HILGARDIA

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOLUME 26 MAY, 1957 NUMBER 12

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Fine sweet table wines of the Sauternes type owe their quality to a fungus, Botrytis cinerea Pers., which causes the affected grapes to shrivel and the percentage of sugar per unit of volume to increase. The quantity of wine produced is relatively small, but its quality is proportionately high.

Although the fungus occurs wherever grapes are grown, climatic conditions favorable to its development prevail in only a few limited regions in Europe. Since California does not provide a suitable field environment for this desirable development of B. cinerea, a plant experiment was designed to provide the necessary conditions of temperature and relative humidity. Harvested grapes were placed on wire trays and sprayed with an aqueous suspension of the spores of the fungus. Most favorable results were obtained when spraying was followed by a 1- or 2-day infection period at 68° F and 95 to 100 per cent relative humidity, then by a dehydration period of 6 to 14 days with a temperature of 68° F and 50 to 70 per cent relative humidity. Musts with Ballings of 30° to 40° were produced, and yields ranged from 50 to 100 gallons per ton (fresh weight).

In California, wine production from grapes infected with B. cinerea would have to be carried out under carefully controlled conditions, and the output would thus be limited. However, the findings of this study indicate that eventually California wine growers may be able to add a new and desirable wine type to their present production.

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THE USE OF BOTRYTIS CINEREA PERS. IN THE PRODUCTION OF SWEET TABLE WINES[1]

KLAYTON E. NELSON[2] and MAYNARD A. AMERINE[3]

INTRODUCTION

THE GREY MOLD *Botrytis cinerea* Pers. is found on grapes wherever they are grown, though it is seldom noted in hot, dry regions. If the relative humidity is too low, little or no infection occurs. In Central European countries where the relative humidity is generally high, it is a common parasite on mature or nearly mature grapes.

Müller-Thurgau (1888) noted that growth of the fungus loosens the skin of the berry. After infection, depending on the climatic conditions, two different effects on the fruit are noted. In rainy weather the infected grapes do not lose water, and the percentage of sugar remains the same or may even decrease. Secondary infections by other organisms may follow. According to Laborde (1908), cold, wet conditions lead to excessive botrytis growth without drying, called *pourriture grise* in France. Such conditions are favorable for the growth of *Penicillium* sp., *Aspergillus* sp., and members of the mucoraceous fungi, which may displace the botrytis. This results in the rapid consumption of the sugar and the production of gluconic and glucuronic acids. It is then called *pourriture vulgaire* in France. Under moist conditions, berries punctured by insects frequently develop undesirable rots (*pourriture acide*). Juice that has exuded from such injuries does not dry, and yeasts transform the sugars to alcohol. *Acetobacter* sp. in turn transforms the alcohol to acetic acid. In contrast, if warm, sunny weather follows infection, the berries lose moisture by evaporation, shriveling occurs, and the percentage of sugar in the juice increases (*pourriture noble*). It is this latter result that has led to the commercial use of *Botrytis cinerea* in the production of sweet white table wines in certain areas of Europe, the high-sugar must being very desirable for the production of high-quality wines of this type.

The general effect of the mold is to reduce the total amount of sugar slightly but to increase markedly the percentage of sugar per unit of volume. Müller-Thurgau (1888) noted that 100 sound White Riesling berries yielded 100 ml of must. The same number of berries attacked by the mold yielded

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only 52 ml. The normal must had 18.24 per cent sugar compared to 30.26 per cent in the molded fruit, and the total acidity was 0.89 per cent compared to 0.79. These acid values mean that *Botrytis cinerea* had reduced not only the total amount of acidity but also the relative amount. This is a very valuable and unique characteristic of this mold, which is not a property of the other common fungi that attack grapes. In addition, botrytised grapes are always high in glycerin, which is formed in the fruit during the metabolism of some of the sugars or acids by the mold. Another noteworthy effect is that this mold does not impart a foreign or moldy odor to the must; in fact, wines of properly botrytised grapes have a special odor, which is one of their most attractive features.

The viticultural industry of California has not been able to produce a Sauternes-type wine, largely because climatic conditions prevent the mold from developing in the manner found so desirable in Europe. If such development could be induced under California conditions, the wine industry of this state might be able to add a distinct and valuable wine type to those now produced. The present study was therefore undertaken—first, to determine the most satisfactory techniques for inoculating grapes with *Botrytis cinerea;* and second, to establish the most favorable conditions for its development. Pilot-plant experiments based on these results could be undertaken. Since it is an expensive operation consumer-acceptance studies should also be made.

HISTORY

France. According to Laborde (1908), the use of botrytised grapes was general in the Sauternes region of Bordeaux as early as 1845. Redding (1861) correctly describes the use of botrytised grapes in Sauternes, Bergerac, and Anjou. Shaw (1863) visited Sauternes, probably during the vintage of 1862, and described the multiple harvesting of botrytised fruit to produce sweet table wines. Thudichum and Dupré (1872) described the practice in that region in 1867, and what they reported is essentially the present French procedure. Limited amounts of similar sweet types are produced in Bergerac, Montbazillac, and other Gironde districts. Gaillac, east of Bordeaux and north of Toulouse, also produces sweet table wines from botrytised grapes and has done so since at least 1860 according to Shand (1928). Shand also notes wines from botrytised grapes in Béarn in southern France.

The practice in Sauternes became so famous that the name Sauternes has become almost a type name for sweet white table wines, although the annual production there is less than a million gallons. The only other French region where this procedure is regularly followed on a large scale in favorable years is in the Anjou-Saumur-Tours district of the Loire Valley, and here only a portion of the wines, even in favorable years, is sweet.

The general practice in Sauternes is to harvest the botrytised fruit when it reaches the desired stage of shriveling. This involves picking off portions of the cluster in successive harvests (*tries*), since not all the berries are infected equally or shriveled sufficiently at the same time. Seven or more pickings have been made in some Sauternes vineyards. However, the number

varies from vineyard to vineyard depending on the degree of sugar desired and on how much volume the proprietor can sacrifice, because the attack of botrytis and the subsequent shriveling always markedly reduce the yield. In the warmer years, such as 1904, musts with a sugar content as high as 50 per cent have been reported, according to Laborde (1908). The more usual result is about 30 to 40 per cent sugar, which yields wines of approximately 13 per cent alcohol and 4 to 14 per cent sugar.

Because of the great reduction in volume, sweet table wines produced from botrytised grapes are always expensive. They have, however, attained enviable consumer acceptance because of their luscious sweet taste and perfumed odor. The French name *pourriture noble* reflects the desirable quality of the mold. The most famous vineyard, Château d'Yquem, has become synonymous with the finest quality in French wines. Laborde (1908) gave the following analyses of Sauternes wines:

	Alcohol *per cent*	Sugar *per cent*	Glycerine *per cent*
Minimum	6.4	1.34	0.70
Maximum	17.5	44.5	2.40
Average	12.9	11.1	1.62

Germany. According to Thudichum and Dupré (1872), the advantage of harvesting grapes very late in the Rheingau district originated accidentally in 1775 when the owner of Schloss Johannisberg, the Bishop of Fulda, forgot to send permission to start the harvest. Müller (1930) dates the beginning of the practice at 1773 but notes that late harvesting in Germany was described in Roman writings. At Steinberg the practice is reported to date from 1822.

The problem of when the late-picking of botrytised grapes originated in Germany is exhaustively treated by Basserman-Jordan (1923). He recognizes the Roman procedures of harvesting dried (raisined) grapes as distinct from the selection of botrytised fruit, but acknowledges the difficulty of exactly dating the latter practice. He dates the practice of multiple pickings in Germany from 1581 at the latest (and perhaps from 1520 at Geisenheim and 1579 at Mainz). The first use of the verb *auslesen* ("to select from") he dates from 1650. The original text in this case, however, simply indicates that green and moldy grapes should be harvested separately from the sound fruit. The noun *Auslese* did not appear in this connection until the nineteenth century. He considers it certain that the practice in its modern form was established by the first decade of the nineteenth century, and gives evidence from labels of the vintage of 1811. This appears reasonable because practical hydrometers did not come into use in Germany until the last half of the eighteenth century.

The current practice there is to separate the botrytised clusters. The wines produced are called *Auslese*. Wines produced from such grapes are usually sweet. If small, heavily botrytised portions of the clusters, or only affected berries, are selected, the wines are called *Beerenauslese* and are always sweet. If the berries are quite shriveled a *Trockenbeerenauslese* wine may be produced. These latter berries sometimes drop to the ground and have to be laboriously picked up. The wines are very sweet and very expensive.

Auslese wines are not produced every year in Germany but only when the climatic conditions are favorable. Even in the most favorable years the production is small. However, these sweeter, highly aromatic types help establish the reputation of German wines for quality.

Botrytis also attacks red grapes, but the wines have a brownish-red color and the sweet German wines produced from Pinot noir are not of high quality. Rentschler and Tanner (1955) have also reported on botrytised red grapes in Switzerland where botrytis attack is considered wholly undesirable.

Hungary. Tokay wine is produced in a small district in northeast Hungary. Not all the wine is sweet. Müller (1930) reported an annual production of sweet types of 21,000 to 80,000 gallons.

Greger (1881) reports that the Tokay wines of Hungary have had a reputation since the thirteenth century. The Tokay *essenz* is made from grapes that have shriveled and nearly become raisins. These produce very sweet musts which are used to sweeten other less sweet musts or are fermented slowly to only about 8 per cent alcohol and a high sugar content. This latter wine is their prized Tokay essence. Szabó and Rakcsányi (1937) report the following analyses in various types of Tokay:

	Alcohol	Total Sugar	Dextrose	Levulose	D/L*
	% by vol.	%	%	%	
Tokajer Szamorodni	13.5	1.6	0.5	1.1	0.45
Mórer Gutedel	13.6	4.6	1.1	3.5	0.31
Tokajer Aszu	13.2	6.7	2.7	4.0	0.67
Tokajer Aszu	12.0	9.5	3.6	5.9	0.61
Tokajer Aszu	11.2	18.8	8.3	10.5	0.79
Tokajer Aszu	9.0	25.2	11.9	13.3	0.89
Essenz, 1888	8.2	23.3	16.6	6.7	2.52
Essenz, 1890	7.9	35.1	21.7	13.4	1.62
Essenz, 1906	4.9	42.6	27.4	15.2	1.80

* Dextrose-levulose ratio.

The prices paid for a bottle of the true Tokay essenz were very high—Gregor reports 3 to 4 pounds in 1881 (and the Tokay bottle holds only about 500 ml)! Berry Brothers and Company (1933) listed a Tokay essence for 79 shillings for sale in London and this was during the depression.

From the description given by Thudichum and Dupré (1872) it is not certain that the Furmint grape of Tokay is attacked by botrytis. They speak of the fruit "cracking" and the juice drying up to form a lump of sugar. They further indicate that not all the fruits crack and dry up, for they note that the pickers separate the plump from the dried berries during harvesting. Müller (1930) specifically says that the climate of the Tokay region is too dry, but he then reports that both raisining and botrytis action are responsible for the sweet types! From observations on the Furmint variety grown in the University of California vineyard at Oakville it would seem that cracking and drying are likely to be more important than botrytis. However, Blaha (1952) believes that botrytis does contribute.

Russia. Maltabar (1951) reported that botrytis did not infect the grapes of the Crimea regularly enough to be used in the production of sweet table

wines. Khovrenko (1910) reported one case of natural botrytis infection of Semillon grapes in the Crimea, which led to the production of a sweet table wine in 1899.

Popova and Puchkova (1947) have studied the use of enzyme preparations of *Botrytis cinerea* in producing table wines (there called chateau-ikem type). They found the enzyme preparation to contain a variety of enzymes, and when added to musts it increased the yield of juice and gave a golden wine (dry?) with an odor unlike that of the wine of untreated grapes. Similar fungal enzyme preparations are widely used in California. It is true that the yield is increased, but specifically beneficial effects on the odor have not been reported here.

Preobrazhanskii (1947) reported on the direct inoculation of grapes with the fungus. Because of the importance of his work it will be described in some detail. He inoculated clusters with spore suspensions or infected berries. In some cases the fruit was sterilized by dipping it into a bactericidal solution and then rinsing it with distilled water. A temperature of 20° C (68° F) and a relative humidity of 92 to 94 per cent were found to be optimum for the development of infection. In inoculation studies in chambers the relative humidity was maintained in this range for four or five days, until considerable infection had taken place, and then the relative humidity was reduced to 72 to 80 per cent. In Semillon grapes left for 18 days a weight reduction of 33 per cent occurred and the sugar content increased from 22.7 to 31.1 per cent.

Preobrazhanskii (1947) also performed a field experiment with Semillon grapes. Infected berries were placed in the clusters and 50 per cent infection occurred in nine days. He did not find the sugar/acid ratio to change much during drying. The pectin content increased. The analyses of his wines show alcohol contents of 3.9 to 14.1 per cent, sugar contents of 5.0 to 13.2 per cent, volatile acidities of 0.122 to 0.222 per cent, and total acidities of 0.69 to 0.88 per cent. These high volatile acidities are very interesting, as we have had the same problem. It was not stated whether the volatile acidities had been corrected for sulfur dioxide, but this correction would not markedly reduce the volatile acidities reported.

Italy. Garino-Canina *et al.* (1951) have described the production of a sweet white table wine in the Caluso region of northern Italy. The practice there is to harvest the grapes (Erbaluce variety) in September and store them until March. During this period general botrytis infection and shriveling occur. It is quite certain that botrytis is primarily responsible for the increased glycerin and pectin contents of the musts, but much of the increase in sugar content is probably due simply to shriveling of the grapes during storage.

Portugal. Everett (1954) states that the sweet white wines of Grandjo in northern Portugal owe their sweetness to the action of *Botrytis cinerea.* They are not, however, always sweet, and the fungus is probably of only limited importance.

California. In California botrytis is seldom observed except very late in the season. Hilgard (1896) reported that it was prevalent in the Napa Valley in 1893 and caused many loads of grapes to be rejected. His experiments

showed that if grapes were only slightly infected with the mold, the quality of the dry table wines produced did not suffer. If, however, the grapes were badly infected, he concluded that it would be better to convert them to distilling material. This latter condition probably corresponded to the *pourriture grise* stage—that is, infection but no shriveling. The disadvantage of this type of infection is that oxidizing enzymes may be derived from the mold and lead to undesirable darkening in color of dry white table wines. Hilgard also produced at least one wine from botrytised grapes from St. Helena, which remained sweet for about a year—the first instance that we have been able to find of the production of such a wine in California.

Amerine and Winkler (1944) produced 90 wines from Semillon grapes from 1935 to 1941. They did not recommend this variety for region I because of its susceptibility to mold. One sample (vintage of 1940) was noted on their cellar records as being heavily infected with botrytis.

Botrytis rot is a serious problem to growers of table grapes in California. Fall rains frequently result in severe outbreaks of the disease, particularly in the Emperor and Flame Tokay districts, according to Nelson (1951). In the early stages of infection the skin slips easily from the pulp when pressed lightly, hence the name "slipskin." This disease is the most troublesome problem in stored table grapes, being the primary reason for periodically fumigating stored fruit.

