FINAL PROJECT REPORT
WTFRC Project #

Project Title: New approaches to decay control of pear
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Lincoln University (Alison Stewart)
Total project funding: 155,372

Budget: 2005-6: 48,862 2006-7: 51,733 2007-8: 54,777

Significant findings:

- A 4-factor gray mold risk prediction model has been developed for the major Oregon and Washington pear districts.
- A real time PCR method to determine the concentration of decay spores (*Penicillium expansum* and *Mucor piriformis*) in dump tank and flume water has been developed.
- 758 new bull's-eye rot isolates have been identified. *N. perennans* is the most common species in Yakima and Wenatchee and *N. alba* the most common in Hood River and Medford.
- A new unnamed species of bull's-eye rot (Neofabraea sp. nova) was found in all major pear districts
- The benzimidazoles thiophanate methyl (Topsin) and thiabendazole (Mertect) appear to have the most effect on *N. alba* and *N. perennans*
- A qPCR method was developed to determine threshold "residues" of the biocontrol agent CIM required on pear fruit for optimum decay control.
- The most effective preharvest spray for overall decay control in 2005-6 was a tank mix of Topsin M and Nutraphos 24.
- Postharvest fungicides Pristine, Penbotec, and Scholar controlled blue and gray mold
- *Muscodor albus,* a biological control agent, significantly controlled blue mold, gray mold, and mucor rot of d'Anjou pear fruit.
- Protective Chemistries paint formulations 1020, 1024, 3020, and 4020 were very effective for prevention of growth of *Botrytis cinerea, Mucor piriformis*, and *Penicillium expansum* on wood and plastic surfaces.

Results and discussion:

1. New model for decay risk prediction

A 4-factor gray mold risk prediction model is shown below in Table 1. The model was developed using data from pear orchards in Oregon, Washington, and New Zealand in 2004-6 (Table 2). Actual decay aligned extremely well with the predicted risk level for fruit from all districts in all three years (Table 2).

Model validation on a larger scale is necessary before the model can be used by the fruit industry. During the 2007-8 season, the model is being validated in orchards in Wenatchee (Dr. Chang–Lin Xiao), Medford (Dr. David Sugar), and the Mid-Columbia (Dr. Bob Spotts) and will use packinghouse cull analyses when available.

Improvements are continually being made and include new, more specific PCR primers for DNA analysis, use of Millipore membranes for high efficiency capture of spores, and scale-up of laboratory methodology to handle large sample numbers necessary for commercialization.

Table 1. Pear gray mold risk prediction model (version 1.00)					
			Orchard rating[4]		
DNA[1]	Fungicide[2]	Rain[3]	1	2	3
L	Yes	No	L	L	M
L	Yes	Yes	L	M	H
L	No	No	L	M	H
L	No	Yes	M	H	E
H	Yes	No	L	M	H
H	Yes	Yes	M	H	E
H	No	No	M	H	E
H	No	Yes	H	E	E

[1]L = *B. cinerea* DNA 0 to 2.2 pg/cm^2; H = over 2.2 pg/cm^2.
[2,3]Yes = fungicide applied within 4 weeks of harvest; 0.02 inches within 2 weeks of harvest.
[4]1 = young to moderate age trees, excellent horticultural and pest/disease practices.
[4]2 = moderate age trees, average horticultural and pest/disease control practices.
[4]3 = old trees, poor horticultural and pest/disease control practices.
[5]Risk levels: L = low, M = moderate, H = high, E = extreme.

Table 2. Pear gray mold predicted risk vs. actual decay in cold stored fruit from Oregon, Washington, and New Zealand

	2004-2005		2005-2006		2006-2007	
Orchard[x]	Predicted risk[y]	Gray mold (%)[z]	Predicted risk[y]	Gray mold (%)[z]	Predicted risk[y]	Gray mold (%)[z]
1	E	14.0	H	8.7	H	7.4
2	H	8.0	H	7.3	H	3.3
3	H	7.0	H	6.6	H	3.0
4	H	5.9	M	5.9	M	3.1
5	H	4.2	M	5.1	M	1.1
6	M	2.6	M	3.8	M	0.9
7	M	2.2	M	3.4	M	0.6
8	M	2.2	L	2.1	---	---
9 Medford					M	1.3
10 Medford					M	1.0
11 Medford					M	0.7
12 Wenatch					M	ND
13 Wenatch					M	ND
14 Wentach					L	ND
15 NZ	M	1.4	L	0.7		
16NZ	L	1.0	L	0.5		
17NZ	L	0.7	L	0.4		
18NZ	L	0.3	L	0.3		
19NZ	L	0.3	L	0.3		
20NZ	L	0.1	L	0.1		

