THE USE OF FUNGICIDAL SPRAYS TO REDUCE MOLD COUNTS IN BLACK RASPBERRIES

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THESIS

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E S Beneke

Major professor

E. S. Beneke

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ABSTRACT

Mold count tolerances for processed black raspberry fruit are difficult, if not impossible to maintain, during hot, humid weather. Because of this fact fungicidal treatments were applied to fruit while still on the bush to ascertain whether or not the mold counts could be kept below the tolerance

Five fungicides, ferbam, nabam, dichlone, glyodin and actidione, were applied to both Cumberland and Logan variety black raspberries at varying intervals before harvest time. These fungicides were used singly and in combination with a spreader-sticker Triton B-1956. Fruit was harvested from these plots at normal picking times and mold counts were made from it to check possible differences in the mold counts.

Growth studies were carried out using these five fungicides and four of the fungi which are most frequently isolated from black raspberry fruit with high mold counts. The fungicides were incorporated into media at field concentrations and at three other concentrations above and below this level. The fungi were grown upon these media and their growth, as indicated by colony diameter increase, was measured and compared against that of a check. An indication of the inhibiting power of the fungicides was thus obtained.

The results of the field studies and growth studies were then compared to see which fungicides, if any, were effective in reducing the growth of the fungi which cause the high mold counts in black respberries. It was found that the results of the growth studies follow quite closely the results of the field studies. Of the fungicides used, however, only ferbam seemed to have merit. It inhibited all fungal growth at all concentrations in the growth studies and it reduced the mold counts in the fruits obtained from the ferbam sprayed plots.

It is believed that a program of preharvest sprays to lower mold counts in black raspberries could be effective but further investigations into the practicality of such a program are necessary.

William Justin Young
Department of Botany
and Plant Pathology

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by

William Justin Young

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CHAPTER I

INTRODUCTION

The Federal Food and Drug Administration in its efforts to keep food products free from decomposition and spoilage has set certain standards for canned and frozen black raspberries. One of these states that the mold count in the packaged product must be less than 40 per cent of the microscopic fields of a standard Howard test chamber. This standard, while reasonable in normal weather, becomes extremely difficult, if not impossible, to maintain during periods of hot, humid weather.

In the fall of 1949 a considerable quantity of canned and frozen black raspberries was seized by the Federal Food and Drug Administration. Their claim was that these berries had excessive mold counts and thereby were spoiled and unfit for human consumption. In the summer of 1950, during the processing period, a hot, humid spell caused mold counts to go so high that the processors were forced to suspend operations. These actions naturally caused worry to both processor and grower alike, for Michigan packs a sizeable portion of the frozen and 80 per cent of the canned black raspberries in the United States.

It was the hope of the processor that some means could be devised for treating the berry at harvest time to reduce the mold count.

Accordingly, field dips were tried as the berries were picked, but these had little apparent effect on the increase in mold count. It was discovered at this time that under the conditions of hot and humid weather the fungal hyphae were present in the ripe berry even while still on the bush (3). As a result it was deemed advisable to see if applications of fungicides to the berries a short time before harvest would have any effect on germination of the fungal spores and their subsequent growth into the raspberry. The writer selected several representatives from the various classes of organic fungicides for application to the raspberry plants. These were applied to the raspberry plants a short time before harvest and their effect on subsequent mold counts was studied.

CHAPTER II

REVIEW OF LITERATURE

The use of the mold count as a criterium for judging food products dates back to 1911. In that year Howard (8) suggested a mold counting method to aid in the judging of quality in tomato products. Briefly he suggested the following procedure. A Thoma-Zeiss blood counting chamber was used and the microscope was adjusted so that each field of the chamber covered 1.5 millimeters. Sufficient pulp was placed in the chamber to cover it to a depth of 0.1 millimeters. This gave a volume of one and one-half cubic millimeters for each field. If an aggregate of not more than three mycelial fragments covered one-sixth or more of the diameter of a field, the field was considered positive. When 25 to 50 microscopic fields were counted and 25 per cent or more of the fields were positive, the canned product could be condemned. In 1917 Howard and Stevenson (9) revised these tolerances to allow a maximum of 66 per cent positive microscopic fields in the total fields counted. A change was made to the Howard mold counting chamber at this time.

In 1924 Needham and Fellers (13) applied the Howard method of mold counting to the detection of spoilage in canned berry products. They worked with canned strawberry and blackberry products and found that from five to ten per cent of decayed or moldy berries in a lot could give mold counts of 40 per cent and higher. They also found that soft overripe fruit could increase mold counts even though no visible mold was present.

In 1939 the mold counting standard was changed to allow only a 50 per cent mold count and in 1938 this was decreased to 25 per cent for tomato juice. In 1940 the standards were changed again, this time a 15 per cent mold count was allowed for tomato juice and 40 per cent for other tomato products. These are the standards that were in effect for tomato products at the time of this work. However, at the same time the indicated range for seizure of canned black raspberry fruits was considered somewhere around 20 to 40 per cent although no definite tolerance was established by the Food and Drug Administration.

Although the mold count was originally intended for use with tomato products its use has been broadened so that all types of canned and frozen berry products are subjected to it. In the fall and winter of 1949 a considerable quantity of black raspberries was condemned on the basis of this application. The Federal Food and Drug Administration claimed that the mold counts were excessive and that the berries were unfit for human consumption. Beneke (2) in 1950 noted, however, that in canned raspberry products when the mold count ran to 100 per cent of the microscopic fields of the test chamber, the volume occupied was only .00162 per cent.

As a result of these excessive mold counts Fabian *et al.* (3) in 1950 began a series of investigations concerning the factors that cause high mold counts. Some possible control measures also were investigated. Using ferbam, captan, copper 3-phen, Dowicide A, and dehydroacetic acid as the fungicides, numerous combinations of dips were tried. Flats of fruit were dipped into the fungicide just after picking and after the fruit had been brought to the cannery. None of these treatments significantly reduced the percentage of mold count. They found that under weather conditions of high heat and humidity the mold was already growing in the berry at picking time. This naturally meant that an original high mold count was present in the berries no matter how carefully they were handled. Beneke (3) found the fungi primarily responsible for the high mold counts to be *Alternaria humicola*, and *Cladosporium* sp. Other fungi frequently isolated were *Pullularia pullulans*, *Botrytis cinerea*, *Penicillium* sp., *Fusarium* sp., *Rhizopus* sp., *Trichoderma* sp., *Aspergillus* sp., *Oospora* sp., *Monilia* sp., and *Mucor* sp.

Since it was known that the fungus may be present in the ripe berry it was thought that perhaps a fungicide applied to the green fruit, before the fungus had a chance to penetrate the berry, might provide a deposit which would prevent spore germination or inhibit mycelial growth. In the tomato industry field sprays have been used with success to keep down fungus growth which leads to high mold counts. In 1944 McNew (10) used preharvest fungicidal sprays in an attempt to reduce losses and trim costs on canned tomato products because of high mold counts caused by Colletotrichum phomoides. He found ferbam to be the most effective fungicide used. In 1948 Fulton (4) found that tomatoes were relatively resistant to Colletotrichum phomoides when they were in the green stage but that susceptibility began ten days before harvest time and increased until the harvest time. In 1949 Schroeder (14) found that zerlate, nabam and ferbam were very effective in reducing Colletotrichum phomoides infections. In 1950 Greenspan and McKellar (6) were able to reduce mold counts by preharvest sprays of peracetic acid.

