Pseudococcus maritimus (Hemiptera: Pseudococcidae) and *Parthenolecanium corni* (Hemiptera: Coccidae) Are Capable of Transmitting Grapevine Leafroll-Associated Virus 3 Between *Vitis* x *labruscana* and *Vitis vinifera*

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ABSTRACT The grape mealybug, *Pseudococcus maritimus* (Ehrhorn), and European fruit lecanium scale, *Parthenolecanium corni* (Bouché), are the predominant species of Coccoidea in Washington State vineyards. The grape mealybug has been established as a vector of Grapevine leafroll-associated virus 3 (GLRaV-3) between wine grape (*Vitis vinifera* L.) cultivars, elevating its pest status. The objective of this study was to determine if GLRaV-3 could be transmitted between *Vitis* x *labruscana* L. and *V. vinifera* by the grape mealybug and scale insects. Three transmission experiments were conducted with regard to direction; from *V. vinifera* to *V.* x *labruscana* L., from *V.* x *labruscana* L. to *V.* x *labruscana* L., and from *V.* x *labruscana* L. to *V. vinifera*. Each experiment was replicated 15 times for each vector species. Crawlers (first-instars) of each vector species were allowed 1-wk acquisition and inoculation access periods. The identities of viral and vector species were confirmed by reverse transcription-polymerase chain reaction, cloning, and sequencing of species-specific DNA fragments. GLRaV-3 was successfully transmitted by both species in all experiments, although *Ps. maritimus* was a more efficient vector under our experimental conditions. To the best of our knowledge, this study represents the first documented evidence of interspecific transmission of GLRaV-3 between two disparate *Vitis* species. It also highlights the potential role of *V.* x *labruscana* L. in the epidemiology of grapevine leafroll disease as a symptomless source of GLRaV-3 inoculum.

RESUMEN En los viñedos del Estado de Washington, las especies dominantes de la Superfamilia Coccoidea son la cochinilla de la uva *Pseudococcus maritimus* (Ehrhorn) y la escama café Europea *Parthenolecanium corni* (Bouché). *Ps. maritimus* se ha establecido como un vector del virus denominado *Grapevine leafroll-associated virus 3* (GLRaV-3) el cual es transmitido a diferentes variedades de uvas viníferas (*Vitis vinifera* L.). Este virus es un agente causal de la enfermedad del enrollamiento de la hoja de la vid (GLRD, por sus sigla en inglés) y debido a su impacto negativo en la economía de la industria de la uva; a su insecto vector se le ha categorizado como plaga. El objetivo del presente estudio fue el de determinar si la cochinilla de la uva y la escama café Europea podrían transmitir el virus GLRaV-3 entre las especies *V. labruscana* y *V. vinifera*, para ello fueron llevado a cabo tres experimentos de transmisión: de *V. vinifera* a *V. labruscana*, de *V. labruscana* a *V. labruscana* y de *V. labruscana* a *V. vinifera*. Cada experimento fue replicado 15 veces por especie vectora. El periodo de adquisición e inoculación del virus por parte del primer estadio de cada especie vectora fue de una semana. La identidad de las especies de virus e insectos fue confirmada mediante RT-PCR, clonación y secuenciación de los fragmentos de ADN específicos para cada especie. GLRaV-3 fue transmitido exitosamente por ambas especies vectoras en todos los experimentos, sin embargo, *Ps. maritimus* resultó ser un vector más eficiente bajo las condiciones de los experimentos. Hasta donde conocemos, el presente estudio representa la primera evidencia documentada de la transmisión interespecífica de GLRaV-3 entre dos especies de *Vitis*. Es también importante subrayar el rol potencial que posee *V. labruscana* en la epidemiología de GLRD por ser un hospedero asintomático de GLRaV-3.