In general, however, botrytis infections are not widespread in California vineyards because of the low relative humidity of our viticultural districts during the period grapes ripen. This fact is supported by results obtained by Nelson (1951), who found that in the absence of free moisture appreciable infection occurred on Emperor and Flame Tokay grapes at 12° C (54° F) only when the relative humidity remained above 90 per cent. Furthermore, in some regions and seasons, the temperature may be high enough to inhibit the growth of this fungus. Nelson (1951) obtained practically no infection of Emperor grapes held at 35° C (95° F), and even at 30° C (86° F) infection was considerably less than at 25° C (77° F). Vineyard temperatures are often in the range of 30° to 35° C or higher.

It thus appears difficult to produce sweet table wines from botrytised fruit under California climatic conditions. To do this it will probably be necessary to control or modify the environment around the fruit by artificial means. Conditions of high humidity would have to be provided for the fungus to become established on the grapes, followed by a dry environment to reduce the moisture content of the fruit. The temperature during the incubation period would also have to be controlled. If the temperature is too high, botrytis will not grow well, and thermophilic fungi such as *Rhizopus nigricans* and *Aspergillus* sp. will predominate, causing unwanted rots and resulting in off-flavors in the wine. If the temperature is too low, the fungus may grow too slowly, and, in addition, the drying rate will be drastically reduced because of the lower vapor-pressure deficit.

MATERIALS AND PROCEDURES

The present paper describes experiments conducted from 1950 to 1953 in which grapes were inoculated with botrytis conidia, both on and off the

vine. The inoculated fruit was held under humid conditions to permit the fungus to infect the grapes, then under dry conditions to accelerate water loss from the diseased berries. Most of the studies were concerned with fruit that was inoculated after harvest, since the incubation period, which includes both the infection (wet) phase and the dehydration phase, could be more critically controlled and evaluated than when the fruit was still on the vine. Various combinations of length, temperature, and relative humidity of the infection and dehydration periods were evaluated in terms of the chemical and organoleptic characteristics of the wine.

In the early experiments several appropriate grape varieties were tested, and the studies dealt with methods of inoculation as well as with the length and environmental factors of the infection period. Semillon, the principal variety from which the natural sweet wines of France are produced, was used predominantly in the later experiments in which interest was focused on the dehydration period.

Cultural Techniques. The inoculum was prepared by growing *Botrytis cinerea* in pure culture on grape-juice agar. This medium produced a more densely sporulating culture than did potato-dextrose-agar medium. Every effort was made to produce cultures with abundant conidia, since the grapes were inoculated with spore suspensions.

Grape juice of about 18 to 20 per cent sugar was adjusted to a pH of 6.5 with sodium hydroxide, then heated to boiling. Two per cent agar-agar was dissolved in the juice, after which the liquid was tubed in 25-ml aliquots, plugged, and autoclaved.

According to Hansen (1938), *Botrytis cinerea* is one of several imperfect fungi which are heterocaryotic—that is, two or more genetically different nuclei occur within the same cell. Such fungi, when single-spored, give rise to three cultural types: an M or mycelial type; a C or conidial type; and an MC type, in general intermediate between the M and C types. Cultures that remain constant in character, either as C or M types, may be obtained by repeated single-spore transfers until all nuclei in the spore being transferred are genetically indentical.

In the present investigation a constant C type of culture of *Botrytis cinerea* was not obtained. However, it was possible to propagate the fungus by mass transfers through two or three cultures—that is, for three or four weeks—before the M type dominated the culture enough to reduce conidial production appreciably. At this time a series of single-spore transfers was made according to the method of Hansen and Smith (1932). From these transfers a C type was selected, which, although it did not remain constant, again produced abundant conidia through three or four mass transfers.

The mass transfers were made by withdrawing a portion of the fungus on a sterile transfer needle and shaking the mass over the surface of 25 ml of solidified grape-juice agar in a 90-mm petri dish. In this way the entire surface of the agar was inoculated with conidia and a uniformly sporulating culture was obtained in 6 to 9 days at room temperature.

Cultures 9 to 14 days old were used for the inoculum. They were flooded with water, then the mycelium and conidia were rubbed from the agar surface with a camel's-hair brush. It was found that a 2 per cent agar medium

was firm enough to withstand the stress of rubbing the culture free from the surface. The suspension thus obtained was decanted through cheesecloth and the resulting filtrate was used as the spore suspension to be sprayed on the fruit. The mycelial mass on the cheesecloth was rinsed into a large con-

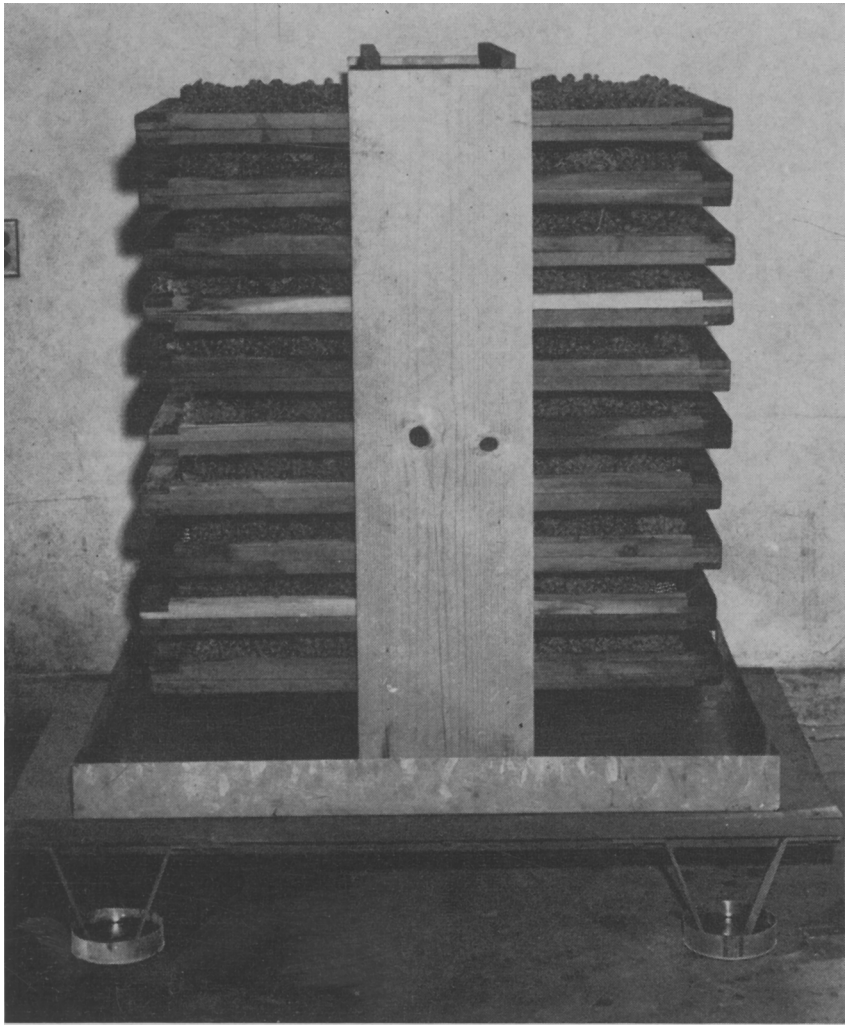

Figure 1. Semillon grapes placed on trays one cluster deep and ready for inoculation. The rack which supports the trays stands in a shallow pan of water.

tainer and used as the crude suspension into which grape clusters were dipped before being sprayed. It was thought that the dipping operation would deposit inoculum in the interior of the cluster where spraying would not.

Inoculation Techniques. The fruit was harvested at about 22° to 24° Balling, cleaned of unsound berries, rinsed in clean water, dipped in the crude suspension, and then sprayed with the spore suspension. Another inoculation

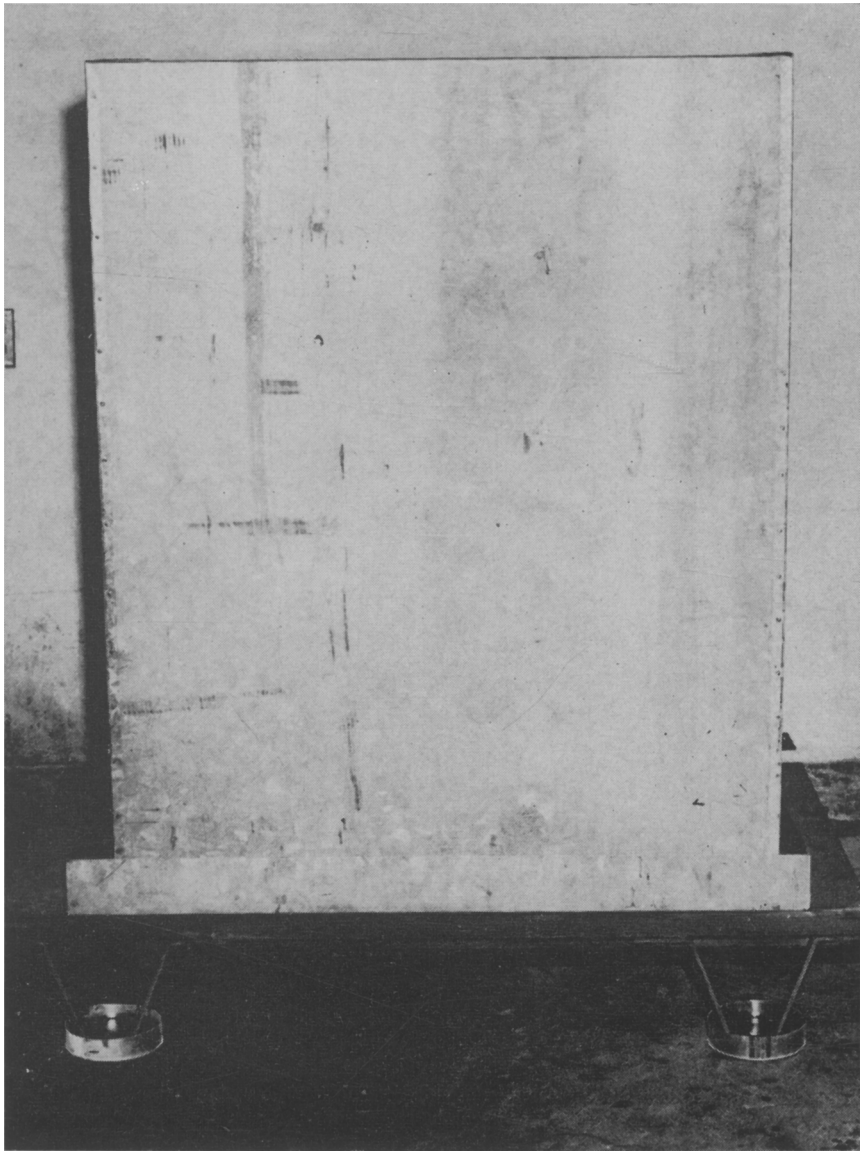

Figure 2. Moistureproof canopy in position over the rack and trays shown in Figure 1.

technique omitted the dip and applied only the spore suspension as a spray. In some experiments dry spores were applied directly to the fruit from cultures by means of an air blast. In the single vineyard experiment a spore suspension was sprayed on the fruit.

The grapes were placed one cluster deep on wooden lattice or metal trays for spraying. Stainless steel ½-inch mesh screen was found very satisfactory for tray bottoms. It allowed good air circulation around the grapes during

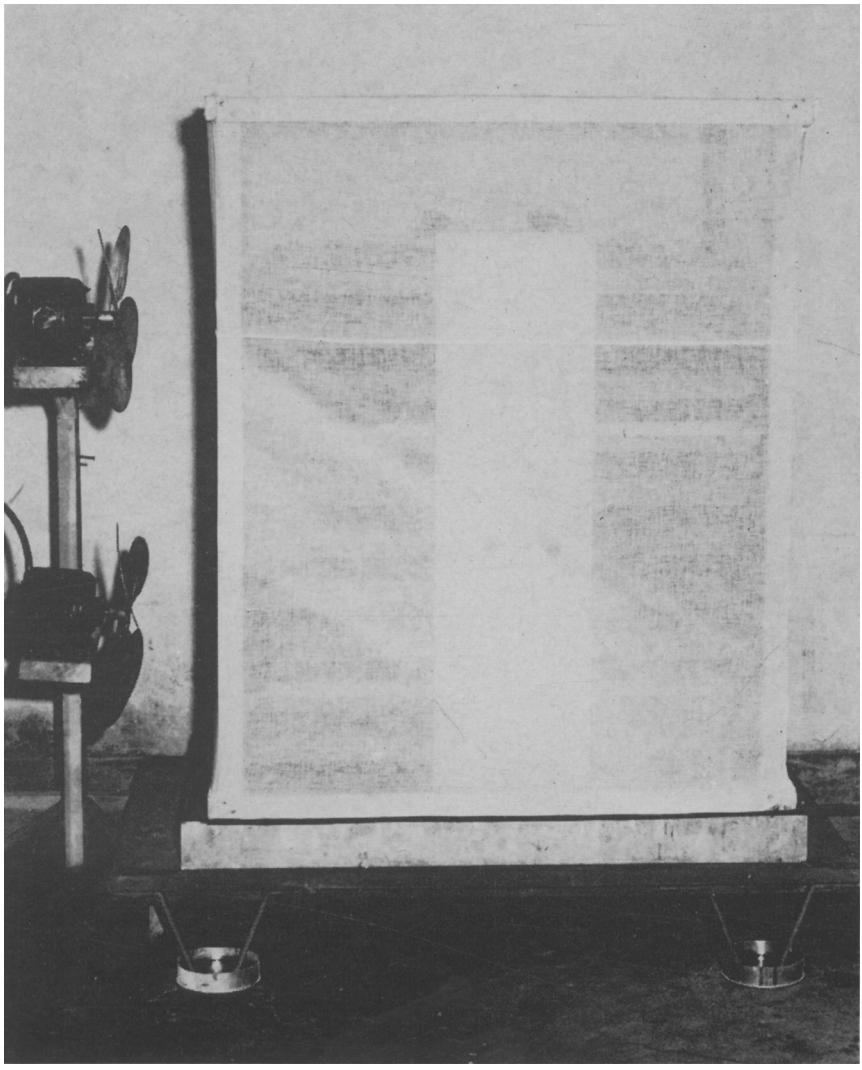

Figure 3. A cheesecloth canopy has been substituted for the canopy shown in Figure 2 to exclude insects and the water has been drained from the pan in preparation for the drying period. The fans force air past the fruit to hasten water loss.

the dehydration period, was easy to clean and sterilize, and also was quite inert to the chemical action of the grape-juice constituents. Tray bottoms made of ½-inch maple dowling spaced 1 inch apart were equally satisfactory and less expensive. Both types of trays were 18 by 36 inches and held 20 to 25 pounds of fruit when covered with a layer of grape clusters.

Infection Period. The trays containing the inoculated fruit were placed one above the other on a rack (fig. 1), which was set in a shallow pan of water. A moistureproof canopy was placed over the rack to maintain the water-

vapor content of the air within the canopy at or near saturation (fig. 2). In various experiments the length of the infection period varied from 6 hours to 4 days, the temperature from 55° F to 80° F, and the relative humidity from 95 to 100 per cent.

