EFFECT OF HARVESTING CONDITIONS ON MOLD COUNT OF BLACK RASPBERRIES

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INTRODUCTION

The production of black raspberries is an important industry in the State of Michigan; approximately 180,000 cases (No. 2) were processed in the year 1949. This represented about 80 per cent of the total processed black raspberries of the United States (21). A substantial portion of the berries for freezing were also grown in Michigan (21).

Several shipments of canned and frozen black raspberries were seized by the United States Government in 1949 and 1950 under the Federal Food, Drug, and Cosmetic Act (9). They were condemned as being adulterated because of excessive mold count. The processors were advised that canned or frozen black raspberries with high mold counts could not be shipped or sold in interstate commerce. The requirements of the law and the presence of high mold counts has posed a serious threat to the black raspberry industry in Michigan, and, as a result, the production dropped to about 140,000 cases in 1950 (21).

Processors claim that the weather conditions in Michigan in 1950 during the latter part of the growing season tended to be favorable for the development of mold on the fruit in the field, thereby making it difficult to produce a product having a sufficiently low mold count to meet government specifications. However, there is a conflict among investigators about the cause of a high mold count in the processed product. Helsel and Eisenberg (13) believed that poor quality, such as overripe or decomposed black raspberries, results in a product having a high mold count. Fabian et al. (8) stated that the presence of a high mold count of 60 per cent per ten samples, or 76 per cent for one sample, is not indicative of fruit decomposition, as they found sound berries that had a high mold count. They also reported a relationship between the weather conditions during harvesting the berries and the mold count. The mold count increased as the temperature and humidity increased.

The author felt that there was a need for further study to determine the factors that might influence the mold count of processed black raspberries.

This investigation was carried out in the vicinity of Lawrence and Keeler, Michigan. A study was made of the mold count at various stages of maturity of the berries, under different weather conditions. The effect of different methods of washing the harvested fruit was studied also, and a study was made to determine the differences that occur in the mold count when various dilution methods were used.

LITERATURE CITED

Molds are of economic importance not only because of their value to industry and medicine, but also because of the damage they cause to food and other products. Molds, a group of the fungi, are microscopic organisms, simple in morphological structure, without differentiation of body into true roots, flowers, or vascular bundles. They are devoid of chlorophyll, and are distinguished by a branched network of filaments or threads known as hyphae. Most bacteria and fungi have rather specific means by which they penetrate the host and relate themselves to the host tissue. They enter the host tissue through natural openings, through wounds, or by direct penetration (33). Berries, like other fruits and vegetables, are naturally protected against molds by a layer of skin which has to be broken or dissolved before the nutrients inside the fruit become available to the organisms. Slate, Braun, and Mundinger (26), and Fabian _et al_. (8) reported that mechanical injury of the skin of berries is one of the most common means by which molds enter and cause decay. Loree (19) stated that soft berries quickly break down and are attacked by molds. _Alternaria humicola_ Oudemans and _Cladosporium_ *epiphyllum* Persoon were reported by Beneke (2) to be the most common molds found in black raspberries (*Rubus occidentalis*), while *Pullaria* sp., *Trichoderma* sp., *Aspergillus* sp., *Oospold* sp., *Monilla* sp., and *Mucor* sp. were found less frequently. In general, berry fruits such as raspberries, blackberries, and dewberries are subject to attack by the same molds (11).

Among the factors affecting the germination of fungous spores are: (1) temperature, (2) moisture, (3) oxygen, (4) the hydrogen-ion concentration, and (5) the viability of the spores. The growth requirements for fungi were reviewed by Lilly and Barnett (18), Walker (33), Clayton (4), Dugger (7), and Lauritzen (17).

Factors that Influence the Mold Count in Berries

Since, in the case of black raspberries, the temperature during the growing season is near the optimum for the growth of molds, the moisture required by molds for germination may be supplied either by rain or by the soft mature fruit, the air furnishes adequate oxygen supply, the hydrogen-ion concentration of the fruit is sufficiently low to be favorable for the growth of molds, and as spores are ubiguitous in nature, it is difficult to produce berries free from molds. Weather plays an important role in influencing the mold count in black raspberries (8, 13, 28). The number of molds in berries was found to be influenced by several factors, which are discussed below.

Temperature. Microorganisms which bring about decay of fruits and vegetables are reduced in growth with the lowering of temperature below the optimum. When these organisms are grown on suitable media over constant temperatures, a correlation is shown between rate of growth and the temperature. For many, the optimum lies between 20° and 30° C., while above and below the optimum, growth rates decline. When the temperature is near the optimum, the enzymatic activity and the rate of spore germination are increased (21). Optimum temperature and growth rates of a given species may differ on various substrates (36). The growing temperature range of *Alternaria solani* is from a minimum of 1° to 3° C., to an optimum of 26° to 28° C., and a maximum of 37° to 45° C. (18). Helsel and Eisenberg (13), Fabian *et al*. (8), and Steinkraus (28) have reported that mold growth in black raspberries was rapid at high temperatures around 80°(±) F.

Humidity requirements for molds. The spores of many species of fungi will not germinate unless they are in contact with free water.

Water is essential to activate certain enzyme systems, to initiate other internal chemical changes, and to increase the volume of the germinating spores of fungi (18). Spores of some species of fungi are capable of germination on dry surfaces in an atmosphere of high humidity, usually 95 per cent or above (18, 11). Others are capable of producing short germ tubes under conditions of extremely low relative humidity (18). Snow, Chrichton, and Wright (27) found the mold growth on locust beans, Scotch beans, bran, oats, bonemeal, and linseed cake in storage was more dependent on relative humidity than on moisture content of the food, and at 75 to 100 per cent relative humidity the molds grew rapidly. Fabian et al. (8), Steinkraus (28), and Helsel and Eisenberg (13) stated that, in black raspberries, mold counts increased in humid weather and warm temperatures. The former two authors mentioned that mold counts in berries were low in hot and dry weather. Steinkraus (28) stated that the mold count in black raspberries increased rapidly during rainy weather. Fabian et al. (8) stated that moist weather favors mold growth by supplying water between the crevices of drupelets where the mold spores are lodged. Also, they stated that the osmotic action resulting from the presence of moisture on the surface of the berries brings nutrients out of the ripe berries for the growth of mold spores.

Increased amounts of water into drupelets causes them to break during handling, and also facilitates the breakdown of the skin of the drupelets by the enzyme system of molds. They found that, at the peak of the black raspberry processing period, mold counts increased on berries when the humidity increased due to rainfall. Slate, Braun, and Mundinger (26) stated that during wet seasons many growers are troubled with fruit rot and various molds, such as _Botrytis_ sp., _Fusarium_ sp., and _Alternaria_ sp., which may cause the fruit to rot just about the time that it becomes ripe. Helsel and Eisenberg (13) reported that a moist environment along with warm temperatures favored growth of molds and other microorganisms which decompose the berries, and also that the rains prevented picking the berries at sufficiently frequent intervals, resulting in the fruit becoming overripe and decomposing on the bushes. Darrow and Waldo (5) mentioned that raspberries become soft due to rainy weather, and are subject to decay by mold fungi.

Maturity of berries and its effect on mold count. It was reported by Loree (19) and Dodge and Wilcox (6) that soft berries break down easily and therefore are attacked by molds. Helsel and Eisenberg (13) stated that soft ripe black raspberries have higher mold counts than firm ripe berries. Fabian _et al._ (8) found higher mold counts in firm ripe black raspberries in some cases than in the berries of more advanced stages of maturity. Needham and Fellers (22) reported that a moderately high mold count might be obtained from soft, mushy, or overripe black berries which showed no visible evidence of mold. Darrow and Waldo (5) stated that soft, overripe berries were attacked easily by fungi which caused decay of the fruit. Rendell (25) reported that forty-eight hours was ample, even under average weather conditions, in Scotland for the raspberries to change from a decidedly unripe state to one of maturity or ripeness, and also that the changes associated with ripening continued even faster after the fruit was actually picked. He also found that loganberries ripened more quickly than other varieties, and occasionally changes were so rapid that berries in red stage turned black and shrivelled the next day.

Effect of harvest on mold count. Raspberries, because of their perishable nature, require very careful handling. The most common cause of decay is mechanical injury resulting from careless handling during harvesting of the fruit (8). Berries injured or bruised in handling are subject to attack by mold fungi which cause decay (5). Special care should be taken to remove the berries from bushes without breaking or crumbling them, and only a few berries should be held in the hand at a time, to avoid bruising. The berries should not be picked when wet unless for immediate use, as they mold quickly, especially in warm weather. Ripe berries left on the bushes become soft and are certain to cause trouble in later pickings (19). Helsel and Eisenberg (13) stated that, with proper handling, the mold count of black raspberries could be reduced, while Fabian et al. (8) reported that proper handling of the berries did not materially aid in reducing the mold count. Loree (19) recommended harvesting of the raspberries on alternate days during the peak of the season. Darrow and Waldo (5) stated that picking of the raspberries must be done at least two or three times each week, depending on locality, variety, and weather conditions. Harvesting of raspberries may be required at least every other day in hot or wet weather, and on every third or fourth day in dry regions of the West (5). Slate, Braun, and Mundinger (26) stated that under normal conditions raspberries should be picked on alternate days; in very hot weather, every day; and once in three days if the weather is cool.

Holding of berries and its effect on mold count. It was reported by Dodge and Wilcox (6) that delay beyond a few hours between picking and sale to the consumer affords an opportunity for the development of fruit rots and molds in raspberries. Loree (19) advised that berries should be kept out of the heat of the sun after they have been picked. Helsel and Eisenberg (13) stated that sound black raspberries picked during rainy weather could be held for a reasonable length of time without an appreciable increase in the mold count. Fabian et al. (8) found a highly significant increase in the mold count of black raspberries by holding the berries for various lengths of time under warm, humid conditions. Stevens and Wilcox (29), working with Rhizopus rot of strawberries, found an increase in decay of berries that had been washed, and then dried by exposure to sunlight.

Washing and sorting. It was reported by Steinkraus (28) that washing and sorting procedures during the processing operation of black raspberries helped in reducing the mold count. Fabian et al. (8) did not find significant decreases in mold count of the berries by washing them. Haynes and his co-workers (12) found significant decreases in mold count of strawberries by treating them with water pressure and detergents. Howard (14) advised that proper sorting and washing procedures would help in reducing the mold count of tomatoes.

Mold Counting

At the beginning of this century, the manufacture of various tomato products became commercially important. As a result, there were radical changes in some of the manufacturing methods, which tended to produce a cleaner and more wholesome product. For the purpose of giving the manufacturer a means of comparing the various methods of handling the products, Howard (14) introduced a method for determining the quality of a tomato pulp or catchup. He felt that the processors handled the product in such a way as to allow the decomposition of the product by bacteria, yeasts, and molds to occur at some time during processing, though the material might be relatively free from decayed or putrid material. Prescott, Burrage, and Philbuck (24) and Darling and Bitting and Bitting (cited by Fabian et al., 8) criticized the procedures used. Their chief criticisms concerned the following: (1) the difficulties and sources of error in distinguishing between plant tissues and microorganisms at low microscopic magnification; (2) only the rod-type bacteria were counted, while the coccus forms were ignored; (3) yeasts and mold spores were counted together, since they could not be distinguished from each other; and (4) the method failed to distinguish between large masses of hyphae and one hypha in the field. Bitting and Bitting (cited by Fabian et al., 8) also reported that no relation existed between the number of organisms and the decomposition of the product, since the number of organisms was not always coincident with putrefactive activity.

Many attempts were made to improve the Howard method. Vincient (32) stated that the direct method of counting bacteria devised by Prescott and Breed in 1911, which also is described in Standard Methods of Bacteriological Analyses of Milk (APHA, 1916), eliminates the sources of error. The advantages of this method are: (1) the bacilli can be distinguished from cocci or inert material, while doubt is often encountered when using the Zeiss counter; and (2) cocci can be counted, since they are stained easily and can be observed when this method is used. Barterelli and Marchelli (1) suggested a modification of both the Howard and Vincient methods, in which the tomato product was diluted, filtered, and the filtrate fixed with alcohol-ether and stained with Loeffler's methylene blue before it was subjected to microscopic count. Another modification of the Howard method was suggested by Miller (20), in which the tomato pulp was boiled with Loeffler's methylene blue and Ziehl-Neelsen's carbofuchsin. With this procedure the microorganisms are stained a deeper color than the tomato tissues, and in addition to this differential effect the combination of the two stains facilitate the differentiation of yeasts from mold spores and bacteria. However, the same criticisms are valid for Miller's method as for the Howard method.

Using the Howard mold count procedure on black raspberries, Steinkraus (28) found that when the samples were diluted beyond a one-to-one ratio with pectin solution there was a decrease in mold count, but that this decrease was not in direct proportion to the dilution. The slope of the dilution curve was found to be greater if the initial mold count was higher.

