Effect of Host Plant Tissue on the Vector Transmission of Grapevine Leafroll-Associated Virus 3

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ABSTRACT Many biotic and abiotic factors affect the transmission efficiency of vector-borne plant pathogens. Insect vector within-plant distribution and host tissue preference are known to affect pathogen acquisition and inoculation rates. In this study, we first investigated whether feeding tissue affects the transmission of Grapevine leafroll-associated virus 3 by *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) and the effect of mealybug within-plant distribution on virus transmission under greenhouse conditions. Results showed no significant effect on transmission efficiency after insect confinement on leaf blades, petioles or stems of virus source or healthy test plants for either acquisition or inoculation trials. Transmission efficiency of a single mealybug varied from 4 to 25% in those trials. Second, we tested whether leaf position affected transmission efficiency due to potentially variable virus populations within acquisition plant tissues. No significant differences of transmission rate among acquisition leaf position were observed, probably because there were no differences in the virus population within source tissues. Finally, we examined the seasonality of the virus in field-collected samples and found that GLRaV-3 prevalence varied along a growing season, such that GLRaV-3 translocated along expanding shoots to leaves. Similarly, mealybug populations are known to increase in spring, and then mealybugs spread to cordons and leaves. This coordination of spatial and temporal dynamics of the virus and its vector may increase the risk of GLRaV-3 transmission during late spring and early summer. Further integration of information about pathogen populations in plants, vector feeding behavior and vector population seasonality could lead to more effective management practices.

KEY WORDS *Ampelovirus*, *Closteroviridae*, *Planococcus ficus*, semipersistent

The transmission ecology of vector-borne plant pathogens is mediated by a complex network of interactions that connect host plant, insect vector and pathogen. Vector feeding behavior, host tissue preference and within-plant distribution may affect the transmission efficiency of pathogens at the level of plant–insect interactions (Fereres and Moreno 2009). Differences in probing behavior can lead to variability in the transmission of nonpersistently transmitted viruses (Wang and Ghabrial 2002, Fernandez-Calvino et al. 2006, Pelletier et al. 2008). Insect vectors also may be unevenly distributed within plants, preferring to colonize specific host tissues with higher or lower pathogen populations, which can be positively correlated to acquisition efficiency and subsequent changes in disease spread (Marucci et al. 2004, Daugherty et al. 2010). Host physiological condition and environmental factors also influence host tissue feeding preference of vectors (Elliott and Hodgson 1996, Tsai et al. 2002, Srinivasan and Alvarez 2007). It is therefore not surprising that a wide range of biological and physical factors have been shown to affect pathogen transmission efficiency. However, the integration of information on vector host tissue preference in relation to pathogen within-plant distribution and its consequences to transmission ecology and disease spread has not been widely performed.

Grapevine leafroll disease occurs in all major grape (*Vitis* spp.)-growing regions worldwide and is one of the most destructive viral diseases of grapevines. The disease is associated with many distinct closteroviruses sequentially named grapevine leafroll-associated viruses (GLRaV-1, -2, -3, and so on; Martelli et al. 2002, Maliogka et al. 2008). Virus species in this complex belong to the family *Closteroviridae*, primarily in the genus *Ampelovirus.* Within this virus complex, GLRaV-3 is the predominant species in most vineyards worldwide (Habili et al. 1995, Sforza et al. 2003, Martin et al. 2005, Cabaleiro and Segura 2006, Coetzee et al. 2010) with reported yield losses up to 40% (Credi and Babini 1997, Woodham et al. 1984). This virus not only impacts vine health and grape quality but also has been implicated in graft incompatibility and young vine failure (reviewed by Charles et al. 2006).

