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Mucor Rot of Pears and Apples



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MUCOR ROT OF PEARS AND APPLES

Paul Bertrand and Janet Saulie-Carter

ABSTRACT

Mucor rot developed to serious proportions in stored Anjou pears in the Mid-Columbia area during the 1975-76 storage season. Direct chemical control of this disease is not currently possible. A series of studies undertaken to learn more about the biology of Mucor rot have shed some light on possible control measures.

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INTRODUCTION

In January 1975, a watery soft rot was observed in stored Anjou[®] pears in the Mid-Columbia area of Oregon and Washington. The decay was most severe in field run pears stored in bins beyond mid-November to early December and in cartons of fruit packed after this time. Pears washed during pre-sizing and returned to clean bins or packed prior to mid November-early December developed much less decay. Primary infection usually occurred at the stem end of the pear. Secondary infections, however, occurred at any point of contact between sound pears and diseased pears or the juice produced as the decaying pears broke down. The decay spread rapidly in loose stored pears. The paper wraps used in packing the fruit appeared to reduce the rate of disease spread. Secondary spread of the disease did not seem to occur in apples even when primary infections were common. The cause of the decay was thought to be Mucor globosus (2, 4). However, isolation, reinoculation and morphological studies have shown the cause to be Mucor piriformis Fisher. This fungus has been previously associated with decay of pears and apples (6, 7, 8). The following studies were initiated to investigate the biology of M. piriformis and the development of Mucor rot.

THE EFFECT OF TEMPERATURE ON GROWTH AND SPORE GERMINATION OF MUCOR PIRIFORMIS

The effect of temperature on the growth of M. piriformis was studied on potato dextrose agar (PDA) (Difco Laboratories, Detroit, Michigan). Cultures of M. piriformis were grown at 20°C. (68°F.) for 3 days. Mycelial plugs, 5 millimeters in diameter, taken from the margins of these colonies were used to inoculate the test plates. Test plates were incubated at constant temperature for 3 days. The diameter of the developing colony in each plate was then measured in two directions. Five plates were used for each of 8 isolates of

M. piriformis in each test. The tests were repeated 3 times. The results are shown in Figure 1. The optimum temperature for growth was 20°C. (68°F.) Some growth occurred at 25°C. (77°F.), but none at 30°C. (86°F.). Measurable growth also occurred at -1°C. (30.2°F.), the recommended storage temperature for pears.

The effect of temperature on spore germination of M. piriformis was studied on PDA. The spore suspensions were prepared as follows:

Cultures were grown at 20°C. (68°F.) for 7 to 10 days. They were then placed in a refrigerator until used. Unused plates were discarded after 4 weeks. During refrigerated storage, the sporangiophores reached a height that resulted in masses of spores being deposited on the inside cover of the culture dishes. The spores were collected by washing the covers with sterile distilled water. Dilutions were prepared in the range of 10^5 to 10^6 spores per milliliter for use in the experiments.

Microscope slides thinly coated with PDA were prepared by dipping slides in 95 percent ethanol, flaming dry and immersing in hot PDA. The PDA-coated slides were then placed in petri dishes on a solid water agar surface. The water agar prevented desiccation of the spore drops during incubation. A small drop of spore suspension was placed at each end of the PDA-coated slides, covers were put on the dishes and they were placed in incubators. At various times slides were removed from the incubators and the percent spore germination was determined by microscopic examination. A spore was considered to have germinated when a germ tube equal in length to one-half the original spore diameter was formed. The tests were continued at each temperature until germination reached completion (95 to 100 percent). Seven isolates of M. piriformis were used. Tests with each isolate were repeated 3 to 5 times. The average time required for complete germination is shown in Figure 2. The

average rate of germination at each temperature is shown in Figure 3. The optimum temperature for germination was 20°C. (68°F.). Germination at 25°C. (77°F.) was nearly as rapid, but abnormal. The spores and germ tubes swelled excessively. The germ tubes formed short branches and little subsequent growth occurred. There was no germination at 30°C. (86°F.). At -1°C. (30.2°F.) germination began to occur in 48 hours and reached completion in 144 hours. All attempts to germinate spores in distilled water failed. There was no evidence of germination or the spore swelling which proceeds germination after 120 hours at 20°C. (68°F.). Spores germinated in non-sterile pear juice as well as they did on PDA. The pear juice quickly became overgrown with a yeast-like growth at temperatures above 15°C. (59°F.) and germination tests in this medium were discontinued.