Dehydration Period. The moistureproof canopy was removed at the end of the infection period, and the rack was enclosed with cheesecloth to exclude insects (fig. 3). An air current from ordinary electric fans hastened the drying rate of the fruit. Dehydration periods varied in length (5 to 17 days), temperature (55° to 95° F), and relative humidity (40 to 84 per cent). In the early experiments a wide range of these conditions was tested; however, during the 1953 vintage season they were maintained nearly constant.

Sulfur dioxide in the form of gas was applied to the fruit of some lots at various times before inoculation. Part of these were fumigated during the dehydration period as well. Other lots were fumigated only during the dehydration period.

When most of the berries had wrinkled considerably, they were pressed. Since the skin of the infected fruit was fragile, it was not necessary to stem or crush the grapes before pressing.

The musts were fermented at 53° F, using a Montrachet strain of yeast (*Saccharomyces cerivasiae* var. *ellipsoideus*). The wines were filtered or given suitable additions of sulfur dioxide to stop fermentation; however, it is difficult to control the extent of the fermentation with small lots, and some wines have an excessive amount of alcohol.

RESULTS

The experiments conducted in 1950 were largely exploratory and involved the following varieties: Semillon, Furmint, Sauvignon blanc, Malvasia bianca, Orange Muscat, Aligote, Tinta Cão, and Mantua de Pilo. Results of the three stages of analysis—the original composition of the grapes, the must from infected fruit, and the wine—are summarized in tables 1, 2, and 3.

The primary problem of this vintage was the very high volatile acidities of most of the new wines. This problem may have been aggravated by infestations of ants and fruit flies on the fruit during the dehydration period. However, it was encouraging to find increases in sugar content ranging from 13 to 97 per cent (table 2). Must yields of 19 to 78 gallons per ton, based on the weight of the fresh fruit, were obtained (table 1). The most promising results in these experiments were the rich, aromatic, "botrytis" odor of several of the wines and the fact that all of the varieties tested became infected and produced musts with high Balling readings.

1951: Methods of Inoculation and Environmental Conditions during Incubation

A total of 38 wines was produced in 1951 from four grape varieties—Semillon, Orange Muscat, White Riesling, and Thompson Seedless. These experiments were designed to test several methods of inoculation and to study the effects of different environmental conditions during the infection and dehydration periods.

The volatile acidity of the musts from 32 lots was determined (table 6),

and only seven had more than 0.110 grams (as acetic) per 100 ml, but in one case the volatile acidity of the must was 0.331 grams per 100 ml! Increases in sugar content ranged from 6 to 142 per cent. Changes in fixed acidity varied from –2.8 to 130.0 per cent. Botrytis obviously did not affect the acids in the samples that showed a high increase in percentage of fixed acidity. No ready explanation occurs for the increase in fixed acidity that exceeded weight loss in five samples, but in several the difference was negligible (table 6). The good organoleptic scores of several of the wines should be noted (table 7).

The various treatments given the fruit, from inoculation to crushing, were evaluated in terms of cracked berries, saprophytic growth, and botrytis-infected berries (table 5) and the Balling degree and volatile acidity of the musts (table 6).

On a fresh-weight basis, yields of 10 to 125 gallons per ton were obtained, the lower yields being for the musts with higher sugar content. On the basis of final weight, yields of 33 to 189 gallons per ton were produced (table 5).

Methods of Inoculation. The method of inoculation was an important factor in controlling the volatile acid content of the must. Three methods were used. The fruit of some lots was dipped in a water suspension of the spores and mycelium of the fungus to bring the inoculum in contact with the berries in the interior of the cluster. The clusters were then sprayed with a spore suspension until the exposed berries were covered with minute droplets of the suspension. In the second method the clusters were sprayed only. The third method was a dry inoculation; the spores were blown directly onto the fruit from cultures of the fungus. In lots carried through the incubation period under parallel conditions, the must from fruit that had been sprayed only had a lower volatile acid content than that from fruit that had been both dipped and sprayed. However, it was found that the volatile acid content of the must from dipped and sprayed fruit could be controlled by varying the conditions of the incubation period.

Lot 18 (table 4), for example, was both dipped and sprayed; the volatile acid content of the must was 0.186 per cent. Lot 19 was sprayed only, and the volatile acid content of the must was 0.074. Water from the dip probably remained between the berries even after the fruit was exposed to an air blast for 3 hours following inoculation. The berries appeared to have imbibed water during the 3-day infection period, for many had split. During the dehydration period yeast and bacteria converted the sugar of part of the free juice into alcohol and acetic acid. The thin film of water deposited as a spray on the fruit in lot 19 was vaporized by a 2-hour air blast before the grapes were exposed to the high humidity of the infection period with the result that less berry cracking occurred. The must of lot 19 had a slightly higher Balling reading than that of lot 18 (table 6), indicating that the spores were able to germinate and infect the fruit just as well whether residual water was present during the infection period or not.

Lots 25 and 26 were similarly treated except that the fruit was not dried after inoculation and the infection period lasted only one day. Again, the volatile acid content of the must from the dipped-sprayed lot (26) was over twice that of the sprayed lot (25). However, the volatile acid content of both

was much less than that of lots 18 and 19 (table 6). Even though no drying took place beforehand, apparently less acetic acid was formed after the 1-day infection period than after the 3-day infection period given lots 18 and 19. Lot 25, which was sprayed only, had a much lower Balling reading than lot 26, which was dipped and sprayed, indicating that with an infection period of only one day the moisture supplied by spraying only was below the opti-

TABLE 1
1950: COMPOSITION OF GRAPES

Variety	Date picked	Balling	Total acid	pH	Incubation period with botrytis	Loss in weight	Yield, based on original weight
		degrees	*% tartaric*		*days*	%	*gal/ton*
Semillon	9/10	22.1	0.605	3.63	9	..	44.2
Furmint	9/4	23.1	0.511	3.93	9	63	77.8
Semillon	9/25	24.7	0.552	3.73	10	68	52.9
Sauvignon blanc	9/29	22.3	0.563	3.30	11	60	34.9
Semillon	9/21	22.9	0.636	3.81	8	..	....
Semillon	10/5	24.6	0.616	3.85	9	..	....
Malvasia bianca	10/16	27.3	0.556	3.72	17	50	54.8
Orange Muscat	10/16	26.2	0.470	4.20	5	34	77.8
Aligote	10/20	24.2	0.556	3.64	13	60	19.2
Tinta Cão	10/25	24.4	0.740	3.72	12	68	19.7
Mantua de Pilo	10/31	16.8	0.369	3.90	5	56	55.1

TABLE 2
1950: COMPOSITION OF MUSTS FROM BOTRYTIS-AFFECTED GRAPES

Variety	Balling	Total acid	pH	Increase in sugar	Increase in acid
	degrees	*% tartaric*		%	%
Semillon	43.6	....	....	97	...
Furmint	42.4	1.01	3.96	84	98
Semillon	36.1	1.34	3.87	46	143
Sauvignon blanc	37.5	1.25	3.70	68	123
Semillon	30.8	1.75	3.50	34	178
Semillon	34.5	1.08	3.90	40	74
Malvasia bianca	38.8	1.15	3.83	42	105
Orange Muscat	29.6	0.841	4.08	13	79
Aligote	31.4	1.77	3.50	30	216
Tinta Cão	31.1	2.76	3.79	27	273
Mantua de Pilo	23.2	3.47	3.29	38	838

mum required for infection. This conclusion is supported by the higher percentage of botrytis-infected berries of lot 26 as compared with lot 25 (table 5).

A comparison of lots 27 and 28 shows that dipping and spraying fruit does not predispose it to any higher volatile acid content in the must than spraying alone when the treatment is followed by a 3-hour drying period and then a 1-day infection period (tables 4 and 6).

Effect of Drying between Inoculation and the Infection Period on the Amount of Infection. A comparison of lots 25 and 27, which were sprayed only, indicates that a dry period between inoculation and the infection period depresses the amount of infection. In lot 27, which was sprayed and

TABLE 3

1950: ANALYSES OF WINES PRODUCED FROM BOTRYTIS-AFFECTED GRAPES

Variety	Total acid	Volatile acid	Fixed acid	pH	Extract	Sugar	Alcohol	Tannin	Color*	Flavor Score	Comments
	% tartaric	*% acetic*	*% tartaric*		*gm/100gm*	*%*	*% by vol.*	*%*			
Semillon	1.11	0.159	0.91	4.00	23.1	17.0	13.2	0.017	28.6	77	Satisfactory
Furmint	1.14	0.201	0.89	4.10	28.6	22.7	11.2	0.023	85.7	74	Too much sugar; slight raisin
Semillon	1.10	0.207	0.85	4.12	18.4	11.8	14.8	0.048	75.0	75	Rich aromatic flavor
Sauvignon blanc	1.12	0.211	0.86	3.93	21.9	16.3	13.7	0.032	77.0	74	Promising; slight raisin
Semillon	1.54	0.199	1.29	3.69	12.2	5.5	16.0	0.049	43.5	70	Too dry; too high total acid; too high alcohol
Semillon	0.927	0.144	0.75	4.20	14.5	7.4	16.0	0.034	66.6	70	Too dry; oxidized; too high alcohol
Malvasia bianca	1.03	0.196	0.78	4.05	27.0	20.8	12.1	0.042	136.0	73	Rich, aromatic flavor
Orange Muscat	0.644	0.078	0.55	4.03	9.2	5.0	16.0	0.020	42.9	74	Rich, aromatic flavor; too high alcohol
Aligote	1.45	0.286	1.09	3.80	13.1	5.8	14.8	0.038	97.0	70	Too dry
Tinta Cão	2.37	0.720	1.47	3.83	25.5	15.9	6.0	0.107	143.0	60	Spoiled grapes
Mantua de Pilo	3.25	1.48	1.40	3.27	22.1	16.4	1.3	0.031	33.0	60	Spoiled grapes

* Color increases as figure increases; color comparator used.

TABLE 4

1951: ORIGINAL COMPOSITION AND TREATMENT OF GRAPES

Lot no.	Variety*	Date harvested	Balling	Total acid	pH	Date inoc.	Inoc. method	Infection period (95–100% r.h. est.)		Dehydration period (70–80% r.h. est.)	
								Days	Temp.	Days	Temp.
			degrees	*% tartaric*					°F		°F
1	Semillon	8/22	23.0	0.68	3.57	8/27	Dipped and sprayed	3	70–80	5	70–80
2	Semillon	8/22	21.2	0.63	3.49	8/27	Dipped and sprayed	3	70–80	5	70–80
3	Orange Muscat	8/22	20.3	0.40	3.83	8/28	Dipped and sprayed	3	70–80	9	70–80
4	Orange Muscat†	8/22	20.3	0.40	3.83	8/28	Dipped and sprayed	3	70–80	9	70–80
5	Malvasia bianca	8/22	24.0	0.60	3.59	8/28	Dipped and sprayed	3	70–80	9	70–80
6	Semillon	8/29	24.6	0.67	3.50	8/31	Dipped and sprayed	4	55	10	55
7	Semillon	9/6	19.2	1.09	3.42	9/18	Dipped and sprayed	3	70–80	6	70–80
8	White Riesling	9/13	23.9	0.75	3.42	9/14	Dipped and sprayed	4	55	8	55
9	Semillon	9/14	22.7	0.70	3.63	9/14	Dipped and sprayed	4	55	8	55
10	Semillon	9/14	22.7	0.70	3.63	9/18	Dipped and sprayed	3	70–80	6	70–80
11	Semillon	9/14	22.7	0.70	3.63	9/18	Dipped and sprayed	2	70–80	7	70–80
12	Semillon	9/14	22.7	0.70	3.63	9/18	Dipped and sprayed	1	70–80	8	70–80
13	Semillon	9/14	21.7	0.72	3.52	9/17	Sprayed only‡	2½	70–80	7	70–80
14	Semillon	9/14	21.7	0.72	3.52	9/17	Sprayed only	2½	70–80	7	70–80
15	Semillon	9/19	21.1	0.56	3.45	10/3	Dipped and sprayed	2	70–80	10	70–80
16	Semillon	10/1	23.5	....	....	10/1	Dipped and sprayed	2	70–80	12	70–80
17	Semillon	10/1	23.5	....	....	10/2	Dipped and sprayed	1⅛	70–80	12	70–80
18	Semillon	10/1	23.5	....	....	10/3	Dipped, sprayed, and dried 3 hrs.	3	70–80	9	70–80

* Source of grapes was as follows: Lots 1–5, Delano; 6, Livingston; 7, Escalon; U.C., Davis; 13–14, Livermore; 15, Ukiah; 31–38, Fresno.
† Lot 4 was made by adding 2 gallons of water to the pomace of lot 3 and pressing.
‡ Fumigated with 0.25 per cent SO_2 for 20 mins. 1½ hrs. before inoculation.

TABLE 4—*Continued*

Lot no.	Variety*	Date harvested	Balling	Total acid	pH	Date Inoc.	Inoc. method	Infection period (95–100% r.h. est.)		Dehydration period (70–80% r.h. est.)	
								Days	Temp.	Days	Temp.
			degrees	*% tartaric*					°F		°F
19	Semillon	10/1	23.5	....	....	10/3	Sprayed and dried 2 hrs.	3	70–80	9	70–80
20	Semillon	10/1	23.5	....	....	10/3	Sprayed	3	70–80	10	70–80
21	White Riesling	10/1	25.0	....	....	10/1	Dipped and sprayed	2	70–80	9	70–80
22	White Riesling	10/1	25.0	....	....	10/2	Dipped and sprayed	1⅛	70–80	9	70–80
23	Semillon	10/16	24.5	....	....	10/16	Dry (dusted with spores)	9	70–80	7§	70–80
24	Semillon	10/16	24.5	....	....	10/16	Dry (dusted with spores)	9	70–80	7‖	70–80
25	Semillon	10/16	24.5	....	....	10/17	Sprayed	1	70–80	11	70–80
26	Semillon	10/16	24.5	....	....	10/17	Dipped and sprayed	1	70–80	11	70–80
27	Semillon	10/16	24.5	....	....	10/18	Sprayed and dried 3 hrs.	1	70–80	11	70–80
28	Semillon	10/17	24.5	....	....	10/18	Dipped, sprayed, and dried 3 hrs.	1	70–80	11	70–80
29	Semillon	10/17	24.5	....	....	10/18	Sprayed and dried 3 hrs.	2	70–80	11	70–80
30	Semillon	10/17	24.5	....	....	10/18	Dipped and sprayed	2	70–80	11	70–80
31	Thompson Seedless	July#	20.9	0.71	3.42	11/5	Dry (dusted with spores)	7	70–80	7**	70–80
32	Thompson Seedless	July	20.9	0.71	3.42	11/5	Dry (dusted with spores)	7	70–80	7††	70–80
33	Thompson Seedless	July	20.9	0.71	3.42	11/5	Dipped and sprayed	1⅛	70–80	10	70–80
34	Thompson Seedless	July	20.9	0.71	3.42	11/5	Dipped and sprayed	3	70–80	8	70–80
35	Thompson Seedless	July	20.9	0.71	3.42	11/15	Dry (dusted with spores)	2	70–80	11	70–80
36	Thompson Seedless	July	20.9	0.71	3.42	11/15	Dry (dusted with spores)	4	70–80	10	70–80
37	Thompson Seedless	July	20.9	0.71	3.42	11/15	Dry (dusted with spores)	6	70–80	12	70–80
38	Thompson Seedless	July	20.9	0.71	3.42	11/15	Dry (dusted with spores)	8	70–80	13	70–80

§ Clusters showing least spoilage pressed.
‖ Rejected clusters of lot 23.
Stored at 32° F until treated.
** Trays of fruit infested with fruit flies segregated and pressed.
†† Trays of fruit relatively free of fruit flies.

then dried for 3 hours, infection developed in only 35 per cent of the berries, whereas in lot 25, which was sprayed and kept wet, 90 per cent of the berries became infected (table 5). The greater amount of infection for the wet lot is reflected in a higher Balling degree and a slightly lower volatile acid content of the must (table 6).