Standards for the Number of Molds Allowed in Food Products

The Federal Food, Drug, and Cosmetic Act of 1906 states that a food product is deemed adulterated "if it consists in whole or in part of a filthy, decomposed or putrid animal or vegetable substance" (31). Five years after the enactment of this act, Howard (14) introduced the first standards for the maximum number of molds, yeasts, and bacteria to be allowed in tomato products; under this standard a tomato product was to be condemned if molds were present in more than 25 per cent of the microscopic fields, twenty-five yeasts and spores in 1/60 milliliter, or twenty-five million of bacteria per milliliter. In 1916 this was modified to permit 66 per cent of molds in the microscopic fields (3); 125 yeasts and spores in 1/60 milliliter, or one hundred million bacteria per milliliter. Howard and Stephenson (15) showed there was a very close relationship between the amount of spoilage of tomato products and faulty cleaning or superficial sorting of the tomatoes. Tice (cited by Fabian et al., 8) stated that when tomatoes were properly handled the mold count was of greater importance in judging the condition of the product than the counts of other microorganisms.

According to FDA standards during 1931, the tomato products were limited to 50 per cent mold count; in 1938 a change was made which limited the mold count in tomato juice to 25 per cent. Standards for catchup, puree, and paste were changed to 40 per cent mold count and 15 per cent for tomato juice in 1940, but later in the same year FDA raised the limits of mold count to 20 per cent for tomato juice, while retaining 40 per cent for the other tomato produces (8).

Application of Howard Method to Berries

Although the mold count procedure originally was designed for tomato products, it has been used for a great number of foods, including red and black raspberries. The application of the Howard mold count method to berries and berry products was first suggested by Needham and Fellers (22). At the present time an unofficial mold standard for black raspberries is 50 per cent, the same as for catchup. Fabian *et al.* (8) suggested that black raspberries, because of their structure, should have a standard of their own and should not be judged according to the standards of other products. They recommended 76 per cent mold count if one sample was used as a representative of a lot, or 60 per cent if at least ten samples were taken and averaged. Helsel and Eisenberg (13) recommended that it should not exceed 50 per cent, even under adverse conditions.

EXPERIMENTAL PROCEDURE

During the seasons of 1951 and 1952 from farms in the vicinity of Lawrence, Michigan, black raspberries were carefully harvested and put in clean quart berry boxes. Some examples were also obtained at Keeler, Michigan, during the season of 1952. The harvested berries were taken directly to the factory, transferred to clean cans, covered, and frozen. Since the mold counts could not be made concurrently with harvest, black raspberry samples obtained near Lawrence were frozen in the Lawrence Frozen Foods freezer, and those collected at Keeler were frozen in the deep freeze at Burnette Farms Packing Company, and later transferred to Lawrence Frozen Foods freezer. All the frozen samples of black raspberries were brought by refrigerated truck to the Horticulture Department of Michigan State College and stored at 0° F. until they were examined.

Experimental Plan

1. Effect of the stage of maturity upon the mold count in black raspberries. Samples of black raspberries were harvested at the following stages of maturity:

(1) Shiny-black: Firm-ripe, shiny-black with occasional red drupelets. Very sound fruit.

(2) Dull-black: Dull appearance, hairs more prominent in crevices between drupelets than in the shiny-black stage, firmer than soft ripe fruit.

(3) Soft ripe: Fruit in very advance stage of ripeness.

(4) Composite: Aggregate sample of all the above stages of maturity.

2. Effect of harvesting black raspberries after rainfall. Samples of berries at each stage of maturity, as well as a composite sample, were collected from eight different fields. These were taken thirteen, seventeen, twenty-four, forty-eight, and seventy-two hours after rainfall.

Samples were also collected from ten different fields at each of the stages mentioned above when there had been little or no rain for four days.

3. Effect of shaking the bushes. Samples of each stage of maturity were collected from Logan and Cumberland varieties. Then the bushes from which these samples were taken, were shaken, and another set of samples collected.

The mold count for each set of samples was determined and a comparison was made between the number of molds in the berries of the two varieties tested. Also, a comparison between the results obtained before and after shaking the bushes was made.

4. Effect of holding black raspberries. On July 17, 1952, six lots of dry black raspberries were collected from six different fields, and each was divided into three portions. One was frozen immediately, another after holding for six hours at 76° to 78° F., and the last one after holding under the same conditions for twelve hours. This treatment was repeated for berries harvested on July 21, 1952.

Similar experiments were conducted for black raspberries harvested immediately after rain on July 18 and 23, 1952.

5. Effect of different methods of washing. Four samples of berries from three regular receipts of a Michigan food processor were taken at the end of the season in 1952 and subjected to three kinds of water-pressure washing to determine the effect of washing on the mold count of berries. The fruit obtained from a shaker-spray washing was taken as a control. The different washing treatments used were shaker and spray washing, shaker and sink washing, and shaker, spray, and sink washing.

6A. Comparison of official mold count method with the modified method. For this study, pulp of seventy-five samples of berries was diluted with 3 per cent pectin solution in the ratio of 1:1 according to the original Howard mold-count method, and the per cent of mold in the microscopic fields in each sample was recorded. Then the mixture of pulp and pectin solution was further diluted with 3 per cent pectin solution to 2:1, and the mold count in each sample was recorded.

6B. Effect of dilution on mold count. Sixty-two samples of black raspberries containing from 20 to 100 per cent mold counts, as determined by the modified technique, were taken to study the effect of dilution with 3 per cent pectin solution on mold count of the berries. The mixture of black raspberry pulp and 3 per cent pectin solution made for observation of mold count was diluted with pectin solution to bring the ratio of pectin to pulp to 1:1, 2:1, 4:1, 6:1, 8:1, 10:1, 12:1, and 14:1, and the per cent of molds in the berries determined at each level of dilution.

A summary of the experimental plan is given in Table I. The official weather data for July, 1951 and 1952, are summarized in Table II.

TABLE I

EXPERIMENTAL PLAN

Code Sample	No. of Samples	Date of Sample Collection	Treatments
Experiment 1. Effect of Maturity on the Mold Count of Berries			
Experiment 2a. Effect of Harvesting After Rain at Different Daily Intervals			
IBa-1	40	7/13/51	Collected 1 day after previous harvest.
IBa-2	40	7/14/51	Collected 2 days after previous harvest.
IBa-3	20	7/15/51	Collected 3 days after previous harvest.
IBa-4	20	7/16/51	Collected 4 days after previous harvest.
IBa-5	20	7/17/51	Collected 5 days after previous harvest.
Experiment 2b. Effect of Harvesting After Rain at Different Hourly Intervals			
IBb-1	32	7/15/52	Collected at 13-14 hrs. after rain.
IBb-2	32	7/15/52	Collected at 17-18 hrs. after rain.
IBb-3	32	7/15/52	Collected at 23-24 hrs. after rain.
IBb-4	32	7/16/52	Collected at 48 hrs. after rain.
IBb-5	32	7/17/52	Collected at 72 hrs. after rain.
Experiment 3. Effect of Shaking Bushes Before Harvesting the Berries			
IC-1	24	7/13/51	Logan variety--no shaking.
IC-2	24	7/13/51	Logan variety--after shaking.
IC-3	24	7/13/51	Cumberland variety--no shaking.
IC-4	24	7/13/51	Cumberland variety--after shaking.

TABLE I (Continued)

Code Sample	No. of Samples	Date of Sample Collection	Treatments
			Experiment 4a. Effect of Holding the Berries After Harvesting Wet
ID-A-1	6	7/18/52	Collected after rain, frozen immediately.
	6	7/23/52	Collected after rain, frozen immediately.
ID-A-2	6	7/18/52	Collected after rain, held for 6 hours before freezing.
	6	7/23/52	Collected after rain, held for 6 hours before freezing.
ID-A-3	6	7/18/52	Collected after rain, held for 12 hours before freezing.
	6	7/23/52	Collected after rain, held for 12 hours before freezing.
			Experiment 4b. Effect of Holding the Berries After Harvesting Dry
I-D-B-1	6	7/17/52	Dry berries frozen after 0 hours holding.
	6	7/21/52	Dry berries frozen after 0 hours holding.
I-D-B-2	6	7/17/52	Dry berries frozen after 6 hours holding.
	6	7/21/52	Dry berries frozen after 6 hours holding.
I-D-B-3	6	7/17/52	Dry berries frozen after 12 hours holding.
	6	7/21/52	Dry berries frozen after 12 hours holding.

TABLE I (Continued)

Code Sample	No. of Samples	Date of Sample Collection	Treatments
			Experiment 5. Effect of Washing
IE-1	12	7/18/52	Control.
IE-2	12	7/18/52	Control + spray-washed.
IE-3	12	7/18/52	Control + sink-washed.
IE-4	12	7/18/52	Control + spray-washed + sink-washed.
			Experiment 6a. Effect of Diluting Black Raspberry Pulp With Pectin Solution
IBb 1	8	7/15/52	Comparison of official method with modified technique.
IBb 2	1	7/15/52	
IBb 3	5	7/15/52	
IBb 4	8	7/15/52	
IBb 5	8	7/15/52	
IDA 1	3	7/23/52	
IDA 2	6	7/18/52 7/23/52	
IDA 3	2	7/23/52	
IDB 1	2	7/17/52	
IDB 2	5	7/17/52 7/21/52	
IDB 3	5	7/17/52 7/21/52	
IE 1	2	7/18/52	
IE 2	4	7/18/52	
IE 3	3	7/18/52	
IE 4	2	7/18/52	
III	16	7/18/52 7/22/52	

TABLE I (Continued)

Code Sample	No. of Samples	Date of Sample Collection	Treatments
			<u>Experiment 6b. Effect of Dilutions on Mold Count</u>
			With above samples. Pectin solution was added to bring the ratio of pectin to pulp as 4:1, 6:1, 8:1, 10:1, 12:1, and 14:1.

TABLE II

OFFICIAL WEATHER DATA FOR JULY, 1951,
IN PAW PAW, MICHIGAN

Date	Temperature		Daily Precip-itation (inches)	Personal Observation at Lawrence, Michigan
	Max. (° F.)	Min. (° F.)		
7/1/51	74	53	0.25	
7/2/51	80	51		
7/3/51	81	56		
7/4/51	78	58		
7/5/51	75	50	0.02	
7/6/51	85	47		
7/7/51	88	58		
7/8/51	80	63	0.68	
7/9/51	83	63	0.87	
7/10/51	85	62		Hot and slightly humid.
7/11/51	78	62	0.03	Light showers in the afternoon and heavy rain in the night.
7/12/51	77	58	0.10	Cool morning, showers till noon.
7/13/51	82	56		Cool morning, slightly warm in the afternoon--no rain.
7/14/51	84	55		Warm day--no rain.
7/15/51	86	59		Hot sunny day, heavy dew in the night--no rain.
7/16/51	88	58		Hot sunny day, heavy dew in the night--no rain.
7/17/51	80	56		Cool in the morning--warm day. Heavy dew in the night--no rain.
7/18/51	87	60		Hot and humid day.
7/19/51	83	57		Hot and humid day.
7/20/51	80	46		Warm and humid day.
7/21/51	86	63		Cloudy and cool day. Light showers. Heavy rain in the night.

[1] Climatological Data (Michigan) W. S. Dept. Commerce, Weather Bureau.

TABLE II (Continued)

Date	Temperature		Daily Precip-itation (inches)	Personal Observation at Lawrence, Michigan
	Max. (° F.)	Min. (° F.)		
7/22/51	84	58	4.85	Cool day. Showers in the morning. Cool night with heavy dew.
7/23/51	77	59	0.03	Cool day.
7/24/51	87	56		Hot and humid. Heavy dew in the night.
7/25/51	88	59		Warm and humid.
7/26/51	89	62		
7/27/51	85	65	0.91	
7/28/51	79	54		
7/29/51	85	62		
7/30/51	90	58		
7/31/51	82	67	0.24	
7/1/52	89	60		
7/2/52	96	68		
7/3/52	89	67		
7/4/52	83	57		
7/5/52	90	53		
7/6/52	92	62		
7/7/52	94	62		
7/8/52	89	60	1.16	Light rain till 2:00 p.m. Then it was cool for the rest of the day.
7/9/52	75	47		Slightly warm and humid.
7/10/52	84	52		Warm and dry.
7/11/52	85	61		Hot and dry.
7/12/52	95	69		Hot and dry.
7/13/52	92	68		Hot and humid, cool and clear night.
7/14/52	87	70	0.35	Cloudy, rained heavily at 4:30 p.m. and again from 5:45 p.m. to 7:00 p.m.