KEY WORDS GLRaV-3, vector, *Vitis*, transmission, mealybug

The grape mealybug, *Pseudococcus maritimus* (Ehrhorn), is one of several mealybug species infesting grapevines (*Vitis* spp.) in the United States (Golino et al. 2002). The species is native to North America (Ben–Dov 1995) and was originally described in 1900 (Ehrhorn 1900). This species has been recorded as an economic pest on grapevines (Flebut 1922), nursery stock of *Taxus* spp. (Neiswander 1949), apricots (Madsen and McNelly 1960), and pears (Madsen and Westigard 1962). However, these records are questionable because of the lack of reliable taxonomic means to differentiate the grape mealybug from the obscure mealybug (*Pseudococcus viburni* Signoret). The de-

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velopment of more reliable taxonomic descriptors (Miller et al. 1984) and molecular methods (Daane et al. 2011) has improved accurate identification of different mealybug species in the vineyards (Daane et al. 2011). The European fruit lecanium scale (*Parthenolecanium corni* Bouché) is a widespread species of soft scale that is known from most of Europe, Turkey, Lebanon, Russia, Algeria, Libya, and New Zealand (Santas 1985). This soft scale insect pest is also widely distributed in the United States (Gill 1988), with records from California, Connecticut, Florida, Illinois, Indiana, Iowa, Kansas, Maine, Massachusetts, New York, Ohio, Rhode Island, and Tennessee (ScaleNet 2012). It is a polyphagous insect (Ben–Dov 1995) and is typically monovoltine (Gill 1988, Sforza 2000).

Historically, it has been the indirect damage from the feeding activities of *Pa. corni* and *Ps. maritimus* that has caused the greatest perceived economic injury in vineyards, because of accumulation of honeydew and, subsequently, growth of sooty mold on berry clusters (Gill 1988, Geiger and Daane 2001). However, it was later discovered that *Ps. maritimus* is a competent vector for Grapevine leafroll-associated virus 3 (GLRaV-3; Golino et al. 2002). GLRaV-3 is the most widespread virus species present in grapevines affected by grapevine leafroll disease (GLRD). In addition, a more recent study also demonstrated that *Pa. corni* is capable of transmitting GLRaV-1 (Hommay et al. 2008), another virus species associated with GLRD. Thus, the economic abundance thresholds of both *Ps. maritimus* and *Pa. corni* have been significantly reduced because of their status as vectors of GLRaVs. Such low abundance thresholds for both *Ps. maritimus* and *Pa. corni* have highlighted the need for faster and more reliable means for their detection (i.e., pheromone-based monitoring) within the vineyards (Bahder et al. 2013). GLRaV-3 is also known to be vectored by other species of Coccoid insects, including *Ceroplastes reusci* (Mahfoudhi et al. 2009), *Heliococcus bohemicus* (Sforza et al. 2003), *Planococcus ficus* (Tsai et al. 2008), *Planococcus citri* (Cabaleiro and Segura 1997), *Phenacoccus aceris* (Sforza et al. 2003), *Pseudococcus calceolariae* (Petersen and Charles 1997‘), *Pseudococcus longispinus* (Petersen and Charles 1997), *Ps. viburni* (Golino et al. 2002), and *Pulvinaria vitis* (Belli et al. 1994). This disease and its vectors are present in all major grape-growing regions of the world (Naidu et al. 2008).

GLRD is the one of the most devastating viral diseases of wine grapes (*Vitis vinifera* L.) in all major grape-growing regions of the world and can cause significant yield losses (Rayapati et al. 2008). The disease is prevalent in Washington State vineyards (Martin et al. 2005), and its symptoms in red-fruited wine grape cultivars include reddening of interveinal areas and downward rolling of leaf margins. Leaves of affected white-fruited wine grape cultivars may show chlorosis and downward rolling of leaf margins. The detection of GLRaV-3, and other GLRaVs, in *Vitis* x *labruscana* L. is difficult because of latent infections, rendering infected vines asymptomatic. Unlike wine grapes, yield losses are not documented in *V.* x *labruscana* L. vines affected by GLRD. As a result of the perceived lack of economic impact of GLRD on *V.* x *labruscana* L., very little attention has been paid to monitoring spread of GLRaVs or control of *Ps. maritimus* and *Pa. corni* in Concord juice grape vineyard blocks in Washington State. However, prophylactic systemic applications of insecticides are conventional practices taken to minimize potential losses because of GLRD in *V. vinifera* vineyards. Both *Pa.* corni and *Ps. maritimus* are primary candidates for studying the epidemiology of GLRD in Washington State because both species have established populations in *V. vinifera* and *V.* x *labruscana* L. vineyards (B.W.B., unpublished data).

