Grape Cluster Architecture and the Susceptibility of Berries to *Botrytis cinerea*

M. E. Vail and J. J. Marois

Former graduate research assistant and associate professor, Department of Plant Pathology, University of California, Davis 95616. Present address of first author: Viticulture Production Specialist, Crop Care Associates, Inc., P.O. Box 288, St. Helena, CA 94574.
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ABSTRACT

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Detached mature grape berries from seven cultivars were inoculated with conidia of *Botrytis cinerea* over a range of 10^2–10^6 conidia per milliliter. The percentage of diseased berries was determined after 7 days at 25 C and 95% relative humidity. The slope of the inoculum density-disease incidence response, used as an index of susceptibility, ranged from 0.07 for Emperor to 2.13 for Muscat of Alexandria in 1988. Cabernet Sauvignon and Muscat of Alexandria berries were more susceptible than expected, considering the low level of disease usually observed in the field. Cluster tightness was also quantified for the seven cultivars. Cabernet Sauvignon and Muscat of Alexandria were less tight than the other cultivars and were below the methodology threshold of 0.0005 N. The cultivars most severely affected by bunch rot in the field had the tightest clusters, while cultivars with loose clusters were least affected in the field. Components of cluster architecture were measured and their respective contribution to cluster tightness determined by path analysis. Cluster weight and the ratio of interior to exterior berries contributed most to cluster tightness, with path coefficients of 0.35 and 0.25, respectively. The number of berries per centimeter of rachis, the component most commonly used by viticulturists to estimate cluster tightness, was least important in its contribution to cluster tightness (path coefficient of 0.09). The rate of surface water loss per hour was also determined for each of the clusters. Cabernet Sauvignon, which had a loose cluster, dried at a higher rate than did Barbera, Colombard, or Semillon, which had tighter clusters.

Additional keywords: Botrytis bunch rot, *Vitis vinifera*.

Botrytis bunch rot of grape, caused by *Botrytis cinerea* Pers.:Fr., is an important disease of grape (*Vitis vinifera* L.). In California, Botrytis bunch rot is a particular problem in the coastal valleys that have a cool, wet maritime macroclimate that is favorable for disease development. However, many microclimate variables within the canopy are also important. Fruit cluster architecture may be an important variable in determining the severity of bunch rot in the field (18). Cultivars with tight clusters develop severe bunch rot (7,12,14,15,20), whereas cultivars with loose fruit clusters are less affected (12). However, no single procedure has been adopted for the quantification of cluster tightness.

Cluster tightness has been estimated visually (2,3,16), volumetrically (16), by the number of berries per centimeter of rachis (2,8), by rachis length (6), by the number and weight of berries per centimeter of rachis, and by the weight of berries per gram of rachis (21). These methods indirectly estimate cluster tightness. Thus, they do not directly account for the many factors that can affect tightness, such as pedicel length or berry diameter, or the interactions among these factors.

The objectives of this study were to quantify cluster tightness, to assess its relationship to berry susceptibility, to determine the effect of cluster tightness on the rate of cluster drying, and to determine the contributions of various components of cluster architecture to cluster tightness.

MATERIALS AND METHODS

To better understand the relationship between fruit cluster architecture and disease development, we selected grape cultivars that showed a range of cluster tightness and disease development in the field. The cultivars were Cabernet Sauvignon, Carignane, Chenin Blanc, Emperor, Muscat of Alexandria, Sauvignon Blanc, and Zinfandel. The cultivars were trained to a bilateral cordon, spur-pruned, and managed under the same production practices in a vineyard at the University of California, Davis.

In 1987 and 1988, 10 mature clusters of each of the cultivars were collected. Five of the clusters were used to determine susceptibility of individual berries to *B. cinerea* and the rest were used to quantify cluster tightness.

Berry susceptibility. Susceptibility of berries to *B. cinerea* was determined in the laboratory with inoculum density-disease incidence (ID-DI) experiments. Three single-spore isolates of *B. cinerea*, one sclerotial and two mycelial, isolated from grape berries were cultured singly on potato-dextrose agar (PDA) at 20–22 C. Conidia were harvested from 10- to 14-day-old colonies in 3–5 ml of sterile distilled water. Conidia were dislodged with a transfer loop. Suspensions were filtered through three layers of cheesecloth to remove mycelia and vortexed for 30–60 sec to disperse the conidia. The conidial suspensions were combined and adjusted to concentrations of 0, 10^2, 10^3, 10^4, 10^5, and 10^6 conidia per milliliter. Germination of conidia on PDA was consistently greater than 90%.