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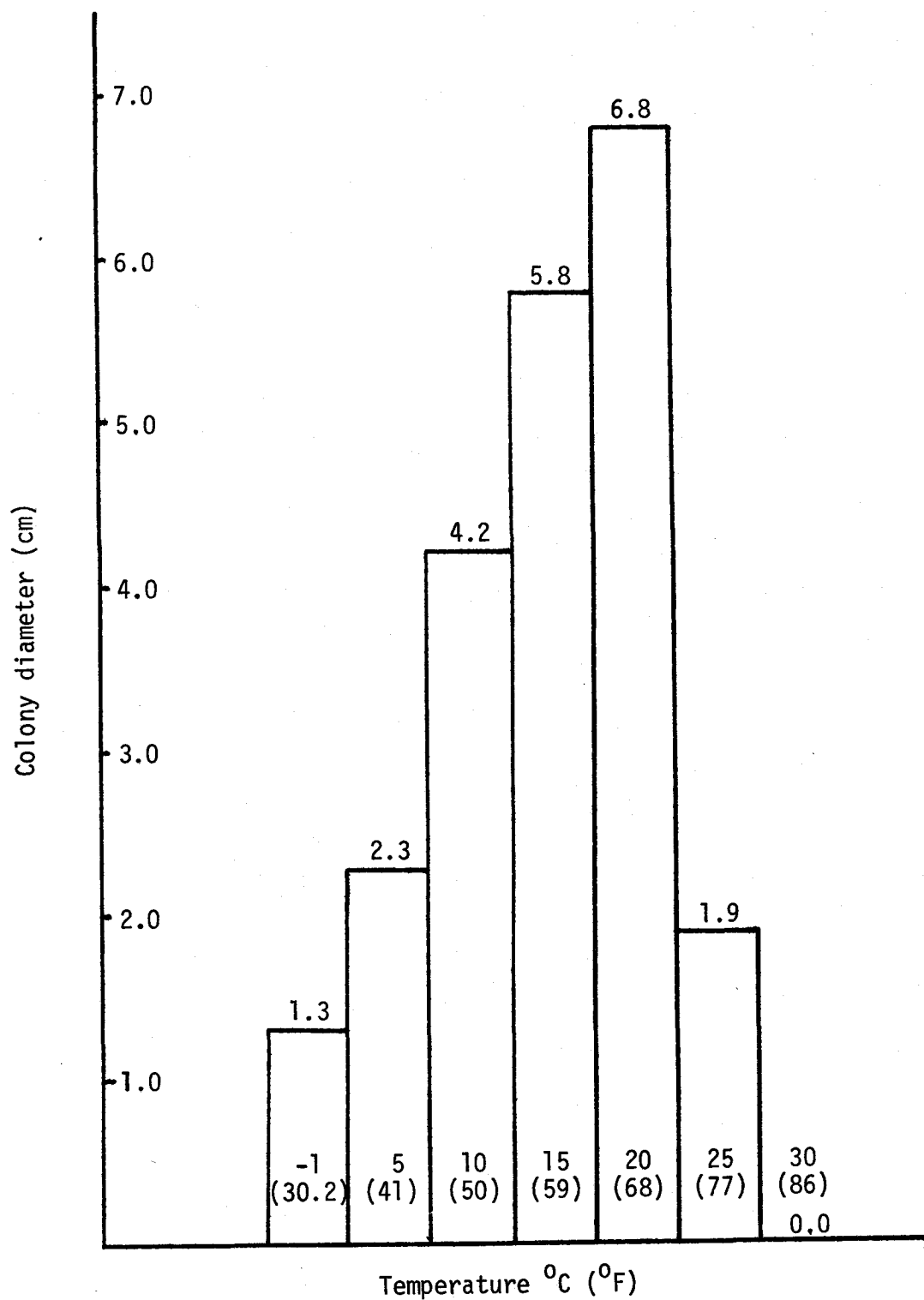


Figure 1. The mean growth of *Mucor piriformis* on potato dextrose agar for 3 days.