Although it has been demonstrated that *Ps. maritimus* is a competent vector of GLRaV-3 (Golino et al. 2002), nothing is known about its role in the epidemiology of GLRD within Concord juice grape (*V.* x *labruscana* L.) vineyards. In Washington State, blocks of *V.* x *labruscana* L. are often grown in proximity to *V. vinifera* blocks within the same vineyard. GLRaV-3 was documented for the first time from samples derived from asymptomatic *V.* x *labruscana* L. grapevines, thus indicating that this virus can infect other *Vitis* species (Soule et al. 2006). In addition, populations of *Ps. maritimus* are known to infest *V.* x *labruscana* L. vineyards in Washington State (Frick 1952). Taken together, it is plausible to assume that *V.* x *labruscana* L. may be playing a role in the epidemiology of GLRD by serving as a source of virus inoculum for spread by a competent vector into adjacent *V. vinifera* vineyards. Thus, investigating the vectoring ability of *Ps. maritimus* to transmit GLRaVs from *V. vinifera* to *V.* x *labruscana* L., and vice versa, would help in the understanding of the epidemiology of GLRD. In addition, although *Pa. corni* has been demonstrated to transmit GLRaV-1 between wine grapes under greenhouse conditions (Hommay et al. 2008), there is a lack of information on its role in the spread of GLRaVs between *V.* x *labruscana* L. and *V. vinifera*, and vice versa.

The primary objective of this research is to determine if *Ps. maritimus* is capable of transmitting GLRaV-3 between *V.* x *labruscana* L. and *V. vinifera* grapevines in a greenhouse environment. The secondary objective is to determine if *Pa. corni* is a competent vector of GLRaV-3 and can also transmit the virus between *V.* x *labruscana* L. and *V. vinifera* grapevines. The results of this study will increase our understanding of the role of *Pa corni* and *Ps. maritimus* in the epidemiology of GLRD in Washington State and, by extension, other major cool climate grape-growing regions of the world. This information could also assist growers in combating the spread of GLRD and its vector.

Materials and Methods

Insect Colonies and Plant Material. Specimens of *Pa. corni* and *Ps. maritimus* were obtained as eggs from *V.* x *labruscana* L. vineyards in Prosser, WA, and reared on *V. vinifera* cultivar ‘Pixie’ (a dwarf Pinot

Table 1. Primer sequences for virus and insect verification

Species	Gene	Direction	Sequence (5′→3′)	PCR product size (bp)
Pa. corni	COI	Forward	CAGGAATAATAGGAACATCAATAAG[a]	550
		Reverse	ATCAATGTCTAATCCGATAGTAAATA[a]	
Ps. maritimus	COI	Forward	CTGATTTCCTTTATTAATTAATTCAAC[b]	400
		Reverse	CAATGCATATTATTCTGCCATATTA[b]	
GLRaV-3	Hsp70	Forward	CGCTAGGGCTGTGGAAGTATT[c]	546
		Reverse	GTTGTCCCGGGTACCAGTTAT[c]	

[a] Deng et al. 2012.
[b] Daane et al. 2011.
[c] Osman and Rowhani 2006.

Meunier) seedlings in a controlled climate greenhouse at 25–30°C with a 14-h day-length and a 10-h dark period. Colonies were housed in separate collapsible observation and rearing cages (Bioquip Rancho Dominguez, CA). Virus-free *V.* x *labruscana* L. plantlets were initially obtained from a commercial nursery (Inland Desert INC., Benton City, WA) and mist-propagated, by using DIP'N GROW (1.0% Indole-3-butyric acid, 0.5% 1-Napthaleneacetic acid; DIP'N GROW, INC, Clackamas, OR) to stimulate root growth. Certified virus-free cuttings of *V. vinifera* cultivar 'Cabernet Sauvignon' were obtained from the National Clean Plant Network at the Washington State University IAREC (Prosser, WA).

