

# Chapter 24

## Vector Transmission of Grapevine Leafroll-Associated Viruses

E. Herrbach, A. Alliaume, C.A. Prator, K.M. Daane, M.L. Cooper, and R.P.P. Almeida

**Abstract** The ampeloviruses causing grapevine leafroll disease are transmitted by mealybug and soft scale insect vectors. Vector transmission of virus species in this complex occurs in a semi-persistent manner, with no current evidence of a strict vector-virus species specificity. However, only a limited number of studies have addressed questions such as virus-vector transmission efficiency, and there are no published studies on virus-vector molecular interactions. Here we present a summary of what is known about the vector transmission of grapevine-associated ampeloviruses. Because the management of grapevine leafroll diseases depends on a robust understanding of how these viruses are disseminated in vineyards, we also highlight research needs and knowledge gaps.

### Introduction

Reported by winegrowers since the turn of the twentieth century, the grapevine leafroll syndrome was regarded as being solely transmitted by cuttings and grafting, so much so that in 1988, Goheen wrote that “no vector for the causal agent of leafroll has been established” (Goheen 1988). However, the ability of the mealybug *Pseudococcus maritimus* to transmit leafroll in California had first been demonstrated, but not published, in 1961 by Dr. L. Chiarappa (Roscioglione et al. 1983; Martelli 2014a). Later on, the natural spread of leafroll in vineyards was reported by Dimitrijevic (1973) and correlated to the presence of scale insect populations, which were therefore suspected to act as the vectors in this disease system (Caudwell and Dalmaso 1985; Engelbrecht and Kasdorf 1985, 1990b; Teliz et al. 1989; Jordan

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E. Herrbach (✉) • A. Alliaume  
SVQV, INRA, Université de Strasbourg, 68000 Colmar, France  
e-mail: [etienne.herrbach@inra.fr](mailto:etienne.herrbach@inra.fr)

C.A. Prator • K.M. Daane • R.P.P. Almeida  
Department of Environmental Science, Policy and Management, University of California at Berkeley, Berkeley, CA, USA

M.L. Cooper  
University of California Cooperative Extension, Napa, CA, USA

1993). The first experimental evidence that mealybugs are vectors of *Grapevine leafroll-associated virus-3* (GLRaV-3) was published by Tanne (1988) in Israel, rapidly followed by Rosciglione and Gugerli (1989) in Switzerland and by Engelbrecht and Kasdorf (1990a) in South Africa. The latter represents the first peer-reviewed paper on the topic.

Within plant-sap-feeding hemipterans with piercing-sucking mouthparts, scale insects form the superfamily Coccoidea, which contains over 8000 species classified in ca. 30 extant families (García et al. 2016). Approximately 35 species in the Coccoidea have so far been identified as vectors of ca. 30 virus species, and they belong to only two families: the Pseudococcidae (mealybugs) and the Coccidae (soft scales) (Herrbach et al. 2016). As to grapevine leafroll viruses, only the members of the genus *Ampelovirus* (GLRaV-1, -3, and -4-like) are transmitted by scale insects, i.e., eleven mealybug and eight soft scale species, as far as known today. Note that the natural vectors (if any) of GLRaV-2 (genus *Closterovirus*) are unknown but would be expected to be aphids based on the evolutionary history of the group (Tsai et al. 2010; Klaassen et al. 2011). GLRaV-7 (genus *Velarivirus*), which has been proposed to be nonpathogenic to grapevines (see Chap. 10), is transmitted through the parasitic dodder plants *Cuscuta* spp. (Mikona and Jelkmann 2010).

Several species recognized as *Ampelovirus* vectors can also transmit the vitiviruses *Grapevine virus A* (GVA), GVB, and GVE, which are involved in the rugose wood disease complex of grapevine (see Chap. 11). The transmission of GVA by *Pseudococcus longispinus* was first demonstrated by Rosciglione et al. (1983). Vitiviruses are often detected in grapevine in coinfection with a leafroll-associated *Ampelovirus*; this frequent association of vitiviruses with ampeloviruses raises questions about their mutual interactions during vector transmission and/or grapevine infection. Nothing is known about the natural vectors, if any, of the vitiviruses GVD and GVF. It should be mentioned that attempts to transmit grapevine leafroll- or rugose wood-associated viruses by other grapevine sap-feeding insects, such as some aphid species including grape phylloxera (Charles et al. 2006; Conti et al. 1980; Kuniyuki et al. 1995; Teliz et al. 1989) and the flatid *Metcalfa pruinosa* (Materazzi et al. 1998), have all failed.

