Potential Interactions Between Rootstocks and Grapevine Latent Viruses

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Among the virus or virus-like agents which cause disease in grapevines are several which produce latent or non-symptomatic infections in *Vitis vinifera*. Research suggests that the severity of disease may vary according to the genotype of the rootstock which is grafted with infected scion wood. This may explain the reports of virus-like diseases which have been observed in California in vineyards where certified rootstock has been grafted with non-certified scion wood selections which appeared disease free when grafted on AXR#1.

KEYWORDS: disease, latent, rootstock, viruses

Factors which affect the horticultural success of a grafted plant include not only genetic interactions between rootstock and scion but also the effects of any graft transmissible disease agents which might be borne by the propagating wood. Although graft incompatibility due to scion and rootstock genotype has been well documented for many horticultural crops (15), the exact causes of this phenomena are not clearly understood. Different physiological, biochemical, and anatomical systems among stocks may contribute to graft failure. Graft failure may be immediate or delayed over years.

Although cases of true genetic incompatibility may exist between *Vitis* species, they have not been well documented by careful experiments using virus-tested clean stock. Since grapevine clonal rootstocks have been selected for their compatibility with *V. vinifera* among other characteristics, it is reasonable to assume that this type of incompatibility would have been noticed if it was common. There are no documented cases of incompatibility between *V. vinifera* and clonal rootstocks where the disease-free status of the propagation materials is established. Unfortunately, much of the work on grapevine scion/rootstock interactions has been done without regard to the disease status of the selections used in the experiments. Since many graft transmissible diseases mimic the symptoms of genetic incompatibility, research done with propagating materials of unknown disease status may not be conclusive.

Most of the graft-transmissible diseases in *Vitis* are caused by viruses, although both bacteria and viroids have been implicated in some cases. Proving the viral etiology of a particular disease is not always possible. Until the actual virus which causes a disease has been identified according to established procedures, a disease may be referred to as "virus-like." This usually implies that the agent can be graft-transmitted and that it is sensitive to elimination by either heat therapy or shoot-tip culture. There is good evidence that some grapevine viruses will cause incompatibility problems, which are known to be more severe on one genotype than another.

A few concepts must be understood in discussing grapevine-virus interactions. A virus is said to infect a plant when it is established in the plant's tissues. It is possible for a virus to infect a plant without the plant exhibiting any obvious symptoms of disease. Often, the symptoms are so subtle that replicated field testing would be required to show the reduction in yield or quality brought about by infection. When the host is carrying a disease agent but not showing any obvious symptoms, these infections are called latent infections. Although symptoms may not be obvious, in a perennial crop like grapes a latent infection which decreases yields even 5% per year may be of economic significance over the lifetime of a vineyard (7). Because the severity of a virus disease is highly dependent upon the genotype of the host, a virus disease which is latent in one grape selection may cause serious disease if graft-transmitted to a different grape genotype. A grape selection which shows severe disease symptoms when inoculated with a particular virus or virus-like agent is called an indicator for that virus. The process of inoculating an indicator to determine whether a selection is infected with a virus or virus-like disease is referred to as indexing.

The extensive work on the grapevine virus and virus-like diseases has been documented in bibliographies published under the auspices of the International Council for the study of Viruses and Virus-like Diseases of the Grapevine (ICVG) (4,5,6,16). In early research in this field, virus detection standards were based on host symptoms, either on the diseased vine or the responses of biological indicators to inoculation. Molecular analysis of diseased grapevines has proven that more than one agent is responsible for some syndromes (see leafroll below as an example). As a result, much of the older literature must be read critically with an appreciation that new techniques have made it possible to set more critical standards for diagnosis (14).

The disease agents which cause pitting and grooving of the wood cylinder of grapes include corky bark, legno riccio, Rupestris stem pitting, and Kober stem grooving. This group of diseases, referred to as the

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"Rugose wood complex" (21), has negative effects upon grafted vines. The relationship between these diseases has not yet been determined because the identity of the causal agents has not been determined. Although some information about the identity of rugose wood diseases can be gained by indexing to specific *Vitis* indicators, individual components of mixed infections are difficult to identify (29). Until this relationship is clarified by unambiguous methods, the determination of disease identity by the author of each publication is probably the most useful identification available and is used in the text below.