Berries on the outside of the clusters were selected for uniformity of size and color and were removed from the rachis with 4–6 mm of pedicel attached. Berries were inoculated by being dipped up to the shoulder region into an inoculum solution and agitated gently for 3–5 sec. Caution was taken not to submerge the pedicel end of the berry because natural wounds occur there. The berries were placed in 92% RH, determined by an aspirated wet-dry bulb psychrometer, and lightly misted once with deionized water from a Chromister (Gelman Instrument Co., Ann Arbor, MI). The inoculated berries were maintained in 92% RH at 20 C for 7 days, and the number of berries with symptoms of infection by *B. cinerea* (water-soaked lesions on the surface of the berry with mycelia formation and sporulation after approximately 3 days) was recorded. Before statistical analysis, the number of infected berries in the uninoculated control treatment was subtracted from the number of infected berries in each of the inoculum densities to correct for background infection.

A split-plot design was employed with cultivar as the main plot and inoculum level as the sub-plot. Sub-plots consisted of 10 berries. The experiment was done three times each year. Slopes were analyzed for significant differences by covariance analysis between each possible combination of regressions with the general

linear model procedure of SAS (13).

Cluster tightness. A standardized, repeatable, quantitative technique was developed to estimate cluster tightness (19). A University of California (UC) Firmness Tester (Western Industrial Supply, San Francisco, CA) equipped with an Ametek gage (Hunter Spring Div., Hatfield, PA) was used to measure the force, in newtons (N), required to separate two berries in contact with one another 2 mm apart. Ten measurements were taken in the middle one-third of each of five clusters per cultivar. The experiment was repeated twice each year. Data were $\log_{10}$ transformed before analysis and back-transformed values are presented in the results. Data were analyzed by analysis of variance (13) with the effect of cultivars assumed to be fixed and the effects of cluster and measurement assumed to be random; measurements were nested within clusters. The percentage of the variance attributable to each source was estimated by the nested analysis of SAS (13). The percentage of variance attributable to the repeated measurements was assumed to be equal to the percentage associated with the error.

The cultivars Semillon, Colombard, Barbera, and Cabernet Sauvignon differ in cluster tightness and were chosen to determine the relative contribution of various components of cluster architecture to cluster tightness. The effect of cluster tightness on the rate of cluster drying was also assessed with these four cultivars. Vines were head-trained, spur-pruned, and managed under the same standard production practices in a vineyard at the University of California, Davis.

Cluster drying rate. The cut end of the rachis was dipped in molten paraffin wax to reduce evaporative loss. Individual clusters were weighed, immersed in deionized water, drained for approximately 1 min and reweighed. Clusters were suspended at approximately 20 C in a 1- × 0.6- × 1-m topless container to reduce air movement, yet still allow for evaporation. Cluster weights were then recorded after 1, 2, and 4 hr and subtracted from the initial wet weight (time 0) to determine the amount of water that remained on the clusters. Five clusters were used for each of the four cultivars and the experiment was repeated twice. Percentage of water retained was regressed on time. For each pair combination of cultivars, slopes of regression lines were compared by analysis of covariance with the general linear model procedure of SAS (13).

Cluster architecture. In determining the number of interior and exterior berries, the same clusters used in the drying-rate experiments were suspended, sprayed with fluorescent paint, and all berries were removed. A berry was considered exterior if more than 25% of its surface was painted. The numbers of interior and exterior berries were used to derive the ratio of interior to exterior berries and the total number of berries. The length of the main rachis was also measured to determine the number of berries per centimeter of rachis.

The relationships of independent variables with one another and to the dependent variable (cluster tightness) were analyzed by using path coefficient analysis (9).

RESULTS

Berry susceptibility. In 1987, the slopes of the ID-DI relationships ranged from 0.67 for the cultivar Emperor to 2.30 for Muscat of Alexandria. Muscat of Alexandria was the most susceptible cultivar and Zinfandel was not significantly different from Emperor. In 1988, the slopes ranged from 0.07 for Emperor to 2.13 for Muscat of Alexandria. Muscat of Alexandria, Carignane, and Cabernet Sauvignon were similar in susceptibility, and Sauvignon Blanc was similar to Emperor (Table 1).

Cluster tightness. In both years, Chenin Blanc, Zinfandel, Sauvignon Blanc, and Carignane had similar cluster tightnesses. Emperor, Muscat of Alexandria, and Cabernet Sauvignon were also similar and had less tight clusters. In 1987, cluster tightness ranged from <0.0005 N for Cabernet Sauvignon to 0.0204 N for Chenin Blanc (Table 2). Similar results were obtained in 1988, when the cluster tightness ranged from <0.0005 N for Cabernet Sauvignon to 0.0199 N for Carignane. In 1987, cultivar and cluster were significant sources of variance ($P < 0.001$). In 1988, cultivar was a significant source of variance ($P < 0.001$). Results from both years were similar (Table 3). No clear relationship between the susceptibility of individual berries to *B. cinerea* and cluster tightness was observed (Figs. 1 and 2).

