Botrytis Bunch Rot: A Disease Requiring Integrated Control

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Botrytis bunch rot (BBR), caused by the fungus *Botrytis cinerea*, causes damage to ripening grape clusters throughout the temperate regions of the world where pre-harvest rains occur. Although pure Botrytis infections free of secondary contaminants can sometimes produce the so-called "noble rot" integral to the production of certain prized dessert wines, a far more common result is a disease that reduces both yield and fruit quality (Figure 1), as infected grapes typically produce wines with substandard flavors and appearance.

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BBR is an amazingly complex disease. Its development is governed by multiple 3-way interactions between the grapevine, the environment, and the Botrytis fungus itself, many of which are poorly understood. With considerable contributions from former graduate student Stella Zitter and technicians Duane Riegel, Judy Burr, and Dave Combs, we have spent a number of years trying to better define some of the individual issues that determine whether and how badly this disease will develop. This article attempts to summarize some of our results within the context of better understanding the fundamental principles underlying BBR development and applying this knowledge to its management.

Disease biology

Botrytis is a "weak" pathogen that primarily attacks highly succulent, dead or injured tissues, or those that are senescing (slowly breaking down). Feeding sites of grape berry moth larvae, powdery mildew scarring of the grape skin, and pre-harvest splitting caused by overcrowding within tight clusters and/or excessive rain are common berry injury sites attacked by Botrytis. Withering blossom parts, aborted fruitlets, and ripening berries as they near maturity are important senescing tissues with respect to BBR development.

The fungus thrives in high humidity and still air, hence the well-known value of cultural practices such as leaf pulling and canopy management to minimize these conditions within the fruit zone. Although the fungus does not grow well in berries until they start to ripen, it can gain entrance into young fruit through senescing blossom parts, old blossom "trash" sticking to berries within the cluster, and scars left by the fallen caps. Such infections typically remain latent (dormant) and unseen while berries are green. However, some of them can resume activity and rot the berries as they start to ripen (senesce) if the conditions are "right", after which further spread can occur as new infections expand from these sites into additional ripening berries. This begs the question, when are damaging infections most likely to occur? And relatedly, when are sprays directed at this disease most important and valuable?

Figure 1. Severe Botrytis bunch rot on clusters of cv. Vignoles.

We began investigating this question some years ago in a block of different Pinot noir clones in cooperation with the late Dr. Robert Pool and his technician, Steve Lerch. Because it is well known that BBR is more severe in cultivars and clones with compacted fruit clusters, we chose to work with tight-clustered clone 29 (PN29) and the loose-clustered Mariafeld clone, which commonly develops lower levels of this disease than most other clones. We added a third "clone", PN29 vines whose clusters were thinned by hand after fruit set so that their architecture resembled that of Mariafeld. This was to help determine whether Mariafeld's relative resistance in the vineyard is due to some chemical or physiological factor specific to the clone or simply to the fact that its clusters are looser than most other Pinot noir clones.

For two consecutive years, clusters of the three clonal treatments were inoculated with spores of the Botrytis fungus and kept wet overnight to promote infection, at four different growth stages: (i) late bloom; (ii) pea-sized berries; (iii) bunch closure; and (iv) veraison. Selected clusters were taken to the lab 10 days later to determine the percentage of berries with invisible latent infections, whereas the remainder were allowed to mature on the vine and were rated at harvest to determine the percentage of the berries that had become rotten by Botrytis. (An interesting side note: latent infections are determined by killing the berries—e.g., by freezing or treatment with certain herbicides—after which the

fungus colonizes the dead berry and forms spores, as if it were growing on inert agar in a petri dish. This indicates that the fungus typically is held in a latent state on the vine through some active process provided by the *living* berries until they begin to senesce = ripen).

The results from these trials are presented in Figures 2–5. There was no consistent effect of inoculation timing on the establishment of latent infections, although a greater percentage of berries did become infected from the late bloom inoculation in Year 2. Similarly, there was no effect of the clonal treatment on latent infection establishment in either of the two years (Figs. 2 & 4). In contrast, both the time of inoculation and clonal treatment had a pronounced effect on the percentage of berries that actually became diseased after they matured. That is, the highest levels of disease resulted from inoculations at veraison, consistent with the preference of the Botrytis fungus to colonize senescing tissues. Also, the greatest number of rotten berries always developed in the naturally compacted clusters of PN29, whereas there were significantly fewer in the naturally looser clusters of the Mariafeld clone or in clusters of PN29 that had been thinned to resemble those of Mariafeld (Figures 3 & 5). Furthermore, it was clear that latent infections often failed to become active and cause berry rot, particularly in the clusters with less compaction. In Year 2 for example, 64 and 76% of the berries developed latent infections when clusters were inoculated at late bloom in the PN29/thinned and Mariafeld treatments, respectively (Figure 4), yet only 2 and 1% of the berries in those same respective treatments actually became diseased by harvest (Figure 5).