TABLE II (Continued)

Date	Temperature		Daily Precip-itation (inches)	Personal Observation at Lawrence, Michigan
	Max. (° F.)	Min. (° F.)		
7/15/52	73	63	0.40	No rain was observed at Keeler, Lawrence, or Benton Harbor. Cloudy in the morning. Slightly warm and humid.
7/16/52	82	62	0.23	Cloudy till late afternoon. Occasional showers between 8:30 a.m. and 1:00 p.m. Sunny from 3:30 p.m. till evening.
7/17/52	84	68		Warm and humid.
7/18/52	89	72	0.85	Rain in the morning from 6:00 to 8:00 a.m., then warm and humid day. Slight drizzling at 3:00 p.m. Heavy rain from 4:30 to 11:00 p.m.
7/19/52	85	70	0.15	Cool morning, warm and humid.
7/20/52	92	68		Hot and humid.
7/21/52	90	77		Hot and humid.
7/22/52	98	68	0.10	Hot and humid. Light rain in the evening.
7/23/52	92	66	0.50	Rain from 5:00 to 8:00 a.m. Warm and humid day.
7/24/52	84	53		Warm and dry.
7/25/52	86	55		Warm and dry.
7/26/52	84	70	0.07	Cloudy day.
7/27/52	90	55		
7/28/52	86	65	0.23	
7/29/52	78	47		
7/30/52	77	55		
7/31/52	77	45		

Methods of Procedure

The following procedures were carried out on each sample unless otherwise stated.

Thawing of berries. The cans containing black raspberries were kept in a container holding boiling-hot water. In this way thawing of berries could be easily attained within fifteen to twenty minutes. All the samples were thawed in this manner, and then pulped.

Pulping of black raspberry samples. Samples were prepared for microscopic examination by pulping the berries with a laboratory pulper[1] having screen openings of 0.027 inch in diameter.

Method for mold counting. One part by weight of the black raspberry pulp was diluted with two parts by weight of 3 per cent pectin solution. The pulped berry samples were examined for mold filaments by the A.O.A.C. Official method (23). All mold counts reported were based on examination of at least one hundred microscopic fields. This method was proposed originally and adopted by Helsel and Eisenberg (13), because the deep color and density of

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[1] Cefaly laboratory pulper.

pulp prepared from fresh and frozen berries made mold counting difficult when pure pulp was used.

The dilution technique described above is a modification of that described in the Methods of Analysis of A.O.A.C. (23), in which the pulp was diluted with equal parts of 3 per cent pectin solution by weight.

The air bubbles incorporated during pulping of frozen raspberry samples were easily removed by adding small amounts of antifoam[1] to the sample and stirring thoroughly with a glass rod, although the air bubbles in the samples could be eliminated if the samples were allowed to settle about thirty minutes. Howard (16), working on the use of different instruments, while making a microscopical examination for mold in tomato products, found it better to use either a large or medium scalpel with pointed end for taking the test material after stirring.

Preparation of pectin solution. A 3 per cent solution of apple pectin (100 grade) was prepared. One hundred milliliters of the distilled water was heated in a beaker on a hot plate, and pectin was added slowly in a sprinkling manner to the liquid, which was agitated

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[1] Antifoam manufactured by Dow Chemical Company.

simultaneously with a glass rod. A small amount of antifoam was added to the hot solution to avoid foam formation. After the solution was cooled, 5 milliliters of 36 per cent formaldehyde per 100 milliliters of solution was added to prevent any microbial growth in the pectin solution. This addition of formaldehyde was a slight modification of the method described in A.O.A.C. (23).

Dilution method for estimating mold count in the berries. The dilutions of the samples were made gravimetrically. Fifty grams of 3 per cent pectin solution was added to 50 grams of black raspberry pulp in order to make a 1:1 ratio of pectin to pulp. The same mixture was diluted further to the desired concentration by adding 3 per cent pectin solution. When the pectin solution was added to the black raspberry pulp, the mixture was stirred thoroughly to make it uniform. As the dilution increased, the intensity of color decreased, and it was easier to examine the samples for mold count.

RESULTS

The tables presented in the text summarize the original data which are given in the Appendix.

Effect of Stage of Maturity of Berries on Mold Count

Berries that were harvested in the shiny-black stage had an average mold count of 4.0, 4.5, 5.5, 9.5, and 11.5 per cent for the time intervals of thirteen, seventeen, twenty-four, forty-eight, and seventy-two hours after rain, respectively. The mean mold count in berries for all intervals was 7 per cent. The dull-black berries were found to contain an average of 10.3, 7.0, 13.5, 16.0, and 22.5 per cent, respectively, for the same intervals with a mean of 13.9 per cent; and the soft berries, 35.8, 50.0, 47.3, 61.0, and 59.3 per cent, respectively, for the same intervals, with a mean of 50.7 per cent (Table III).

Examination of Table IV shows that, as the berries advanced in maturity from the shiny-black to the soft-black stage, the mold count increased. This increase was found to be highly significant, and can be explained by the fact that the skin of the advanced mature

TABLE III

AVERAGE MOLD COUNT OF BERRIES AT DIFFERENT STAGES OF MATURITY, COLLECTED AT DIFFERENT INTERVALS AFTER RAIN, DURING THE 1952 SEASON[1]

Stages of Maturity	Hourly Intervals		Daily Intervals		
	13-14 (%)	17-18 (%)	1 day (%)	2 days (%)	3 days (%)
Shiny-black	4.0	4.5	5.5	9.5	11.5
Dull	10.3	7.0	13.5	16.0	22.5
Soft	35.8	50.0	47.3	61.0	59.3
Composite sample	24.3	34.0	40.3	38.5	41.8
Mean value	18.6	23.9	26.6	31.3	33.8

[1] Original data in Table XII (Appendix).

berries was easily broken or dissolved by the molds, so that water and nutrients became available for the growth of the mold. The composite samples were found to contain an average mold count of 24.3, 34.0, 40.3, 38.5, and 41.8 for the periods of thirteen, seventeen, twenty-four, forty-eight, and seventy-two hours after rain, with a mean of 35.8 per cent. Averages for composite samples were found to be always significantly lower than that of the soft ripe stage, and

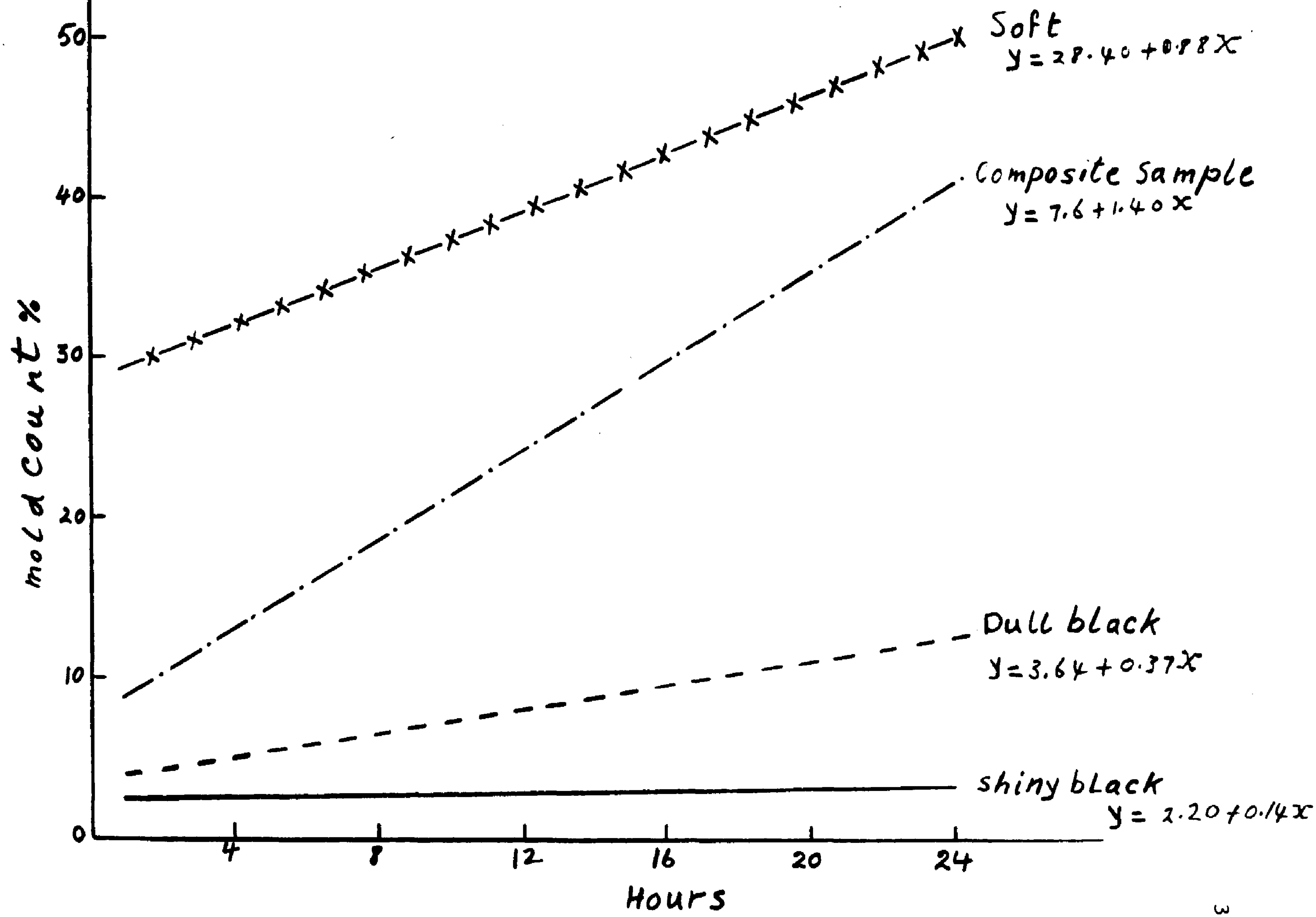
TABLE IV

AVERAGE MOLD COUNT OF BERRIES COLLECTED AT DIFFERENT STAGES OF MATURITY, AND ANALYSIS OF VARIANCE

Average Mold Count of				No. of Sam-ples	F Value	L. S. D.	
Shiny-Black (%)	Dull (%)	Soft (%)	Com-posite (%)			5%	1%
7.0	13.9	50.7	35.8	160	57.9**	3.7	4.88

** Highly significant.

higher than that of the shiny- and dull-black stages (Table IV). The rate of increase in the mold count of the composite sample was greater than the rate of increase in the soft berries--the dull-black or shiny-black berries (Figure 1). The shiny-black had the lowest rate of mold increase with time, while the dull-black raspberries had the next lowest rate of increase. The higher rate of mold increase with time for the composite sample may be the result of mixing the soft fruit--which have high mold counts--with shiny-black and dull-black fruit having low mold counts, and so giving the mold more surface area and media to grow upon. Also, it is commonly believed that the empirical methods for counting mold would give such on time after rain



results when soft fruit having high mold count is mixed with fruit in other stages of maturity containing a small number of molds.

Data obtained seem to indicate that the soft berries were responsible for the high mold count in the composite samples.

Effect of Harvesting After Rain at Different Intervals

Hourly intervals. For this experiment the samples were collected on July 15, 1952. Examination of Table II shows that on July 12 and 13, prior to the collection of samples, the temperature ranged from 68° to 95° F., with an average of 81° F. No rain fell untill the evening of the fourteenth. This period was not favorable for the germination and growth of mold spores. After the rain on the fourteenth, the temperature ranged from 62° to 84° F., with an average of 72° F. during the three-day period of sample collection. The humidity was high due to the rainfall, and weather conditions during this period seemed to be favorable for the growth of molds.

Examination of Table V shows that the samples which were collected thirteen hours after rain had an average of 4.0, 10.3, 35.8, and 24.3 per cent, respectively, for the shiny-black, dull, soft, and composite samples.

TABLE V

ANALYSIS OF VARIANCE OF THE MOLD COUNT IN BERRIES COLLECTED AT DIFFERENT INTERVALS AFTER RAIN[1]

Stage of Maturity	Average Mold Count in			
	13 hrs.	17 hrs.	24 hrs.	48 hrs.
Shiny-black	4.0	4.5	4.5	6.9
Dull-black	10.3	7.0	12.4	20.8
Soft-black	35.8	50.0	61.3	61.4
Composite sample	24.3	34.0	31.2	29.1
Total average	18.6	23.9	27.4	29.6

[1] Original data in Tables XII and XIII (Appendix).

* Significant.