Various aspects of the role of scale insects in transmitting grapevine ampeloviruses and vitiviruses have been reviewed or summarized earlier in Charles et al. (2006), Laimer et al. (2009), Oliver and Fuchs (2011), Daane et al. (2012), Almeida et al. (2013), Maree et al. (2013), Martelli (2014a, b), Naidu et al. (2014, 2015), Hull (2016), and Herrbach et al. (2016). Here we focus exclusively on the vector transmission of grapevine leafroll-associated viruses. We also direct the reader to chapters in this book addressing grapevine leafroll-associated viruses (Chaps. 6 and 8) and vitiviruses (Chap. 11), topics relevant to this chapter but not covered here.

## The Vectors

### *Mealybug and Soft Scale Species Identified as Vectors*

After the reports of *Planococcus ficus* and *Ps. longispinus* as vectors of GVA and GLRaV-3, respectively (Roscioglione et al. 1983; Tanne 1988), research has demonstrated that many other grapevine-colonizing pseudococcid species transmit grapevine leafroll-associated viruses, including insects in the genera *Pseudococcus*, *Planococcus*, *Phenacoccus*, *Heliococcus*, and *Ferrisia* (Golino et al. 1994; Sforza et al. 2003; Tsai et al. 2010; Wistrom et al. 2016). Several species in the family Coccidae have also been identified as vectors of grape ampeloviruses and GVA, especially the grapevine-dwelling species *Parthenolecanium corni*, *Pulvinaria vitis*, and *Neopulvinaria innumerabilis* (Belli et al. 1994; Fortusini et al. 1997; Sforza et al. 2003). All vector species presently known as able to transmit at least one grape ampelo- or vitivirus are listed in Table 24.1. The fact that grape ampelo- and vitiviruses can be transmitted by members of two scale insect families is unique among vector-transmitted plant viruses and suggests a broad specificity or even a general lack of vector-virus specificity (Tsai et al. 2010). Therefore, additional vector species are expected to be identified in the future, even among insects that do not colonize grapevine and are unlikely to have any epidemiological relevance. Indeed, South African workers (Krüger and Douglas 2009; Krüger and Douglas-Smit 2013) obtained GLRaV-3 transmission events with the coccids *Coccus longulus*, *Parasaissetia nigra*, and *Saissetia* sp., when forced to feed on grapevine under laboratory conditions; however, these species did not survive on grapevine, a plant they rarely colonize in vineyards (García et al. 2016).

### *Vector Cycle and Ecology*

Although morphologically similar, each vector species has distinct biological characteristics and generally unique geographic origins that result in differing host plant preferences and present regional distributions (Daane et al. 2012). In general, vineyard mealybugs and soft scales have two or three larval instars for the female, and three or four instars for the male, with the last instar male going through a cocoon or pupal stage before the winged adult emerges (McKenzie 1967; Ben-Dov 1995; Wakgari and Giliomee 2005). Most species of mealybugs and soft scales lay eggs in an ovisac; however, some species like *Heliococcus bohemicus* are ovoviviparous. The general body shape is elongate-oval, and the body is covered with a protective wax secretion (mealybugs) or a chitinized shield (soft scales). Following each successive molt, the instars increase in size and amount of wax secreted. The first instar – which is commonly referred to as a crawler since it is considered the dispersal stage – measures ca. 0.6 mm, with the female growing to a length of 4–5 mm, depending on the species. The winged male typically measures ca. 1.5 mm in length.

