the 1970s. The acreage planted to grapes has fluctuated as markets have changed. Grower prices have fluctuated in response to climatic variations and cyclic shortages and surpluses. The magnitude and duration of these shortages and surpluses suggest that grape supply and wine demand have never been effectively coordinated.

Wine and grape prices have changed reasonably with changes in the Consumer Price Index. Production costs, on the other hand, appear to have grown at a substantially higher rate. The wine and grape industry has remained a vigorous part of California's agricultural economy, presumably in large part because improved efficiency has offset significant cost changes. A major part of this gain was from University research directed toward improved varieties, better cultural practices, and superior winemaking technology.

Many research challenges remain if California's industry is to meet future wine demands. Among these are economic studies directed toward more complete wine demand forecasts and their implication for grape variety requirements; analysis of trade policies that will facilitate market development for California wines; and more complete analysis of wine production and marketing strategies and costs in order to improve efficiency.

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# 100 years of wine microbiology Ralph E. Kunkee George M. Cooke

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t can be said that wine microbiology began about 100 years ago, if we think of it as being concerned mainly with alcoholic fermentation and selection of yeasts, malo-lactic fermentation and the bacteria involved, and problems with microbiological spoilage. Louis Pasteur's first edition of *Etudes sur le Vin* was published in 1866, and in 1889 Kulisch presented the first evidence that malolactic fermentation was microbiological. However, the major contributions by the University of California in wine microbiology did not begin until early in this century.

As new vineyards were planted at the turn of the century, the University wine microbiologists were able to look at the ecology of the recently established microflora (the yeast and the bacteria on the grapes) and to compare these studies with those from the well-established vineyards of the European wine regions.

#### Yeast culture collection

Athough no enological research was done, as such, at the University during Prohibition, apparently some attention was paid to establishing a wine yeast culture collection and, later, a bacteriological culture collection. Because information was not published, however, it is difficult to pinpoint the actual origins of the various strains in the present University of California enology collection at Davis. Professor William Cruess had a collection of yeast originally obtained by Dean Eugene Hilgard. We presume that some of our present culture collection came from these strains. We know that a "Burgundy" and a "Champagne" strain arrived in the collection from an institute in France.

Professor Cruess also studied flor sherry production. Because the yeast strains needed were not available in California, he obtained them from Spain and added them to the collection. This research on flor sherry was continued later and led to the utilization of the submerged flor process, now widely practiced in California.

Apparently the present system of numbering the yeast strains did not begin until after 1938, when Professor John Castor systematized the yeast collection. It seems to have been Castor who introduced the strain "Montrachet."

The use of pure yeasts as starter cultures, as recommended by University enologists, became common practice in California after Prohibition. One strain of choice was Montrachet because of its fast fermentation rate, consistency in fermentation, and pleasant end-products. A few other strains also have been considered exceptionally good.

The use of a limited number of wine yeast strains, some of which came from the University's collection, led to commercialization of yeast starter culture preparation, first in the pressed cake form and then as active dry preparations. Early research in the preparation of frozen and frozen dried yeast was the basis for the later development of these active dry yeast cultures.

Another important development by the University was the isolation, cultivation, and description of a wine spoilage bacterium known as hair bacillus or cottony mold, *Lactobacillus trichodes* (that is, "hairlike"). The organism is resistant to ethanol and thrives in dessert wines—its most common natural habitat. Fortunately, it is also extremely sensitive to sulfur dioxide and thus has not been an important spoilage problem in California wine since publication of the research results in 1943.

## **Fermentation studies**

Castor began the studies at the University on controlled fermentations with various wine yeast strains, and studied rates of yeast growth and fermentation. These latter studies led recently to formulations of computerized fermentation equations. The fermentation control research was continued; a lengthy series of papers was published following Castor's work, which described on a practical basis the factors that influence the alcoholic fermentation.

Research in the late 1950s on formation of the higher alcohols (fusel oil) showed that they do not arise exclusively from amino acids in the grape juice itself, as had been thought previously. The higher alcohols can also come directly from the sugars in the grape juice during alcoholic fermentation by yeast. Information obtained from bacteriological research at that time led to the proof, with the use of mutant yeast strains, of the metabolic pathways of the formation of the higher alcohols and suggested the kinds of metabolic control that should be applicable.

