Infectious diseases and certification of grapevines

Martelli G.P.

in

Martelli G.P. (ed.), Digiaro M. (ed.).
Proceedings of the Mediterranean network on grapevine closteroviruses 1992-1997 and the viroses and virus-like diseases of the grapevine a bibliographic report, 1985-1997

Bari : CIHEAM
Options Méditerranéennes : Série B. Etudes et Recherches; n. 29

1999
pages 47-64

Infectious diseases and certification of grapevines

G.P. Martelli

Dipartimento di Protezione delle Piante, Università degli Studi and Centro di Studio del CNR sui Virus e le Virosi delle Colture Mediterranee, 70126 Bari, Italy

---

SUMMARY - The world-wide sanitary deterioration of vegetatively propagated crops, like grapevines *(Vitis* spp.), calls for the enforcement of preventive measures for producing propagating material with a health status compatible with the expected economic returns. Improvement of the sanitary level can be achieved through selection and sanitation, which are best performed in the framework of certification programmes. Although certified stocks guarantee also trueness to type, their major and qualifying trait is constituted by a well-established sanitary status. The demand for certified material has grown steadily over the years, concomitantly with increased awareness that unrestricted domestic and international trade of sanitarily uncontrolled plant material has caused a highly threatening world-wide distribution of infectious diseases and their agents (primarily viruses, viroids, and intracellular prokaryotes). Quarantine measures, even the most effective, are not sufficient to stop the trend. However, modern technology has developed efficient tools for producing sanitarily improved stocks, detecting infectious agents and establishing effective certification schemes.

Key words: grapevine, viruses, virus-like, certification, diagnosis

RESUME *- La dégradation sanitaire des cultures multipliées par voie végétative telles que la vigne (Vitis spp.) impose le recours à des mesures préventives pour produire du matériel de multiplication dont l'état sanitaire permette d'obtenir le rendement économique attendu. L'état sanitaire peut être amélioré à travers la sélection et l'assainissement, si possible dans le cadre d'un programme de certification. Nul doute que le matériel certifié assure l'authenticité variétale, mais il garantit, en premier lieu, un état sanitaire approprié. Ces dernières années, la demande de matériel certifié a connu une augmentation considérable, vu qu'on est de plus en plus persuadé que les nombreux échanges de matériel végétal non contrôlé du point de vue sanitaire, à l'échelon national et international, ont favorisé la distribution dans le monde entier des maladies infectieuses et de leurs agents (principalement, des virus, des viroïdes et des procaryotes intracellulaires). Les mesures de quarantaine, même les plus efficaces, ne sont pas suffisantes pour arrêter la circulation de ce matériel. De toute façon, la technologie moderne a mis au point des outils fiables pour produire du matériel amélioré du point de vue sanitaire, détecter des agents infectieux et lancer des programmes de certification efficaces.*

Mots-clés *: vigne, virus, certification, diagnostic*

As repeatedly reviewed (Martelli, 1995, Martelli and Walter, 1988) the reasons that have favoured the sanitary decay of grapevines *(Vitis vinifera* and American *Vitis* species and hybrids used as rootstocks) are to be found in: (i) the increased domestic and international demand and trading of nursery products, to comply with the requirements of the expanding world viticulture; (ii) the insufficient knowledge of the sanitary problems affecting grapevines; (iii) the existence of *Vitis* species and/or cultivars that carry infectious disease agents symptomlessly; (iv) the lack of appropriate sanitary control of propagating material being marketed; (v) the inefficiency of quarantine systems. All this has contributed to the generalised dissemination of a number of diseases and pathogens among which graft-transmissible infectious agents prevail by far.

The awareness of the world-wide disastrous conditions of the grapevine industry, the alarm signals launched by the scientific community, and the increased demand for high quality planting material, has compelled many countries to implement measures aimed at producing propagating material with a health status compatible with the economic returns expected from the crop. Improvement of the sanitary level can be achieved through selection and sanitation which, however, are best performed in the framework of certification programmes.

Viruses and other disease agents of grapevines

To date, more than 60 different infectious agents (viruses, viroids and intracellular prokaryotes) have been identified in grapevines. This is indeed the highest number of pathogens encountered in any single woody species. It represents a veritable record, both for the variety of the findings and the relatively short time in which they have occurred, considering that the first isolation of a grapevine virus dates back to the early 1960s (Cadman *et al*., 1960). Some of these viral species, i.e. Cucumber mosaic (CMV), Tobacco mosaic (TMV), Broad bean wilt (BBWV), Potato X (PVX), Artichoke Italian latent (AILV), and Alfalfa mosaic (AMV) viruses are serious pathogens of several important crops but, for grapevines, they represent mere scientific curiosities, either because they are rare, or because the damage induced is negligible. By converse, other viruses, especially members of the *Nepovirus, Closterovirus* and *Vitivirus* genera, are veritable pathogens and the agents of diseases that have an undoubted negative impact on the quality and quantity of the yield (Walter and Martelli, 1996).