TABLE 5

1951: BERRY CONDITION, WEIGHT LOSS AT CRUSHING, AND JUICE YIELD

Lot no.	Cracked berries	Saprophytic growth	Botrytis-infected grapes (per cent)	Loss in weight (per cent)	Gal/ton Fresh wt.	Gal/ton Final wt.
1	....	....	....	29.0	97.0	136.8
2	....	....	....	28.5	94.6	142.9
3	....	....	....	69.5	10.0	32.8
4	....	....	....	....	....	.....
5	....	....	....	54.6	55.0	121.2
6	....	....	....	22.9	125.6	162.9
7	....	....	....	60.7	95.7	159.6
8	....	....	....	26.9	116.3	159.2
9	much	....	....	23.6	110.3	144.5
10	much	....	....	38.2	84.9	110.0
11	....	....	....	33.5	113.2	170.2
12	....	....	....	29.4	68.8	97.4
13	....	....	....	16.9	69.8	83.9
14	....	....	....	16.7	64.6	77.9
15	....	....	....	50.3	31.9	64.1
16	....	....	....	53.1	47.4	101.2
17	....	....	....	49.4	56.5	111.6
18	....	....	....	49.6	57.8	114.8
19	....	....	....	53.6	64.0	138.0
20	....	....	....	50.4	38.8	78.1
21	....	....	....	50.0	71.5	143.0
22	....	....	....	45.1	82.0	149.1
23	much	much	....	....	....	.....
24	....	much	....	....	....	.....
25	....	....	90	35.6	74.1	115.1
26	....	....	100	56.8	43.2	100.0
27	....	....	35	26.8	56.3	76.9
28	....	....	85	37.2	53.6	85.4
29	....	....	40	24.2	80.6	106.6
30	....	....	98	47.2	48.0	145.5
31	trace	much	....	42.5	91.6	159.3
32	trace	much	....	47.3	74.7	142.0
33	none	none	....	35.8	103.7	161.2
34	much	much	....	42.2	109.4	189.1
35	some	trace	80	37.9	60.6	97.5
36	trace	little	95	46.4	51.0	95.2
37	trace	much	100	64.8	20.4	58.0
38	trace	much	100	71.5	10.2	35.7

The infection results of lots 26 and 28, which were dipped as well as sprayed, support the conclusions drawn from lots 25 and 27. Aside from the method of inoculation, all four lots were handled in the same manner. The drying after inoculation to which lot 28 was exposed depressed the amount of infection only 15 per cent below that of lot 26. This indicates that the residual water from the dip in lot 28 apparently maintained favorable moisture conditions for infection in spite of the 3-hour drying period.

Unfortunately, no critical comparisons can be made between lots inoculated with wet spores and those given dry inoculations. A comparison of the Balling readings of the musts of lots 31 and 32 (inoculated with dry spores) with those of the musts from lots 33 and 34 (inoculated with wet spores) does

TABLE 6

1951: COMPOSITION OF MUSTS FROM BOTRYTIS-AFFECTED GRAPES

Lot no.	Variety	Balling	Total acid	Volatile acid	Fixed acid	pH	Increase in sugar	Fixed acid
		degrees	*% tartaric*	*% acetic*	*% tartaric*		%	%
1...	Semillon	26.7	....	....	....	....	16	....
2...	Semillon	28.0	....	....	....	....	32	....
3...	Orange Muscat	47.2	....	....	....	....	133	....
4...	Orange Muscat	32.8	0.60	....	....	4.26	62	....
5...	Malvasia bianca	47.4	....	....	....	....	97	....
6...	Semillon	26.8	0.505	....	....	3.82	9	....
7...	Semillon	26.1	1.55	0.128	1.39	3.32	36	17.3
8...	White Riesling	25.3	1.03	0.026	1.00	3.53	6	33.3
9...	Semillon	25.5	1.03	0.029	0.99	3.62	11	41.4
10...	Semillon	32.2	1.045	0.050	0.98	3.70	142	40.0
11...	Semillon	33.0	0.935	0.019	0.91	3.71	45	30.0
12...	Semillon	30.7	0.76	0.013	0.74	3.81	35	5.7
13...	Semillon	26.2	0.78	0.016	0.76	3.50	21	5.5
14...	Semillon	24.9	0.77	0.013	0.75	3.50	15	4.2
15...	Semillon	34.8	1.27	0.225	0.99	3.43	65	76.8
16...	Semillon	35.3	1.92	0.308	1.53	3.52	..	....
17...	Semillon	41.0	1.16	0.045	1.10	3.89	..	....
18...	Semillon	37.2	1.52	0.186	1.29	3.70	..	....
19...	Semillon	39.4	1.12	0.074	1.03	3.80	..	....
20...	Semillon	34.3	1.38	0.206	1.12	3.70	..	....
21...	White Riesling	26.6	2.21	0.331	1.80	3.42	..	....
22...	White Riesling	30.8	1.41	0.109	1.27	3.51	..	....
23...	Semillon	37.9	1.31	0.051	1.25	3.62	..	....
24...	Semillon	34.7	1.75	0.141	1.57	3.50	..	....
25...	Semillon	31.3	0.70	0.029	0.66	3.90	..	....
26...	Semillon	41.8	1.13	0.069	1.04	4.10	..	....
27...	Semillon	28.8	0.81	0.042	0.76	3.79	..	....
28...	Semillon	34.5	0.83	0.045	0.77	4.02	..	....
29...	Semillon	29.0	0.85	0.077	0.75	3.80	..	....
30...	Semillon	39.0	1.20	0.102	1.07	4.00	..	....
31...	Thompson Seedless	35.7	0.88	0.038	0.83	4.28	71	18.3
32...	Thompson Seedless	34.9	0.78	0.010	0.77	4.02	67	8.5
33...	Thompson Seedless	29.2	0.73	0.013	0.71	3.80	40	0.0
34...	Thompson Seedless	30.0	1.01	0.019	0.99	3.66	44	39.5
35...	Thompson Seedless	31.0	0.74	0.016	0.72	4.02	48	1.4
36...	Thompson Seedless	32.3	0.70	0.010	0.69	4.05	55	−2.8
37...	Thompson Seedless	42.7	1.17	0.010	1.16	3.90	104	63.5
38...	Thompson Seedless	47.2	1.65	0.019	1.63	3.78	126	130.0

show, however, that dry inoculations can produce high Balling readings, provided high humidity conditions follow the inoculation.

Relation of Length of Infection Period to Level of Infection Produced. The percentage of botrytis-infected berries (table 5) and the Ballings of the musts (table 6) of lots 26 and 30 were little influenced whether the infection period lasted 1 or 2 days. However, the volatile acid content of the must from lot 30, with a 2-day infection period, was higher than that of lot 26, with a 1-day infection period. The same relationship exists between lots 27 and 29

and lots 21 and 22. Lots 10, 11, and 12, with infection periods of 3, 2, and 1 days respectively, produced musts with volatile acid contents proportional to the length of the infection period. The Balling degree was not affected, however, indicating that an infection period of one day is enough for satisfactory botrytis infection. Beyond this the volatile acid problem becomes progressively worse.

Figures 4, 5, and 6 are photographs of the same cluster from lot 10 (table 4) taken at different times during incubation, showing why the volatile acid problem is aggravated by prolonged infection periods. Figure 4 shows the cluster just after inoculation. No infection has taken place. Figure 5, after a 3-day infection period, shows the development of saprophytic activity (*C*). Dead stems and floral parts, particularly in the interior of the cluster, became wet at inoculation and remained so long enough for undesirable saprophytic growth (*Rhizopus nigricans*) to become established. The split berry at *A* may contribute to a high volatile acid content in the must, since the exuded juice is an excellent medium for the yeast-Acetobacter complex. Wounds as infection courts for botrytis are not undesirable as long as microbiological activity associated with them is restricted to this organism. In figure 6, one berry, *C*, has been spoiled by Rhizopus and will impart a moldy flavor to the wine. The berry at *B*, though well infected with botrytis and well shriveled, may be a source of volatile acidity. Berries such as that shown at *A* offer less opportunity for saprophytic activity than those illustrated by *B*.

Lot 16, with a 2-day infection period, produced a must with more than six times the volatile acid content of that of lot 17, with an infection period of 1⅛ days. The must of lot 20 (3-day infection period) had a volatile acid content between that of lots 16 and 17. However, this lot had been sprayed only.

The length of the infection period following the dry application of spores is correlated with the percentage of botrytis-infected berries and the Balling degree of the must. This is demonstrated by lots 35 to 38. Lot 35, with only a 2-day infection period, developed 80 per cent infected berries (table 5) and produced a must of 31.0° Balling (table 6). Lots 36, 37, and 38, with infection periods of 4, 6, and 8 days respectively, showed progressively more infection and higher Balling readings for the musts. It is unfortunate that the dehydration periods for the four lots were not of the same length; however, it is reasonable to expect that had the infection periods for lots 37 and 38 been as long as those for lots 35 and 36, the Balling readings would still have shown a correlation with the length of the infection period. The volatile acid content of the lots did not show such a relationship, remaining low for all four lots.

Effect of Incubation Temperature on Balling of the Must. The effect of the incubation temperature on the Balling degree of the must is shown by a comparison of lot 9, incubated at 55° F, with lots 10, 11, and 12, incubated at 70°–80° F. Although lot 9 had an infection period of 4 days and a dehydration period of 8 days, its Balling degree was still much less than that of the other three lots with shorter infection and dehydration periods. The volatile acid content of the must of lot 9 was lower than that of lot 10

TABLE 7

1951: ANALYSES OF WINES PRODUCED FROM BOTRYTIS-AFFECTED GRAPES

Lot no.	Variety	Total acid	Volatile acid	Fixed acid	pH	Alcohol	Extract	Sugar	Tannin	Color*	Flavor score	Comments
		% tartaric	*% acetic*	*% tartaric*		*% by vol.*	*gm/100 gm*	%	%			
1...	Semillon	0.51	0.062	0.43	4.12	15.6	6.5	3.0	0.03	11	76	Alcoholic; slight botrytis
2...	Semillon	0.56	0.074	0.47	4.12	15.0	7.1	3.6	0.02	8	76	Alcoholic; little botrytis
3...	Orange Muscat†	0.88	0.174	....	....	....	....	....	....	..	..	.....
4...	Orange Muscat	0.72	0.115	0.58	3.98	9.7	20.7	15.8	0.03	11	77	Medium botrytis; tart
5...	Malvasia bianca	0.71	0.180	0.49	4.30	8.5	40.2	32.8	0.03	32	80	Very rich; distinct muscat
6...	Semillon	0.53	0.064	0.45	3.85	12.1	8.2	5.1	0.03	21	77	More sugar and botrytis
7...	Semillon	1.30	0.186	1.07	3.48	13.4	6.7	1.1	0.04	11	70	Too tart; musty
8...	White Riesling	0.92	0.125	0.61	3.60	13.5	5.8	0.6	0.03	10	74	Medium botrytis; moldy
9...	Semillon	0.80	0.089	0.58	3.72	14.5	5.2	0.4	0.03	9	76	Too dry; fair botrytis
10...	Semillon	0.93	0.154	0.54	3.90	15.1	10.8	5.0	0.03	13	80	Medium botrytis; promising
11...	Semillon	0.93	0.180	0.68	4.00	14.0	13.3	8.5	0.02	10	80	Medium botrytis; slight mold
12...	Semillon	0.72	0.129	0.40	4.05	14.9	9.1	5.0	0.02	6	79	Slight botrytis; too dry
13...	Semillon	0.67	0.077	0.57	3.58	13.1	6.4	3.6	0.03	7	79	Slight botrytis; too dry
14...	Semillon	0.66	0.071	0.57	3.42	9.7	10.0	6.8	0.03	6	79	Slight botrytis; SO_2
15...	Semillon	1.14	0.276	0.79	3.72	14.1	15.3	11.0	0.02	12	74	Medium botrytis; high volatility
16...	Semillon	1.61	0.337	1.19	3.85	9.9	23.3	16.1	0.03	50	70	Medium botrytis; high volatility
17...	Semillon	1.05	0.192	0.81	3.31	9.2	30.1	27.6	0.06	50	78	Medium botrytis; slight raisin
18...	Semillon	1.21	0.257	0.89	4.08	12.1	21.2	15.6	0.03	37	72	Slight botrytis; high volatility

* Color increases as figure increases; color comparator used.
† Sample too small for complete analysis.

TABLE 7—*Continued*

Lot no.	Variety	Total acid	Volatile acid	Fixed acid	pH	Alcohol	Extract	Sugar	Tannin	Color*	Flavor score	Comments
		% tartaric	*% acetic*	*% tartaric*		*% by vol.*	*gm/100 gm*	*%*	*%*			
19...	Semillon	0.92	0.161	0.72	4.29	13.4	21.7	15.8	0.02	23	76	Slight botrytis; high volatility
20...	Semillon	1.24	0.257	0.92	4.22	15.1	14.4	7.1	0.02	33	71	Slight botrytis; moldy
21...	White Riesling	1.98	0.456	1.41	3.75	11.3	14.1	6.8	0.03	30	67	Slight botrytis; acetic; too dry
22...	White Riesling	1.27	0.244	0.96	3.72	12.6	13.8	7.6	0.03	22	70	Slight botrytis; acetic; tart
23...	Semillon‡	1.01	0.199	0.76	4.28	9.1	31.4	28.3	0.03	55	73	Slight botrytis; moldy
25...	Semillon	0.68	0.148	0.49	4.12	16.1	9.0	3.6	0.01	12	71	Slight botrytis; moldy
26...	Semillon	0.95	0.199	0.70	4.45	11.1	26.7	18.5	0.04	57	76	Medium botrytis; moldy
27...	Semillon	0.66	0.122	0.51	4.15	16.1	5.0	0.9	0.01	19	71	Slight botrytis; too dry
28...	Semillon	0.67	0.158	0.47	4.60	15.1	13.6	7.5	0.01	13	76	Slight botrytis
29...	Semillon	0.66	0.154	0.47	4.17	14.2	7.7	3.3	0.02	10	74	Slight botrytis
30...	Semillon	0.84	0.212	0.57	4.45	12.8	21.1	14.9	0.03	25	77	Medium botrytis
31...	Thompson Seedless	0.79	0.167	0.58	4.55	16.5	12.6	6.5	0.02	16	76	Slight mold; alcoholic
32...	Thompson Seedless	0.74	0.112	0.60	4.05	15.3	14.4	9.9	0.01	16	77	Slight botrytis
33...	Thompson Seedless	0.66	0.096	0.54	3.83	16.0	6.4	2.0	0.01	13	71	Slight botrytis; too dry
34...	Thompson Seedless	0.74	0.129	0.58	3.92	15.1	14.5	9.6	0.01	4	78	Slight botrytis; moldy (slight)
35...	Thompson Seedless	0.69	0.167	0.48	4.05	15.8	8.8	3.5	0.01	8	71	Medium botrytis; moldy
36...	Thompson Seedless	0.69	0.174	0.47	4.09	14.8	12.1	6.0	0.01	14	74	Medium botrytis; moldy
37...	Thompson Seedless	1.24	0.276	0.89	3.82	10.0	27.8	20.8	0.02	38	70	Medium botrytis; high volatility
38...	Thompson Seedless	1.75	0.270	1.41	3.65	6.0	42.1	32.6	0.03	109	70	Medium botrytis; raisined; high volatility

* Color increases as figure increases; color comparator used.
‡ Includes best clusters from lots 23 and 24.

