RESEARCH UPDATE

Grapevine trunk diseases in California

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Grapevine trunk diseases are responsible for significant economic losses to the wine industry worldwide. Symptoms of these diseases include dead spurs, arms, and cordons and eventual vine death due to canker formation in the vascular tissue. In Eutypa dieback, deformed leaves and shoots occur as the pathogen invades spur positions. As cankers develop, yield reductions occur due to the loss of productive wood. The impact of grapevine wood diseases can be significant in older vineyards, and usually becomes more severe as vineyards become older.

Eutypa dieback, caused by *Eutypa lata* was originally thought to be responsible for most canker development in California vineyards. However, recent findings have highlighted the importance of other fungi involved in the death and decline of grapevines in California. In this regard,

Figure I: Fruiting bodies (perithecia) of *E. lata* on a grapevine previously grafted for variety change. The large pruning wound favored formation of *E. lata* perithecia on the dead trunk of the old variety.

Botryosphaeria species have also been recovered from cankers, and were determined to be the main cause of canker diseases in some California vineyards.

Recent research has also indicated the occurrence of several new fungal trunk disease pathogens of grapevine belonging to the family Diatrypaceae (the same family as *Eutypa*). These include *Eutypa leptoplaca*, *Cryptovalsa ampelina*, *Diatrype* species, and *Diatrypella* species. We will present current information on the epidemiology and control strategies of fungal organisms responsible for grapevine spur, cordon, and trunk dieback in California.

Figure II: Black stroma bearing perithecia (fruiting bodies) of *E. leptoplaca* on dead trunk of big leaf maple collected in St. Helena, Napa County, CA.

Eutypa dieback

Eutypa dieback was responsible for a loss in net income for California wine grapes estimated to be over $260 million in 1999. Many growers consider Eutypa to be the most significant disease of grapevines.

Typical symptoms of *E. lata* include formation of a wedge-shaped canker and stunted shoots with cupped, tattered, chlorotic, and necrotic leaves that are best seen in spring time. Foliar symptoms are due to toxins produced by *E. lata.* Differences in susceptibility of grapevine cultivars to infection have been reported, although no cultivars are immune. Cankers develop downward at a faster rate than toward the end of cordons and also increase in diameter over time. Extended infection of grapevines by *E. lata* leads to vine death.

Eutypa lata spreads to new pruning wounds by wind-driven and water-splashed ascospores released during rain events. Ascospores develop inside perithecia (fungal fruiting bodies) that form when the fungus enters its sexual stage. The sexual stage develops on dead wood, where masses of perithecia are produced in a black substrate referred to as stroma (Figure II). The sexual stage develops in regions that receive over 16 inches of rain. It is common to find stroma and perithecia on old grapevines and other types of wood in the North Coast and Delta production areas.

Ascospores infect grapevines through fresh pruning wounds during the dormant season. They germinate, invade xylem vessels, weaken the plant by producing toxins and cause wood decay by excreting cell wall degrading enzymes.

Figure IV: Typical wedge shape canker formed by *Botryosphaeria* in the cordon of grapevine.

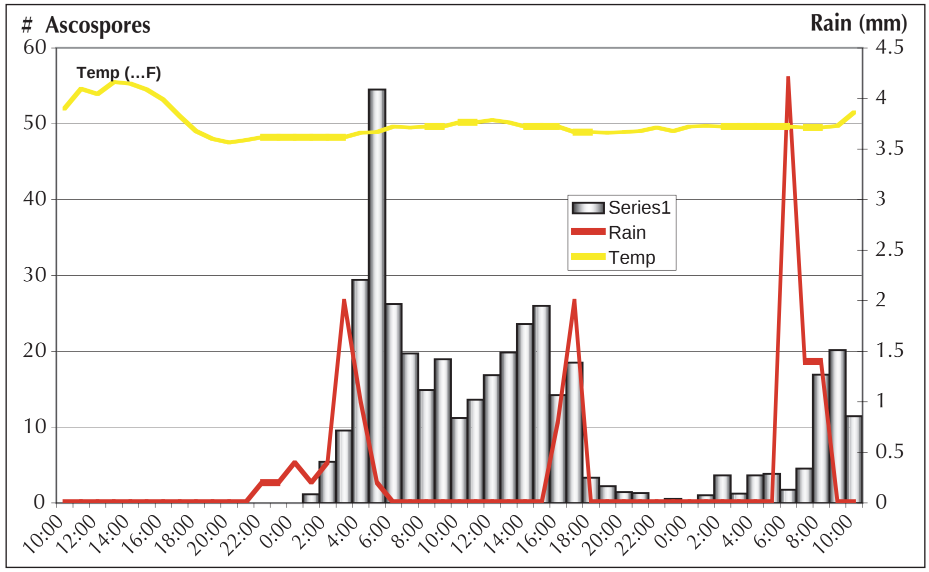

Figure III: Ascospore release curve of *E. lata* during a period of rainfall recorded in December 2001 in Davis using a Burkard spore trap placed around dead grapevine trunk bearing perithecia.

Eutypa lata also produces asexual spores called conidia. These are formed inside pycnidia (another type of fruiting body) that develop on wood, but these conidial spores do not play a role in disease epidemiology.

In California, ascospore discharge of *E. lata* occurs from the first rain of the early fall until the last rains of the spring (Figure III). Ascospore discharge decreases significantly in late February and remains low to nil by early March (Figure IX).