But why are *Vitis* species so prone to viral infections? The explanation resides in a number of reasons among which, the intrinsic susceptibility of grapevines to viruses, the way in which grapevines are multiplied, and the variety of geographical and climatic environments under which they are grown. In *Vitis,* like in all vegetatively propagated plant species, there is a progressive accumulation of infectious agents that are acquired by the plants with exposure to inoculum in different places and times, and are "clonally" perpetuated with them. This allows the survival and dissemination also of those viruses to which individual vines are little susceptible, but that have found the way to infect them, sometimes quite by chance through the fortuitous inoculation by a passing vector.

Infectious diseases of grapevines

As compared with the very high number of potential pathogens infecting grapevines, the list of recognised diseases is short (Tab. 1). The reason is twofold: (i) several of the viruses recovered may cause latent infections; (ii) the same disease can be induced by multiple agents.

Table 1. Infectious diseases of grapevines

A. MAJOR VIRUS DISEASES

1. Degeneration (GFLV and European nepoviruses)
2. Decline (American nepoviruses)
3. Leafroll (closteroviruses)
4. Rugose wood complex (vitiviruses, foveaviruses)
5. Fleck

B. MINOR VIRUS DISEASES

6. Yellow mottle (alfalfa mosaic alfamovirus)
7. Line pattern (grapevine line pattern ilarvirus)
8. Stunt (grapevine stunt virus)
9. Ajinashika (grapevine ajinashika virus)
10. Berry inner necrosis (grapevine berry inner necrosis virus)
11. Roditis leaf discoloration (carnation mottle carmovirus and GFLV)

C. VIRUS-LIKE DISEASES (agents unknown)

12. Enations
13. Vein necrosis
14. Vein mosaic
15. Summer mottle
16. Asteroid mosaic
17. Bushy stunt
18. Graft-incompatibility disorders

D. VIROID DISEASES

19. Yellow speckle

E. PROKARYOTIC DISEASES

20. Flavescence dorée (elm yellows phytoplasma group)
21. German EY-type yellows (elm yellows phytoplasma group)
22. Yellows induced by the stolbur phytoplasma group (e.g. Bois noir and Vergilbungskrankheit)
23. Yellows induced by the X-disease phytoplasma group
24. Pierce's disease *(Xylella fastidiosa)*

Virus diseases

Degenerative diseases and decline

Nepoviruses are the agents of these diseases (Tab. 2). The degenerative condition caused by European nepoviruses is universally known as fanleaf, whereas comparable disorders elicited by American nepoviruses are referred to as decline (Goheen, 1977; Martelli, 1993). Several of the European nepoviruses (GFLV, ArMV, TBRV, GCMV) possess distorting and chromogenic strains that induce malformation of leaves and canes or chrome yellow discoloration of the foliage, respectively. Reduction in vigour and in the quantity and quality of the yield is associated with infection by both types of strains.

American nepoviruses evoke responses that vary with the grapevine species and climatic conditions. In cold climates European grapes infected by TRSV or ToRSV show stunted growth, distortion of leaves and canes, low yield, and rapid decline. In warmer climates, yield but not vigour is affected, and the leaves may show chrome yellow flecking along the veins. Grapevine nepoviruses are disseminated over medium and long distances by propagating material. Their field transmission is mediated by longidorid nematodes, i.e. *Xiphinema* and *Longidorus* (Tab. 2).

Table 2. Nepoviruses agents of grapevine diseases and their vectors

VIRUS	VECTOR
A. AGENTS OF DEGENERATION	
1. Grapevine fanleaf virus (GFLV)	*Xiphinema index*
2. Arabis mosaic virus (ArMV)	*X diversicaudatum*
3. Grapevine chrome mosaic virus (GCMV)	Unknown
4. Strawberry latent ringspot virus (SLRV)	*X. diversicaudatum*
5. Raspberry ringspot virus (RRV)	*Longidorus macrosoma, Paralongidorus maximus* *L. elongatus*
6. Tomato black ring virus (TBRV)	*L. elongatus, L. attenuatus*
7. Artichoke Italian latent virus (AILV)	*L. attenuatus, L. fasciatus*
8. Grapevine Bulgarian latent virus (GBLV)	Unknown
B. AGENTS OF DECLINE	
9. Peach rosette mosaic virus (PRMV)	*X americanum, L. elongatus L. diadecturus* *X californicum, X. rivesi*
10. Tomato ringspot virus (ToRSV)	*X. americanum*
11. Tobacco ringspot virus (TRSV)	Unknown
12. Blueberry leaf mottle virus (BBLMV)	
C. UNDETERMINED PATHOGENICITY	
13. Grapevine Tunisian ringspot virus (GTRV)	Unknown

Leafroll

Closteroviruses are currently regarded as agents of leafroll. Seven serologically distinct such viruses have been identified so far in leafroll-affected grapevines (Tab. 3). Although all of these viruses continue to be cautiously called grapevine leafroll-associated (GLRaVs) (Boscia *et al.,* 1995), there is experimental evidence that some (e.g., GLRaV-1, -2, -3, and -7) induce leafroll-type responses in vines infected by grafting or vectors. This prompted the suggestion that the word "associated" be dropped from the name of the first three GLRaVs of the series (Martelli *et al.,* 1997). Disease symptoms are those typically induced by viruses multiplying in the phloem and affecting its functionality. Leaves are thicker than normal, brittle, with margins rolled downwards and discoloured, i.e., yellowish in white-berried cultivars and reddish to deep purple in red-berried cultivars. A great variability in symptom expression is commonly observed in the field, symptomatological variants being probably determined by different viral combinations and differential varietal responses (Martelli, 1993; Martelli *et al.*, 1997).