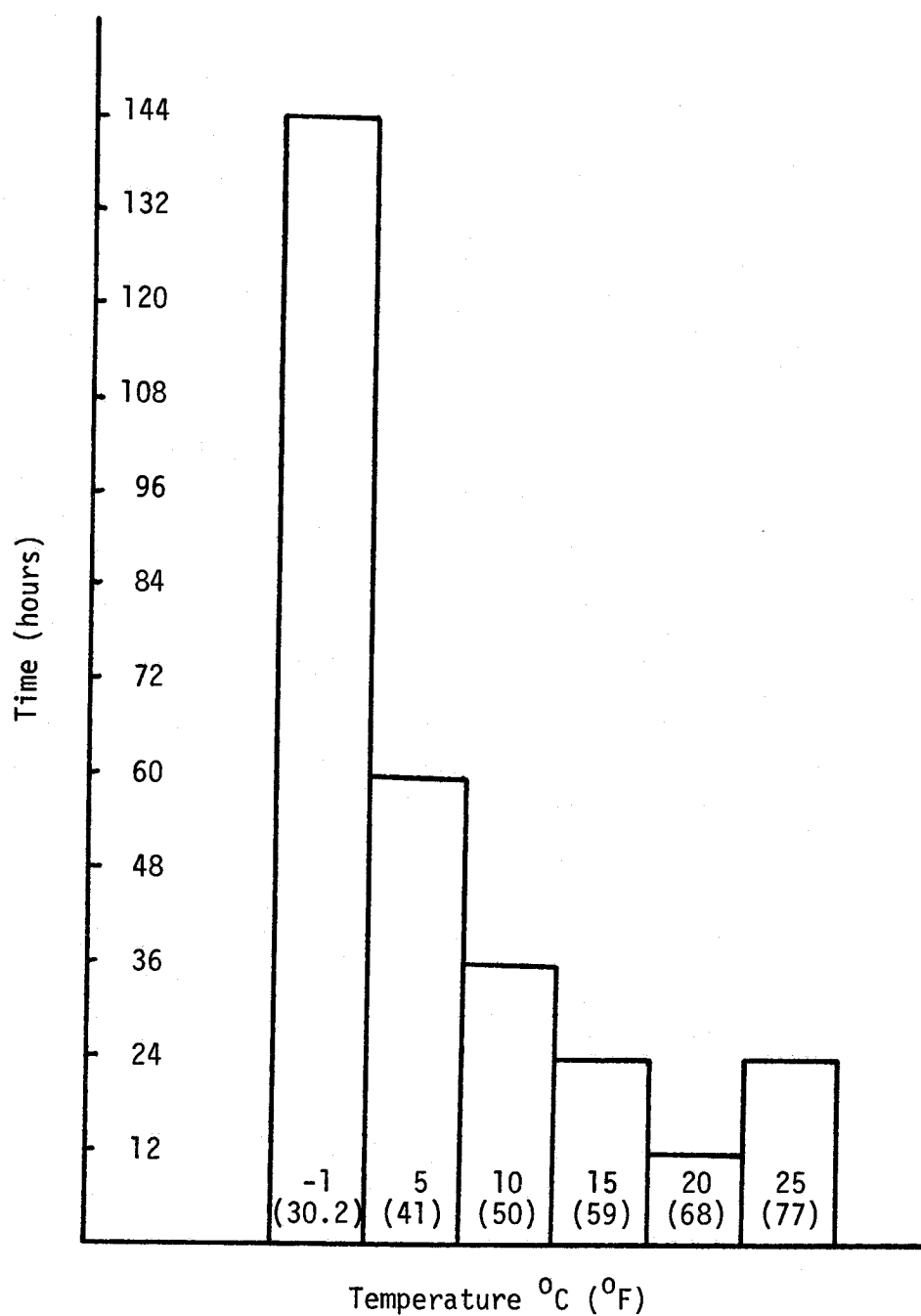


Figure 2. The average time required for spores of *Mucor piriformis* to reach complete germination on potato dextrose agar.