These results tend to show that high mold counts can be reduced by preharvest sprays on the green and semi-ripe fruit, at least in the tomato industry. This present work was carried out in the summer of 1951 to ascertain if preharvest field sprays might be applicable to the raspberry as well as the tomato industry as a means of reducing mold counts.

CHAPTER III

FIELD METHODS

A grower was selected in the Benton Harbor area who grew both the Cumberland and the Logan varieties of black raspberry, these being the varieties in current commercial use in Michigan. Identical spray plots were set up in plantings of each variety and consisted of single row strips 100 feet in length. In the case of the Cumberland variety, a second spray was applied to the southern 50 feet of the plots. The actidione plots of the Cumberland variety received three sprays with the third spray having been applied to the southern 25 feet of the plot. The Logan plots received one spray each of all the fungicides twelve days before harvest and a second spray on the actidione plots four days before harvest. The Cumberland plots received two sprays of all fungicides, one sixteen and the other eight days before harvest. A third spray of actidione was applied four days before harvest. The berries were harvested at normal picking times, brought to the cannery, and frozen for storage until mold counts could be made.

The five fungicides used in these tests were ferbam which is the ferric salt of dimethyldithiocarbamate, nabam which is the disodium salt of ethylene bisdithiocarbamate, dichlone which is 2:3-dichloro-1:4-naphthoquinone, glyodin which is 2-heptadecylglyoxalidine, and actidione which is the antibiotic cyclohexamide. These were used singly and in combination with a spreader sticker Triton B-1956 which is a phthalic glycerol alkyd resin. The dosages were as follows: ferbam, one and one-half pounds per 100 gallons; nabam, two quarts per 100 gallons; plus one-half pound of $ZnSO_4$; glyodin, one and one-half quarts per 100 gallons plus one-half pound of lime; dichlone, one-eighth pound per 100 gallons and actidione two parts per million or 0.76 grams. These were all recommended dosages except for dichlone where a lower rate was applied to avoid possible injury.

The materials were applied with a John Bean sprayer with a seven and one-half gallon pump at about 250 pounds pressure per square inch. A Friend brand spraygun was used for application. The bushes were sprayed until the run-off point was reached. This amounted to about 400 gallons per acre.

The picking of the raspberry fruits on the plots was carried on at the same time that the grower picked his planting. One and one-half quarts were picked at random from each spray plot. The berries were placed in polyethylene bags and taken immediately to the processor. Here they were held for several hours to simulate actual processing time and then frozen for storage until the mold counts could be made. The counts were made at the Benton Harbor plant of the Michigan Fruit Canners, Incorporated and were made by the inspector employed by the company. A Cefaly power pulper with a 0.027 inch screen was used to grind the berries for a puree and the counts were made with the Howard mold count chamber in accordance with official Association of Official Agricultural Chemists methods.

CHAPTER IV

FIELD RESULTS

When all the mold counts had been made from the berry samples taken from the various fungicide plots it was noticed that there was wide variation in the mold counts between plots. Some of these variations in the mold counts may be due to weather conditions at the time of picking and differences in fungicides, but sometimes they may be due to variation in samples used for mold counts. Fabian et al. (3) in 1950 found mold counts ranging from 8 to 52 per cent in various samples taken from a single 30 pound tin of frozen black raspberries.

The mold counts, which were made from the Logan variety of raspberries picked July 6, 1951, ran from zero to twelve per cent (Table 1). This showed very little variation between plots at that time and was no doubt due to: (1) the lack of rainfall before picking and the resultant lack of high humidity (Table 2), and (2) the fact that this was the first picking reduced the possibility of overripe berries being included. A few overripe berries can noticeably increase the per cent of mold count in a given quantity of berries. The mold counts, as shown in Table 1, made on the Logan variety raspberries which were picked on July 10, 1951 began to show wider differences. There had been one and one-half inches of rain during July eighth and ninth (Table 2) and this provided better growing conditions for the fungi.

TABLE 1.

EFFECT OF FUNGICIDES ON BLACK RASPBERRY

MOLD COUNTS

Fungicides	Logan		Cumberland	
	July 6	July 10		July 13
Ferbam	4[1]	8	4 4	16 24
Ferbam - B 1956	6	4	10 10	10 4
Actidione	0 4 2	14 18 14	-- 10 22	-- 10 6
Actidione - B 1956	10 4 0	8 4 24	10 18 6	6 28 28
Nabam	12	8	16 8	38 20
Nabam - B 1956	0	14	26 16	34 42
Glyodin	6	44	10 10	23 44
Glyodin - B 1956	12	8	14 16	84 8
Dichlone	2	24	6 16	64 35
Dichlone - B 1956	4	26	13 4	4 92
Control	6	20	4	18

1. First number appearing is the mold count for one spray, the second for two sprays, and the third for three sprays.

TABLE 2.

OFFICIAL WEATHER BUREAU DATA

United States Weather Bureau
Benton Harbor, Michigan

Date	Median	High	Low	Rainfall	Time
June 25	64	79	52		
26	73	83	89	Trace	
27	76	83	60	Trace	
28	63	76	60		
29	72	74	50		
30	72	74	52		
July 1	71	73	54		
2	76	79	51		
3	79	82	79		
4	58	79	55	Trace	
5	61	66	54	Trace	
6					
7	84	86	45		
8	69	84	63	.55"	1:00 - 6 a.m. 4:00 - 6 p.m.
9	81	82	63	1.00"	1:00 - 4 a.m. 6:30 - 11 p.m.
10	69	82	58	Trace	
11	67	73	62		
12	66	71	58		
13	73	76	51		

Also this was the second picking of these plots and the chances for overripe berries to be included was greater. Table 1 shows that the mold counts made from the fruit in the ferbam and nabam sprayed plots were quite low while the fruit from the actidione and glyodin sprayed plots gave mold counts which showed wide variation. The mold counts from the actidione treated berries ranged from 0 to 24 and those on the glyodin treated fruit ranged from 6 to 44. The dichlone treated berries gave moderately high counts of 24 and 26 per cent.

The first picking of the Cumberland variety plots was also made on July 10, 1951. Again as with the Logans (see Table 1) there was wide variation between spray plots. When the Cumberlands had two sprays of fungicide before picking there did not seem to be any indication that a second spray had been any more effective in reducing mold counts than just one spray. The ferbam sprayed plots continued to show the lowest mold counts. The nabam sprayed plots still gave low mold counts although somewhat higher than the counts made on the Logans. The glyodin and dichlone sprayed plots gave considerably lower mold counts than those on similarly tested Logans picked on the same date. The mold counts for the actidione plots remained about the same on the Cumberlands as they had on the Logans. One would normally expect slightly lower mold counts on the Cumberlands picked on July 10, 1951 because it was the first picking for the Cumberlands and the last picking for the Logans.