However, ascospore release may occur during rains in March and April if they are preceded by several weeks of no rain and sunny, warm weather. Such releases may occur because perithecia are able to recover in productivity during the dry period, or because spores that would have been released in the winter months are released in the spring simply because they were not released in the winter. This scenario more often occurs in years when there is little rainfall during the winter months.

Ascospore release from individual perithecia may occur continuously for approximately 24 hours during periods of rainfall, starting a few hours after the onset of a rain (Figure IX).

Over 80 plant species around the world have been reported to be potential hosts for *E. lata*. In California, many of these species were found to be infected with *Eutypa lata* and bore perithecia producing ascospores in the vicinity of vineyards. We now know that these species serve as natural reservoirs of *E. lata* inoculum.

In the 1970s, grapevine, apricot, and *Ceanothus* were found to be natural hosts of *E. lata*. More recently we have identified kiwifruit, blueberry, and cherry to be hosts in California and they were shown to bear the fruiting bodies (perithecia) of *E. lata.*

Additionally, a recent survey identified almond, crab apple, and pear trees as new fruit crop hosts for *E. lata*, and showed the presence of

Figure V: Mature *Botryosphaeria obtusa* conidia.

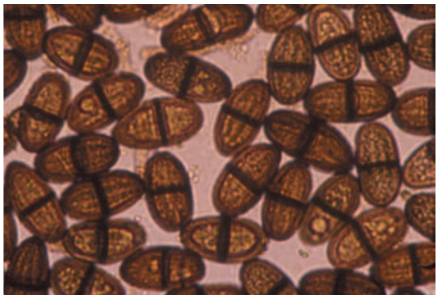

Figure VI: Mature *Botryosphaeria theobromae* conidia.

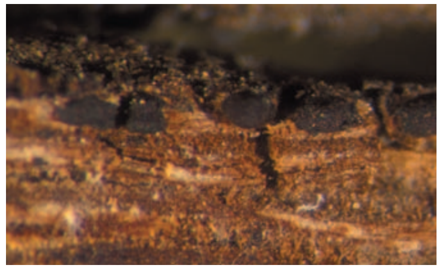

Figure VII: *Botryosphaeria obtusa pycnidia* found under the bark on the arm of an infected grapevine.

Figure VIII: *Botryodiplodia theobromae pycnidia* found on old grapevine pruning wood left on the vineyard.

perithecia of *E. lata* on several other new hosts in California including California buckeye, big leaf maple, willows, and oleander (Figure X).

Perithecia of *E. lata* were found to be particularly well established on dead branches of various willow species occurring along natural creeks and irrigation waterways.

It appears likely that the flora surrounding vineyards is potentially a key factor in disease epidemiology and surely acts as an inoculum reservoir. Sanitation of the dead wood of potential hosts of *E. lata* in areas surrounding vineyards is advised in order to decrease the inoculum level. Sanitation can be accomplished by removing the infected tissues on plants that show disease. Generally speaking, this disease can be identified on other hosts by the black stroma that is produced (Figure II). By removing the stroma-infected wood and burning it, the number of spores released in winter can be reduced.

Surveys inside vineyards and apricot and cherry orchards have revealed an abundance of inoculum in plantings of approximately 20 years and older. Only a few perithecia have been found in almond orchards.

Perithecia of *E. lata* were found to be prevalent in vineyards or surrounds in the counties of Napa, Sonoma, Yolo, Sacramento, Contra Costa, San Benito, El Dorado, Mendocino, San Joaquin, Stanislaus, and Merced. Perithecia of *E. lata* were not found in Madera, Fresno, Kings, Tulare, and Kern counties.

Large amounts of viable inoculum were found in several old vineyards near Healdsburg, Sonoma County. Perithecia were particularly well-developed on vines that had been previously top-worked for variety change. Stroma on those vines had developed on old wood below the grafting wound down to the union with the rootstock (Figure I).

While *E. lata* appears to be the primary Eutypa species involved in dieback in California, we have identified a second Eutypa species that can also cause dieback in grapevines (Figure XIII). This species is *E. leptoplaca*, a slower growing fungus in culture but with the capability to do the same kind of damage as *E. lata*. This new fungus occurs in the North Coast but seems to be more limited in its distribution.

It is our opinion after this study that even though *E. lata* and *E. leptoplaca* ascospores can travel over considerable distances and cause disease, Eutypa dieback is primarily a disease of local origin, developing in the vicinity where the ascospores are released.

Control

Disease management should include removal of the dead parts of grapevines and other host plants because of the potential risk of increased inoculum. We currently have no proof that sanitation will reduce the disease level in vineyards, but it seems appropriate if local production of inoculum is considered to be a key to disease development.