** Highly significant.

TABLE V (Continued)

Berries (%)			No. of Samples	Sample F Value	L. S. D.	
72 hrs.	96 hrs.	120 hrs.			5%	1%
9.5	2.4	4.0	75	2.602*	0.52	
16.3	2.4	4.0	75	3.02*	4.06	
70.3	9.2	22.5	75	7.39**	7.82	10.38
46.2	4.8	8.4	75	6.11*	5.45	7.24
35.6	4.7	9.7				

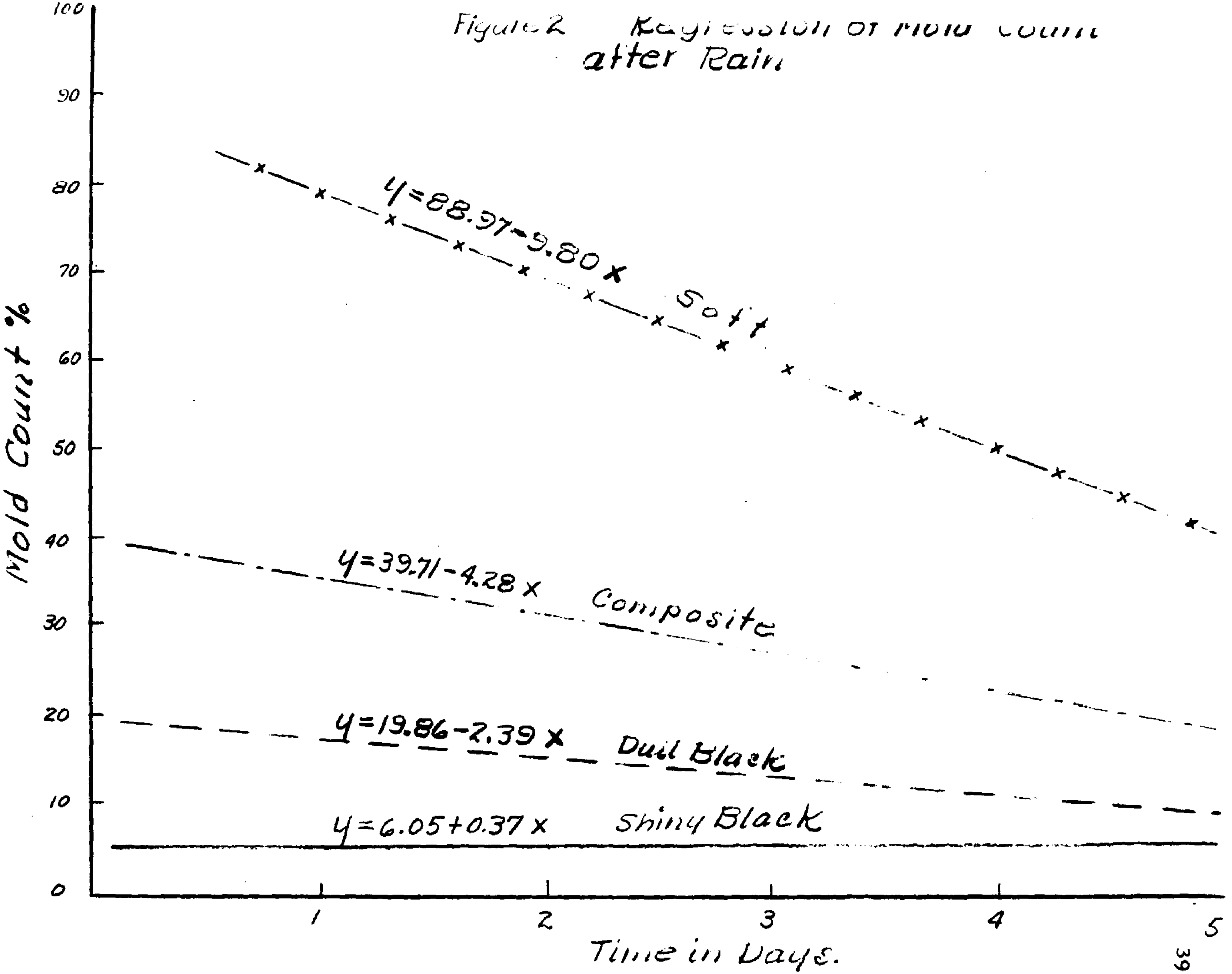
The next collection, seventeen hours after the rain, had an average of 4.5, 7.0, 50.0, and 34.0 per cent for the same stages of maturity, respectively. Analysis of variance (Table V) indicated that there was no significant difference in the mold count of the shiny-black and dull-black berries collected between thirteen and seventeen hours after rain, but that there were highly significant differences in the soft-black and composite samples. Black raspberries collected twenty-four hours after rain had an average of 5.5, 13.5, 47.25, and 40.25 per cent for the shiny-black, dull-black, soft, and composite samples, respectively. Analysis of variance showed that there was no significant difference between the mold count of shiny-black and dull-black berries collected twenty-four hours after rain and those collected thirteen hours after rain. There were significant differences in the soft and composite samples.

The regression curve (Figure 1) indicates that the number of molds in black raspberries increases with the increase of time following rainfall. This was more noticeable for the soft fruit and the composite samples than for the shiny-black and dull-black fruit, where the increase was negligible.

Day intervals. The average mold count in the black raspberries one, two, three, four, and five days after rain was 27.4, 29.6, 36.6, 4.7, and 9.7, respectively (Table V). Analysis of variance showed that there were significant differences between the mold counts in berries after these intervals (Table V). There was a noticeable increase in mold count of the berries up to the third day after rain; then the mold counts decreased rapidly (Figure 2). It was observed that shiny-black berries took twenty-four hours to reach the dull stage during the growing season, and another twenty-four hours to become soft. On this basis, the mold count for the berries that were shiny black twenty-four hours after rain increased from 4.5 to 20.8 per cent after forty-eight hours, which was the count for the dull-black, and to 70.3 per cent after seventy-two hours, which was the count for the soft berries. On the fourth day, they shriveled and dried up, and so were eliminated. The samples collected on the fourth and fifth days represented the fruits that were in the green and red stages during the rainy period, and so they matured under normal dry conditions and might be expected to have lower mold count. The data obtained during the fourth and fifth days correspond with data previously obtained during dry conditions.

From the results obtained, the author feels that the best time for harvesting the berries in rainy weather to get low mold count would be as soon as possible after rain.

Figure 2 Regression of Mold Count after Rain



Effect of Variety on the Mold Counts

Data obtained (Table VI) showed that the Logan variety berries had an average mold count of 12.7, 39.7, 87.0, and 79.7 per cent for the shiny-black, dull-black, soft, and composite samples, respectively. The averages for the Cumberland variety were 12.0, 24.0, 67.7, and 53.3 per cent for the same stages of maturity. The berries of the Logan variety had significantly higher mold counts than of the Cumberland variety at all the stages of maturity except the shiny-black stage (Table VI). The results obtained seemed to indicate that the Cumberland variety berries were more resistant to molds than the berries of Logan variety. Therefore, it would seem that the Cumberland variety, or any other variety which proves to be more resistant to molds, should be grown in areas that have trouble with mold count in berries.

Effect on the Mold Count in Berries of Shaking the Bushes Before Harvesting

Experiment 1 showed that soft berries had the highest mold count, and it was concluded that they might be the cause of the high mold count in the composite sample. The easiest way to eliminate the soft berries seemed to be by shaking the bushes before harvesting.

TABLE VI

AVERAGE MOLD OF LOGAN AND CUMBERLAND BLACK RASPBERRIES IN DIFFERENT STAGES OF MATURITY BEFORE AND AFTER SHAKING THE BUSHES FOR HARVEST[1]

Variety	Shiny-Black	
	Before Shaking	After Shaking
Logan	12.7	3.3
Cumberland	12.0	6.0
Total means	12.3	4.7

F Value	
Treatment	14.60**
Variety	0.25

[1] Original data in Table XIV (Appendix).

* Significant.

** Highly significant.

TABLE VI (Continued)

Dull-Black		Soft		Composite	
Before Shaking	After Shaking	Before Shaking	After Shaking	Before Shaking	After Shaking
39.7	10.0	87.0	59.3	79.7	41.0
24.0	10.7	67.7	40.0	53.3	31.3
31.8	10.3	77.3	49.7	66.5	36.2
39.33**		43.55**		83.48**	
4.79*		21.27**		29.40**	

Samples that were harvested before shaking the bushes had an average of 12.3 per cent mold for the shiny-black berries, 31.8 per cent for the dull-black berries, 77.3 per cent for the soft berries, and 66.5 per cent for the composite samples. After shaking the bushes, the berries in the shiny-black stage had an average of 4.7 per cent mold count; the dull-black stage, 10.3 per cent; the soft stage, 49.7 per cent; and the composite samples, 36.2 per cent (Table VI). The decrease in number of molds was 62.2 per cent for the shiny-black, 67.5 per cent for the dull-black, 35.8 per cent for the soft-ripe, and 45.6 per cent for the composite samples (Table VII).

Shaking the bushes before harvest significantly decreased the mold counts in berries at all stages of maturity. The greater per cent decreases in the shiny-black and dull stages were due to lower initial mold counts of the berries (Figure 3).

These results are in agreement with the statement of Helsel and Eisenberg (13), that:

> Several instances were reported in which growers removed overripe berries by shaking the bushes before beginning to pick the crop. This was said to have resulted in their picking only sound berries for the packing plants. There was other evidence to indicate that some growers were aware of the moldy condition of some of their berries and that they were interested in delivering only sound fruit to packers.

TABLE VII

REDUCTION IN MOLD COUNT OF LOGAN AND CUMBERLAND BLACK RASPBERRIES OF DIFFERENT STAGES OF MATURITY AFTER SHAKING THE BUSHES

Variety	Shiny-Black (%)	Dull-Black (%)	Soft-Ripe (%)	Composite (%)
Logan	73.6	74.8	31.8	48.5
Cumberland	50.0	61.1	40.9	41.3
Combined (both varieties)	62.2	67.5	35.8	45.6

Effect on the Mold Count of Holding Dry and Wet Black Raspberries After Harvesting

The time that elapses between harvesting and processing has been found to have a great effect on the mold count of the final product (8). Data obtained showed that when berries were harvested and processed immediately, they had an average mold count of 45.2 per cent; when held for six hours before processing, the mold count increased to an average of 60 per cent; and when held for twelve hours, it increased to 77.2 per cent. These increases were found to be significant (Table VIII). A high correlation was found to exist between the holding time and the number of molds in berries (Figure

Histogram of the Mold Count of Berries Harvested before and after Shaking the Bushes

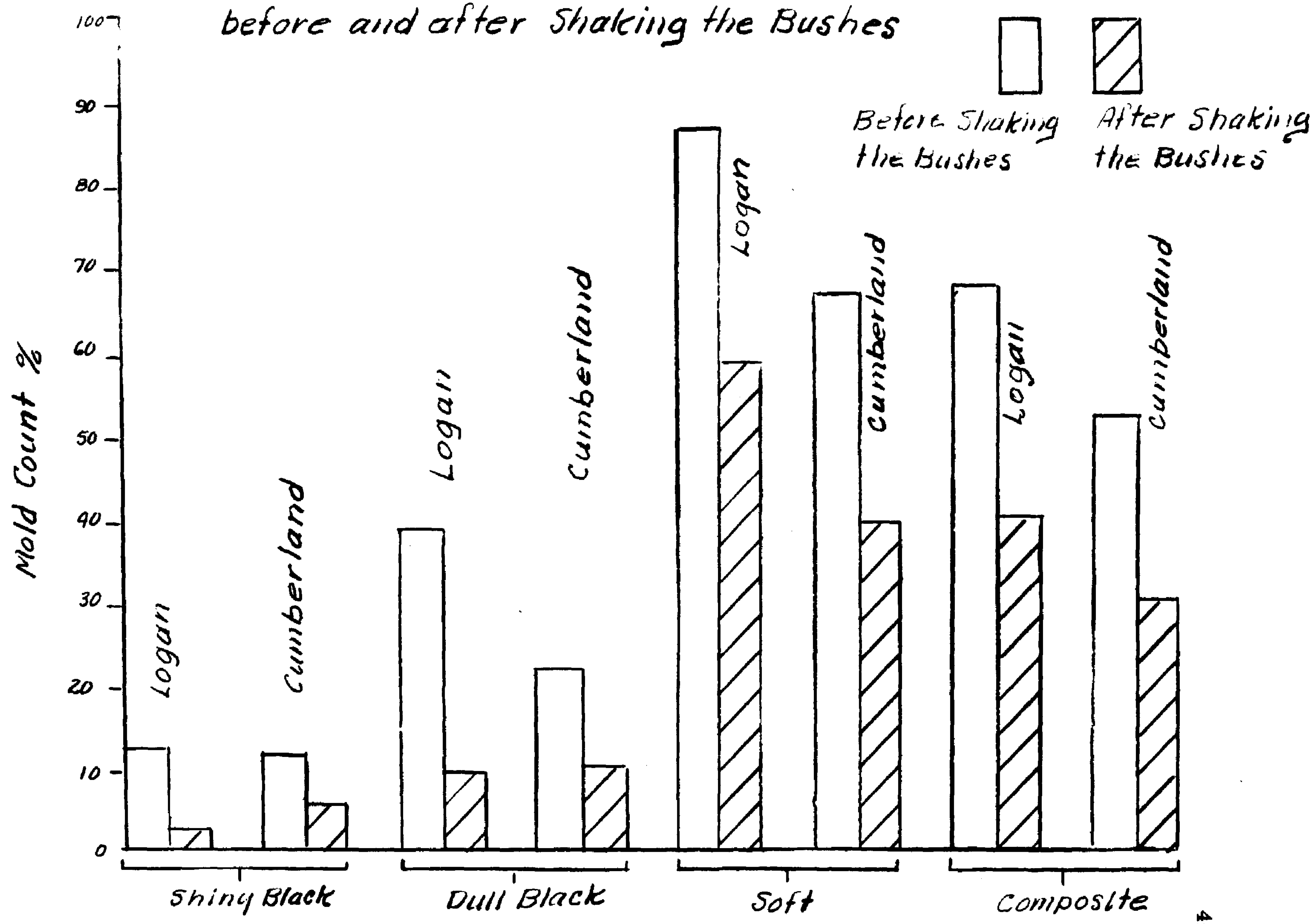


TABLE VIII

MOLD COUNT OF DRY AND WET BLACK RASPBERRIES FROZEN IMMEDIATELY AFTER HARVEST, AND FROZEN AFTER HOLDING FOR SIX AND TWELVE HOURS AT 76° TO 78° F.[1]

	Holding Time (hours)						No. of Samples	Sample F Value	L. S. D.	
	0		6		12				5%	1%
	Dry	Wet	Dry	Wet	Dry	Wet				
Mold Count Avg. Pct.	45.0	45.3	61.2	58.8	74.3	79.8	72	6.21**	17.40	23.10

[1] Original data in Tables XV and XVI (Appendix).