Cluster drying rate. The slope of the relationship between the percentage of water retained on clusters and time was −0.111 for Colombard, −0.130 for Semillon, −0.158 for Barbera, and −0.226 for Cabernet Sauvignon. Covariance analysis of the slopes indicated that all cluster drying rates were significantly different from one another ($P < 0.05$) (Fig. 3). With the same clusters, no significant differences were obtained for cluster tightness among Colombard (0.0059 N), Semillon (0.0064 N), and Barbera (0.0057 N). The cluster tightness of Cabernet Sauvignon was less than the lower threshold of the technique (<0.0005 N).

Cluster architecture. Cabernet Sauvignon had significantly fewer interior berries (1.0), exterior berries (92.3), and total berries (93.3) than the other cultivars and had the lowest ratio of interior to exterior berries (0.005) (Table 4). Semillon had a significantly higher ratio of interior to exterior berries (0.203) than Barbera (0.140). Cabernet Sauvignon had significantly fewer berries per centimeter of rachis than did Barbera, Semillon, or Colombard ($P < 0.05$). Rachis lengths did not differ significantly among cultivars.

Path analysis indicated that, of the factors considered, cluster weight had the greatest effect on cluster tightness, with a path coefficient of 0.35. There was a wide range in the number of interior berries in the clusters. Therefore, the number of interior berries had a large positive influence (path coefficient of 1.07) on the interior to exterior berry ratio, while the number of exterior berries showed a small negative influence (path coefficient of −0.18). The total number of berries had a large positive effect on the number of berries per centimeter of rachis (path coefficient of 0.84), whereas rachis length had a large negative influence (path coefficient of −0.78). The ratio of interior to exterior berries had a greater impact on tightness (path coefficient of 0.25) than

TABLE 1. Slope of the linear relationship between inoculum density of *Botrytis cinerea* and disease incidence (ID-DI) of grape berries

Cultivar	1987 ID-DI slope	1987 Coefficient of determination	1988 ID-DI slope	1988 Coefficient of determination
Muscat of Alexandria	2.30 a[z]	0.94	2.13 a	0.79
Carignane	1.96 b	0.74	2.00 ab	0.81
Cabernet Sauvignon	1.80 b	0.61	1.93 ab	0.69
Chenin Blanc	1.73 b	0.72	1.23 b	0.49
Sauvignon Blanc	1.43 bc	0.77	0.20 c	0.12
Zinfandel	1.23 bcd	0.48	1.47 ab	0.72
Emperor	0.67 d	0.36	0.07 c	0.02

[z]Values in columns followed by different letters are significantly different ($P < 0.05$). Slopes were analyzed for significant differences by covariance analysis between each possible combination of regressions.

TABLE 2. Quantification of grape cluster tightness

Cultivar	Force (newtons) 1987	Force (newtons) 1988
Chenin Blanc	0.0204 a[y]	0.0137 a
Zinfandel	0.0132 ab	0.0064 b
Sauvignon Blanc	0.0130 ab	0.0045 b
Carignane	0.0084 b	0.0199 a
Emperor	0.0007 c	NA[z]
Muscat of Alexandria	0.0006 c	NA
Cabernet Sauvignon	NA	NA

[y]Means in a column followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).
[z]The cluster tightness was below the limit of the methodology (<0.0005 N).

did the number of berries per centimeter of rachis (path coefficient of 0.09). The residual effect (0.65) indicates that cluster tightness is dependent on other factors that were not measured. The path diagram in Figure 4 indicates the relative importance and interdependency of the factors and their contribution to cluster tightness. Correlations are indicated by bidirectional arrows, and unidirectional arrows indicate possible causal relationships.

DISCUSSION

The lack of a clear relationship between individual berry susceptibility and cluster tightness suggests that these variables are not directly related. Carignane, Chenin Blanc, and Zinfandel, which are severely affected by Botrytis bunch rot in the field (12), all had tight clusters and highly susceptible berries. Sauvignon Blanc, a cultivar that has a tight cluster and is moderately affected by Botrytis bunch rot in the field, had moderately susceptible individual berries. Emperor, a cultivar with a loose cluster architecture and relatively unsusceptible berries, is little affected by Botrytis bunch rot in the vineyard. However, Muscat of Alexandria and Cabernet Sauvignon, both of which have a loose cluster architecture and are only slightly affected by Botrytis bunch rot in the field, had highly susceptible berries. This indicates that cluster architecture has a dramatic influence on Botrytis bunch rot epidemics. Cultivars that have berries that are highly susceptible to *B. cinerea* and a tight cluster architecture may be predisposed to the development of more severe Botrytis bunch rot epidemics than cultivars with highly susceptible berries and a loose cluster architecture. This indicates the importance of cluster architecture and its impact on the microclimate at the berry surface.