The mold counts made from the berries picked on July 13, 1951 showed an increase in percentage (see Table 1). This was probably due to the favorable conditions for good fungal growth after the rains of July eighth and ninth and the fact that this was the last picking. Also there may have been washing off of the fungicide during the rainy period. The ferbam sprayed fruit again showed the lowest mold counts. These were somewhat higher than the preceding counts for the ferbam plots but not enough to make a significant difference. Mold counts made from nabam sprayed fruit showed a substantial increase at this time. It is thought that this may have resulted from washing off of the fungicide. Mold counts made from the actidione sprayed fruit continued to be erratic while both the glyodin and the dichlone sprayed plots yielded fruit which had higher mold counts.

The results obtained from the use of the spreader sticker were inconclusive. In some cases the mold count showed a reduction when the spreader was incorporated with the fungicide and in other cases the mold count was higher. The spreader sticker was, however, of considerable help in wetting the green fruit and therefore would seem to have a place in the spray program.

CHAPTER V

LABORATORY METHODS

This project was initiated late in the fruiting season of the black raspberry and consequently no time was available to check the effect of the fungicides chosen on the fungi known to occur at high frequencies in the black raspberry. As it was desirable to know the effect of the fungicide on the organisms *in vitro*, a series of growth rate comparisons were run later in which varying amounts of the fungicides used were incorporated into an agar medium. The rate of colony growth on the fungicide was compared with that of untreated checks.

The organisms used in this experiment were *Alternaria humicola* Oudemans, *Cladosporium* sp., *Botrytis cinerea* Persoon, and *Fusarium* sp. *A. humicola* and *Cladosporium* sp. are the most frequently isolated fungi from black raspberry fruits according to Beneke (3). *B. cinerea* and *Fusarium* sp. are also quite regularly found in black raspberry fruit.

The fungicides used were nabam, ferbam, glyodin, dichlone and actidione. These were tested at concentrations which were equivalent to those used in the field tests and at concentrations above and below this amount. In the case of nabam concentrations of 1250, 2500, 5000, and 7500 parts per million were used, 5000 being the normal field concentration. Dichlone was used at concentrations of 150, 250, 500 and 1000 parts per million. One hundred and fifty parts per million was the concentration used in the field. Glyodin was used at concentrations of 500, 1000, 3700 and 5000 parts per million, 3700 being the concentration used in the field. Ferbam was used at 5000, 1000, 1800, and 2500 parts per million. Eighteen hundred parts per million was the concentration used in the field. Actidione was used at 2, 5, 10, and 20 parts per million. Two parts per million was the concentration used in the field.

A two per cent malt extract agar was selected for the growth medium. This was sterilized in an autoclave at 15 pounds pressure for 15 minutes and the fungicide added aseptically to the cooled medium just before it was poured into Petri plates. The plates were inoculated with five and one-half millimeter agar plugs cut with a flame sterilized cork borer from the periphery of five-day old cultures of the fungi used. These tests were run at 24 and 28 degrees Centigrade and the daily increase in the diameter of the colony was measured for four days. Three replications were made of each combination and the final figures are an average of these.

CHAPTER VI

LABORATORY RESULTS

The results obtained from the growth studies when the fungicides were incorporated into the media proved to be quite interesting. These results are shown in Tables 3, 4, 5 and 6. The fungicides which had been used on the spray plots whose fruit gave the lowest mold counts usually showed the most inhibition of fungal growth when used for the studies.

Ferbam was quite inhibitory to fungus growth when incorporated into an agar medium. The fruit from the plots which were sprayed with ferbam also had low mold counts. The concentration used in the field test was 1800 parts per million or one and one-half pounds per 100 gallons while the concentrations used in the growth studies were 500, 1000, 1800, and 2500 parts per million. No fungal growth was observed on any of the plates during the period of observation and measurement, as shown in Figure I. Some sparse aerial mycelium was noted on the surface of the inoculating plug but no growth was observed on the surface of the agar containing the fungicide.

Nabam (see Figure II) which showed results in the field similar to those of ferbam also inhibited all fungi tested at all concentrations used. These were 1250, 2500, 5000, and 7500 parts per million. Apparently the cause of the high mold counts obtained from the fruit on the last

TABLE 3.

EFFECT OF FUNGICIDES ON GROWTH RATES OF ALTERNARIA HUMICOLA

Chemical	Hours	Temp.	Control	Concentration in parts per million			
				150	250	500	1000
Dichlone	24	24°	11[1]	12	11	11	-
		28°	12	12	13	12	7
	48	24°	17	19	18	17	8
		28°	18	20	21	19	10
	72	24°	26	27	27	25	14.5
		28°	27	29	29	26	16
	96	24°	34	37	34	32	20
		28°	35	38	36	34	22
				500	1000	3200	5000
Glyodin	24	24°	13	8	7	-	-
		28°	14	10	7	-	-
	48	24°	21	12	9	8	-
		28°	21	13.5	9	8	6
	72	24°	32	17	12	11	8
		28°	31	19	11	11.5	9
	96	24°	40	24	15	13	11
		28°	40	25	14	14	13
				1250	2500	5000	7500
Nabam	24	24°	9	-	-	-	-
		28°	11	-	-	-	-
	48	24°	13.5	-	-	-	-
		28°	19	-	-	-	-
	72	24°	21	-	-	-	-
		28°	25	-	-	-	-
	96	24°	31	-	-	-	-
		28°	37	-	-	-	-

1. Figures represent diameter of colony in millimeters.

TABLE 3. (continued)

Chemical	Hours	Temp.	Control	Concentration in parts per million			
				500	1000	1800	2500
Ferbam	24	24°	11	-	-	-	-
		28°	14	-	-	-	-
	48	24°	20	-	-	-	-
		28°	22	-	-	-	-
	72	24°	30	-	-	-	-
		28°	32	-	-	-	-
	96	24°	40	-	-	-	-
		28°	41	-	-	-	-
				2	5	10	20
Actidione	24	24°	6	-	-	-	-
		28°	7	-	-	-	-
	48	24°	14	18	15	14	14
		28°	15	16	16	13	14
	72	24°	22	25	24	24	21
		28°	28	25	25.5	23	23
	96	24°	29	31	32	33	29
		28°	35	34	34	32	32

TABLE 4.

EFFECT OF FUNGICIDES ON GROWTH RATES OF BOTRYTIS CINEREA

Chemical	Hours	Temp.	Control	Concentration in parts per million			
				150	250	500	1000
Dichlone	24	24°	15[1]	12	11	9	-
		28°	10	9	9	8	-
	48	24°	35	27	26	23	-
		28°	28	20	20	20	6
	72	24°	56	38	38	35	11
		28°	36	31	29	27	10
	96	24°	78	47	46	40	13
		28°	43	39	36	34	12
				500	1000	3700	5000
Glyodin	24	24°	16	8	7	6	-
		28°	10	-	-	-	-
	48	24°	34	14	11.5	10	8
		28°	19	10	8	7.5	-
	72	24°	60	28	16	14	10
		28°	28	17	13	10	-
	96	24°	77	39	25	18	13
		28°	40	22	20	12	-
				1250	2500	5000	7500
Nabam	24	24°	14	-	-	-	-
		28°	10	-	-	-	-
	48	24°	35	-	-	-	-
		28°	24	-	-	-	-
	72	24°	58	-	-	-	-
		28°	33	-	-	-	-
	96	24°	80	-	-	-	-
		28°	41	-	-	-	-

1. Figures represent diameter of colony in millimeters.

TABLE 4. (continued)

Chemical	Hours	Temp.	Control	Concentration in parts per million			
				500	1000	1800	2500
Ferbam	24	24°	17	-	-	-	-
		28°	11	-	-	-	-
	48	24°	37	-	-	-	-
		28°	20	-	-	-	-
	72	24°	62	-	-	-	-
		28°	34	-	-	-	-
	96	24°	80	-	-	-	-
		28°	46	-	-	-	-
				2	5	10	20
Actidione	24	24°	16	13.5	10	9	-
		28°	13	10	10	-	-
	48	24°	36	30	24	19	11
		28°	24	19	19	11	9
	72	24°	59	49	40.5	30.5	24
		28°	34	30	29	20	12
	96	24°	80	61	52	51	36
		28°	45	40	37	27	17

TABLE 5.