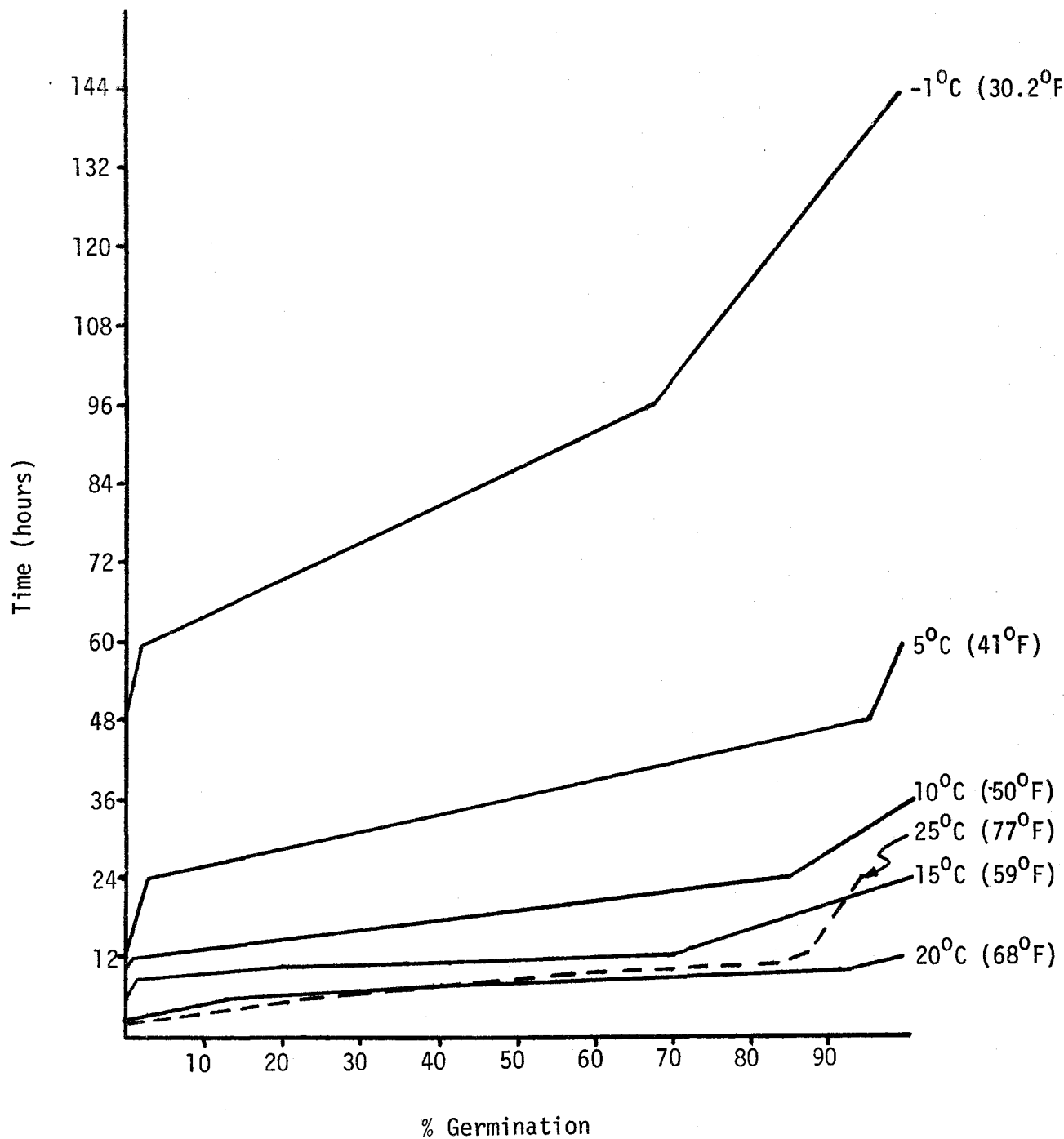


Figure 3. The rate of germination of spores of Mucor piriformis on potato dextrose agar at various temperatures.