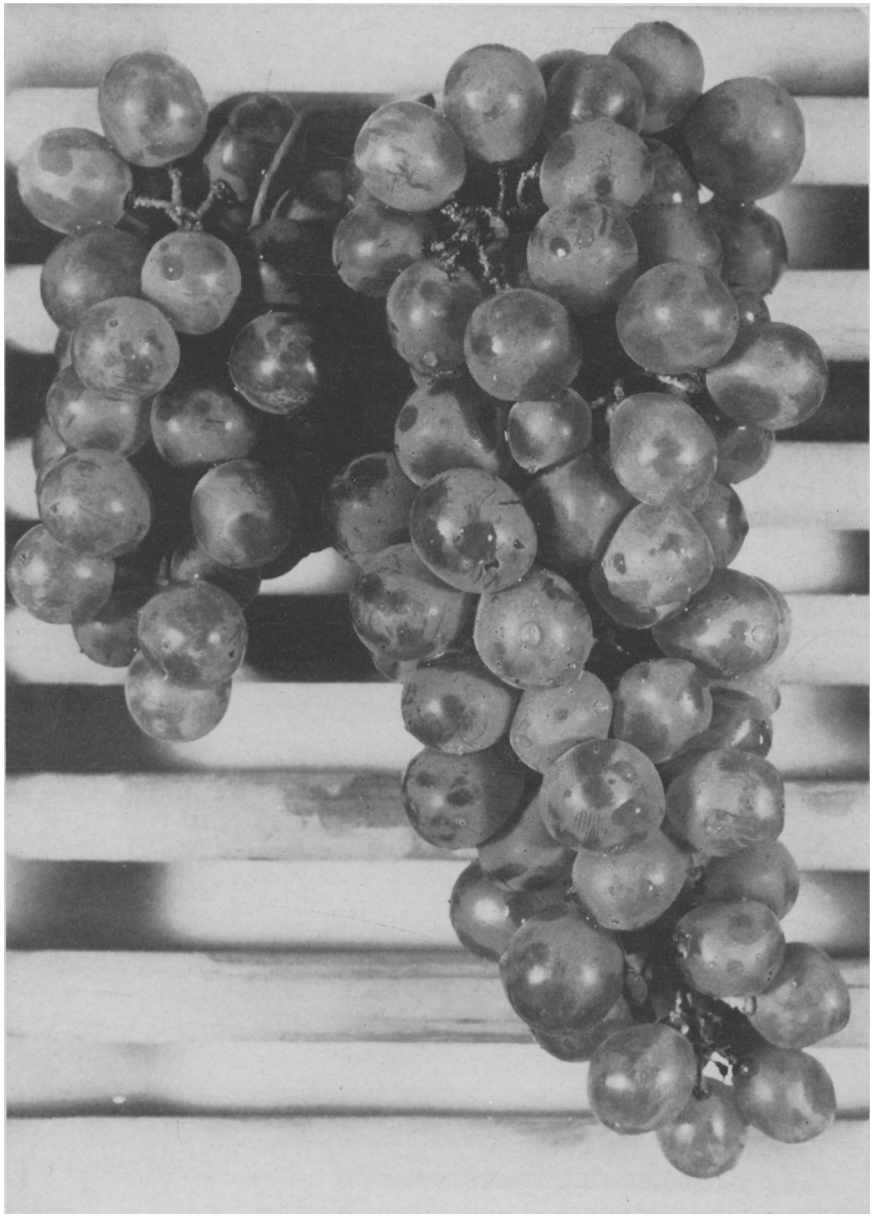

Figure 4. Cluster of Semillon grapes from lot 10, table 4, immediately after being dipped in a suspension of *B. cinerea* spores, then sprayed with a spore suspension.

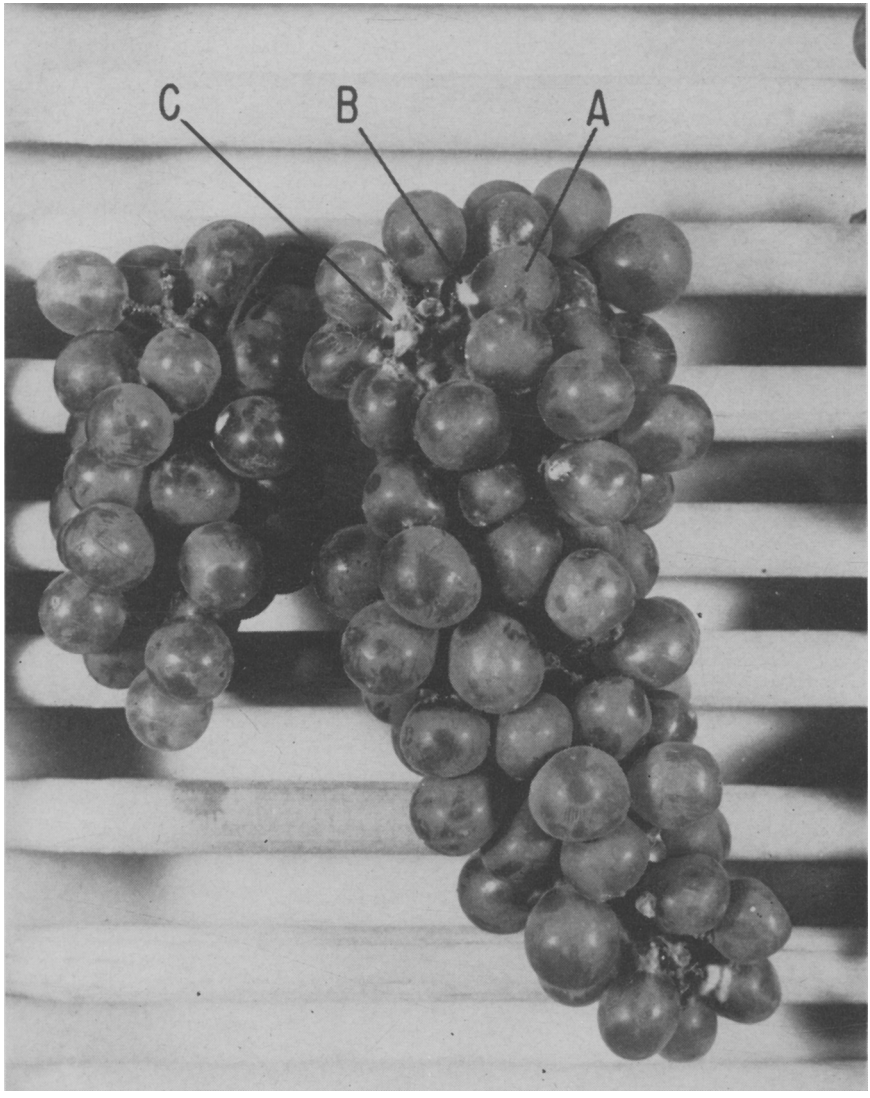

Figure 5. The same cluster of grapes as that in figure 4 after a 3-day infection period at 70° to 80° F and 95 to 100 per cent relative humidity. A) Berry split from imbibing water. B) Botrytis growth in the berry split and on the capstems. C) Growth of *Rhizopus nigricans* on dead stems and floral parts.

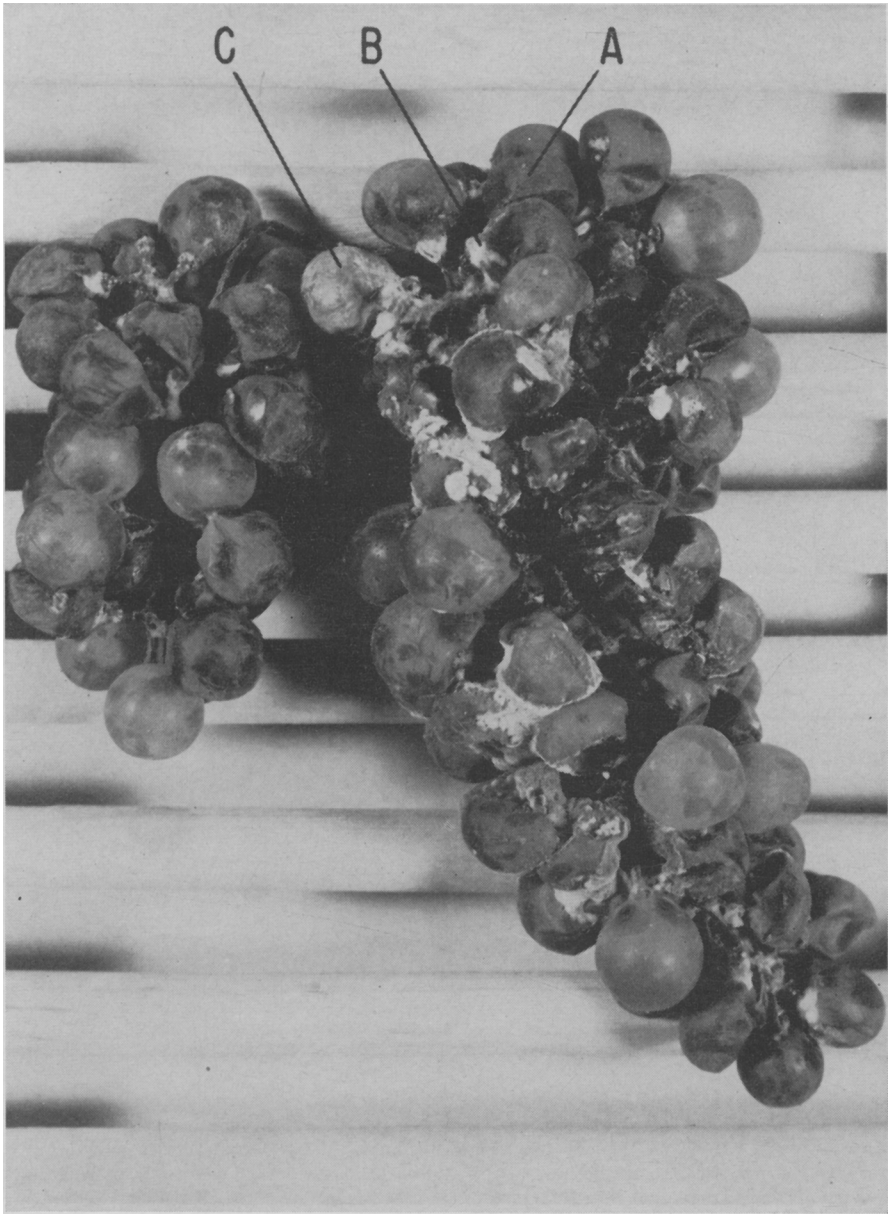

Figure 6. The same cluster of grapes as that of figure 5 after 6 days at 70° to 80° F and 70 to 80 per cent relative humidity. A) Desirable type of botrytis infection; berry was infected through the uninjured skin and has lost considerable water. B) Less desirable type of infection. Berry was infected through a split in the skin. There is more danger of spoilage with wounds present. C) Berry spoiled by *R. nigricans.*

with a shorter infection period; however, it was more than those of lots 11 and 12, which had the shortest infection periods.

The lower temperature of 55° F appears to be less satisfactory than the range of 70°–80° F, particularly if the infection period at the higher temperature lasts no longer than 1 to 2 days.

Effect of Fumigation with Sulfur Dioxide on Volatile Acid Content. Lot 13 was fumigated with sulfur dioxide shortly before inoculation to determine if the fungicide would reduce the volatile acid content of the musts by re-

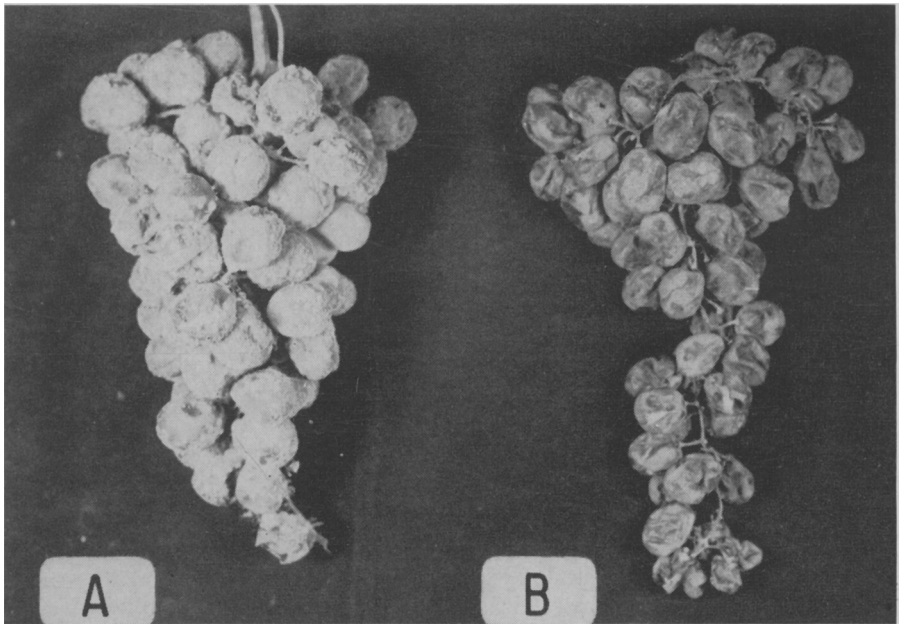

Figure 7. Effect of the relative humidity during the drying period on the host (Muscat grapes) and on the facultative saprophyte (*B. cinerea*). A) Representative cluster from lots 36 to 40 (table 11) which were inoculated and infected during a wet period of one day, then held 9 days at a temperature of 67° to 70° F and 70 to 84 per cent relative humidity. B) Cluster from lots 31 to 35 similarly treated, but held at 49 to 59 per cent relative humidity for 7 days.

ducing the population of undesirable microörganisms present on the fruit. Lot 14 was not fumigated. The volatile acid content of the musts of the two lots was about the same, and both were low.

