Baozhong Meng · Giovanni P. Martelli
Deborah A. Golino · Marc Fuchs *Editors*

Grapevine Viruses: Molecular Biology, Diagnostics and Management

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Preface

Grapevine is one of the most important fruit crops throughout the world. With evidence of its cultivation in the Middle East over 8000 years ago, it is also one of the most ancient grown horticultural crops. Based on the International Organisation of Vine and Wine, in 2014 alone, grapevine was grown on 7.5 million hectares, producing 75 million metric tons of grapes. Together, the grape and wine industry represents a major economic cornerstone for many countries. Interestingly, grapevine hosts the largest number of viruses known to infect a crop plant. Since the initial identification and characterization of grapevine fanleaf virus in degenerated grapevines almost 60 years ago, nearly 70 distinct virus species that belong to a wide range of taxonomic groups (17 families and 27 genera) have been reported in grapevine. From an economic perspective, many grapevine viruses are important because they are highly pathogenic and responsible for widespread disease complexes, such as infectious degeneration, leaf roll, rugose wood, and graft incompatibility and decline, and can be of regulatory concern. More recently, emerging viruses such as grapevine red blotch-associated virus and grapevine Pinot gris virus have been identified in association with economically relevant diseases. Most of the viruses identified in grapevine infect only *Vitis* spp.

Many of the grapevine viruses have unique attributes compared to more extensively studied plant viruses that infect annual, herbaceous crop plants. Our understanding of the molecular biology, evolution, and pathological properties of the grapevine viruses in general, those involved in the aforementioned disease complexes and especially those of the families *Closteroviridae* and *Betaflexiviridae*, is very limited. Much further work is required in the years to come.

The advent and application of recombinant DNA methodologies and, more recently, of high-throughput sequencing (HTS) technologies have advanced, at an unprecedented speed, the field of grapevine virology in the past two decades. Such advances include the development and refinement of rapid and highly sensitive nucleic acid-based assays for the detection of a large number of grapevine viruses, as well as the discovery of new viruses and viral strains. Further, HTS technologies have enabled the characterization of viral communities (virome) in an infected grapevine or even a commercial vineyard. This sets the foundation for the elucidation

and understanding of the collective impact of multiple, coinfecting viruses on the grapevine host. This is very important because grapevine, as a woody perennial species, is commonly infected simultaneously with multiple viruses. Therefore, it is critical that we understand the biology of individual viruses, but we also need to understand how a certain combination of viruses interacts and exerts an even greater effect on the grapevine host.

Several books have been published on various aspects of grapevine virology in the last century. The most recent book, entitled *Graft-Transmissible Diseases of Grapevines: Handbook for Detection and Diagnosis*, by Dr. G. P. Martelli, was published in 1993. Much information has been generated since then. A new and comprehensive book on this subject, entitled *Grapevine Viruses: Molecular Biology, Diagnostics and Management*, was deemed necessary and beneficial for diverse readership communities. This book comprises four sections. Section I starts with a brief account on grapevine, a brief history of viticulture and winemaking, and an overview chapter on grapevine viruses, viroids, and the associated diseases. This is followed by 17 chapters each focusing on a specific virus or a group of related viruses and viroids. Section II includes three chapters on the methods currently in use for the detection of grapevine viruses and the diagnosis of viral diseases. In Sect. III, topics include effects of viruses and their diseases on the grapevine host, as well as on fruits and wine products, the transmission of viruses by vectors, and management strategies that are either currently used or novel strategies that are explored. The last section describes methodologies and applications of high--throughput sequencing technologies, the potential applications of viruses as beneficial vectors for protein expression and functional genomics, as well as speculations on the origin and evolution of major grapevine viruses. This book ends with a conclusion chapter that points out some future research directions in grapevine virology.

This book is intended for a broad audience, including researchers and students interested in grapevine virology, extension educators, viticulturists, vintners, service providers, and regulatory agencies, as well as diagnostic laboratories. Many of the chapters are also comprehensible to avid grape growers and nurseries that are directly impacted by viruses and the diseases they cause and have to deal with the resulting hardship. The inclusion of color photographs to illustrate typical disease symptoms caused by major grapevine viruses should render this book helpful to a wide readership.

We would like to thank the large number of authors who has participated in, and made significant contributions to, this project. Without their support, this book project would not have come to fruition. A special thank-you goes to Dr. Kenneth K. Tang, publishing editor at Springer, for his assistance with the initiation of this project. A meeting at the University of Guelph, Ontario, Canada, in June 2014 made this project possible. We also thank Ms. Mariska Van Der Stigchel, editorial assistant at Springer, for guiding us through the various technical and editorial requirements throughout this project. Finally, we are expressing our gratitude to you, the reader of this book, for your interest and curiosity. We sincerely hope the content of

the book will expand your knowledge and solicit a desire to join us in further exploring the fascinating field of grapevine virology.

Lastly, as this is the first attempt to compile such a comprehensive book, mistakes and insufficiencies are inevitable. Suggestions and constructive criticisms for further improvement are most welcome.

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Part I
An Overview on Grapevine Viruses, Viroids and the Diseases They Cause

Chapter 1
The Grapevine, Viticulture, and Winemaking: A Brief Introduction

A.G. Reynolds

Abstract Grapevine is one of the longest-domesticated species with evidence of winemaking found in Anatolia dating from ca. 6000 BCE. Its spread throughout the Near East and Europe relied upon: (1) cultivar and later clonal selection and (2) vegetative propagation. Both of these processes encouraged the spread of viruses and increased the potential for infections that might result in yield reductions, compromised fruit composition, and reduced wine quality. This chapter describes how *Vitis vinifera* became a widespread crop species throughout the Near East and Europe during Neolithic, Bronze Age, Iron Ages, and thereafter and the implications that were brought by vegetative propagation of existing cultivars, new cultivars, and new clones in terms of vine vigor, yield, berry composition, and wine quality.

Keywords Neolithic • Iron age • Bronze age • Transcaucasia • Anatolia • Mesopotamia • *Vitis vinifera sylvestris*

Introduction

Grapevine (*Vitis* spp.) is among the most widely grown of fruit crops worldwide. Recent worldwide production estimates (2014) are 7.6 million hectares and 74 million metric tons (MT; OIV 2016). Its main use is for wine production (270 million hL, MhL), but grapes are also grown for fresh fruit (25 MT), raisins (5.2 MT), juice (30 MhL), vinegar, seed oils, and other products (OIV 2016). Five countries presently represent 50% of the world's vineyards (thousands of ha): Spain (1038), China (799), France (792), Italy (690), and Turkey (502) (OIV 2016; Table 1.1). Major wine-producing countries include (MhL) France (46.7), Italy (44.7), Spain (38.2), the USA (22.2), and Argentina (15.2) (OIV 2016; Table 1.1). Wine presently occupies 55% of grape usage, followed by 35% (fresh grapes), 8% (raisins), and 2% (juice, etc.) (OIV 2016).