**Table 24.1** Scale insect species of the families Pseudococcidae and Coccidae known as vectors of leafroll-associated ampeloviruses and of rugose wood-associated vitiviruses of grapevine

Scale insect species	GLRaV-1	GLRaV-3	GLRaV-4 strain 4	GLRaV-4 strain 5	GLRaV-4 strain 6	GLRaV-4 strain 9	GVA	GVB	GVE	References
<b>Pseudococcidae (mealybugs)</b>										
<i>Ferrisia gilli</i> (Gill's mealybug)		X								Wistrom et al. (2016)
<i>Heliococcus bohemicus</i> (Bohemian mealybug)	X			X			X			Sforza et al. (2003), Zorloni et al. (2003, 2004, 2006a) and Bertin et al. (2016b)
<i>Phenacoccus aceris</i> (apple mealybug)	X		X	X	X	X	X	X		Sforza et al. (2003) and Le Maguet et al. (2012)
<i>Planococcus citri</i> (citrus mealybug)	X						X	X		Agran et al. (1990), Pedroso et al. (1991), Cabaleiro and Segura (1997), Golino et al. (2000, 2002), Ioannou et al. (2000), Cid et al. (2007), Scotto et al. (2009) and Bertin et al. (2016a)

<i>Planococcus ficus</i> (vine mealybug)	X	X	X	X	X	X	X	X	X	Engelbrecht and Kasdorf (1985, 1990a), Rosciglione and Castellano (1985), Rosciglione and Gugerli (1989), Tanne et al. (1989a, 1989b, 1993), Boscia et al. (1993), Acheche et al. (2000), Ioannou et al. (2000), Goszczynski and Jooste (2003), de Borbon et al. (2004), Zorloni et al. (2004), Douglas and Krüger (2006), Krüger et al. (2006, 2015), Douglas and Krüger (2008), Tsai et al. (2008, 2010, 2011), Elbeaino et al. (2009), Mahfoudhi et al. (2009), Blaisdell et al. (2012, 2015), Jooste and Krüger (2015) and Bertin et al. (2016a)
<i>Pseudococcus calceolariae</i> (citrophilus mealybug)	X									Petersen and Charles (1997)
<i>Pseudococcus comstocki</i> (Comstock mealybug)	X								X	Nakano et al. (2003) and Nakaume et al. (2008)

(continued)

**Table 24.1** (continued)

Scale insect species	GLRaV-1	GLRaV-3	GLRaV-4 strain 4	GLRaV-4 strain 5	GLRaV-4 strain 6	GLRaV-4 strain 9	GVA	GVB	GVE	References
<i>Pseudococcus longispinus</i> (long-tailed mealybug)	X			X		X	X	X		Rosciglione et al. (1983), Tanne (1988), Tanne et al. (1989b, 1993), Golino et al. (1994, 1995, 2000, 2002), La Notte et al. (1997), Petersen and Charles (1997), Sim et al. (2003), Krüger et al. (2006), Kuniyuki et al. (2006), Sciancalepore et al. (2006), Douglas and Krüger (2008), Tsai et al. (2010), Sandanayaka et al. (2013) and Krüger et al. (2015)
<i>Pseudococcus maritimus</i> (grape mealybug)		X								Golino et al. (2000, 2002), Martin et al. (2005), Soule et al. (2006) and Bahder et al. (2013)
<i>Pseudococcus viburni</i> ( <i>Ps. affinis</i> ) (obscure mealybug)		X					X	X		Garau et al. (1995), Golino et al. (1994, 1995, 2000, 2002) and Blaisdell et al. (2015)
<b>Coccidae (soft scales)</b>										
<i>Ceroplastes rusci</i> (fig wax scale)	X			X						Mahfoudhi et al. (2009)
<i>Coccus longulus</i> (long brown scale)	X									Krüger and Douglas (2009) and Krüger and Douglas-Smit (2013)

<i>Neopulvinaria innumerabilis</i> (cottony maple scale)	X	X							X			Fortusini et al. (1997), Zorloni et al. (2006b) and Le Maguet (2012)
<i>Parasaissetia nigra</i> (nigra scale)		X										Krüger and Douglas (2009) and Krüger and Douglas-Smit (2013)
<i>Parthenolecanium corni</i> (European fruit lecanium)	X	X			X				X			Fortusini et al. (1997), Sforza et al. (2003), Hommay et al. (2008) and Bahder et al. (2013)
<i>Parthenolecanium persicae</i> (European peach scale)		X										Habili (2015)
<i>Pulvinaria vitis</i> (cottony vine scale)		X							X			Belli et al. (1994), G. Hommay (INRA Colmar, F), unpublished
<i>Saissetia</i> sp.		X										Krüger and Douglas (2009) and Krüger and Douglas-Smit (2013)

Mealybug females emit sex pheromones to attract males, and it is generally accepted that mating is probably necessary for vineyard mealybugs (Zaviezo et al. 2010; Waterworth et al. 2011). Identification of the sex pheromones of multiple species has facilitated the synthesis of compounds used in monitoring and mating disruption programs (Daane et al. 2012; Zou and Millar 2015). It should be added that many soft scale species are parthenogenetic throughout their distribution area or only in specific regions (Danzig 1997; Kosztarab and Kozar 1988).