Readers should be cautioned, however, that the name of the diseases and/or disease agents frequently includes a description of the disease symptom. This does not mean that the same symptoms can not be caused by more than one agent. For example, stem pitting is a symptom which can be caused by a number of different viruses or virus-like agents on a diversity of *Vitis* species including, but not limited to, the uncharacterized agent which causes Rupestris stem pitting disease. Rupestris stem pitting disease is defined by the response of *V. rupestris* St. George which develops stem pitting symptoms below, but not above, the inoculation point when grafted with a selection infected with this disease (13). Stem pitting symptoms on other indicators such as LN33 or most *V. vinifera* cultivars are not usually caused by Rupestris stem pitting.

Many of the grapevine virus and virus-like diseases cause a general decline in vigor and productivity but have not been observed to produce any special problems in grafted vines. For some viruses, differences in disease response have been documented among species of *Vitis* and also among individual selections within a species (21). It is expected that some variation in disease response would exist due to variation among isolates of each disease agent but only limited research has been published in this area. Grapevine virus and virus-like diseases which cause a general decline, but have not been associated with reactions which could adversely affect rootstock-scion combinations (*i.e.,* ajinashika, asteroid mosaic, nepovirus diseases, fleck, yellow mottle, *etc.*), are not discussed in this article but have been well reviewed elsewhere (3,10,13,17,21).

Grapevine Virus and Virus-like Diseases Likely to Affect the Success of Certain Rootstock-Scion Combinations

Bushy stunt: Savino and his colleagues (30) have described a graft-transmissible disease which can be eliminated by heat treatment. This disease shows no symptoms of any kind on the rootstock 140Ru, a *V. berlandieri* × *V. rupestris* hybrid, but causes stunting, shoot deformity, and reduced yield when infected 140Ru rootstock is grafted with scions of *V. vinifera* cvs. Italia and Sangiovese. This disease, which has been named grapevine bushy stunt, is suspected to have a viral origin. Interestingly, the selection of 140Ru which was carrying bushy stunt disease had passed all normal tests for certified stock in its country of origin. It was then widely distributed before it was found to be carrying the bushy stunt agent. This exemplifies some of the difficulties faced by grapevine clean stock programs which must determine which tests are necessary and practical to use for certification.

Corky bark: This disease has been associated with closteroviruses (13,21,25,26), but the etiology of the disease is not yet established. The diagnostic host is LN33 (Couderc 1613 × Thompson Seedless). Martelli (21) differentiates between a phloem proliferation reaction on LN33 (corky bark disease) and a stem pitting reaction without phloem proliferation (LN33 stem grooving).

Many infected selections of *Vitis* are symptomless when propagated on their own roots. Profound disruption of the graft union may result in certain rootstock/scion combinations. Diagnosis is most readily achieved in the field during the active growing season (late spring to mid-summer) by removing a "window" of bark over the graft union and looking for xylem disruption. This disease is noted for causing serious decline or even the death of affected vines in certain scion-rootstock combinations.

Infectious graft incompatibility: This is a disease which has been reported exclusively from France and for which the causal agent is still unknown (9,20). A virus etiology is suspected because the agent is graft transmissible. It can cause reduced growth, decline or rapid death of vines when *V. vinifera* is grafted onto sensitive rootstocks. Kober 5BB is especially sensitive and is used as an indicator host.

Kober stem grooving: This disease is included by Martelli as part of the rugose wood complex (21). The agent is as yet uncharacterized, but the disease can be diagnosed by indexing on Kober 5BB, St. George, and LN33. Pitting and grooving symptoms will develop on Kober 5BB, but no obvious symptoms are found on the other two indicators, separating Kober stem grooving from similar diseases.

One study on the viticultural effects of Kober stem grooving has demonstrated that the disease can produce significant symptoms on both Kober 5BB and *V. rupestris* (11). Graft survival was greatly reduced when infected wood was propagated on the rootstock Kober 5BB as opposed to *V. rupestris*. On vines where the grafts survived, severe and extended stem grooving was observed after three years on the Kober 5BB and pitting symptoms were common on *V. rupestris*.

The identity of this disease, as distinct from other components of the rugose wood complex, has only been established in recent years (11). To the author's knowledge, no US grape clean stock program currently tests for Kober stem grooving.