Continuing some of this earlier work, we have recently obtained a mutant of Montrachet that is deficient in the pathway for the production of a higher alcohol. This mutant yeast strain ferments as rapidly as the control strain and produces low levels of fusel oil. We have also studied the enzymatic formation of ethanol itself by the important enzyme alcohol dehydrogenase and have studied the control of this enzyme's activity.

#### Malo-lactic fermentation

The secondary malo-lactic fermentation, which occurs normally in many wines in the spring, a few months after the alcoholic fermentation, was originally considered rare and undesirable in California wines. However, a high incidence was found in a 1960 survey of wines. When the role of malo-lactic fermentation was examined, it was found to be desirable, because it provided bacteriological stability to the wine and in some cases seemed to add complexity to the flavor.

An important malo-lactic organism, Leuconostoc oenos ML 34, was isolated and cultivated in the early 1960s. A wine consultant in the Napa Valley obtained the organism from the University and distributed it to several California wineries to induce malo-lactic fermentation. The organism was also sent to at least one enological institute in Europe. Much of the excellent malo-lactic research done at the University of Bordeaux has been with Leuconostoc gracile Cf-34, which is, in fact, the "ML 34" organism from Napa Valley.

Another development was a simplified paper chromatographic procedure for detecting the malo-lactic fermentation. Although

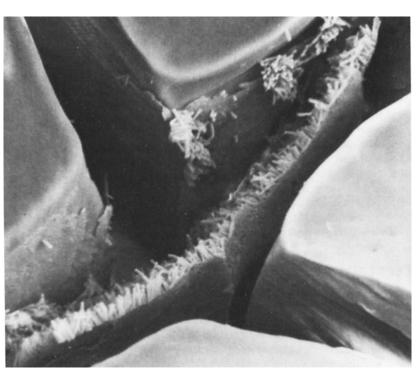
the procedure is qualitative and requires several hours for obtaining the results, it is now widely used in California winery laboratories.

The biochemistry of the utilization of malic acid was also studied. It was found that the initial growth rate of malo-lactic bacteria was stimulated by an incomplete utilization of malic acid to give an intermediate, pyruvic acid, and probably acetyl phosphate, which could serve as hydrogen acceptors needed to stimulate the beginning of the fermentation.

One drawback in the use of L. oenos is that microbiological technical skill is needed to prepare a starter culture. Although much less malo-lactic starter culture is needed than when using a yeast starter culture, it is still a large amount if large quantities of wine need to be inoculated. We are working on methods to simplify starter culture preparation and enable the winemaker to obtain the malo-lactic fermentation during the alcoholic fermentation.

### Production of semi-dry table wine

In the late 1960s and early 1970s, production of "mellow" (semi-dry) white wines increased in California, either to emulate the German Rhineland-style wines or to appeal to America's sweet tooth. In any case, secondary yeast fermentation of the wine after bottling had to be prevented. Several practical methods were avail-



t rained hard and long during the winter and spring of 1884 in Anaheim...and those extraordinary rains turned out to be harbingers of the first recorded serious disease epidemic in California vineyards. A new and mysterious malady of the vines, later to be known as Pierce's disease, dealt damaging blows to the grape industry established by German settlers around the little town southeast of Los Angeles, and local wineries had to close down as more than 35,000 acres of vines disappeared over the following

Newton B. Pierce, a young U.S. Department of Agriculture plant pathologist assigned to the West Coast to study the problem,

hopper, as shown here in electron micrograph, helped explain unusual characteristics of transmission of the destructive disease.

Discovery in 1979 by U. C. entomologist Alex Purcell that Pierce's disease bacteria attach to the mouth and throat lining of grape leaf-

reported in 1892 on detailed studies and observations which are the basis of our present-day understanding of this serious disease.

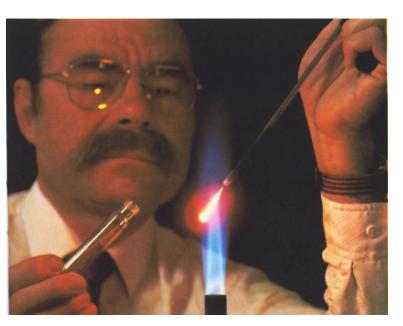
Virus diseases of plants were a new concept in the early 1900s, and following the early groundwork of Pierce, who incidentally was strongly inclined to attribute the California vine disease to bacteria, it became generally accepted that the disease was caused by a virus. In many ways it behaved like a virus disease, especially in its transmissibility by budding and its manner of spread.

