The Role of Cleistothecia in Grapevine Powdery Mildew in California.

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The role of cleistothecia in powdery mildew of grape was investigated in California. A regional survey of the grape growing regions indicated that cleistothecia occur abundantly and are viable in all of the regions surveyed. However, cleistothecia which form in vineyards in the center of the San Joaquin Valley demonstrate lower viability than those which form in vineyards in the extreme southern or northern San Joaquin Valley or Coastal Production regions. Cleistothecia are washed during fall rains to positions below the point of origin. Trellis systems which supported the majority of the canopy directly over the cordon resulted in larger populations of cleistothecia captured in the bark of the cordon. Cleistothecia which washed to the soil were not viable, spring wet periods resulted in ascospore release from cleistothecia residing and resulting on cordons in disease expression 7-10 days later. Dormant lime sulfur and micronized sulfur

applied at 2 to 4 inch shoot growth controlled early season ascospore infection.

Additional key words: *Vitis vinifera*, *Oidium tuckeri*, *Uncinula necator*, plant disease, fungicide control.

Grapevine powdery mildew caused *by Uncinula necator* (Schw.) Burr., is a major disease in California (2). *U. necator* has been shown to survive the winter in California as mycelium in dormant buds (Sall 1982). Repeated observations of uniform epidemics of powdery mildew in the absence of observable bud infections suggested that another source of primary inoculum may be present. Pearson and Gadoury demonstrated that cleistothecia are the sole source of primary inoculum in New York vineyards (3). Cleistothecia have long been observed in California but the role of the sexual state in the disease cycle was not known (1).

This study was undertaken to quantify presence of cleistothecia in four grape production regions in California, determine their viability, determine overwintering locations on the vine and mechanisms of transport to these locations, and test the effect of early season and dormant application of two sulfur fungicides as potential control measures.

Materials and Methods

Survey. A survey of the general viability of ascospores in various regions was conducted. Exfoliating bark or leaves bearing cleistothecia were removed from grapevines in 28 vineyards, representing 4 regions: the San Joaquin Valley, the North Coast, the Central Coast, and Temecala, Calif., were transported to the laboratory where number and viability of cleistothecia was determined. Cleistothecia were collected from bark or leaves onto filter paper as described by Pearson and Gadoury (3).

Approximately 50 g of exfoliating bark was removed from 20 - 30 random selected grapevines in each vineyard tested. Samples were placed in 2L flasks with approximately 300 ml of tap water. Flasks were vigorously shaken by hand for 3 min., after which the water was passed successfully through 20, 40, and 170 mesh screens. Cleistothecia were then washed onto a 9 cm Whatman #1 filter paper held in a Buchner funnel. The filter paper was cut into 8 equal sections and each section was affixed to the top of a petri dish, misted with water and placed over 1% water agar (WA) or detached grape leaves held in double petri dish (4). Ascospore viability was measured as percent of ascospores released which germinated or as number of detached grape leaves infected.

In addition, six vineyards were retested at various times during the fall, winter and spring 1988 to 1989.

Location of cleistothecia. During the fall of 1989 and 1990, cleistothecia were trapped during rain events to determine the relative distribution of

cleistothecia on the vine. Funnel traps were made by folding Whatman #9 cm filter paper circles (Fisher Scientific, Pittsburgh, PA 15219-4785) into quarters and thumbtacking in place on the vine.

During the fall of 1990, Cleistothecia also were trapped during rain events. Funnels, as described above, were attached to grapevine cordons at three positions along the cordon. The three positions were next to the trunk, half-way along the cordon and on the outer tip of cordon. Funnels also were attached to wooden stakes placed in three positions at cordon height at 25 cm spacing from the plane formed by the trunk and the cordon: 1) midway between the cordon and drip line, 2) at the canopy drip line, and 3) outside the canopy. Each of the 12 positions were replicated three times per vineyard. The experiment was conducted in three T-bar trellis vineyards, two two-wire vertical vineyards, and two vertically shoot positioned vineyards. Funnels were collected after each rain until leaf fall. Cleistothecia were rinsed from funnels onto 9 cm Whatman #1 filter paper and total number counted using a binocular dissection microscope.