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Table 1.1 Top grape-producing countries in the world

Country	Vineyard area (kha)	Wine production (mhL)
Spain	1038	38.2
China	799	11.2
France	792	46.7
Italy	690	44.7
Turkey	502	[a]
USA	425	22.3
Argentina	228	15.2
Portugal	224	6.2
Chile	211	10.5
Romania	192	3.7
Australia	154	12.0
Moldavia	133	4.1
South Africa	132	11.3
Greece	110	2.9
Germany	102	9.2
Brazil	89	2.7
Hungary	78	2.6
Ukraine	69	2.0
Russia	47	5.4
Austria	44	2.3

Source: OIV (2016)
[a]Mostly table grapes and raisins

Evolution of *Vitis* spp. in the Near East and Europe

Grapevines belong to the family Vitaceae, which contains 12 genera and >700 species (Galet 2000). Most species of Vitaceae are climbing vines and include genera such as *Ampelocissus*, *Ampelopsis*, *Cayratia*, *Cissus*, *Clematicissus*, *Parthenocissus*, *Tetrastigma*, and *Vitis* (Galet 2000). It has been speculated that prior to separation of our present continents in the late Jurassic Period (165 million years ago), there existed a northern portion of the landmass, Laurasia, and a southern portion, Gondwanaland. Modern members of Vitaceae in former Laurasia mostly have chromosome number of 19 or 20 (e.g., *Vitis*, *Ampelopsis*, *Parthenocissus*), whereas those native to former Gondwanaland (e.g., *Ampelocissus*, *Cissus*, *Cayratia*) have $n = 11$ or 12. Putative early members of Vitaceae (*Cissites, Vitiphyllum*) likely evolved during the Cretaceous Period, and fossils have been discovered in Nebraska and Portugal (Galet 2000). Confirmed species of Vitaceae are associated with the beginning of the Tertiary Period to Early Eocene (50 million years ago) (*Ampelopsis*, *Cissus*). The first *Vitis* fossils date from the Eocene in England (*V. subglosa*) and France (*V. sezannensis*) and from the Miocene in Germany (*V. teutonica*). Fossils in Provence dated to the Early Quaternary Period include *V. ausoniae*, which resemble *V. vinifera*. Prehistoric grapevines are known in Europe from the Paleolithic/

Mesolithic periods onward (Galet 2000; Renfrew 1996). Neolithic evidence includes seeds from several locations in Switzerland and wood from Italy and Belgium. Grapevine seeds and canes from the Bronze Age were discovered in numerous locations throughout Italy, Greece, and elsewhere (Galet 2000; Renfrew 1996).

A fairly large number of *Vitis* species (≈60) have evolved worldwide, of which *V. vinifera* has become the most widespread for wine and table use. However, North America is considered as a major center of origin of numerous *Vitis* species. Numerous interfertile dioecious *Vitis* spp. are native to North America, Mexico, and the Caribbean (Olmo 1976). All *Vitis* species contain 19 chromosome pairs and all are capable of hybridization. Among North American species, *V. labrusca* (fox grape) has been used to develop several cultivars widely used for juice production (e.g., Concord, Niagara, Catawba), and these are widely grown in the Great Lakes region (Fig. 1.1a). Most of these cultivars are considered as *V. labruscana*, since their genetic background likely contains species other than exclusively *V. labrusca* (Cattell and Stauffer Miller 1980; Hedrick et al. 1908; Reynolds and Reisch 2015). Recently introduced wine grape hybrids, such as Frontenac, La Crescent, and Marquette, contain up to 50% *V. riparia* (Riverbank grape; Fig. 1.1b) (Hemstad 2015). Herbemont and Black Spanish cultivars, grown in Texas and other parts of the US Gulf Coast due to their resistance to Pierce's disease (*Xylella fastidiosa*), are thought to be hybrids of *V. cinerea*, *V. aestivalis*, *and V. vinifera* (Munson 1900). *V. riparia*, *V. rupestris* (Sand Grape; Fig. 1.1c), and *V. berlandieri* (mountain grape) have also been widely used for rootstock breeding and well-known rootstocks such as Couderc 3309, Millardet et de Grasset 101-14, and MdG 101-15 (*riparia* X *rupestris*), as well as Kober 5BB, 5C, and SO 4 (*berlandieri* X *riparia*). The cultivar Norton (Cynthiana), grown widely in the US Midwest, is likely a pure clone of *V. aestivalis* (Fig. 1.1d; Hedrick et al. 1908). *V. candicans* (mustang grape), native to Texas, gave rise to *V. champini* (*candicans* X *rupestris*) and was the basis for several rootstocks with phylloxera (*Daktulosphaira vitifoliae*) and salinity resistance, e.g., Couderc 1613 and C.1616 (based on *V. candicans;* Fig. 1.1e), and Salt Creek and Dog Ridge (based on *V. champini*) (Munson 1900).

A related genus, *Muscadinia*, contains one major species, *M. rotundifolia* (formerly *V. muscadinia*; Fig. 1.1f). *Muscadinia* contain 20 chromosome pairs and cannot hybridize successfully with other *Vitis* species by conventional breeding. A well-known cultivar, scuppernong, dates to the seventeenth century. Breeding programs in southern USA have led to the introduction of several cultivars (Stafne et al. 2015). *Muscadinia* cultivars are well-known as having immunity to phylloxera, and efforts have taken place to use them in rootstock breeding (Olmo 1996; Walker et al. 1985).

Eastern Asia is considered, with the Near East and North America, a major center of origin of many grape species. Liu and Liu (2015) indicate there are 37 species, one subspecies, and 10 variation species of grape in China alone. Among eastern Asian species, perhaps the best known is *V. amurensis* (Liu and Liu 2015). It is extremely cold-resistant but has no resistance to fungal diseases introduced from North America such as powdery mildew (*Uncinula necator*), downy mildew (*Plasmopara viticola*), black rot (*Guignardia bidwellii*), and phomopsis (*Phomopsis viticola*). Numerous *V. vinifera* X *V. amurensis* hybrids have been produced from crosses dating back to 1951 (Liu and Liu 2015).