Collectively, these results led us to hypothesize that the higher levels of disease occurring in the tight-clustered PN29 clone resulted from a relative few latent infections becoming active during the post-veraison period and then spreading to a much greater degree than when such clusters were thinned or in the loose Mariafeld clusters. To examine this possibility, 10 days after veraison we inoculated either 1, 3, or 5 berries on various PN29 clusters, which were either naturally compacted or had been thinned by hand at fruit set as before. To do so, we used a hypodermic needle to inject the designated berries with Botrytis spores, thereby producing individual rotten berries within clusters about 1 week later. These served as initial "point sources" of the disease from which it could spread, and were meant to simulate the occasional post-veraison activation of latent infections.

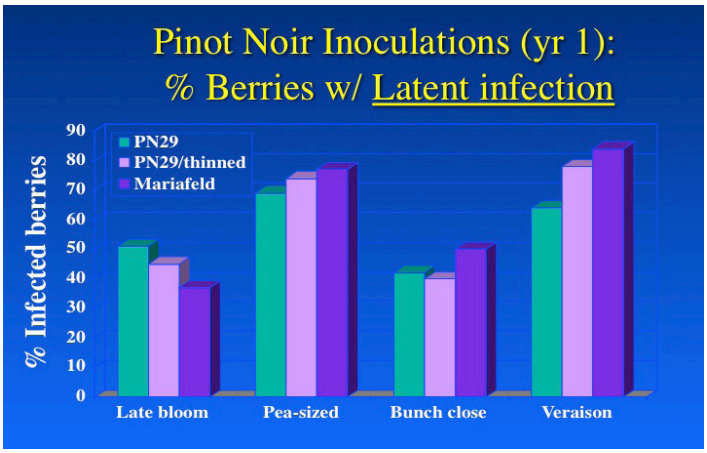

Figure 2. Effect of clonal treatment and inoculation timing on the frequency of Pinot noir berries with invisible latent infections in Year 1 of the study.

As Figure 6 shows, the disease was able to spread extensively throughout the natural, unthinned PN29 clusters: from a single rotten berry that first developed 2.5 weeks after veraison, the disease subsequently spread to an average of 50 additional berries by harvest. In contrast, disease spread was minimal within the thinned clusters in which a single berry was inoculated and only modestly greater when three or five berries were inoculated.

In a related experiment the following year, bunches of a

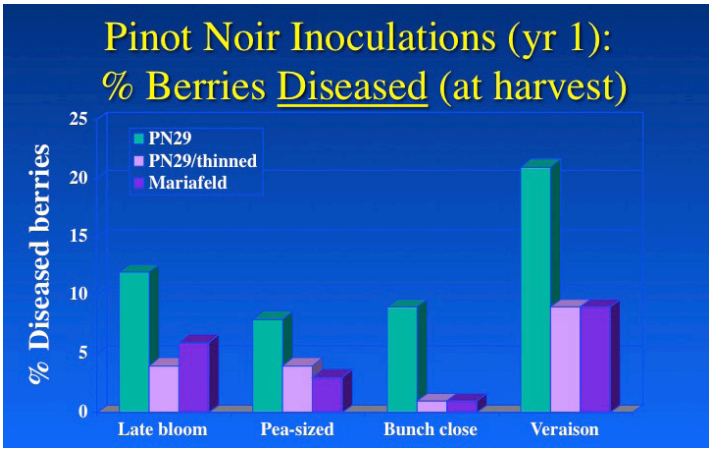

Figure 3. Effect of clonal treatment and inoculation timing on the frequency of Pinot noir berries with symptoms of Botrytis bunch rot at harvest in Year 1 of the study.