Cleistothecia were retrieved from soil in March from 2 vineyards in which hundreds of viable cleistothecia were trapped on cordons of Chardonnay grapevines. The top 2.5 cm of soil was collected from an area approximately 30 cm away from the cordon and 15 cm long using a soil spade. Soil was placed into plastic bags and returned to the lab in an ice chest with no ice ? g %, soil was placed in 2 L flasks with ? ml of tap water and shaken by hand for 2-3 minutes after which cleistothecia were

separated by pouring the solution through 20, 40, and 170 mesh soil screens. Cleistothecia were rinsed from the 170 mesh screen onto 9 cm Whatman #1 filter paper in a Buchner funnel. The filter paper containing cleistothecia were cut into 4 equal pieces and either placed over 1% water agar (WA) by affixing the paper to the inside of a petri dish lid or placed inside the top of a 30 cm X 7.5 cm plastic tube in which a disease free seedling, Carignane grapevine resided. Paper containing cleistothecia were misted with distilled water each 48 hours for approximately 1 month. After each misting, the petri dish lid was placed over new plates of WA and the old plates were stained with 1% acid Fuchoin and examined for released ascospores. Cleistothecia over plants maintained for 1 month. Cleistothecia were collected in March of 1991 from leaf litter under vines from Chardonnay vineyards located in the North Coast and Central Coast production avas. Fifty of litter was used to collect cleistothecia as previously described from bark.

Fungicide trials. Fungicide trials aimed at controlling ascospore primary inoculum were conducted in two Chardonnay vineyards in 1989, 1990, and 1991. Tests were established in Monterey and Santa Barbara Counties. Treatments included a dormant lime sulfur application (93.5 1/ha, Oithorix in 1989, Chevron. . . , Best Sulfur Products, Fresno, CA.) and a micronized sulfur application (4.5 kg/ha). Thiolux Sandoz, Chicago, ILL or Microthiol Special, Atochem, UAP, Fresno, CA. at 5 to 10 cm. shoot growth and an unsprayed control. Ten-vine treatment plots were replicated five times in a

randomized complete block design at each site. Handgun applications were made at 120 psi in 935 1 per ha using a Nifty-Fifty sprayer utilizing a D-5 full cone nozzle (Spray Systems, Inc., Wheaton, ILL., Rears Manufacturing Co., Eugene, OR.).

Assessment of disease incidence and severity in the field was made by randomly removing 20 basal leaves along the cordon from each of the center eight vines in each 10 vine replicate and recording the number of mildew colonies present. Disease incidence and severity was reported as the percentage of vines with mildew and the total number of mildew colonies per 160 leaves, respectively.

Results

Survey. The results of the regional survey indicated that cleistothecia form in all regions surveyed (Table 1). While there were apparent differences in viability, cleistothecia from all regions produced viable ascospores based on spore germination (Table 1). Ascospore viability was greatest in the North

Coast and Central Coast Production areas with average germination rates of 70 and 73 percent, respectively. Ascospore infection of grape leaves also was observed in these tests. The greatest number of infections occurred from ascospores produced in Central Coast Vineyards followed by North Coast San Joaquin Valley and Temecula, respectively.

Ascospore viability was lowest in the fall and increased during the winter and spring with maximum viability occurring in March (Table 2). Though ascospores were released from cleistothecia obtained in the fall from leaves, no germination was observed from any of the locations. Subsequent sampling from bark during February and March showed a rather dramatic increase in the percentage of spore germination between the February and March sample dates. The high of 20 percent germination in the Kern County Vineyard was attained on 2/16/89 and was not further sampled because budbreak had occurred. The end germination efficiency coincided with budbreak in the remaining vineyards.

Location of cleistothecia. Most cleistothecia washed off the leaves and into the funnels during the first few rains of the season. Funnels placed in the vineyard from November 21, 1989 to December 21, 1989 trapped an average cleistothecia per funnel of 82 on the cordon, 53 on the spur, and 56 on the croion. A few cleistothecia continued to be washed into each funnel with subsequent rains for the duration of the winter. During the peak displacement periods in the first rains, as many as 1400 cleistothecia were trapped in one funnel.