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Grapevine leafroll disease has emerged as a threat to the grape industry in the past two decades due to the reported rapid spread of the associated viruses in vineyards worldwide (Habili et al. 1995, Habili and Nutter 1997, Cabaleiro et al. 2008, Golino et al. 2008), as well as perceived increases in damage to wine grape quality (Credi and Babini 1997, Guidoni et al. 2000, Borgo et al. 2003). Mealybug (Pseudococcidae) and soft scale (Coccidae) species have been shown to transmit various GLRaV species (Engelbrecht and Kasdorf 1990, Belli et al. 1994, Cabaleiro and Segura 1997, Petersen and Charles 1997, Sforza et al. 2003, Tsai et al. 2010). *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a cosmopolitan mealybug species, first invaded Coachella Valley vineyards of California in the early 1990s (Godfrey et al. 2003). After introduction, *P. ficus* spread rapidly throughout the entire Coachella Valley and most grape-growing counties in California (Daane et al. 2006). The invasive *P. ficus* has higher reproduction rate, more generations per year and wider host range than already established mealybug species (Walton et al. 2006, Daane et al. 2008) and may result in the increased prevalence of grapevine leafroll disease in California. Despite growing interest in the transmission of GLRaVs, the transmission biology, ecology, and vector specificity have not been well studied for this virus complex. Based on a limited number of studies, transmission of GLRaVs seems to occur in a semipersistent manner (Tsai et al. 2008), and there is no evidence of mealybug-GLRaV specificity for virus transmission (Tsai et al. 2010).

There are 26 species of mealybugs worldwide that feed on grapes, and 17 of them are regarded as vineyard pests (Charles et al. 2006). Current control practices for mealybugs in California vineyards are based on suppressing mealybug populations to levels below economic thresholds for direct damage to grape clusters (Daane et al. 2008). Mealybugs colonize grape clusters, reducing yield and excreting copious honeydew that result in sooty mold infections on berries and leaves (Daane et al. 2008). However, as vectors of grapevine-infecting pathogens, neglecting low-level infestations of mealybugs on vines may facilitate the spread of GLRaVs (Golino and Almeida 2008). For example, in most North Coast vineyards, *Pseudococcus maritimus* (Ehrhorn) is present at low densities and rarely causes economic damage to the fruit (Bentley et al. 2011). However, low mealybug populations may still result in disease spread in the area (Golino et al. 2008). To reduce vector transmission of GLRaVs, control measures must target key developmental stages of insect vectors and incorporate within-plant distribution and population seasonality data. Such a framework would indicate when vectors are most likely to acquire and inoculate GLRaVs from vine to vine. In this study, we investigated from which plant tissues *P. ficus* is more likely to acquire and inoculate GLRaV-3, the effect of mealybug within-plant distribution on virus transmission, and the seasonal dynamics of GLRaV-3 population in infected grapevines in the field to identify a window with high risk of disease spread.

Materials and Methods

Insects, Virus, and Plants. *P. ficus* colonies that were derived from field-collected mealybugs in vineyards near Del Rey, CA, were established and reared on butternut squash (*Cucurbita moschata* Duchesne ex Poir.) in a growth chamber at 22 ± 2°C. First-instar nymphs were used in all experiments because they have been found to be the most competent vectors of GLRaV-3 (Tsai et al. 2008). Virus-infected grapevines, *Vitis vinifera* 'Italia' (LR101 accession) were obtained from a virus collection at the University of California (UC)–Davis. LR101 was infected with GLRaV-3 only. Source vines used for virus acquisition were rooted from dormant cuttings and grown in a greenhouse. All source plants were tested by reverse transcription-polymerase chain reaction (RT-PCR), as described below, to ensure they were infected with GLRaV-3. Healthy grapevines ('Cabernet Franc') used for mealybug transmission experiments were kindly provided by the Foundation Plant Services (FPS) at the UC–Davis, and were rooted from dormant cuttings until they reached ≈20 cm in height with six expanded leaves. Cabernet Franc is frequently used as a biological indicator for leafroll virus infection because of its high susceptibility to this virus complex (Martelli and Boudon-Padieu 2006). All experiments included greenhouse negative control plants, which were grapevines from the same group of test plants that we did not expose to mealybug vectors. None of the controls were positive for GLRaV-3.