Table 3. Leafroll and rugose wood viruses and their vectors

DISEASE	VIRUS	VECTOR
A. Leafroll	GLRaV-1	*Parthenolecanium, corni, Neopulvinaria innumerabilis*
	GLRaV-3	*Planococcus ficus,Pseudococcus longispinus, Ps. affinis, Pulvinaria vitis*
	GLRaV-4	Unknown
	GLRaV-5	Unknown
	GLRaV-6	Unknown
	GLRaV-7	Unknown
B. Leafroll and graft incompatibility	GLRaV-2	Unknown
C. Rugose wood complex		
a. Rupestris stem pitting	RSTaV	Unknown
b. Kober stem grooving	GVA	*Pl. citri, Pl. ficus, Ps. longispinus, Ps. affinis, Pulvinaria vitis*
c. Corky bark	GVB	*Ps. longispinus, Pl. ficus, Ps. affinis*
d. LN33 stem grooving	Unknown	Unknown

All grapevine closteroviruses are spread primarily by propagative material but two (GLRaV-1 and GLRaV-3) are transmitted by pseudococcid mealybugs and scale insects (Tab. 3), and there is some evidence that GLRaV-2 may also have pseudococcid vectors (Martelli *et al.,* 1997).

Rugose wood

Rugose wood is a complex disease in which four different disorders denoted Rupestris stem pitting, Corky bark, Kober stem grooving, and LN33 stem grooving can be recognised and sorted out by graft transmission to *Vitis* indicators (Martelli, 1993). In the field, rugose wood is characterised primarily by alterations of the woody cylinder. Affected vines may be dwarfed and less vigorous than normal, have delayed bud opening in spring, and some decline and die within a few years after planting. Grafted vines often show swelling above the bud union and a marked difference between the diameters of scion and rootstock. Sometimes, the bark of the scion above the graft union is exceedingly thick and corky (corky rugose wood) (Bonavia *et al.*, 1996), has a spongy texture and rough appearance. The woody cylinder is typically marked by pits and/or grooves which may occur on the scion, the rootstock, or both. In most cases no specific symptoms are shown by the foliage, but the crop may be reduced. Four serologically distinct vitiviruses and a foveavirus (Martelli and Jelkmann, 1998) were found to be associated with rugose wood-affected vines. For GVA, GVB, and RSPaV there is evidence of the involvement in the aetiology of three of the disorders of the complex, i.e. Kober stem grooving (GVA), Corky bark (GVB) and Rupestris stem pitting (Boscia *et al.*, 1997; Meng *et al.*, 1998). Rugose wood is distributed over long distances by propagative material, whereas the spread at a site of at least two of the viruses involved in its aetiology (GVA and GVB) is mediated by pseudococcid mealybugs (Tab. 3).

Fleck

Fleck is latent in European grapes and in most American rootstocks. Symptoms are expressed in *Vitis rupestris,* a self-indexing species, and consist of clearing of the veins of third and fourth order that produce localised translucent spots. Leaf deformity and stunting may also occur (Martelli, 1993). Fleck is a widespread and damaging disease (Walter and Martelli, 1996). Its causal agent is a phloem-limited non mechanically transmissible isometric virus (Boscia *et al.*, 1991), likely belonging to a still undescribed genus. No vectors are known, thus spreading is through infected propagating material. Recently, circumstantial evidence was gathered of natural spread in the field (Fortusini *et al.*, 1996).

Viroid diseases

Yellow speckle

There are six grapevine viroids but only two, denoted grapevine yellow speckle viroid 1 and 2 (GYSVd-1 and GYSVd-2), seem to be pathogenic to grapevines (Martelli, 1993; Semancik, 1993). Yellow speckle, the disease they induce, is elusive as its outward expression is conditioned by climatic and, perhaps, varietal factors. Symptoms consist of a few to many minute chrome yellow spots or flecks scattered over part or the whole leaf surface, or gathering along the main veins. The latter condition, known as "vein banding", is enhanced by the synergistic effect resulting from concomitant infection by GFLV and GYSVd-1 (Krake and Woodham, 1983; Szychowski *et al.,* 1995). Typical yellow speckle may not be too harmful, whereas vein banding has a severe detrimental effect on the yield of certain varieties. As with other viroids, GYSVd does not have a vector but it is perpetuated in propagating material with which it spreads. Mechanical transmission to field-grown vines through surface-contaminated cutting tools is possible, though with a low efficiency (Staub *et al.,* 1995).

Phytoplasma-induced diseases

Grapevines are affected by several yellow diseases elicited by different phytoplasmas (Tab. 1), and called with different names: flavescence dorée, bois noir, Vergilbungskrankheit, Mediterranean, subtropical, or North American grapevine yellows (Caudwell, 1993). Regardless of the disease and the causal agent, the symptoms are very similar: leaf rolling, yellowing or reddening of the leaves (according to whether the vines are white- or red-berried), necrosis along the veins, incomplete wood ripening, withering of the berries and drying up of the bunches. Severely affected vines may die (Martelli, 1993). Phytoplasma infections are spreading in epidemic form in several European countries but vectors have been identified only for flavescence dorée and Vergilbungskrankheit. These are transmitted by the leafhoppers *Scaphoideus titanus* and *Hyalestes obsoletus,* respectively (Caudwell, 1993; Maixner, 1994). Medium and long distance spread is through infected wood.

