SOUR ROT: ETIOLOGY, BIOLOGY, AND MANAGEMENT

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SOUR ROT is a term that sometimes is used to describe a general decay of nearly-ripe grape clusters that may be associated with various bacteria, yeasts, and/or filamentous fungi that are not species considered to be primary decay pathogens, e.g., *Botrytis cinerea*, Colletotrichum spp., Greenaria uvicola, etc. (McFadden and Gubler, 2015). Unfortunately, this means that different people (and in the United States, various fungicide labels) often use this one term imprecisely to refer to a condition that has different causes. For this discussion we will define sour rot as a syndrome that involves pre-harvest cluster decay accompanied by the smell of vinegar (acetic acid).

The characteristic visual symptom of sour rot is a tan to occasionally reddish discoloration of the rotting berries, which eventually lose their integrity and begin to decompose, and no moldy growth needs to be present (Figs. 1 and 2). Whereas various molds, including Botrytis, are sometimes found on sour-rotted clusters (Fig. 3), these organisms are not necessary for sour rot to develop. Although some potential role for them in specific cases cannot be dismissed entirely, such molds usually occur coincidentally with the organisms that cause sour rot, since they utilize the same food source and are favored by the same environmental conditions as the yeasts and bacteria that cause the disease, although these causal organisms are not visible to the naked eye. One additional group of organisms characteristically associated with sour-rotted clusters, which are highly visible and appear to be an important if not essential component of the disease, are Drosophila “fruit flies” or “vinegar flies” (Fig. 4), as discussed later.

Fig. 1. Pre-harvest sour rot on cv. Riesling. Note almost complete lack of mold growth on the diseased berries.

Fig. 2. Pre-harvest sour rot on cv. Riesling. Note complete lack of mold growth and the breakdown of diseas

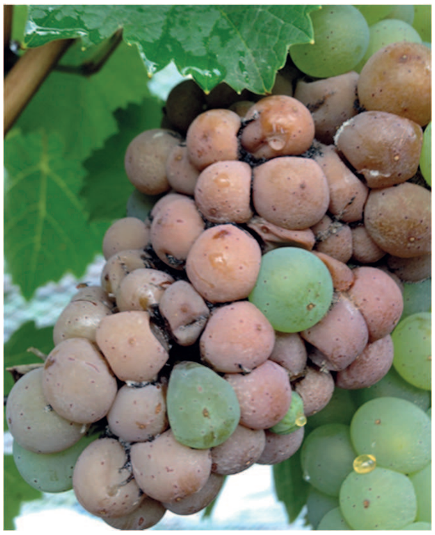


Fig. 3. Pre-harvest sour rot on cv. Riesling. Note coincidental presence of additional black, secondary mold fungi

Fig. 4. Pre-harvest sour rot on cv. Riesling. Note lack of mold growth and presence of numerous Drosophila fruit flies (arrows).

Dr. Wendy McFadden-Smith, working with the provincial government of Ontario in Canada, has shown that the measure of volatile acidity in crushed grapes harvested from different vineyards is strongly correlated with the pre-harvest severity of sour rot present in these same vineyards. It's generally accepted that the vinegar in such clusters is produced by certain acetic acid-forming bacteria (species of *Acetobacter* and *Gluconobacter* are most often implicated), and that various wounds are necessary for infection to occur and disease to develop subsequently. Occasionally, these bacterial infections are accompanied or followed by infections by several wild yeasts that produce ethyl acetate (which smells like nail polish remover or varnish), although this does not occur typically.