Tissue Specificity for Virus Transmission. The effect of acquisition host tissue on GLRaV-3 transmission efficiency by *P. ficus* was tested. Mealybugs were allowed to move onto source vine cuttings (20 cm) laid on mealybug-infested squashes. The stems and petioles of these cuttings were sealed with vinyl electrical tape (Ace, Oak Brook, IL), which only left exposed the leaf blades for mealybug feeding. For other sets of source cuttings, leaves were detached and the stems or petioles were sealed with vinyl tapes and only exposed petioles or stems for mealybug feeding. After 2 h, the cuttings were removed from the mealybug colonies, and tissue hydration was maintained by placing the base of the stem in a flask of water. After mealybugs were given an acquisition access period (AAP) of 24 h, insects were gently shaken off the specific plant source tissue onto paper disks (0.5 cm in diameter). Five potentially viruliferous mealybugs were then transferred to each healthy test plant and caged on the leaf blades; therefore, plant tissue varied for virus acquisition but not for virus inoculation. Clip cages used to confine insects were described previously (Tsai et al. 2008). After a virus inoculation access period (IAP) of 24 h, mealybugs were removed from the test plants with a fine brush and plants were treated with insecticide (Safari) to kill all remaining insects.

A similar experiment was conducted to determine the effect of 'inoculation tissue' on GLRaV-3 transmission efficiency by *P. ficus.* Mealybugs were only allowed to feed on leaf blades of source vine cuttings

for virus acquisition. After an AAP of 24 h, mealybugs were transferred onto healthy test plants. Five mealybugs were transferred to each test plant and caged on leaf blades or petioles of test plants. Groups were also confined on stems of test plants using 3-cm straws with Parafilm-sealed ends. Therefore, plant tissue was the same for virus acquisition but different for virus inoculation. After an IAP of 24 h, mealybugs were removed from the test plants with a fine brush and these plants were treated with an insecticide (Safari) to kill all remaining insects.

Effect of Virus Population on Transmission Efficiency. Under field conditions, leaves located basally on a stem generally harbor higher GLRaV-3 populations than apical leaves in an infected grapevine (Ling et al. 2001). The effect of leaf position on GLRaV-3 transmission efficiency by *P. ficus* was investigated under greenhouse conditions. Using the methods described above, mealybugs were allowed to feed on source tissues for a 24-h AAP followed by a 24-h IAP. For acquisition, insects were confined onto leaves from apical, middle and basal parts of greenhouse-grown GLRaV-3–infected shoots (120 cm). Groups of five mealybugs per plant were used for virus inoculation. To avoid potential confounding effects of host tissue, mealybugs were only allowed to feed on the leaf blades of source and test plants. Leaf blade tissue samples from the individual source leaves were taken after mealybug acquisition, and the population of GLRaV-3 in these tissues was estimated through quantitative RT-PCR (C.-W.T., et al. unpublished). After the 24-h IAP, mealybugs were removed from the test plants with a fine brush, and then these plants were treated with an insecticide (Safari) to kill all remaining insects.

All greenhouse experiments (effects of host tissue and virus population on transmission) were performed three times, and 10 replicate test plants of each treatment were inoculated in each trial. After vector inoculation, all test plants were maintained in a greenhouse and sprayed with insecticides and fungicides as needed until RT-PCR assay. The greenhouse was subjected to natural light, which was supplemented with 400 W sodium lamps from 0600 to 2200 hours during the winter; temperature ranged from 17 to 26°C. All plants were periodically pruned to avoid overgrowth. Transmission rate was determined by RT-PCR assay 5 mo after inoculation, although GLRaV-3 can be readily detected in the test plants 2–4 mo after inoculation (Tsai et al. 2008). The data were analyzed by binomial generalized linear model and the effect of treatment was examined with likelihood ratio tests.

Seasonal Progress of In-Vine Virus Distribution. To study the virus population dynamic in infected grapevines during a growing season, the presence of GLRaV-3 was examined in field-collected samples from a virus-infected clone of *V. vinifera* Cabernet Sauvignon (FPS accession VIS 29) maintained in the field at UC–Davis. During the 2007 growing season, leaf samples were collected monthly from apical, middle and basal parts of shoots of VIS 29 grapevines. Except for April, 36 leaf samples in total (three leaf positions × four selected shoots × three plants) were collected and stored at −80°C until RT-PCR assay. Samples were only collected from apical and basal parts of shoots in April because the shoots were very short. Petiole samples were used for RNA extraction and RT-PCR assay because petioles generally harbor higher populations of GLRaV-3 (Ling et al. 2001). Presence or absence of GLRaV-3 in the leaf petioles of these infected vines was used as a measure of virus population fluctuation within plants.