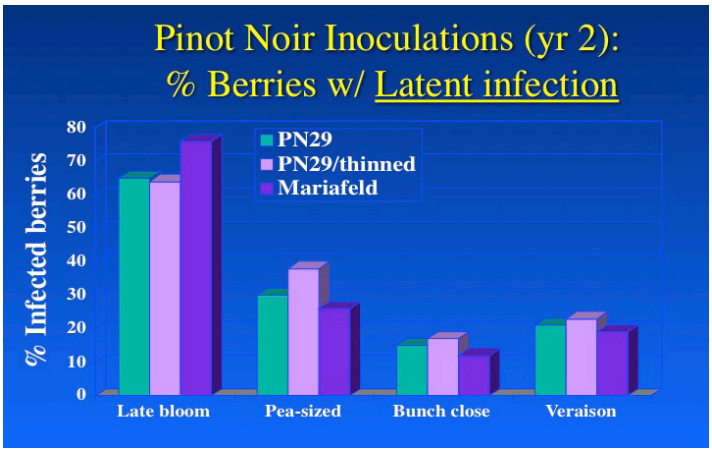

Figure 4. Effect of clonal treatment and inoculation timing on the frequency of Pinot noir berries with invisible latent infections in Year 2 of the study.

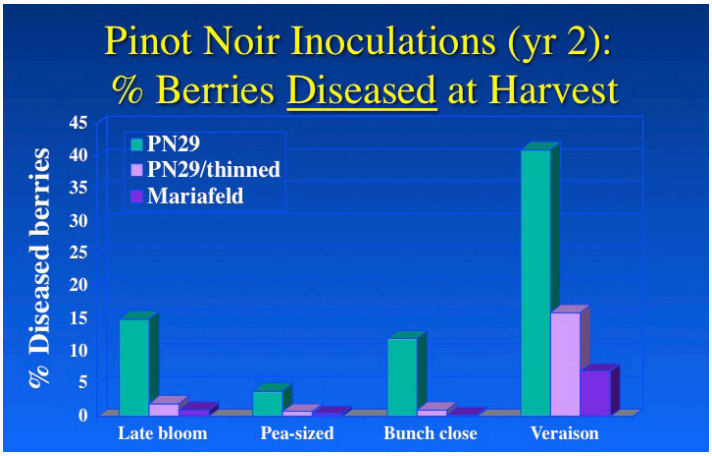

Figure 5. Effect of clonal treatment and inoculation timing on the frequency of Pinot noir berries with symptoms of Botrytis bunch rot at harvest in Year 2 of the study.

tight-clustered Chardonnay clone were similarly thinned (or not) and inoculated. Additionally, based upon a phenomenon we had observed years ago with Botrytis infections of strawberries, some vines received four weekly sprays of urea (8 lb/A) starting at veraison, to see if high berry N content would affect disease spread. (Note that due to its late application, this treatment increased assimilable N in the must without increasing canopy growth.) Once again, little disease spread occurred in the thinned clusters regardless of nitrogen treatment, whereas significant spread did occur in the naturally compacted clusters. Furthermore, elevated berry N also increased spread within these clusters when the system was not "saturated" with the maximum number of inoculated berries. For example, when three berries per cluster were inoculated, the disease spread to three additional berries in the thinned clusters with or without post-veraison N sprays; in contrast, it spread to 31 and 11 additional berries in the compacted clusters on vines that did or did not receive the N applications, respectively (Figure 7).

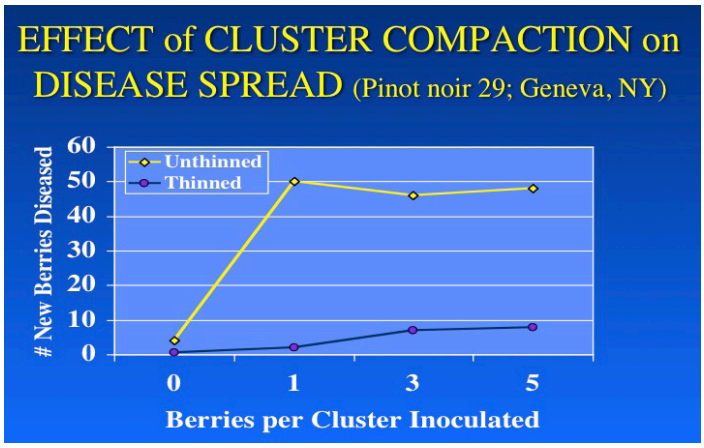

Figure 6. Effect of cluster tightness on disease spread. Selected clusters on vines of Pinot noir clone 29 were hand-thinned after fruit set to approximate the looseness of those of the Mariafeld clone. Either 0, 1, 3, or 5 berries per cluster were inoculated at veraison and disease was present on those initial "point sources" 1 wk later. Data reflect the number of additional berries to which the disease had spread by harvest.