In our work over the past 4 years, we've discovered a far more important contribution of yeast species to sour rot development. When we began sampling multiple sour rotted clusters from vineyards throughout the Finger Lakes region of New York and elsewhere, we always detected high population of *Saccharomyces cerevisiae* yeasts in the rotten fruit, and significant concentrations of ethanol in addition to acetic acid. For example, in one series of sour rot samples from 16 different affected vineyards, high-performance liquid chromatography (HPLC) and gas chromatography (GC) analysis for acetic acid and ethanol, respectively, revealed an average acetic acid content of 2.4 g/L in juice of the affected clusters and an average ethanol content of 0.23% (v/v), with some samples as high as 4.6 g/L and 0.48%, respectively. In comparisons among the individual simples we also found an inverse relationship between the two products, i.e., as the amount of acetic acid increased there was less ethanol and vice versa. In retrospect, this isn't surprising, since ethanol is the substrate that the abovementioned bacteria convert to acetic acid. Thus, sour rot appears to be the culmination in a step-wise process that begins with injury to the berries, which allows entry of both the yeasts that convert the grape's juice to ethanol and the bacteria that subsequently convert this into acetic acid. We have reproduced both the visual and accompanying olfactory symptoms of sour rot in the lab by wounding ripe berries and co-inoculating them with *S. cerevisiae* and *Gluconobaceter oxidans* or *Acetobacter aceti*. However, to reliably produce typical sour rot symptoms, including acetic acid production within diseased fruit, we also have found that we must simultaneously expose the inoculated clusters to *Drosophila* flies. Both the common species, *D. melanogaste*r, and the so-called "spotted wing" Drosophila, *D. suzukii*, are equally effective in this regard.

***The role of* Drosophila *fruit flies*.** Many people have observed the association of *Drosophila* flies with sour-rotted clusters. Because these insects are attracted to the smell of both ethanol and acetic acid, it has been thought that they are secondary colonizers of rotten berries attracted to an abundant food source—a good place to lay their eggs--and that they might help to spread the disease passively by moving the responsible microbes on their bodies as they travel within the vineyard. However, a study from Portugal published shortly before we began our project (Barata et al., 2012) suggested that the flies might actually play a direct role in the development of the disease, leading us to examine this hypothesis.

The results from one illustrative experiment are shown in Figs. 5 and 6. In this experiment, we inoculated berries in different petri dishes with (i) the standard wine yeast, *S. cerevisiae*, and an acetic acid bacterium (*A. aceti*), or (ii) water; and simultaneously (a) introduced *D. melanogaster* fruit flies into the dishes, or (b) omitted insects from them. We measured ethanol and acetic acid accumulation on each of the next 5 days. As shown in Fig. 5, ethanol began to accumulate significantly by Day 4 in the inoculated berries, with or without flies. One day later (Day 5), ethanol accumulation doubled in the inoculated treatment when flies were not present, whereas there was little additional accumulation in the inoculated treatment that included flies (arrow). Why? As shown in Fig. 6, ethanol was not being converted to acetic acid when the flies were not also present, whereas this did occur when flies were present (arrow). The two most likely explanations for such a phenomenon are (i) the flies are introducing microbes from their gut, which are also involved in the process of oxidizing ethanol to acetic acid; and (b) the insects are catalyzing this process through some non-microbial (e.g., enzymatic) mechanism. It is possible that both mechanisms are involved, and we have experimental evidence for each of them.

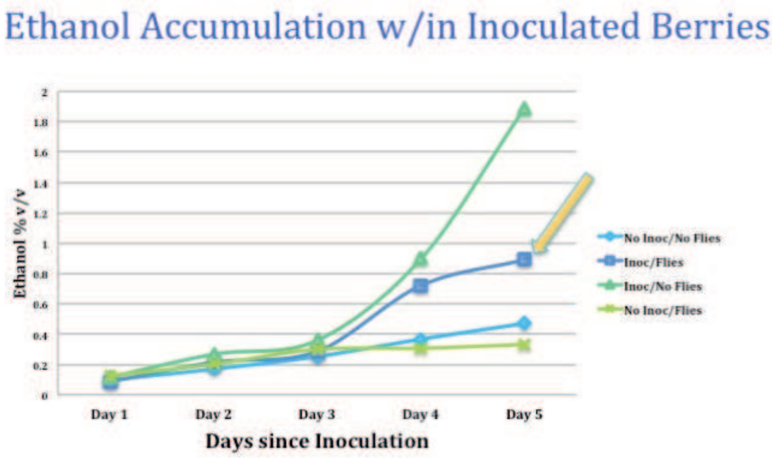


Fig. 5. Ethanol accumulation over the course of 5 days after grape berries were inoculated in the lab with a combination of S. cerevisiae and A. aceti and exposed or not to D. melanogaster fruit flies.