Leafroll viruses: Leafroll disease has been demonstrated in the 1980s to be caused by a complex of different viruses including grapevine virus A (GVA), grapevine virus B (GVB), grapevine potyvirus (GPV), and a series of closteroviruses known as grapevine leafroll associated viruses (GLRaV I, II, III, IV, and V)

(17). Most of the information on the effects of leafroll virus on vines was published prior to our current understanding of the complex etiology of this disease. Therefore, very limited data are available about the effects of individual causal agents upon rootstock/scion combinations.

Legno riccio: The term "legno riccio" translates literally to mean rugose wood. The term is a general one which describes the symptom of severe stem pitting or grooving on the woody cylinder of a grapevine when the bark is removed. These symptoms can be caused by a number of graft-transmissible agents. Unequivocal molecular methods are not yet available to distinguish between these agents. *Vitis* indicators can be used for this purpose (29) but do not always give consistent or definitive results.

LN33 stem grooving: Martelli has named this disease to differentiate between two symptom types observed on the indicator selection LN33. By his definition, corky bark disease must produce bark swelling and phloem proliferation which is externally visible on LN33. If examination of the inoculated indicator LN33 externally does not indicate disease, but stripping of the bark from the wood cylinder reveals pitting and grooving symptoms, LN33 stem grooving is diagnosed. Little information is available about the viticultural effects of this disease but it seems likely that it can possibly cause girdling disorders in grafted grapevines.

Rupestris stem pitting: This disease is believed to be viral in origin because it is graft-transmissible and has been associated with dsRNAs (2). Detection is accomplished by a woody index on St. George (13). A positive test for Rupestris stem pitting is indicated by the development of a small row of pits below the chip bud of the selection being tested without the development of pits above the bud shield. *V. rupestris* and its hybrids are particularly susceptible, although other American species have been determined to develop symptoms after inoculation (26). Pitting caused by Rupestris stem pitting cannot be differentiated from pitting caused by corky bark on St. George when infected scion wood is bench grafted to this indicator. Although replicated field trials on this disease have not been completed, it is reported to cause effects which are similar to those of leafroll disease in regards to fruit quality and vine yields (13).

Vein mosaic: This disease causes feathering or banding along the veins of leaves. The agent is graft-transmissible and can be eliminated by thermotherapy. No information is available on the severity of this disease or the identity of the causal agent. A number of grape species have been shown to be symptomless carriers. The indicator for the disease is *V. riparia* Gloire de Montpellier (19).

Vein necrosis: This virus-like disease is symptomless on most European and American species. The rootstock 110R develops necrosis of the veinlets on the underside of the leaf blade and sometimes necrosis of the shoot tips (19). The disease agent can be eliminated by thermotherapy (28).

Selected Studies

Studies have documented grapevine virus interactions with rootstock selections. Applications of the results of these studies should be made with an awareness that results may vary with each virus isolate, clonal source of rootstock and scion, and location. In many cases, no voucher specimen of the virus isolate is available for determining the identity of the disease isolate by definitive tests.

Prudencio (26) investigated the relative effects of grapevine corky bark disease and Rupestris stem pitting disease in 68 grape selections from University of California Davis grapevine collections. The grape selections were chip-budded with wood from vines infected with each disease agent. The inoculated plants were observed for 18 months before they were undercut and the wood cylinder examined for pitting and grooving symptoms. The two diseases could be differentiated on some grape selections. *V. rupestris* St. George was the only selection which expressed symptoms to both diseases. Severe responses to the corky bark isolate were observed on LN33 and *V. rupestris* Constantia; moderate symptoms on Couderc 1613 and Harmony; and mild symptoms on Richter 110. Several other varieties showed disruptions which could not be clearly attributed to corky bark inoculation. Rupestris stem pitting produced strong symptoms in Paulsen 1103 and Castel 216-3; moderate symptoms in Paulsen selections 1447, 1045, and 779; and mild or questionable symptoms in Couderc 161-49, *V. smalliana,* and *V. rupestris* Tiefenbach. Fifty-three of the selections appeared healthy 18 months after inoculation, suggesting that they are less sensitive to these two disease isolates, although symptoms may have developed if the experiment had been carried on longer. Unfortunately, the exact identities of both these virus isolates was not recorded for future use as reference material, but it is likely that both are included in the Davis Grapevine Virus collection (14).