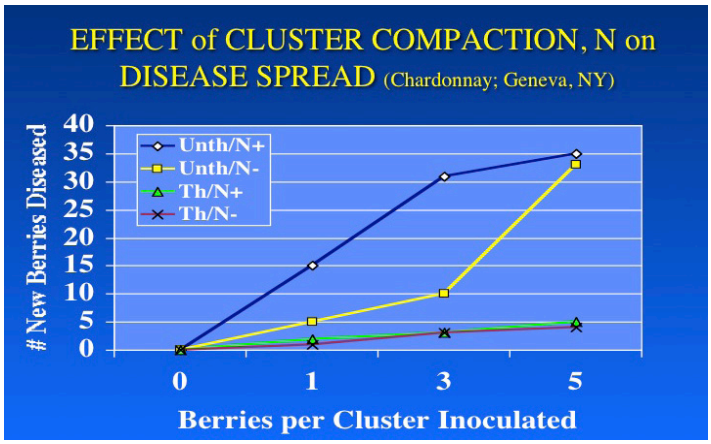

Figure 7. Preharvest spread of Botrytis on Chardonnay berries as affected by the number of initial infection sites, cluster architecture, and berry nitrogen status. Selected clusters were hand-thinned after fruit set to minimize contact among berries as they matured (Th) whereas others were allowed to retain their natural tightness (Unth). Selected vines received four weekly foliar sprays of urea (8 lb/A) starting at veraison (N+), whereas others did not (N-). Either 0, 1, 3, or 5 berries per cluster were inoculated at veraison, providing individual diseased berries 1 wk later. Data reflect the number of additional berries diseased after harvest.

Thus, it appears that latent infections that occur during the bloom and post-bloom period probably result in relatively few rotten berries in and of themselves, but instead serve the role of "primary" infections, providing a foothold for the pathogen from which damaging levels of secondary spread can occur should they become active and conditions be favorable for disease development pre-harvest. Such conditions include not only climatic factors but others, including high berry nitrogen levels and compacted clusters, as shown above. Cluster compaction appears to be extremely influential, since the fungus can spread through tight clusters from just a single initial rotten fruit, via berry-to-berry contact (Figure 8). Thus, finding a means to reduce cluster compaction safely and reliably is something of a "Holy Grail" in terms of Botrytis management. Several techniques have been tried, two of the more successful being well-timed applications of gibberellic acid and cluster-zone leaf removal at the very beginning of bloom. However, these and other techniques have their own drawbacks and potential pitfalls, and deserve far more discussion than this article will allow.

Figure 8. Spread of Botrytis via berry-to-berry contact within a compacted cluster of Chardonnay grapes.

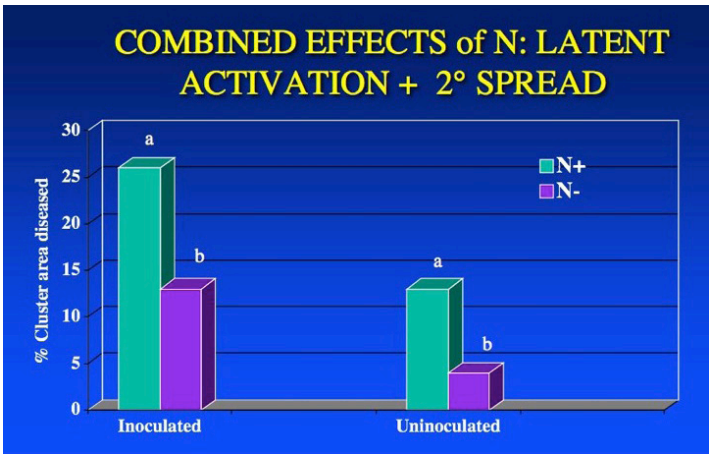

Figure 9. Effect of nitrogen on latent infection activation. Selected clusters on field-grown Chardonnay vines were inoculated with Botrytis spores at bloom to initiate latent infections, and some vines were sprayed with urea (8 lb/A) five times at weekly intervals beginning 1 week before veraison (N+), whereas others were not (N-). Data show the percentage of clusters with at least one diseased berry at harvest, on both the inoculated clusters and uninoculated neighboring clusters flagged at bloom for comparison.