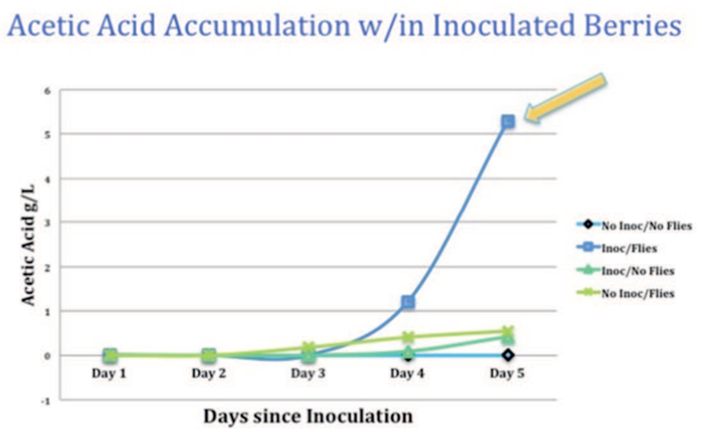
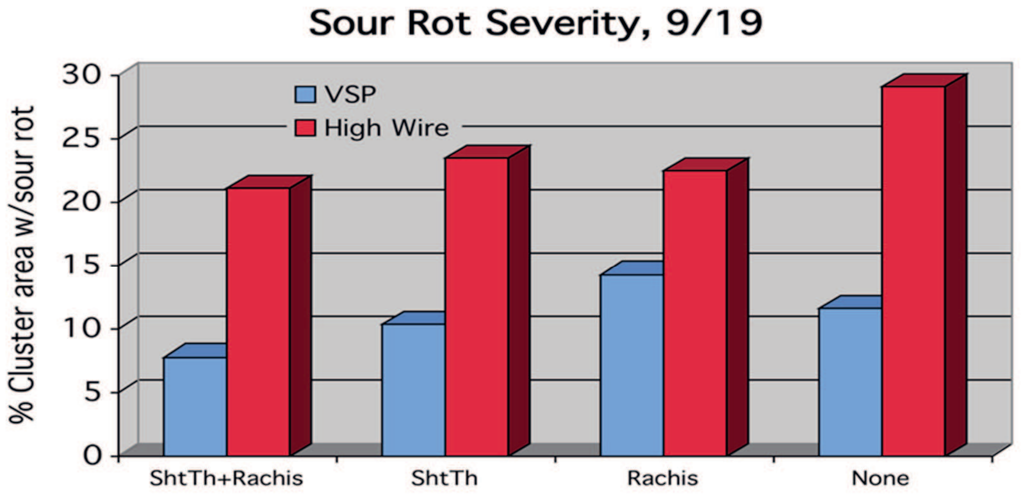


Fig. 6. Acetic acid accumulation over the course of 5 days after grape berries were inoculated in the lab with a combination of S. cerevisiae and A. aceti and exposed or not to D. melanogaster fruit flies.

Disease management. Based on what we know about its biology, an integrated management program for sour rot might include: (1) Provide a berry microclimate within the canopy that's less conducive to pathogen growth; (2) Minimize berry injuries; (3) Minimize populations of the responsible microbial pathogens; and (4) Minimize populations of the responsible Drosophila flies.

Canopy microclimate. Before starting this present study, there was the opportunity to measure the effect of canopy management on sour rot in a field trial on the interspecific hybrid 'Vignoles', a tight-clustered variety that is very susceptible to pre-harvest rots. The experiment was designed to study how fruit composition and Botrytis development are affected by different canopy management practices, including shoot thinning and the complete removal of the previous season's rachises that remained after mechanical harvesting, and these treatments were imposed on adjacent rows of vines trained to either a High Wire Cordon or Vertical Shoot Position (VSP) system. The pre-harvest weather conditions were very wet and favorable for sour rot development,and the effects of these management factors effects on disease development were significant, as shown in Fig. 7 below.



• Effect of training system was greater than that of canopy manipulation: across all four treatments, average of 11.0% cluster area w/sour rot for VSP, 22.2% for Top Wire.
• Effects of training system and canopy manipulation were additive: best treatment = Shoot Thin + Rachis Removal/VSP (7.8%), worst treatment = Check/Top Wire (29.1%)

Fig. 7. Average sour rot severity at harvest in Vignoles clusters from vines trained to either a VSP or high-wire cordon system and subjected to three different early-season canopy management treatments (or none).

In September 2014, we returned to this vineyard to assess sour severity in a different season. No variable canopy management treatments were imposed, but the effect of training system was significant once again, with twice as much disease in the High Wire vines versus VSP. (Likely reason: with the High Wire system, the vigorous shoots grow down almost to the ground, essentially enclosing the clusters within a “tent” of leaves). The data are presented below in Fig. 8.