Organoleptic Analyses of the Wines. The presence of high volatile acidity in the musts continued to hamper the organoleptic analysis, but several wines of outstanding quality that were low in volatile acidity were produced. The scores of several lots would have been higher had the fermentation been stopped with a higher percentage of sugar and at a lower percentage of alcohol. This, however, is difficult to do when small lots are handled. The moldy (penicillium) odor of several lots was objectionable. Also, the tasters objected to the raisin-like (caramel?) flavor of lots that were allowed to

become very high in sugar. With age, the volatile acidities became less objectionable, and the taste scores improved 3 to 5 points.

Field Experiment. Some fruit of the White Riesling variety was inoculated on the vine and kept wet for as long as three days with periodic wettings from a spray rig. The results were unsatisfactory, since it was difficult to keep the fruit wet without washing off the spores. It was also difficult to prevent the outer berries of the cluster from drying off between sprayings during the warmer periods of the day. Saprophytic organisms, including *Penicillium* sp., *Rhizopus nigricans,* and *Aspergillus* sp., were quite troublesome, partly because of the high daytime temperatures and partly because the excess spray tended to accumulate in the interior of the clusters. Split berries provided infection courts for these organisms, and old, diseased, dried-up berries were hydrated and became excellent media to spread the rots to healthy berries, chiefly by way of the stems.

Very little infection by botrytis could be ascertained. The results indicate that the vines would probably have to be covered during the infection period so that the amount of water used and the number of sprayings could be reduced. This would probably not be feasible during the day if the sun were shining because the accumulation of heat under the cover would produce temperatures above that tolerated by *Botrytis cinerea.* From the work of Nelson (1951), it is doubtful if the period from sundown to sunrise would be long enough to allow the fungus to infect enough of the berries. This appears to be the only period when excessive temperatures would not be a problem and moisture conditions could be maintained at a level sufficient for infection.

1952: Effect of Wet and Dry Methods of Inoculation and Varying Infection Periods on the Composition of Must and Wine

The experiments during the 1952 season were designed largely to study more critically the influence of wet and dry methods of inoculation and infection periods of varying length on the composition of the must and wine. The trials were confined to 25 lots of Semillon grapes. The treatments are listed in table 8, the composition of the botrytised fruit is summarized in table 9, and the composition of the wine and the organoleptic data are presented in table 10. Somewhat better control of the humidity during incubation resulted in musts of low volatile acidity, with few exceptions. However, moldy odors were found in some of the wines. The chief problem was to keep fermentations from exceeding 14 per cent alcohol. The scores would have been much higher if the wines had been sweeter.

Wet and Dry Methods of Inoculation. The wet and dry methods of inoculation were compared by spraying a spore suspension on lot 2 and blowing spores from a culture onto the fruit of lot 1 (table 8). Both were exposed to an infection period of 2 days at 54° F and 95 per cent relative humidity. The dehydration period extended through 9 days. It is interesting that the Balling degree of the must of the dry (dusted) lot was four degrees higher than that of the sprayed lot (table 9). The total and volatile acid contents were slightly higher. With a wet period of 2 days at 54° F, dry inoculation seems to compare very favorably with the wet method.

In addition it was found that the normal amount of inoculum present on the fruit when harvested was not sufficient to produce enough infection for satisfactory water loss during the dehydration period. The Balling degree in the original analysis of lot 3 (table 8), which was not inoculated, is practically the same as that of the must (table 9). The low Balling degree of the must was probably due to the absence of inoculum before the infection period. The lot was treated as a wet lot except that the spray contained no spores. After a 2-day infection period at 54° F and

TABLE 8

1952: ORIGINAL COMPOSITION AND TREATMENT OF SEMILLON GRAPES

Lot no.	Original analysis			Inoculation method	Infection period			Length of dehydration period at 68-80° F and 70-80% r.h.
	Balling	Total acid	pH		Days	Temp.	r.h.	
	degrees	*% tartaric*				*°F*	*%*	*days*
1	22.5	0.87	3.49	Dusted	2	54	95	9
2	22.5	0.87	3.49	Sprayed	2	54	95	9
3	25.0	0.73	3.60	Not inoculated	2	54	95	7
4	24.6	0.63	3.67	Sprayed	¼	68	98-100	10
5	24.6	0.63	3.67	Sprayed	½	68	98-100	10
6	24.6	0.63	3.67	Sprayed	¾	68_80	98-100	10
7	24.6	0.63	3.67	Sprayed	1	68-80	98-100	10
8	24.4	0.69	3.60	Dusted	¼	68-80	95	16
9	24.4	0.69	3.60	Dusted	½	68-80	95	16
10	24.4	0.69	3.60	Dusted	¾	68-80	95	15
11	24.4	0.69	3.60	Dusted	1	68-80	95	15
12	20.7	0.69	3.50	Dusted	1	54	95	10
13	20.7	0.69	3.50	Dusted	2	54	95	9
14	20.7	0.69	3.50	Dusted	3	54	95	8
15	23.8	0.70	3.63	Sprayed	¼	68-80	98-100	17
16	23.8	0.70	3.63	Sprayed	½	68-80	98-100	17
17	23.8	0.70	3.63	Sprayed	¾	68-80	98-100	17
18	23.8	0.70	3.63	Sprayed	1	68-80	98-100	17
19	23.8	0.54	3.79	Dusted	¼	68-80	90	14
20	23.8	0.54	3.79	Dusted	½	68-80	90	14
21	23.8	0.54	3.79	Dusted	¾	68-80	90	14
22	23.8	0.54	3.79	Dusted	1	68-80	90	14
23	24.2	0.58	3.72	Dusted	1	54	95	15
24	24.2	0.58	3.72	Dusted	2	54	95	14
25	24.2	0.58	3.72	Dusted	3	54	95	13

95 per cent relative humidity and 7 days of dehydration, the Balling degree of the must was only 1.4 degrees higher than that of the original analysis. Unfortunately, a check lot inoculated with spores was not included; however, it was apparent that the small water loss was near normal for grapes under the conditions of exposure.

Length of Infection Periods. Infection periods longer than one day did not consistently increase the subsequent drying rate of fruit inoculated with dry spores, as indicated by the Balling readings of the musts. Lots 12, 13, and 14, with infection periods of 1, 2, and 3 days respectively (table 8), produced musts with Ballings of 27.9°, 27.3°, and 26.9° respectively (table 9). Although the dehydration period of lot 14 was 2 days less than that of lot 12, it is still apparent that its rate of dehydration was not appreciably faster. Had the dehydration period been equal to that of lot 12, the Balling reading

would probably have been little, if any, higher than that of lot 12. There was a positive correlation between the length of the infection period and the Balling readings of lots 23, 24, and 25; however, the dehydration periods for these lots were 5 days longer than for lots 12, 13, and 14. The rise in Balling degree as the infection period was extended would undoubtedly have been greater had all three lots been exposed to the same dehydration period. Also, a relative humidity greater than 95 per cent during the infection period might well have resulted in a more pronounced correlation between length

TABLE 9

1952: COMPOSITION OF MUSTS FROM BOTRYTIS-AFFECTED SEMILLON GRAPES

Lot no.	Balling	Total acid	Volatile acid	pH
	degrees	*% tartaric*	*% acetic*	
1	34.9	0.88	0.018	3.99
2	30.9	0.84	0.015	3.98
3	26.4	0.79	0.028	3.53
4	27.0	0.77	0.012	3.65
5	23.0	0.87	0.021	3.61
6	27.2	0.90	0.018	3.69
7	27.4	0.81	0.030	3.64
8	30.3	0.65	0.018	3.69
9	30.8	0.66	0.018	3.77
10	30.6	0.72	0.021	3.68
11	31.5	0.67	0.012	3.80
12	27.9	0.79	0.030	3.69
13	27.3	0.74	0.040	3.75
14	26.9	0.72	0.061	3.81
15	30.5	0.68	0.012	3.75
16	31.6	0.76	0.018	3.75
17	33.1	0.74	0.018	3.80
18	34.2	0.80	0.030	3.82
19	33.0	0.87	0.082	3.75
20	29.2	1.13	0.203	3.68
21	30.6	0.93	0.085	3.68
22	29.9	1.07	0.180	3.69
23	30.9	0.86	0.049	3.70
24	32.8	0.93	0.083	3.79
25	31.2	1.00	0.086	3.74

of infection period and the Balling degree of the must, since higher relative humidities are probably nearer the optimum for infection in the absence of liquid water.

The volatile acid content of the musts showed a positive correlation with length of infection period for lots 12, 13, and 14 and for lots 23, 24, and 25 (tables 8 and 9). Apparently the high humidity during the infection period, beyond one day at least, promoted volatile acid formation even though the fruit was inoculated with dry spores. This is interesting since the 1951 experiments showed the same results when the lots were inoculated with spores in a water spray. Apparently, water may aggravate the volatile acid problem although it is not primarily responsible for it.

Infection periods shorter than one day were tested to determine their influence on the amount of infection and on the composition of the must. Lots 4, 5, 6, and 7 were inoculated with spores in a water spray and kept wet

for ¼, ½, ¾, and 1 day respectively, then dried for 10 days. The Balling readings of the musts showed little relationship to the treatments. Lots 4, 6, and 7 showed progressively higher Balling readings but only to a slight degree. No explanation can be offered for the low Balling degree of lot 5 (23.0°). It is, in fact, lower than the Balling degree of the original analysis (24.6°). The volatile acid relationship is quite irregular, probably because of the short infection period for the four lots. Lots 15, 16, 17, and 18 were inoculated in the same manner as lots 4, 5, 6, and 7. It was 17 days before the 1-day lot was dry enough to press, since the relative humidity was about 80 per cent during the dehydration period. The Balling readings of the musts from these lots were correlated with the length of the infection periods, ranging from 30.5° for lot 15, ¼ day, to 34.2° for lot 18, 1 day (table 9). The volatile acidities showed a similar relationship, although those of lots 16 and 17 were the same. It was encouraging that the volatile acid content was low for the four lots.

Visual observations support these results: that it takes about one day at room temperature to obtain maximum infection when water is present on the surface of the berries. Lots incubated for 18, 12, and 6 hours developed progressively less infection, both as to number of berries infected and number of infections per berry. This is reflected in the relatively slower drying rates and the lower Balling readings of the musts as well as in the lower percentage of alcohol and reducing sugar content of the wines.

Dry Inoculations in Relation to Varied Conditions during Incubation. Dry inoculations were made on lots 19, 20, 21, and 22, which were subsequently incubated at 90 per cent relative humidity for ¼, ½, ¾, and 1 day respectively. Unfortunately, the fruit became infested with fruit flies and was spoiled during the treatment. This is indicated by the irregular Balling readings and the high, irregular volatile acidities of the musts. A low amount of botrytis infection developed, indicating that the combination of infection-period conditions used in this experiment was below the minimum necessary for satisfactory infection.

Lots 8, 9, 10, and 11 were treated in the same manner as lots 19 to 22 except that the relative humidity during the infection period was 95 per cent. Neither the Balling readings nor the volatile acid content of the musts could be correlated with the treatment. This indicates that a relative humidity of 95 per cent for a period of one day or less is still too low to produce satisfactory amounts of infection when no liquid water is initially present to promote germination of the spores.

Other Factors Affecting Results. Lack of consistent results in the 1952 experiments was due to several factors. Lots of about 25 pounds apiece (fresh weight) introduced considerable sampling error. The small must samples from these lots did not appear to ferment as uniformly as the larger samples obtained in other experiments.

Lack of close temperature and humidity control during the incubation period also hampered the results. Fluctuating temperatures and humidities made it difficult to compare experiments. High humidities during the dehydration period necessitated prolonging this period until excessive botrytis growth on the outside of the berries and growth of saprophytes probably

TABLE 10

1952: ANALYSES OF WINES PRODUCED FROM BOTRYTIS-AFFECTED SEMILLON GRAPES*

Lot no.	Total acid	Volatile acid	pH	Alcohol	Extract	Sugar	Tannin	Color†	Flavor score	Comments
	% tartaric	*% acetic*		*% by vol.*	*gm/100 gm*	*%*	*%*			
1	0.68	0.132	4.16	18.6	6.6	2.20	0.047	12	71	Moldy
2	0.62	0.081	4.50	18.1	3.9	0.32	0.133	13	72	Flat, alcoholic
3	0.61	0.041	3.42	14.8	2.9	0.95	0.045	9	73	Nearly dry, alcoholic
8	0.56	0.054	3.50	17.3	2.7	0.60	0.013	11	72	Vinous, alcoholic
9	0.57	0.067	3.70	17.1	3.0	0.60	0.013	12	71	Vinous, alcoholic
10	0.63	0.058	3.47	17.4	2.9	0.56	0.013	11	72	Vinous, alcoholic
11	0.58	0.064	3.72	18.6	3.2	0.76	0.013	13	73	Medium distinct, alcoholic
12	0.60	0.032	3.52	15.3	3.0	0.44	0.026	10	70	Slightly moldy
13	0.59	0.039	3.62	15.2	3.0	0.52	0.029	12	70	Slightly oxidized and moldy
14	0.60	0.061	3.77	15.9	3.3	0.45	0.034	14	70	Moldy
15	0.52	0.100	3.78	17.2	3.6	0.95	0.056	10	72	Slightly moldy
16	0.63	0.088	3.72	17.7	5.3	2.76	0.029	10	71	Yeasty
17	0.59	0.104	3.95	16.9	7.8	4.10	0.029	11	75	Slightly moldy
18	0.58	0.099	4.00	17.0	7.2	3.90	0.029	10	77	Moldy
23	0.80	0.139	3.74	16.7	8.9	4.84	0.017	20	68	Medium-plus moldy
24	0.79	0.135	3.74	16.6	9.0	4.76	0.026	13	74	Medium mold and medium botrytis
25	0.68	0.097	3.70	17.6	4.6	1.48	0.021	15	74	Medium mold and medium botrytis

* No wine was made from lots 4–7 and 19–22.
† Color increases as figure increases; color comparator used.

masked differences that would have appeared if the drying rates could have been faster. The berries often became quite "moldy" in appearance. Cracks in the berries became filled with ridges of conidiophores and conidia of botrytis until many of the berries were completely covered with the fungus. Injured berries or dead stems were consistently courts of infection for *Aspergillus* sp., *Penicillium* sp., *Rhizopus nigricans,* and yeast-Acetobacter activity. The "moldy" odor of the wines was probably due to the action of the saprophytes. Berries so infected were very sour and unpalatable, whereas those infected by botrytis only were very sweet.

1953: Further Studies of the Variables Affecting the Chemical Composition of Must and Wine under More Controlled Conditions

The closer control of temperature and humidity in the 1953 tests is reflected in a more consistent relationship between the treatments and the chemical composition of the musts and wines. Forty lots of the three most promising grape varieties—Semillon, Muscat, and Malvasia bianca—were tested. The results of previous experimentation made it possible to narrow the range of temperature and relative humidity during both the infection and dehydration periods with greater assurance of satisfactory results. It was also possible to estimate more closely optimum lengths for both periods.