Identification of *Pa. corni* and *Ps. maritimus*. Species identifications were made by Dr. Gillian Watson at the Plant Pest Diagnostic Branch of the California Department of Agriculture (Sacramento, CA). Voucher specimens of *Pa. corni* and *Ps. maritimus* from Washington State were taken from established colonies and stored at Washington State University's Irrigated Agriculture Research and Extension Center (Prosser, WA). Sequence data were obtained from the COI region of both species by using primers designed by Daane et al. (2011) and Deng et al. (2012) (Table 1) to complement the morphological data. Because of the difficulty in using morphological characters for species-level identification in the Coccoidea, a result of high levels of morphological variation even within species, sequence data were analyzed to ensure species identity. For this purpose, genomic DNA was extracted by grinding individual, adult insects in 25 μl of GES buffer (0.1M glycine, 50 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, and pH 6.93) containing a 1% mercaptoethanol. The homogenate was denatured at 95°C for 10 min, and then cooled on ice for 5 min (Rowhani et al. 2000). All reactions were a total volume of 12.5 μl, by using 1 μl of the denatured extract of genomic DNA, regardless of its concentration. Concentrations for reagents were as follows: 4 mM $MgCl_2$, 0.25 μM for each primer, 0.1 mM of each dNTP per microliter, and 0.75 U *Taq*DNA polymerase (Roche Diagnostics, Mannheim, Germany). Amplification was performed in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Carlsbad, CA) with the following conditions: initial denaturation at 94°C for 30 s followed by 35 cycles of melting at 94°C for 30 s, annealing at 55°C for 45 s, extension at 72°C for 30 s, and a final extension at 72°C for 7 min. To visualize amplification, 5 ml of polymerase chain reaction (PCR) product was run on 1.5% agarose gel stained with GelRed (Biotium, Hayward, CA). Samples that produced strong bands were sequenced directly from the PCR product, whereas those that produced weak bands were first cloned by using TOPO TA Cloning kit (Invitrogen, Carlsbad, CA) before sequencing.

Transmission Bioassays. Ninety transmission bioassays were conducted from 2011 to 2012. Three different experiments were conducted for both species of insect, *Pa. corni* and *Ps. maritimus*. For both species, acquisition and inoculation access periods of 1 wk each were tested due the semipersistent transmission mode of GLRaVs, where 10 first-instar individuals were allowed to feed on the virus source plant for a 1-wk acquisition access period (AAP) and were then transferred to a virus-free recipient plant for a 1-wk inoculation access period. This was accomplished by cutting the section of the leaf where the insects were feeding and placing the leaf section on the recipient plant, allowing the insect to naturally change hosts and avoiding direct contact with the insect so as to reduce mortality. The transfer of 10 individuals from source to recipient was replicated 15 times across all experiments. Transmission bioassays consisted of transferring individuals from an infected *V. vinifera* plant to virus-free *V.* x *labruscana* L. plants, an infected *V.* x *labruscana* L. plant to virus-free *V.* x *labruscana* L., and from an infected *V.* x *labruscana* L. to virus-free *V. vinifera* plants. All recipients were tested multiple times at 2-wk, 1-mo, 3-mo, and 6-mo intervals postinoculation access period. Specimens of both *Pa. corni* and *Ps. maritimus* were collected from the GLRaV-3-positive source plant and extracts from these insects were tested by reverse transcription-polymerase chain reaction (RT-PCR) to verify that both insects could acquire the virus. As a positive control to validate the transmission protocol, five transmissions for each species of vector were conducted between plants of *V. vinifera* by using the same technique used for the interspecific transmission assays, as both insect species have previously been shown to transmit GLRaVs between plants of *V. vinifera* (Golino et al. 2002, Sforza et al. 2003, Hommay et al. 2008, Tsai et al. 2010). To test for the presence or absence of GLRaV-3 in recipient plants, the positive source plant, and in each vector species, single-tube RT-PCR reactions were performed by using primers targeting a portion of the heat-shock protein-70 (hsp-70) homolog gene of

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Pmar_COI_Daane TTCAATTTTCATTAAATGTATGATTTAAATTTGGATTATTAT-TTAATCATTCAGGATTAAAAAATTTTATTTTAAAT
Pmar_COI       ..A.....................................-.....................................
Pvib_COI       AAT..A.A.A...TTTGC...A....C....G.A...T.AGGA..T..TG.ATGA.C.C.TC.T..---....ACT..

Pmar_COI_Daane AAAATTAAACGTTTAGAAATTAA-AGATTCAATGATAATAAAAATGAATATAATAGTAAATAAAATAGTTATAAAAGA
Pmar_COI       ......................-.........A.....................................
Pvib_COI       TGG....G.TA..G.T.CTCA.TT.T...TT..ATC.GC..CT..A.T...--.GC..TTCC..C...AAT.....TT

Pmar_COI_Daane TCCAATAGAAGAAATATTGTTTCATAAAATAAAATAATCTGAATATATAATATATCGACGTGGTATACCATTTAATCC
Pmar_COI       .................A.....................................................
Pvib_COI       .TT.G.T...T..TA.C.-..AA..GG..A.....GT..AA.T.CATC...T.TATT.T.ATCA...GG.....T.AT