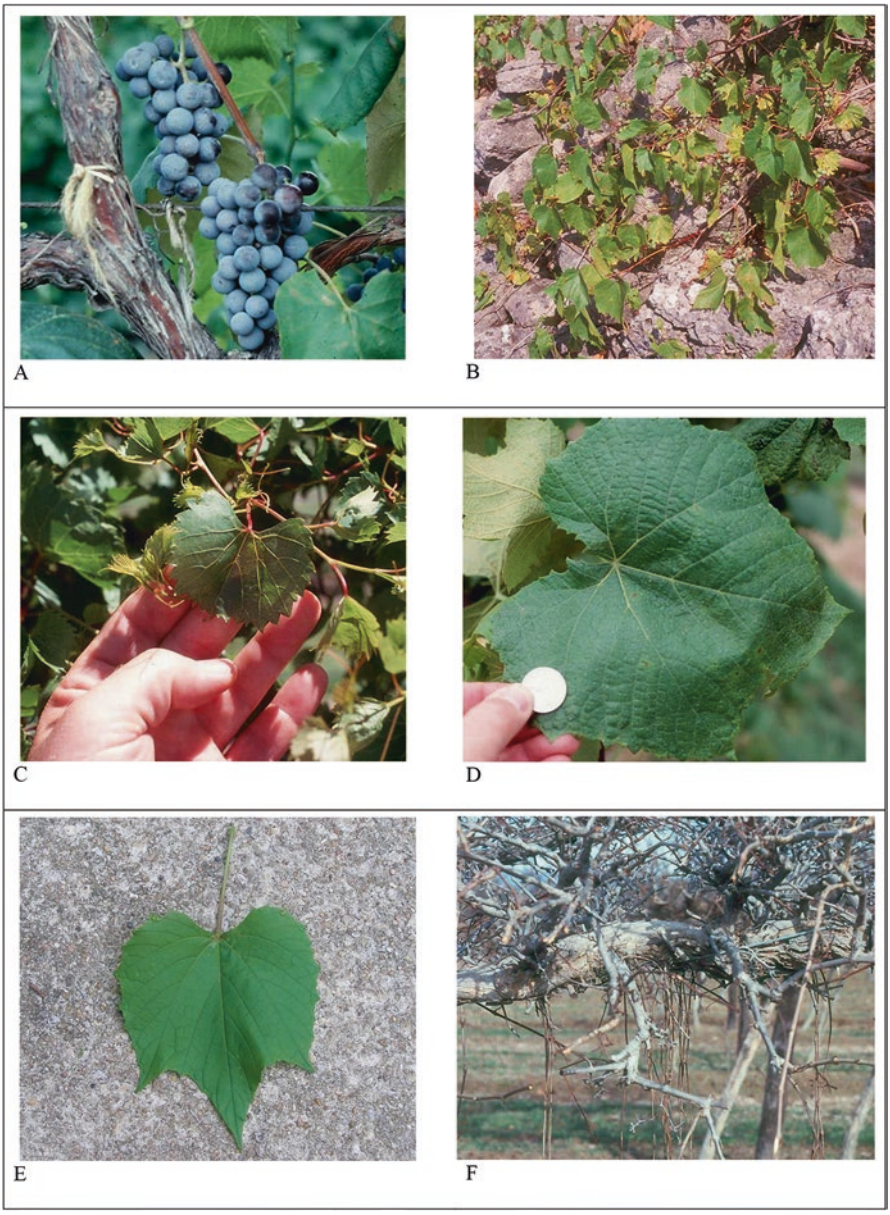

Fig. 1.1 Examples of North American Vitaceae species. (**a**) *V. labruscana* Concord; (**b**) *V. riparia*; (**c**) *V. rupestris*; (**d**) *V. aestivalis* Norton; (**e**) *V. candicans*; (**f**) *Muscadinia rotundifolia* (Photos: A.G. Reynolds)

Europe and Central Asia has a single species, *V. vinifera*, which is frequently subdivided into *V. vinifera* ssp. *sativa* (hereinafter *V. vinifera*; cultivated grape) and *V. vinifera* ssp. *sylvestristypica* (hereinafter *V. sylvestris*; wild grape) (Olmo 1996). Present cultivars likely arose initially by collection and planting seeds. These seed-propagated populations would have been highly heterozygous, and specific cultivars could not have been selected until vegetative propagation (by cuttings or layering) was introduced. One very practical basis for selection was hermaphrodism, since *V. sylvestris* was by nature dioecious. Grape seeds recovered from early archeological sites tend to be round with short beaks (*V. sylvestris*), whereas late Neolithic and Early Bronze Age sites have revealed seeds that are longer with elongated beaks (*V. vinifera*). It is highly likely that the emergence of *V. vinifera* corresponded with vegetative propagation, cultivar selection, and establishment of vineyards of uniform hermaphroditic cultivars. Singleton (1996) has suggested that vegetative propagation may have begun as early as ca. 8000 BCE, which predates the Neolithic.

There are now >10,000 grapevine cultivars recognized, and this number has expanded substantially over the past 150 years as a result of grapevine breeding programs. Several attempts have been made to classify *V. vinifera* on the basis of ampelographic traits. Perhaps most widely accepted is that of Negrul (1946), who divided all *V. vinifera* into three classes, or *proles* (descendance) based on a combination of ampelographic, ecological, and geographical criteria. *Proles occidentalis* includes most small-clustered wine cultivars of western Europe, e.g., Pinot noir, Chardonnay, Riesling, etc.; they share common traits such as high sugar, high acidity, small clusters, and in most cases slightly hairy shoot tips. *Proles pontica* includes those cultivars that originated on the banks of the Black Sea near the purported center of origin, most of which have hairy growing tips and include Furmint, Rkatsiteli, Black Corinth, etc. This group has been subdivided into *sub-proles balkanica*, which includes most small-clustered wine grapes such as Furmint, and *sub-proles georgica*, which encompasses large-clustered wine grapes, e.g., Rkatsiteli. *Proles orientalis* has glabrous shoot tips, large clusters, and frequently muscat flavor and seedlessness. Typical cultivars are Thompson Seedless, Muscat blanc, Muscat of Alexandria, etc. This group has been subdivided into *sub-proles caspica*, which includes large-clustered wine grapes (e.g., Alicante), and *sub-proles antasiatica*, which includes large-clustered table grapes (e.g., Thompson Seedless).

The Evolution of Viticulture in Neolithic Times (10200–2000 BCE)

V. vinifera has often been said to have evolved in the Transcaucasia region, between the Black and Caspian Seas (Olmo 1996). However, there is considerable paleontological evidence to dispute this. At the end of the Tertiary Period (ca. 66 million to 2.6 million years ago) or Early Quaternary Period (ca. 2.6 million years ago), *V. sylvestris* was already present in western Europe and Asia Minor (Galet 2000;

Olmo 1996). During the Pleistocene Epoch (2.6 million to 11,700 years ago), *V. sylvestris* survived in forests throughout the Mediterranean and south of the Caspian Sea. During the Neolithic Period, *V. sylvestris* occupied a similar distribution although its range was somewhat diminished due to climate change resulting from glaciation. This wild grapevine (*V. vinifera* ssp. *sylvestris typica*) picked by humans were from dioecious vines spread by birds and other animals and are referred to as Lambrusco (i.e., lambrusque; wild) vines (Zohary 1996). Although these feral vines were substantially reduced in population by phylloxera beginning in the mid-nineteenth century, they are still widely distributed throughout Mediterranean Europe and North Africa (Zohary 1996). Seeds, canes, and other materials found in Neolithic encampments in Switzerland and Italy suggest that grapes were already becoming an important food source. There are likewise several paleobotanical finds in Greece, one of which dates to the Paleolithic Period (11000 BCE), and a lengthy list of grape-related archeological sites that date to the Early (6400–5300 BCE), Middle (5300–4300 BCE), and Late (4300–2800 BCE) Neolithic Period. Seeds from *V. sylvestris* are typically round and squat, whereas *V. vinifera* seeds are more elongated. Although the Paleolithic and Early/Middle Neolithic finds are exclusively *V. sylvestris*, some seeds from the Late Neolithic sites are definitely *V. vinifera*, strongly suggesting that viticulture had begun during this period and that vegetative propagation was being used to establish vineyards.

Godin Tepe

The Godin Tepe site in Iran may be the first archeological site that provides evidence of winemaking and wine consumption in Neolithic times (Badler 1996; McGovern and Michel 1996). This site in the Zagros Mountains dates from the late fourth millennium BCE—3500–3100 BCE for the early phase and 3100–2900 BCE for the late and final phase. The clay jars recovered from the site are inverted teardrop shapes with narrow openings at the tops to facilitate pouring (Fig. 1.2a). Small holes drilled on the sides of the jars above the base are speculated to be for draining of finished wines (i.e., decanting, racking) or for release of CO_2 during fermentation. Residue from jars has included tartaric acid and a red deposit that is presumably anthocyanin pigments. Additional information from the site suggested that inhabitants traded extensively with southern Mesopotamia and southwestern Iran. Of perhaps greater significance is the fact that Mesopotamia was a beer-drinking culture, with no evidence of native *Vitis*. Consequently, initial access to grapes must have come from further north, likely Transcaucasia. Whether the vineyards planted in Godin Tepe were seed-propagated or vegetatively propagated is unknown.