Table 1. Transmission rate of Grapevine leafroll-associated virus 3 by groups of five *P. ficus* among different acquisition and inoculation plant tissues

	Leaf blade	Petiole	Stem
Virus acquisition tissue, followed by leaf blade inoculation			
Transmission rate[a]	23/30[a]	22/30	15/30
Ps[b]	0.25	0.23	0.13
Virus inoculation tissue, with virus acquisition from leaf blade			
Transmission rate[a]	8/30	6/30	7/30
Ps[b]	0.06	0.04	0.05

[a] Number of plants positive for GLRaV-3/number of plants tested. Data represent the combined results of three independent experimental replicates.

[b] Estimated probability of an individual insect transmitting the virus (following Swallow 1985).

Virus Detection. For all greenhouse experiments, test plants were assayed for GLRaV-3 infection by using a modified version of an RT-PCR procedure optimized for grapevine tissues (Osman et al. 2007). The virus extraction and RT-PCR procedures used in this study were described in Tsai et al. (2008). For field samples, total RNA was extracted from leaf petioles with RNeasy Plant Mini kit (QIAGEN, Valencia, CA). The presence of GLRaV-3 was detected by RT-PCR with the same reagents and conditions as described in Tsai et al. (2008), except total RNA was used instead of crude extract as templates.

Results

Tissue Specificity for Virus Transmission. Under the greenhouse conditions used, neither acquisition nor inoculation host tissue affected GLRaV-3 acquisition or inoculation efficiency by *P. ficus.* There were no significant differences of transmission rate whether mealybugs acquired the virus from leaf blade, petiole or stem of source vines ($P = 0.061$; likelihood ratio test). Similarly, there were no significant differences of transmission rate whether mealybugs inoculated the virus to leaf blade, petiole or stem of test plants ($P = 0.830$; likelihood ratio test). The estimated virus transmission rate for a single mealybug based on the Swallow estimator (Swallow 1985) was 0.11 for the combined data set (81 transmission events out of 180 test plants, Table 1). The probability that a single insect transmits the virus was ≈4 times lower in the assays on the role of host tissue on inoculation (Table 1). This difference may be due to low virus population in source tissues used in that experiment.

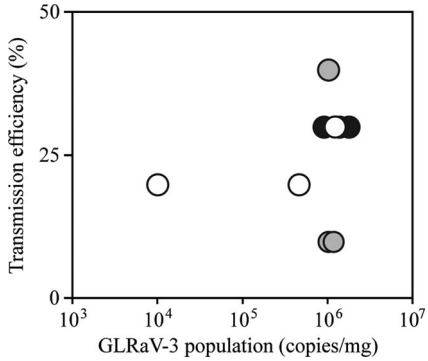

Fig. 1. Correlation between the population of Grapevine leafroll-associated virus 3 in source vines and virus transmission rate by *P. ficus.* Circles represent results from three independent experimental repetitions; white circle, acquisition from apical leaf; gray circle, acquisition from middle leaf; black circle, acquisition from basal leaf.

Effect of Source Leaf Position on Virus Transmission. There were no significant differences of transmission rate whether mealybugs acquired GLRaV-3 from leaves at basal, middle or apical parts of virus-infected shoots ($P = 0.660$; likelihood ratio test). Because there were no significant effects of source leaf position on virus transmission, virus populations in the virus source tissues were estimated through quantitative RT-PCR. Except for one sample of virus source tissue, all leaves harbored similar populations of the virus (Fig. 1). Within the range of 10^4–10^7 genome copies per mg of plant tissue, there was no correlation between virus population and transmission efficiency (Fig. 1).

Seasonal Progress of In-Vine Virus Distribution. The seasonal progress of GLRaV-3 population in infected grapevines was studied on a virus collection maintained in the field on UC–Davis. The seasonal dynamic of GLRaV-3 prevalence in infected vines showed that the virus was readily detected in May but not April in basal leaves (Fig. 2). In middle leaves, the infection incidence increased from April to May and reached the highest rate in May. In basal and apical leaves, the infection incidence increased from April to June and reached the highest rate in June and July, respectively. The infection incidence began to decrease after June or July. At the end of the growing season, 20–40% of leaves still had detectable virus populations.

