Chapter 20
Biological Assays

A. Rowhani, P. La Notte, J.K. Uyemoto, S.D. Daubert, and V. Savino

Abstract Biological indexing is a methodology for the detection of grapevine virus diseases that relies on specific responses from panels of indicator host plants. Grafting to a set of four *Vitis* indicators and rub inoculation on a set of herbaceous hosts are routinely used in clean plant centers to transmit virus diseases to indicator hosts. Dormant bud chips from accession plants are grafted to the woody stems of indicator vines. Green grafting of first year canes to young indicator material is also used. When candidate buds are diseased, indicator vines develop typical foliar or stem symptoms 2–4 years post-grafting. Sap from accession plants that are infected with mechanically transmissible viruses can be screened by rub inoculation to a panel of herbaceous indicator plants, on which positive reactions develop in a matter of weeks. Biological indexing is seldom diagnostic for a given virus species. The presence of well-characterized species can be confirmed by ELISA, PCR, or RT-PCR. The more recently developed technique of high-throughput sequencing (HTS) can detect both well-characterized and novel viruses. Those molecular-based methodologies in general are more reliable, more sensitive, and more rapid than bioassays. However, the classic biological assay cannot yet be completely replaced by the more modern laboratory analyses. The bioassay is still used to demonstrate the presence of unidentified graft-transmissible agents, especially diseases such as 110R necrotic union or 3309C stem necrosis distortion, where the causative pathogens have not yet been specifically identified by other means.

Keywords Biological host indicators • Chip-bud inoculation • Green grafting • Mechanical inoculation • Virus assay

A. Rowhani (✉) • S.D. Daubert
Department of Plant Pathology, University of California, Davis, CA 95616, USA
e-mail: akrowhani@ucdavis.edu

P. La Notte • V. Savino
Dipartimento di Protezione delle Piante e Microbiologia Applicata,
Universita degli Studi and Istituto di Virologia Vegetale, CNR, Sezione di Bari,
Via Amendola 165/A, 70126 Bari, Italy

J.K. Uyemoto
USDA-ARS, Department of Plant Pathology, University of California,
Davis, CA 95616, USA

© Springer International Publishing AG 2017

B. Meng et al. (eds.), *Grapevine Viruses: Molecular Biology, Diagnostics and Management*, DOI 10.1007/978-3-319-57706-7_20

Introduction

Biological indexing with indicator hosts is a classic testing strategy that has been used for decades for the detection of virus diseases in many different crops. One of the first applications of biological assays in pathogen detection was in viticulture. Scheu (1935) first demonstrated the graft transmissibility of leafroll disease in Germany. Later in California, a detection assay was initiated by Olmo and Rizzi (1943) who were working at the University of California on a disease that affected Red Emperor grapevines. The disease decreased the vigor of the vines and caused such poor color in the grapes that it was named "White" Emperor disease. Olmo and Rizzi demonstrated that the disease was graft transmissible, implicating an infectious agent. (The viral nature of this agent would not be understood for decades after their demonstration.) Later, Austin Goheen transmitted White Emperor disease to Mission grapevines by grafting. Mission was first cultivated by the Spanish at the California missions. Goheen et al. (1959) found that Mission reliably produced distinct leafroll symptoms after graft inoculation. Hence, Mission vines became one of the first indicator host plants used in the diagnosis of grapevine viral disease.

Goheen (1989) went on to optimize a set of grapevine cultivars for use as indicator hosts for the identification of each of the major viral diseases of California grapevines. He worked with the Native American *Vitis rupestris* St. George rootstock. *V. rupestris* reproducibly produced uniform foliar symptoms following grafting with *Grapevine fanleaf virus* (GFLV)-infected material. (The symptoms in source plant cultivars were unreliable, varying widely from "fanleaf degeneration" to "grape yellow vein" presentations.) Goheen found that those same *V. rupestris* St. George indicator plants also produced diagnostic stem markings in response to grafts from plants carrying a disease he named "*rupestris* stem pitting." The causative virus induced no significant effect on the growth of most *V. vinifera* European grape cultivars. Further assays demonstrated that most if not all European grapevines were infected with *rupestris* stem pitting disease. Furthermore, Goheen identified a complex *V. berlandieri* hybrid grape cultivar LN33 as a reliable indicator of "corky bark" disease. *Grapevine virus B* (GVB), the virus closely associated with the disease, was otherwise difficult to detect, being latent in many *V. vinifera* cultivars. In addition, Goheen demonstrated that *V. vinifera* cultivar Cabernet Franc was the most reliable index host for leafroll disease detection.

