Effect of Fungus Infection on Respiration and Reducing Sugar Accumulation of Sugarbeet Roots and Use of Fungicides to Reduce Infection[1]

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Received for publication August 9, 1976

In recent years, the covering of sugarbeet storage piles has greatly reduced losses due to freezing and thawing. This practice, however, has not reduced losses from fungus deterioration of stored roots. In fact, pile covering often provides a favorable environment for fungus growth.

The objective of this research was to obtain information on the effect of fungus infection on sugarbeet roots and to determine whether fungicides would be an effective control measure.

Materials and Methods

The fungi used in these studies were isolates of *Penicillium* and *Botrytis* obtained from infected beet roots from a storage pile at Quincy, Washington. To study the effect of infection by these fungi, a method was developed to obtain predictable amounts of infection on roots. Roots were injured, using a small 2.5x5x15 cm board pierced by 12 small nails within a circular area 3 cm in diameter and protruding 3 mm through the board. The nail points were pressed against the root surface and then rotated to produce a circular injury 3 cm in diameter and 3 mm deep. The injured area was inoculated, and the roots were stored under humidity (98%) and temperature (15°C) conditions favorable for infection. Fungus inoculum was prepared from colonies grown in petri dishes on potato dextrose agar (PDA). The agar disk with the fungus culture was chopped in a blender for a very brief period of time so as not to liquify the agar. The agar was then separated from the mycelium and spores by straining through cheesecloth.

Respiration rates were determined using a flow-through system. Carbon dioxide concentrations in the air stream were determined with an automated switching system and an infrared analyzer. The amount of

[1]Cooperative Investigations of the Agricultural Research Service, U.S. Department of Agriculture; the Beet Sugar Development Foundation; and the Utah State Agricultural Experiment Station. Approved as Journal Paper No. 2103. Utah Agricultural Experiment Station, Logan, Utah 84322.

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reducing sugar accumulated was determined using dinitrosalicylic acid on leaded juice (1).[3] Measurements of respiration rate and reducing sugars were made three weeks after roots were inoculated, during which time the roots were held at 15°C.

Fungicides were initially evaluated by measuring inhibition of fungus growth on an agar medium. Disks of filter paper saturated with a particular concentration of fungicide were positioned equidistantly around the outer edge of a petri dish containing 20 ml of PDA. The fungus was seeded in the center and allowed to grow toward the filter paper disks (Fig. 1). Inhibition of growth was measured after two to three days.

Superior fungicides selected by this method were tested directly on roots. Injured roots were inoculated and then treated with spray applications of 100, 250, 500, 750, 1000, and 1500 ppm of fungicide. Evaluation of fungicide effectiveness was based on visual observation of fungus growth and measurement of root respiration rate.

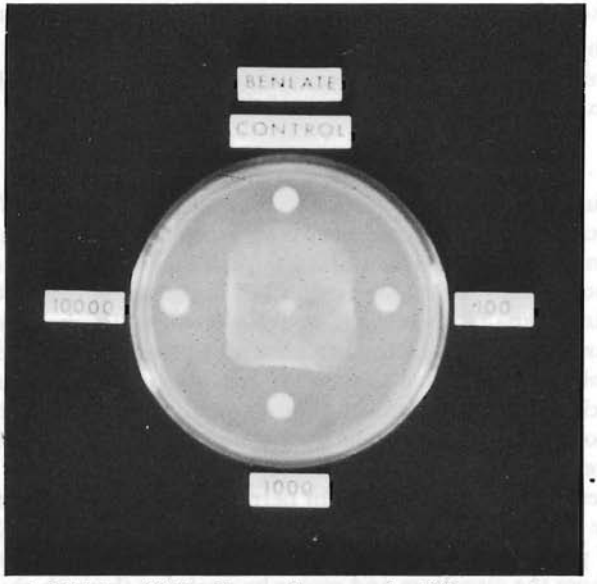

Figure 1.—Inhibition of *Botrytis* growth on agar by different concentrations of fungicide.

Results

Effect of fungus infection on root respiration rate and reducing sugar accumulation. Respiration rate increased as the percentage of surface area infected increased, as shown in Fig. 2. A correlation of .93 was obtained between these two factors. Roots with 20% of their surface area infected with *Botrytis* had a 100% higher respiration rate than injured but uninfected controls. Similar results occurred with roots infected with *Penicil-*

[3]Numbers in parentheses refer to literature cited.

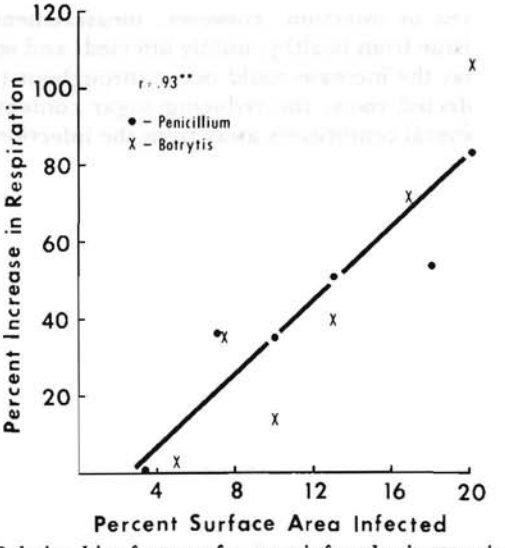

Figure 2.—Relationship of root surface area infected to increase in respiration rate.

lium. Injured but noninoculated roots, which had little or no fungus infection, had less than a 5% increase in respiration rate.