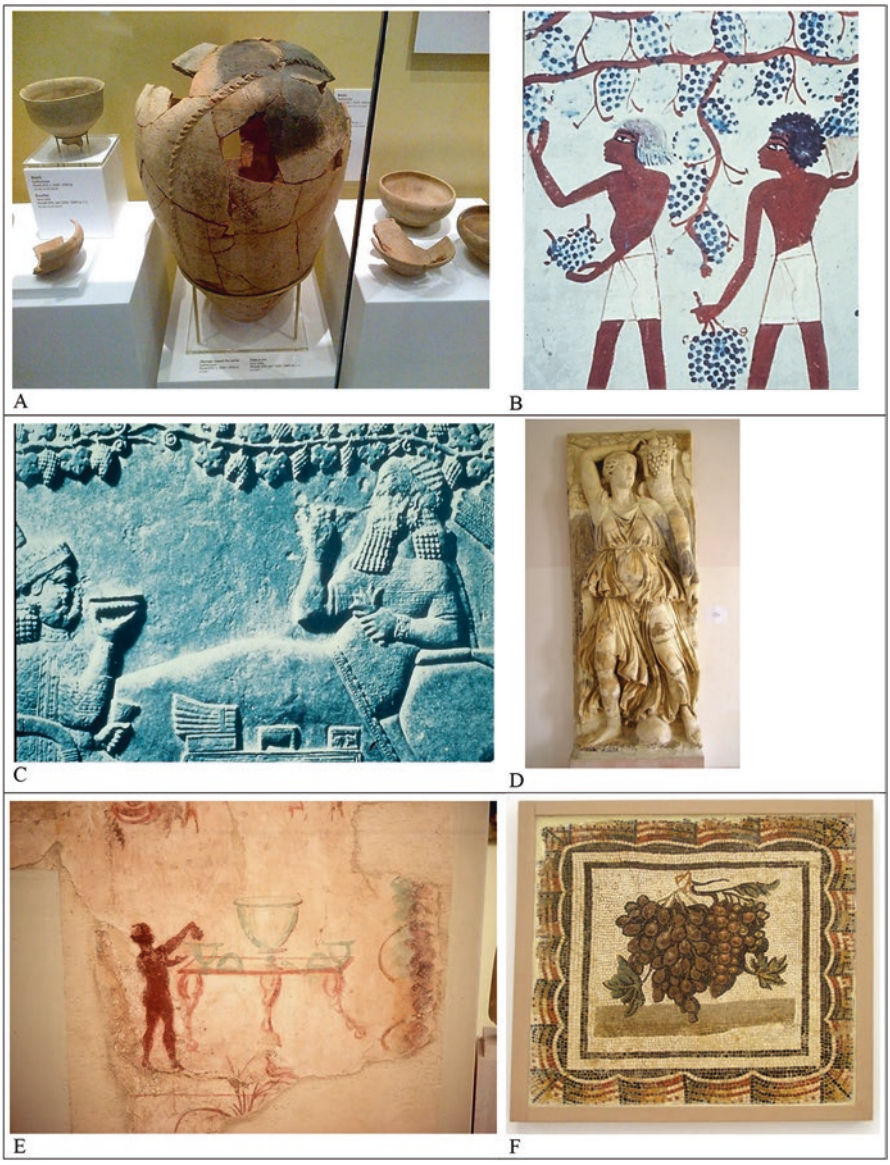

Fig. 1.2 Images of the ancient history of wine. (**a**) Godin Tepe vessel (ca. 4000 BCE), Royal Ontario Museum; (**b**) ancient Egyptian harvest scene; (**c**) King Ashurbanipal of Assyria (668–630 BCE) consuming wine; (**d**) wine-related statue, Carthage Museum, Tunis; (**e**) wine service fresco, Delos, Greece; (**f**) Roman mosaic, Bordo Museum, Tunis (Photographs: **a**, **d**, **e**, **f**: A.G. Reynolds; **b**, **c**: Wilhelm Nassau)

Mesopotamia

Evidence of grape consumption dates to pre-Bronze Age Mesopotamia. The Neolithic site Abu Hureyra revealed grape seeds that presumably came from *V. sylvestris* growing along the upper Euphrates (Algaze 1996). The Uruk Period in ancient Mesopotamia beginning in the fourth millennium BCE included numerous urban areas in valleys throughout the Zagros Mountains and in the alluvial zone between the Tigris and Euphrates rivers (Algaze 1996). These outposts extended as far north as the Taurus Mountains in eastern Anatolia and included well-known cities such as Aleppo and Nineveh, in addition to outposts of archeological significance such as Arslan Tepe in the Taurus Mountains. It is clear that these urban areas were widely engaged in trade—wood, flint, copper, precious stones, textiles, pottery, and also agricultural products that included beer and wine. Several examples of Uruk spouted bottles have been discovered in Arslan Tepe and were likely used for containment of wine and olive oil. It is speculated that these products were shipped on the Tigris and Euphrates downstream and westward into Anatolia.

Anatolia

No historical discussion of wine would be complete without a short treatise on Anatolia. Eastern Anatolia, which includes modern Armenia, Georgia, and Azerbaijan, is widely believed to be the center of origin of *V. vinifera*, and Herodotus indicated Armenia to be the source of winemaking, although evidence for this is limited (Gorny 1996). Noah is mentioned in the Bible as planting vineyards on the slopes of Mount Ararat. Neolithic evidence of wild grape consumption includes seeds uncovered in a ninth millennium BCE site in Çayönü and Can Hasan III (7200–6500 BCE) in eastern Turkey and suggests the potential for rudimentary viticultural experimentation (Gorny 1996). Invention of pottery ca. sixth millennium BCE permitted production and storage of wines. The earliest viticulture in the region has been credited to the spread of Transcaucasian culture in eastern Anatolia in the fourth millennium BCE; however, wild grapes were identified from this period throughout coastal Turkey as well as interior river valleys, which casts doubt on a Transcaucasian origin of viticulture. Chalcolithic (4500–3500 BCE) seed evidence has also been uncovered at Korucutepe, a possible cultivar from Tepecik, and Late Chalcolithic (3500–3200 BCE) seed and charcoal remains at Kurban Höyük (Gorny 1996). The majority of archeological evidence of viticulture dates from the Early Bronze Age and thereafter.

Bronze Age Viticulture (3300–1200 BCE)

Mesopotamia

The first evidence of systematic writing appeared in Mesopotamia ca. 3000 BCE with the introduction of cuneiform (Powell 1996). This is noteworthy because it is also during the Early Bronze Age (third millennium BCE) that evidence of actual viticulture emerged (Algaze 1996). Cuneiform symbols ca. 3000 BCE exist in early Sumerian texts for grape, as well as date palm, apple, and fig. However, evidence of viticulture from Babylonia in the lower Tigris-Euphrates is not extensive. Part of the reason for this is climatic—prior to the advent of irrigation, culture of temperate fruit crops was likely unsuccessful due to high summer temperatures and lack of rainfall. Moreover, the southern Tigris-Euphrates region contains poorly drained sites with highly saline soils, which do not facilitate viticulture. Those grapevines grown in the region were trained to trees—an advent of modern trellising—and also grown in raised beds. Most viticulture consequently was primarily limited to areas north of the present Syrian-Iraq border, in river valleys, and higher elevation sites. However, in the third millennium BCE, seeds and charcoal were identified in Malyan in Iran, which is outside the range of wild grape, suggesting that actual viticulture was being practiced. Sumerian texts describe production of a multitude of grape-based products, including wine, juice, concentrated grape syrups, and raisins. It also appears likely that grape syrups were used as a sugar source to facilitate beer fermentations, since the Babylonian civilization was largely a beer-drinking culture.

The Middle Bronze Age in Mesopotamia included the reign of Hammurabi (ca. 1792–1750 BCE). His destruction of the city of Mari in Assyria on the Euphrates has left evidence of viticulture in northern Mesopotamia (Powell 1996). As with southern Mesopotamia, the extent of wine production in the region does not appear to be large, and much of the wines mentioned in texts originated further north and west. There is also text evidence of wine trade between Antioch on the Mediterranean and Aleppo through the town of Alalakh in northern Assyria. However, just as agricultural limitations restricted viticulture in the lower Tigris-Euphrates and consequently defined wine as a luxury good, Middle to Late Bronze Age wines were likewise regarded as items for the elite.