General principles of certification

To attain sanitary improvement of any crop a system of preventive, protective and, often, curative measures has to be established and implemented, encompassing that complex series of interventions currently referred to as "certification".

Speaking of certification, five major questions arise:

1. *What is it?* Certification can be defined as a procedure whereby candidate mother plants to be used as source of material for propagation, undergo controls and, whenever necessary, treatments to secure trueness-to-type and absence from any number of pathogens, as specified by regulations officially issued, or endorsed, by competent governmental agencies.

   There are two major kinds of certification:

   (i) Voluntary. A widespread form of certification largely propitiated by the growers' increasing demand of material of known sanitary status for establishing their plantations, especially if these are long-lasting woody crops. Voluntary certification is regimented by regulations issued by a "certifying authority" (i.e. usually an organ of the country's Ministry of Agriculture, or the equivalent) but, by definition, cannot be forcefully imposed.

(ii) Compulsory. A type of certification enforced whenever it becomes essential to prevent the dissemination of destructive diseases, whose dispersal through propagative material may establish infection foci that, in turn, may favour explosive vector-mediated spread at a site (e.g., Pierce's disease, tristeza, plum pox).

2. *What does it apply to?* In principle, certification can be applied to any cultivated plant species, regardless of whether it is propagated vegetatively (cuttings, buds, tubers, bulbs, sets, offshoots, etc.) or through seeds. Thus, both vegetable and woody crops are liable to enter certification schemes, which, in fact, they do. However, with few exceptions (e.g. potatoes), the most popular and widespread certification programs are those concerning fruit trees, vines, and small fruits. There are no limitations to the kind and number of pathogens that may be considered for exclusion in a certification program. Several species of bacteria, fungi, and nematodes are certification organisms in a number of schemes but, most certainly, graft-transmissible infectious agents like viruses, viroids and intracellular prokaryotes (phytoplasmas and fastidious bacteria) are those of major concern.

3. *Is there a need for it?* Certification is largely justified by the afore-mentioned progressive sanitary deterioration grapevines, the same as others vegetatively propagated crops. Infectious diseases are widespread throughout the world, both in developed and developing countries. For example, an extensive 4-year survey (1984-87) carried out in Southern Mediterranean and Near East countries under the FAO patronage by J. Dunez, G.P. Martelli and A.A. Salibe, unravelled a significant deterioration of the health status of stone fruit trees, grapevines and citrus, due to continued propagation of infected planting material and to the lack of provisions for its sanitary amelioration (Anonymous, 1988).

4. *What are the conditions needed for its implementation?* To secure the success of a certification program there are a number of conditions that must be met before venturing into its launch, such as:

(i) Existence of the problem: i.e. occurrence of phytosanitary conditions objectively calling for specific interventions;

(ii) Compelling request by the grower associations, so as to create a "political consent";

(iii) Involvement and convinced participation of nurserymen;

(iv) Commitment of governmental authorities to support the program legally, logistically, and, whenever possible, financially;

(v) Adequate legislation: i.e. emanation of regulations that finalise the scheme to be enforced and that regiment the production and marketing of certified material;

(vi) Appointment of the "certifying authority", i.e. a public service entrusted with control duties and delivery of certification labels.

(vii) Unfailing support by scientific institutions;

(viii) Availability of technology for the reliable detection of diseases and their agents and for the effective elimination of diseases and pathogens (sanitation).

5. *How long for should it last?* Certification is an integral part of sanitary improvement programs and is often the only way to curb certain diseases of woody crops. Hence, it is by no means a temporary operation. Rather, it is long-lasting endeavour that must continue indefinitely, first to attain the desired health level of the crop taken into the scheme, then to maintain this level through time.

Present status of grapevine certification in Europe

Infectious diseases of the grapevine occur throughout the Old World, including the viticultural countries of the European Union (EU), i.e. Portugal, Spain, France, Luxembourg, Germany, Austria, Italy, and Greece. In these countries, the incidence of virus and virus-like diseases is high and their spread has been rapid, consequent also to the uncontrolled distribution and use of infected scions and rootstocks that took place especially in the post-war period. The alarming sanitary deterioration of the crop, which was only in part counterbalanced by the interventions of individual Members States (France, Germany, Italy), prompted the EU Council to issue in 1968 and 1971 Directives for the improvement of the Union's grapevine industry.

Directive 68/93 on the "*Marketing of vegetatively propagated material of grapevines*" classified propagative material into categories denoted "basic", "certified", and "standard", and contained indications encompassing the sanitary characteristics of mother vineyards destined for the production of these materials. Directive 68/93 was modified by Directive 71/40, which defines the sanitary requirements of current EU certification as follows:

"*In the vineyards producing basic material, harmful virus diseases, notably fanleaf and leafroll, must be eliminated. Vineyards producing materials of other categories must be kept free from plants showing symptoms of virus diseases*"

Bylaws generated by these Directives were promulgated in EU Member States, except for Austria which has joined the Union only recently. National certification schemes are now under way in all these countries (Martelli, 1992). However, whereas in France, Germany and Italy these programs have been in operation for many years, yielding already a substantial number of certified clones, their enforcement in Spain, Greece and Portugal is more recent.