The advent of a set of grapevine cultivars optimized for use as indicator hosts enabled the identification of clean stocks for the production of certified planting material. As such, it facilitated the early elimination of diseased stocks from the propagation scheme. This enabled grape growers to clean plant material sources and reduce disease incidence in vineyards. Should virus diseases and viruses go undetected in foundation stocks, they would be vegetatively perpetuated by plant propagators. Moreover, planting material derived from diseased stock and released to growers for establishing vineyards would provide the endogenous arthropod vectors with a pathogen source to spread disease beyond the newly planted vineyards. Biological assays, applied at the first steps in the process of vegetative propagation

of clean material, are designed to preclude such infection amplification problems and to identify clean foundation stocks for certification programs.

Two different biological assays are used for the screening of virus diseases in grapevine stocks. Herbaceous indicator plants are used to detect the presence of mechanically transmissible viruses such as nepoviruses, some vitiviruses, and some closteroviruses. Inoculation of herbaceous plants with grapevine sap extract can induce diagnostic symptoms in greenhouse indicator plants in a matter of weeks.

Vitis indicator plants are used to detect virus diseases for which associated viruses are not sap transmissible, including most closteroviruses, *Grapevine rupestris stem pitting-associated virus* (GRSPaV), marafiviruses, and maculaviruses. These biological assays involve inoculation from accession sources to indicator vines by bud chip or green grafting. But, as opposed to herbaceous plant indexing which takes 3–4 weeks, biological assay using a panel of grapevine indicator hosts requires up to 3 years to complete. Biological indexing methods specific to some of the most recently identified novel viruses, such as *Grapevine red blotch-associated virus* (GRBaV), *Grapevine vein clearing virus* (GVCV), or some members of the genus *Vitivirus*, have not been established yet.

Sap-Transmissible Virus Analysis

Biological indexing on herbaceous hosts is used to detect sap-transmissible viruses, primarily nepoviruses, in test plants. In some cases, viruses in the genus *Vitivirus* such as *Grapevine virus A* (GVA) and GVB (Garau et al. 1993; Boscia et al. 1993) or in the genus *Closterovirus* such as Grapevine leafroll-associated virus 2 (GLRaV-2, Goszczynski et al. 1996) can be detected with difficulty when inoculated to *Nicotiana occidentalis* or *N. benthamiana.* When that is possible, the herbaceous host test offers a more rapid assay compared to graft inoculations onto woody grape indicators. Detectable grapevine nepoviruses include *Arabis mosaic virus* (ArMV), GFLV, *Raspberry ringspot virus* (RpRSV), *Tobacco ringspot virus* (TRSV), *Blueberry leaf mottle virus* (BLMoV), *Tomato ringspot virus* (ToRSV), and other viruses such as *Strawberry latent ringspot virus* (SLRSV). Commonly used herbaceous indicators include *Chenopodium quinoa*, *C. amaranticolor*, *N. tabacum*, and *Cucumis sativus*.

With sap inoculations, succulent tissues (young leaves and tips of actively growing shoots of a candidate grapevine specimen) are triturated in 10 mM phosphate buffer pH 7.0–7.5 containing 2% nicotine or 3% polyvinyl pyrrolidone 40 (PVP40) or insoluble PVP (approximately 1:10, wt. tissue-ml buffer). The liquid slurry is applied to herbaceous plants pre-dusted with an abrasive powder (Jones 1993) such as carborundum (silicon carbide), corundum (aluminum oxide), or celite (diatomaceous earth). The tissue extract is gently rub-inoculated onto cotyledons and leaves of indicators using a pestle, cotton tip swab, or one's finger (Fig. 20.1). The rubbed leaves and cotyledons are then gently rinsed with water to prevent leaf damage resulting from prolonged exposure to the additives in the buffer. The ages of the