Anatolia

Botanical evidence exists for Early Bronze Age viticulture in Anatolia at the beginning of the third millennium BCE. Seed evidence has been uncovered at several sites in Anatolia including Korucutepe (Early Bronze Age), Tepecik, Arslan Tepe (Early Bronze Age), Kurban Höyük (Early Bronze Age), Tell es-Sweyhet, Tell Hadidi (Late Bronze Age), and Tell Selenkahiyah (Early Bronze Age) (Gorny 1996).

Introduction of writing in the Middle Bronze Age documented development of viticulture in the region. The period referred to as the Old Assyrian Colony Age (ca. 2000–1750 BCE) provides evidence of grape harvesting and wine production, although it is apparent that the Assyrian colonists likely derived their horticultural skills from their Anatolian natives. Bronze Age Anatolia is dominated by the Hittite culture (1600–1200 BCE). This is noteworthy for several reasons—written texts in the second millennium BCE described the role of viticulture, wine production and consumption became more commonplace by the first millennium BCE, and new vessel shapes were closely linked to wine storage and transport. Wine, more than any other food, became central to the Hittite way of life and came to symbolize life itself. The development of vocabulary associated with viticulture included terms associated with a young plant, a mature vine, cane, cluster, and roots. A detailed set of laws described penalties for theft of a grapevine, burning a vineyard, release of sheep into a vineyard, etc. In their summation, they provide ample evidence that viticulture was extant throughout the Anatolian Peninsula, with the likelihood that individual cultivars had been selected, and vegetative propagation was standard practice. Other grape-derived products included raisins, raisin wine, and *basduk* (boiled-down juice dried into a leather-like substance).

Egypt

Grape seeds have been reported from predynastic fourth millennium sites south of Cairo east of the Nile River (James 1996). Dynastic Egypt began in the Early Bronze Age ca. 3100 BCE. Viticulture was likely introduced to Egypt from western Asia in predynastic times (James 1996; Zohary 1996). Old Kingdom (Second Dynasty; ca. 2890–2686 BCE) hieroglyphs depict a wine press, several variations of trellises, and vines growing from pots (Fig. 1.2b). Later evidence from the Third Dynasty (ca. 2686–2613 BCE) includes seeds in tombs, raisins, hieroglyphic representations of wine presses, and numerous storage jars. The tomb of Metjen of the Fourth Dynasty is significant insofar it describes the establishment of a vineyard in addition to detailed hieroglyphic descriptions of the winemaking process, including harvest, treading, pressing, and the filling of wine jars. However, no details appear to be available as to whether vineyard establishment occurred by use of cuttings or planting of seedlings. Scenes of winemaking are likewise found in tombs from the Fifth Dynasty (ca. 2494–2345 BCE) onward.

Archeological evidence of wine production in Middle Kingdom tombs is abundant but not necessarily more informative than Old Kingdom hieroglyphs with respect to viticulture. Late 11th Dynasty (ca. 2050–2000 BCE) tombs depict various stages of winemaking, including one where men sieve pressed juice through a cloth into a jar. In the New Kingdom 18th Dynasty (ca. 1570–1544 BCE), wine jars were marked with information such as vintage, winemaker, and vineyard (Lesko 1996).

Aegean

As in ancient Anatolia, predynastic Egypt, and much of Mesopotamia, viticulture was prevalent in Greece prior to 3000 BCE (Leonard 1996). The change from wild to domesticated grapes took place during the Early Bronze Age. Several storage jars from Crete and the Cyclades dated from this period contain images of grape leaves, providing evidence that they likely contained wine. Numerous Early, Middle, and Late Bronze Age paleobotanical sites exist throughout mainland Greece, Crete, and other Aegean islands. Most of these contain a significant percentage of *V. vinifera* seeds admixed with those from *V. sylvestris*, which could suggest that vineyards had been established simultaneously with grape harvest from wild vines. Renfrew (1996) suggests that by the Late Neolithic Period and most certainly by the Early Bronze Age, viticulture was extant to the point that propagation by cuttings and possibly grafting was typical, as was pruning, hybridization between *V. sylvestris* and *V. vinifera*, and cultivar selection for specific purposes such as for wine, table grapes, and raisins. Overall, seed evidence indicates that viticulture and wine production existed in Greece at least 1500 years before the establishment of the Mycenaean period ca. 1600 BCE, and it is likely that grapes, olives, wheat, and barley were grown in Crete and Mainland Greece ca. 2170 BCE.

Iron Age Viticulture (1200–500 BC) and Relevance to Spread of Viruses

Eastern Mediterranean and North Africa

It is highly likely that *V. sylvestris* was native to higher elevation areas in North Africa in addition to Mediterranean Europe and Transcaucasia. Moreover, although there was a vigorous wine trade during the Bronze Age among Greece, Egypt, Anatolia, and the Levant (Fig. 1.2c), there is no reason to believe that viticulture did not occur in North Africa prior to the arrival of Phoenician traders. However, there is apparently no hard evidence supporting this; so therefore, one must accept that Phoenicians established Carthage ca. eighth century BCE (Greene 1996). Earliest evidence of *V. vinifera* in Carthage dates to the fourth century BCE (Fig. 1.2D). During this period, Carthage contained vast areas of olive groves and vineyards. Writings of Mago the Carthaginian agronomist include suggestions on vine planting, pruning, site selection (planting on north-facing slopes), and production of raisin wine. It was also likely that the Phoenicians brought cuttings from the Levant for vineyard establishment in Carthage and elsewhere in North Africa.

Greece and Rome

Phoenicians traded vigorously with Greece, Carthage, and also with the developing Roman Empire (Fig. 1.2e, f). Vines were first transported to southern France and the Iberian Peninsula shortly after the Punic Wars in the second century BCE. As the empire expanded, grapevines were transported to interior river valleys of Europe, and soon viticulture was prevalent in the Loire, Rhine, and Danube river valleys. Viticulture was introduced to France (Gaul) by the Greeks ca. 600 BCE and by the Romans ca. 125–118 BCE into the Languedoc and Rhone Valley and in the second century into Bordeaux and Burgundy (Mullins et al. 1992). Grape growing was introduced later into the river valleys of Germania (Germany), and the first reference to wine production in the region was in 370. Viticulture reached Brittania (Britain) in the first century. Writings of Roman authors such as Columella and others (e.g., Cato the Elder, Pliny, Quintillian) describe viticulture in great detail, but it is interesting that in Columella's famous treatise, there are no mentions of diseases and pests except those pertaining to cattle and sheep and only a single mention of insects (Columella 1745). Extensive description is dedicated to propagation methods, particularly grafting, in which it is described as "…how a cluster of grapes may have berries of different kinds." Propagation by cuttings and through layering is also described.

Propagation and Its Relevance to Spread of Viruses

There are substantial descriptions in Roman writings with respect to propagation of grapes by cuttings, layering, and grafting (Mudge et al. 2009). It is also highly likely that as hermaphroditic *V. vinifera* cultivars were selected sometime during the Bronze Age, vegetative propagation became commonplace for the establishment of vineyards. Consequently, if we are to assume that viruses were part of the ecosystem in ancient times, and moreover that insects capable of vectoring these viruses were likewise common, then it is plausible to suggest that viruses were being spread by vegetative propagation and subsequent vineyard establishment as early as the fourth millennium BCE or before. However, although we have fossil records of *Vitaceae* as far back as the Jurassic Period, and paleobotanical records of grape canes and seeds dated to the Neolithic, there is no record of insects other than those in amber (not soft-bodied insects capable of vectoring viruses, e.g., mealybugs or leafhoppers), and certainly there is no possible historical evidence of viruses.