Pmar_COI_Daane TAAAAA-ATGTTGAGGAAAAAAAGTTAAATTAATTGATAAAAATAAATTTATAAAATTAATTTTTA-ATCAATTATTA
Pmar_COI       ......-.........G.....................................................-..........
Pvib_COI       ..T.TTT.CAA.AG.TGGTTT..CAGG.....T.CTT.CT..T.C....AT.G.T........AC.TGAT.C.....T

Pmar_COI_Daane T-TTAAAGTTGAATTAATTAATAAAGGAAATCAG
Pmar_COI       .-.............................
Pvib_COI       .G...TT.C.C.T..TCA.T..GTTCTTTC.ATA
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Fig. 1. Sequence data for the COI gene of *Ps. maritimus*; Pmar_COI_Daane represents sequence data for *Ps. maritimus* provided by Daane et al. 2011, Pmar_COI represents COI sequence data obtained from individuals used in transmission bioassays, and Pvib_COI represents COI sequence data from *Ps. viburni*, the sister species to *Ps. maritimus*.

GLRaV-3 (Table 1). Extraction of virus genetic material from plants was accomplished by grinding 25 mg of petiole tissue in 5 ml of general extraction buffer (GEB; 1.59 g/L Na_2CO_3, 2.93 g/L $NaHCO_3$, 2% polyvinylpyrrolidone mw 40,000, 0.2% bovine serum albumin, 0.5% Tween 20, and pH 6.9), and the extracts were stored at −80°C until use. To extract viral RNA, 4 μl of the GEB extract was denatured in 25 μl of GES solution (containing 1% mercaptoethanol), as described earlier. All RT-PCR reactions, reagent concentrations, and visualization steps for verifying the presence or absence of GLRaV-3 are the same as described by Rowhani et al. (2000). Samples that produced strong bands were sequenced directly from the PCR product, whereas those that produced weak bands were first cloned by using TOPO TA Cloning kit (Invitrogen) before sequencing.

Data Analysis. Sequence data were analyzed by using Vector NTI (Life Technologies, Grand Island, NY) and MEGA 5 (Tamura et al. 2011). Logit analysis was used to determine whether probability of infection was affected by vector (*Pa. corni* vs *Ps. maritimus*) and by direction of the transmission (*V. vinifera* to *V.* x *labruscana* L., *V.* x *labruscana* L. to *V.* x *labruscana* L., and *V.* x *labruscana* L. to *V. vinifera*). The analysis was done by using PROC GENMOD in SAS. The saturated model with main effects and the interaction term was fitted. The ilink option was used to transform means back to proportions. The odds ratio for the vector effect was calculated by using the estimate option.

Results

Species Identity. In pair-wise comparisons, sequence data for the COI region of *Ps. maritimus* from the colony used in the transmission bioassays (KC679784) shared 99% nucleotide identity with *Ps. maritimus* sequence data obtained from GenBank (JN112800) and 81% nucleotide identity with *Ps. viburni* (JN112803; Fig. 1). Furthermore, sequence data for the COI region of *Pa. corni* from the colony used in the transmission bioassays (KC679783) shared 99% nucleotide identity with *Pa. corni* sequence data obtained from GenBank (JQ795617) (Fig. 2). Finally, hsp-70 homolog-derived sequences of the GLRaV-3 isolate (KC113195) obtained from the virus-infected source plant shared 99% nucleotide identity with the WA isolate (DQ780890). Gene fragments of COI for *Pa. corni* were 399 bp (Fig. 1), gene fragments of COI for *Ps. maritimus* were 543 bp (Fig. 2), and gene fragments of hsp-70 from GLRaV-3 isolated from source plant, vector, and recipient were 546 bp (Fig. 3). Based on nucleotide identity and fragment sizes produced, these results confirmed the identities of the virus and vector species used in this study.