DEVELOPMENT OF MUCOR ROT IN STORAGE

To measure the development of Mucor rot of individual fruit at -1°C . (30.2°F .), Anjou pears, Bosc pears and Red Delicious apples were inoculated by introducing a spore suspension of M. piriformis into a 3 millimeter x 3 millimeter puncture. Five polyethylene-lined boxes of each pear variety and four polyethylene-lined boxes of apples inoculated in this manner were stored at -1°C . (30.2°F .). At 10-day intervals a box of each variety was removed from storage and the extent of decay of each fruit was measured. The results are shown in Tables 1, 2 and 3. These data show the rapidity of fruit destruction by Mucor rot. At -1°C . (30.2°F .) measurable decay was present in only 10 days.

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TABLE 1. The Development of Mucor Rot in Anjou Pears During 50 Days at -1°C . (30.2°F .)

	Days in Storage				
	10	20	30	40	50
Mean fruit weight (grams)	196.20	200.64	190.74	189.68	197.15
Mean lesion diameter (centimeters)	0.66	1.95	4.02	5.58	9.22
Mean weight decayed tissue (grams)	0.33	2.82	16.35	40.86	100.44
Mean % tissue decayed	0.17	1.38	8.57	21.54	50.95

TABLE 2. The Development of Mucor Rot in Bosc Pears During 50 Days at -1°C . (30.2°F .)

	Days in Storage				
	10	20	30	40	50
Mean fruit weight (grams)	187.40	213.05	192.55	210.53	210.96
Mean lesion diameter (centimeters)	0.68	1.88	3.89	6.21	8.34
Mean weight decayed tissue (grams)	0.54	2.12	13.48	48.18	92.24
Mean % tissue decayed	0.29	1.00	7.00	23.00	43.73

TABLE 3. The Development of Mucor Rot in Red Delicious Apples During 40 Days at -1°C . (30.2°F .)

	Days in Storage			
	10	20	30	40
Mean fruit weight (grams)	148.18	155.47	167.72	155.49
Mean lesion diameter (centimeters)	0.81	1.41	2.70	6.17
Mean weight decayed tissue (grams)	0.32	1.46	9.83	53.75
Mean % tissue decayed	0.21	0.99	5.90	36.26

ORCHARD SOIL AS A RESERVOIR OF MUCOR PIRIFORMIS

Mucor piriformis has been recovered from soil in the eastern United States (5). A survey to determine the presence and distribution of M. piriformis in orchard soils was conducted in the Hood River Valley. Soil samples were collected and processed as follows:

A one-pint composite sample of the top two inches of orchard floor litter (when present) and soil from 4-6 sites was collected in each orchard. The soil was thoroughly mixed and 100 grams of it was added to a blender containing 200 milliliters of distilled water. The soil and water were mixed in the blender for one minute. A 0.1 milliliter sample of this solution was spread on each of two acidified PDA plates. Another 0.1 milliliter sample was diluted with 5 milliliters of distilled water. A 0.1 milliliter sample of this solution was spread on each of 2 acidified PDA plates. The plates were incubated at 20°C. (68°F.) for 3 days and evaluated for M. piriformis. An additional 100 grams of soil was dried to constant weight (10 days at 110°C. (230°F.)) so M. piriformis could be expressed as number of viable units per gram of dry soil. The results shown in Table 4 indicate that M. piriformis is present in orchard soils throughout the Hood River Valley. The fungus was detected in 21.4 percent of the orchards sampled. The amount of M. piriformis detected varied from 1.4×10^1 to 1.6×10^4 viable units per gram of dry soil. In making these calculations, each colony counted on the assay plates was considered to represent one viable unit. The nature of the viable units was not determined. All isolates of M. piriformis recovered from the orchard survey were found to cause typical Mucor rot in Anjou pears.