Nonetheless, viruses were likely with us in Neolithic times. There is speculation that nepoviruses such as grapevine fanleaf were present in the first vineyards in present-day Syria, Iraq, and Turkey and likely were spread with the propagation and transplantation of vines (Hewitt 1968). Therefore, as viticulture spread from the Fertile Crescent to Egypt, the Aegean, and throughout the Mediterranean, rooted vines likely had both the nepoviruses in their vascular system and the vector

(i.e., nematodes) present on the root systems. Fresco paintings in Pompeii (ca. 79) suggest presence of fanleaf virus (Fuchs; personal communication 2016); however, one cannot be sure whether the leaf shape was entirely accurate or whether it can be attributed to artistic license. Assuming viruses—both nepoviruses and closteroviruses/ampeloviruses—were present in vineyards during the time of the Roman Empire, they would have been transported throughout the Empire to Britain, France, Germany, and down the Danube to central and eastern Europe. However, there is no indication until the widespread use of phylloxera-resistant rootstocks that viruses were in any way debilitating to grapevines (Hewitt 1968; Vuitennez 1962).

Grapevine Fanleaf

Reports of viruslike symptoms in grapevines are found in mid-19th literature, although this predates the actual discovery of viruses by nearly a century. Numerous reviews are extant that describe the history of grapevine viruses in substantial detail (Bovey 1958; Hewitt 1968; Martelli 2014; Martelli and Boudon-Padieu 2006). The latter reference mentions that >5400 papers related to nearly 70 grapevine viruses and viruslike agents had been published as of 2004, and many more have been appeared in literature since then. For brevity, we have confined our discussion in this chapter to the two major viral diseases, Grapevine fanleaf (GFL) and Grapevine leafroll (GLR). Grapevine fanleaf was described in France (Cazalis-Allut 1865), Austria (Rathay 1882), Italy (Ruggeri 1895; cited in Martelli 2014), and Germany (Cholin 1896), and herbarium specimens suggest GFL was present in Sicily in the late nineteenth century (Martelli and Piro 1975). Baccarini (1902) first suggested that GFL may be due to a virus. Two important conclusions were reached shortly thereafter, when graft transmission of grapevine fanleaf virus (GFLV) was confirmed (Schiff-Giorgini 1906), and transmission through the soil was established (Pantanelli 1910). Several later studies speculated that GFL was viral in origin (Petri 1929; Arnaud and Arnaud 1931) but without necessary technology, this remained unproven. Introduction of electron microscopy permitted the ability to see GFLV and other plant viruses, and the first description of GFLV as well as its transmission from grapevine to herbaceous hosts was reported by Cadman et al. (1960). Prior to this Hewitt (1951, 1954) had concluded that GFLV and several other viruses were widespread in California vineyards. It was also widely believed that GFLV was transmitted by phylloxera (Arnaud 1937; Branas et al. 1937) until Hewitt et al. (1958) concluded that the nematode *Xiphinema index* was the vector. Control has been attempted by use of nematicides (Raski and Goheen 1988; Raski and Schmitt 1972; Raski et al. 1971, 1981, 1983), heat treatment (Bovey 1958; Gifford and Hewitt 1961; Goheen et al. 1965), and subsequent micropropagation (Barlass et al. 1982; Galzy 1964). The ultimate goal of virus elimination was the establishment of clean stock programs such as those in California (Olmo 1951). Detailed information on GFLV is available in Chaps. 3 and 4 of this book).

Grapevine Leafroll

Besides GFL, GLR is perhaps the second most common viral disease globally (Martelli 2014). An excellent detailed historical summary of GLR can be found in Hoefert and Gifford (1967). The first description of leafroll symptoms, called *rougeau*, was by Fabre (1853), who noted that red wine cultivars failed to develop color. A similar disease, *brunissure*, was described by Pastre (1891), which produced a brownish discoloration in autumn. Symptoms similar to what we now know as GLR were later described in California (red leaf; Butler 1905), Italy (Sannino 1906), France (*flavescence*; Ravaz and Roos 1905; Ravaz and Verge 1924), and Germany (Scheu 1935, 1936) early in the twentieth century. It was also likely, based on herbarium specimens, that GLR occurred in Sicily as early as the late nineteenth century (Martelli and Piro 1975). Scheu (1935) concluded that the GLR symptoms were viral in origin, and, graft transmissible, which suggests that grapevine leafroll-associated virus (GLRaV), may have been spread many millennia ago as a result of vegetative propagation. A disease in California known as "White Emperor Disease" (so known because the normally red Emperor fruit did not develop pigmentation) was described (Harmon and Snyder 1946) and was likewise considered as viral in origin and graft transmissible. Grapevine leafroll symptoms were described by Hewitt (1951, 1954), and subsequently Goheen et al. (1958) concluded that "White Emperor" and GLR were identical diseases.

Descriptions of GLR were subsequently published by authors in Australia (Fraser 1958), France (Vuittenez 1958), Czechoslovakia (Blattny et al. 1960), New Zealand (Chamberlain 1967), Italy (Belli and Cesati 1967), Switzerland (Bovey 1968), Hungary (Lehoczky et al. 1969), Yugoslavia (Dimitrijevic 1970), and Israel (Tanne and Nitzany 1973). Goheen and Cook (1959) reviewed the pre-1960 literature, as well as synthesized relevant work that attempted to explain the causal organism of the malady variously known as *brunissure*, *rougeau*, red leaf, and *flavescence*. Their own experiments indicated that all of these aforementioned diseases were very likely grapevine GLRaV. It was later confirmed that GLRaV was present in the original rootstocks imported into California in 1890 (Luhn and Goheen 1970), once again suggesting its graft transmissibility. In addition to graft transmissibility, GLRaV can be transferred by several species of mealybugs such as *Planococcus ficus* (Rosciglione and Gugerli 1989), *P. longispinus* (Teliz et al. 1989), and *P. citri* (Cabaleiro and Segura 1997); *Pseudococcus affinis* (Golino et al. 1995), *Ps. longispinus*, *Ps. viburni*, *Ps. maritimus*, and *Ps. affinis* (Golino et al. 2000); and *Heliococcus bohemicus* and *Phenacoccus aceris* (Sforza et al. 2000). Transmission also occurs by scale insects such as *Pulvinaria vitis* (Belli et al. 1994; Sforza et al. 2000). Control of GLRaV has been based upon much the same strategies as for GFLV, particularly heat therapy (Diaz-Barrita et al. 2007; Goheen et al. 1965; Savino et al. 1991) and micropropagation (Barlass et al. 1982; Diaz-Barrita et al. 2007; Savino et al. 1991) followed by the establishment of clean stock programs. Strategies are also extant that involve a combination of pesticides to control mealybug vectors, as well as use of herbicides to control weeds in the vineyard perimeters that may harbor insect vectors (Pietersen et al. 2013).

Impact of Viruses on Yield, Berry Composition, and Wine Quality

Resistance and Susceptibility

Both GFLV and GLRaV produce significant debilitating effects in grapevines, particularly *V. vinifera*. However, there is evidence that some species and cultivars are at least partially resistant to GFLV (Martelli 2014). As Hewitt (1968) mentioned, GFLV is a very old virus that appears to coexist with *V. vinifera* when it is planted on its own roots. Accessions of *V. vinifera* obtained from the Middle East were reported to be GFLV resistant (Vuittenez 1962). Walker and Meredith (1990) and Walker et al. (1985) identified two *V. vinifera* accessions from Afghanistan and Iran resistant to GFLV and indicted the resistance to be based upon two unlinked recessive genes.