EFFECT OF FUNGICIDES ON GROWTH RATES OF *CLADOSPORIUM* SP.

Chemical	Hours	Temp.	Control	Concentration in parts per million			
				150	250	500	1000
Dichlone	24	24°	6.5[1]	7	7	6	-
		28°	5.5	-	-	-	-
	48	24°	9	10	9	8	-
		28°	6.5	7	6	6	-
	72	24°	13	13	12	10	-
		28°	10	10	7	7	-
	96	24°	16	16	14	12	-
		28°	12	11	9	10	-
				500	1000	3700	5000
Glyodin	24	24°	7	-	-	-	-
		28°	-	-	-	-	-
	48	24°	9	6	5.5	-	-
		28°	-	-	-	-	-
	72	24°	11	8	7	6.5	-
		28°	7	6	-	-	-
	96	24°	14	11	9	8	-
		28°	8	8	7	6	-
				1250	2500	5000	7500
Nabam	24	24°	6	-	-	-	-
		28°	-	-	-	-	-
	48	24°	9	-	-	-	-
		28°	-	-	-	-	-
	72	24°	12	-	-	-	-
		28°	6	-	-	-	-
	96	24°	14	-	-	-	-
		28°	7	-	-	-	-

1. Figures represent diameter of colony in millimeters.

TABLE 5. (continued)

Chemical	Hours	Temp.	Control	Concentration in parts per million			
				500	1000	1800	2500
Ferbam	24	24°	6	-	-	-	-
		28°	-	-	-	-	-
	48	24°	10	-	-	-	-
		28°	6	-	-	-	-
	72	24°	14	-	-	-	-
		28°	8	-	-	-	-
	96	24°	16	-	-	-	-
		28°	9	-	-	-	-
				2	5	10	20
Actidione	24	24°	7	-	-	-	-
		28°	-	-	-	-	-
	48	24°	9	8	7	7	6
		28°	6	6	6	-	-
	72	24°	18	15	12	10	9
		28°	7	8	8	7	6
	96	24°	21	18	15	13	11
		28°	10	10	9.5	8	7.5

TABLE 6.

EFFECT OF FUNGICIDES ON GROWTH RATES OF *FUSARIUM* SP.

Chemical	Hours	Temp.	Control	Concentration in parts per million			
				150	250	500	1000
Dichlone	24	24°	-[1]	7	6	-	-
		28°	6	8	6.5	-	-
	48	24°	6	11	10	6	-
		28°	13	15	11	7	-
	72	24°	15	24	22	10	-
		28°	20	28	25	16	-
	96	24°	24	33	30	14	-
		28°	30	39	32	23	-
				500	1000	3700	5000
Glyodin	24	24°	13	10	8	7	6.5
		28°	18	11	9	8	7
	48	24°	29	23	16	14	14
		28°	34	23	20	15	14
	72	24°	42	35	25	20	18
		28°	49	37	32	24	19
	96	24°	60	45	33	24	21
		28°	64	50	45	30	23
				1250	2500	5000	7500
Nabam	24	24°	9	-	-	-	-
		28°	11	-	-	-	-
	48	24°	18	-	-	-	-
		28°	20	-	-	-	-
	72	24°	29	-	-	-	-
		28°	31	-	-	-	-
	96	24°	42	-	-	-	-
		28°	44	-	-	-	-

[1] Figures represent diameter of colony in millimeters.

TABLE 6. (continued)

Chemical	Hours	Temp.	Control	Concentration in parts per million			
				500	1000	1800	2500
Ferbam	24	24°	-	-	-	-	-
		28°	9	-	-	-	-
	48	24°	12	-	-	-	-
		28°	21	-	-	-	-
	72	24°	24	-	-	-	-
		28°	36	-	-	-	-
	96	24°	35	-	-	-	-
		28°	44	-	-	-	-
				2	5	10	20
Actidione	24	24°	-	-	-	-	-
		28°	9	-	-	-	-
	48	24°	9	6.5	6	-	-
		28°	14	8	7	8	-
	72	24°	18	11	7	-	-
		28°	25	14	9	7	-
	96	24°	35	19	11	7	-
		28°	39	22	13	10	-