Effect of Inoculum Potential on Infection Level. To test the hypothesis that the inoculum must be applied to the fruit to secure satisfactory infection and, in turn, satisfactory drying rates, lot 14 was not inoculated, whereas lots 12 and 13 were (table 11). Subsequently, all three lots were held under the same environmental conditions. The Balling degree of the must of lot 14 was 5.6 degrees less than that of lots 12 and 13 (table 12), indicating that the fruit of lot 14 did not become infected to any extent. Apparently, the inoculum, if any, present on the fruit when it was harvested was not sufficient for satisfactory results.

Relation of Length of Infection Period to Volatile Acid Content of Must. The length of the infection period had a marked effect on the volatile acid content of the must. Lots 1 to 5, with an infection period of 1⅔-days, produced musts with volatile acid content considerably higher than that of all the other lots, which were exposed to a 1-day infection period (tables 11 and 12). This agrees with the results of previous seasons and supports the conclusion that infection periods longer than one day are unnecessary and may even be undesirable, provided the incubation temperature is at or near 68° F, the relative humidity is close to 100 per cent, and the spores are applied wet.

Relative Humidity during the Dehydration Period. The relative humidity during the dehydration period had a marked effect on the length of the dehydration period required to produce musts with satisfactory Ballings. Lot 1, held for 6 days at 40 per cent relative humidity, produced a must of 38.5° Balling, whereas lot 5, held for 8 days at about 80 per cent relative humidity, produced a must of only 35.7° Balling (tables 11 and 12). The difference between the Ballings would have been even greater had the dehydration temperatures been the same, since it is reasonable to expect a must of

TABLE 11

1953: COMPOSITION AND TREATMENT OF GRAPES

Lot no.	Variety	Date harvested	Original analysis			SO_2* days before inoculation	Date inoculated	Infection period			SO_2* days after inoculation	Dehydration period		
			Balling	Total acid	pH			Days	Temp.	r.h.		Days	Temp.	r.h.
			degrees	*% tartaric*					°F	%			°F	%
1	Semillon	10/19	20.5	0.59	3.62	...	10/19	1⅔	68	100	...	6	68	40
2	Semillon	10/19	20.5	0.59	3.62	¼	10/19	1⅔	68	100	4	6	68	40
3	Semillon	10/19	20.5	0.59	3.62	...	10/19	1⅔	68	100	...	6	95	40
4	Semillon	10/19	20.5	0.59	3.62	¼	10/19	1⅔	68	100	4	6	95	40
5	Semillon	10/19	20.5	0.59	3.63	...	10/19	1⅔	68	100	...	8	75†	80†
6	Malvasia bianca	10/19	21.0	0.62	3.65	...	10/27	1	77†	98–100	...	8	77†	55†
7	Malvasia bianca	10/19	21.0	0.62	3.65	1	10/27	1	77†	98–100	...	8	77†	55†
8	Malvasia bianca	10/19	21.0	0.62	3.65	...	10/27	1	77†	98–100	4	8	77†	55†
9	Malvasia bianca	10/19	21.0	0.62	3.65	1	10/27	1	77†	98–100	4	8	77†	55†
10	Semillon	10/25	25.9	0.66	3.79	...	10/28	1	75†	98–100	...	7	73–78	60–80
11	Semillon	10/25	25.9	0.66	3.79	...	10/28	1	75†	98–100	...	7	73–78	60–80
12	Semillon	11/4	25.3†	0.61†	3.64†	...	11/5	1	75†	98–100	...	6	72	55
13	Semillon	11/4	25.3†	0.61†	3.64†	...	11/5	1	75†	98–100	...	6	72	55
14	Semillon	11/4	25.3†	0.61†	3.64†	...	not inoc.	1	75†	98–100	...	6	72	55
15	Semillon	11/2	25.3	0.61	3.64	⅙	11/4	1	72†	98–100	5	7	72†	60–67
16	Semillon	11/2	25.3	0.61	3.64	...	11/4	1	72†	98–100	5	7	72†	60–67
17	Semillon	11/2	25.3	0.61	3.64	...	11/4	1	72†	98–100	...	7	72†	60–67
18	Semillon	11/2	25.3	0.61	3.64	⅙	11/4	1	72†	98–100	...	7	72†	60–67
19	Semillon	11/2	25.3	0.61	3.64	...	11/4	1	72†	98–100	⅚	7	72†	60–67

* 0.5 per cent by volume for 10 minutes; blanks indicate lot was not fumigated before (seventh col.) or after (twelfth col.) inoculation.
† Approximate; for temperature, plus or minus 2 degrees.

TABLE 11—*Continued*

Lot no.	Variety	Date harvested	Original analysis			SO_2* days before inoculation	Date inoculated	Infection period			SO_2* days after inoculation	Dehydration period		
			Balling	Total acid	pH			Days	Temp.	r.h.		Days	Temp.	r.h.
			degrees	*% tartaric*					°F	%			°F	%
20	Semillon	11/2	25.3	0.61	3.64	...	11/11	1	70†	98–100	3	7	70	60
21	Semillon	11/2	25.3	0.61	3.64	...	11/18	1	70†	98–100	3	6	70	57–61
22	Semillon	11/2	25.3	0.61	3.64	...	11/18	1	70†	98–100	3	6	70	57–61
23	Semillon	11/2	25.3	0.61	3.64	⅔	11/18	1	70†	98–100	3	6	70	57–61
24	Muscat‡	11/7	—§	—§	—§	¾	11/19	1	70†	98–100	...	6	70	58–60
25	Muscat	11/7	—	—	—	¾	11/19	1	70†	98–100	4	6	70	58–60
26	Muscat	11/7	—	—	—	...	11/19	1	70†	98–100	4, 5	6	70	58–60
27	Muscat	11/7	—	—	—	...	11/19	1	70†	98–100	4	6	70	58–60
28	Muscat	11/7	—	—	—	...	11/19	1	70†	98–100	...	6	70	58–60
29	Muscat	11/17	—	—	—	3¼	11/24	1	70†	98–100	2	7	69–71	63–72
30	Muscat	11/17	—	—	—	...	11–25	1	70†	98–100	...	7	72–73	56–66
31	Muscat	11/17	—	—	—	...	12/3	1	70†	98–100	...	7	68–74	49–59
32	Muscat	11/17	—	—	—	¼	12/3	1	70†	98–100	2, 4	7	68–74	49–59
33	Muscat	11/17	—	—	—	...	12/3	1	70†	98–100	2, 4	7	68–74	49–59
34	Muscat	11/17	—	—	—	...	12/3	1	70†	98–100	2	7	68–74	49–59
35	Muscat	11/17	—	—	—	¼	12/3	1	70†	98–100	...	7	68–74	49–59
36	Muscat	11/17	—	—	—	...	12/1	1	70†	98–100	...	9	67–70	70–84
37	Muscat	11/17	—	—	—	¼	12/1	1	70†	98–100	...	9	67–70	70–84
38	Muscat	11/17	—	—	—	¼	12/1	1	70†	98–100	2, 4	9	67–70	70–84
39	Muscat	11/17	—	—	—	...	12/1	1	70†	98–100	2, 4	9	67–70	70–84
40	Muscat	11/17	—	—	—	...	12/1	1	70†	98–100	2	9	67–70	70–84

* 0.5 per cent by volume for 10 minutes; blanks indicate lot was not fumigated before (seventh col.) or after (twelfth col.) inoculation.
† Approximate; for temperature, plus or minus 2 degrees.
‡ Lots 24–40 Muscat of Alexandria.
§ Dashes indicate data not available.

lower Balling from grapes held at 68° instead of 75° F. The effect of the relative humidity during the dehydration period is also demonstrated by a comparison of lots 31 to 35, held at 49–59 per cent relative humidity, with lots 36 to 40, held at 70–84 per cent relative humidity (table 11). Both groups reached about the same range of Balling readings; however, the group incubated at the higher relative humidity required a 9-day dehydration period as compared with 7 days for the other.

The contrast in appearance of the two groups at the end of the dehydration period was striking. Figure 7*A* shows a representative cluster from the lots incubated in the high humidity range. Botrytis growth was very profuse both inside and outside of the berries. The skin of each infected berry was ruptured in a dozen or more places, each break being filled with the conidia and conidiophores of the fungus. The berries had wrinkled considerably, although this condition is masked somewhat by the fungus growth. The fruit in figure 7*B* from the lots dehydrated in the lower humidity range shows little or no mycelial growth. Nearly all of the growth was confined to the inside of the berries.

Temperature during Dehydration and Its Effect on Rate of Water Loss. The temperature during the dehydration period appears to affect the rate of water loss from the fruit in at least two ways. First, temperature is correlated with the length of the dehydration period; that is, the higher the temperature, the shorter the dehydration period necessary to produce musts with high Balling readings, owing to a correlation between temperature and vapor-pressure deficit. Second, temperature affects the growth rate of *Botrytis cinerea* and thus, indirectly, the rate of water loss from fruit. This effect is demonstrated by a comparison of lots 1 and 2, dehydrated at 68° F, with lots 3 and 4, dehydrated at 95° F (tables 11 and 12). In spite of the higher temperature, lots 3 and 4 produced musts with lower Balling readings than those of lots 1 and 2. This anomaly can be accounted for by the fact that a temperature of 95° F is considerably above the optimum necessary for the growth of *B. cinerea* (Nelson, 1951). The fruit of the 95° F lots showed very little evidence of botrytis activity at the time of crushing even though the fungus was well established by optimum temperature and moisture conditions during the infection period immediately preceding the high-temperature dehydration period. It appears, then, that the fungus predisposes the fruit to rapid water loss—to the extent that the well-infected fruit of lots 1 and 2 had a higher moisture-loss rate than did lots 3 and 4 in spite of a much lower vapor-pressure deficit (tables 11 and 12).

The Effect of Fumigation with Sulfur Dioxide. A comparison of the various treatments of sulfur dioxide shows that the gas had no consistent effect on the volatile acid content of the must (tables 11 and 12), which was, however, quite low for all lots. Apparently, when temperature and moisture conditions are optimum for botrytis infection and are maintained just long enough for the fungus to infect the fruit, the level of undesirable microbiological activity is so low that the depressing effect of sulfur dioxide on this activity is not apparent in the volatile acid content of the musts.

It is possible that fumigation of the fruit before inoculation may have a depressing effect on the amount of botrytis infection, as indicated by the

Balling degree of the must. Lot 18 was fumigated, whereas lot 17 was not. The Balling of the must from the nonfumigated lot was 34.4° as compared with 30.9° for the lot that was fumigated (table 12). Similar results were obtained with lots 28 and 24, 31 and 35, and 36 and 37. When lots fumigated

TABLE 12

1953: COMPOSITION OF MUSTS FROM BOTRYTIS-AFFECTED GRAPES

Lot no.	Balling	Total acidity	Volatile acidity
	degrees	*% tartaric*	*% acetic*
1	38.5	1.10	0.023
2	38.0	1.00	0.030
3	37.6	0.95	0.076
4	33.8	0.89	0.075
5	35.7	0.82	.....
6	33.3	0.86	.....
7	31.7	0.74	.....
8	29.6	0.79	.....
9	34.5	0.72	.....
10	34.9	0.59	.....
11	34.8	0.67	.....
12	31.2	0.65	0.009
13	31.2	0.65	0.009
14	25.6	0.63	0.006
15	34.9	0.69	0.006
16	34.4	0.60	0.007
17	34.4	0.60	0.009
18	30.9	0.59	0.008
19	29.8	0.66	0.008
20	32.1	0.74	0.012
21	29.5	0.51	0.012
22	31.4	0.62	0.018
23	31.6	0.63	0.015
24	27.3	0.70	0.012
25	27.9	0.73	0.015
26	28.9	0.75	0.015
27	30.6	0.73	0.015
28	30.1	0.78	0.013
29	31.5	0.73	0.009
30	33.0	0.86	0.009
31	32.5	0.81	0.006
32	34.1	0.71	0.006
33	31.8	0.74	0.006
34	31.0	0.72	0.006
35	36.1	0.82	0.007
36	32.0	0.90	0.006
37	29.3	0.72	0.006
38	27.0	0.62	0.006
39	33.4	0.79	0.009
40	32.2	0.72	0.006

before inoculation are compared with lots fumigated afterward, the depressing effect of the gas on the Balling degree is apparent in three comparisons out of four. Lots 18 and 16, 24 and 27, and 37 and 40 show this difference. However, the Balling degree of lot 35, which was gassed before inoculation, is higher than that of lot 34, which was gassed afterward.

Comparisons of lots gassed both before and after inoculation with corresponding checks that were not gassed are very inconclusive as to the effect of the gas on the Balling degree of the must. Lots 1 and 2, 28 and 25,

TABLE 13

1953: ANALYSES OF WINES PRODUCED FROM BOTRYTIS-AFFECTED GRAPES*

Lot no.	Total acid	Volatile acid	pH	Alcohol	Extract	Reducing sugar	Tannin	Color†	Balling	Flavor score	Comments
	% tartaric	*% acetic*		*% by vol.*	*gm/100 gm*	*%*	*%*		*degrees*		
1	1.11	0.131	3.31	14.2	19.1	14.6	0.025	14.9	14.9	78	Very distinct botrytis character
2	0.93	0.126	4.05	15.2	17.5	12.0	0.025	13.3	12.5	77	Good botrytis odor
3	0.79	0.147	4.30	15.0	17.2	12.5	0.025	34.5	13.8	78	Good botrytis, noticeable volatility
4	0.69	0.114	4.40	16.3	11.8	6.5	0.025	23.2	6.4	75	Fair botrytis, but oxidized
5	0.64	0.091	4.32	16.2	13.4	7.5	0.021	12.5	7.6	75	Low botrytis, slightly oxidized
6	0.68	0.087	4.48	15.8	11.0	6.4	0.008	22.0	6.7	78	Medium muscat, smooth, alcoholic
7	0.57	0.066	4.51	17.6	8.2	2.0	0.025	16.5	1.2	73	Medium muscat, slightly off (oxidized?)
8	.63	0.091	4.49	15.4	7.3	2.5	0.025	20.3	2.2	72	Medium muscat, bacterial?
9	0.61	0.087	4.42	15.6	12.3	6.5	0.025	18.8	7.5	76	Medium muscat, slight mold?
10	0.55	0.103	4.37	14.8	14.1	10.0	0.017	19.6	10.3	77	Low botrytis, smooth
11	0.63	0.150	4.41	15.7	13.0	7.6	0.017	8.2	8.2	79	Low botrytis
12	0.59	0.091	4.33	15.5	8.8	5.1	0.017	16.2	4.9	78	Low botrytis, smooth
13	0.57	0.074	4.32	15.3	9.1	4.8	0.017	18.1	3.0	80	Low botrytis, slightly alcoholic
14	0.51	0.033	4.18	14.2	4.2	1.4	0.017	13.3	0.0	77	Low botrytis, lacks character
15	0.63	0.099	4.38	15.2	13.7	9.6	0.004	16.7	9.8	80	Low botrytis, lacks character
16	0.53	0.084	4.42	14.7	13.0	6.9	0.025	21.2	7.5	73	Distinct, oxidized, rancio
17	0.52	0.105	4.40	14.6	13.8	8.7	0.021	23.3	9.2	73	Distinct, oxidized, rancio
19	0.51	0.063	4.32	14.8	7.6	3.7	0.008	19.1	3.8	76	Lacks botrytis character, alcoholic
20	0.57	0.081	4.52	15.3	10.9	6.2	0.025	20.0	6.4	72	Slight off-odor (mold)

* Lot 18 lost.