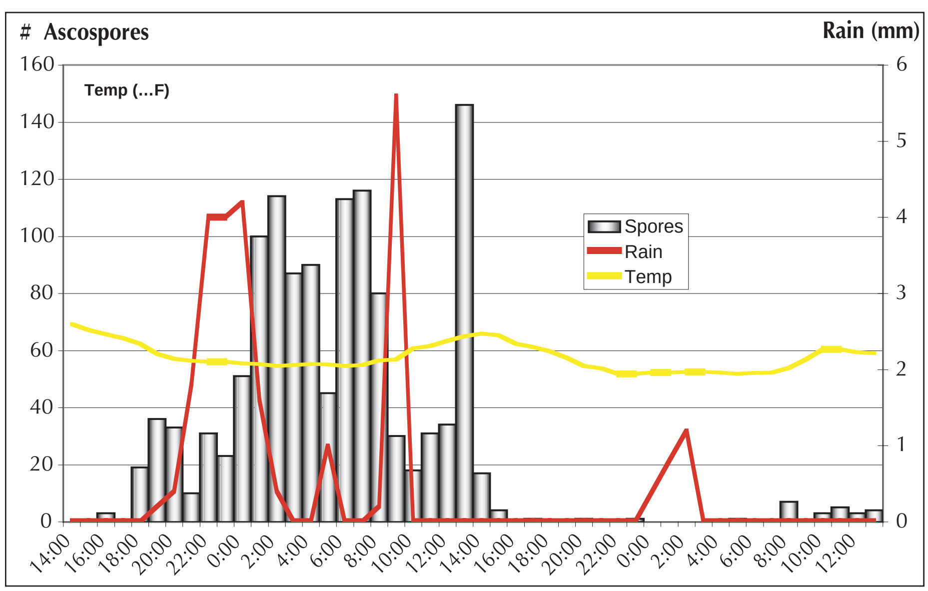

Figure IX: Ascospore release of *E. lata* recorded on the 12th, 13th and 14th of April 2000 in a vineyard in Healdsburg, Sonoma County, CA, with rainfall and temperature data. Ascospore release occurred for 24 hours during the rainfall period.

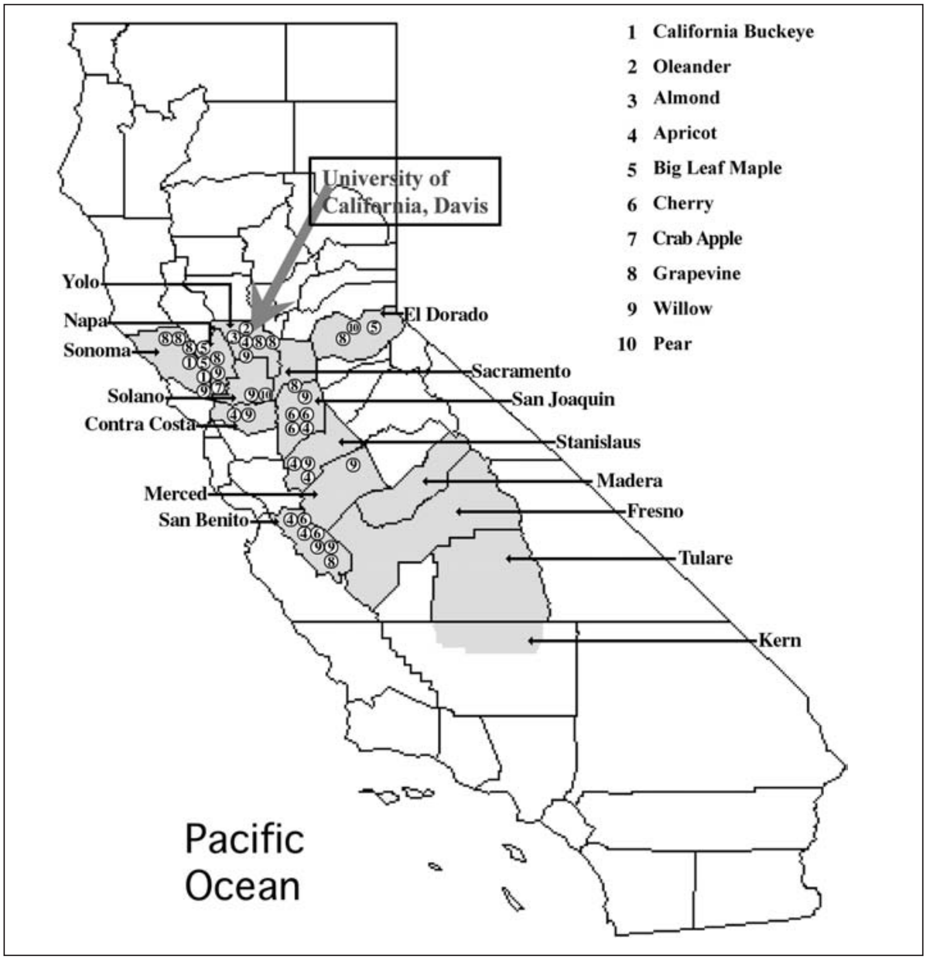

Figure X: Geographical area and new hosts of the pathogen *E. lata* in California. Survey was conducted in 2002. Areas represented in gray correspond to the areas that were surveyed.

Grapevine pruning wounds can be susceptible to infection by *E. lata* for as long as seven weeks, but the length of this period varies with the time of pruning, size of the wound, and age of the wood pruned. Wound susceptibility to *E. lata* declined faster under higher (70º to 90ºF) temperatures.

Wound healing occurs by deposition of polymerized phenolic compounds in the opened wood vessels, and concomitantly, natural establishment of a microbial population on the wound surface provide a natural "bio-barrier" to infection. These natural organisms (epiphytes), including *Cladosporium herbarum* and *Fusarium lateritium*, grow over the surface of a pruning wound and prevent infection by *E. lata* spores.

In a previous study, more than 1300 species of fungi and bacteria were found to colonize grapevine wounds in spring conditions.

Wound healing and colonization by epiphytic fungi and bacteria have been shown to occur more readily in the warmer spring months. Ascospore release, germination, and infection are also decreased during this time. Therefore, significant wound protection can be readily achieved simply by late pruning of vineyards.