[x]Orchards 1-8 in Mid-Columbia; Orchards 9-11 in Medford; Orchards 12-14 in Wentachee; Orchards 15-20 in Motueka, New Zealand.
[y]Risk levels: L = low, M = moderate, H = high, E = extreme.
[z]Decay after 6 months storage at 30°F.

The average incidence of gray mold at each risk level varied from year-to-year (Table 3). This appears to be related to yearly changes in susceptibility of fruit and matches the annual susceptibility index determined from a standardized lab test (Table 4). For example, the percent gray mold was highest in 2005-6 and lowest in 2006-7 in both actual decay (Table 3) as well as in the standard test (Table 4).

Table 3. Amount of gray mold from decay model risk levels

	Risk level[y]			
	Low	Moderate	High	Extreme
Year	Percent gray mold[z]			
2004-5	0.5	2.1	6.5	14.0
2005-6	0.6	4.5	7.5	---
2006-7	---	1.3	3.7	---

[y]Risk levels from gray mold risk prediction model.
[z]Gray mold is average of all orchards in each risk level.

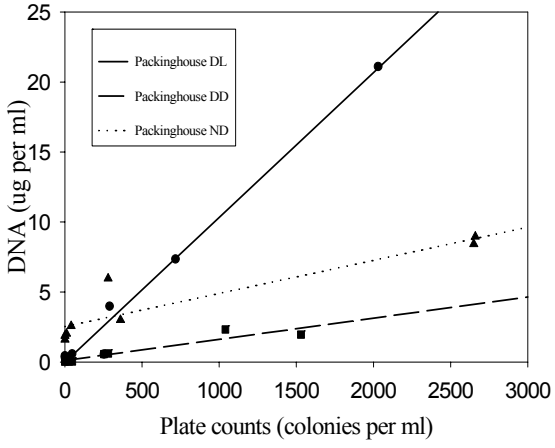
Table 4. Susceptibility of Anjou pear fruit to decay in standardized laboratory conditions

	Infection index[z]		
Year	Gray mold	Blue mold	Mucor rot
2004	22.7b	28.2a	48.4b
2005	28.0b	28.0a	58.2b
2006	13.0a	31.4b	49.2b
2007	32.9c	26.5a	21.6a

[z]Index is calculated as lesion diameter (mm) x proportion of fruit infected. Numbers followed by the same letter within columns are not significantly different at P = 0.05.

2. DNA techniques for rapid, accurate detection of decay spores in packinghouses

Excellent agreement was found between the amount of *P. expansum* DNA in "spiked" dump tank water from three packinghouses and the spore counts from traditional dilution plates. A new method was introduced in 2007 using a Millipore membrane rather than centrifugation to remove spores from the water prior to DNA extraction. The new method is much faster and more accurate than the old method. The experiment needs to be continued with additional water samples from Oregon and Washington packinghouses.