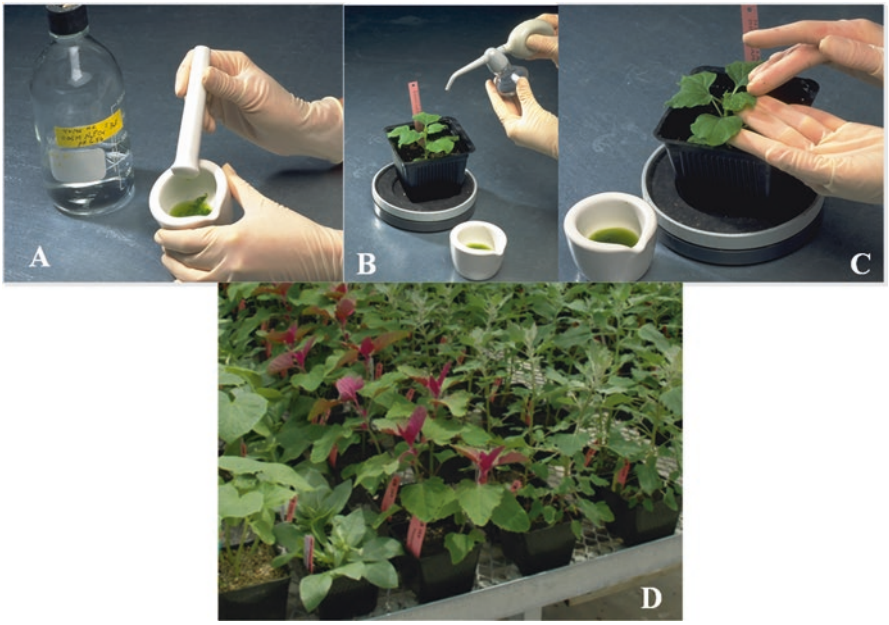

Fig. 20.1 Steps in biological index using herbaceous host indicators. (**A**) Homogenization of leaf tissue from a candidate grapevine, (**B**) application of carborundum on the indicator host, (**C**) application of leaf homogenate on the leaf of the indicator host, and (**D**) maintenance of inoculated indicators in the greenhouse for symptom development

indicators that give best results are *C. quinoa* and *C. amaranticolor*, both at 4–6 leaf stages; *N. tabacum*, at 2–4 leaf stages; and cucumber, cotyledon seedling stage. The additives nicotine and PVP are used to neutralize the inhibitory effects of polyphenolic compounds and other host cell inhibitors of virus infectivity. As such, they facilitate virus transmission (Jones, 1993; Authors, unpublished data).

When inoculation is successful, symptoms develop in 7–10 days. In general, *Nepovirus*-infected *C. quinoa* and *C. amaranticolor* develop diagnostic chlorotic and/or necrotic local lesions and systemic mottling (Uyemoto et al. 1976); some nepoviruses also induce leaf deformation and shoot tip necrosis. *Nicotiana* spp. may show variable symptoms depending on the indicator cultivar, the virus species, and the growing conditions. Those symptoms can include local chlorotic lesions, chlorotic or necrotic rings and systemic mottling, ringspot and line patterns, and/or leaf deformation. Cucumber may be asymptomatic for GFLV, while developing chlorotic local lesions and systemic mottling for TRSV and ToRSV. As infected cucumber seedlings grow, newly expanding leaves may be asymptomatic (Cadman et al. 1960; Dias 1963; Hewitt et al. 1970; Martelli 1993; Ramsdell and Gillett 1998; Ramsdell and Stace-Smith 1983; Stace-Smith 1984, 1985; Uyemoto et al. 1976).

Non-Sap Transmissible Virus Analysis

Biological indexing on grapevine indicator hosts is a classical approach in plant pathology used to detect diseases caused by virus infection (Martelli et al. 1993). Grapevine viruses in the families *Closteroviridae*, *Betaflexiviridae*, and *Tymoviridae* (Martelli et al. 2002) cause diagnostic symptoms on indicators such as St. George (*V. rupestris*), LN33 (1613 Couderc x *V. vinifera* cv. Thompson seedless), Kober 5BB (*V. berlandieri* x *V. riparia*), and *V. vinifera* cv. Cabernet Franc indicator hosts. Grapevine red blotch-associated virus (GRBaV) from the family *Geminiviridae* also causes disease symptoms in *V. vinifera* hosts such as Cabernet Franc (unpublished data).

V. rupestris cv. St. George produces diagnostic symptoms in response to infections by GFLV, *Grapevine fleckvirus* (GFkV), *Grapevine asteroid mosaic-associated virus* (GAMaV), and GRSPaV. Symptoms induced by GFLV infection consist of leaf vein clearing, chlorotic ringspots, oak leaf patterns, short internodes, and/or distortion of leaf blades (Bovey et al. 1980; Brunt et al. 1996; Krake et al. 1999; Martelli 1993; Wilcox et al. 2015). GFLV leaf symptoms develop in early spring, are ephemeral in nature, and fade with the rise in ambient temperatures (Golino et al. 1991). GFkV leaf symptoms consist of a "clearing" of third- and fourth-order veinlets and localized translucent spots. In severe cases, leaves may wrinkle, twist, and curl upward. Further, a diffused mosaic pattern may develop on mature leaves (Bovey et al. 1980; Brunt et al. 1996; Krake et al. 1999; Martelli 1993; Wilcox et al. 2015). With GAMaV, chlorotic star-shaped spots, which may cluster irregularly, develop on leaves (Martelli 1993; Wilcox et al. 2015). Finally, symptoms ascribed to GRSPaV include stem markings, i.e., distinct basipetal pitting extending downward from the grafted chip bud. Occasionally, stem pits encircle the woody cylinder (Martelli 1993; Wilcox et al. 2015). GRSPaV does not show symptoms on grapevine virus indicators LN33 and Kober 5BB.

LN33 is an indicator for corky bark disease, to which GVB is associated. Symptoms include grooves and pits on the woody cylinder, trunk bark split, and red leaves due to swelling of canes and proliferation of spongy callus tissues (hence the name corky bark) (Krake et al. 1999; Martelli 1993; Wilcox et al. 2015). Kober 5BB and St. George are asymptomatic hosts for corky bark disease and GVB.