A distinctive trait of EU certification schemes is that they are not mere clean stock programs applied to pomologically uncontrolled mother vines. Rather, only sources true to type and with a well-established clonal nature can be registered and certified. The identification of clones is a lengthy procedure regulated by EU Directive 72/169, and outlined in a recent Resolution (Viti 1/91) of the Office International de la Vigne et du Vin (OIV) (2). Thus, as conceived in EU Member States, clonal and sanitary selection is an interdisciplinary activity requiring the joint effort of viticulturists, virologists and, in the case of wine grapes, technologists.

Candidate clones are selected in vineyards with desirable characteristics in the typical area of cultivation of each variety. Selection is based on varietal conformity, vegetative vigour, bud fertility, quality (e.g. sugar content, titratable acidity) and quantity of the yield, timing and uniformity of ripening, general sanitary conditions. Vines are kept under observation for two to four years and the best performing and least infected ones are chosen as candidate clones. These are grafted onto two different rootstocks and planted in two sister performance plots established in two diverse ecological environments. Candidate clones are kept under scrutiny in these plots for no less than five years. The whole process thus requires 8 to 10 years, or more, if clones must undergo sanitation and be re-indexed for health assessment.

Newly identified clones are described and submitted for registration to governmental authorities, together with a statement defining their sanitary status, issued by recognised laboratories. In France new clones are approved by the "Comité Technique Permanent de la Selection des Plantes Cultivées", in Germany by the "Bundessortenamt", in Italy by the "Comitato Nazionale per la Valutazione delle Varietà di Vite", and in Spain by the "Instituto de Semillas y Plantas de Vivero" which are organisms of the respective Ministries of Agriculture. Approved clones are registered in National Catalogues (Martelli, 1992).

Although national certification schemes implemented in EU countries are inspired by, and more or less conform to, EU Directives, they differ to varying extents from one another. Some of these differences pertain to the pomological aspects of clonal selection (e.g., in Germany it lasts longer than in France or Italy), others concern sanitary requirements. In most countries, these requirements are stricter than those contained in EU Directives, which prescribe freedom from fanleaf and leafroll only. However, there is little uniformity among individual schemes as the health status of certified clones may vary from country to country. Thus, for instance, exemption from rugose wood is required in Portugal, France, Italy and in Spain (limitedly to Rupestris stem pitting and Corky bark) but not in Germany where no tests are made for its detection. France and Germany, contrary to all other countries, require absence of fleck only in rootstocks (Martelli, 1992).

Although additional differences may exist in the way in which certified material is maintained, propagated and distributed, certain steps are common to the various schemes. Registered clones (primary sources or nuclear stocks) are maintained by the juridical or physical person who owns them ("obtenteur" or conservative breeder) and undergo a first multiplication in specialised outfits (premultiplication or foundation blocks). These distribute propagating material to nurseries for the establishment of certified mother vine plots which, in turn, are used for the production of certified budwood, rooted cuttings, or grafted vines for commercial purposes. Official, or officially authorised organisations carry out controls on the health, origin and amount of certified plants and issue certification labels.

Proposed scheme for grapevine certification in the European Union

The sanitary provisions of the EU Directives discussed above are largely inadequate for two order of reasons. First, they do not guarantee an acceptable sanitary status of propagative material of any category. Second, they are outdated, failing to take into account recent developments of grapevine virology. Furthermore, the Directives do not provide guidelines for the implementation of a standardised certification protocol in the Union. As a consequence, propagative material of *Vitis* currently produced in the EU is not sanitarily uniform, its health status being determined by national certification schemes, which differ from country to country. All this contrasts with the criteria inspiring the agricultural policy of the EU and is prejudicial to the free circulation of this material within the Union.

Faced with such a regrettable situation and sharing the concern of growers nurserymen and their association (Comité International des Pépinieristes), a group of European grapevine virologists members of the International Council for the Study of Virus and Virus Diseases of the Grapevine (ICVG), reviewed the state-of-the-art of certification in the Union (Martelli, 1992) and outlined a scheme mediating between the procedures implemented in EU Member Countries (Martelli *et al.*, 1993). A similar move was made shortly afterwards by the European and Mediterranean Plant Protection Organisation (EPPO), which proposed a certification scheme conforming to the same technical criteria used by ICVG virologists (Anonymous, 1994).

Both the above protocols took notice that propagative material of grapevines in the EU is classified into categories with a colour coding system:

(i) "*Primary source*" originally obtained by the conservative breeder (i.e. the physical or juristic person who has identified the plant selection or clone). Primary sources are grown under the responsibility of the breeder, in a repository under conditions ensuring freedom from re-infection, usually in an insect-proof screenhouse.

(ii) *Pre-basic.* Material propagated from the primary source, grown under the responsibility of a public agency in a national repository, under conditions ensuring

freedom from re-infection, better if under screen. In Italy, pre-basic material may identified by a white label with a purple band.