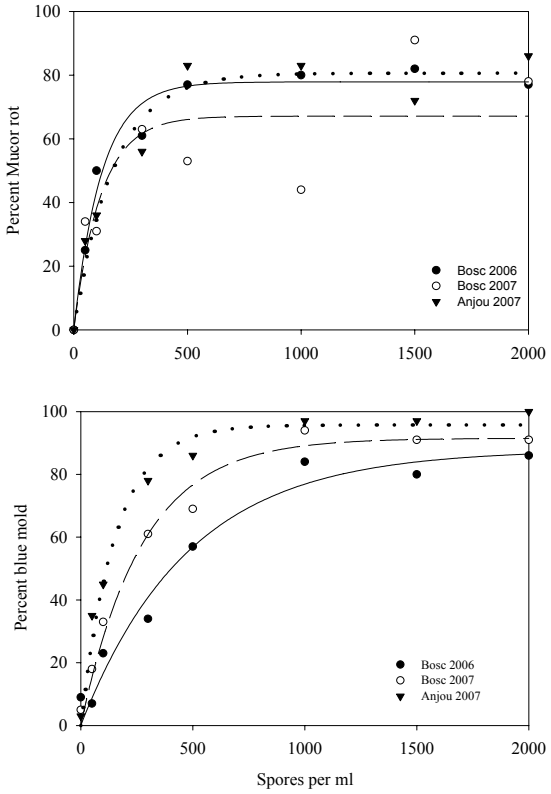


Relationships between decay and spore loads in water

Mucor rot and blue mold decays are closely related to spore loads in packinghouse water systems. We found that the relationship is similar for Bosc pear in 2006(solid line), Bosc in 2007 (dashed line), and Anjou in 2007 (dotted line). The steep curve for Mucor rot between 0 and 500 spores per ml of water indicates that reduction of spore numbers in this part of the curve will result in significant reductions in Mucor rot.

For blue mold, the curve increases more gradually than for Mucor rot, and reductions between 0 and 1,000 spores per ml of water will result in gradual decreases in the amount of decayed fruit.

These results emphasize the importance of good sanitation in the packinghouse. Spore loads in packinghouse water should be reduced as much as possible to reduce decay in storage.



3. Bull's-eye rot species in Washington and Oregon and fungicide sensitivity

We have identified 758 isolates of *Neofabraea* from decayed pear (Table 5) and apple (Table 6) fruit. *N. perennans* is the most common species in Yakima and Wenatchee and *N. alba* the most common in Hood River and Medford. *N. malicorticis* was not found in any Oregon or Washington orchards but is known to occur on the west side of the Cascade Mountains. The new, unnamed species of *Neofabraea* was found in all four districts and was most common on apples from Yakima and Wenatchee (Xiao collection).

Table 5. Summary of *Neofabraea* (bull's-eye) collection in pears

	Percent of total			
Location	*N.alba*	*N.perennans*	New species	Number samples
Yakima	0.0	100.0	0.0	20
Wenatchee	15.8	84.2	0.0	101
Mid Columbia	64.6	34.9	0.6	175
Medford	78.2	16.0	5.8	312

Table 6. Summary of *Neofabraea* (bull's-eye) collection in apples

	Percent of total			
Location	*N.alba*	*N.perennans*	New species	Number samples
Yakima	0.0	81.5	18.5	27
Wenatchee	0.0	88.5	11.5	113
Mid Columbia	0.0	0.0	0.0	0
Medford	90.0	0.0	10.0	10

Table 7. Summary of *Neofabraea* (bull's-eye) entire apple and pear collection

	Percent of total			
Location	*N.alba*	*N.perennans*	New species	Number samples
Yakima	0.0	89.4	10.6	47
Wenatchee	7.5	86.4	6.1	214
Mid Columbia	64.6	34.9	0.6	175
Medford	78.6	15.5	5.9	322

Effect of fungicides on *Neofabraea alba and N. perennans in vitro*

In order to achieve satisfactory control of bull's-eye rot, it is critical to know the *Neofabraea* species profile in each district and use fungicides that have good activity against those species.

We found that the four most effective fungicides for bull's-eye rot control on all four species of *Neofabraea* were the two benzimidazoles, Mertect and Topsin M, Penbotec (pyrimethanil), and Pristine (pyraclostrobin + boscalid) (Table 8). In addition to these four fungicides, other fungicides gave acceptable control of some species of *Neofabraea* but not other species. For example, Scholar controlled bull's-eye rot caused by *N. malicorticis* but not by the other three species.

Copper and ziram have been used routinely for many years in the Pacific Northwest for control of Neofabraea cankers on trees and bull's-eye rot on fruit. We found that ziram was moderately effective, but copper gave poor control of all species except N. malicorticis (Table 8).