The results relating amount of reducing sugars accumulated with percentage of root surface area infected are presented in Fig. 3. When 15% of the root surface area was infected, there was a three-fold increase in reducing sugars compared to uninfected roots. This increase was highest in

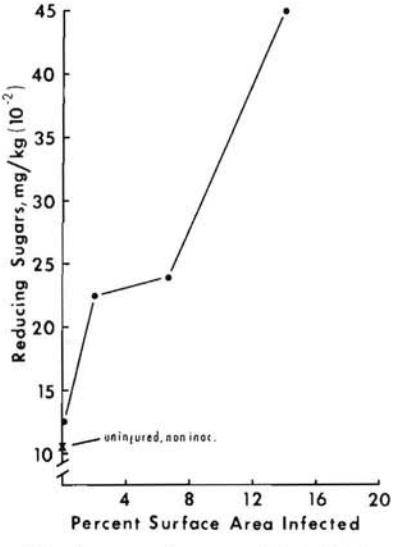

Figure 3.—Relationship of root surface area infected to increase in reducing sugars accumulated.

the immediate area of infection. However, measurements of reducing sugar content of tissue from healthy, mildly infected, and severely infected roots indicated that the increase could occur throughout the root (Table 1). In severely infected roots, the reducing sugar content is greatly increased in tissue several centimeters away from the infection site, as shown in Table 1.

Table 1. Reducing sugar content of apparently healthy tissue from infected roots.

Root Condition	Tissue Location	Reducing Sugar (mg/gm Fresh Wt.)
Healthy	2 cm from surface	1.9
	Center of root	1.9
Mildly Infected	2 cm from infected area[1]	7.4
	Center of root	1.8
Severely infected	2 cm from infected area	15.0
	Center of root	13.6

[1]All tissue showing discoloration symptoms of infection was removed before sampling.

Evaluation of fungicides in reducing infection of sugarbeet roots by Penicillium and Botrytis. Sixteen fungicides (Table 2) were evaluated by the agar plate method. Of the four fungicides causing the greatest inhibition of fungus growth (Table 3), benomyl and thiabendazole were selected for testing on sugarbeet roots.

Table 2. Fungicides tested as a control for *Penicillium* and *Botrytis*.

Benlate (benomyl)	OAC 5
Botran	OAC 258
Bravo 6F	Pyrocatechol
Dowicide A	Steri-Seal "D" (SOPP)
Dowicide 1	Steri-Seal D-D-400
Fisons NC16598	Terraclor (PCNB)
Hydrogen peroxide	Terrazole
Mertect (thiabendazole)	Zinc Omadine

Table 3. Inhibition of fungus growth on agar medium by four fungicides.

		Inhibition in percentage of control for each concentration		
Fungus	Fungicide	100ppm*	1,000ppm	10,000ppm
Botrytis	Benomyl	44	51	56
	Thiabendazole	27	54	57
	SOPP	0	23	54
	PCNB	13	18	26
Penicillium	Benomyl	21	31	49
	Thiabendazole	0	27	41
	SOPP	0	14	33
	PCNB	0	7	9

*Concentrations were adjusted to comparable amounts of active ingredient for each fungicide.

Figure 4.—Transverse and surface views of injured roots inoculated with *Penicillium*. No infection is present on root treated with spray application of 500 ppm thiabendazole.

Based on visual observation of fungus growth (Fig. 4) and measurement of root respiration rate, complete control of infection was obtained by a spray application of either fungicide at a concentration of 500 ppm. A spray application of 500 ppm similar to the one we used was estimated by Merck and Company to leave about 0.5 ppm fungicide on an average (0.45 kg) root. Fungus growth on roots treated with concentrations as low as 100 ppm was greatly reduced compared to untreated roots.

Our observations indicated that injury was essential for fungus infection. Many uninjured roots were inoculated, but none became infected. It was also noted that inoculation was necessary to obtain high levels of infection when using washed roots but was not necessary when using unwashed roots. This indicated an abundance of inoculum present in soil adhering to roots, as they would normally go into storage.

Conclusions

The results of these experiments indicate that within a period of one month the respiration rate of stored sugarbeet roots will double if approximately 20% of their surface area is infected by fungi. The data also indi-

cate that there will be over a three-fold increase in reducing sugars with similar amounts of infection.

A spray application of benonyl, or thiabendazole, at a concentration of 500 ppm will prevent infection by *Penicillium* and *Botrytis* of injured sugarbeet roots during the initial storage period.

Root injury before storage is probably the most significant factor determining the extent of fungus infection. There is probably sufficient fungus inoculum in soil adhering to roots to initiate infection when conditions are favorable for fungus growth during root storage.

Literature Cited

(1) MILLER, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31:426.