Kober 5BB is an indicator for Kober stem grooving disease to which GVA is associated. Symptoms include wood necrosis, pits, and grooves, often accompanied by yellowish spots on the leaves (Martelli 1993; Garau et al. 1994). St. George and LN33 are asymptomatic hosts for Kober stem grooving disease and GVA.

V. vinifera cv. Cabernet Franc is diagnostic for leafroll disease and GRBaV. Other leafroll disease indicators include *V. vinifera* cvs. Pinot noir, Mission, Cabernet Sauvignon, Gamay, and Barbera. The choice of indicator depends upon professional experience of the investigator with local climatic and environmental conditions (Krake et al. 1999; Martelli 1993; Wilcox et al. 2015). On Cabernet Franc, leafroll virus symptoms are interveinal reddening of the leaf blade, beginning in early fall and intensifying thereafter, with primary veins prominently green, although these

green veins fade late in the season. Leaf margins may roll downward. Often internodes are shortened and stunting is apparent. The currently characterized viruses associated with leafroll are members of the family *Closteroviridae*. To date, five *Grapevine leafroll-associated viruses* (GLRaV) have been reported (Martelli et al. 2012). Redglobe strain of GLRaV-2 and GLRaV-7 are asymptomatic on *V. vinifera* cv. Cabernet Franc. Symptoms of GRBaV on Cabernet Franc include development of red blotches on the leaves.

Inoculation Methods

Chip-Bud Inoculation

Self-rooted indicator plants grown in pots are inoculated with dormant bud chips from accession canes of interest. The bud chips are placed onto matching cut areas on stems of the indicator plants, overlaid with a plastic strip and secured with budding rubber (Fig. 20.2). Three replicates of two or three bud chips are grafted per indicator plant. A set of healthy indicator plants and another set grafted with known disease sources are included as controls in these tests.

Chip-bud grafts are usually made in late winter or early spring. The inoculated plants are maintained in a greenhouse for a month and bud chips are evaluated for viability. Then indicator plants are acclimated for a few weeks in a lathhouse prior to being transplanted to an isolated field site. While growing in the field, indicator vines are visually inspected annually during spring and fall of the following and subsequent years. Examination for wood markings involves uprooting indicator

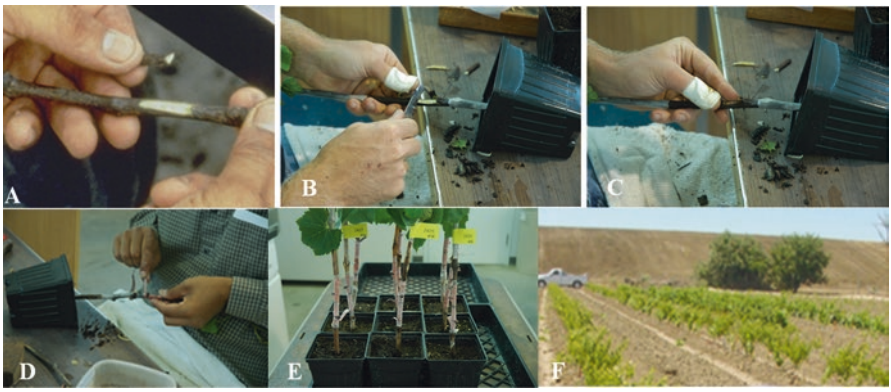

Fig. 20.2 Steps in biological index using grape indicator host. (**A**) Selection of bud chip from a dormant cane collected from a candidate plant, (**B**) preparing a matching cut on the indicator plant, (**C**) placement of the bud chip from the candidate plant onto the matching cut produced on the indicator host, (**D** and **E**) securing the bud chip on the indicator host by wrapping it with a rubber band, and (**F**) planting of the grafted indicators in the field for symptom development observation

plants and removing the bark to expose the woody cylinders. This is usually done during the second or third growing season post graft inoculation. How quickly definitive symptoms develop is dependent on the climate. In warmer areas where strong growing conditions exist, symptom development is faster.

Green Grafting

The indicator host on its own roots can be green grafted with accession sources (Taylor et al. 1967; Walter et al. 1990). With this procedure, both the accession vine, as the scion, and the indicator are used as herbaceous cuttings during the growing season. They are fitted together with a cleft graft. All grafted vines must be maintained under greenhouse conditions. This technique is used to screen for virus in certification programs (Pathirana and McKenzie 2005; Tanne et al. 1993). Infection of the indicator host through green grafting results in diagnostic symptoms expressed more rapidly (Taylor et al. 1967; Pathirana and McKenzie 2005) than would be expressed by field indexing on woody indicator vines. Although green grafting is not as sensitive as dormant grafting for diseases that cause wood markings (Lahogue et al. 1995), this drawback must be balanced against its potentially more rapid development of foliar symptoms and its independence from environmental conditions and seasonal time frame constraints in the field (Cirami et al. 1988).