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TABLE 4. The Occurrence of Mucor piriformis in the Orchard
Soils of the Hood River Valley

Location	Number of Samples	
	Collected	With <u>M. piriformis</u>
Pine Grove	29	13
Odell-Willow Flat	44	8
Parkdale-Dee	68	11
Oak Grove	48	9
Hood River	17	3
Total	206	44

THE INCIDENCE OF MUCOR PIRIFORMIS IN DUMP TANK WATER

The water used in dumping and moving fruit in packing and canning plants was sampled for M. piriformis. Water samples were collected by submerging uncapped 100 milliliter bottles to a depth of 1 to 2 inches. In the laboratory, 0.1 milliliter aliquats were spread evenly on acidified PDA. Two to 4 plates were used for each sample. The plates were incubated at 20°C. (68°F.) to 25°C. (77°F.) for 2 to 5 days. Each distinct colony was considered to represent a single viable spore. The results are shown in Table 5. Mucor piriformis was detected periodically in all the sampled plants.

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TABLE 5. The Incidence of Mucor piriformis in Dump Tank and Flume Water in Packing Houses and Canneries

Spores per milliliter per sample ¹	No. samples in this range
0-2.5	66
2.5-9	16
10-99	39
100-999	23
1000	0

¹The sampling procedure could not detect less than 10 spores per milliliter divided by the number of plates for that sample.

THE EFFECT OF HARVESTING WET AND DRY FRUIT ON THE INCIDENCE OF MUCOR ROT

A Canadian report suggested that avoiding harvest of wet fruit might aid in reducing losses from Mucor rot (8). The following experiment was done on 4 adjacent Anjou pear trees to test this suggestion.

At 8:00 p.m. on September 7, 1977 two trees were sprayed to run-off with a suspension containing 3.8×10^5 spores of M. piriformis per milliliter. The other two trees were sprayed in a like manner with water. At 8:00 a.m. on September 8, 1977 all 4 trees were sprayed to run-off with water. While the trees were still wet, one each of the trees sprayed the previous day with M. piriformis or water only were harvested. The other M. piriformis sprayed and water only sprayed trees were allowed to dry completely and then harvested. All fruit was harvested directly into polyethylene-lined boxes and placed in storage at -1°C . (30.2°F .). The incidence of primary infection was evaluated on December 20, 1977 and the results are shown in Table 6.

Harvesting wet fruit resulted in a higher incidence of Mucor rot even when trees had been sprayed with water only. These results support the suggestion of the Canadian workers.

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TABLE 6. The Effect of Harvesting Anjou Pears Wet or Dry on the Incidence of Primary Infection of Mucor Rot

Treatment of September 7, 1977	No. Pears	% Primary Infection
<u>Mucor piriformis</u> sprayed; picked dry	820	0.7
<u>Mucor piriformis</u> sprayed; picked wet	1093	14.1
Water sprayed ; picked dry	626	0.0
Water sprayed ; picked wet	782	6.8

SECONDARY SPREAD OF MUCOR ROT

The most destructive aspect of Mucor rot is its rapid secondary spread in pears stored in bins. The following experiment was done to determine the rate of secondary spread of Mucor rot in Anjou pears.

Fifteen polyethylene-lined boxes of pears were used for each of two replications. Each box was sorted to remove all pears injured in previous handling. Ten pears inoculated at the stem end with 8.0×10^6 spores of M. piriformis per milliliter to simulate natural primary infection were placed at random in each box as it was packed. The polyethylene liner was folded over and the fruit was stored at -1°C . (30.2°F .). At four-week intervals, 3 boxes from each replication were selected at random and evaluated for secondary spread of Mucor rot. The results are shown in Table 7. Traces of secondary spread were observed by the 8th week. This observation may have bearing on our earlier observation that fruit packed, washed or processed within 8 weeks of harvest tended to have fewer problems with Mucor rot than fruit handled more than 8 weeks after harvest.

While secondary spread of Mucor rot seemed to be rapid in bins of pears, it appeared to be greatly reduced in packed cartons where the fruit had been individually wrapped in packing tissue. An experiment was designed to determine the effect of the paper wraps used in commercial fruit packs on the secondary spread of Mucor rot.