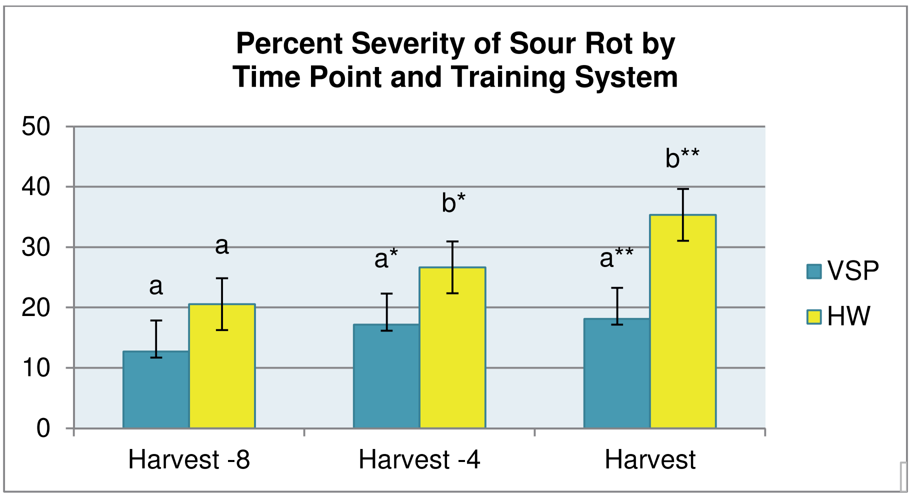


Figure 8. Effect of training system (VSP and High Wire [HW]) on the development of sour rot in a commercial vineyard of cv. Vignoles, Finger Lakes NY, 2014. Disease severity represents the average percent of the cluster area affected with sour rot, assessed on the day of harvest plus 4 and 8 days before.

We returned to this vineyard again in 2015 and found the same effect for a third time. As shown in Fig. 9, by 8 days pre-harvest (14 Sep) 29% of the berries in the high-wire system had sour rot whereas 16% were diseased in VSP vines in the row next to them. At that point, the grower sprayed a labeled insecticide active against fruit flies (Mustang Maxx, zeta-cypermethrin) and a labeled antimicrobial, Oxidate (dilute hydrogen peroxide), after which the disease essentially stopped progressing.

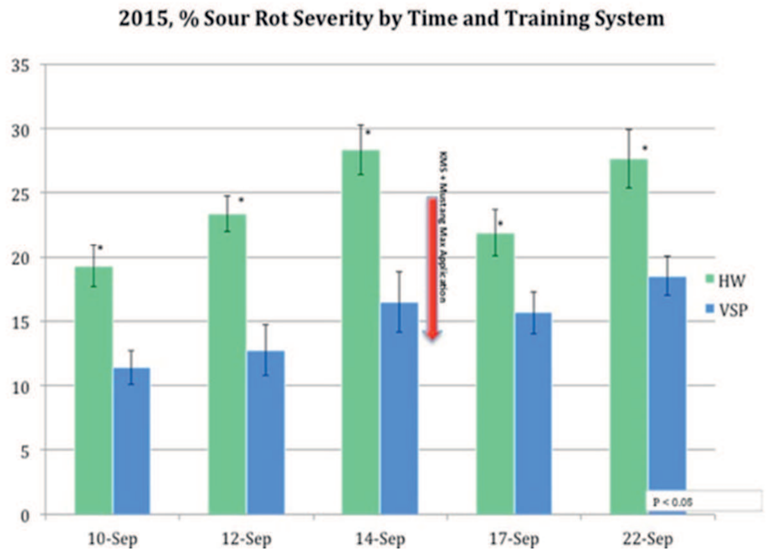


Figure 9. Effect of training system (VSP and High Wire [HW]) on the development of sour rot in a commercial vineyard of cv. Vignoles, 2015. Disease severity represents the average percent of the cluster area affected with sour rot, assessed over 12 days before harvest on 22 September. Note the effect on disease progression after the grower applied an insecticide (Mustang Maxx, zet-cypermethrin) and antimicrobial (Oxidate, hyrdrogen peroxide) after the 14 Sep assessment.