† Depth of color increases as figures increase.

TABLE 13—*Continued*

Lot no.	Total acid	Volatile acid	pH	Alcohol	Extract	Reducing sugar	Tannin	Color†	Balling	Flavor score	Comments
	% tartaric	*% acetic*		*% by vol.*	*gm/100 gm*	%	%		*degrees*		
21	0.52	0.054	4.20	15.5	7.5	2.8	0.013	11.7	3.4	80	Medium botrytis odor
22	0.58	0.072	4.37	16.2	8.9	5.5	0.021	15.1	4.3	79	Medium botrytis, alcoholic
23	0.54	0.066	4.43	16.2	10.1	5.7	0.021	16.8	5.0	79	Low botrytis, slightly oxidized
24	0.66	0.061	4.18	14.6	6.4	3.0	0.017	12.0	1.5	72	Medium muscat, flat
25	0.68	0.060	4.22	14.0	8.1	4.2	0.017	8.1	3.6	72	Low muscat, bitter
26	0.67	0.063	4.28	14.4	8.4	4.3	0.017	7.6	3.3	73	Medium muscat, bitter
27	0.71	0.071	4.20	15.9	8.1	4.4	0.008	13.2	3.2	73	Medium muscat, bitter
28	0.71	0.084	4.19	15.0	8.8	4.3	0.021	10.0	4.0	71	Low muscat, bitter
29	0.65	0.083	4.29	15.2	10.1	6.2	0.013	14.4	5.9	75	Low muscat, bitter
30	0.75	0.109	4.26	14.6	12.2	8.3	0.013	18.1	8.2	74	Low muscat, bitter
31	0.82	0.095	4.30	15.1	11.6	6.9	0.025	20.0	4.0	75	Medium muscat, slight botrytis
32	0.69	0.099	4.32	15.3	13.1	8.1	0.021	15.0	8.7	75	Medium muscal bitter
33	0.75	0.072	4.10	14.1	11.2	7.0	0.008	14.3	7.4	75	Medium muscat, bitter
34	0.71	0.069	4.23	15.3	9.2	5.5	0.021	14.4	5.0	73	Medium muscat, bitter
35	0.86	0.091	4.25	14.7	15.7	11.7	0.025	21.9	12.3	73	Medium muscat, bitter
36	0.58	0.090	4.38	13.6	12.4	7.2	0.021	18.6	8.3	78	Medium muscat, medium botrytis
37	0.65	0.066	4.29	14.7	7.3	3.7	0.017	10.6	3.2	72	Slight muscat, bitter
38	0.48	0.060	4.41	14.1	6.6	2.9	0.017	10.0	1.9	71	Medium muscat, flat
39	0.72	0.073	4.38	15.4	12.8	7.9	0.017	15.5	8.7	75	Medium muscat, slightly fruity
40	0.67	0.081	4.38	15.1	10.5	6.0	0.017	14.4	5.8	75	Medium muscat, slightly fruity

† Depth of color increases as figures increase.

30 and 29, and 36 and 38 show the depressing effect, whereas lots 9 and 6, 17 and 15, and 31 and 32 show the reverse (tables 11 and 12). When fruit fumigated only after inoculation was compared with non-fumigated checks, no depressing effect on infection was apparent. This is indicated by comparing the Balling reading of lot 17 with those of lots 16 and 19; lot 28 with 26 and 27; 31 with 33 and 34; and 36 with 39 and 40 (tables 11 and 12).

The Influence of Variety. No critical comparisons can be made among the musts of the three varieties tested because of differences in the initial Balling readings of the fruit and the post-inoculation environment. However, satisfactory Balling levels for the musts were obtained from lots of all of these varieties. This supports the results of the 1952 experiments and indicates that the critical factors for a must with a high Balling degree are the inoculum, the inoculation method, and the post-inoculation environment of the fruit.

Significant Chemical Aspects of Natural Sweet Musts. The composition of the musts before and after treatment is given in tables 11 and 12. In the four groups of varietal pickings for which original Balling readings are available (lots 1–5, 6–9, 10–11, and 12–23), sugar increased 79.0, 53.8, 27.4, and 26.9 per cent. The percentage increase was less the later the harvesting and the greater the original sugar content. Adding the Muscat group (lots 24–40), the average Ballings attained were 36.7°, 32.3°, 33.0°, 32.1°, and 31.1°. The total acidities all increased, but except for the first Semillon group the increase was not excessive. The percentage increase in total acidity for the four groups was 61.0, 25.4, –3.0, and 2.7 per cent.

Except for the first Semillon group, the data show that botrytis infection need not result in an undue increase in acidity, even with increases in sugar of 25 to 79 per cent. There seems to be a limit to which the percentage of sugar in detached grapes can be allowed to increase without incurring too great an increase in acidity. This is very promising, since it was believed that during botrytis growth on the berries, contact with the vine was also necessary to allow for potassium translocation and acid neutralization in the fruit.

Less promising is the large increase in pH. The pH increased from 3.62, 3.65, 3.79, and 3.64 to 4.0 or over. Sweet table wines made from musts with such a high pH are less satisfactory than those from musts with a pH of 3.6 to 3.8. The free acids and some of the tartrates have been destroyed by the botrytis growth. At pH's of 4.0 and over potassium acid tartrate is not an important part of the buffer system ($pK_1 = 2.99$; $pK_2 = 4.34$).

The volatile acidity developed in the must was very small except for two samples in the first series. The amounts of acetic acid formed in the other samples would be partially utilized during alcoholic fermentation and would not inhibit alcoholic fermentation.

Wine Analyses. The composition of the wines that were produced is given in table 13. Mainly because of the long, slow fermentation, more than the usual amount of volatile acidity developed during fermentation. This was excessive in about seven lots. However, many French Sauternes are as high in volatile acidity as those produced here, or higher. The pH's increased during fermentation as expected; all except one were over 4.05 and one was 4.52! Greater success in stopping the fermentations was achieved by filtering the wines. However, 22 wines, or about half of the lots, were over 15 per cent in

alcohol. The sugar contents were variable but encouraging, ranging from 1.4 to 14.6 per cent. Tannin content and color were moderately low. Many of the wines had a full gold color, which is considered desirable with this type of wine.

The organoleptic data are also given in table 13. Practically all of the Semillon wines had a botrytis odor. The muscat wines (Malvasia bianca and Muscat of Alexandria) did not, in most cases, reveal a recognizable botrytis odor, probably because of the predominant muscat aroma. The scores of the muscat wines were also lower than those of the Semillon wines. The high alcohol contents probably reduced the scores 2 to 5 points.

DISCUSSION AND CONCLUSIONS

Natural sweet white table wines of high quality, which possess the characteristic aroma and bouquet associated with the metabolic activity of *Botrytis cinerea* in the fruit before crushing, can be produced only as a result of the critical integration of many factors. These may be considered in five groups: 1) environmental and nutritional factors favorable to the facultative saprophytic activity of *B. cinerea* as well as the saprophytic activity of a number of other microörganisms; 2) factors of handling that cause or permit mechanical and insect injury to the fruits; 3) moisture, temperature, and air-movement conditions that lead to berry splitting and drying; 4) temperature conditions from infection to crushing that influence the flavor of the wine; and 5) fermentation and cellar practices that affect the chemical balance of the final product.

Considering that under natural conditions most of these factors operate uncontrolled in the vineyard, it is not surprising that great wines of this type are produced in only a few localities (and not every year in these). Only after the fruit is harvested and crushed can any degree of control be effected, although harvesting practices do permit some selection of botrytised fruit.

Musts with a high Balling degree and good chemical balance can be consistently obtained by harvesting only mature fruit and holding it under controlled conditions during the incubation period. High levels of botrytis infection can be obtained with very little saprophytic activity if certain factors are closely controlled:

1. A high concentration of inoculum (botrytis spores) well distributed over the fruit is essential.
2. The spores require liquid water, or a relative humidity of 95 per cent or more, to germinate and infect healthy, uninjured fruit.
3. A temperature of about 68° F is near the optimum for botrytis infection.

It is important to provide an environment that will enable botrytis to infect the fruit as quickly as possible since the moist conditions necessary for botrytis also favor the growth of many undesirable saprophytes. Prolonged infection periods allow these organisms to become established in dead or dying grape tissue, or in the juice of berries split from imbibed water. If the wet conditions are replaced with a dry environment just after the germ tubes of the botrytis spores have penetrated the grape skin and are no longer dependent on a wet external environment for growth, the degree of undesir-

able saprophytic activity is minimized. Thus, by careful manipulation of the environment, the skin of the grape becomes a microbiological screen—permitting the penetration of botrytis as a facultative saprophyte but preventing the entrance of undesirable organisms, all of which are saprophytic.

Once established, the fungus not only contributes to the quality of the wine with the end products of its metabolic activity, but it also predisposes the fruit to a more rapid water loss. The temperature during the dehydration period should, therefore, be optimum for growth of the fungus even though higher temperatures would result in a greater vapor-pressure deficit. Short dehydration periods are desirable, but water-loss rates apparently should be accelerated by very low relative humidities rather than by excessively high temperatures. It is interesting that the rate of water loss was slightly less at 95° F than at 68° F (tables 11 and 12), even though the vapor-pressure deficit was much greater at the higher temperature. Apparently the higher vapor-pressure deficit at 95° F, coupled with little or no botrytis activity, did not compensate for the great botrytis activity at 68° F coupled with a lower vapor-pressure deficit insofar as rate of water loss is concerned.

It is quite possible that faster drying rates may be obtained at temperatures of 80° F or more, since botrytis grows well in this range. If dehydration periods were shorter, the production of end products of metabolism by botrytis might be curtailed enough to diminish the characteristic aroma and bouquet of the wine owing to these materials. Conversely, slower drying rates, brought about by lower temperatures or higher relative humidities, would lengthen the dehydration period and possibly enhance the characteristic aroma and bouquet of the wine. However, there are very definite limits within which this technique can be applied without undesirable side effects. A relative humidity of 70 to 80 per cent at 68° F resulted in the fungus growing through breaks in the skin of the berries. Juice often exuded through these breaks, providing an excellent medium for the yeast-Acetobacter complex and other undesirable saprophytic activity. Wines with high volatile acid content and moldy flavors frequently resulted. Humidities below 60 per cent appeared to confine the fungus to the interior of the berries, and very few cracks in the skin appeared. Wines from this fruit had just as much "botrytis" aroma and bouquet as did those from grapes that were covered with botrytis growth at the time of crushing.

It is quite probable that the exterior growth of botrytis and the saprophytic growth are correlated with the relative humidity. Presumably it is the growth of botrytis *within* the berries that makes the fruit suitable for drying at moderate temperatures and produces the characteristic flavor and texture so desirable for natural sweet wines. According to Laborde (1908), it is the wrinkled, brown, botrytis-infected berries showing no external signs of the fungus that are considered the most desirable.

Only fruit of the highest quality should be treated. It should be as ripe as possible for three reasons. First, the sugar content is high; it should be 23° Balling or more. Second, the total acid content is reduced as the fruit ripens. A moderately low total acidity is desirable since there may be some increase during the drying period. Third, as grapes ripen they become more susceptible to botrytis infection. Overripe fruit, however, is very undesirable

because raisin flavors are frequently associated with overripeness, and decay is more apt to be present as maturity advances.

Maturity should be as uniform as possible throughout the lot. Slight differences in maturity often result in great differences in the response of the fruit during the treatment period. Small clusters are more desirable than large, as there is less danger of bunch rot remaining undetected in the middle of the bunch. All decayed or otherwise injured berries should be removed. The clusters must be carefully handled, not only to prevent injury but also to prevent juice from injured berries being smeared over the surface of adjacent berries and thus becoming a medium for saprophytic growth during the infection period. Actually, the clusters should be handled more like table grapes than wine grapes and laid carefully in the picking box, preferably not more than one cluster deep.

The climate in California vineyards makes it unlikely that high-quality wines of this type can be produced from fruit inoculated on the vine. Liquid water or a relative humidity of 95 per cent or more must be maintained on or around the fruit for at least 24 hours after inoculation. This is difficult though not impossible to do, either by nearly continuous spray application or by covering the vines with a waterproof canopy. The first method has the serious disadvantage of washing off the inoculum before infection is established, and the second results in an unduly high temperature under the canopy. It appears that only in those districts with the coolest, cloudiest weather during the harvest season would the treatment have much chance of success.

SUMMARY

It was found that the mature harvested fruit of several varieties of grapes could be heavily infected with *Botrytis cinerea* if it was held under saturated conditions for one day or more after application of the spores. Dense sporulating colonies of *B. cinerea* were obtained by growing the fungus on grape-juice agar and making single-spore isolates every three to six weeks to recover the conidial type. Musts with Ballings as high as 47° were obtained from this infected fruit when it was held under dry conditions for a week to 10 days at room temperature. Natural sweet white wines derived from these musts possessed the distinct Sauternes type of aroma and bouquet associated with European wines of this type.

The relationships of various techniques and conditions to the final product were investigated, and the following conclusions were reached:

1. The fruit could be inoculated by either dry or wet application of spores.

2. If the spores were applied in a water spray, an infection period (95 to 100 per cent relative humidity) of 24 hours at 68° F was long enough to obtain a satisfactory level of infection. Allowing water to remain on the fruit longer than this caused the berries to split from imbibed water, and the exuded juice promoted the growth of undesirable microörganisms. Excessive yeast-Acetobacter activity was reflected in a high volatile acid content of the must.

3. Dry spores on the fruit required relative humidities of 95 per cent or more during the infection period. Satisfactory levels of infection necessitated

infection periods lasting two days or longer at 68° F. Little or no berry splitting occurred.

4. The length of the dehydration period necessary to produce musts with a high Balling degree was inversely proportional to the vapor-pressure deficit, so long as the temperature was about 68° F or less. At 95° F the drying rate was no more rapid than at 68° F in spite of a much higher vapor-pressure deficit because the fungus does not grow at this temperature. Growth of the fungus in the fruit predisposed the fruit to more rapid water loss, even when cracks in the berries were not apparent.

5. A dehydration temperature of 95° F produced a raisin-like flavor in the wine, whereas a temperature of 54° F resulted in moldy flavors.

6. Fumigation of the fruit with sulfur dioxide before inoculation did not depress the volatile acid content of the must, but it did depress the amount of botrytis infection. Fumigation of the fruit during the dehydration period had no effect.

7. Musts of 30° to 40° Balling may be expected. The acidity increases, but much less than the sugar content. Yields of only 50 to 100 gallons per ton were obtained.

8. The extent of the fermentation must be carefully controlled to prevent formation of an excessively high percentage of alcohol.

9. Pilot-plant experiments and consumer acceptance of the admittedly expensive products should be undertaken.

10. Attempts to produce high-Balling musts from grapes inoculated on the vines were unsuccessful because of high daytime temperatures. It was difficult to maintain the moisture conditions necessary for infection for a 24-hour period, and thermophilic saprophytes were a serious problem.

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4*m*-5,'57(B9666)BEB

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