** Highly significant.

4). No significant difference was found between holding the wet and dry berries (Table VIII and Figure 4). The average mold count for berries that were processed immediately after harvesting was 45.0 per cent for the dry berries and 45.3 per cent for the wet berries. Berries that were held for six hours had an average mold count of 61.2 per cent for the dry berries and 58.8 per cent for the wet berries, and those held for twelve hours contained an average of 74.3 per cent mold count for the dry berries and 79.8 for the wet berries.

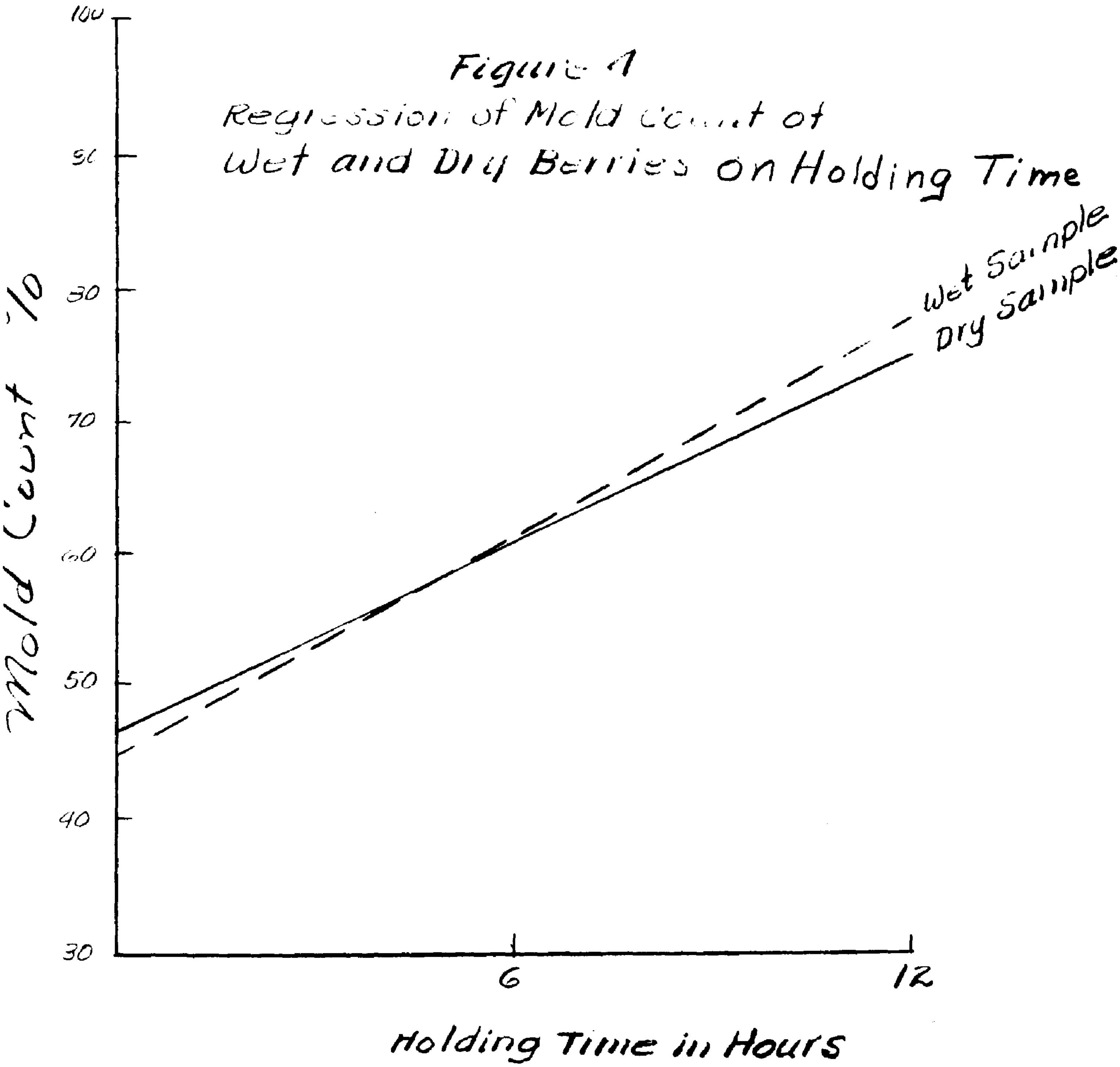


Figure 1
Regression of Mold Count of Wet and Dry Berries on Holding Time

The lack of significant differences between the held wet and dry berries may have been due to the use of individual quart samples. It might be expected that in large stacks of crates, where the evaporation would be decreased, the mold count of wet berries would increase at a more rapid rate than that of the dry berries.

The results obtained for holding are in general agreement with those of Fabian <u>et al</u>. (8), who found that the mold count doubled when black raspberries were held for 8-1/4 hours under warm, humid conditions, and increased from 2 to [illegible] times when stored for twenty-eight to thirty hours. Stevens and Wilcox (29), working on strawberries, stated that once the fungus is inside the berry no amount of external drying is sufficient to stop its development. They were of the opinion that the moisture inside the fruit was enough to allow the fungus to grow sufficiently to destroy the berry. Their experiments indicated that the rate of growth of <u>Rhizopus nigricans</u> increases very rapidly with the increase of temperature above 10° C. (50° F.), and when contaminated berries were held for a few hours at a relatively high temperature (i.e., at 30° C.), the fungus developed in the tissues to such an extent as to cause the breakdown of the berries.

Data obtained are not in agreement with the results obtained by Stevens and Wilcox (29), who stated that the higher shipping quality of strawberries packed wet seemed to be due to the fact that the temperature of the fruit was lowered by washing in cold water, and that by evaporation the berries were kept cool for a considerable time. They also found that the drying of washed berries, especially by exposure to sunlight, increased decay caused by <u>Rhizopus</u> sp., as the subsequent exposure of the dry berries to prevailing high temperatures favored development of the fungus within the tissues.

Effect of Washing Treatment on the Mold Count in Berries

It is a well-known fact that washing the product is necessary, not only for the removal of dirt, but also for the reduction in the number of microorganisms, and thus a product with lower mold count is obtained. The better and more thorough the method of washing, the fewer the number of organisms that will remain on the product. In commercial practice there are several methods in which different principles are used for the removal of dirt and microorganisms. Soaking in water, treating the product in a shaker-spray washer and using high-pressure sprinklers are among the methods employed. One of these methods might be effective in one or more of the products but less effective or unsuitable for others. Each processor uses the method which proves to give the best results under his conditions. In this experiment, some of the methods were used and the results obtained were compared.

Three lots of berries of different mold counts were subjected to the washing treatments. The first lot had an average mold count of 37.0 per cent; the second lot, 55.5 per cent; and the third lot, 76.5 per cent when washed in a shaker washer before processing. These results were considered as controls with which the results of other treatments were compared.

When the berries were washed by a spray washer, the mold count was reduced to 33.5, 48.5, and 71.0 per cent for the first, second, and third lots, respectively (Table IX).

The mold count in berries washed in the shaker washer, followed by a sink washer, had an average mold count of 31.0, 44.0, and 55.0 per cent for the first, second, and third lots, respectively. Berries washed by this method had lower mold counts than those washed in the shaker washer alone, or those washed in a shaker washer and then spray-washed.

When the berries were washed in a shaker washer, then in a spray washer, and finally in a sink washer, they had a mold count of 27.0, 41.0, and 45.5 per cent, respectively.

TABLE IX

EFFECT OF WASHING ON MOLD COUNT OF BLACK RASPBERRIES[1]

Treatments	Shaker Washed A	Shaker Washed and Spray Washed B	Shaker Washed and Sink Washed C	Shaker Washed, Spray Washed, and Sink Washed D	F Value	L. S. D.	
						5%	1%
Avg. of lots	(%)	(%)	(%)	(%)			
I	37.0	33.5	31.0	27.0	23.44**	2.28	3.27
II	55.5	48.5	44.0	41.0	16.44**	4.97	7.13
III	76.5	71.0	55.5	45.5	256.37**	3.56	4.08
Combined	56.3	51.0	43.5	37.8	103.98**	2.30	3.15

[1] Original data in Table XVII (Appendix).

** Highly significant.

The data obtained showed that the greatest reduction of mold count occurred in berries which had the highest initial mold count (Lot III). Since the soft berries seemed to be the cause for a high mold count in a composite sample, it might be expected that any treatment bringing about their removal, such as a high water-pressure wash, would result in a lower mold count for the remaining intact firm berries. This may explain the greater reduction in mold count when berries having a high mold count are washed.

The analysis of variance of Lot II indicated no significant differences between Treatments B and C, and C and D.[1] This lot indicated that it made no difference in mold count whether the berries were sprayed or sink-washed after shaker-washing, and that berries subjected to shaker- and sink-washing had a similar mold count as those subjected to shaker-, sink-, and spray-washing consecutively. From this data alone it might be inferred that spray-washing is of no additional value in the reduction of mold count where sink-washing is practiced, and that, with shaker-washed berries, sink-washing contributes no more to the mold count reduction than spray-washing.

The analysis of variance of Lots I and III indicated that all treamtments differed with respect to their influence on the mold count. In contrast to Lot II, significant differences were found

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[1] A - Shaker washer treatment.
B - Shaker washer treatment and spray washer treatment.
C - Shaker washer treatment and sink washing treatment.
D - Shaker washer treatment and spray and sink washing treatments.

between treatments B and C, and C and D. These data showed that sink-washing was more effective than spray-washing and that the combination of shaker-, sink-, and spray-washing was more effective than shaker- and sink-washing.

When all three lots were combined, it was found that the additional washing treatments resulted in the reduction of mold counts. The consistency of the treatment effects from replication to replication and lot to lot, particularly when the replication and lot means varied, emphasized the value of the treatments over a rather wide range of initial mold counts.

In general, these results would indicate for the reduction of mold count in black raspberries that spray-washing after shaker-washing is not greatly effective, and that for effective reduction in mold count the berries should be sink-washed with high pressure (Figure 5).

Lot III, in which the greatest initial mold count and the highest reduction was obtained, had a steeper slope than the other two curves (Figure 5).