Our work, and other research (Moller and Kasimatis), has shown that pruning wounds made in March heal more rapidly when compared to mid-winter. In our trials, late season pruning wounds that were artificially inoculated showed only approximately 10% of the infection rate of pruning wounds inoculated in mid-winter.

However, the large acreage of individual vineyards in California makes late pruning impractical for many growers who need to schedule their labor force throughout the winter months. One way to allow for more efficient late season pruning is to double-prune a vineyard. This involves going through the vineyard twice during the pruning season.

The first "pre-pruning" pass is made early in the season at which time the canes are simply trimmed back to a set length, usually 12 to 18 inches. This step can be performed mechanically or by a hand crew. "Final" pruning to spurs would take place late in the season (March) when the chances for infection are reduced.

Table I. Field trials in evaluating control of *E. lata* in San Joaquin and Yolo Counties in 2001 to 2002 and in Napa and San Joaquin Counties in 2002 to 2003. Pruning wounds were inoculated with 1000 ascopores of *E. lata* one and 10 days after application of test material.

	First timing of inoculation		Second timing of inoculation	
Treatments	Mean percent infection	Mean percent disease control	Mean percent infection	Mean percent disease control
Non-inoculated control	3	—	3	—
Inoculated control	64	—	32	—
Boron-free paste	61	4.7	19	40.6
C. herbarum	32	50	17	46.9
Boric acid	2	96.9	9	71.9
Bioshield	4	93.8	7	78.1
Biopaste	1	98.4	2	93.8

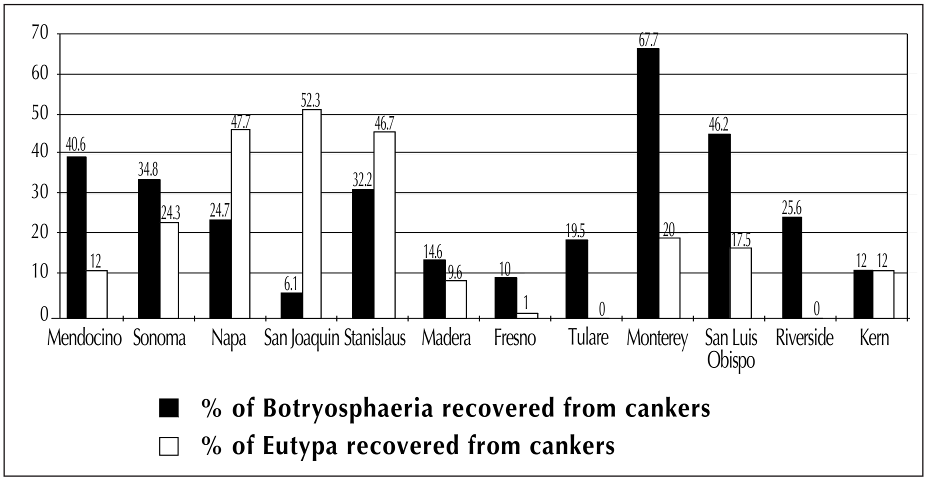

Figure XI: Relative occurrence of *Eutypa spp.* and *Botryosphaeria spp.* in California grapevine production regions.

By eliminating most of the brush early in the winter, final pruning should go more quickly, thereby allowing the final cuts to be made late in the season. Double-pruning is not appropriate for cane-pruned vineyards where long canes are retained each year.

Double-pruning creates an opportunity for wood pathogens such as *E. lata* to colonize wounds made early in the dormant season during pre-pruning. However, these infections will be eliminated during the final pruning step. We inoculated pre-pruning cuts throughout the winter months and showed that by March, *E. lata* had not grown downward more than four to five cm, which was well above the point where final pruning cuts were made.

Late pruning, either in a single step or as the final pass in double-pruned vineyards, is not a guarantee that Eutypa infections will not occur. Late spring rains can trigger ascospore releases and increase the risk of infection of newly pruned vineyards. However, late pruning certainly reduces the overall chances for infection and is a standard recommendation for disease control.

Disease management was historically achieved by treating pruning wounds with fungicides. Benlate® (DuPont de Nemours & Co., U.S.) was registered for *E. lata* for 30 years, and field trials showed good efficacy in preventing Eutypa dieback. However, long-term protection of pruning wounds was not achievable because the product did not persist in woody tissue for a sufficient time period. Therefore, multiple applications of Benlate would have been required to provide protection until pruning wounds were completely healed.

Benlate was withdrawn from the market in 2001 leaving growers without an efficacious chemical treatment to control *E. lata*. Topsin M was registered in 2003 under section 18 (emergency exemption) in California, but this product also belongs to the same class of fungicides as Benlate, a benzimidazole, and therefore has the same drawback (short-term protection) as Benlate.

Biocontrol agents have been tested as an alternative method for control of *E. lata. Bacillus subtilis*, *Fusarium lateritium*, and *Cladosporium herbarum* all showed some potential activity in limiting the establishment of the pathogen.

However, unlike chemical applications, which have an immediate protective effect, maximum protection from biocontrol agents requires colonization of the surface of the wound. Thus, there is a window of susceptibility after treatment, until the biocontrol agent is established well enough to prevent development of *E. lata* in the wounded tissue.

Biocontrols tested as alternatives to fungicides showed mixed success, but both *F. lateritium* and *C. herbarum* worked well when they were applied two to three weeks before infection occurred.