Professor W. B. Hewitt and coworkers at the University of California accumulated an impressive array of alternative host plants and information on the epidemiology of Pierce's disease as it made further inroads into the northern vineyards during the 1930s. The true nature of the microscopic causal agent was not revealed, however, until the 1970s, when the electron microscope enabled U. C. scientists to accurately visualize minute bacteria in the water-conducting tissues of diseased vines and subsequently to culture the organism on artificial media.

Consistent control of Pierce's disease in the vineyard still eludes researchers, but rapidly expanding knowledge of the relationship between insect vectors and grapevines, and of the nature of these newly recognized, xylem-inhabiting bacteria, promises better management techniques for the future.

#### Mildew

Blessed with a consistently dry, humidity-free growing season, the typical western vineyard climate has always been unfavorable to downy mildew of the vine (*Plasmopara viticola*), unlike many other temperate grape-producing regions of the world, and this devastating water mold has never been seen on vinifera grapes in California. However, radical man-made changes in vineyard



The use of pure yeasts as starter cultures, recommended by U. C. enologists, was a major step for California vintners. Ralph Kunkee sterilizes loop before inoculating a growing medium with one of the many yeasts in the University collection.

able for this: use of diethylpyrocarbonate (now banned); addition of sorbic acid; or sterile filtration followed by sterile bottling. Research at the University was carried out on each of these methods to make them practical for the California wine industry. For the sterile process, a method for quick detection of faulty procedures was developed.

## Summary

The results of University research in wine microbiology during the past century have led to the widespread application in the wine industry of several important technologies: submerged culture flor sherry production; the use of pure yeast starter cultures in active dry form; the elimination of spoilage in dessert wines by Lactobacillus trichodes; controlled alcoholic fermentations including refrigeration; the ability to induce or inhibit malo-lactic fermentations; and quality control procedures for monitoring aseptic bottling of table wine containing residual sugar. These authors look forward to continued significant contributions in wine microbiology at the University of California during the next 100 years.

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# Milestones in grape pathology

microclimate, coupled with some unusual weather patterns, might still create a suitable environment for the disease, and growers need to keep it in mind. A strain of downy mildrew occasionally occurs on the state's wild grape, *Vitis californica*.

California vineyards still have their mildew—the powdery form (*Uncinula necator*)—first recognized in a northern grape district as early as 1859. Pierce reported on its "rapid spread" in the 1860s to southern California vineyards. Oidium, as it is sometimes called (a generic term for all the surface mildews), needs little moisture; in fact, it is favored by a warm, dry climate. Frederic Bioletti once wrote that (powdery) mildew "is the only serious fungus disease of the vine in California." Some 65 years later, his statement is still close to the mark.

Other important fungal diseases of grapevines have since been recognized, but powdery mildew is the most widespread and economically significant. Few serious growers neglect routine mildew treatments, and table grapes might receive as many as 12 treatments each season.

Sulfur has been a safe, inexpensive, and reliable mildewcide on vinifera grapes since it was first used successfully in California in 1861. New chemicals have been regularly tested, and some have shown great promise, only to prove unacceptable because of cost or health hazards. Sulfur is still the mainstay of any grape mildew control program in California—either as a finely micronized wettable powder, or more commonly as a dust. Agricultural dusting is becoming less acceptable in many areas, however, and there are also increasing concerns about the energy required for a regular sulfur dust program. University researchers are hoping to improve forecasting of critical mildew periods, permitting more timely and less frequent chemical applications.

A widespread vineyard disease in low-rainfall districts appeared early in the state's grape-growing history. Known variously as measles, black measles, Spanish measles, and black mildew, it is probably the same disorder that Europeans refer to as esca or apoplexy. Over the years it has been ascribed to attacks of parasitic organisms, especially fungi entering via pruning wounds. Although there is general agreement on the symptoms of leaf burn and fruit

William J. Moller

Striking autumn vineyard colors are caused by leafroll virus, the most common grape virus disease in California. Such colors are less often seen as growers use virus-free stock.