Three treatments, each consisting of 3 cartons of Anjou pears were compared: 1) loose pack, no paper wraps used; 2) standard pear wraps containing 0.4 percent 6-ethoxy-1,2-dihydro-2,2,4 trimethyl quinoline (ethoxyquin); and 3) copperized pear wraps containing 1.3 percent copper as basic copper carbonate and 0.1 percent ethoxyquin. The ethoxyquin in the wrap aids in the control of superficial scald (9). All wraps used in this work are products of Crown

Zellerbach Corporation, San Francisco.

Primary inoculum was supplied by placing pears inoculated in one cheek with 0.3 milliliter of a suspension containing 1.3×10^4 spores of M. piriformis per milliliter in the cartons. The pears were packed into polyethylene-lined boxes in 4 layers. The second layer from the bottom in each carton contained 31 inoculated pears.

In the treatments involving wraps, all pears included inoculated pears were individually wrapped with a single 27.9 centimeter x 27.9 centimeter sheet of the proper tissue. The packed cartons were stored at -1°C . (30.2°F .) from November 7, 1975 until February 2, 1976, when decay counts were made. The effect of paper wraps on decay control is shown in Table 8. Wrapping the fruits individually significantly ($P = 0.01$) reduced the spread of decay from fruit to fruit, the copperized wrap being significantly ($P = 0.01$) better than the standard wrap. The copperized wraps were made of somewhat heavier paper (1.43 kilograms/1000, 30.48 centimeter x 30.48 centimeter sheets) than the standard pear wrap (1.13 kilograms/1000, 30.48 centimeter x 30.48 centimeter sheets). Whether the weight of the paper was a factor in disease control was not determined. Copperized wraps were developed in 1931 to control fruit to fruit spread of gray mold rot caused by Botrytis cinerea (3).

Though secondary spread of Mucor rot occurred commonly in bins of pears, there appeared to be no significant secondary spread in bins of apples even though primary infections were common at times. Unlike pears, infected apples do not break down and release liquid, even though the flesh decays completely. It appeared that the thicker skin of the apples held the rotted fruit intact. A small study was set up to see if the absence of secondary infection in apples was due to failure of the fungus to move out of the primary infections or a failure to move into adjacent sound fruit.

The test consisted of 4 treatments: 1) inoculated pears packed with sound pears; 2) inoculated apples packed with sound pears; 3) inoculated pears packed with sound apples; and 4) inoculated apples packed with sound apples. The fruit was packed in polyethylene-lined boxes and stored at -1°C . (30.2°F .) for 90 days. The results are shown in Table 9. The expected secondary spread in pears (Treatment 1) was observed. There was no secondary spread out of infected apples (Treatments 2 and 4) into either pears or apples. There was some spread into apples from pears (Treatment 3), especially when apples contacted the liquid produced from disintegration of the pears.

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TABLE 7. The Rate of Secondary Spread of Mucor Rot in Anjou Pears¹

Time	No. Pears	Secondary Infection	
		No.	%
4 weeks	666	0	0.0
8 weeks	684	8	1.2
12 weeks	642	53	8.2
16 weeks	657	117	17.8
20 weeks	660	162	24.5

¹The data from the two replications have been combined.

TABLE 8. The Effectiveness of Paper Wraps in Controlling Secondary Spread of Mucor Rot in Anjou Pears During 3 Months at -1°C. (30.2°F.)

Treatment	No. Pears ¹	% Secondary Decay ²
No wrap	274	62.5x
Standard wrap ³	281	24.5 y
Copperized wrap ⁴	283	7.7 z

¹Total number of pears excluding preinoculated pears in each treatment.

²Mean for three cartons. Means followed by a different letter are significantly different (P = 0.01).

³Crown Zellerbach Corporation Crownoil^R wrap containing 0.4% ethoxyquin.

⁴Crown Zellerbach Corporation Super Copperized^R wrap containing 1.3% copper as basic copper carbonate and 0.1% ethoxyquin.

TABLE 9. Comparison of Secondary Spread of Mucor Rot in Pears and Apples

Treatment	No. Fruit ¹	Secondary Infections	
		No.	%
Inoculated pears packed with sound pears	41	19	46.3
Inoculated apples packed with sound pears	50	0	0.0
Inoculated pears packed with sound apples	44	5 ²	11.4
Inoculated apples packed with sound apples	53	0	0.0

¹The number of initially sound fruit.