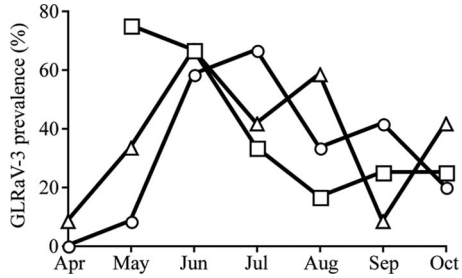

Fig. 2. Seasonal progress of Grapevine leafroll-associated virus 3 within-vine distribution in a virus-infected clone VIS 29 (*V. vinifera* Cabernet Sauvignon) under field conditions. Virus infection prevalence as estimated by RT-PCR for different leaf positions in each month along growing season; circles, apical; squares, middle; and triangles, basal.

Discussion

Our experiments demonstrated that GLRaV-3 transmission by *P. ficus* was not affected by host plant acquisition or inoculation tissues (i.e., leaf blade, petiole, and stem) under greenhouse conditions. Similarly, leaf position on virus-infected source shoots did not influence acquisition efficiency by *P. ficus.* Consistently, within a small range of virus population size in source tissues, transmission efficiency was not affected. These results suggest that virus acquisition rates were equivalent for different plant tissues because the source tissues harbored similar virus populations above a threshold sufficient for virus acquisition by mealybug vectors. In addition, potential differences in vector feeding behavior due to confinement on various plant tissues did not affect acquisition or inoculation efficiency, as virus acquisition and inoculation opportunity was equivalent for all insects. These results suggest that feeding behaviors associated with virus acquisition and inoculation do not vary significantly among host tissues.

P. ficus infests a variety of plant tissues, including leaves (feeding on both leaf surfaces), trunk, cordons, canes and clusters, especially roots and under loose bark (Gutierrez et al. 2008). During a growing season, GLRaV-3 translocates along growing shoots, reaching populations sufficient for insect acquisition in leaves, petioles and stems. Petersen and Charles (1997) also reported that an increase in GLRaV-3 population with grapevine growth did not affect the virus transmission efficiency by two mealybug species, *Pseudococcus longispinus* (Targioni-Tozzeti) and *Pseudococcus calceolariae* (Maskell). Our previous work showed that first instar nymphs of *P. ficus* are more likely to transmit GLRaV-3 compared with later stages (Tsai et al. 2008); therefore, GLRaV-3 transmission in vineyards should be associated with populations of first instars and their dispersal within and between vineyards. In the San Joaquin Valley in California, *P. ficus* populations increase in spring and remain high until August (Daane et al. 2008); therefore, virus spread would be highest during summer. Nevertheless, because this mealybug colonizes grapevines year-round, the risk of GLRaV-3 transmission is not restricted to the summer months. Although our results suggest that GLRaV-3 prevalence (i.e., availability of virus-positive leaves) decreases as a growing season progresses, a more detailed study will be necessary to tightly link pathogen populations within plants to vector transmission efficiency through the use of quantitative RT-PCR to analyze virus populations within plants under field conditions throughout the year.

P. ficus is very prolific; a female can deposit ≈300 eggs (Walton and Pringle 2004) and has four to seven

overlapping generations per year in California compared with two separate generations for *P. maritimus* (Daane et al. 2008, Gutierrez et al. 2008). Due to its higher reproduction potential, more generations per year, and year-round persistence on grapevines, the invasive *P. ficus* is likely to be the most effective mealybug vector species of GLRaV-3 in California. Moreover, the high population density and prevalence of crawlers throughout the growing season makes this pest easily dispersed by wind, animals and farm equipments. Currently, standard control practices for mealybugs in California vineyards are based on suppressing populations below the economic thresholds for direct damage to grape clusters (Bentley et al. 2011, Daane et al. 2011), whereas the tolerance threshold for *P. ficus* as a vector of GLRaV-3 should be much lower. The seasonal dynamics of *P. ficus* population match the seasonal abundance of GLRaV-3 within infected vines observed here. The population of *P. ficus* increases in spring and remains at a high level until August (Daane et al. 2008); the frequency of GLRaV-3–positive leaves seems to increase from spring to summer but decrease during fall. These results are similar to those obtained by Fiore et al. (2009) in Chile, where the frequency of GLRaV-3 detection in leaf petioles by RT-PCR decreased toward the end of a growing season. Therefore, in addition to planting healthy certified vegetative material, virus monitoring may be an important parameter to incorporate into strategies to reduce leafroll virus spread.