Minimize injury. In addition to standard practices designed to reduce damage to clusters from birds, insects, powdery mildew, etc., loosening the density of berries within clusters is likely to reduce mechanical injuries that often occur from compaction in tight-clustered cultivars and clones. Practices to reduce cluster compaction such as leaf removal at the start of bloom and application of specific growth regulators will reduce sour rot in the same way that they reduce Botrytis, although there are potential problems with all of these approaches, which are still being investigated by many different researchers and growers.

Minimize the pathogen population. In several field trials, we have obtained significant control of sour rot with two general antimicrobials: the dilute formulation of hydrogen peroxide mentioned above and a 0.5 to 1.0% (0.5 to 1.0 kg/100 L) solution of potassium metabisulfite (KMS), applied weekly once rains begin and berries reach 15° Brix. Wendy McFadden-Smith's research group in Ontario also has obtained significant control with KMS. Although KMS is used widely in wineries both to sanitize equipment and as a food-grade additive to musts and wines to kill wild microorganisms and prevent oxidation, it is NOT registered for spraying onto vines to control diseases, either in the US or Canada. Nevertheless, these results validate the concept of utilizing general antimicrobials to help control sour rot. Furthermore, in our trials, the antimicrobial treatments are always more effective when combined with an insecticide treatment effective against Drosophila flies, as discussed next.

Field trial results. We looked at a combination of insecticide and antimicrobial sprays in a Cornell University vineyard of the hybrid cv. 'Vignoles' near Geneva, NY in 2013, '15, and '16. Alternate rows were sprayed with the insecticide zeta-cypermethrin weekly beginning at 15° Brix, with the remaining rows receiving no insecticide. Then, within the “insecticide +” or “insecticide –“ rows, we applied various antimicrobial treatments, also on a weekly schedule. These treatments included KMS (0.5% or 1.0%), copper hydroxide (2013 only), hydrogen peroxide, and a biorational product (BLAD peptide, derived from Luipnus seeds; the North American trade name is “Fracture”). Most antimicrobial treatments began at 15° Brix, before symptoms were present, but a few were not applied until symptoms were visible.

In 2013, the antimicrobial treatments applied with insecticide provided an average of 50% control relative to the untreated check; antimicrobials without insecticide provided an average of 9% control; and insecticide without antimicrobials provided 15% control (data not shown).

In 2015, the insecticide application itself had a major effect: across the seven individual antimicrobial treatments, there was an average of 43% fewer diseased berries when insecticide was applied relative to the same treatment that did not receive an insecticide application, and a 50% reduction in disease severity resulting from insecticide application when no antimicrobial was applied. When combined with insecticide sprays, the three antimicrobial products provided additional control if begun at 15°Brix, before symptoms were present, with approximately 70 to 80% fewer diseased berries relative to vines that received no insecticide or antimicrobial spray. Antimicrobial sprays that did not begin until disease symptoms were present provided no significant additional control beyond that provided by the insecticide (Fig. 10). Due to the logistics of the experimental design, we could not include a treatment where insecticide + antimicrobial was applied only after symptoms first appeared.