American species are generally susceptible to *X. index* and consequently are not GFLV resistant (Martelli 2014). However, Harris (1983, 1988), among others, indicated a wide range of American species with *X. index* or *X. americanum* resistance (see Oliver and Fuchs 2011 for a detailed list). *Muscadinia rotundifolia* has partial resistance and resists *Xiphinema index* transmission (Bouquet 1981, 1983), but becomes infected by graft transmission. Rootstock selections derived from *V. vinifera* x *M. rotundifolia* showed good GFLV resistance (Walker et al. 1985, 1989, 1994), as did hybrids of *V. rupestris* × *M. rotundifolia* (Walker and Jin 2000). The *rotundifolia*-based resistance is due specifically to a resistance to *X. index* feeding rather than host plant resistance to the virus and is controlled by a single dominant gene. More recent nematode-resistant rootstock introductions from the Davis, CA, program include *M. rotundifolia* parentage but also *V. champini*, *V. riparia*, *V. rufotomentosa*, *and V. rupestris* (Ferris et al. 2012).

V. labrusca is highly susceptible to GFLV but asymptomatic (Martelli 2014) and is quite susceptible to other eastern North American nepoviruses such as peach mosaic viruses (Ramsdell and Gillet 1985; Ramsdell and Myers 1978). Several French-American hybrids are highly sensitive to tomato ring spot virus, including Baco noir (Gilmer and Uyemoto 1972; Uyemoto and Gilmer 1972; Uyemoto et al. 1977), de Chaunac (Dias 1977; Uyemoto et al. 1977), Cascade (Uyemoto 1975; Uyemoto et al. 1977), and most rootstocks, e.g., C.3309, SO 4, 5BB (Gonsalves 1982; Uyemoto et al. 1977).

Unlike GFLV, there are no apparent sources of genetic resistance to GLRaV in *V. vinifera* (Martelli 2014), suggesting perhaps that it may not be an old virus. In fact, among 223 European, American, and Asian *Vitis* accessions tested, none were resistant to either GLRaV-1 or GLRaV-3 (Lahogue and Boulard 1996). Others, however, have suggested that despite this lack of host plant resistance that GLRaV is indeed an ancient virus that evolved with *V. vinifera* in the center of origin, mainly due to its common occurrence in own-rooted vines throughout the Middle East (Maree et al. 2013).

Effects on Physiology, Yield, and Berry Composition

Viruses most certainly cause debilitating effects in grapevines, and the impacts of GFLV and GLRaV have been widely researched. Grapevine fanleaf virus reduced photosynthetic rate, leaf chlorophyll concentration, trunk diameter, shoot length, berry diameter, and yield of Thompson Seedless vines in Chile compared to their virus-free counterparts (Auger et al. 1992). Yamakawa et al. (1987) reported that an unidentified virus infection of Merlot vines was associated with reduced cluster weight, berry volume and weight, and juice Brix compared to virus-free vines.

Early GLR literature described associations with reduced crops (Ravaz 1904), lack of pigmentation (Fabre 1853), delayed fruit maturity (Scheu 1936), potassium deficiencies (Ravaz et al. 1933), calcium deficiencies (Ravaz and Roos 1905), and impaired water relations (Butler 1905), although it was not clear whether these nutritional and vine water status issues were hypothesized solely as causes or effects. Cook and Goheen (1961) indeed reported lower potassium levels in leaves from GLRaV-infected vines, while Goheen and Cook (1959) reported that yields, cane lengths, and soluble solids (Brix) of five wine grape cultivars were reduced by GLR infection. Alley et al. (1963) similarly showed debilitating effects of GLR on Ruby Cabernet vines and also showed that wine color, alcohol, and tannin tended to be inversely related to severity of GLR symptoms. However, wine quality was not reduced, perhaps as a result of yield reductions in GLR-infected vines. There have been occasional reports of compromised physiology such as reduced photosynthesis (Bertamini et al. 2004; Endeshaw et al. 2014; Gutha et al. 2012) as well as other physiological metrics, e.g., stomatal conductance, transpiration, quantum efficiency of PS II, maximum carboxylation efficiency, rate of photosynthetic electron transport, and triose phosphate use (Endeshaw et al. 2014). Others have enumerated the negative effects of GLRaV including reduced yields (Alabi et al. 2016; Credi and Babini 1997; Endeshaw et al. 2014; Komar et al. 2007, 2010; Lider et al. 1975; Mannini et al. 2012; Over de Linden and Chamberlain 1970), cluster numbers (Alabi et al. 2016; Mannini et al. 2012), cluster weights (Komar et al. 2007, 2010; Mannini et al. 2012), berry weight (Hale and Woodham 1979), vine size (Credi and Babini 1997; Endeshaw et al. 2014; Guidoni et al. 1997; Komar et al. 2007, 2010; Lider et al. 1975; Mannini et al. 2012), Brix (Alabi et al. 2016; Cabaleiro et al. 1999; Endeshaw et al. 2014; Kliewer and Lider 1976; Komar et al. 2007, 2010; Lider et al. 1975; Martinson et al. 2008; Over de Linden and Chamberlain 1970; Wolpert and Vilas 1992), pH (Cabaleiro et al. 1999; Credi and Babini 1997), anthocyanins (Alabi et al. 2016; Guidoni et al. 1997; Over de Linden and Chamberlain 1970), and phenolics (Alabi et al. 2016; Guidoni et al. 1997), as well as increased titratable acidity (TA) (Cabaleiro et al. 1999; Kliewer and Lider 1976; Komar et al. 2007; Lider et al. 1975). Endeshaw et al. (2014) reported GLRaV-induced reductions in shoots per vine, shoot growth, shoot leaf area and internode length, and cane lignification. Kliewer and Lider (1976) also measured reductions in Burger fruit proline and arginine and increases in potassium, malate, and tartrate. Hale and Woodham (1979) likewise measured higher potassium, malate, and tartrate in

GLRaV-infected Sultana in Australia. Heat treatment of GLRaV-infected Nebbiolo vines led to increases in several anthocyanins as well as quercetin (Guidoni et al. 1997). Wines produced from GLRaV-infected Merlot vines in Washington State had less ethanol, anthocyanins, and phenols than those produced from virus-free vines and had less color, astringency, and red fruit aroma (Alabi et al. 2016).

GLRaV also impacts performance of hybrid vines. Kovacs et al. (2001) in Missouri found that virus infection did not reduce vine size but reduced berry weight slightly, decreased Brix and pH, and increased TA in St. Vincent and Vidal blanc. They attributed this low magnitude of effect to host tolerance. Milkus and Goodman (1999) reported the widespread occurrence of GLRaV-3 in French-American hybrids Seyval blanc (20–75% incidence) and Vignoles (0–100% incidence) in the region. Among six commercial vineyards sampled, four had GLRaV-3-infected plants. Disease incidence was also high for Norton (*V. aestivalis*) and Catawba (*V. labruscana*). The Finger Lakes region in NY also had a high percentage of sites infected with either GLRaV-1, -2, or -3 (Fuchs et al. 2009). Other indications of GLRaV infection is the exhaustive survey in Canada (Mackenzie et al. 1996) that sampled 1091 vineyards in Ontario, Quebec, and Nova Scotia and concluded that 560 had GLRaV-3-positive grapevines, of which 14.8% contained infected French-American hybrids. The common occurrence of GLRaV-3 in *V. labruscana* grapevines was also documented by Wilcox et al. (1998), who identified infected Concord, Catawba, Elvira, and Niagara grapevines in New York. None of the reports noted visual disease symptoms.