The roles of vector and pathogen seasonality, within-plant distribution, and population numbers on disease spread have been poorly explored for most vector-borne plant disease systems. Interconnecting these factors is somewhat simpler for systems in which infective vectors migrate from areas adjacent to focal crops at specific times of the year, as in diseases driven by primary spread such as Tomato spotted wilt (Groves et al. 2002) and grapevine Pierce's disease (Purcell 1974). However, for plant diseases in which secondary spread drives epidemics, the role of pathogen populations within crop plants in relation to vector feeding behavior and disease spread is likely to impact disease spread. Information regarding pathogen populations in plants, vector feeding behavior and disease spread is available for many plant diseases but is fragmented and needs to be integrated through experimental and modeling approaches.

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References Cited

Belli, G., A. Fortusini, P. Casati, L. Belli, P. A. Bianco, and S. Prati. 1994. Transmission of a grapevine leafroll associated closterovirus by the scale insect *Pulvinaria vitis* L. Riv. Patol. Veg. 4: 105–108.

Bentley, W. J., K. M. Daane, P. A. Phillips, M. C. Battany, and W. Peacock. 2011. Psuedococcus mealybugs. Pest Management Manual, 3rd edition. Agricultural Natural Resources Publ. 3343. University of California, Oakland.

Borgo, M., E. Angelini, and R. Flamini. 2003. Effects of grapevine leafroll associated virus 3 on main characteristics of three vineyards. L'enologo 3: 99–110.

Cabaleiro, C., and A. Segura. 1997. Field transmission of grapevine leafroll associated virus 3 (GLRaV-3) by the mealybug *Planococcus citri.* Plant Dis. 81: 283–287.

Cabaleiro, C., and A. Segura. 2006. Temporal analysis of *grapevine leafroll associated virus* 3 epidemics. Eur. J. Plant Pathol. 114: 441–446.

Cabaleiro, C., C. Couceiro, S. Pereira, M. Cid, M. Barrasa, and A. Segura. 2008. Spatial analysis of epidemics of Grapevine leafroll associated virus-3. Eur. J. Plant Pathol. 121: 121–130.

Charles, J. G., D. Cohen, J.T.S. Walker, S. A. Forgie, V. A. Bell, and K. C. Breen. 2006. A review of the ecology of grapevine leafroll associated virus type 3 (GLRaV-3). N Z Plant Prot. 59: 330–337.

Coetzee, B., M. J. Freeborough, H. J. Maree, J. M. Celton, D. J. Rees, and J. T. Burger. 2010. Deep sequencing analysis of viruses infecting grapevines: virome of a vineyard. Virology 400: 157–163.

Credi, R., and A. R. Babini. 1997. Effects of virus and virus-like infections on growth, yield and fruit quality of Albana and Trebbiano Romagnolo grapevines. Am. J. Enol. Vitic. 48: 7–12.

Daane, K. M., W. J. Bentley, V. M. Walton, R. Malakar-Kuenen, J. G. Millar, C. A. Ingels, E. A. Weber, and C. Gispert. 2006. New controls investigated for vine mealybug. Calif. Agric. 60: 31–38.

Daane, K. M., M. L. Cooper, S. V. Triapitsyn, V. M. Walton, G. Y. Yokota, D. R. Haviland, W. J. Bentley, K. E. Godfrey, and L. R. Wunderlich. 2008. Vineyard managers and researchers seek sustainable solutions for mealybugs, a changing pest complex. Calif. Agric. 62: 167–176.