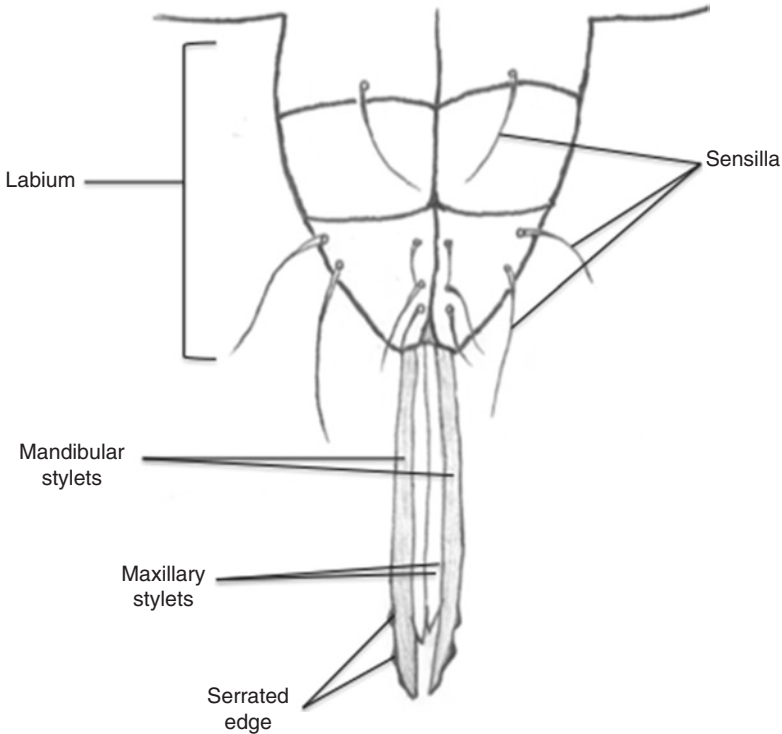
The number of generations developing per year is influenced chiefly by temperature; development time and critical temperature thresholds differ by species. For example, in California's interior valleys, *Ps. maritimus* completes two generations per year (Geiger and Daane 2001), whereas *Pl. ficus* may complete up to seven generations, but only three to five in coastal regions in northern California (Gutierrez et al. 2008). In Europe, all grapevine mealybug and soft scale species are monovoltine in northeastern France and in Germany (Le Maguet 2012), whereas *Pa. corni* and *H. bohemicus* are bivoltine in Mediterranean regions (Kosztarab and Kozar 1988; Duso 2013). Similarly, feeding location on the vine also varies by season, a phenomenon that is likely driven both by temperature and access to a high-quality food source. For instance, during the dormant (winter) period, mealybugs are more likely to be found under the bark of permanent woody structures such as the trunk or cordon, while around harvest (summer, late fall), they are more likely to be found on leaves or in the bunches (Geiger and Daane 2001), whereas adult soft scales prefer the woody canes in the spring, and their larval progeny then invades leaves (Marotta and Tranfaglia 1997). The vineyard mealybug and soft scale pests often have a wide host range, e.g., *Ps. viburni* and *Ps. longispinus* are well-known pests of ornamental plants, whereas *Phenacoccus aceris* is a pest on apple. Still, each species has a number of differences in their biological characteristics and complex of natural enemies; monitoring and management programs (see Chap. 26) must consider each of these targeted vector species.

