A Laboratory Study on the Ability of Fungicides to Control Beet Rotting Fungi

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Abstract

A laboratory procedure for testing the capability of fungicides to control beet rotting fungi was selected. A temperature at which to test the fungicides was chosen, and fourteen fungicides or fungicide combinations were tested for their ability to control four different beet rotting fungi.

Introduction

Storing of beets for prolonged periods of time has created a fungal problem in the storage piles. Species of *Phoma, Botrytis, Fusarium, Penicillium, Rhizopus,* and *Mucor* have been reported to rot sugarbeets (2, 3, 4, and 5)[2]. The experiments in these studies were conducted for the purpose of selecting a method and a temperature for screening fungicides in the laboratory and the screening of several fungicides for their effectiveness in controlling the fungi which cause sugar loss and rot in stored beets.

Materials and Methods

Twenty isolates involving species of *Rhizopus, Botrytis, Fusarium, Penicillium,* and *Mucor* were isolated from beets and tested for pathogenicity. One isolate of each of the first four genera were selected for use in this study. *Mucor* was eliminated because it was not considered a serious problem in this area. From previous work by Beck and Nielson (1), three fungicides were selected for the preliminary tests. The fungicides selected were 2-(4-Thiazolyl) benzimidazole (TBZ), 2-6-Dichloro-4-nitroniline (DCNA), and 1-(4 chlorophenoxy) 3,3-dimethyl-1-(1H-1, 2,4-Triazol-1-yl)-2-butanone (Bay Meb 6447). Additional fungicides were tested in later experiments.

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[2]Numbers in parentheses refer to literature cited.

1. Three methods of screening fungicides were tested:

A. Fungicide Agar (FA). 250 ppm, 500 ppm, or 1000 ppm of active ingredient of fungicide was added to mycophil agar just prior to pouring into 15 x 100 mm petri dishes. Plain mycophil agar plates were used as controls. A 4 mm agar plug containing one of the four selected fungi was transferred to the center of the petri dish containing the fungicide agar or the plain mycophil agar. For convenience, the plates were held at room temperature (23°C). Measurements, in mm, of the amount of growth from the edge of each fungus plug were made three, six, eight, ten, and sixteen days after the transfer. The treatments were replicated five times.

B. Paper Discs (PD). Filter paper discs 4 mm in diameter were soaked in 250 ppm, 500 ppm, and 1000 ppm active ingredient of the selected fungicides. Paper discs soaked in sterile H_2O served as the controls. The paper discs were transferred to mycophil agar plates 15 x 100 mm on which one of the four selected fungi had been transferred approximately one hour earlier. Four paper discs, at the same concentration, were placed on each petri dish. Each treatment was replicated five times. The zone of inhibition was measured in mm one, three and nine days after transfer.

C. Beet Tissue (BT). A core 70 mm in diameter was cut from beets which had been in storage for 90 days. This core was then cut into slices 6 mm thick. The slices were then dipped in one of the selected fungicides at 250 ppm, 500 ppm, or 1000 ppm for two minutes, then placed in a petri dish. The controls were dipped in sterile H_2O. Three milliliters of a suspension of one of the selected fungi were placed on the beet slice and spread over the upper surface of the tissue. The fungus suspensions were made by putting a culture (one petri dish) of a fungus that had grown for approximately two weeks into 200 milliliters of sterile de-ionized water and blending for three minutes. The treatments were replicated five times and evaluations were made visually three and seven days after inoculation of the slices.

2. Temperature Study

The (FA) method was selected for a temperature study. Four temperatures were selected: 5°C, a target storage temperature; 10°C, a temperature commonly reached late in the storage period; 23°C (room temperature); and a treatment in which the temperature varied from 1.5°C to 15°C (the temperature which occurred at the top of one of our storage piles). The procedure was the same as described under Fungicide Agar. The same three chemicals were

used at the same concentrations with the same fungi. There were five replications per treatment. Growth of the fungi, from the edge of the 4 mm plug, was measured in millimeters seven and twenty days after initiation of the experiment.

3. Screening of Fungicides

The (FA) method at 23°C was selected as the most suitable method for the screening of various fungicides. Fourteen fungicides or fungicide combinations were selected and tested at 250 ppm, 500 ppm, and 1000 ppm active ingredient. There were five replications of each treatment, and the fungal growth was measured in millimeters four, eight, and twenty-one days after the transfer of the fungi to the fungicide agar.

Results and Discussion

1. Comparison of Methods. The methods gave similar results with two exceptions: (1) Bay Meb 6447 controlled the growth of *Botrytis* in the (FA) method where it did not in the other two methods, and (2) DCNA controlled *Rhizopus* in the (PD) method, where it did not in the (FA) and the (BT) methods.

A contamination problem was encountered with the (BT) method, probably due to use of beets which had been in storage for approximately 90 days. This made it difficult to evaluate the method.

An indication of the fungicide's effectiveness could be made at one day for the (FA) and the (PD) methods. Readings, however, were taken after longer periods of time, at our convenience.

2. Temperature Study. The effect of temperature on the growth of beet rotting fungi on an agar medium containing 1000 ppm of selected fungicides is shown in Table 1. As shown in the table, the effectiveness of the fungicides does appear to be altered slightly by the temperatures used in this study. The temperature, as expected, does affect the growth of the fungi. At 5°C *Rhizopus* and *Fusarium* did not grow and *Botrytis* and *Penicillium* growth was at a reduced rate.