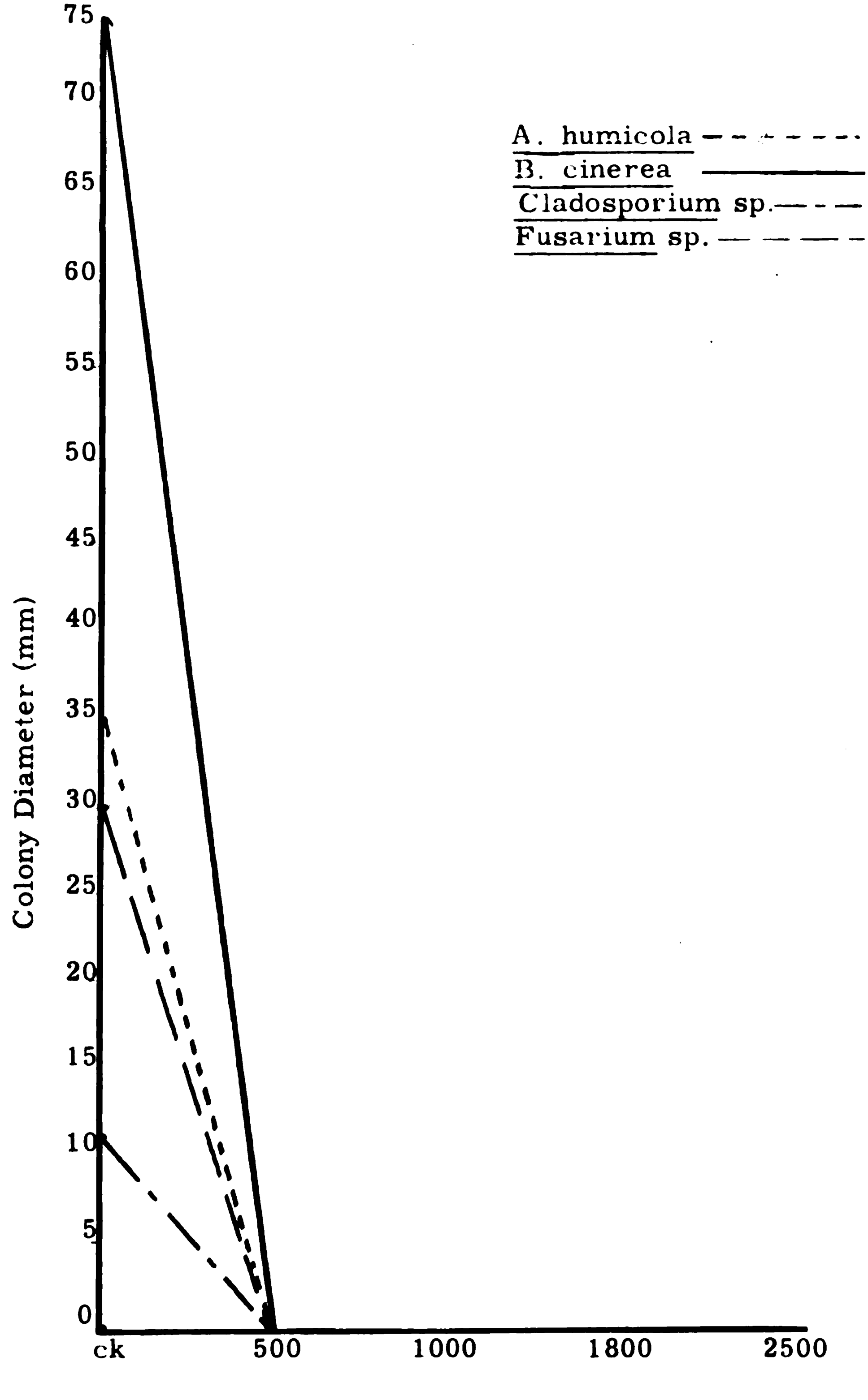

Figure I. Fungal colony growth at 96 hours with various concentrations of ferbam

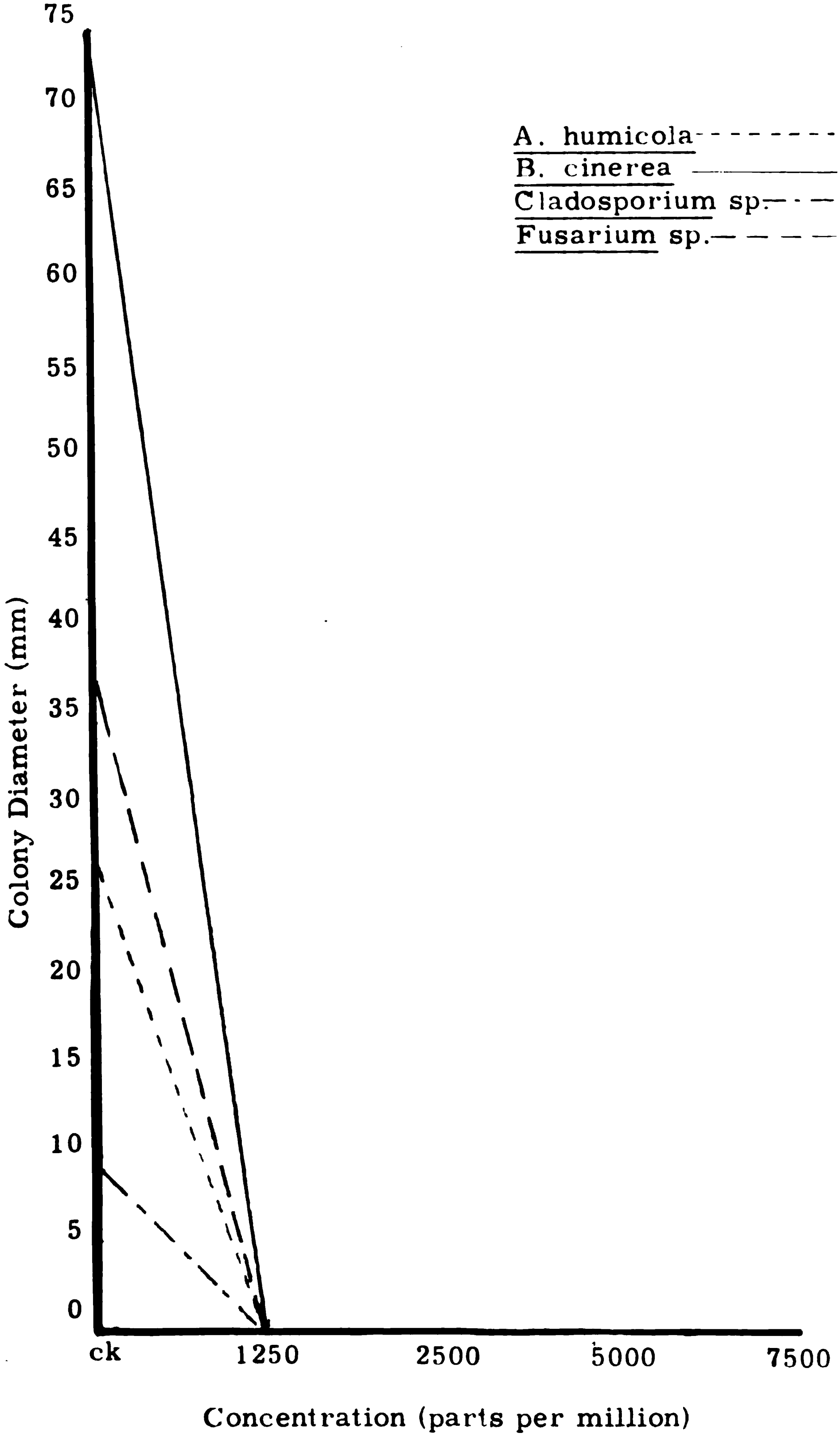

Figure II. Fungal colony growth at 96 hours with various concentrations of nabam picking date was due to the fungicide being washed from the fruit by rain or from natural breakdown of the fungicide rather than its inability to inhibit fungal growth.

Glyodin (as shown in Figure III) reduced the growth of all organisms tested but inhibited only Cladosporium sp. at 5000 parts per million, a concentration above the 3700 parts per million used in the field. At 3700 parts per million the growth of Cladosporium sp. was reduced by 50 per cent while the growth of A. humicola was reduced by 66 per cent. There was still some growth at 5000 parts per million however. Wellman and McCallan (16) in 1947 reported that the ED 50 of glyodin for spore germination of A. solani and S. fructicola was twelve parts per million. Generally, however, the concentration of a compound needed to inhibit mycelial growth is greater than the concentration needed to inhibit spore germination. Also there may be absorption effects occurring between the compound and the media.