Because most latent infections initiated during and after bloom do not become active and rot berries before harvest, it would be helpful to predict when pre-harvest activation might occur to potentially start an epidemic. Although the factors that stimulate activation are not well understood, we have identified three that appear to be involved: high berry nitrogen content, high atmospheric relative humidity (RH), and high plant water content.

Since we had already determined that increasing berry nitrogen levels could increase secondary spread of the disease, we decided to see whether it might also promote the activation of latent infections. Chardonnay vines were inoculated with Botrytis spores at bloom to initiate latent infections, some were sprayed with urea (8 lb/A) five times at weekly intervals beginning 1 week before veraison, and the effect was evaluated at harvest in two different ways. In the first, we determined the percentage of clusters that had at least one diseased berry, presumably the result of a latent infection initiated at bloom that had become active; while doing so, we separately evaluated both the inoculated clusters and uninoculated neighboring clusters that were subject only to natural infection. In both cases, the incidence of diseased clusters was nearly half-again as great on vines receiving the urea sprays versus those that did not (Figure 9). We also determined the percentage of the cluster area that was diseased on these bunches (essentially, the percentage of diseased berries), which integrates the effect of N on both the activation of latent infections and their subsequent spread through the affected bunches. This measure of disease severity was doubled and tripled on the inoculated and uninoculated clusters, respectively, for vines treated with urea versus those that were not (Figure 10).

To examine the effects of high RH, we utilized potted Chardonnay vines. These were inoculated with Botrytis spores at bloom to initiate latent infections and then maintained in a covered screenhouse, where they were subject to ambient environmental conditions while being protected from rain. At either veraison or 10 days pre-harvest, 25 plants were moved to a large humid chamber (95% RH), and five of these were removed either 1, 3, 5, 7, or 9 days later and returned to the screenhouse. At harvest, we then determined the percentage of clusters that had at least one diseased berry, presumably the result of a latent infection initiated at bloom that had become active. As shown in Figure 11, imposing high RH for as long as nine consecutive days had no effect on latent infection activation if the treatment began at veraison. However, prolonged humid conditions during the pre-harvest period markedly increased the frequency of clusters with active infections by harvest, from 10% with 0 or 1 day of exposure to 30 and 80% after 3 and 9 days of highly humid conditions.

Although latent infections usually do not become active until after veraison, we occasionally see pea-sized berries with Botrytis symptoms when extensive rainfall occurs during the post-bloom period. Again, based upon what is known about the interaction of

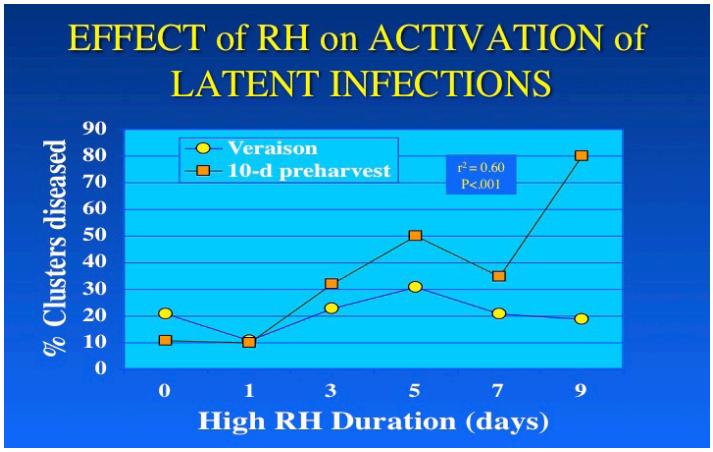

Figure 10. Combined effects of nitrogen on latent infection activation and secondary spread. Selected clusters on field-grown Chardonnay vines were inoculated with Botrytis spores at bloom to initiate latent infections, and some vines were sprayed with urea (8 lb/A) five times at weekly intervals beginning 1 week before veraison (N+) whereas others were not (N-). Data show the percentage of the cluster area diseased at harvest, on both the inoculated clusters and uninoculated neighboring clusters flagged at bloom for comparison.