Boron was tested as an alternative control method because this element was proven to be effective at controlling other wood decay fungi. It is also used by growers as a fertilizer spray to improve fruit set. The application of boron as a fungicide may not yet be legal to control Eutypa dieback of grapevines, however, boron can be applied to correct nutritional problems.

We evaluated boric acid solutions and two boric acid-based products as pruning wound treatments. These included biopaste (5% boric acid [wt/wt] in a polyvinyl paste) and bioshield (5% liquid boric acid [wt/vol] plus a spore suspension of *Cladosporium herbarum)*.

Boric acid reduced ascospore germination and mycelial growth of *E. lata* under controlled conditions in the laboratory, and the boron-based products yielded excellent disease control on artificially inoculated grapevines in both field trials and lab studies in comparison to *C. herbarum* and boron-free paste treatments (Table I).

However, in one observation, the bud located at the first node below the pruning wound failed to push following treatment with liquid boric acid. This effect was also reported in peach and nectarine orchards sprayed with toxic amounts of boron-based fertilizers.

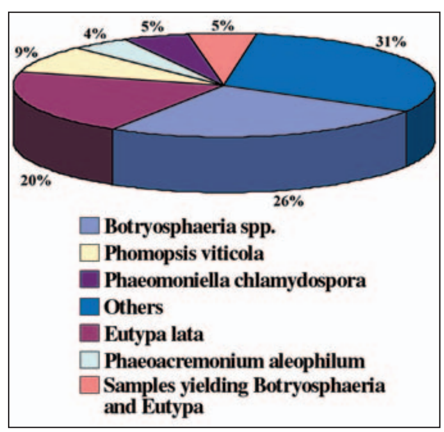

Figure XII: Comparison between percentages of cankers infected by *Botryosphaeria* spp. and other fungi in relation with grapevine dieback in California grapevines.

Mechanisms involved in bud failure are not understood, and the economic impact has not been assessed. However, we do not recommend liquid boric acid for Eutypa dieback control.

Treatment of pruning wounds with boron-based products offers an effective, economical, and environmentally-safe management strategy to control Eutypa dieback in vineyards. However, formulations have to be optimized in order to increase control over longer time periods on the surface of pruning wounds, and to limit possible effects on bud failure of grapevines.

We believe strongly that if boron is used, it should be put in a paste so that it stays where it is placed.

In earlier studies, soaps also provided good protection of pruning wounds against *E. lata*. However, most soaps, including dishwashing detergents, were phytotoxic. One laundry detergent, Dreft, not only provided excellent control of *E. lata,* but has never shown any phytotoxicity. This detergent, when used at 30% aqueous suspension (wt/vol), provided excellent disease control.

Further fungicide testing is underway, and we are trying to convince the chemical industry that Eutypa control products are worth registering for California viticulture.

BOTRYOSPHAERIA canker diseases

"*Botryosphaeria* canker disease" of grapevine is a wood disease caused by as many as four different *Botryosphaeria* species in California. Although it is well accepted that some *Botryosphaeria* species have been shown to be the causal agent of canker diseases of various crops and woody plants in California, the importance of these fungi as pathogens of grapevines has been largely ignored throughout the state.

Various species of *Botryosphaeria*, causing different symptoms, have been reported in the last decade as pathogens on grapevines in the U.S., South Africa, Australia, France, Italy, Portugal, Egypt, India, Mexico, Chile, and Brazil. In California, wedge-shaped cankers caused by *Botryosphaeria* can be found on vines 10 years old and older, especially where large pruning wounds have been made in retraining vines.