Table 8. Control of bull's-eye rot of d'Anjou pear fruit with postharvest dip of fungicides

		Percent bull's-eye rot caused by[x]			
Treatment	Rate[y]	*N. perennans*	*N. alba*	*N. sp. nova*	*N. malicorticis*
Penbotec 400 SC	1000 ppm	0.0a	0.0a	1.7ab	0.0a
Penbotec 400 SC	500 ppm	3.3ab	0.0a	4.2b	0.0a
Topsin M 70WP	1.0 lb/200 gal	0.8ab	1.7ab	0.0a	1.9a
Mertect 340F	16 oz/100 gal	0.8ab	7.4bc	2.6a	0.0a
Pristine 38%	2000 ppm	7.5b	0.0a	0.8a	0.0a
Procure 480SC	12 oz/200 gal	85.8d	13.3c	3.3a	23.4cd
Sovran 50WDG	6.4 oz/200 gal	80.8d	29.0d	18.0b	4.2ab
Ziram 76DF	8.0 lb/200 gal	66.1c	55.8ef	22.4b	18.6cd
Scholar 230 SC[z]	300 ppm	68.3	28.3	58.3	0.0
Scholar 230 SC	150 ppm	91.7ef	44.2de	82.5d	9.4bc
Flint 50WDG	2.5 oz/200 gal	90.6de	67.5f	32.5b	19.3bc
Cuprofix Ultra 40	16 lb/200 gal	96.4fg	98.1g	63.5c	26.9d
Pencozeb 75DF	3.0 lb/200 gal	93.9ef	69.5f	60.8c	45.6e
Water control	---	100.0g	97.4g	79.2d	68.0f

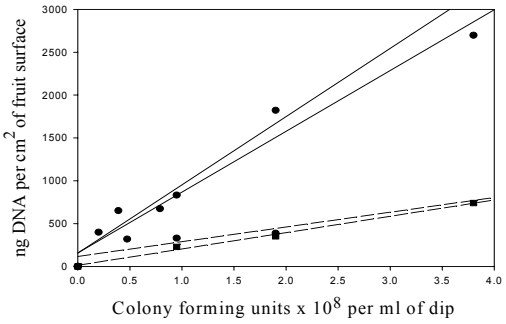
[x]Numbers within columns followed by the same letter are not different according to protected LSD test at P = 0.01.

[y]Rate per 200 gal is the per acre rate.

[z]Single trial results, no statistics.

4. Use of qPCR to determine "residues" of a biocontrol agent on pear fruit

The recommended concentration of the biological control yeast CIM for decay control is 2 x 10^8 cfu/ml. When Anjou (solid lines for 2006 and 2007) and Bosc (dashed lines for 2006 and 2007) fruit were treated with this concentration, the amounts of DNA on the fruit surfaces were about 1700 and 460 ng per cm^2, respectively. This method can be used to assure that CIM is being properly applied to pear fruit on the packing line or in the drench and will result in optimum decay control. Negotiations are underway to license CIM and obtain EPA registration.



5. Preharvest and postharvest fungicides for decay control

Preharvest treatments. For blue mold control, two treatments (Ziram and the foliar nutrient Nutraphos) were ineffective. The most effective preharvest spray for blue mold was Topsin M, either alone or combined with Nutraphos 24 or Ziram.

All fungicides controlled gray mold, but the foliar nutrient Nutraphos was not effective. Pristine was effective when applied twice but not as a single application.

Table 9. Preharvest fungicides to control postharvest decay of d'Anjou pears

		Blue mold (%)[y]			Gray mold (%)[y]		
Preharvest treatment and rate/A	PHI[z] (wk)	2004	2005	2006	2004	2005	2006
Control	--	23b	36d	23b	9c	9b	3ab
Pristine 38 WG 14.5 oz	2	8a	---	---	7bc	---	---
Pristine 38 WG 14.5 oz	1	5a	35d	---	3ab	9b	---
Pristine 38 WG 14.5 oz	2+1	---	23c	10a	---	3a	1a
Topsin M 70WSB 1 lb	2	10a	25c	9a	1a	2a	3ab
Topsin M 70WSB 1 lb	1	8a	11b	10a	2a	2a	2a
Ziram 76DF 8.0 lb	2	19b	45e	22b	2ab	2a	6b
Nutraphos 24 15 lb	2	---	33d	23b	---	8b	5b
Topsin M 70WSB 1 lb + Nutraphos 24 15 lb	2	---	3a	8a	---	2a	1a
Topsin M 70WSB 1 lb + Ziram 76DF 8.0 lb	2	---	---	8a	---	---	2a
Ziram 76DF 8.0 lb + Nutraphos 24 15 lb	2	---	---	24b	---	---	1a

[y]Numbers followed by the same letter within columns are not significantly different at P = 0.05 according to ANOVA and protected LSD of square root transformed data.
[z]PHI = preharvest spray interval in weeks.