A third method used for the biological assay of grapevine viruses is micropropagation and acclimatization of indicator plants for green grafting with petioles or secondary shoots from accession plants (Vindimian et al. 1998). In this method the indicator plant is micropropagated in vitro and then subcultured by nodal cuttings on culture media. After the plants produce sufficient roots, they are transferred to soil and acclimatize to greenhouse conditions. These plants are then green grafted with leaves (petiole attached) or secondary shoots collected from accession plants. The grafted plants are kept in the greenhouse and inspected periodically for symptom development.

A fourth grafting method used for the detection of grapevine viruses involves micrografting. Tanne et al. (1996) used tissue culture technology to mimic indexing and to enhance symptom development in vitro. This allows for rapid diagnosis of grapevine viral diseases by grafting in vitro onto cultured indicators. In this technique, the grafted plants are put under mild stress by adding sorbitol at 4% to the culture medium. A variety of distinct symptoms result within 4–8 weeks.

Detection of Diseases of Unknown Etiology

110R Necrotic Union

Biological indexing was used in 2012 to investigate a disease of Pinot noir (PN) observed in California. The affected vineyards had been established with PN clones 02A, 667, 777, and UCD 04, each cultivated on rootstock 110 Richter (110R; *V. berlandieri* × *V. rupestris*). Diseased vines exhibited solid red canopies and necrosis of the graft union. Two distinct disease stages were evident: diseased vines showing no stunting and normal-sized grape clusters were designated "acute disease stage" vines; stunted vines with short shoots and straggly grape clusters were designated "chronic stage" vines (Al Rwahnih et al. 2012a, b). Similar canopy symptoms (showing chlorotic instead of solid red leaves) along with necrosis at the graft union were found on accessions Chardonnay 04 and Pinot gris 152. Disease progress from 2004 to 2009 in one PN 02A vineyard established in 1997 revealed an increase from 2.1% (14 of 664 vines) to 21.9% (145 of 664 vines) in 2009, suggesting the occurrence of secondary spread (see also Chap. 13 in this volume).

None of the diseased vines induced typical reactions on the panel of four conventional indicators. Repeated bud-chip inoculations or extended lengths of diseased canes side-grafted onto test plants cultivated on 110R produced viable grafts, but no disease transmission was observed. However, some bench grafts using asymptomatic sources of the disease in Pinot noir and Chardonnay accessions cultivated on rootstocks other than 110R produced necrotic union symptoms.

RT-PCR failed to detect virus, phytoplasma, or *Xylella fastidiosa* association with this disease (Al Rwahnih et al. 2012a, b). Samples from PN clones 02A and UCD 04 were tested by high-throughputsequencing (Rowhani, unpublished data). Viruses found in PN 02A were GRSPaV and *Grapevine redglobe virus* and in Pinot noir UCD 04, GRSPaV and *Grapevine rupestris vein feathering virus*. The role of these viruses in inducing 110R necrotic union has yet to be demonstrated.

3309C Stem Necrosis Distortion

In several California vineyards planted with PN clone 23 (PN23) cultivated on 3309 Couderc rootstock, grapevines were observed in decline. Decline symptoms consisted of severe stunting, red canopy, and poor berry development, with severe stem necrosis and distortion on the rootstock. Biological assays failed to identify a transmissible disease with typical symptomatology on the four conventional indicators. RT-PCR failed to detect the presence of known viruses, phytoplasmas, or *Xylella fastidiosa* in diseased material. However, double-stranded RNA(dsRNA) extracts from diseased grapevines were found to contain high molecular weight bands about 8.7 kb in size (Lima et al. 2009). A cDNA library was constructed from the dsRNA, and its sequencing revealed a viral genome with similarities to GRSPaV. The

genome was found to be 8724 nt in length (excluding the poly A tail) with an identity of 76–78% with GRSPaV sequences listed in GenBank. This sequence was designated as the PN strain of GRSPaV. Its role in inducing stem necrosis distortion on 3309C has yet to be demonstrated.

Strengths and Limits of Biological Indexing

Serological and molecular analyses, such as ELISA, RT-PCR, or HTS analyses, are designed for the specific identification of virus species and strains. These analyses are constrained by their need for prior characterization of the viruses they detect. Information about the antigenicity of the virion (required for ELISA) or about the viral genomic sequence (necessary for PCR primer design, or for identification of HTS reads for contig construction) is required for the molecular analyses. In contrast, biological assays are broad spectrum; they detect diseases but do not identify the causative agents of those diseases to the species level (Al Rwahnih et al. 2015). For example, to date four virus species and their multiple strains are associated with grapevine leafroll disease. In California, the grapevine indicator Cabernet Franc responds to inoculations with any of them by producing the same generic leafroll disease symptoms irrespective of the identity of the causative GLRaV species or strains. (The bioassay would not detect GLRaV-2 RG strain or GLRaV-7, since they are asymptomatic on Cabernet Franc.)