A few workers carried out similar experiments. Fabian <u>et al.</u> (8) stated that berries washed under pressure did not show a significant reduction in mold count. These results do not agree with the

Figure 5

Effect of Washing Treatment on the Mold Count in Berries

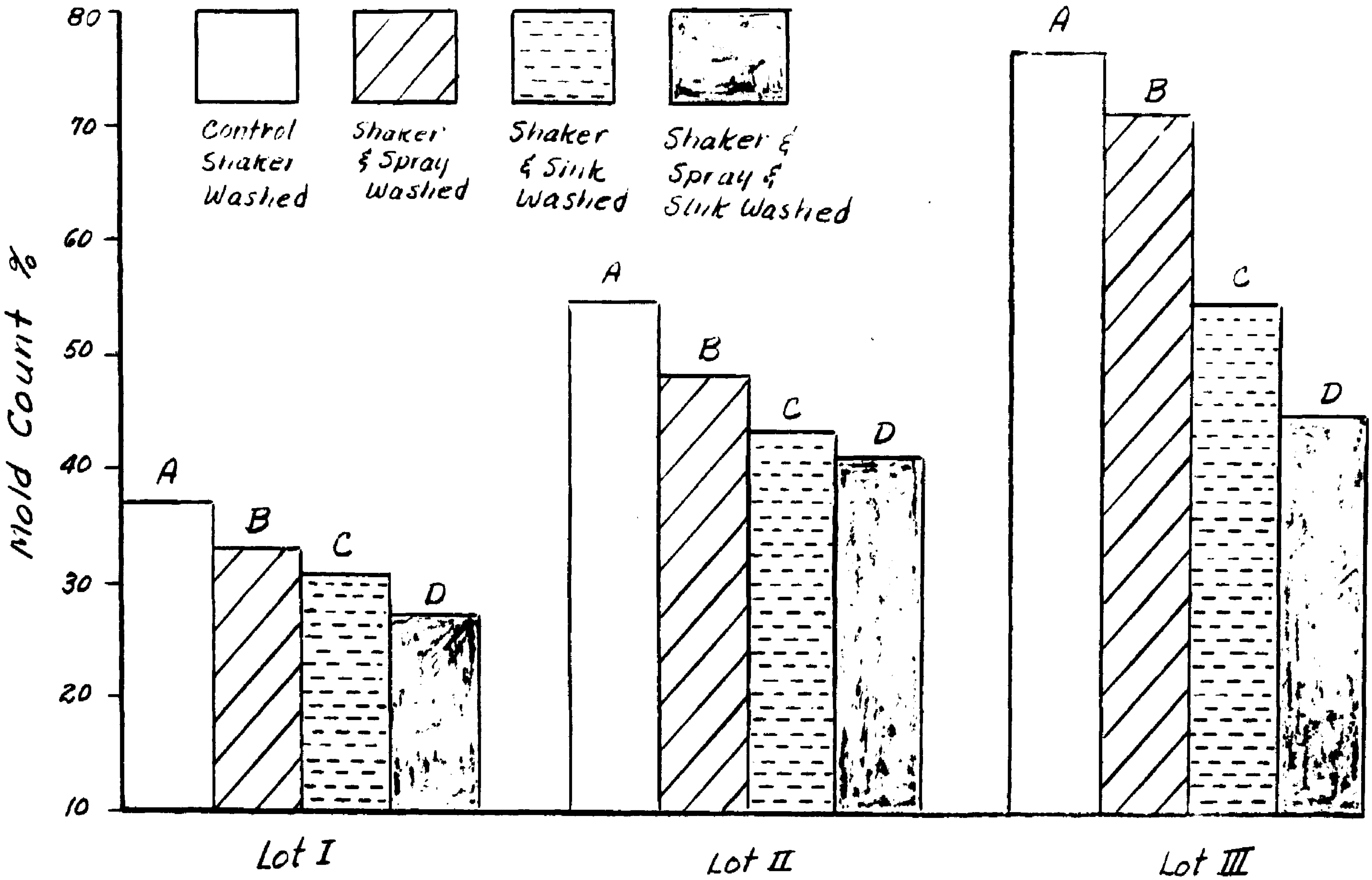
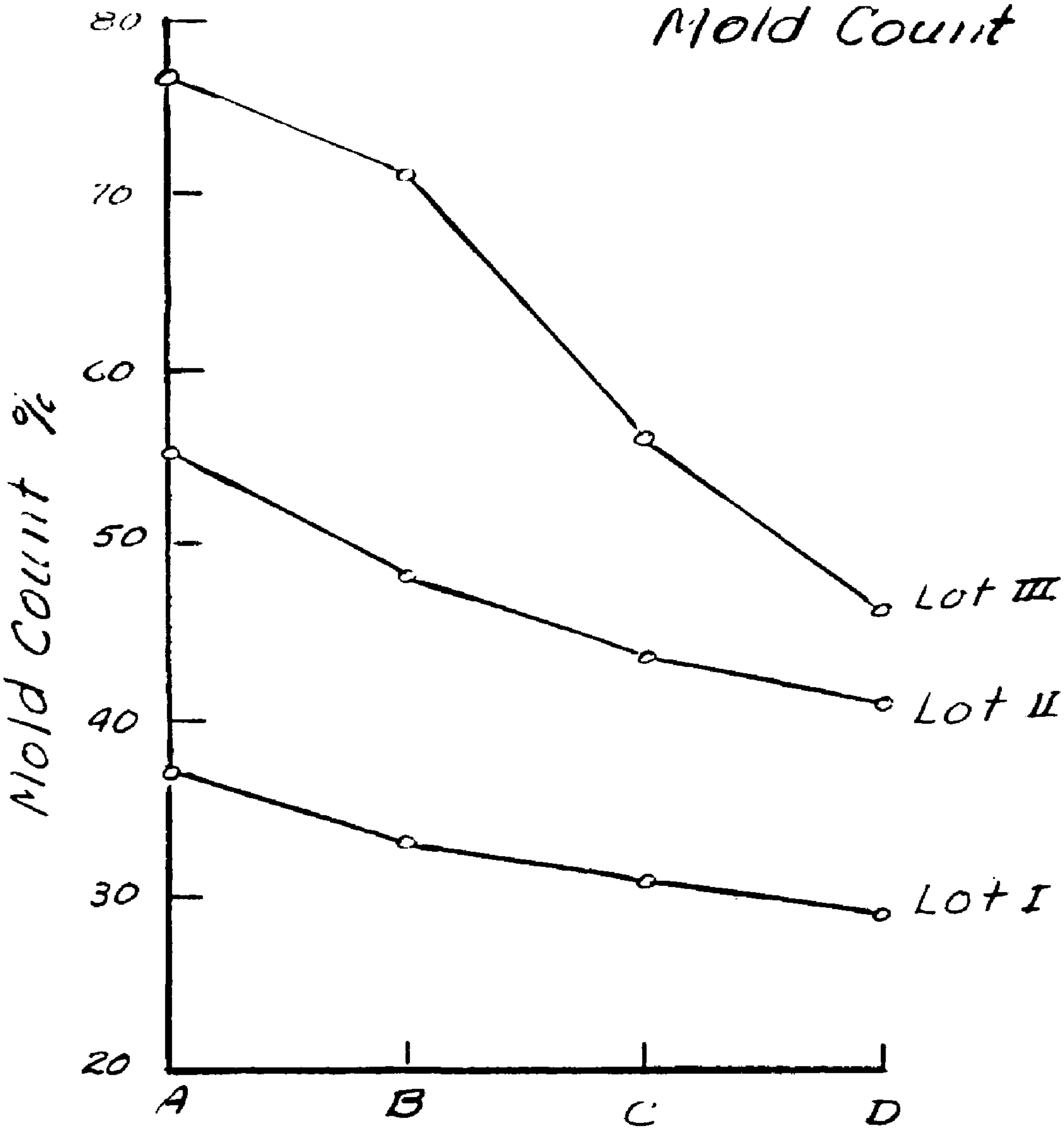


Figure 6
Effect of Washing Treatment on Mold Count



A. Shaker Wash Treatment

B. " " and Spray Wash Treatment

C. Shaker Wash Treatment and Sink Wash Treatment

D. Shaker Wash Treatment, Spray Wash and Sink Wash Treatment data obtained by the author. It might be possible that the samples which Fabian and his co-workers used had a very low initial mold count when compared to that of the berries used by the author.

The data obtained during this work are in general agreement with those of Haynes et al. (12), who found 54 per cent reduction in mold count of strawberries when they were washed with Santomerse in a soaker washer, followed by rinsing with water in a shaker-spray washer. They did not find consistent reduction of mold count either with or without a detergent when the berries were sprayed on a stationary screen, which may be considered comparable to the action of the conveyer-belt-spray washer used in this work. Further, they noted 51 per cent mold-count decrease when the berries were treated with Santomerse under forced immersion washer, comparable to sink-washing procedure in this work.

Effect of Dilutions on Mold Count of Black Raspberry Pulp

Comparison of official method with modified technique for measuring mold count in black raspberries. The mold count as originally devised by Howard (14) was intended to count the number of fields containing hyphae in tomato products, and the same method was later adopted to determine the number of fields containing hyphae in berries and berry products.

For determining mold count more accurately in black raspberries, Helsel and Eisenberg (13) suggested the modified technique in which pectin solution was added to berry pulp in a ratio of 2:1, instead of equal parts by weight employed in the modified Howard method. This procedure was adopted during the course of this work to determine the mold count of black raspberries.

A comparison of mold counts by both of the above-mentioned methods was made to determine whether there were any significant differences. Seventy-five representative samples of black raspberries with mold counts ranging from 6 to 100 per cent were examined for the comparison.

The experimental results showed a linear relationship between the mold count and the dilution levels (Figure 7).

Analysis of variance indicated highly significant differences between mold counts at the levels ranging from 30 to 70 per cent, and significant differences at the 20 to 30 and 80 to 90 per cent levels. No significant differences were obtained between mold counts of the 6 to 20, 70 to 80, and 90 to 100 per cent levels and when the average of all levels was compared, no significant differences were found (Table X).

Figure 7

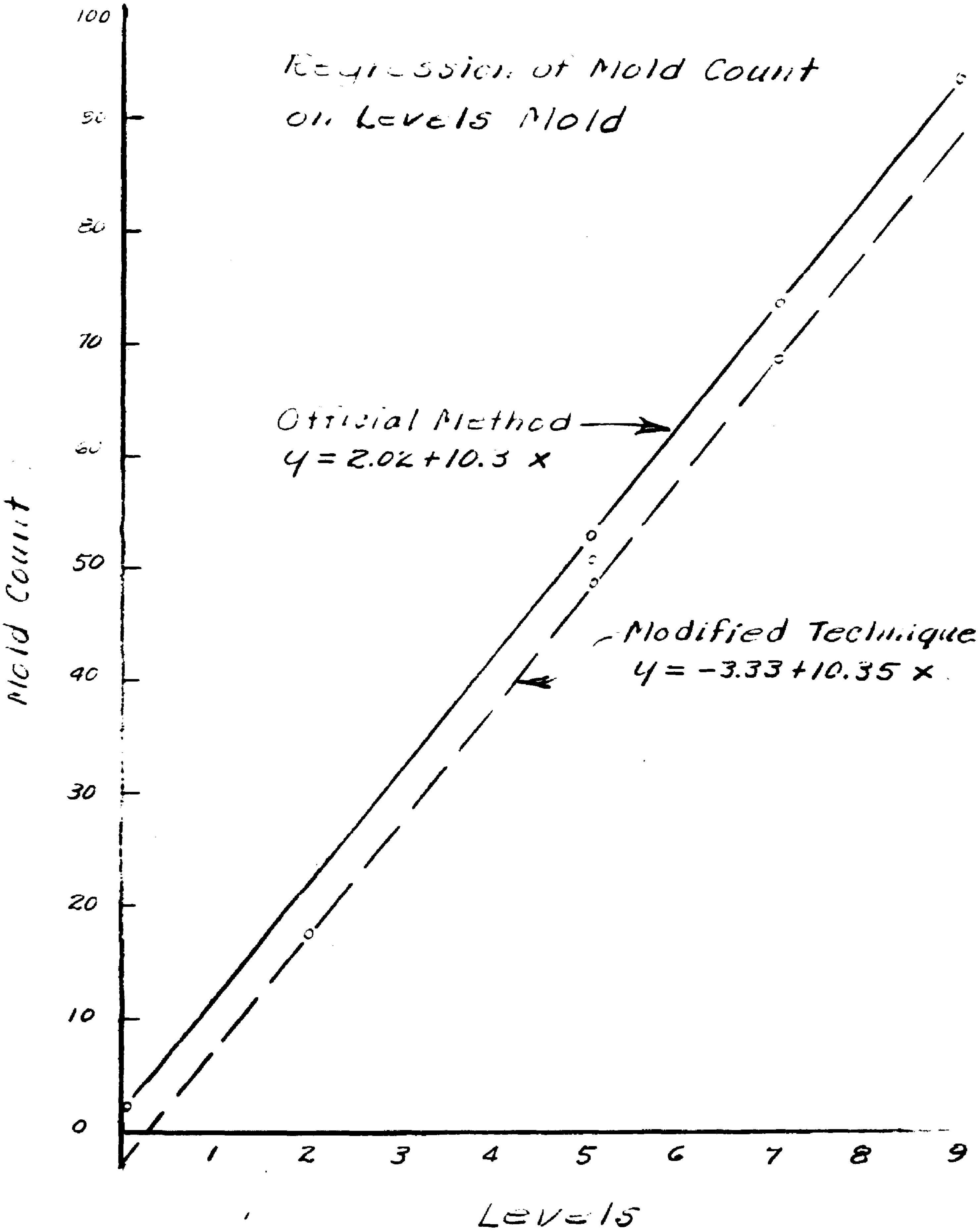

Regression of Mold Count on Levels Mold
Official Method
y = 2.02 + 10.3 x
Modified Technique
y = -3.33 + 10.35 x
Mold Count
Levels
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9

TABLE X

ANALYSIS OF VARIANCE OF MOLD COUNTS ESTIMATED BY OFFICIAL METHOD AND MODIFIED TECHNIQUE FOR MEASURING MOLD IN BLACK RASPBERRIES[1]

Level	Mold Count (%)	Average Mold Count: Official Method 1:1 (%)	Average Mold Count: Modified Technique 2:1 (%)	F Value
1	6-20	11.0	8.0	3.24^{-}
2	20-30	22.5	18.5	6.40*
3	30-40	34.0	29.1	10.44**
4	40-50	44.6	33.7	38.34**
5	50-60	53.5	46.5	18.54**
6	60-70	64.9	57.1	15.19**
7	70-80	73.6	71.4	2.03^{-}
8	80-90	85.0	80.0	6.73*
9	90-100	93.8	90.0	2.27^{-}
	6-100	54.34	49.25	1.26^{-}

[1] Original data in Table XVIII (Appendix).