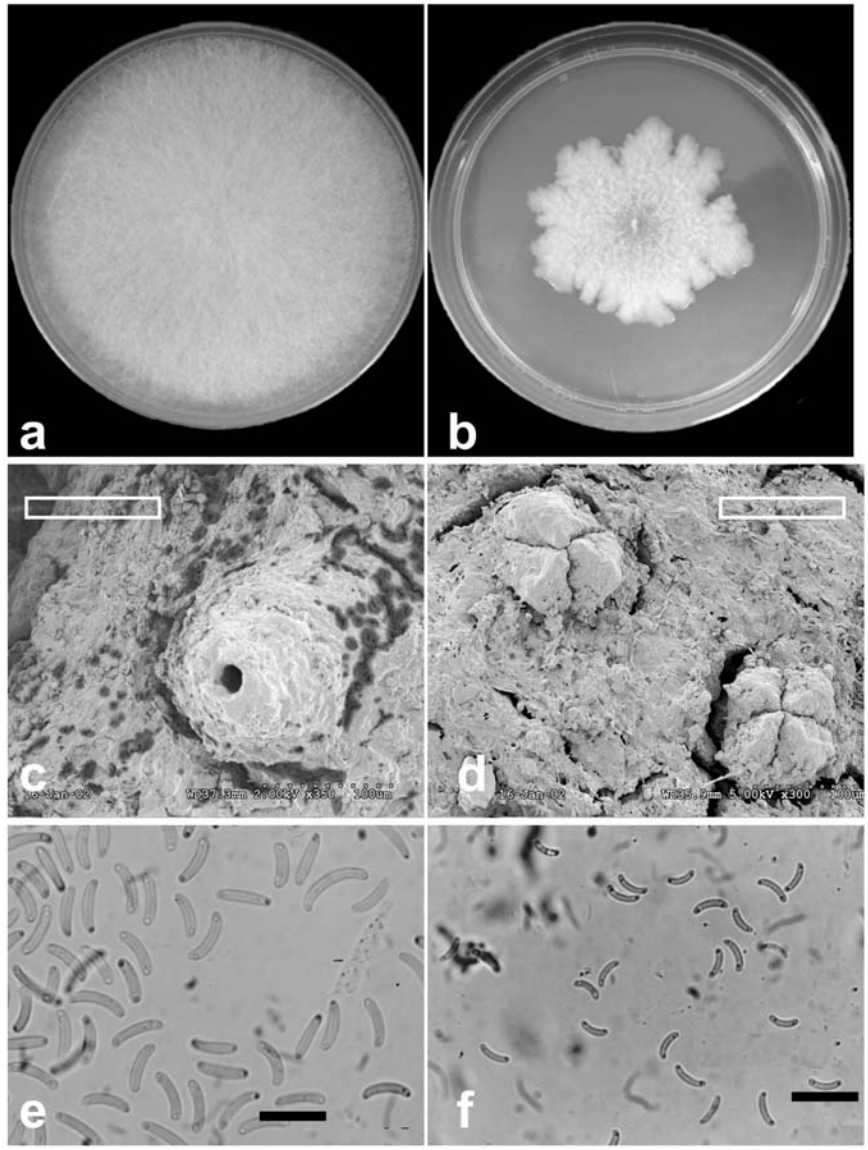

Figure XIII: Fungal colonies of *Eutypa lata* (a) and *E. leptoplaca* (b) growing on PDA-tet dishes incubated at 24ºC under intermittent fluorescent lighting (12 h) for 22 d. Scanning electron microscope images of perithecial ostiola showing the roundish ostiole of *E. lata* (c) and the 3 to 4 sulcate ostioles of *E. leptoplaca* (d) (Bars = 100µm). Compound microscope images of the ascospores of *E. lata* (e) and smaller ascopores of *E. leptoplaca* (f) at same magnification (Bars = 10 µm).

For several years, *Botryosphaeria rhodina* has been known to cause wedge-shaped canker symptoms in California. This species was previously known as *Botryodiplodia theobromae*, and the disease it causes was referred to as "Bot canker."

Fungi often produce both an asexual (imperfect) stage and a sexual (perfect) stage, and they may have different names. *Botrysphaeria rhodina* is the name for the sexual stage of this fungus; *Botryodiplodia theobromae* is the name of the asexual stage.

We prefer to use the name *Botryosphaeria* instead of *Botryodiplodia*. The disease is still known as Bot Canker.

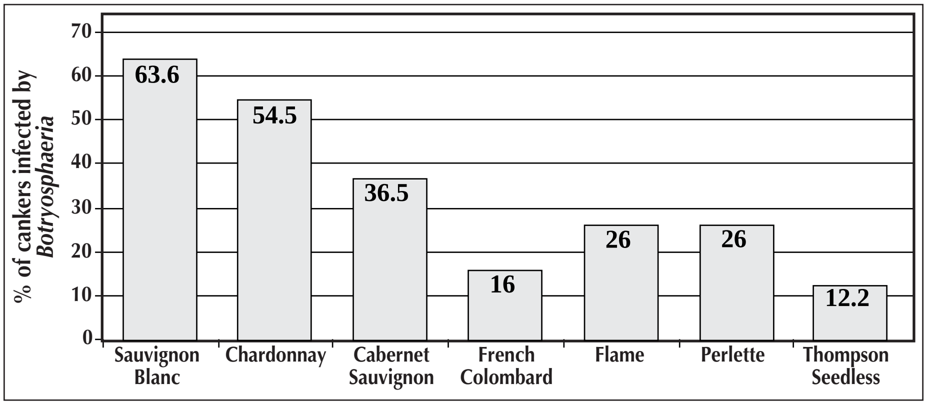

Figure XIV: % of cankers infected by *Botryosphaeria* in wine and table grape varieties in California.

The fungus is now considered to be an endemic species in many vineyards in warm and hot climate areas in California.

Typical symptoms caused by *Botryosphaeria* on grapevines in California are wedge-shaped cankers in the trunk and cordons (Figure IV) and dead spur positions. No foliar symptoms associated with *Botryosphaeria*-induced canker diseases have been observed in California grapevines. This is in contrast to other areas in the world where foliar symptoms have been observed on grapevines infected by different species of *Botryosphaeria* (*B. stevensii, B. obtusa* and *B. dothidea*).

The wedge-shape cankers caused by *Botryosphaeria* are visually indistinguishable from those formed by *Eutypa lata* or *E. leptoplaca*. All three fungi can cause cankers and can be detected in the vine at the same time. The best distinguishing characteristic is the presence of cankers and the absence of the stunted or chlorotic spring growth which is typical of infections by *Eutypa lata*.

Botryosphaeria spp. are wound pathogens entering the vine through fresh pruning wounds. Large numbers of *Botryosphaeria* conidia are exuded from black fruiting bodies (pycnidia) found on diseased vine parts, under the bark of cordons, trunks, and spurs (Figure VII) or on the residual pruning wood left in the vineyards.

In addition, we also know that many of these species cause disease on different hosts around the vineyards. The formation of numerous fruiting bodies provides an excellent source of spores for further infections in the vineyard. Conidia may be easily distributed over the vineyard due to wind, or they may be waterborne in splashed drops from rain or sprinkler irrigation, but very little information is available concerning the *Botryosphaeria* disease cycle.