## **Vector Feeding**

### **Scale Insect Mouthparts and Foregut**

Scale insects are plant-sap feeders and with specialized piercing-sucking mouthparts that play key roles in host choice, feeding, and virus transmission (Blanc et al. 2014; Whitfield et al. 2015). Similar to that of other hemipterans, these mouthparts are composed of a clypeolabral shield and a labium containing the stylet bundle, which is composed of two maxillary stylets and two mandibular stylets (Fig. 24.1) (Bronn 1939; Calatayud and Le Rü 2006; Ahmad et al. 2012; Cicero et al. 2015). The labium bears a variable number of sensilla, probably playing a mechano- and/or chemoreceptive role as in other hemipterans (Calatayud and Le Rü 2006; Alliaume 2016). When the mealybug does not feed, the stylet bundle is retracted as a loop in the body cavity of the insect inside a sheath called "crumena" (Heriot





**Fig. 24.1** Schematic diagram showing the general structure of the mouthparts of scale insects (Drawn by Antoine Alliaume)

1936). The crumena allows the redirection of the stylet bundle from a posterior to a ventral direction to initiate penetration of host tissues (Pollard 1969). The mandibular stylets provide support to the maxillary stylets, which are sealed against each other longitudinally by means of parallel ridges through the length of each stylet; two grooves are generated by pairing the maxillary stylets, the food canal and the smaller salivary canal. Recent observations of the tips of maxillary stylets of *Ph. aceris* (Alliaume 2016) do not provide evidence of a typical “acrostyle” as found in aphids (Uzest et al. 2010). The mandibular stylets surround the coapted maxillary stylets and display serrated ridges or “mandibular teeth” at their tip, with one longitudinal canal containing one or more dendrites, likely of mechanoreceptive function (Ahmad et al. 2012; Alliaume 2016). The food canal is connected to the foregut, comprising the precibarium, the cibarium, equipped with a muscular pump, and the esophagus (Balachowsky 1937; Pesson 1944). The alimentary tract then follows with the midgut and the hindgut. Adult males of scale insects are devoid of mouthparts and therefore are unable to feed. The feeding apparatus of soft scales is very similar to that of mealybugs (Foldi 1997).

## Mealybug Feeding Behavior

Calatayud and Le Rü (2006) proposed that the first step in identifying a suitable feeding site involves physical and probably chemical cues perceived by receptors in the antennae and labium, followed by the insertion of the stylets into plant tissues. The insertion site is usually marked by a small deposit of gelling saliva, the salivary flange (Ahmad et al. 2012), which may facilitate the penetration of plant tissue by providing structural support and protection for the maxillary stylets. The use of histology (Ahmad et al. 2012) and electropetrography (EPG) (Calatayud et al. 1994; Calatayud and Le Rü 2006; Huang et al. 2012; Sandanayaka et al. 2013) has revealed that the stylets penetrate plant tissues in a perpendicular and mainly intercellular pathway associated with secretion of a salivary sheath, similar to that described for other phytophagous hemipterans (e.g., Leopold et al. 2003; Pollard 1973; Tjallingii 1988). Eventually the stylet tips reach the vascular tissues where ingestion and presumably watery salivation start. Epidermal short probes, which are common in aphids and involved in nonpersistent virus transmission, have not been reported in scale insects (Herrbach et al. 2016). Pseudococcids and coccids feed primarily on phloem tissue (Calatayud et al. 1994; Sandanayaka et al. 2013), but xylem-sap feeding has also been reported (Cid and Fereres 2010).

## Effect of Scale Insects Feeding on Plants

Mealybugs and soft scales usually have no direct negative effect on grapevines, except when populations reach high-density levels, in which case plant vigor is reduced and honeydew excretion leads to the development of sooty mold on leaves and fruit; the presence of any mealybugs is also an issue for table grapes because of cosmetic damage and exportation regulations (Daane et al. 2012). In addition, a recent study suggests that grapevine infested by *Pl. citri* responds only weakly at the transcriptional level (Timm and Reinecke 2014). Citrus and tomato infested by a coccid displayed a decreased photosynthetic activity (Huang et al. 2013; Golan et al. 2015); however, whether a similar effect exists in coccid-infested grapevine is not known.