(iii) *Basic.* Material derived from the multiplication of pre-basic stocks, propagated under conditions ensuring freedom from re-infection. Mother plants of basic category are usually grown in the field (propagation blocks) in special outfits under the direct management and control of a public agency. Basic material is intended for delivery only to nurseries that have the necessary qualifications and is identified by a white label.

(iv) *Certified.* Material which is produced from basic stocks by authorised nurseries under appropriate growing conditions. Certification is granted at this stage and labels (blue tags) are issued by the certifying authority. Then, the material is delivered to the growers and leaves the control of the certification system.

There is a fifth category of propagating material called "*standard*" (usually identified by a yellow-orange label) which is true to type but has no officially recognised health status because has never entered the certification system. Because of this, standard material may represent a sanitary hazard.

Selected stocks that have undergone certification are highly valuable, they must be grown with care and be protected as much as possible from re-infection, especially while in the multiplication and nursery phase. Thus, multiplication plots and nurseries producing certified material must be established in soils of good quality free from soil-borne virus vectors and be under superior management by highly qualified technicians. These and other requirements pertaining to safety distances and cultural practices are usually codified by official regulations.

Outline of the grapevine certification scheme

To be certified, grapevine varieties and rootstocks must undergo the following stepwise procedure:

1. Identification of candidate clones through selection for pomological and health quality of individual vines.
2. Establishment of candidate clone repositories in soil without nematode vectors. Grape selections can either be grown on their own-roots or can be grafted on virus-tested rootstocks.
3. Assessment of the health status of visually selected candidate clones by indexing and laboratory assays. Selections that do not meet the sanitary requirements of the scheme undergo sanitation and are re-tested for health assessment. If the totality of selections of

a given variety or rootstock are expected to be infected, it is advisable to proceed directly with sanitation.

4. Establishment of performance plots in which virus-tested candidate clones are evaluated for ultimate selection and identification of clones.
5. Application to governmental authorities for official recognition and registration of clones.
6. Maintenance of registered clones (nuclear stocks, pre-basic material) under conditions (e.g. insect-proof screenhouse) ensuring freedom from re-infection by soil or aerial vectors. Nuclear stocks are tended by their conservative breeders and checked each year for virus symptoms. ELISA is also used for the detection of certain viruses (e.g. GFLV and ArMV). Re-indexing is advisable if novel or more efficient detection techniques, antisera or indicators become available, or whenever visual inspections suggest tests to be carried out.
7. Multiplication of nuclear stocks in outdoor plantings (propagation blocks) under conditions ensuring freedom from re-infection. Propagation blocks should have a safety distance of 15 to 20 mt from vineyards planted with material of lower category ("certified" or "standard") and be established in soils with no grapevine history or where grapevines were not grown for at least six years. Propagation blocks are the source of "basic material". Mother vines are checked visually each year for virus symptoms and re-indexed regularly so that, according to the size of the plot, all are tested in a 5 to 8 year period.
8. Distribution of basic material to qualified nurseries under official control.
9. Establishment of commercial stands for production of certified material for delivery to growers (certified blocks). These are planted with budwood coming directly from propagation blocks, at a minimum distance of 8 to 10 mt from other vineyards and in soils in which virus-transmitting nematodes are not detected.
10. Certification and labelling. Labels are supplied by the certifying authority which may be either a government agency or an officially recognised private organisation.

Steps 1-3 are considered to be carried out by a government agency or an official organisation; steps 4, 6, 7, 8, and 9 by or under the strict control of an official organisation; step 10 under strict control only.

Evidently, certification schemes do not embrace standard material, a category which, in principle, is due to disappear. Trueness to type is guaranteed by all categories. Basic and certified categories guarantee also the sanitary status, as declared for individual scion cultivar, clone or rootstock type. In grafted vines both scion and rootstock must belong to the same category, failing which the resulting grafted combination will have the status of the lower category: i.e., basic/basic = basic; certified/certified = certified; basic/certified or vice versa = certified; basic or certified/standard or vice versa = standard.

Minimum sanitary requirements

To date, only the diseases and viruses listed below are regarded as undesirable in the UE. Their occurrence is incompatible with registration and certification of selected clones:

1. Degeneration, including GFLV and other European nepoviruses
2. Leafroll and related agents
3. Rugose wood complex and related agents
4. Fleck
5. Yellows diseases

Absence of other disorders, among which some widespread virus-like (vein mosaic, vein necrosis, enations) and viroid diseases (yellow speckle) is optional. Their presence does not impair registration and certification but efforts should be made for their elimination.

Testing for disease freedom and sanitation procedures

Diagnostic tests

The type of detection tests, their application and reliability have been the object of extensive debate in the EU. For verifying the efficiency of diagnostic procedures and harmonising their application, an EU-supported Network of grapevine virologists was established in 1993 (Walter, 1996). The results of comparative testing carried out seem to confirm the validity of most of the protocols currently used, with reference to the following main procedures (Walter, 1997):

Indexing on Vitis *indicators.* The use of woody differential hosts is a compulsory step of grapevine certification programs, for there are diseases that can be identified only by the reaction of indicators (Tab. 4). Inoculation is by: (i) Whip or cleft grafting in the field; (ii) Chip-bud grafting, a technique recommended for detection of rupestris stem pitting. The pits induced by this disease develop on the indicator stem below the grafted chip, and extend basipetally in a band or stripe; (iii) Machine or bench grafting; (iv) Green grafting, a technique to be encouraged because of the distinct advantages over other grafting systems (Walter *et al*., 1990).