Cleistothecia were usually washed, during rainfall, to locations directly below the point of origin of the cleistothecia (Table 3). T-bar trellis systems have the majority of the canopy suspended away from the cordon and had only 11 percent of cleistothecia trapped on the cordon. Whereas 36, 40, and 5 percent of cleistothecia were trapped below the middle cordon, dripline and outside the drip line, respectively. In the case of the 2-wire vertical trellis systems, though most of the canopy is not directly above the cordon, the funnels attached to the cordon collected 25 percent of the total cleistothecia while 75 percent were caught in funnels placed away from the cordon.

Cleistothecial trapping in shoot positioned canopies resulted in 41 percent being caught in funnels attached to the cordons while 59 percent were washed into funnels placed in positions which would have allowed them to fall to the ground.

Retrieved cleistothecia from soil did not release ascospores when placed over 1% water agar and did not result in disease when placed over seedling Carignance plants.

Cleistothecia collected from leaves of grapevine at monthly intervals beginning in July and ending in October of 1991 released viable ascospores at each sample date. A high percentage of released ascospores germinated and produced appressoria on 1% WA.

Cleistothecia removed from vineyard leaf litter in February 1991 released viable ascospores.

Fungicide Trials. Trials conducted to observe the effects of control strategies aimed at cleistothecia as sources of primary inoculum showed that both micronized DF sulfurs at 4.5 Kg/ha applied at budbreak (5-10 cm. shoot length) or lime sulfur applied at 93.5 L/ha resulted in significant disease control when compared to the non-treated control (Table 4). Both disease incidence and severity were reduced. In every case micronized DF sulfurs resulted in significantly better disease control than lime sulfur.

Discussion

Cleistothecia are an important source of overwintering inoculum for *Uncinula necator* in California vineyards. Though they function in every production area except the Coachilla Valley their role as the main source of primary inoculum is greater in the Coastal Production areas. In both the North Coast and Central Coast, cleistothecia are produced in large numbers and the ascospore germination and infection efficiency is high. In addition, grapevine canopies are smaller and more upright resulting in more cleistothecia being trapped on cordons and therefore potentially capable of functioning in the disease cycle.

Production of cleistothecia in the Central Valley occurs rather uniformly in most years but there are areas where cleistothecia function with regularity versus areas where they seldom function even though produced. In the upper San Joaquin and low Sacramento Valley and the extreme southern San Joaquin Valley, viable cleistothecia are abundant and can be found on cordons and canes in most vineyards. In these areas late summer and/or fall, weather conditions are conducive for cleistothecial production and survival with the main factor in their functioning being rainfall occurrence before leaf fall. In the center of the San Joaquin Valley the main form of overwintering appears to be bud perennation (5). Though cleistothecia form, ascospore efficiency is low. In addition, late summer and fall temperatures are often above 35 C and previous work (3?) has shown that high temperatures result in cleistothecial abortion. Another negative factor in this region is early freezing which results in dead foliage at just the time when temperatures become moderate enough to allow for disease increase and fruiting body production on young leaves.

As shown in New York studies (?) our data indicate that cleistothecia wash to positions directly below their location in the canopy. This in general results in most cleistothecia being washed into the soil especially from vines with large, containing canopies. Our work has shown that cleistothecia which are washed into soil die and therefore no longer function in the disease cycle. However, cleistothecia residing on leaf litter at the time of budbreak do release viable ascospores and though data is lacking

we suspect that these spores have been responsible for disease in some coastal vineyards.

Cleistothecia forming on leaves during the summer and early fall have not been shown to release viable ascospores in other areas but in the coastal regions of California we have documented release of viable ascospores as early as July in three vineyards. This has major implications in terms genetic recombination as relating to strain development and fungicide resistance and we suspect that in some vineyards there could be two ascospore derived generations per year in sprinkler irrigated vineyards.

Fungicide trials in California have shown similar results to New York studies (?) in that dormant applications of lime sulfur greatly reduce the incidence and severity of disease at disease onset. Additionally, our data has shown significantly better disease control with the use of micronized DF Sulfurs and Copper DF + Sulfur DF combination (Kocide 4045) when applied at budbreak and used at 10 day to 14 day intervals during cool, wet springs.