The bioassay would be crucial in cases of previously uncharacterized leafroll agents. The biological assay could still reveal the appearance of leafroll disease symptoms, even though the inducing agent itself was unknown and could not be detected by laboratory analytics. The use of biological indexing analysis for the certification of clean stock nursery material is discussed in Chap. 27 of this volume.

A disadvantage of the bioassays, compared to serological and molecular methods, is the time and expense required. Bioassays on woody indicators can take years to yield results, they are expensive and labor intensive, and they require considerable greenhouse and field space (Al Rwahnih et al. 2015). Furthermore, test results may be influenced by seasonal environment and growth conditions (Constable et al. 2013). Also, specific biological assay is not available for some viruses of significance found in grapevine, including some members of the *Maculavirus*, *Marafivirus*, and *Vitivirus* genera. Some members of the family *Closteroviridae* are asymptomatic in biological index hosts that are currently employed in clean stock programs.

Conclusion and Directions for Future Research

Biological indexing was once the mainstay of grapevine viral disease diagnosis programs. However, ELISA followed by RT-PCR and now HTS have replaced it in many applications. The newer techniques show greater specificity and sensitivity and lower cost (Al Rwahnih et al. 2015). In the future, biological indexing may be eclipsed as a primary diagnostic by the advent of more modern laboratory and computer techniques of pathogen detection.

Biological indexing is still necessary in many grapevine virus characterization capacities. These would include the diagnosis of disease associated with a novel virus for which no serological reagent is available and no nucleotide sequence is known (Rowhani et al. 2005; Martelli and Walter 1998). In that case, modern diagnostic methods will not be available for identification by comparison with known sequence datasets. Biological analysis would be the fallback diagnostic in that scenario.

Modern diagnostic technologies, particularly HTS, do not predict pathogenicity. The possibility that a newly discovered virus can be pathogenic or have differential virulence in various cultivar backgrounds (Alkowni et al. 2011) can only be evaluated through biological analysis. The possibility that a novel virus could synergize in combination with a second virus (Rosa et al. 2011) will also require a biological assay to demonstrate the synergy.

When HTS analysis discovers a putative viral grapevine pathogen, biological assay is used to validate the discovery and to characterize the graft transmissibility of the agent. The validity of the viral sequence data is demonstrated by graft transmission of the infection from the discovery host plant into another vine. HTS provides the sequence information used to design PCR diagnostics for newly characterized viruses. The PCR diagnostics designed from HTS data are then employed to confirm virus transmission to the recipient plant.

HTS has the capacity to discover a wide range of new grapevine viruses. In the past 8 years, previously unknown viruses identified by HTS have included Grapevine Syrah virus-1 (Al Rwahnih et al. 2009), Grapevine virus F (Al Rwahnih et al. 2012a, b), Grapevine red blotch-associated virus (Al Rwahnih et al. 2013), Grapevine Cabernet Sauvignon reovirus (Al Rwahnih et al. 2015), and Grapevine geminivirus (Al Rwahnih et al. 2017). When a novel virus is identified, biological assays will be employed to demonstrate the agronomic significance of the find, particularly its pathological potential. The biological assay would be required to demonstrate that a cloned viral sequence derived from HTS sequence data is infectious, which is proof that the data from the sequencing analysis is correct.

Plant viruses exist as quasispecies mixtures of genetic variants. The biological activity of such a mixture is readily demonstrated by biological assays. Eventually, modern techniques will (a) identify novel viral pathogens by their genomic sequences and (b) in vitro synthesize full-length genome copies of those newly identified viruses, so that (c) the synthetic genomes can be inoculated into index grapevine plants to characterize their infectivity. In the process of sequencing a full-length viral genome and then synthesizing it in vitro, all of the quasispecies

diversity is lost to the production of the single cloned genome sequence. The difference in infectivity between that single-sequence genome, and the multitude of variant sequences that make up the genome of a viral field isolate, will then be readily apparent in the comparison of their respective biological assays.

References

Alkowni, R., Y.-P. Zhang, A. Rowhani, J.K. Uyemoto, and A. Minafra. 2011. Biological, molecular, and serological studies of a novel strain of grapevine leafroll-associated virus 2. *Virus Genes* 43: 102–110.

Al Rwahnih, M., S. Daubert, D. Golino, and A. Rowhani. 2009. Deep sequencing analysis of RNAs from a grapevine showing Syrah decline symptoms reveals a multiple virus infection that includes a novel virus. *Virology* 387: 395–401.

Al Rwahnih, M., A. Rowhani, R.J. Smith, J.K. Uyemoto, and M.R. Sudarshana. 2012a. Grapevine necrotic union, a newly recognized disease of unknown etiology in grapevines grafted on 110 Richter rootstock in California. *Journal of Plant Pathology* 94: 149–156.