Inoculation to herbaceous hosts. Herbaceous indicators detect mechanically transmissible viruses, including those of minor relevance. Their use is complementary to other diagnostic procedures. Sap transmission may be useful for preliminary screening and random testing.

Serological tests. Serology is to be regarded as a complement to, but not as a substitute for, other diagnostic procedures. However, the use of ELISA is recommended for the identification of GFLV and other European nepoviruses, for GFkV, closteroviruses and trichoviruses antisera to which are available. Source of antigens for ELISA can be grapevine buds, roots, leaves, and cortical scrapings from mature canes. Cortical scrapings are advantageous because they can be used throughout the year without apparent loss of efficiency due to seasonal variation of antigen titer in vegetating organs (Walter and Etienne, 1987). Furthermore, cortical scraping extracts give low background readings and are much more reliable for detection of closteroviruses in American rootstocks, especially V. *rupestris* and its hybrids (Boscia *et al.,* 1991).

Double-stranded RNAs. Electrophoretic detection of dsRNAs from tissue extracts may complement other diagnostic procedures. It may be useful in evaluating the outcome of sanitation treatments (Habili *et al.,* 1992).

Molecular assays. Molecular probes and primers for target nucleic acid amplification (PCR) have been produced to several grapevine nepoviruses (Martelli, 1993; Walter, 1993), closteroviruses (Martelli *et al.,* 1997), vitiviruses (Boscia *et al.,* 1997), GFkV (Sabanadzovic *et al.,* 1996), viroids (Walter, 1993)), and grapevine phytoplasmas (Caudwell, 1993; Davis and Prince, 1993; Walter, 1993). Whereas molecular tools have not yet found generalised application for virus detection and identification, they have become the method of choice for phytoplasmas (Caudwell, 1993).

Table 4. Main indicators for identification of virus and virus-like diseases of grapevines

INDICATOR	DISEASE IDENTIFIED
1. Vitis rupestris St. George	Degeneration[a], fleck, Rupestris stem pitting, asteroid mosaic.
2. Vitis vinifera	Leafroll
Cabernet franc,	
Pinot noir,	
or other red-fruited cultivars[b]	
3. Kober 5BB	Kober stem grooving
4. LN 33	Corky bark, LN 33 stem grooving, enations
5. Vitis riparia Gloire de Montpellier	Vein mosaic
6. 110 R	Vein necrosis

[a] In countries where degeneration is caused also by nepoviruses other than GFLV, Siegfriedrebe (FS4 201- 39) may be used as an indicator.

[b] The choice of the most suitable testing indicator for leafroll depends on climatic conditions of the place of testing.

Sanitation procedures

The high incidence of infectious diseases in commercial vineyards of EU Member States makes sanitation highly desirable, if not compulsory. The main techniques currently available are:

1. Hot water treatment of dormant canes at 50 °C for 45 min, for eliminating prokaryotes.
2. Hot air treatment of vegetating vines at constant temperature of about 38°C, excision and rooting of shoot tips, for eliminating virus and virus-like diseases.
3. Meristem tip culture *in vitro,* for eliminating virus and virus-like diseases. *In vitro* culture can be used in combination with heat therapy or chemotherapy . The consensus is that *in vitro* culture is more efficient than hot air treatment.
4. Micrografting of meristem tips onto *in vitro*-grown seedlings

Regardless of the procedure used, testing of treated material for assessment of its health status must follow, when a suitable period from the end of the sanitation treatment has elapsed.

References

Anonymous, 1988. Fruit crop sanitation in the Mediterranean and Near East region. Status and requirements. United Nations Development Programme/Food and Agricultural Organisation of the United Nations, Rome, Italy.

Anonymous, 1994. Certification scheme. Pathogen-tested material of grapevine varieties and rootstocks *EPPO Bulletin,* **24**: 347-367.

Bonavia, M., Digiaro, M., Boscia, D., Boari, A., Bottalico, G., Savino, V, and Martelli, G.P., 1996. Studies on "corky rugose wood" of grapevine and on the diagnosis of grapevine virus B. *Vitis*, **35**: 53-58.

Boscia, D., Minafra, A. and Martelli, G.P. 1997. Filamentous viruses of the grapevine: putative trichoviruses and capilloviruses. In: *Filamentous Viruses of Woody Plants.* Monette, P.L. (ed), Research Signpost, Trivandum, India: 19-26.

Boscia, D., Martelli, G.P., Savino, V. and Castellano, M.A., 1991. Identification of the agent of grapevine fleck disease. *Vitis,* **30***:* 97-105.

Boscia, D., Savino, V., Elicio, V., Jebahi, S.D. and Martelli, G.P., 1991. Detection of closteroviruses in grapevine tissues. *Proceedings 1Oth Meeting ICVG, Volos 1990:* 52-57.