Postharvest treatments - Drench application (Table 10). Control fruit (water drench) had 2.7% blue mold. All Pristine and Penbotec drench treatments significantly reduced blue mold and gave 100% control. Ethoxyquin increased blue mold, probably by acting as a wetting agent to increase penetration of spores into wounds. Gray mold in control fruit was 13.1%, and ethoxyquin was not significantly different from the control. Pristine and Penbotec gave 100% control of gray mold. Ethoxyquin caused slight

phytotoxicity, and Pristine at 2000 ppm caused very slight phytotoxicity. Phytotoxicity appeared as dark spotting at lenticels where fruit was in contact with the polyliner and was slow to dry.

Table 10. Control of decay of d'Anjou pear fruit with postharvest drenches of Pristine at MCAREC, Hood River, OR in 2006-7

		Percent fruit infected[z]	
Treatment	Rate product per 100 gal	Blue mold	Gray mold
Pristine 2000 ppm	70.0 oz	0.0a	0.0a
Pristine 1000 ppm	35.0 oz	0.0a	0.0a
Pristine 500 ppm	17.5 oz	0.0a	0.0a
Pristine 250 ppm	8.75 oz	0.0a	0.0a
Penbotec 1000 ppm	1.0 quart	0.0a	0.0a
Ethoxyquin 2700 ppm	2.0 quarts	9.5c	14.7b
Water control	---	2.7b	13.1b

[z]Numbers followed by the same letter within columns are not significantly different at P = 0.05.

Postharvest treatments – Line spray application (Table 11). Water and wax control fruit had 27.5 and 5.8% of wounds infected with blue mold, respectively. The wax contains morpholine, which probably contributed to the reduction in decay. All rates of Pristine in both water and wax gave excellent control of blue mold, and there were no significant differences among rates. Penbotec in water and wax also gave excellent control of blue mold.

Gray mold incidence was 35.8 and 8.3% for the water and wax controls, respectively. All rates of Pristine in both water and wax gave excellent control of gray mold, and there were no significant differences among rates. Penbotec in water and wax also gave excellent control of gray mold. In wax, the Penbotec treatment had significantly less gray mold than the lowest rate (250 ppm) of Pristine. No phytotoxicity was observed with any of the treatments in water or wax.

Table 11. Control of blue mold and gray mold of d'Anjou pear fruit with postharvest line spray application of Pristine at MCAREC, Hood River, OR in 2006-7

		Percent wounds infected[y]	
Treatment[z]	Rate (ppm a.i.)	Blue mold	Gray mold
1. Water control	---	27.5c	35.8d
2. Pristine	250	0.0a	0.2ab
3. Pristine	500	0.2a	0.5ab
4. Pristine	1000	0.0a	0.0a
5. Penbotec	1000	0.2a	0.0a
6. Wax control	---	5.8b	8.3c
7. Pristine	250	0.0a	0.7b
8. Pristine	500	0.0a	0.2ab
9. Pristine	1000	0.2a	0.4ab
10.Penbotec	2000	0.0a	0.0a

[y]Numbers followed by the same letter within columns are not different at P = 0.05.
[z]Treatments 2 to 5 are water suspensions, treatments 7 to10 are wax suspensions.

6. Evaluation of *Muscodor* for decay control

The biological control fungus *M. albus* significantly controlled blue mold, gray mold, and mucor rot of d'Anjou pear fruit at 30° F to 41° F. Phytotoxicity needs to be reduced before *M. albus* can be used commercially on pear fruit. As storage temperature was reduced, phytotoxicity decreased. No phytotoxicity was observed at the 4 gram per box rate at 30° F.

Table 12. Control of pear decays with *Muscodor albus* in 2004-2005

	Percent gray mold[y]			Percent mucor rot[y]			Percent blue mold[y]		
Rate[z]	41° F	32° F	30° F	41° F	32° F	30° F	41° F	32° F	30° F
0	97b	97b	100c	100c	100c	100c	100c	100c	100d
0.5	6a	50a	72b	72b	39a	50b	61b	89bc	78bc
1	6a	47a	31a	70b	58b	45b	19a	84b	53a
2	3a	56a	67b	51b	61b	22a	14a	55a	69ab
4	3a	56a	53ab	26a	53ab	8a	28a	78b	89cd

[y]Numbers followed by the same letter within columns are not significantly different at P = 0.05 according to least significant difference test.