Al Rwahnih, M., M.R. Sudarshana, J.K. Uyemoto, and A. Rowhani. 2012b. Complete genome sequence of a novel vitivirus isolated from grapevine. *Journal of Virology* 86: 9545–9545.

Al Rwahnih, M., A. Dave, M.M. Anderson, A. Rowhani, J.K. Uyemoto, and M.R. Sudarshana. 2013. Association of a DNA virus with grapevines affected by red blotch disease in California. *Phytopathology* 103: 1069–1076.

Al Rwahnih, M., S. Daubert, D. Golino, C. Islas, and A. Rowhani. 2015. Comparison of next generation sequencing vs. biological indexing for the optimal detection of viral pathogens in grapevine. *Phytopathology* 105: 758–763.

Al Rwahnih, M., O. Alabi, N.M. Westrick, D. Golino, and A. Rowhani. 2017. Description of a novel monopartite geminivirus and its defective subviral genome in grapevine. *Phytopathology* 107 (2): 240–251.

Boscia, D., V. Savino, A. Minafra, S. Namba, V. Elicio, M.A. Castellano, and G.P. Martelli. 1993. Properties of a filamentous virus isolated from grapevines affected by corky bark. *Archives of Virology* 130: 109–120.

Bovey, R., W. Gartel, W.B. Hewitt, G.P. Martelli, and A. Vuittenez. 1980. *Virus and virus-like diseases of grapevine, color atlas of symptoms*, 181pp. Paris: La Maison Rustique.

Brunt, A.A., K. Crabtree, M.J. Dallwitz, A.J. Gibbs, and L. Watson. 1996. *Viruses of plants, description and lists from the VIDE database*, 1484 pp. Wallingford: CAB Int.

Cadman, C.H., H.F. Dias, and B.D. Harrison. 1960. Sap-transmissible viruses associated with diseases of grapevines in Europe and North America. *Nature* 187: 577–579.

Cirami, R.M., R.J. van Velsen, and J. Niejalke. 1988. Grapevine virus indexing in the South Australian vine improvement scheme, 1974–1987. *Australian Journal of Experimental Agriculture* 28: 645–649.

Constable, F.E., J. Connellan, P. Nicholas, and B.C. Rodoni. 2013. The reliability of woody indexing for detection of grapevine virus-associated diseases in three different climatic conditions in Australia. *Australian Journal of Grape and Wine Research* 19: 74–80.

Dias, H.F. 1963. Host range and properties of grapevine fanleaf and grapevine yellow mosaic viruses. *Annals of Applied Biology* 51: 85–95.

Garau, R., V.A. Porta, D. Boscia, R. Piredda, and U. Porta. 1993. Studies on grapevine virus B isolates from corky bark-affected vines in Sardinia. *Rivista di Patologia Vegetale* 3: 83–89.

Garau, R., V.A. Porta, R. Piredde, and U. Porta. 1994. On the possible relationship between Kober stem grooving and grapevine virus A. *Vitis* 33: 161–163.

Goheen, A.C. 1989. Virus diseases and grapevine selection. *American Journal of Enology and Viticulture* 40: 67–72.

Goheen, A.C., W.B. Hewitt, and C.J. Alley. 1959. Studies of grape leafroll in California. *American Journal of Enology and Viticulture* 10: 78–84.

Golino, D.A., A. Rowhani, and M.A. Walker. 1991. Fanleaf in the vineyard. *Practical Winery and Vineyard*. November/December 21–23.

Goszczynski, D.E., G.F. Kasdorf, G. Pietersen, and H. Tonder. 1996. Detection of two strains of grapevine leafroll-associated virus 2. *Vitis* 3: 133–135.

Hewitt, W.B., G. Martelli, H.F. Dias, and R.H. Taylor. 1970. *Grapevine fanleaf virus*. CMI/AAB description of plant viruses No. 28. Association of Applied Biologists. Wellesbourne, UK.

Jones, A.T. 1993. Experimental transmission of viruses in diagnosis. In *Diagnosis of plant virus diseases*, ed. R.E.F. Matthews, 59–62. London: CRC Press.

Krake, L.R., N.S. Scott, M.A. Rezaian, and R.H. Taylor. 1999. *Graft-transmitted diseases of grapevines*, 137pp. Canberra: CSIRO.

Lahogue, F., G. Boulard, and C. Schneider. 1995. Comparaison de differentes techniques de greffage vis-à-vis de leur efficacite de transmission virale sur vigne. *Vitis* 34: 177–183.

Lima, M., R. Alkowni, J.K. Uyemoto, and A. Rowhani. 2009. Genomic study and detection of a new variant of Grapevine rupestris stem pitting associated virus in declining California Pinot Noir grapevines. *Journal of Plant Pathology* 91: 153–160.