Daane, K. M., W. J. Bentley, R. J. Smith, D. R. Haviland, E. Weber, C. Gispert, M. C. Battany, and J. G. Millar. 2011. Vine mealybug. Pest Management Manual, 3rd edition. Agricultural Natural Resources Publ. 3343. University of California, Oakland.

Daugherty, M. P., J.R.S. Lopes, and R.P.P. Almeida. 2010. Vector within-host feeding preference mediates transmission of a heterogeneously distributed pathogen. Ecol. Entomol. 35: 360–366.

Elliott, D. I., and C. J. Hodgson. 1996. The distribution of the vetch aphid on bean stems in relation to stylet length and phloem depth. Entomol. Exp. Appl. 78: 175–180.

Engelbrecht, D. J., and G.G.F. Kasdorf. 1990. Transmission of grapevine leafroll disease and associated closteroviruses by the vine mealybug *Planococcus ficus.* Phytophylactica 22: 341–346.

Fereres, A., and A. Moreno. 2009. Behavioural aspects influencing plant virus transmission by homopteran insects. Virus Res. 141: 158–168.

Fernandez-Calvino, L., D. Lopez-Abella, J. J. Lopez-Moya, and A. Fereres. 2006. Comparison of *Potato virus Y* and *Plum pox virus* transmission by two aphid species in relation to their probing behavior. Phytoparasitica 34: 315–324.

Fiore, N., S. Prodan, and A. M. Pino. 2009. Monitoring grapevine viruses by ELISA and RT-PCR throughout the year. J. Plant Pathol. 91: 489–493.

Godfrey, K. E., J. Ball, D. Gonzalez, and E. Reeves. 2003. Biology of the vine mealybug in vineyards in the Coachella Valley, California. Southwest. Entomol. 28: 183–196.

Golino, D. A., and R. Almeida. 2008. Studies needed of vectors spreading leafroll disease in California vineyards. Calif. Agric. 62: 174.

Golino, D. A., E. A. Weber, S. T. Sim, and A. Rowhani. 2008. Leafroll disease is spreading rapidly in a Napa Valley vineyard. Calif. Agric. 62: 156–160.

Groves, R. L., J. F. Walgenbach, J. W. Moyer, and G. G. Kennedy. 2002. The role of weed hosts and tobacco thrips, *Frankliniella fusca,* in the epidemiology of *Tomato spotted wilt virus.* Plant Dis. 86: 573–582.

Guidoni, S., F. Mannini, A. Ferrandino, N. Argamante, and R. Di Stefano. 2000. Effect of virus status on leaf and berry phenolic compounds in two wine grapevine *Vitis vinifera* cultivars. Acta Hortic. 526: 445–452.

Gutierrez, A. P., K. M. Daane, L. Ponti, V. M. Walton, and C. K. Ellis. 2008. Prospective evaluation of the biological control of vine mealybug: refuge effects and climate. J. Appl. Ecol. 45: 524–536.

Habili, N., C. F. Fazeli, A. Ewart, R. Hamilton, R. Cirami, P. Saldarelli, A. Minafra, and M. A. Rezaian. 1995. Natural spread and molecular analysis of grapevine leafroll-associated virus 3 in Australia. Phytopathology 85: 1418–1422.

Habili, N., and F. W. Nutter, Jr. 1997. Temporal and spatial analysis of grapevine leafroll-associated virus 3 in Pinot Noir grapevines in Australia. Plant Dis. 81: 625–628.

Ling, K. S., H. Y. Zhu, N. Petrovic, and D. Gonsalves. 2001. Comparative effectiveness of ELISA and RT-PCR for detecting grapevine leafroll-associated closterovirus-3 in field samples. Am. J. Enol. Vitic. 52: 21–27.

Maliogka, V. I., C. I. Dovas, and N. I. Katis. 2008. Evolutionary relationships of virus species belonging to a distinct lineage within the *Ampelovirus* genus. Virus Res. 135: 125–135.

Martelli, G. P., A. A. Agranovsky, M. Bar-Joseph, D. Boscia, T. Candresse, R.H.A. Coutts, V. V. Dolja, B. W. Falk, D. Gonsalves, W. Jelkmann, et al. 2002. The family *Closteroviridae* revised. Arch. Virol. 147: 2039–2044.