Transmission Bioassays. Transmission efficiency of *Ps. maritimus* varied between 26.7 and 53.3%, whereas efficiency of *Pa. corni* was substantially lower at 6.7%. We observed transmission in all three directions. The logit analysis revealed a highly significant vector effect ($df = 1, \chi^2 = 12.10, P = 0.0005$.). The odds ratio for the vector effect was 7.1 (confidence interval of 2.0–24.4), indicating that the likelihood of infection under controlled greenhouse conditions was about sevenfold higher if *Ps. maritimus* was the vector than if *Pa. corni* was the vector. Transmission success was not affected by direction of the transmission ($df = 2, \chi^2 = 0.11, P = 0.9453$) among grape species. The interaction between vector and transmission direction among grape species also was not significant ($df = 2, \chi^2 = 1.81, P = 0.4041$). Of the 100 individuals of *Pa. corni* that were tested for presence of GLRaV-3 from the positive source plant, 41% tested positive for GLRaV-3, whereas 17% of the 100 *Ps. maritimus* sampled tested positive for GLRaV-3. For the positive control bioassays, there was 20% successful transmission for *Pa. corni* and 40% successful transmission for *Ps. maritimus*.

Both species of vector were capable of transmitting GLRaV-3 from *V. vinifera* to *V.* x *labruscana* L. under experimental conditions. Of the 15 replicates performed for each vector species, there were six suc-

Fig. 2. Sequence data for the COI gene of *Pa. corni*; Pcor_COI_Deng represents sequence data for *Pa. corni* provided by Deng et al. (JQ795617.1) and Pcor_COI represents sequence data for *Pa. corni* used in transmission bioassays.

cessful transmissions for *Ps. maritimus* (Table 2), whereas *Pa. corni* had one successful transmission (Table 2). Both species of vector were capable of transmitting GLRaV-3 from *V.* x *labruscana* L. to *V.* x *labruscana* L. under experimental conditions. Of the 15 replicates performed for each vector species, there were four successful transmissions for *Ps. maritimus* (Table 3), whereas *Pa. corni* had one successful transmission (Table 3). Both species of vector were capable of transmitting GLRaV-3 from *V.* x *labruscana* L. to *V. vinifera* under experimental conditions. Of the 15 replicates performed for each vector species, there were eight successful transmissions for *Ps. maritimus* (Table 4), whereas *Pa. corni* had one successful transmission (Table 4). All samples that tested positive for GLRaV-3 were cloned and sequenced to verify identity.

Discussion

Based on the results of this study, it can be concluded that both *Pa. corni* and *Ps. maritimus* are competent vectors of GLRaV-3 between *V.* x *labruscana* L. and *V. vinifera* under controlled greenhouse conditions. The control transmissions of GLRaV-3 between *V. vinifera* by *Ps. maritimus* yielded a 40% success rate, which is comparable with the 41% success rate achieved by Golino et al. (2002). This is the first report of *Pa. corni* successfully transmitting GLRaV-3 between *Vitis* species, and it appears that the transmis-

Fig. 3. Sequence data for hsp-70 gene of GLRaV-3 used in transmission bioassays; sequence data obtained from virus in source plant (1), sequence data obtained from virus within the insect vector (2), and sequence data obtained from virus in recipient Concord plant (3).

Table 2. Number of *V.* x *labruscana* L. grapevines that tested positive for GLRaV-3 after exposure to vector species that had been feeding on a virus-infected grapevine; insects moved from *V. vinifera* to *V.* x *labruscana* L.

Species	Inoculation	Test date			
		28 Sept. 2011	14 Oct. 2011	14 Dec. 2011	14 Mar. 2012
Pa. corni	14 Sept. 2011	0/15	0/15	5/15	8/15
Ps. maritimus	14 Sept. 2011	0/15	0/15	0/15	1/15

Table 3. Number of *V.* x *labruscana* L. grapevines that tested positive for GLRaV-3 after exposure to vector species that had been feeding on a virus-infected grapevine; insects moved from *V.* x *labruscana* L. to *V.* x *labruscana* L.

Species	Inoculation	Test date			
		28 Sept. 2011	14 Oct. 2011	14 Dec. 2011	14 Mar. 2012
Pa. corni	14 Sept. 2011	0/15	0/15	4/15	4/15
Ps. maritimus	14 Sept. 2011	0/15	0/15	1/15	1/15

Table 4. Number of *V.* x *labruscana* L. grapevines that tested positive for GLRaV-3 after exposure to vector species that had been feeding on a virus-infected grapevine; insects moved from *V.* x *labruscana* L. to *V. vinifera*