The growth of Fusarium sp. was reduced by over 50 per cent at 3700 parts per million but there was still considerable growth at 5000 parts per million, which was over one-third the amount of growth in the control. The growth of B. cinerea was greatly reduced at 500 parts per million. Concentrations of 1000 and 3700 parts per million did not reduce growth much more than 500 parts per million. However, at 5000 parts per million there was another great reduction in the rate of

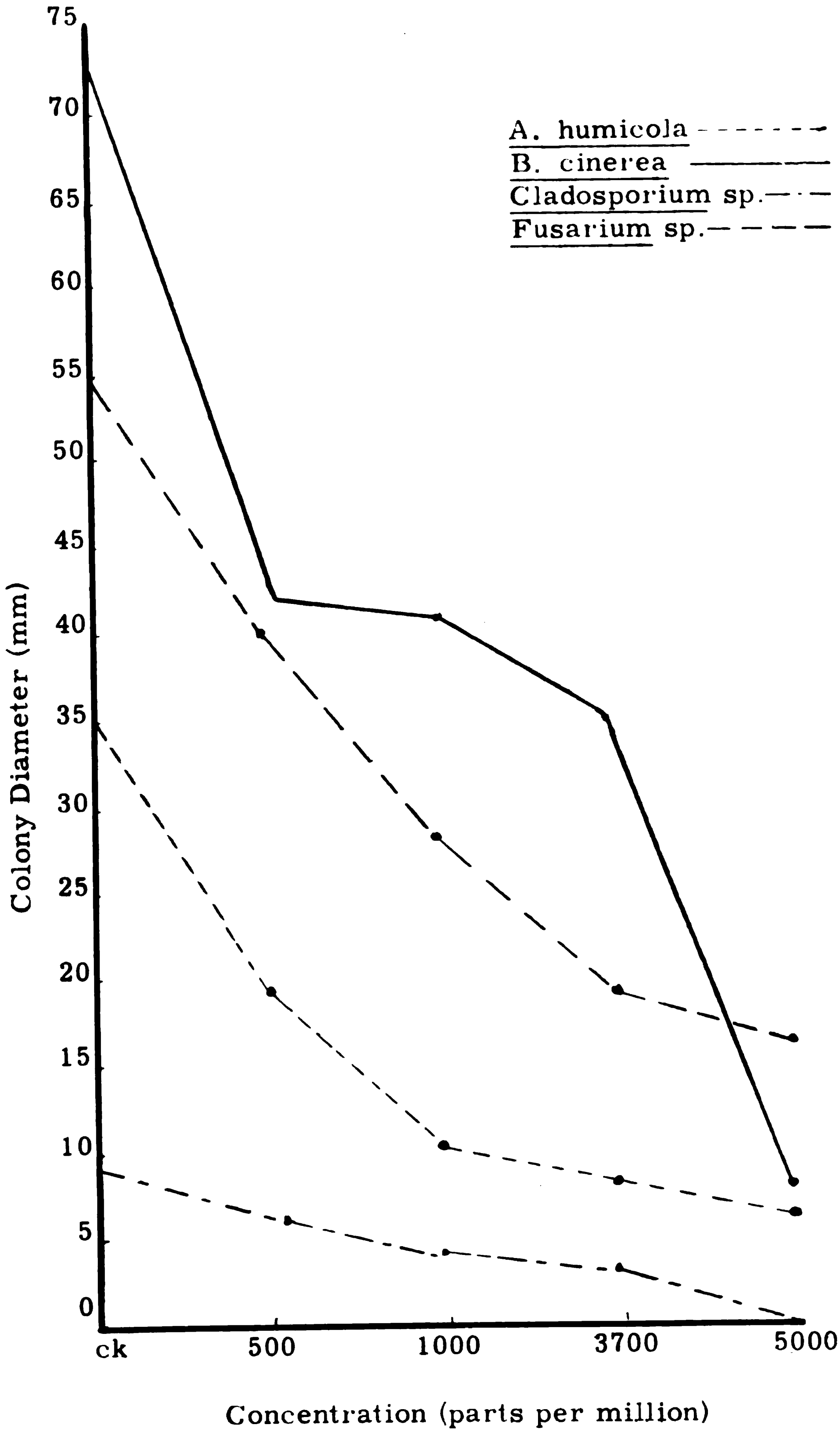

Figure III. Fungal colony growth at 96 hours with various concentrations of glyodin growth. The effect of different concentrations was also noticed when dichlone and glyodin were used in the growth studies. It is interesting to note that these three compounds showed similar sharp reduction in the growth of B. cinerea at the lowest concentrations used, with a leveling of growth rates through the intermediate concentrations and then a further sharp reduction at the highest levels.

Actidione gave very little inhibition at two parts per million (see Figure IV) which was also the concentration used in the field. The field results would indicate that actidione was somewhat better than indicated in vitro so there may be an absorption of the compound by the agar medium. Gottlieb et al. (5) reported adsorption of actidione by soil and in agar there may be a similar type action. Thanos (15) reported stimulation effects of actidione between one-half and ten parts per million on the germination of spores of C. cucumerinum and B. cinerea. In these studies the growth of A. humicola was stimulated to some extent by actidione. This stimulation was noticed at two, five, and ten parts per million. Above ten parts per million there was a decrease in growth. The growth of B. cinerea was reduced by 18 per cent at two parts per million but at 20 parts per million there was only a 50 per cent reduction in growth as compared to the control. The growth of Fusarium sp. was reduced by half at two parts per million and completely inhibited at twenty parts per million. Growth of