²Four of the five secondary infections were related to areas of apples standing in juice produced as the inoculated pears disintegrated.

DISCUSSION

The temperature studies show that spore germination, infection and disease development can occur at temperatures used for the storage of pears and apples. Disease development at cold storage temperature is quite rapid.

Secondary spread of Mucor rot occurs quite readily in pears, but seems very limited, if occurring at all, in apples. The thicker skin of the apple may be partially responsible by preventing the complete breakdown of the primary infected fruit.

In experiments with Anjou pears, only traces of secondary spread were evident after 8 weeks of storage, even though visible primary infections might be present in as little as 10 days at -1°C . (30.2°F .). These results indirectly support our earlier observation that packing, presizing or canning fruit within 8 weeks of harvest appeared to result in reduced Mucor rot. There is evidence that directly supports this observation. One grower delivered all his Anjou pears between the 9th and 22nd of September 1975. His fruit was packed in two separate runs. The pears packed between the 4th and 8th of October 1975 (12 to 29 days from harvest) held up well in storage. However, the pears packed between the 16th and 19th of November 1975 (56 to 71 days from harvest) required repacking in early 1976 because of Mucor rot. The benefits gained from packing or pre-sizing fruit within 8 weeks of harvest could be twofold: 1) primary infections may be sorted out before secondary infection occurs, and 2) the washing that accompanies pre-sizing and packing may remove spores from the fruit to prevent further primary infection. While these results and observations suggest the advantages of immediate handling, they do not prove early handling will control Mucor rot.

The paper fruit wraps used during packing have been shown to greatly reduce the secondary spread of Mucor rot. Copperized paper was particularly

effective. Other results, not reported here, support this conclusion. These paper wraps are gradually being phased out in favor of less costly packing methods. As long as a portion of the fruit continues to be individually wrapped, some consideration should be given to Mucor rot in selecting those lots.

Soil may be the major reservoir of M. piriformis. The fungus was found in all parts of the valley. The mechanisms of fruit contamination and primary infection of M. piriformis are not known. The fungus could get to the fruit from the soil by rising dust and/or splashing rain or sprinkler irrigation water. Soil caked on bins from careless handling in the orchard can result in fruit contamination when the bins are stacked for hauling and/or storage. Soon after harvest, fruit lying on the ground is commonly found to be decayed by M. piriformis. Fruit may become contaminated on striking the ground. Fruit lying on the ground even for a short time may be infected. Collecting this fruit may increase the amount of primary infection.

Harvesting fruit while wet would provide the moisture needed for infection. The water films also may allow fungus spores to spread and create increased difficulties in keeping the fruit clean.

The specific significance of finding M. piriformis in dump tank water has not been determined. Most Mucor rot in fruit stored in bins has developed in fruit that has never passed through a dump tank. Passing injured fruit through contaminated water, however, can result in infection and decay (9). Some injured fruit is missed during sorting. Mucor rot found in packed fruit, in some cases, may be related to contaminated dump tank water.

SUMMARY

There is no direct chemical control for Mucor rot of pears and apples. In the course of this work, observations and experimental results pointed out several measures that should aid in reducing of losses from Mucor rot:

- (1) Avoid harvesting wet fruit.
- (2) Keep dust in the orchard to a minimum.
- (3) Do not pick up fruit from the ground and put it in the bins.
- (4) Avoid contaminating bins with orchard soil. Do not skid them along the ground when moving them around.
- (5) Pack or pre-size pears from orchards with a Mucor rot history within 8 weeks of harvest. Plants with pre-size capability should try to pre-size all pears within 8 weeks of harvest.
- (6) Individually wrapping fruit can reduce secondary spread of Mucor rot. Lots with Mucor rot present at packing time may benefit from handling in this manner.
- (7) Dump tank sanitation may be important. Either chlorine or sodium o-phenylphenate can be added to the dump tank water (9). One of these materials should be used if Mucor rot is present in the fruit being run.

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