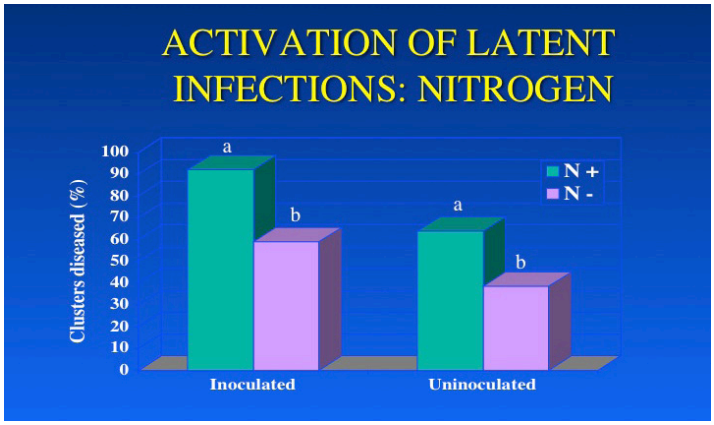

Figure 11. Effect of relative humidity on the activation of latent infections. Potted Chardonnay vines were inoculated with Botrytis spores at bloom to initiate latent infections and maintained in an outdoor screenhouse, protected from rain. At either veraison or 10 days pre-harvest, selected pots were moved to a large humid chamber (95% RH) and returned to the screenhouse either 1, 3, 5, 7, or 9 days later. Data show the percentage of clusters that had at least one diseased berry at harvest.

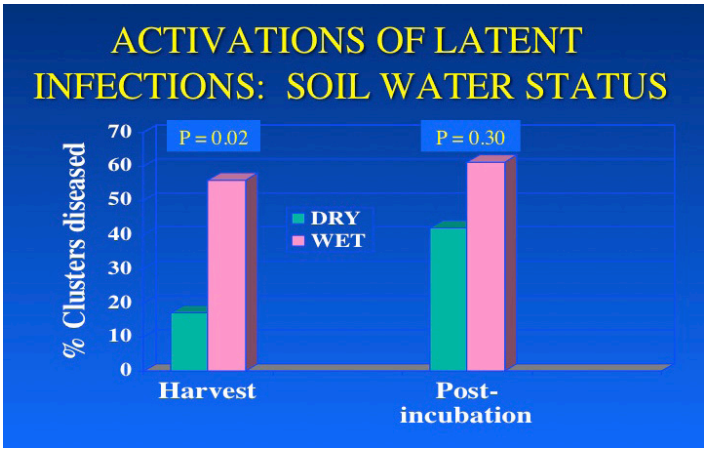

Figure 12. Effect of soil water status on the activation of latent infections. Potted Chardonnay vines were inoculated with Botrytis spores at bloom to initiate latent infections, then were maintained in a covered outdoor screenhouse and watered regularly with a hose until veraison. Subsequently, vines were either: (i) similarly watered to keep the soil wet (WET), or (ii) watered only when the shoot tips began to wilt (DRY). The percentage of clusters with at least one diseased berry was determined at harvest and again after the harvested clusters were incubated at 95% RH for an additional 4 days

the Botrytis fungus with other crop plants such as strawberries, it seemed possible that this might be due in part to berries becoming more susceptible to colonization by the pathogen (latent infections becoming active) when vines are provided unrestricted access to water in the soil. To examine this, we again inoculated potted Chardonnay vines with Botrytis spores at bloom and maintained them in a covered screenhouse. The vines were watered regularly until veraison, then the pots were split into two groups, which were: (i) similarly watered (with a hose) almost daily to keep the soil wet (WET), or (ii) watered only when the shoot tips began to wilt (DRY). The percentage of clusters with at least one diseased berry was determined at harvest, after which the harvested clusters were incubated at 95% RH for an additional 4 days to see whether additional latent infections might become active.

As shown in Figure 12, latent infections had become active by harvest in approximately three times as many clusters in the WET treatment as in the DRY, although the only difference between the two was the amount of water added directly to the soil (the foliage and berries did not get wet in either). When the harvested clusters were then incubated under high RH conditions, the percentage of diseased clusters more than doubled in the DRY treatment, whereas it was virtually unchanged in the WET (Figure 12). These results suggest that in the former treatment, a significant number of viable latent infections had failed to become active by harvest but did so once conditions became more favorable during the subsequent high-RH incubation. In contrast, the pre-harvest conditions were much more favorable for latent infection activiation when vines were provided high amounts of water, so subsequent incubation under high RH conditions had little effect.