The work of Credi *et al.* (8) provided information on the susceptibility to legno riccio of 13 clonal rootstocks (225 Ruggeri, Cosmo 2, SO4, Kober 5BB, 125AA, Teleki 5C, 157-11 Couderc, 420A, 140 Ruggeri, 1103 Paulsen, Golia, 41B, and *V. rupestris* Du Lot). A selection of the red wine grape *V. vinifera* cv. Lambrusco grasparossa which was infected with an isolate of legno riccio was grafted to the rootstocks. A randomized block design (13 rootstocks × 13 replications × 4 sub-replications) was planted. All the vines were scored individually for disease incidence and severity. Data on yield and vigor were collected for seven years. An average of 73% of the vines developed legno riccio symptoms; this incidence varied with rootstock selection with a range from 26% (140 Ruggeri, 41B) to 100% (Kober 5BB, SO4). Severity of symptoms depended on rootstock and was correlated with reduced yield and vigor. Mild effects were seen in the rootstock 140 Ruggeri, 41B, and Cosmo 2; severe responses were noted in 420A, Kober 5BB, SO4, and 157-11 Couderc; the most productive rootstocks when

infected with legno riccio were Golia, Cosmo 2, 225 Ruggeri, and 1103 Paulsen.

The California Situation

In 1991, many viticulturists noticed unusual virus-like symptoms in young vineyards throughout California. These symptoms included severe stunting, internode shortening, leaf discoloration, leaf rolling, and disorders of the graft union. The common element in the history of these vineyards was, first, the rootstock selection was new to the vineyard, second, the rootstock source was certified (virus-tested), and, third, the scions were obtained from non-certified selections (not virus-tested) which appeared virus-free on A×R#1.

Grape virologists have shown that some virus diseases can have severe effects on vines, depending on the genotype of both the rootstock and scion selections (see sections above). Since systemic infection of a vine with a virus can occur if either the rootstock or the scion is infected, non-certified field selections used to graft certified rootstocks may infect a vine with a virus. These field selections can harbor viruses as latent infections without showing any obvious disease symptoms.

Only a few California vineyards are propagated with certified scion wood. There are a number of reasons for this, including the difficulties in obtaining certified wood in large quantities for fall budding, the desirable wine making characteristics of some field selections, and cost. However, for years, field scion selections have been evaluated for their success on A×R#1. A×R#1 is believed to be resistant to many latent viruses (A. Goheen, personal communication). It is reasonable to hypothesize that California growers, who have used A×R#1 to the exclusion of other rootstocks, have empirically selected for scion materials which produce mild or no virus symptoms on A×R#1. Many of these selections could harbor latent viruses which did not cause disease symptoms on A×R#1 but do cause symptoms on more sensitive rootstocks that are being planted today.

The extent and severity of this problem is not yet known. Research efforts in the future should help clarify the importance of this issue. If this epidemic is severe, faster virus detection techniques, such as green grafting (23,31) and advanced molecular techniques (21,24,27), will be needed to screen field scion selections. In addition, valuable California field scion selections which are not certified may need to be treated for virus infection, using either heat treatment or tissue culture techniques, and released as certified selections.

Recommendations and Conclusions

Rootstock and scion selections can easily be infected with one or more viruses or virus-like agents when used for propagation. The source of the virus may be either scion or rootstock. Disease symptoms do not always occur with infection. The severity of a virus disease will depend upon which virus is present, the genotype of both rootstock and scion, and the cultural conditions. The apparent lack of symptoms in a scion selection grafted on one rootstock does not insure against virus infection. Disease may be more serious, even lethal, when the virus is transmitted to other rootstock genotypes.

Certified wood has been tested for specific viruses. The best insurance against virus infection and disease is to use both certified scion and rootstock selections. It is possible that California certification standards, based on detection and elimination of economically important diseases, may have to be reviewed and upgraded now that genetically diverse rootstocks are being widely planted. However, at this writing, all of the cases of virus-rootstock union disorders have involved non-certified wood.

Certified rootstocks are in good supply. If a grower is unable to obtain certified scion wood and is concerned about possible virus effects, a small trial of the exact rootstock/scion combination which is proposed may provide information about possible problems. It is important to remember that individual vine sources of a scion selection may differ in virus status. Grapevine certification programs are based on determining the disease status of an individual vine and using that vine as the source of all progeny. To the extent that this is possible, vineyard selections should be tested in the same way.

Ultimately, the problem of rootstock interactions with grapevine viruses will be solved by grape clean stock programs. Our industry needs well-documented clonal material from around the world which has been qualified as disease free by state-of-art diagnostic techniques.

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