The data on the 250 ppm and 500 ppm concentration of the fungicides follow a similar pattern.

3. Screening of Fungicides. The results of the activity of the fourteen fungicides or fungicide combinations against the selected fungi in agar media are shown in Table 2. As can be seen in the table, Propionic acid at 1000 ppm and SOPP were the only chemicals which completely controlled all fungi tested. The benzimidazoles were effective against all fungi tested except *Rhizopus*.

Table 1.—The effect of temperature on the growth of beet rotting fungi on an agar medium containing 1000 ppm fungicides.

Chemical	Fungus	Temperature 5°C	10°C	23°C	Pile[a] (1.5-15°C)
Control	Rhizopus	0[b]	43[c]	43	43
	Fusarium	0	10	43	11
	Penicillium	38	43	43	35
	Botrytis	33	43	43	43
TBZ	Rhizopus	0	4	43	3
	Fusarium	0	0	0	0
	Penicillium	1	1	0	1
	Botrytis	0	0	0	0
DCNA	Rhizopus	0	4	43	2
	Fusarium	0	1	29	1
	Penicillium	2	6	13	4
	Botrytis	0	1	1	0
Bay Meb 6447	Rhizopus	0	5	43	5
	Fusarium	0	1	18	5
	Penicillium	7	17	17	20
	Botrytis	0	0	7	1

Standard Deviation of Mean/Cell = 1.27

[a]Plates were set on top of a canopy-covered storage pile.

[b]Numbers represent the average growth in mm of 5 replicates.

[c]43 mm = plate completely covered.

Readings were taken 21 days after transfer of fungi to the medium.

Discussion

In this paper, data have been presented on three different methods of testing fungicides in the laboratory. Of the three methods, the fungicide agar method was preferred for several reasons:

A. The concentration of the fungicide was known. The agar medium and the beet tissue have a diluting effect in the other methods making a precise determination of fungicide concentration impossible.

B. There was more contamination with the (BT) and the (PD) methods than with the (FA) method. There was a severe contamination problem with the (BT) method, possibly because the beets used had been in storage.

C. The manipulations involved in the technique are simpler to perform than those of the other two methods.

From the temperature study, the 23°C temperature was selected for further study. At this temperature, the fungicide effec-

Table 2.—The effect of fungicides and fungicide combinations at 250 ppm, 500 ppm, and 1000 ppm active ingredient of the fungicide on four beet rotting fungi.

	Rhizopus			Fusarium			Penicillium			Botrytis		
Chemical	250	500	1000	250	500	1000	250	500	1000	250	500	1000
Captafol*	43[a]	43	43	7	8	17	8	9	6	9	12	12
Captan	43	43	43	29	18	22	11	5	7	8	9	8
DCNA	43	43	43	29	24	22	0	11	6	1	1	1
Ferbam	43	43	43	21	3	0	24	9	9	0	0	0
Bay Meb 6447	43	43	43	24	24	23	22	20	20	18	12	16
Sulfur	43	43	43	43	43	43	38	38	38	43	43	43
Propionic Acid	43	43	0	41	0	0	33	11	0	43	0	0
SOPP	0	0	0	0	0	0	1	0	0	0	0	0
Thiophanate	43	43	43	5	4	7	2	5	1	0	0	0
Benomyl	43	43	43	0	0	0	1	1	1	0	0	0
TBZ	43	43	43	0	0	0	1	1	1	0	0	0
TBZ & DCNA	43	43	43	0	0	0	1	1	1	0	0	0
Benomyl & Captan	43	43	43	0	0	0	1	1	1	0	0	0
DCNA & Bay Meb 6447	33	20	13	13	11	13	10	10	8	4	4	4
Control 0 ppm		43			43			39			43	

Standard Deviation of Mean/Cell = 0.71

[a]Numbers represent the average growth in mm of 5 replicates.

*Scientific names of above chemicals:

CAPTAFOL - cis-N-(1,1,2,2,-tetrachloroethylthio)-4-cyclohexene-1,2-dicarboximide. CAPTAN — N-(trichloromethylthio)-4-cyclohexene-1,2 dicarboximide. DCNA — 2,6-dichloro-4-nitroaniline. FERBAM - ferric dimethyldithiocarbamate. BAY-MEB-6447 — 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butonone. SOPP — Sodium-o-phenyl phenate or the sodium salt of o-phenyl phenol. THIOPHANATE — Diethyl 4,4'-o-phenylenebis 3-thioallophanate. BENOMYL - methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate. TBZ — 2-(4-thiazolyl) benzimidazole.

Readings were taken 21 days after transfer of fungi to the media.

tiveness could be determined more precisely and rapidly because of higher fungal growth rates.

The pathogens, *Penicillium* and *Botrytis*, were the only fungi tested that would grow at 5°C, a target for our storage piles. The fact that these species were the only organisms to grow at 5°C agrees with observations that these two organisms account for the majority of losses caused by beet rotting organisms in beet storage piles.

From the screening of several fungicides in the laboratory, it appears that propionic acid, SOPP, and the benzimidazoles show promise for controlling the problem fungi in beet storage piles.

Literature Cited

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