* Significant.

** Highly significant.

\- Not significant.

These results are in agreement with the observation of Helsel and Eisenberg (13) that mold counts by either method were about the same for any particular sample of berries.

Effect of adding different amounts of pectin solution to black raspberry pulp on mold count. After comparing the official method with modified technique suggested by Helsel and Eisenberg (13) for measuring mold count in black raspberries, a study was made to determine the effect of diluting black raspberry pulp with greater amounts of pectin solution.

Dilution curves were determined for mold counts ranging from 20 to 100 per cent in black raspberries, by diluting the mixture of pulp and pectin solution with additional amounts of the latter. The additions of pectin solution to berry pulp were made so that the proportion of pectin to pulp was 1:1, 2:1, 6:1, 8:1, 10:1, 12:1, and 14:1, in which the per cents of pulp were 50.0, 33.0, 20.0, 14.3, 11.1, 9.1 7.7, and 6.6, respectively (Table XI).

The results obtained showed highly significant correlation ratios between mold counts and dilutions (Figure 8). The reduction in mold count of berries was not in direct proportion to the dilution of the sample with pectin solution.

TABLE XI

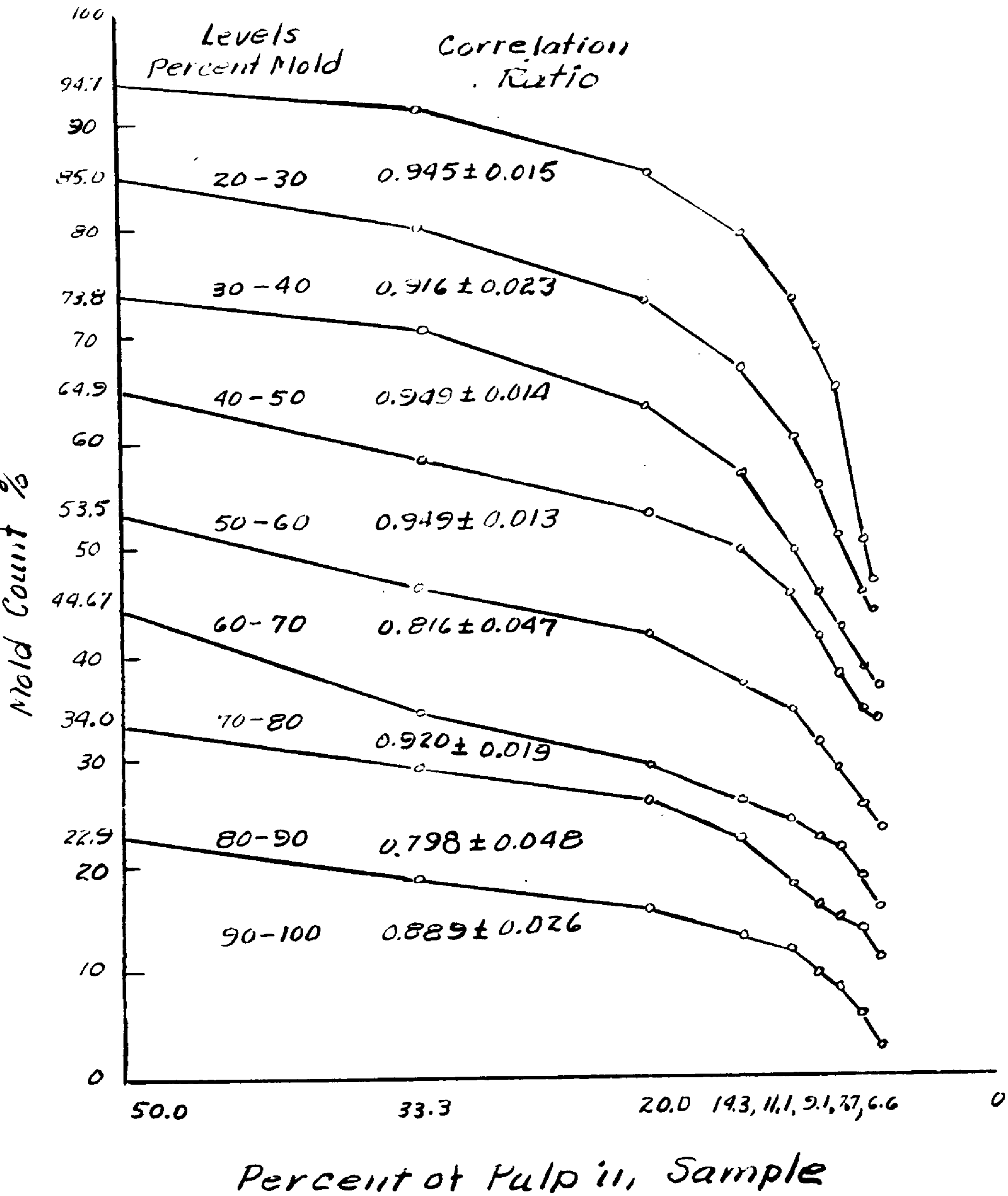
EFFECT OF ADDING DIFFERENT AMOUNTS OF PECTIN SOLUTION TO BLACK RASPBERRY PULP ON MOLD COUNT[1]
(average mold count in various dilutions)

Dilution (pectin to pulp)	Pct. of Pulp in Sample	Levels of Mold Count							
		20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100
1:1	50	22.9	34.0	44.6	53.5	64.9	73.8	85.0	94.7
2:1	33.3	19.1	29.1	34.3	47.3	58.0	70.9	80.0	91.1
4:1	20.0	16.0	26.3	29.4	41.3	52.6	63.1	72.5	86.0
6:1	14.3	12.9	22.0	25.7	37.0	49.7	56.2	67.5	78.9
8:1	11.1	11.3	18.0	24.3	33.3	44.3	50.7	59.5	72.7
10:1	9.1	8.3	15.4	21.7	29.3	39.1	43.8	55.5	65.8
12:1	7.7	6.0	13.7	18.9	27.3	35.4	38.2	49.5	54.9
14:1	6.6	3.7	10.3	14.9	23.0	33.1	34.9	43.5	46.7

[1] Original data in Table XIX (Appendix).

Figure 8

Effect of Adding Different Amounts of Pectin Solution on Mold Count



These results are in general agreement with those obtained by Steinkraus (28).

DISCUSSION

Experimental results indicated a close correlation between stage of maturity and mold count of black raspberries. It has been recognized that soft fruits are more susceptible to attack by fungi and other microorganisms, and the experimental work demonstrated that berries in advanced stages of maturity always had a higher mold count than those in earlier ripening stages under similar conditions. This may be due to mechanical injury of the skin caused by wind or rain while the berries were on the bushes, or by the dissolving of the skin between individual drupelets by mold enzymes, permitting the molds to enter the fruit. The presence of sugar and acids in the fruits favors rapid development of molds after they once enter the berries under proper environmental conditions. A close correlation also existed between the mold counts in soft berries and of composite samples which were a mixture of soft fruit having a high mold count, with dull or shiny-black raspberries having lower mold counts. The berry maturity and the time interval between ripening and harvesting of the berries for delivery to the factory seemed to be dominant factors influencing the mold count of black raspberries.

There was no doubt that weather conditions during the harvesting period of black raspberries affected the conditions of the fruit. The experimental results indicated a definite trend towards higher mold counts with a delay of harvest following a rain. It was observed that the farmers waited too long after a rain to pick the berries, affording an opportunity for the molds to develop. The pickers were found to leave foliage-hidden fruits unharvested, which then became overripe, infected, and served as a source of further infection. It was noted that rain hastened the maturity of the berries. Heavy rain not only made the fruit soften more quickly, but sometimes bruised the berries, which then became more susceptible to attack by molds. In 1951, there was a heavy rain on July 21. After two days, a slight fungus growth was observed on bruised drupelets near the caps of most shiny-black berries. At the same time, berries in more advanced stages of maturity had profuse external mycelial growth. Uninjured, firm shiny-black raspberries did not show any visible symptoms of mold, though they were among heavily infected fruit in the same clusters. In many cases the first visible symptoms of mold attack either in the dull or shiny-black stages often were found to be on the drupelets near the place where the fruit is detached from the receptacle. This indicates that the mold might have entered the berry through the basal portion of the fruit when it was loosened from the cap. In dry weather, profuse mycelial growth was noted near the basal portions of dull-black berries just maturing into the soft stage.

The data obtained showed significant increase in mold count of soft ripe and composite sample of berries within twenty-four hours after rain. After twenty-four hours, the mold count increased considerably. The greatest change in the mold count of berries was noted in black raspberries that were picked from bushes which had not been harvested for four days. This undoubtedly was due to the dropping-off of many of the older heavily infected fruits, and the fact that weather conditions were unfavorable for germination and growth of mold spores. Observations made at the factories showed that the mold count in the black raspberries was low at the beginning of the season, when the weather was hot and dry, but mold count in the berries increased later in the season, following rainfall.

Therefore, it would seem to be essential to develop a system of harvesting which would insure the collection of berries before they become too mature. At times, such a system may require the harvesting of berries when they are wet from dew or rain. On the basis of the data obtained during the course of this investigation, it is justifiable to assume that the harvesting of black raspberries should be made within twenty-four hours after rainfall.

Farmers have often reported that they had a major problem in procuring help in harvesting berries when they were wet, and as a result they were left unharvested for some time. Thus, molds would have a chance to attack the fruit during a wet period and would develop rapidly inside the fruit if the weather became hot following the rain.

Besides harvesting the fruit within twenty-four hours after rainfall, another method could be employed to remove soft, overripe fruits with high mold counts. From the experimental results it was found that shaking the bushes of Cumberland and Logan varieties before harvest caused appreciable reduction in mold count. To make practical use of these findings, one must either supply leather gloves to pickers in order to obtain their cooperation in the shaking operation, or devise a mechanical means of shaking the bushes before harvest. It is a common belief of the growers that the growing of the Logan variety would avoid high mold count, since this variety of berries matures earlier than the Cumberland variety. However, the factual data do not support this view, and the mold count of black raspberries is more dependent on weather conditions during the harvesting period than on the variety.

The time interval between harvesting the berries and processing them is generally recognized by everyone concerned with the industry as an important factor in influencing the mold count. There was definite evidence from these experiments that the mold count increased as the time of holding the berries at the factory was prolonged before processing. The data showed no significant difference in the increase of mold count between dry and wet berries when they were held under identical conditions. When the mold was present inside the berries, it seemed to find a favorable medium for rapid development under proper environmental conditions. In addition, it was difficult to estimate the mold count in the berries by visual appearance of the fruit.

When fruit was received at the factory, it was found possible to reduce the mold count by applying high water pressure during processing of the berries. A combination of treatment with shaker washer, water spray on the belt, and washing with water in sink by thorough agitation seemed to be the most effective method for reducing mold count in berries under normal factory operations. When high water pressure was used only the firm berries having lower mold counts.

The mold-count method has been used for many years as an objective test for determing the relative amount of decomposition in fruit products. The mold-count proceedure is a convenient and often necessary test for proper evaluation of the quality of frozen or canned raspberries, because the original appearance of the fresh fruit is so altered by the processing as to make direct visual examination difficult and inadequate. For proper evaluation of the mold population it is necessary to prepare the samples in a way that facilitates accurate examination. The greater dilution of two parts pectin solution to one part of pulp prepared the sample for easier examination than the official method now in general use. This change in the method caused a lighening of the deep color and decreased the density of pulp taken from frozen raspberries. The experimental results showed no significant differences in the overall mold count between the official and modified methods, with the modified method generally showing slightly lower mold counts. When the mixture of raspberry pulp and pectin solution was diluted further, it was noted that there was a progressive decrease with dilution, but the reduction in mold count was not in proportion to the amounts of dilution. Highly significant correlation ratios at all levels of dilutions were obtained, indicating a nonlinear relationship between mold count in the berries and dilutions of the samples.

SUMMARY AND CONCLUSIONS

A study was made to determine: (1) the mold count of black raspberries collected at various stages of maturity and at different intervals after rain; (2) the effect of holding dry and wet berries on the mold count; (3) the effect of shaking the black raspberry bushes before harvesting; (4) the effect of different methods of washing on the mold count; and (5) the relationship between various dilution methods and the mold count.

The data obtained during the 1951 and 1952 seasons showed that:

1. Soft, ripe black raspberries had higher mold counts than any other stage of maturity, and eliminating these fruits would greatly reduce the mold count.

2. Weather conditions play an important part in the mold count. and the fruit should be harvested immediately after a rain to keep the mold count at a lower level.

3. The mold counts of the Cumberland black raspberry were significantly lower at all stages of maturity than the Logan variety.

