weller – CSIRO

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Mr Carl Kenmir – CSIRO

IX Sub-Project Name: Assessment of a lowered dosage of methyl bromide for finished product fumigation (2005)

Background

Information has suggested that packaging is creating a major barrier between fumigant and rice intended for fumigation. This risks unsuccessful fumigation if not properly understood. With the phase out of methyl bromide and the suggestions from the MeBr committees to halve our usage of methyl bromide, we may find that the dosage is not enough to perform a successful fumigation.

Objective

To assess products and their permeability to fumigant

Progress

Over 50 trials have been conducted on all pack sizes at different dosage rates of methyl bromide in normal fumigation facilities. These have been set-up during normal fumigation operations and have included monitoring of gas concentration inside packs. Information gathered this far indicates that some pack sizes are not as permeable to gas as is required as some dosage rates.

These trials will be ongoing, however are showing that packaging permeability needs further study.