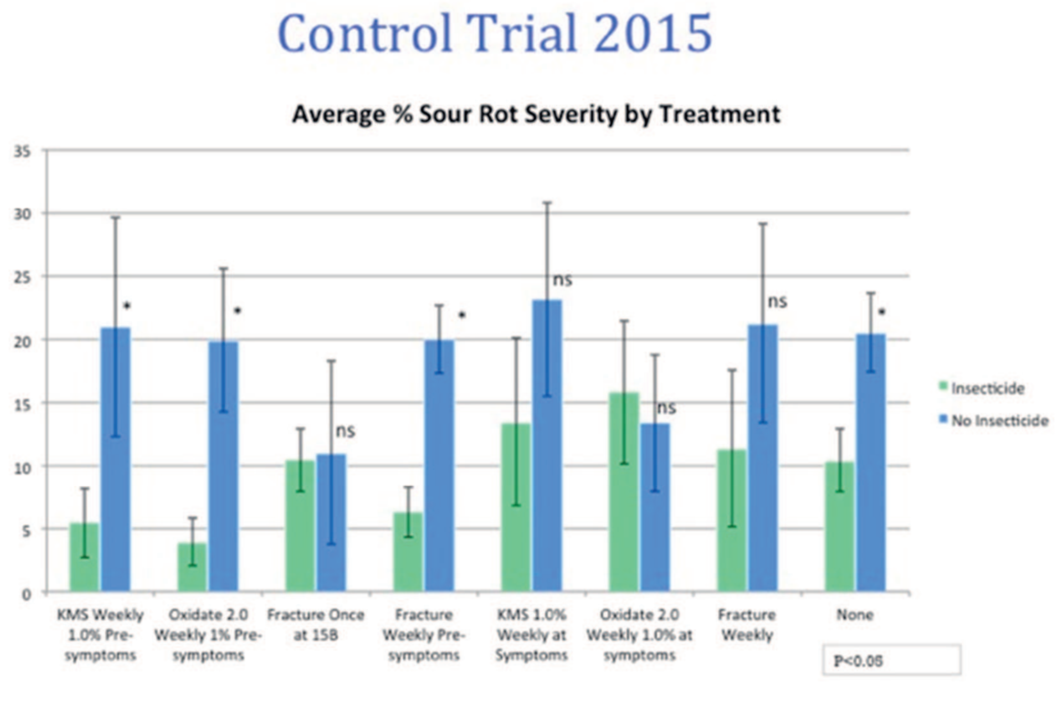


Figure 10. The effect of antimicrobial and insecticide (zeta-cypermethrin) sprays on sour rot control in an experimental ‘Vignoles’ vineyard; Geneva, NY 2015. Potassium metabilsulfite (KMS) in a 1.0% solution or labeled rates of Oxidate (hydrogen peroxide) or Fracture (BLAD peptide) were applied at weekly intervals beginning either at 15° Brix (Pre-symptoms) or after symptoms first appeared; Fracture was also applied once at 15° Brix. Insecticide was applied at weekly intervals beginning at 15° Brix.

Summary

We believe that we have shown that sour rot results from the presence in injured fruit of both a fermentative yeast (note that *S. cerevisiae* is commonly associated with inured fruit in vineyards, whether they have developed sour rot or not) and an acetic acid-forming bacterium. In warmer climates such as California and South Australia, species of the Aspergillus fungus have been reported to be associated with sour rot and they have sometimes have been assumed to help cause the disease. However, this has never been demonstrated experimentally and what role they do play, if any, is not clear.

We also believe that disease development is strongly catalyzed, and may be dependent upon, the presence of Drosophila fruit flies in conjunction with the appropriate yeast and bacteria. Although there are some European reports that sour rot is often associated with *D. suzukii* and that this pest may be an important component of the disease complex, we seldom find this species associated with diseased clusters in New York, nor have colleagues in neighboring states. Rather, nearly all of the flies that we find in diseased vineyards or rear from diseased berries are D. melanogaster, and we have seen no difference between the activities of the two Drosophila species in the types of laboratory tests that we've conducted to assess sour rot development.

Our spray trials have been designed as a "proof of concept"—we have sprayed the vines more intensively than most growers would care to, especially pre-harvest, in order to see whether insecticide plus antimicrobial sprays can have an effect. They seem to, provided that we start spraying about 15°Brix, before the disease becomes visible, and continue applying them regularly. Although our less-intensive spray programs have been less effective, it's important to realize that in our trials we're treating just a few rows that are embedded within a 0.6-ha solid block of grapes. None of the other rows receive these sour rot treatments although they almost always get the disease. Thus, our treated rows are surrounded by >1,000 vines where flies and sour rot microbes build to very high levels, putting much more pressure on our treated vines than would occur if the entire block were treated, as would happen in a commercial vineyard. In this case, a limited number of sprays would probably be much more effective. For example, note how a single spray applied in the commercial vineyard shown in Fig. 9 stopped disease progression during the last week before harvest that year, whereas disease severity doubled over the final 8 days in that same vineyard the year before.

REFERENCES

Barata, A., Santos, S. C., Malfaeto-Ferreira, M., and Loureiro, V. 2012. New insights into the ecological interaction between grape berry microorganisms and Drosophila flies during the development of sour rot. Microb. Ecol. 64:416-430.

Mcfadden-Smith, W. and Gubler, W. D. 2015. Sour Rot. in: Compendium of Grape Diseases, Pests, and Disorders 2nd Ed. W. F. Wilcox, W. D. Gubler, and J. K. Uyemoteo, eds. APS Press, St. Paul, MN. 232 pp.