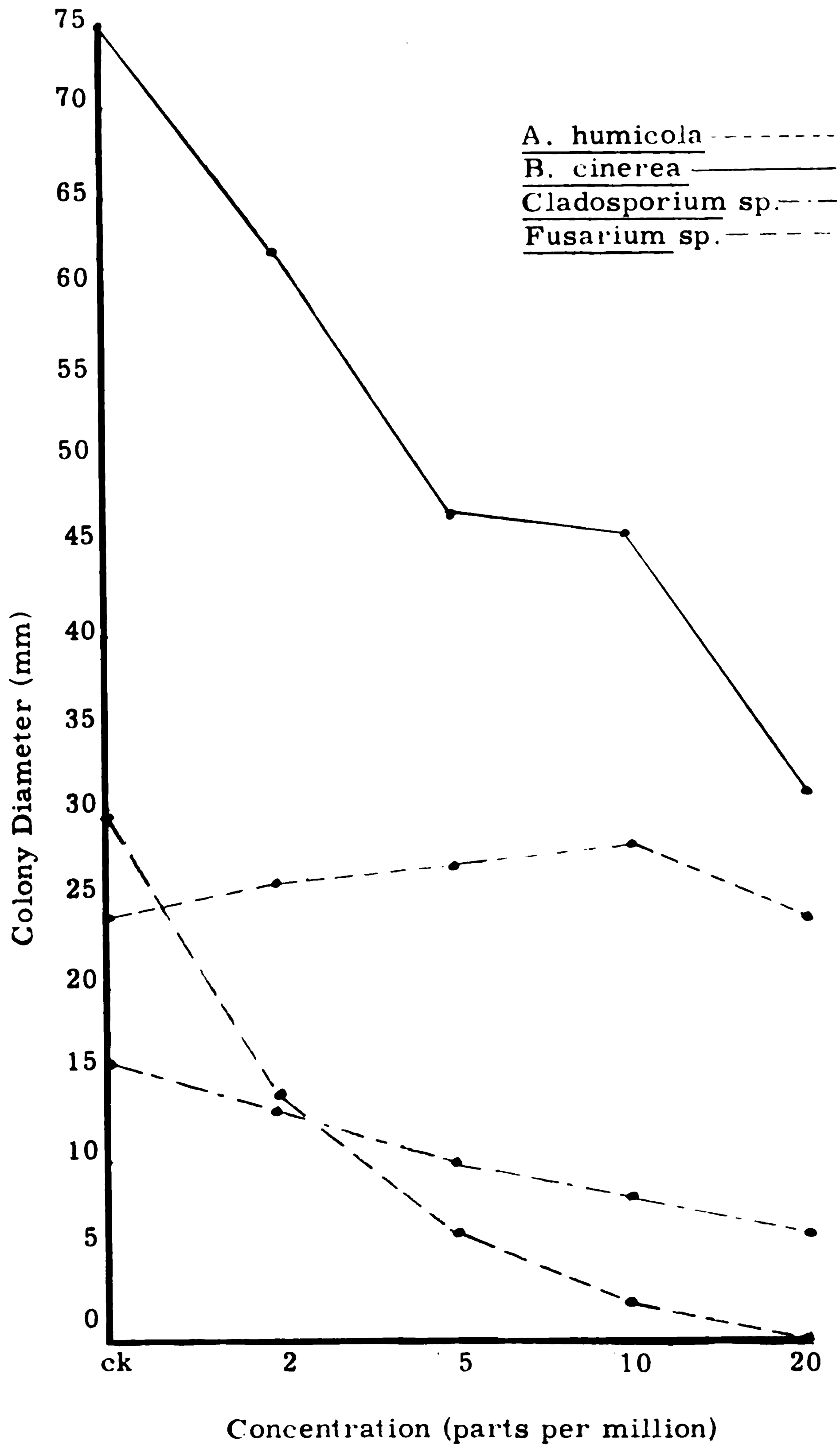

Figure IV. Fungal colony growth at 96 hours with various concentrations of actidione

Cladosporium sp. was slightly reduced at two parts per million but at twenty there was a 50 per cent reduction.

Dichlone (Figure V) stimulated both *Fusarium* sp. and *A. humicola* at 150 parts per million which was equivalent to the concentration used in the field. At 1000 parts per million *Fusarium* sp. was inhibited completely and the growth of *A. humicola* had been reduced by 50 per cent. McNew and Burchfield (11) reported the ED 95 of dichlone against *A. solani* on tomato foliage to be 100 parts per million. They also regard dichlone as a AAA compound which means that the ED 50 values for spore germination range from 0.001 to 0.01 parts per million.

The growth of *Cladosporium* sp. was not reduced at 150 parts per million of dichlone but with increasing concentrations of the fungicide showed gradually decreasing rates of growth until complete inhibition was reached at 1000 parts per million. *B. cinerea* showed sharp reduction in growth at 150 parts per million. At 250 and 500 parts per million there was only slightly more reduction in the amount of growth, however, between 500 and 1000 parts per million there was another sharp reduction in growth.

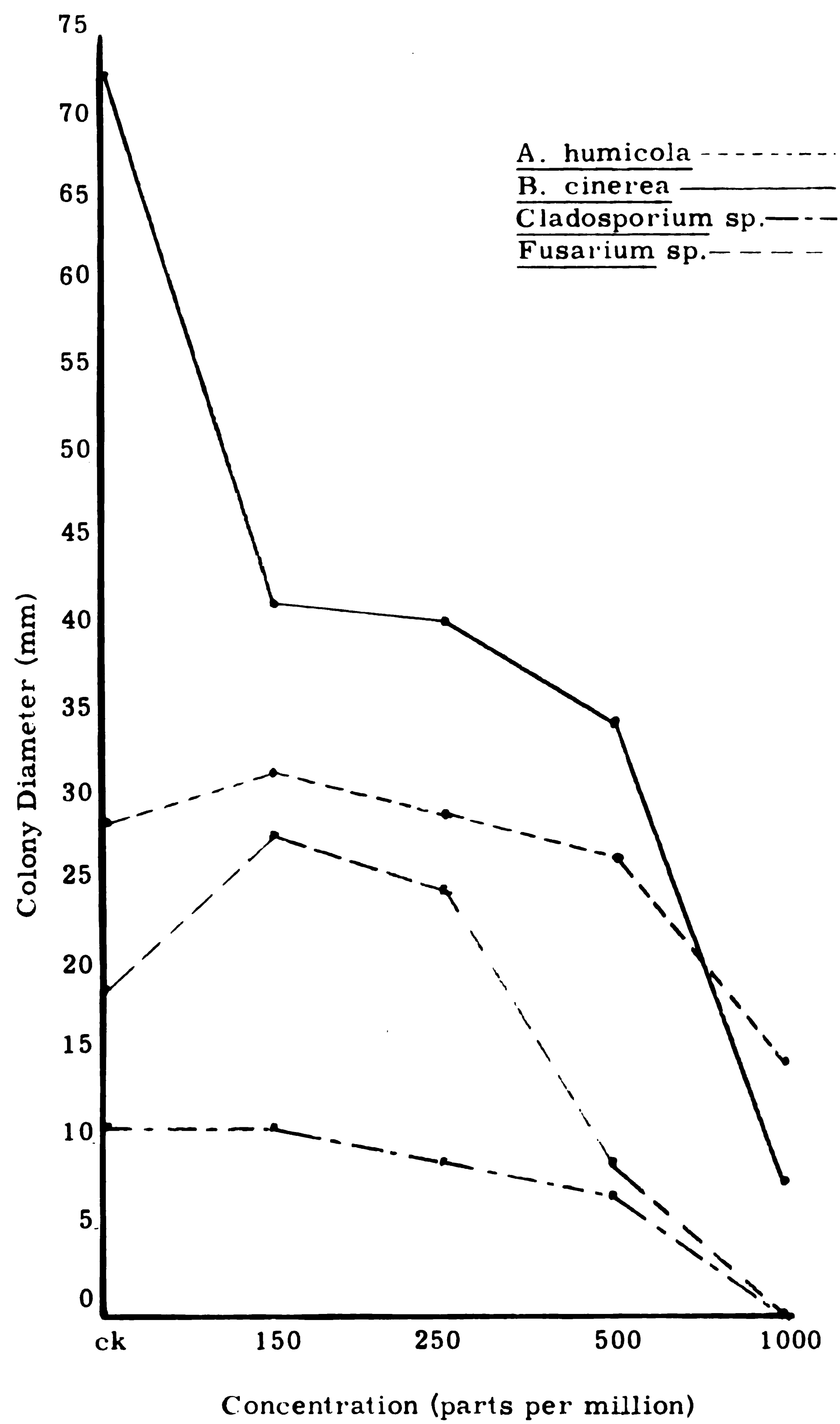

Figure V. Fungal colony growth at 96 hours with various concentrations of dichlone

CHAPTER VII

DISCUSSION

Throughout all experiments the fungicide ferbam gave good results. The fruit which was harvested from the ferbam sprayed plots had very uniform, and for the most part, low mold counts (Table 1). The mold counts made on the fruit picked July thirteenth were slightly higher but this no doubt was due to the more optimum conditions for mold growth brought about by the rains of July eighth and ninth. In addition there probably was some washing of the fungicide although the fruit from the plots where a spreader was used had counts that were just as low as those on any other day. Results of *in vitro* studies (as shown in Figure I) in which ferbam was incorporated in various concentrations into a two per cent malt extract agar to measure inhibitory action against various fungi, were highly satisfactory. Ferbam completely inhibited the growth of all four fungi against which it was tested at all concentrations used.