[z]Rate of *M. albus* grams per liter.

7. Evaluation of paint formulations for control of apple and pear postharvest decay fungal pathogens on plastic and wood surfaces

Paint formulations (Protective Chemistries 1020, 1024, 3020, and 4020) were very effective for prevention of growth of *Botrytis cinerea*, *Mucor piriformis*, and *Penicillium expansum* on wood and plastic surfaces. Mortality of spores of these fungi was 100% in most time/temperature combinations on wood and plastic. Use of these paints on apple and pear bins and on surfaces in packinghouses and cold storage rooms will significantly reduce or eliminate spores and growth of several important postharvest fungal pathogens and may be a key component in an integrated decay control program.

Table 13. Effect of three paint formulations on sporulation and growth of *Botrytis cinerea* on wood and plastic chips

			0°C			
	1 month @ 20°C		2 months		3 months	
Treatment	Cfu/chip	Rating[y]	Cfu/chip	Rating[y]	Cfu/chip	Rating[y]
			Plastic			
3020	0a[z]	0.0a	0a	0.0a	0a	0.0a
Unpainted	845b	2.0b	198a	1.1b	344a	1.1b
			Wood			
1020	0a	0.0a	0a	0.0a	0a	0.0a
4020	0a	0.0a	0a	0.0a	0a	0.0a
Unpainted	12a	0.4a	115a	0.3a	198a	1.8c

[y]Rating 0 = no fungal visible growth; 1 = some diffuse growth; 2 = moderate to heavy growth.

[z]Each value is the mean of 3 replications with 3 chips per replication. Numbers followed by the same letter are not significantly different at P = 0.05 according to ANOVA and the protected least significant difference test. Initial cfu/chip = 8,000.

Table 14. Effect of three paint formulations on sporulation and growth of *Penicillium expansum* on wood and plastic chips

			0°C			
	1 month @ 20°C		2 months		3 months	
Treatment	Cfu/chip	Rating[y]	Cfu/chip	Rating[y]	Cfu/chip	Rating[y]
			Plastic			
3020	0a[z]	0.0a	0a	0.0a	0a	0.0a
Unpainted	89,246a	0.7a	2,908b	0.3a	25,666b	1.1b
			Wood			
1020	3,595a	0.0a	0a	0.0a	0a	0.0a
4020	0a	0.0a	0a	0.0a	0a	0.0a
Unpainted	56,343a	0.2a	175a	0.9b	6,950a	1.4b

[y]Rating 0 = no fungal visible growth; 1 = some diffuse growth; 2 = moderate to heavy growth.
[z]Each value is the mean of 3 replications with 3 chips per replication. Numbers followed by the same letter are not significantly different at P = 0.05 according to ANOVA and the protected least significant difference test. Initial cfu/chip = 7,000 at 20°C, 10,000 at 0°C.

Table 15. Effect of paint formulations 1020 (New) and 1024 on sporulation and growth on wood of three decay fungi of pear fruit, OSU MCAREC, Hood River, 2007

			0°C[x]			
	1 month at 20°C[x]		2 months		3 months	
	CFU/chip	Rating[y]	CFU/chip	Rating[y]	CFU/chip	Rating[y]
			Penicillium expansum			
1020 (New)	nd[z]	0a	0a	0a	328a	0a
1024	nd	0a	0a	0a	297a	0a
Control	nd	2b	355,402b	2b	1,814,302b	2b
			Mucor piriformis			
1020 (New)	0a	0a	8a	0a	0a	0a
1024	0a	0a	8a	0a	0a	0a
Control	2,777,667a	2b	283,333b	2b	257,990b	2b
			Botrytis cinerea			
1020 (New)	nd	0a	0a	0a	nd	0a
1024	nd	0a	0a	0a	nd	0a
Control	nd	1b	19a	0.4b	nd	2b

[x]Each value is the mean of 3 replications with 3 chips per replication. Numbers followed by the same letter are not significantly different at P = 0.05 according to ANOVA and the protected least significant difference test. Initial CFU/chip = 7,261 for P. expansum, 1,845 for M. piriformis, and 5,777 for B. cinerea.
[y]Rating 0 = no fungal visible growth; 1 = some diffuse growth; 2 = moderate to heavy growth.
[z]nd = contamination and/or sporulation not determined.