## Transmission of Grapevine Leafroll Viruses

### *Transmission Biology*

There is limited information available on the mode of vector transmission of grapevine leafroll-associated viruses. So far, only one vector-pathogen combination (*Pl. ficus* – GLRaV-3) has been sufficiently studied to conclude that transmission occurs in a semi-persistent manner (Tsai et al. 2008). Earlier efforts generated compatible

yet inconclusive results in regard to a semi-persistent mode of transmission (e.g., Cabaleiro and Segura 1997; Golino et al. 2002; Le Maguet et al. 2012; Alliaume 2016). Transmission of GLRaV-3 by *Pl. ficus* increased in efficiency as a function of plant access period up to 1 day, either for inoculation or acquisition; no latent period was required for transmission, and inoculum as well as infectivity were lost 4 days post-acquisition (Tsai et al. 2008). Even though no other studies have addressed this question in as much detail to our knowledge, there is the expectation that other grapevine-infecting ampeloviruses will be transmitted in a similar manner, as transmission characteristics are generally conserved among members of a viral genus, and other viruses in the *Closteroviridae*, including in the genus *Closterovirus*, are also semi-persistently transmitted (Tsai et al. 2010).

Even though evidence for a semi-persistent and non-circulative mode of transmission by grapevine ampeloviruses by mealybugs seems now established for specific virus-vector combinations (Tsai et al. 2008; Le Maguet et al. 2012; Alliaume 2016), the report of the presence of GLRaV-3 virions, at least components thereof, in the salivary glands of *Pl. citri*, raised the idea of a hypothetical circulative transmission (Cid et al. 2007). However, these reports, whether artifactitious or not, have not been confirmed by others. The short plant access time required for virus acquisition or inoculation (1 h being the shortest period tested) and the absence of a latent period for transmission are generally not considered to be compatible with a circulative mode of transmission. Moreover, it has been suggested, on the basis of in vitro results and of the above unconfirmed findings by Cid et al. (2007), that GLRaV particles could interact with obligate symbiotic bacteria harbored in the hemolymph of mealybugs (Gatehouse et al. 2012; Iasur-Kruh et al. 2015). However, this hypothesis seems improbable since non-circulative virions are by nature not present in the hemolymph and therefore unlikely to interact in vivo with these symbionts, or products thereof.

Despite our limited knowledge of the transmission biology of these ampeloviruses, virus-vector specificity has been characterized. Petersen and Charles (1997) reported on differences in transmission efficiency among mealybug life stages, an observation that was later reproduced with different vectors as well as different viruses, including GVA (Tsai et al. 2008; Mahfoudhi et al. 2009; Le Maguet et al. 2012). The finding that early instars of mealybug and soft scales are more efficient vectors, when compared to adults, makes biological sense; adult females are largely immobile, and adult males do not have functional mouthparts. Therefore, virus transmission from plant to plant by adults would be unlikely. However, there are concerns over improper insect handling and the possibility of stylet bundles breaking during experimental manipulations. While this may occur, these observations have now been reproduced by different groups working on distinct insect species as well as virus species. We hypothesize that the observed differences in transmission among life stages are not experimental artifacts but reflect the differences in probing behavior, possibly in relation to the retention site of the virus in the foregut (which remains unknown).

## ***Possible Interactions Between Ampelovirus and Vitivirus During Transmission***

The frequent coinfection of grapevine by phloem-limited viruses belonging to two distinct viral genera (*Ampelovirus* and *Vitivirus*) raises interesting questions about their potential mutual effects in virus-plant-vector interrelations. It is known that GVA and other vitiviruses are frequently found together with GLRaV-1, -3, or both (e.g., Engelbrecht and Kasdorf 1990a; Fiore et al. 2011; Goszczynski and Jooste 2003; Ipach and Kling 2008; Zorloni et al. 2004, 2006a), although that is not always the case (Agran et al. 1990; Gribaudo et al. 2009; Milkus et al. 2000). GVA, GVB, and GVE were often transmitted along with GLRaV-1 or -3 by scale insects fed on co-infected vines (Herrbach et al. 2016). However, GVA can also be transmitted alone to grapevine (Bertin et al. 2016a, b), at least when recipient vines were analyzed for infection 4–5 months post inoculation, which may be insufficient to detect the possible presence of the *Ampelovirus*. Engelbrecht and Kasdorf (1990a) and Hommay et al. (2008) suggested that GVA could depend on the presence of a co-infecting *Ampelovirus* for transmission, while the opposite situation, i.e., GLRaV-1 depending on GVA to be transmitted, has also been reported (Fortusini et al. 1997). To date, it is not known whether these results are merely circumstantial or reflect a possible “dependent transmission” or “hetero-assistance” phenomenon, at least for specific virus-vector combinations. If confirmed, such a phenomenon would imply that a putative factor (a helper component or a structural peptide bearing a transmission determinant) of one virus, and necessary to its own transmission, would assist the transmission of the other.