The removal of basal leaves alters the microclimate within the grapevine canopy (4,5) and reduces the development of Botrytis bunch rot (5,7). Modification of the grapevine canopy by removal of the basal leaves 2–4 wk after set, resulted in a reduction of bunch rot incidence and severity (5,7). English et al (4,5) found this disease reduction to be associated with microclimatic differences. Canopy microclimates were most completely distinguished by canonical discriminant analysis when temperature, vapor pressure, wind speed, and leaf wetness were considered together (5). Evaporative potential, the capacity of the atmosphere to evaporate moisture, has pronounced effects on the biology of *B. cinerea*. Under controlled conditions, wind speed and relative humidity, both of which strongly influence evaporative potential, are important in the development of aerial mycelia and conidia of *B. cinerea* (17). Also, Marois et al (11) found that susceptibility of rose flowers to *B. cinerea* was inversely correlated to the vapor pressure deficit for the 5-wk growth period before harvest.

Cluster architecture influences the length of time that a cluster retains water. For the maximum germination of conidia, up to 12 hr of free water may be required, depending on the temperature (1). Tight clusters, from cultivars such as Barbera, Semillon, and Colombard, dry at a slower rate and take more time to dry completely. Whereas, loose clusters, like those of Cabernet Sauvignon, dry at a more rapid rate.

Although there was no clear relationship between susceptibility and cluster tightness, cluster tightness may affect the propensity for individual berries to become infected. This study found that tight clusters have a higher ratio of interior to exterior berries than loose clusters, and consequently many berry-to-berry contact sites. Marois et al (10) demonstrated that these contact areas are more susceptible to *B. cinerea* due to reduced deposition of cuticle and epicuticular wax.

A new method, using the UC Firmness Tester, provided a means to quantify cluster tightness. This method will allow cluster

TABLE 3. Sources of sampling variance

Variance source	Percentage of total variance	
	1987	1988
Cultivar	70.1	62.3
Cluster	9.9	0.9
Replicate measure	20.0	36.8

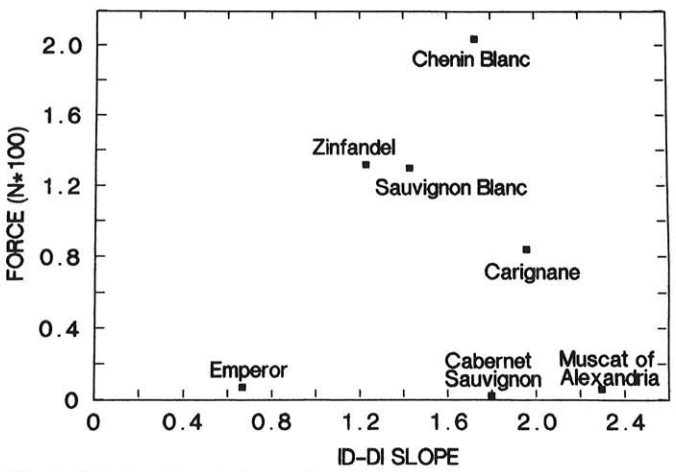

Fig. 1. Relationship of cluster tightness with the slope of the inoculum density-disease incidence (ID-DI) relationship in 1987. The slope of the ID-DI relationship was used as an index of berry susceptibility; the greater the slope, the more susceptible the berry. N = newtons.

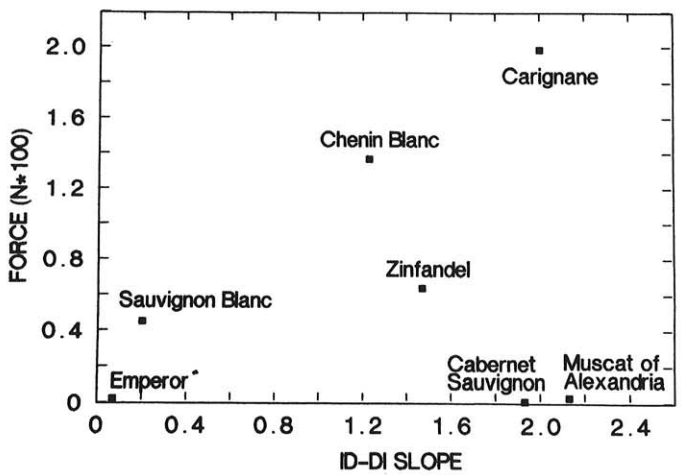

Fig. 2. Relationship of cluster tightness with the slope of the inoculum density-disease incidence (ID-DI) relationship in 1988. The slope of the ID-DI relationship was used as an index of berry susceptibility; the greater the slope the more susceptible the berry. N = newtons.