These effects of GLRaV on yield components, berry composition, and vine size have economic implications. Atallah et al. (2012) estimated a $25,000–$40,000 per hectare economic loss based on a 30% infection rate. They estimated that the impact of GLRaV could be substantially reduced (to $3000–$23,000 per ha) through rogueing if levels of disease prevalence are moderate (1–25%). However, the best response to GLRaV levels >25% is removal of the entire vineyard. Binzen Fuller et al. (2015) suggested that a virus screening program could save the North Coast region of California >$50 million. Research into vine responses to viticultural practices has been limited and mixed. Cluster thinning of GLRaV-infected Burger vines increased yield (Lider et al. 1975), Brix (Kliewer and Lider 1976; Lider et al. 1975), and proline (Kliewer and Lider 1976) and reduced TA (Lider et al. 1975). Basal leaf removal has been reported to increase Brix in fruit from GLRaV-affected vines (Pereira-Crespo et al. 2012). Rootstock choice appears not to have any impact on the magnitude of effect of GLRaV on yield components, vine size, and berry composition (Komar et al. 2010).

A recent study in Ontario examined several treatments including cluster thinning, basal leaf removal, exogenous abscisic acid (ABA), brown algal extract, and soluble silicon, alone and in various combinations on GLRaV-infected Cabernet franc (Hébert-Haché 2015). None of the treatments had any beneficial impact on yield components (Table 1.2), but leaf removal increased total anthocyanins and total phenols, while both the algal extract and a combination of cluster thinning + ABA + algal extract likewise increased phenols (Table 1.3).

Table 1.2 Yield and berry weights of Cabernet franc with confirmed GLRaV-1 and/or -3 infection, Beamsville, Ontario

Treatment[a]	Clusters/vine	Yield/vine (kg)	Cluster weight (g)	Berries/cluster	Berry weight (g)
Control	12 a[b]	1.48 ab	122.7 ab	101 ab	1.22
LR	12 a	1.37 ab	106.7 b	93 b	1.19
CT	**6 bc**	0.69 bc	110.7 b	93 b	1.18
SM	12 a	1.36 abc	108.3 b	86 b	1.24
2SM	13 a	1.68 a	126.1 ab	100 ab	1.21
ABA	16 a	1.47 a	146.0 a	126 a	1.19
SIL	15 a	2.02 a	125.4 ab	98 ab	1.27
SM+CT	**5 c**	0.71 bc	128.9 ab	107 ab	1.28
SM+CT+ABA	**5 c**	**0.61 c**	100.3 b	83 b	1.20

Hébert-Haché (2015)

[a]Treatments: control, leaf removal (LR), cluster thinning (CT), Stella Maris (SM; extract from marine brown algae *Ascophyllum nodosum*), double concentration Stella Maris (2SM), abscisic acid (ABA), Silamol (SIL; soluble Si), SM+CT and SM+CT+ABA

[b]Means followed by different letters are significantly different at $p \leq 0.05$ with Tukey's multiple comparison test. Means that are boldfaced are significantly lower than the control, $p \leq 0.05$

Table 1.3 Berry composition of Cabernet Franc with confirmed GLRaV-1 and/or -3 infection, Beamsville, Ontario

Treatment	Brix	Titratable acidity (g/L)	pH	Hue	Color intensity	Total anthocyanins (mg/L)[b,c]	Total phenols (mg/L)[d]
Control[a]	21.2	7.89	3.42	0.58 ab	12.3	665 b	2979 c
LR	22.7	7.55	3.44	0.56 b	15.9	**889 a**	**4086 a**
CT	21.6	7.97	3.46	0.56 b	15.4	729 ab	3473 abc
SM	21.7	7.94	3.42	0.59 ab	13.5	701 b	3288 bc
2SM	22.3	7.77	3.45	0.60 ab	13.3	740 ab	**3776 ab**
ABA	21.9	7.54	3.44	0.60 ab	13.4	736 ab	3644 abc
SIL	22.0	7.60	3.49	0.64 a	12.2	742 ab	3231 bc
SM + CT	22.4	7.78	3.47	0.59 ab	14.0	790 ab	3687 abc
SM + CT + ABA	21.9	8.37	3.46	0.58 ab	14.6	694 b	**3829 ab**

Hébert-Haché (2015)

[a]Treatments: control, leaf removal (LR), cluster thinning (CT), Stella Maris (SM; extract from marine brown algae *Ascophyllum nodosum*), double concentration Stella Maris (2SM), abscisic acid (ABA), Silamol (SIL; soluble Si), SM + CT and SM+CT+ABA

[b]Means followed by different letters are significantly different at $p \leq 0.05$ with Tukey's multiple comparison test. Means that are boldfaced are significantly higher than the control, $p \leq 0.05$

[c]Total anthocyanins measured in malvidin-3-glucoside equivalents

[d]Total phenols measured in gallic acid equivalents

Interest has increased in the detection of GLRaVs and possibly Grapevine red blotch-associated virus by the use of unmanned aerial vehicles (UAVs) as well as by proximal sensing (Reynolds et al. 2015). Data were collected by GreenSeeker proximal sensing in a GLRaV-affected Cabernet franc vineyard between July and September 2014, and GIS maps were created from the data. The Cabernet franc vineyard showed clear expansion of the zones with GLRaV (Fig. 1.3). Affected areas (designated by the red-colored map zones) were largely confined to the northwest corner of the property but spread significantly from mid-July until the final sampling in mid-September. These red zones corresponded to GLRaV symptoms. Work is now underway to use both proximal sensing and UAVs to produce spectral fingerprints of vineyards, along with quantitative PCR to confirm presence and titer of the virus.

Occasionally, there are reports of desirable effects of viruses on grapevines when compared to virus-free material. A report from Australia indicated that a mild strain of GLRaV increased berry weight and volume of Emperor table grapes (Anonymous 1985), while lower TA was measured in juices from virus-infected Merlot grapevines grown in Japan (Yamakawa et al. 1987). Auger et al. (1992) showed that GFLV-infected vines produced berries with higher Brix. Wolpert et al. (1996) demonstrated that infection of Cabernet Sauvignon with yellow speckle viroids led to lowered TA and higher pH. Reynolds et al. (1997) demonstrated that *rupestris* stem pitting-infected vines at two locations generally had lower TA and higher pH than their virus-free counterparts. In the Piedmont region in Italy, GLRaV-infected Dolcetto vines produced wines that were slightly different from their virus-free counterparts and displayed lower red berry aroma and softness, but higher plum aroma, astringency, body, and violet color (Mannini et al. 2012).

Conclusions

Members of the Vitaceae family can be traced to the Jurassic Period (165 million years ago) prior to the continental drift. Modern Vitaceae in the Northern Hemisphere include *Vitis*, *Ampelopsis*, and *Parthenocissus*, whereas Southern Hemisphere genera include *Ampelocissus*, *Cissus*, and *Cayratia*. Early members of Vitaceae (*Cissites*, *Vitiphyllum*) likely evolved during the Cretaceous Period, with confirmed Vitaceae (*Ampelopsis, Cissus*) associated with the beginning of the Tertiary Period to Early Eocene (50 million years ago). The first true *Vitis* fossils date from the Eocene in England (*V. subglosa*) and France (*V. sezannensis*) and from the Miocene in Germany (*V. teutonica*). Fossils in Provence dated to the Early Quaternary Period include *V. ausoniae*, which resemble *V. vinifera*. Prehistoric grapevines are known in Europe from the Paleolithic/Mesolithic periods onward.

The center of origin of *V. vinifera* is widely considered to be the Transcaucasian region but archeological evidence casts doubt on this. Substantial paleobotanical evidence exists of both *V. vinifera* ssp. *sylvestris typica* (*V. sylvestris*; wild European grape) and *V. vinifera* ssp. *sativa* (cultivated grape; *V. vinifera*) throughout the