Like *Eutypa lata* and *E. leptoplaca*, the canker formed by *Botryosphaeria spp.* grows more rapidly basipetaly (or toward the root from the point of infection). The canker develops for several years in the trunks and cordons depending on where the infection was located. Death of the infected vine part occurs when the last live wedge of tissue is killed by the growth of the fungus.

Eutypa dieback and "Bot canker disease" decrease the life of the vineyard, reduce yields and increase production costs due to the application of control treatments, cultural practices to prevent infections, pruning out

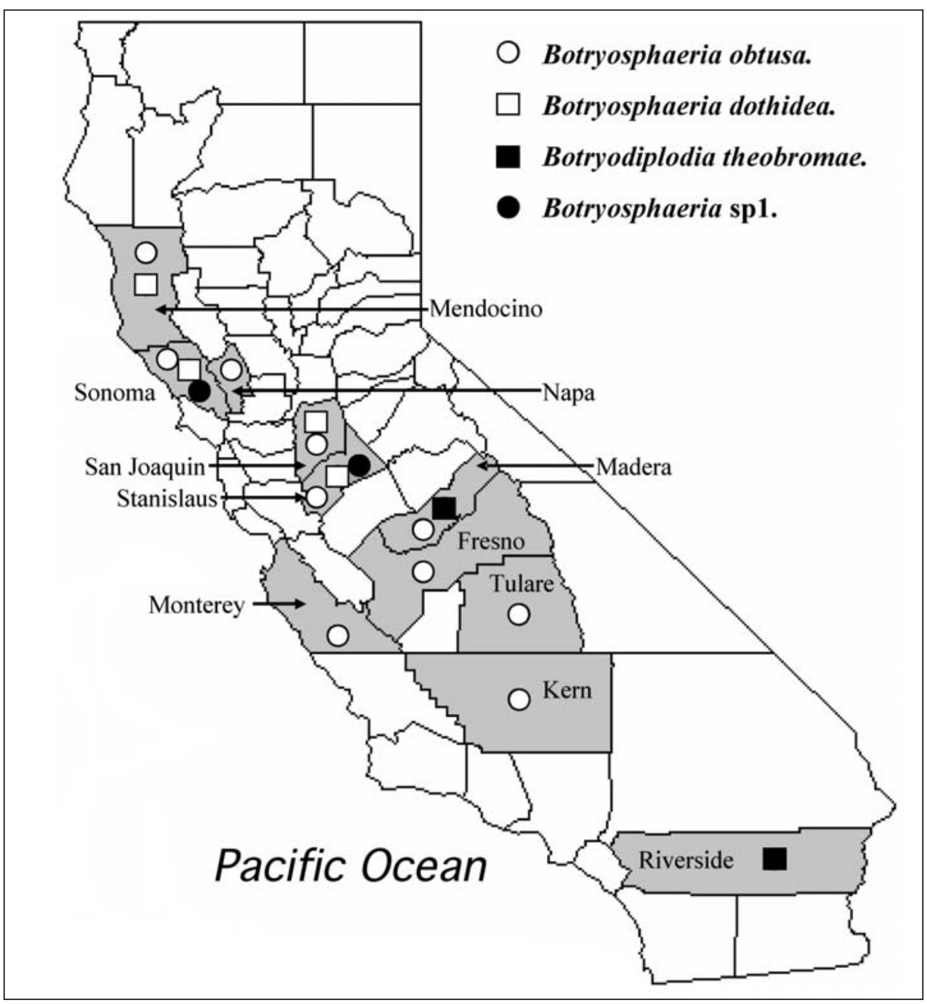

Figure XV: Geographical distribution of *Botryosphaeria* species found in the grapevine production areas in California during 2003 and 2004.

of diseased tissue, and training new cordons and spur positions to replace those killed by the disease.

Field survey of Botryosphaeria

In a recent field survey (2003–2004), over 1,100 samples showing the typical wedge-shaped cankers were collected from 110 vineyards in 12 counties (Mendocino, Napa, Sonoma, Monterey, San Luis Obispo, Madera, Stanislaus, San Joaquin, Fresno, Kern, Tulare, and Riverside County). *Botryosphaeria* species were found in every grape growing area surveyed (Figure XV), showing that *Botryosphaeria* species are prevalent in cankered cordons and spurs in grapevines in California.

Botryosphaeria was the main fungus recovered from cankers in five counties: Mendocino, Sonoma, Monterey, San Luis Obispo, and Riverside. *Botryosphaeria* was also isolated in a higher percentage than Eutypa in Madera, Fresno, and Tulare counties (Figure XI). *Eutypa lata* was the main fungus isolated from cankers in Napa, San Joaquin, and Stanislaus County. In Mendocino, Sonoma, Napa, Monterey, San Luis Obispo, Stanislaus, and San Joaquin counties both fungi were occasionally isolated from the same canker.

It is important to note that *Phomopsis viticola* was the principal fungus found in cankers from Fresno and Tulare counties. We have yet to initiate a project there to evaluate the significance of this finding but plan to do so.

The fungi that cause Esca or black measles (*Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum)* were also isolated in a low percentage in this study. These pathogens produce different types of internal symptoms and were not a target of this work, but they reside in the same vascular tissue as the canker pathogens and thus will be isolated from time to time.

Four different *Botryosphaeria* species are associated with the wedge-shape canker symptom in California; *Botryosphaeria obtusa*, *B. rhodina (Botryodiplodia theobromae*), *Botryosphaeria dothidea* and an as yet unidentified *Botryosphaeria* species called *Botryosphaeria* sp1 (Figure XV).