## ***Vector-Virus Molecular Interactions***

The non-circulative transmission of plant viruses can be split into two strategies. While many viruses interact directly with insect vectors by means of their coat protein, others require an intermediate nonstructural protein, or helper component, to act as a molecular bridge between virus and the vector (reviewed in Whitfield et al. 2015). The strategy used by grapevine ampeloviruses has yet to be determined; however, potential leads to follow are provided by members of other genera in the *Closteroviridae* family. Firstly, a direct capsid strategy has been described for the transmission of the whitefly-borne *Lettuce infectious yellows virus* (LIYV), a member of the genus *Crinivirus* (Tian et al. 1999; Stewart et al. 2010), and the aphid-borne *Citrus tristeza virus* (CTV, *Closterovirus* genus) (Killiny et al. 2016). Secondly, using an immunofluorescent localization assay, Chen et al. (2011) showed that the minor coat protein (CPm) of LIYV, a component of the virion “tail” (a structure probably common in all closterovirids; Alzhanova et al. 2007; Dolja et al. 2006), is the major determinant of transmission by whiteflies and that LIYV virions were localized to a specific retention site in the foregut of *Bemisia tabaci*. Using

similar approaches, Killiny et al. (2016) confirmed the involvement of the CPM and other tail components of CTV in its transmission by the aphid *Toxoptera citricida*, as well as in retention in the foregut. These authors proposed that this foregut retention is mediated by direct interactions between the vector foregut and the CPM on the virion and is associated with transmission success of LIYV and CTV. This is in contrast to an unrelated non-circulative virus, *Cauliflower mosaic virus* (CaMV, *Caulimovirus*), which localizes to the tip of maxillary stylets in aphids via the helper strategy (Uzest et al. 2007). The viral determinants of transmission and retention sites for any *Ampelovirus*-vector species combination remain undescribed.

## Prospects

Although information about the transmission of grapevine-infecting ampeloviruses by mealybugs and soft scales remains limited and fragmented, recent efforts to close many of these current knowledge gaps have provided many insights into the mechanisms underlying the transmission of grapevine leafroll-associated ampeloviruses, as well as the rugose wood-associated vitiviruses. In view of these insights, we propose the following hypotheses and questions, which we hope will be tested and addressed, respectively, by the community at large.

First, we hypothesize that all grapevine-infecting ampeloviruses are transmitted in a semi-persistent manner. This mode of transmission has been demonstrated in the case of GLRaV-3 and *Pl. ficus* (Tsai et al. 2008), and findings reported with other vector-virus species combinations tested are generally compatible with this mode. However, it cannot be ruled out that the transmission mode may possibly differ in some combinations, e.g., with soft scale vectors as compared to mealybugs. Moreover, we hypothesize that differences in transmission efficiency among life stages of the insect vectors are not experimental artifacts but rather reflect an adaptation driven by the fact that adult insects are unlikely virus vectors. Furthermore, we hypothesize that the observed differences in transmission efficiency among various vector-virus species combinations that were investigated are due to a combination of factors, which include viral populations available in source plants, differences in vector probing behaviors and/or in intraspecific vector ability, as well as a variable affinity of virion binding sites.

Finally, the following questions need to be addressed to fill significant knowledge gaps in this research field. The determinants of virus-vector molecular interactions are still unknown: What is/are the viral peptide(s) involved in the recognition by a virus retention site in the vector? Is this *Ampelovirus* retention site located in the vector's foregut, like for LIYV and CTV? Most studies on transmission biology by scale insects were performed in controlled cabinet or greenhouse conditions. However, Blaisdell et al. (2016) reported that transmission efficiency may be lower in the vineyard; therefore, the extrapolation of transmission efficiency data gathered in controlled conditions to vineyards should be investigated. Moreover, how to apply transmission biology data to disease epidemiology is an important prerequisite

for better understanding and forecasting of the spread and impact of grapevine leafroll and rugose wood diseases in vineyards.

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