Martelli, G. P., and E. Boudon-Padieu. 2006. Directory of infectious diseases of grapevines and viruses and virus-like diseases of the grapevine: bibliographic report 1998–2004. Options Méditerranéennes, Ser. B, N. 55. Mediterranean Agronomic Institute of Valenzano, Bari, Italy.

Martin, R. R., K. C. Eastwell, A. Wagner, S. Lamprecht, and I. E. Tzanetakis. 2005. Survey for viruses of grapevine in Oregon and Washington. Plant Dis. 89: 763–766.

Marucci, R. C., J.R.S. Lopes, J. D. Vendramim, and J. E. Corrente. 2004. Feeding site preference of *Dilobopterus costalimai* Young and *Oncometopia facialis* (Signoret) (Hemiptera: Cicadellidae) on citrus plants. Neotrop. Entomol. 33: 759–768.

Osman, F., C. Leutenegger, D. Golino, and A. Rowhani. 2007. Real-time RT-PCR (TaqMan) assays for the detection of *Grapevine leafroll associated viruses* 1–5 and 9. J. Virol. Methods 141: 22–29.

Pelletier, Y., X. Nie, M. McClure, S. Whitney, and M. A. Giguère. 2008. Behavior of bird cherry-oat aphid and green peach aphid in relation to Potato virus Y transmission. J. Econ. Entomol. 101: 728–735.

Petersen, C. L., and J. G. Charles. 1997. Transmission of grapevine leafroll-associated closteroviruses by *Pseudococcus longispinus* and *P. calceolariae.* Plant Pathol. 46: 509–515.

Purcell, A. H. 1974. Spatial patterns of Pierce's disease in the Napa Valley. Am. J. Enol. Vitic. 25: 162–167.

Sforza, R., E. Boudon-Padieu, and C. Greif. 2003. New mealybug species vectoring grapevine leafroll-associated viruses-1 and -3 (GLRaV-1 and -3). Eur. J. Plant Pathol. 109: 975–981.

Srinivasan, R., and J. M. Alvarez. 2007. Effect of mixed viral infections (Potato virus Y-Potato leafroll virus) on biology and preference of vectors *Myzus persicae* and *Macrosiphum euphorbiae* (Hemiptera: Aphididae). J. Econ. Entomol. 100: 646–655.

Swallow, W. H. 1985. Group testing for estimating infection rates and probabilities of disease transmission. Phytopathology 75: 882–889.

Tsai, C. W., J. Chau, L. Fernandez, D. Bosco, K. M. Daane, and R.P.P. Almeida. 2008. Transmission of *Grapevine leafroll-associated virus* 3 by the vine mealybug (*Planococcus ficus*). Phytopathology 98: 1093–1098.

Tsai, C. W., A. Rowhani, D. A. Golino, K. M. Daane, and R.P.P. Almeida. 2010. Mealybug transmission of grapevine leafroll viruses: an analysis of virus-vector specificity. Phytopathology 100: 830–834.

Tsai, J. H., J. J. Wang, and Y. H. Liu. 2002. Seasonal abundance of the Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae) in southern Florida. Fla. Entomol. 85: 446–451.

Walton, V. M., and K. L. Pringle. 2004. Vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a key pest in South African vineyards. A review. South Afr. J. Enol. Vitic. 25: 54–62.

Walton, V. M., K. M. Daane, W. J. Bentley, J. G. Millar, T. E. Larsen, and R. Malakar-Kuenen. 2006. Pheromone-based mating disruption of *Planococcus ficus* (Hemiptera: Pseudococcidae) in California vineyards. J. Econ. Entomol. 99: 1280–1290.

Wang, R. Y., and S. A. Ghabrial. 2002. Effect of aphid behavior on efficiency of transmission of *Soybean mosaic virus* by the soybean-colonizing aphid, *Aphis glycines.* Plant Dis. 86: 1260–1264.

Woodham, R. C., A. J. Antcliff, L. R. Krake, and R. H. Taylor. 1984. Yield differences between sultana clones related to virus status and genetic factors. Vitis 23: 73–83.

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