Identification of cleistothecia as a primary inoculum source lead to new disease control strategies. In addition, the potential for development of new biotypes through sexual recombination would indicate the more judicious use of selective fungicides such as the group of sterol biosynthesis inhibitors. Budbreak in the vineyards tested usually occur in March at time when cleistothecia are mature and ascospore viability is highest.

Table 1. Summary of a regional survey of the viability of ascospores released from cleistothecia collected from 28 vineyards in 4 grape growing regions of California.

Grape Production Region	No. of Vineyards Sampled	Average # of Cleistothecia per 10 g/park	Average Percent Germination	Total No. of Infections	Samples with no Release
Central Coast	5	690	70	26	0
North Coast	4	400	73	6	0
San Joaquin	15	28	18	3	8
Temecula	4	28	17	1	1

1, Central Coast vineyards represent Monterey, San Luis Obispo and Santa Barbara Counties. North Coast represent Napa and Sonoma Counties. . . San Joaquin Valley represents Madera, Fresno, Kern and Tulare Counties. Temecula is a small winegrape region located approximately 60 miles north of San Diego.

2. Exfoliating bark (50g) was removed from 20-30 randomly selected grapevines in December and January and processed as described previously.

3. Percent of ascospores released onto water agar which germinated within 48 hrs. ____

4. Number of infections occurring on detached leaves 2 weeks following ascospore release.

Table 2. Effect of sample date on viability ascospore released from cleistothecia collected from six Chardonnay vineyards in three grape production regions in California.

Location	Source of [a] Cleistothecia	Date	Percent [b] Ascospore Germination
San Joaquin Valley Region	Leaves	12/4/88	0
Kern County	Bark	2/16/89	20
North Coast Region	Leaves	11/19/88	49
Sonoma County	Bark	3/21/89	93
Central Coast Region	Leaves	10/27/88	0
Monterey County A	Bark	2/27/89	27
	Bark	3/15/89	93
Monterey County B	Leaves	10/27/88	0
	Bark	2/27/89	68
	Bark	3/15/89	86
Monterey County C	Leaves	10/27/88	0
	Bark	2/27/89	35

	Bark	3/23/89	77
Santa Barbara County	Leaves	11/10/88	0
	Bark	2/9/89	79
	Bark	3/11/89	86

[a] Collection of cleistothecia was made on 2-3 dates beginning with leaves in fall or early winter. Cleistothecia were washed from bark or leaves by vigorously shaking sample in water and filtering through mesh screens.
[b] Percent germination based on the number of germinated ascospores/total # released onto 1% water agar from cleistothecia on filter paper wedges adhered to the inside top of a plastic petri dish lid. Test run 48 hours.

Table 3. Percent of total cleistothecia trapped in filter paper funnels during fall rains in 1990 for three trellis types. Values are means of 9 traps placed under three vines.

	Number of cleistothecia [b] Trapping Location [c]			
Trellis Type	Cordon	Middle Canopy	Drip Line	Outside of Drip Line
T-bar	11	36	40	5
Shoot positioned	41	36	19	3
2-wire vertical	25	43	29	3

[a]
[b]
[c]

Table 4. Effect dormant lime sulfur and early-season micronized sulfur on early season incidence and severity of grapevine powdery mildew in four vineyards in California in 1989-1990.

Treatment	Rate/ha	Disease Incidence[a]	Disease Severity[b]
Monterey County			
Thiolux DF	4.5 kg	7.4 A	3.8 A
Liquid Lime Sulfur	93.5 l	7.2 B	5.4 B
Unsprayed Control	-	69.9 C	3.8 C
Santa Barbara County			
Thiolux DF	4.5 kg	2.5 A	0.2 A
Liquid Lime Sulfur	93.5 l	6.3 B	1.8 B
Unsprayed Control	-	18.8 C	8.3 C

[a] Percent disease incidence based on 5 replications, 8 vines per rep. Values followed by the same letter do not differ significantly according to Duncan's Multiple Range Test, $P < 0.05$.

[b] Values are average number of colonies per replication determined by randomly sampling 20 basal leaves along both arms of the cordon of each of 8 vines per replication and counting individual colonies. Values followed by the same letter do not differ significantly according to Duncan's Multiple Range Test, $P < 0.05$.

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