Boscia, D., Greif, C., Gugerli, P., Martelli, G.P., Walter, B and Gonsalves, D., 1995. The nomenclature of the leafroll-associated putative closteroviruses. *Vitis,* **34**: 171-175.

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

Caudwell, A., 1993. Advances in grapevine yellows research since 1990. *Extended Abstracts 11th Meeting ICVG, Montreux 1993:* 79-83.

Davis, R.E. and Prince, J.P., 1993. Grapevine yellows diseases: diverse etiologies indicated by new DNA-based methods for pathogens detection and identification - Implications for epidemiology. *Extended Abstracts 11th Meeting ICVG, Montreux 1993:* 93-94.

Fortusini, A. Scattini, G., Cinquanta, S. and Prati, S., 1996. Diffusione naturale del virus 1 (GLRaV-1) e del virus 3 (GLRaV-3) dell'accartocciamento fogliare e della maculatura infettiva o "fleck" della vite. *Informatore Fitopatologico,* **46** (12*):* 39-43.

Goheen, A.C., 1977. Virus and virus-like diseases of grapes. *Hort. Science,* **12**: 465-469.

Habili, N., Krake, L.R., Barlass, M. and Rezaian, M.A., 1992. Evaluation of biological indexing and dsRNA analysis in grapevine virus elimination. *Annals of Applied Biology,* **121**: 277-283.

Krake, R.L. and Woodham, R.C., 1983. Grapevine yellow speckle agent implicated in the aetiology of vein banding disease. *Vitis,* **22***:* 40-50.

Maixner, M., 1994. Transmission of German grapevine yellows (Vergilbungskrankheit) by the planthopper *Hyalestes obsoletus* (Auchenorrhynca: Cixiidae). *Vitis,***33***:* 103-104.

Martelli, G.P. (ed.), 1992. Grapevine viruses and certification in EEC countries: State of the art. Quaderno No. 3, Istituto Agronomico Mediterraneo, Bari, Italy.

Martelli, G.P. (ed.), 1993. Graft-transmissible diseases of grapevines. Handbook for detection and diagnosis. FAO Publication Division, Rome, Italy.

Martelli, G.P. and Walter, B., 1988. Virus certification of grapevines. In: Plant Virus Disease Control. A. Hadidi, R.K. Khetarpal and H. Koganezawa (eds.). APS Press, St. Paol. USA: 261-276.

Martelli, G.P. and Jelkmann W., 1998. *Foveavirus,* a new plant virus genus. *Archives of Virology,* **143**: 1245-1249.

Meng, B., Pang, S.Z., Forsline, P.L., McFerson, J.R and Gonsalves, D., 1998. Nucleotide sequence and genome structure of rupestris stem pitting-associated virus 1 reveals similarities to apple stem pitting virus. *Journal of General Virology,* **79***:* 2059-2069.

Martelli, G.P., Saldarelli, P. and Boscia, D., 1997. Filamentous viruses of the grapevine: closteroviruses. Pages 1-9. In: Filamentous viruses of woody plants. Monette, P.L, (ed.) Research Signpost, Trivandum, India.

Martelli, G.P., De Sequeira, O.A., Kassemeyer, H.H., Padilla, V., Prota, U., Quacquarelli, A., Refatti, E., Rudel, M., Rumbos, I.C., Savino, V. and Walter, B., 1993. A scheme for grapevine certification in the European Economic Community. Page 279-284 In: Plant health and the European single market. Ebbels, D. ed. British Crop Protection Council Monograph No.54.

Sabanadzovic, S., Saldarelli, P. and Savino, V., 1996. Molecular diagnosis of grapevine fleck virus. *Vitis*, **35**: 137-140.

Semancik, J.S., 1993. Current status of research on grapevine viroids. *Extended Abstracts 11th Meeting ICVG, Montreux 1993:* 34-36.

Staub, U., Polivka, H., Herrmann, J.V. and Gross, H.J., 1995. Transmission of grapevine viroids is not likely to occur mechanically by normal pruning. *Vitis,* **34**: 119-123.

Szychowski, J.A., McKenry, M.V., Walker, M.A., Wolpert, J.A., Credi, R. and Semancik, J.S., 1995. The vein-banding disease syndrome: a synergistic reaction between grapevine viroids and fanleaf virus. *Vitis,* **34**: 229-232.

Walter, B., 1993. Advances in virus disease diagnosis since 1990. *Extended Abstracts 11th Meeting ICVG, Montreux 1993:* 127-130.

Walter, B., 1996. Harmonisation dans l'Union Européenne des protocoles de dépistage de maladies virales de la vigne: les virologues sont à l'ouvrage. *Progres Agricole et Viticole,* **112**: 460-461.

Walter, B. (ed.), 1997. Sanitary selection of the grapevine. Protocols for detection of viruses and virus-like diseases. Les Colloques, No. 86. INRA Editions, Paris, France.

Walter, B. and Etienne, L., 1987. Detection of grapevine fanleaf viruses away from the period of vegetation. *Journal of Phytopathology,* **120**: 355-364.

Walter, B. and Martelli, G.P., 1996. Sélection clonale de la vigne: sélection sanitaire et sélection pomologique. Influence des viroses et qualità. *Bulletin OIV*, **69**: 945-971.

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