4. Shaking the bushes before harvesting significantly reduced the mold count.

5. Holding the berries after harvesting caused highly significant increases in the mold count, and the results obtained indicated that the fruit should be processed less than six hours after picking.

6. Washing seemed to be effective in reducing the mold count of berries. Subjecting berries to water washing by shaker washer, water spray on the conveyer belt, and sink washing with thorough agitation of the fruit was found to be the most effective method in reducing the mold count.

7. No significant differences were found between the official and modified methods of mold counting.

8. Adding different amounts of pectin solution to black raspberry pulp lowered the mold count, but its decrease was not in direct proportion to the amounts of pectin solution.

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APPENDIX

TABLE XII

MOLD COUNT ON FRUIT HARVESTED AT DIFFERENT HOURLY INTERVALS AFTER RAINS (1952 SEASON)

Stages of Maturity	Field No.	Hours After Rain				
		13-14 (%)	17-18 (%)	23-24 (%)	48 (%)	72 (%)
Shiny-black . . .	1.	2	0	2	8	6
	2.	0	2	0	0	12
	3.	4	0	0	8	14
	4.	0	2	0	10	16
	5.	14	16	20	20	14
	6.	6	4	4	6	8
	7.	0	4	8	10	12
	8.	6	8	10	14	10
Dull-black	1.	8	8	8	12	24
	2.	4	6	0	6	30
	3.	8	0	6	8	14
	4.	12	2	6	20	22
	5.	28	22	38	40	36
	6.	8	0	14	12	18
	7.	4	6	20	12	14
	8.	10	12	16	18	22

TABLE XII (Continued)

Stages of Maturity	Field No.	Hours After Rain 13-14 (%)	17-18 (%)	23-24 (%)	48 (%)	72 (%)
Soft	1.	12	24	40	62	56
	2.	72	72	22	24	42
	3.	16	42	20	72	54
	4.	14	12	18	40	42
	5.	28	62	90	46	68
	6.	70	90	56	78	100
	7.	46	60	86	100	24
	8.	28	38	46	66	88
Composite	1.	32	56	30	20	30
	2.	22	20	12	40	52
	3.	14	20	26	18	40
	4.	10	24	32	30	28
	5.	24	38	56	36	50
	6.	52	62	84	52	62
	7.	24	32	50	66	20
	8.	16	20	32	46	52

TABLE XIII

EFFECT OF HARVESTING BLACK RASPBERRIES AT DIFFERENT INTERVALS AFTER RAIN ON JULY 11 AND 12, 1951

Interval Since Previous Harvest	Field No.	Mold Count (per cent)			
		Maturity Stages			Composite Samples
		Shiny-Black	Dull-Black	Soft-Ripe	
1 day . .	1.	4	18	74	22
	2.	4	4	62	8
	3.	4	12	100	20
	4.	4	10	80	20
	5.	2	12	76	20
	6.	0	14	46	30
	7.	8	10	70	26
	8.	6	14	92	48
	9.	4	12	64	24
	10.	2	10	62	22
2 days . .	1.	0	2	50	14
	2.	6	18	68	30
	3.	8	20	80	26
	4.	4	16	52	20
	5.	6	20	46	24
	6.	4	18	100	22
	7.	4	4	72	8
	8.	4	12	60	20
	9.	4	74	48	22
	10.	8	62	42	30

TABLE XIII (Continued)

Interval Since Previous Harvest	Field No.	Mold Count (per cent)			
		Maturity Stages			Composite Samples
		Shiny-Black	Dull-Black	Soft-Ripe	
3 days . .	1.	0	0	100	6
	2.	8	40	100	76
	3.	0	18	56	68
	4.	8	20	90	54
	5.	16	26	94	62
4 days . .	1.	0	8	8	4
	2.	4	4	12	6
	3.	2	4	10	4
	4.	2	12	10	4
	5.	4	6	6	6
5 days . .	1.	2	4	10	4
	2.	4	8	16	12
	3.	4	10	20	6
	4.	6	18	24	10
	5.	4	12	42	10

TABLE XIV

EFFECT OF SHAKING BUSHES ON MOLD COUNT OF BERRIES

Variety	Field No.	Mold Count (per cent)							
		Shiny-Black		Dull-Black		Soft		Composite	
		Before	After	Before	After	Before	After	Before	After
Logan . .	1.	16	6	34	10	82	60	90	50
	2.	14	4	40	12	76	54	84	40
	3.	4	0	46	8	88	60	76	38
	4.	20	4	52	14	94	62	82	40
	5.	4	0	28	4	92	60	70	38
	6.	18	6	38	12	90	60	76	40
Cumberland . . .	1.	2	6	20	8	86	56	60	40
	2.	10	4	24	6	70	40	56	30
	3.	16	8	44	16	68	38	62	34
	4.	10	4	24	14	82	52	60	32
	5.	20	8	12	12	54	28	40	26
	6.	14	6	20	8	46	26	42	26

TABLE XV

EFFECT OF HOLDING WET BERRIES FOR ZERO, SIX, AND TWELVE HOURS AT 76° TO 78° F.

Date of Collection	Sample No.	Mold Count (per cent)		
		0 Hours	6 Hours	12 Hours
July 18, 1952	1.	78	74	100
	2.	22	28	98
	3.	56	72	94
	4.	36	42	52
	5.	62	84	100
	6.	42	66	82
July 23, 1952	1.	44	68	84
	2.	10	16	24
	3.	54	70	92
	4.	42	60	78
	5.	62	80	94
	6.	36	46	60

TABLE XVI

EFFECT OF HOLDING DRY BLACK RASPBERRIES FOR ZERO, SIX, AND TWELVE HOURS AT 76° TO 78° F.

Date of Collection	Sample No.	Mold Count (per cent)		
		0 Hours	6 Hours	12 Hours
July 17, 1952	1.	32	76	86
	2.	60	84	90
	3.	72	40	56
	4.	22	30	42
	5.	44	68	72
	6.	30	56	66
July 21, 1952	1.	52	70	92
	2.	64	80	96
	3.	76	86	100
	4.	32	54	68
	5.	26	36	58
	6.	30	54	66

TABLE XVII

EFFECT OF WASHING WITH WATER ON MOLD COUNT OF BLACK RASPBERRIES

Lot	Sample No.	Mold Count (per cent)			
			Shaker Washed and Spray Washed	Shaker Washed and Sink Washed	Shaker Washed, Spray Washed, and Sink Washed
I	a.	30	28	26	24
	b.	42	38	36	32
	c.	50	42	40	38
	d.	26	26	22	22
II	a.	60	56	48	40
	b.	44	32	32	34
	c.	52	48	42	40
	d.	66	58	54	50
III	a.	82	76	60	56
	b.	74	70	56	44
	c.	74	68	52	40
	d.	76	70	54	42

TABLE XVIII

MEASUREMENT OF MOLD IN BLACK RASPBERRIES ESTIMATED BY ORIGINAL HOWARD METHOD AND MODIFIED TECHNIQUE

Mold Count (%)		Mold Count (%)		Mold Count (%)	
Official Method	Modified Method	Official Method	Modified Method	Official Method	Modified Method
6	6	40	32	72	70
6	4	42	38	74	64
6	6	42	28	74	72
10	8	46	32	74	76
10	6	46	36	76	74
12	8	48	34	76	76
12	10	48	36	78	76
14	6	50	42	82	74
16	12	50	46	82	76
18	14	52	46	84	76
20	18	52	48	84	80
20	16	54	42	86	84
20	20	56	46	86	78
22	12	56	50	88	84
22	18	58	52	88	88
24	20	62	52	90	88
24	20	62	56	90	86
28	24	64	58	92	88
30	28	64	54	92	86
32	26	66	58	94	74
32	28	68	68	94	94
36	32	68	54	94	92
36	26	70	68	96	96
36	30	70	70	96	96
36	34	72	68	100	100

TABLE XIX

EFFECT OF DILUTING BLACK RASPBERRY PULP WITH PECTIN SOLUTION

Mold Count (per cent) at Dilution:							
1:1	2:1	4:1	6:1	8:1	10:1	12:1	14:1
20	18	14	12	8	8	6	4
20	16	12	10	8	8	4	4
22	18	16	12	10	8	6	4
22	18	14	12	10	8	6	4
24	20	18	14	10	6	6	2
24	20	16	12	10	8	6	4
28	24	22	18	16	12	8	4
30	28	22	20	16	14	10	6
32	26	22	18	16	12	12	8
32	28	20	20	14	12	10	6
36	32	34	26	22	20	18	18
36	30	28	24	20	18	16	12
36	34	30	26	22	18	14	14
36	26	28	20	16	14	16	8
40	32	24	18	24	18	14	8
42	38	32	28	22	22	18	14
42	32	24	24	22	20	20	16
46	32	28	24	22	20	18	16
46	36	34	30	28	24	20	16
48	34	30	28	28	24	22	18
48	36	34	28	24	24	20	16

TABLE XIX (Continued)

Mold Count (per cent) at Dilution:							
1:1	2:1	4:1	6:1	8:1	10:1	12:1	14:1
50	42	36	32	30	26	24	20
50	46	38	32	32	24	26	20
52	46	40	36	34	26	26	22
52	48	40	38	32	30	26	22
54	42	38	34	30	30	24	24
56	50	46	42	36	32	30	26
56	52	46	40	36	34	32	24
58	52	46	42	36	32	30	26
62	52	44	42	38	34	32	28
62	56	50	44	40	32	30	28
64	58	52	48	42	36	30	28
64	54	48	44	40	38	32	30
66	58	52	48	40	36	32	32
68	60	54	50	46	40	36	34
68	68	68	72	64	58	56	52
70	68	58	56	52	38	32	28
70	70	60	58	54	42	36	34
72	68	58	50	46	42	38	38
74	72	66	54	46	44	36	22
74	64	52	42	40	36	30	26
74	76	68	54	42	40	38	38
76	74	70	64	58	50	42	40
76	70	66	62	58	48	44	42

TABLE XIX (Continued)

Mold Count (per cent) at Dilution:							
1:1	2:1	4:1	6:1	8:1	10:1	12:1	14:1
78	76	70	66	60	54	48	46
82	74	72	68	62	58	52	44
82	76	70	62	56	54	46	46
84	76	58	48	28	24	16	14
84	80	72	64	56	50	48	42
86	84	78	74	66	64	60	56
86	78	74	68	66	60	56	42
88	84	74	78	68	64	62	58
88	88	82	78	74	70	56	46
90	88	76	72	68	62	58	50
90	86	80	74	70	64	60	52
92	88	86	82	76	66	60	52
94	74	76	70	68	64	50	48
94	94	92	90	80	74	58	44
96	96	94	92	72	72	66	44
96	96	92	56	52	38	32	28
100	98	84	86	84	76	52	48
100	100	94	88	84	76	58	54

EFFECT OF HARVESTING CONDITIONS ON MOLD COUNT OF BLACK RASPBERRIES

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AN ABSTRACT

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KUCHIBHATLA LAKSHMI NARASIMHAM ABSTRACT

A study was made to determine various factors that might influence the mold count of processed black raspberries in Michigan during the seasons of 1951 and 1952. Effects of different stages of maturity of berries harvested at different intervals after rains, shaking the black raspberry bushes before harvesting the fruit, holding wet and dry berries for different intervals before processing and subjecting the berries to various water-pressure washings were studied. The relationship between various dilution methods and mold count in berries was also determined. The berries were carefully harvested to prevent any mechanical injury. They were frozen at the factories and brought to Michigan State College for further examination.

The frozen black raspberries were thawed by keeping the cans containing them in a vessel holding boiling-hot water. After thawing, the samples were pulped by a laboratory pulper with screen openings of 0.027 inch in diameter. One part of black raspberry pulp obtained as above was diluted with two parts by weight of 3 per cent pectin solution. The pulped berry samples were examined for mold filaments by the modified procedure of the official mold-count method.

Soft ripe black raspberries were found to have higher mold counts than any other stage of maturity, and seemed to be responsible for the higher mold counts of the composite samples. Highly significant differences between maturity stages and a definite increase in the mold count with time following rainfall were observed, indicating that weather conditions play an important role in influencing the mold count of berries. Highly significant reduction of mold count in berries was obtained by shaking the black raspberry bushes before harvesting the fruit. The berries of Logan variety had higher mold counts than those of the Cumberland variety at all stages of maturity except the shiny-black stage.

The time that elapses between harvesting the berries and processing them was found to have a great effect on the mold count of the final product. A significant increase in the mold count of the berries and a high correlation between holding time and the number of molds in berries were found. No significant difference between holding the wet and dry berries was observed in this study.

Washing seemed to be effective in reducing the mold count of berries. Black raspberries subjected to water washing by shaker washer, water spray on the conveyor belt, and sink washing with thorough agitation of the fruit was found to be the most effective method for reducing the mold count.

No significant differences were obtained when mold counts determined by modified technique were compared with those obtained by the official method. Adding different amounts of pectin solution to black raspberry pulp lowered the mold count, but its decrease was not in direct proportion to the amounts of pectin solution added.