Species	Inoculation	Test date			
		28 Sept. 2011	14 Oct. 2011	14 Dec. 2011	14 Mar. 2012
Pa. corni	14 Sept. 2011	0/15	0/15	8/15	8/15
Ps. maritimus	14 Sept. 2011	0/15	0/15	0/15	1/15

sion success of GLRaV-1 by this vector species is slightly higher with a 33% success rate between plants of *V. vinifera*, whereas our experiments with GLRaV-3 between plants of *V. vinifera* yielded a 6% success rate. Whereas *Pa. corni* appears to be more efficient at acquiring GLRaV-3, *Ps. maritimus* seems to be more efficient at transmitting the virus under controlled greenhouse conditions. The differences in the abilities of these two species to acquire and transmit GLRaV-3 may be a result of the biology of each insect or the result of stress from changing host plants. *Ps. maritimus* is typically more mobile, migrating to different regions of the vine throughout the season and among generations, whereas *Pa. corni* are typically sedentary once a suitable feeding site is found. This behavior may have caused *Pa. corni* to be more prone to stress, translating into lower survival or transmission rate when they were moved to a new food source in these studies. Although *Pa. corni* and *Ps. maritimus* were shown to be competent vectors of GLRaV-3 between *V.* x *labruscana* L. and *V. vinifera* under experimental conditions, it is not known if this transmission occurs in a natural, vineyard setting and if it does, if it is economically significant. There are variables present in a natural setting, such as presence of insect predators and parasitoids, adverse weather conditions, and pesticides that may prevent successful, frequent transmission or movement of first-instar crawlers among vineyards. In this light, future research will be needed to evaluate the economic impact of spread of GLRaV-3 by mealybugs and scale insects between *V.* x *labruscana* L. and *V. vinifera.* However, even if the movement of crawlers between vineyard types is very low and the transmission rate of GLRaVs in the field is very low, there is a potential that insect transmission of GLRaV-3 can still pose a threat to sustainability of the grape industry in the long-term. Furthermore, knowing that transmission is possible in both directions, *V. vinifera* to *V.* x *labruscana* L. and *V.* x *labruscana* L. to *V. vinifera*, will necessitate a more inclusive monitoring and management plan that includes all vineyards infected with GLRD, not only *V. vinifera.*

Because of the impact of GLRD on the wine grape industry, a more collaborative effort may be required between growers of *V.* x *labruscana* L. and *V. vinifera* to more effectively manage the spread of this disease in Washington State. One major aspect in the prevention of the spread of GLRD is control of vectors. In Washington State, this is accomplished through chemigation, a technique where a systemic insecticide is introduced through a drip irrigation system. Chemigation is the preferable means to control *Ps. maritimus* in vineyards because it does not disrupt populations of natural enemies that are effective at keeping *Ps. maritimus* populations low and it targets all feeding instars. Although successful in *V. vinifera* vineyards, this technique is not applicable to *V.* x *labruscana* L. in Washington State because the majority of vineyards of *V.* x *labruscana* L. are irrigated by overhead sprinklers, necessitating traditional spray insecticides to control pest populations. Thus, vineyards of *V.* x *labruscana* L. could be harboring populations of *Ps. maritimus* that could transmit GLRD to neighboring *V. vinifera* vineyards. Alternatively, the spray regimens being applied in *V.* x *labruscana* L. vineyards may have a negative impact on populations of natural enemies of *Ps. maritimus* and *Pa. corni*, leading to a build up of these pests in neighboring vineyards.

The results obtained in this study demonstrate for the first time that interspecies transmission of GLRaVs in grapes, *V.* x *labruscana* L. and *V. vinifera*, by *Pa. corni* and *Ps. maritimus* is possible under controlled greenhouse conditions in Washington State. While the degree to which this is occurring under natural conditions is unknown, the risk that GLRD poses to the sustainability of the wine industry highlights the need for immediate steps in expanding integrated pest management programs to include *V.* x *labruscana* L. Because the biological possibility exists for GLRaV-3 to be transmitted from *V.* x *labruscana* L. to *V. vinifera* under experimental conditions, it is likely that this transmission is occurring under field conditions. Because of this risk, future work should focus on evaluating the frequency at which GLRaV-3 is moving among vineyards of *V.* x *labruscana* L. and *V. vinifera* as well as the economic and biological significance of GLRaV-3 moving between vineyards of *V.* x *labruscana* L. and *V. vinifera*, so that responsible management decisions can be made that will allow for the sustainability of the wine grape industry in Washington State.

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