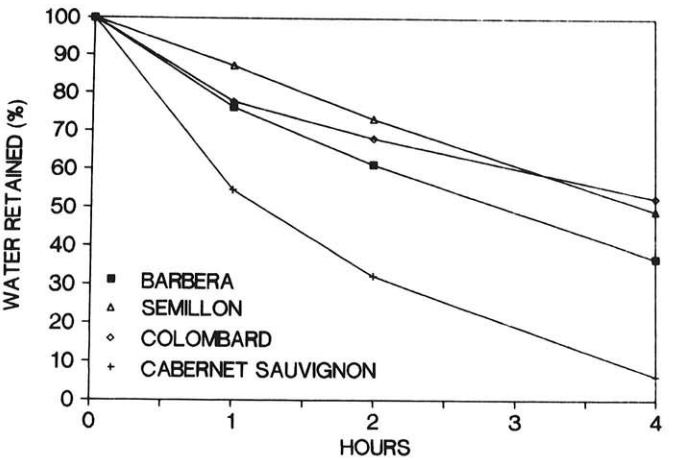

Fig. 3. Relationship of surface water retention (expressed as percentage of water remaining from time 0) of clusters and time for selected grape cultivars.

TABLE 4. Components of cluster architecture for selected grape cultivars

Cultivar	Components					
	Interior berries	Exterior berries	Interior/ exterior ratio	Total berries	Rachis length (cm)	Berries per centimeter of rachis
Semillon	37.7 a[z]	175.9 a	0.203 a	213.7 a	23.7 a	11.1 a
Colombard	33.7 ab	180.0 a	0.166 ab	212.9 a	22.1 a	10.7 a
Barbera	21.9 b	145.6 a	0.140 b	167.5 a	19.8 a	9.1 a
Cabernet Sauvignon	1.0 c	92.3 b	0.005 c	93.3 b	19.1 a	4.6 b

[z]Means in a column followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

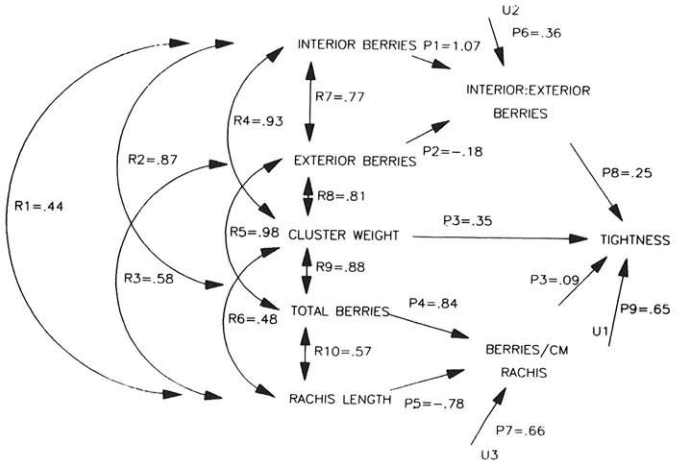

Fig. 4. Path diagram that indicates the relationship of the components of cluster architecture to cluster tightness. P_i = path coefficients; R_i = correlation coefficients; U_i = residual effect of undefined factors.

tightness to be used as a quantitative tool in grape disease resistance breeding programs, in determining the suitability of a particular clone for a given site, and in determining the response of grapevine reproductive growth to viticultural practices.

The most appropriate model determined by path analysis indicated that cluster weight made the largest contribution to cluster tightness; the ratio of interior to exterior berries was next in importance. The number of berries per centimeter of rachis, a way most commonly used by viticulturalists to estimate cluster tightness, was least important.

It is generally accepted that cultivars with tight clusters are severely affected by Botrytis bunch rot in the field and that cultivars with loose clusters are not. However, berries from some cultivars with loose clusters were more susceptible to *B. cinerea* than berries from cultivars with tight clusters. Although this relationship was not absolute, it does indicate the effect of cluster architecture on the microclimate at the berry surface and on the development of epidemics of Botrytis bunch rot.

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