The consistently low mold counts obtained from the fruit sprayed with ferbam and the complete inhibition of fungi in *in vitro* studies would seem to indicate that ferbam may be a suitable fungicide for use on fruit to reduce mold counts. Other considerations such as residue and effect on the canned product were not studied and will not be discussed here.

When used during in vitro studies, nabam (Figure II) also gave complete inhibition of all fungi tested and at all concentrations. It would appear thus to have good potentialities, although in the field, the results were somewhat erratic (Table 1). The mold counts made from fruit harvested from plots sprayed with nabam only were low, with the exception on mold counts made from fruit harvested on July thirteenth. This can be accounted for by the rains of July eighth and ninth which may have washed the fungicide deposit from the fruit. Also there may have been a compound breakdown although nabam is considered to have a good residual action, at least for five days. The mold counts made from the fruit harvested from the plots which were sprayed with nabam plus B 1956 were higher on both the tenth and the thirteenth of July. This may, however, be due to some interaction between the fungicide and the spreader which reduces the effectiveness of the fungicide. Since these high mold counts were from the Cumberland fruit only, there may be the possibility of variation in varietal susceptibility, although more likely explanation would be that the conditions were less favorable for fungal growth during the period when the Logan variety was maturing and being harvested.

On the basis of mold counts made from harvested fruit it would not seem that nabam would be a desirable chemical to use to reduce raspberry fruit mold counts. This would not be due to the fact that the compound is ineffective against fungi, as can be seen by *in vitro* studies, but due rather to a lack of ability to maintain a lethal residue or an inability to act under these particular circumstances.

Mold counts made from the fruit harvested from the actidione (as shown in Table 1) sprayed plots showed considerable variation. The highest count obtained, which was 28 per cent of the microscopic fields, was not extreme but the wide variation on any one day would indicate that the compound is not completely effective. Natural variation in the sampling method for the mold count, of course, would enter into this to some extent. The number of sprays seemed to have had little effect on the sample taken for the final mold count. Fruit which had received only one spray often had a lower mold count than fruit which had had two or three sprays. In general, one would assume that at the concentration used, two parts per million, actidione was not as effective as could be desired. The *in vitro* studies (Figure IV) showed that at two parts per million there was little inhibition of *Cladosporium* sp. and a stimulation of *Alternaria humicola*. These two organisms are the ones isolated most frequently from black raspberry fruit and if we can assume that they are chiefly responsible for causing high mold counts then the erratic mold counts are explained. *Botrytis cinerea* also showed much growth at two parts per million, and while found less frequently, its great volume of growth should also contribute to the mold count total. The in vivo results are lower than the in vitro studies would indicate, but an undetermined amount of adsorbtion of actidione by the agar medium must be allowed for. This adsorbtion would reduce the effective amount of compound present in the medium and thereby allow greater growth than an actual concentration of two parts per million. There was considerable variation in the mold count between actidione and actidione plus B 1956 sprayed fruit. There may be an interaction between these two chemical compounds which reduces the effective amount of actidione present.

On the basis of the present tests actidione would not seem a desirable compound for the reduction of high mold counts on black raspberry fruits. However, since it is apparently adsorbed into plant tissue according to Hamilton (7), it thereby would not be as likely to be washed off by rain. If a reasonably higher dosage could be used without undue toxicity and expense, it might be a desirable compound to use in reducing black raspberry mold counts.

The results obtained with the use of dichlone were unsatisfactory. In the laboratory growth studies (Figure V) 150 parts per million, equal to the field concentration, had no effect on the growth of Cladosporium sp. and it stimulated the growth of Alternaria humicola. At this concentration the growth of Botrytis cineria was reduced and the growth of Fusarium sp. was stimulated. Since Cladosporium sp. and Alternaria humicola are isolated about 70 per cent of the time from raspberry fruits with high mold counts, one would expect that fruit harvested from dichlone sprayed plots might have higher mold counts. Such was the case. When the weather conditions were adverse, that is hot and humid, the mold counts tended to run high; when normal weather conditions were present the mold counts were also normal (see Table 1).

It is probable that dichlone would produce better results at higher concentrations although in the growth studies there was considerable fungal growth present at 500 parts per million which was very close to a normal field concentration.

Glyodin performed very much the same as dichlone. Although it did not stimulate the growth of any fungus tested it allowed growth of all of them at a concentration equivalent to that used in the field tests. In the field tests (Figure III) it apparently failed to inhibit the growth of the fungi which are responsible for high mold counts brought about by hot, humid weather. Low mold counts were obtained from glyodin sprayed fruit only when it was picked during periods when conditions were unfavorable for fungus growth and when one would normally expect low mold counts (Table 1). On the basis of this experiment it would seem that glyodin is not a suitable chemical for inhibiting the growth of the fungi causing high mold counts in black raspberries.

Of the five fungicides ferbam seems to offer some possibility of reducing the fungi which are causing the high mold counts in black raspberries. It inhibited the growth of all fungi tested _in vitro_ and the fruit harvested from ferbam sprayed plots showed consistently low mold counts.

There are many other factors to be considered, however, when discussing the feasibility of applying preharvest sprays to control high mold counts in black raspberry fruits. The primary consideration to the grower would of course be expense; would the use of a preharvest spray be insurance against the closing of canneries during the periods when high mold counts occur and would the extra cost of such a spray be returned. Another consideration is that of moving spray equipment through the planting so close to harvest time. Would the berries be bruised or broken from the bushes thus lowering quality of fruit and yield.

Of primary interest to the canner is the problem of residue. Will the normal washing procedures remove the chemical so that legal tolerances may be met. Another important factor is whether the fungicide would have any effect on the canned product. Adams _et al._ (1) working with canned black currants found that zinc nabam, glyodin, dichlone, ziram and thiram residues stimulated hydrogen production in the canned product even at very low concentrations and that these chemicals may adversely affect the flavor of the canned product. Moore (12) however stated that canned sour cherries which had been sprayed with ferbam had been kept for two years with no adverse affect on flavor or keeping quality.

The results obtained here are merely indications. The work should be repeated under more adverse conditions to see if comparable results are obtained. Extensive tests are also required to determine whether residue tolerances could be met. If these problems are resolved one remaining is economic feasibility. However, if by the use of preharvest sprays fruit mold counts can be held to a level which would forestall closing the canneries, then such sprays would no doubt be extremely important.

CHAPTER VIII

SUMMARY

Five fungicides were applied on black raspberry bushes as pre-harvest sprays in an attempt to lessen or prevent the fungal growth which causes high mold counts in the harvested fruits. Mold counts were made with fruit harvested from the spray plots to determine the effectiveness of the spray.

Growth studies were carried out in which varying concentrations of the fungicides were incorporated into media. Upon these media were grown two fungi which are most frequently isolated from raspberry fruits with high mold counts and two others which are commonly isolated. The amount of increase in colony diameter was used as an index of fungicide effectiveness.

The results of field studies and growth studies were compared to see which fungicides, if any, were effective in reducing the growth of the fungi which cause high mold counts in black raspberries.

Ferbam, ferric dimethyldithiocarbamate, was found to inhibit all fungal growth in *in vitro* studies and to bring about a reduction in black raspberry mold counts by inhibiting or killing the fungi which cause the higher counts.

It is suggested that further experiments be conducted under more adverse conditions. Explorations should be made to determine the economic necessity and practical possibilities of such a spray program.

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