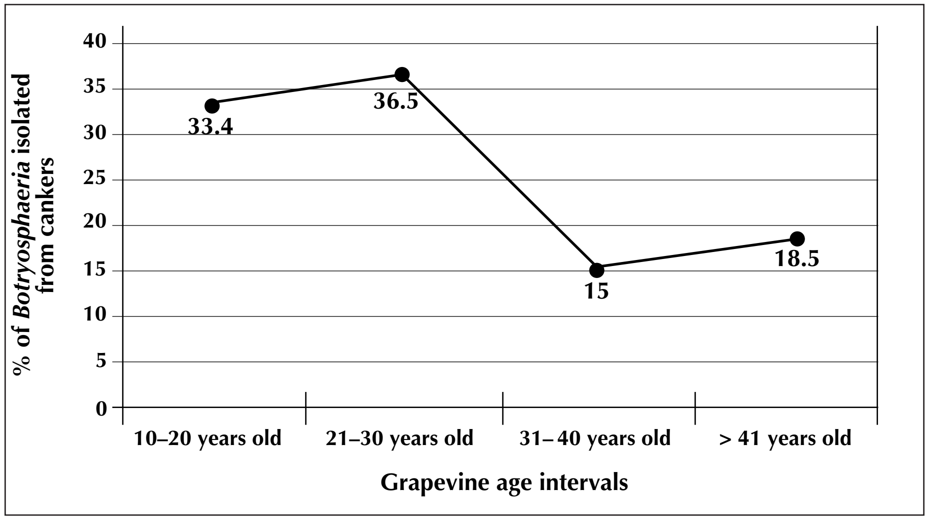

Figure XVI: Effect of vineyard age on the occurrence and incidence of *Botryosphaeria spp.*

It is also important to emphasize that only *Botryosphaeria rhodina (Botryodiplodia theobromea)* was recovered from cankers in the desert area of Coachella Valley. No other *Botryosphaeria* species or other wood invading, canker-causing fungi were isolated from the wedge shape cankers in this region. Only *Botryosphaeria rhodina* has been proven to cause a dieback disease causing a wedge-shape canker. The precise role of the other species isolated from cankers is still not clearly understood.

In the 2003–2004 survey, (Figure XIV), *Botryosphaeria* was isolated most often from Sauvignon Blanc (63.6% recovery from cankers tested) followed by Chardonnay (54.5%). Cabernet Sauvignon had the most cankers with *Eutypa* (57.7%).

Botryosphaeria was isolated in the same percentage from cankers of Perlette and Flame seedless (26%). Fewer cankers with *Botryosphaeria* were found in Thompson seedless (12.2%). Thompson Seedless appears highly susceptible to *Phomopsis viticola* and the pathogen was isolated from 29% of Thompson Seedless cankers tested.

Botryosphaeria was found in vineyards of all ages sampled (Figure XVI), but was isolated most frequently from cankers in 21 to 30-year-old grapevines (36.5%), followed by those grapevines that were 10 to 20 years old (33.4%).

The main fungi isolated from all wedge-shaped cankers collected in California have been *Botryosphaeria* (25.5%), followed by Eutypa (20%), *Phomopsis viticola* (8.7%), *Phaeomoniella chlamydospora* (5.2%) and *Phaeoacremonium aleophilum* (4.3%) (Figure XII). However, the latter two pathogens do not cause cankers and were picked up in these studies as hitchhikers on wood showing symptoms of other cankers. In a parallel study, *Eutypa leptoplaca* was also isolated from vines in the North Coast but was not part of this survey.

Other wood decay fungi

Besides *Eutypa lata* and *Botryosphaeria* species, several other fungi were recovered from cankers of grapevines. These include *Phomopsis viticola*, *Phaeomoniella chlamydospora,* and *Phaeoacremonium aleophilum.* Others, such as *Diatrypella* sp., *Diatrype* sp., *Crytovalsa ampelina* and *E. leptoplaca* were also isolated from the margins of infected tissue.

Recently, another Eutypa species (*E. leptoplaca*) and *Cryptovalsa ampelina* were found to be pathogenic on grapevines as were species of *Diatrypella* and *Diatrype*. These fungal species all belong to the same family as *E. lata* and closely resemble *E. lata* in their spore shape, size, and by their appearance in culture (Figure XIII). These fungi also have been found commonly on various host plants in the vicinity of vineyards.

Eutypa lata and the other diatrypaceous fungi have the same morphological characteristics in culture including spore shape and color. It is apparent that over the last several years, *E. lata* was not the only pathogen being isolated from cankers. Not until this study did we realize that more than one pathogen was capable of causing the same type of disease.

Given these results, it is evident that *Eutypa lata* is not the only cause of grapevine canker diseases in California vineyards. More studies should be conducted to elucidate the impact of the different species of *Botryosphaeria*, and other fungi detected in cankers. The virulence of many of these species remains to be determined.

These recent discoveries have led to the conclusion that grapevine trunk diseases are more complicated than initially thought, and that a complex of fungi may be involved. Development of information regarding the biology, epidemiology and control of each of these fungi and diseases is underway. Nevertheless, we know that vineyard sanitation through the removal of infected parts of vines is highly advised, and where possible, sanitation of surrounding areas where other potential hosts of these pathogenic fungi reside. ■

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