Martelli, G.P. 1993. *Graft-transmissible diseases of grapevines, handbook for detection and diagnosis*, 263pp. Rome: Food and Agriculture Organization of the United Nations.

Martelli, G.P., and B. Walter. 1998. Virus certification of grapevines. In *Plant virus disease control*, ed. A. Hadidi, R.K. Khetarpal, and H. Koganezawa. St. Paul: APS Press.

Martelli, G.P., V. Savino, and B. Walter. 1993. Indexing on *Vitis* indicators. In *Graft-transmissible diseases of grapevines, handbook for detection and diagnosis*, ed. G.P. Martelli. Rome, 263pp: FAO.

Martelli, G.P., S. Sabanadzovic, N. Abou-Ghanem Sabanadzovic, M.C. Edwards, and T. Dreher. 2002. The family *Tymoviridae*. *Archives of Virology* 147: 1837–1846.

Martelli, G.P., N. Abou Ghenem-Sabanadzovic, A.A. Agranovsky, M. Al Rwahnih, V.V. Dolja, C.I. Dovas, M. Fuchs, P. Gugerli, J.S. Hu, W. Jelkmann, N.I. Katis, V.I. Maliogka, M.J. Melzer, W. Menzel, A. Minafra, M.F. Rott, A. Rowhani, S. Sabanadzovic, and P. Saldarelli. 2012. Taxonomic revision of the family *Closteroviridae* with special reference to the Grapevine leafroll-associated members of the genus *Ampelovirus* and the putative species unassigned to the family. *Journal of Plant Pathology* 94: 7–19.

Olmo, H.P., and A.D. Rizzi. 1943. Selection for fruit color in the Emperor grape. *Proceedings of the American Society of Horticultural Science* 4: 395–400.

Pathirana, R., and M. McKenzie. 2005. Early detection of grapevine leafroll virus in *Vitis vinifera* using *in vitro* micrografting. *Plant Cell, Tissue and Organ Culture* 81: 11–18.

Ramsdell, D.C., and J.M. Gillett. 1998. CMI/AAB description of plant viruses No. 364. Association of Applied Biologists. Wellesbourne, UK.

Ramsdell, D.C., and R. Stace-Smith. 1983. CMI/AAB description of plant viruses No. 267. Association of Applied Biologists. Wellesbourne, UK.

Rosa, C., J.F. Jimenez, P. Margaria, and A. Rowhani. 2011. Symptomatology and effects of viruses associated with rugose wood complex on the growth of four different rootstocks. *American Journal of Enology and Viticulture* 62: 207–213.

Rowhani, A., J.K. Uyemoto, D.A. Golino, and G.P. Martelli. 2005. Pathogen testing and certification of *Vitis* and *Prunus* species. *Annual Review of Phytopathology* 43: 261–278.

Scheu, G. 1935. Die Rollkrankheit des Rebstockes. D.D. Weinbau 14: 222–358.

Stace-Smith, R. 1984. *Tomato ringspot virus*. CMI/AAB Description of Plant Viruses No. 290. Association of Applied Biologists. Wellesbourne, UK.

———. 1985. *Tobacco ringspot virus*. CMI/AAB Description of Plant Viruses No. 309. Association of Applied Biologists. Wellesbourne, UK.

Tanne, E., N. Shtanovitz, and P. Spiegel-Roy. 1993. Rapid diagnosis of grapevine corky bark by in vitro micrografting. *Horticultural Science* 28: 667–668.

Tanne, E., P. Spiegel-Roy, and N. Shlamovitz. 1996. Rapid *in vitro* indexing of grapevine viral diseases: the effect of stress-inducing agents on the diagnosis of leafroll. *Plant Disease* 80: 972–974.

Taylor, R.H., R.C. Woodham, and A.W. Allen. 1967. Green-grafting: a useful technique for detecting grapevine viruses. *Australian Journal of Experimental Agriculture and Animal Husbandry* 7: 91–95.

Uyemoto, J.K., A.C. Goheen, C.F. Luhn, and L.J. Peterson. 1976. Use of *Chenopodium quinoa* in indexing for *Grapevine fanleaf virus*. *Plant Disease Reporter* 60: 536–538.

Vindimian, M.E., A.M. Ciccotti, U. Malossini, and I. Rancador. 1998. Biological indexing in greenhouse with the use of micropropagated material of genus *Vitis*. *Acta Horticulturae* 473: 151–153.

Walter, B., P. Bass, R. Legin, C. Martin, R. Vernoy, A. Collas, and G. Vesselle. 1990. The use of a green-grafting technique for the detection of virus-like disease of the grapevine. *Journal of Phytopathology* 128: 137–145.

Wilcox, F.W., D.G. Gubler, and J.K. Uyemoto. 2015. *Compendium of Grape Diseases, Disorders and Pests, pp 114–134*. St. Paul: APS Press.