Management

Cultural practices to improve air flow around the clusters, such as canopy management and leaf pulling, are well known and widely practiced. Removal or destruction of vineyard debris, particularly old cluster stems that serve as a major source of overwintering inoculum, is useful as well and worth employing to whatever extent is practical. Minimizing cluster compaction through cultivar and clone selection at planting, and perhaps by utilizing some experimental techniques such as gibberellic acid application and trace bloom leaf removal, can have a major positive impact. Excessive levels of nitrogen application (and pre-harvest irrigation, where that is practiced) should be avoided. Fungicide sprays targeted specifically at BBR also can be beneficial on susceptible cultivars and/or clones, particularly in a wet year. However, it's important to remember that unlike some of our other common fungal diseases, it is very difficult to control Botrytis simply by using a good spray program. Integrating fungicide applications with cultural control techniques is a necessity when managing this disease.

The fundamental questions regarding fungicides are which materials, and when? Traditional BBR spray programs call for possible applications at bloom (or late bloom), just as bunches are closing, veraison, and pre-harvest. The earlier timings are designed to prevent the initial establishment of infections through susceptible blossom parts and blossom trash, whereas the later sprays are designed to prevent both initial infections through injured ripening berries and the spread of active infections throughout the ripening clusters. Despite some pronouncements to the contrary, none of these timings are necessarily better than the others

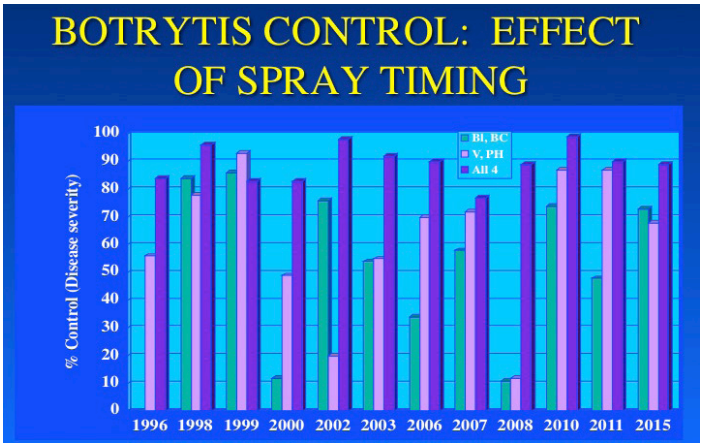

Figure 13. Influence of spray timing on the control of Botrytis bunch rot in Geneva, NY (cv. Aurore, 1996-2000; cv. Vignoles, 2002-2015). Sprays were applied at (i) Bloom + bunch closure (Bl, BC); (ii) Veraison and 2-3 wk later (Ve, PH); or (iii) at all four of these stages. Data are expressed as percent reduction of diseased berries relative to vines in the same trial that received no Botrytis fungicides.

since either, both, or neither end of the seasonal spectrum can be important, depending on the infection pressure at that time.

This concept is nicely illustrated by data that we have gathered over 12 different seasons since 1996. Figure 13 shows the control provided by Botrytis sprays applied early (late bloom plus bunch closure), late (veraison plus 2 weeks pre-harvest), or at all four of those stages, expressed as a percent reduction in disease severity relative to vines in the same trial that received no Botrytis sprays. Note that in some years (e.g., 1998, 1999, 2007, 2015), either two early sprays or two late sprays provided as much or nearly as much control as all four. In 2002, the two early sprays alone provided most of the control provided by the full program, whereas the two late sprays alone provided very little. In contrast, the two late sprays were as effective as the full program in 2011, whereas the two early sprays provided only half as much control. And in the remaining years, the full program was superior to those confined to either the first or last two applications, with the relative contributions of the early and late timings varying among years.

The activities of the fungicides used against Botrytis could be the topic of another full article, although much of this information is presented in the annual NY and PA Pest Management Guidelines for Grapes. Remember that these materials can vary not only in their general efficacy but also in their physical mode of action. That is, whereas most provide fair to excellent protective activity, only some have the capacity to enter flowers and berries and fight the fungus after infection has occurred. Generally speaking, this activity is relatively common (but not universal) among the newer synthetic fungicides but is not common among the biological and biologically-derived products. When known, these activities are noted in appropriate sections of the Guidelines. And remember, the need to rotate among the various fungicide groups cannot be overemphasized if we want to maintain the utility of all of them.

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