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Serological detection of viruses included in certification protocols for stone fruits

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SUMMARY - Stone fruits are affected by many viruses which are routinely detected by serological methods. The paper focuses on ELISA and other serological techniques reviewing reports for Trichoviruses, Potyviruses, Ilarviruses, and Nepoviruses affecting stone fruits. Diagnostic problems that disturb the reliability of ELISA results are also discussed.

Key words: stone fruits, serology, ELISA , plant viruses, plant certification.

RESUME *- Les essences à noyaux sont affectées par bon nombre de virus qui sont détecés par des méthodes sérologiques de routine. Dans le présent travail, l'accent est mis sur l'ELISA et d'autres techniques sérologiques, en passant en revue les Trichovirus, les Potyvirus, les Ilarvirus et les Nepovirus signalés sur les essences à noyaux. On discute également les problèmes qui inerfèrent avec la fiabilité des résultats de l'ELISA.*

Mots-clés: *essences à noyaux, sérologie, ELISA, virus des végétaux, certification des plantes.*

I – Introduction

Serology is a traditional technique for virus detection, and based on the use of antibodies, proteins of the immunoglobulin type, raised in animals and capable of specific binding to

antigens. Early serological research was of great importance in identificating and classifying viruses, but, in the case of woody plants, they lacked the sensitivity for routine diagnosis. A tremendous improvement in sensitivity was achieved with the development of immunoenzymatic techniques, i.e. ELISA (enzyme linked immunosorbent assay), which employs antibodies conjugated to an enzyme, to greatly amplify and signal the presence of amounts of viral antigens.

The potential application of the serological diagnosis was limited for which viruses specific antibodies were produced. Consequently only known viruses, not diseases of unknown origin, can be detected by serology. Moreover, since the antigenic properties reside in the coat proteins, viroids cannot be detected by this means.

II – Serological methods

1. ELISA

ELISA is a diagnostic techniques utilised for identifying plant viruses. The presence of viral specific antigens in infected sap is detected through a colorimetric reaction, that develops because of the reaction of an enzyme (alkaline phosphatase or horseradish peroxidase) conjugated to antibodies in the presence of an appropriate substrate (paranitrophenylphosphate or tetrametylbenzidine, respectively). Among several variants of ELISA (Fig. 1), DAS (double antibody sandwich) ELISA is a standard procedure. Schematically, viral antigens are first trapped by virus-specific antibodies coating the internal surfaces of polystyrene wells, and, then, covered by enzyme-conjugated virus antibodies. Finally, the addition of the substrate induces a colorimetric reaction in the presence of the antigen-enzyme antibody-conjugate complex.

The success of ELISA, adopted in most diagnostic laboratories, is due to the numerous advantages that this technique offers in comparison with others (Clark and Bar Joseph, 1984). For example:

- sensitivity for detecting very small amounts of virus, i.e., antigen concentrations of 1-10 ng/ml;
- speed of reaction – results are usually available within 6-24 hr;
- scale of operation – several hundred samples can be readily handled, either individually or in groups;
- use with plant extracts and purified virus preparations;
- specificity for differentiating serotypes;
- suitability for both intact and fragmented virions of different size or morphology;

- possibility of obtaining quantitative measurements;
- possibility of automation and of standardising tests by the production and availability of commercial kits;
- low cost and relatively long shelf life of reagents;
- basic requirement for accessory equipment and supplies;
- economical technique.

ELISA has been applied to viruses of stone fruit trees since its first introduction into plant virology in 1976. The first approach was with arabis mosaic virus (ArMV) and plum pox virus (PPV), representatives of isometric and filamentous viruses, respectively (Voller *et al.*, 1976; Clark *et al.*, 1976). The technique was applied later to the majority of the viruses for which antisera were already available.

Up to now, almost 30 viruses are reported as actual or putative agents of diseases affecting stone fruits. Considering that some occur only in North America, there are still 16 viruses (Tab. 1) for which tests have to be done for the production of certified plant propagating material in the EPPO countries. These include members of the Trichovirus (ACLSV); Potyvirus (PPV); Ilarviruses (ApMV, PDV and PNRSV); Nepoviruses (ArMV, CLRV, CRLV, MLRSV, RRSV, SLRSV, TBRV ands TomRSV); Foveavirus (CGRMV) Closterovirus (LCV) and, Tombusvirus (PeAMV) groups.

The above viruses, belonging to different genera or families, possess different antigenic properties and, consequently, induce the production of antisera and serological reagents characterised by different levels of sensitivity that, in some cases, are of low diagnostic power. This aspect, together with others important parameters, as in the choice of sampled tissue and the season samples are collected, are of major importance in order to consider the ELISA results as be reliable and satisfactory the evaluation of the sanitary status of plants.

1.1. Monoclonal antibodies (MAbs)

For the absolute majority of viruses that have been isolated, purified, and characterised as causal agents of a disease, serology was performed using polyclonal reagents and more recently, monoclonal antibodies. Monoclonal antibodies have been produced against several stone fruit viruses (Halk *et al.*, 1982a, b; 1984; Hsu, 1985; Poul and Dunez, 1989; Cambra *et al.*, 1994; Boari *et al.*, 1998b; Myrta *et al.*, 1998). Particularly important was the possibility to identify and differentiate between virus strains of PPV with strain-specific monoclonal antibodies (Cambra *et al.*, 1994, Boscia *et al.*, 1997; Boscia *et al.*, 1998b; Myrta *et al.*, 1998; Navràtil *et al.*, 1998). Beside obvious advantages of monoclonal antibodies (e.g. specificity, unlimited production, reproducibility of results, easier immunization, possible utilisation of

mix infected virus sources) these reagents should not be used exclusively. The reason for this is because of its extreme specificity and some false negatives may be realised. A good example concern PDV and PNRSV, due in part to a high level of serological variability among virus isolates, can not be safely detected by single monoclonal antibodies, unless appropriated cocktails are used (Boari *et al.*, 1998b).

1.2. Artificial polyvalent antisera

To overcome limitations of strain specific antibodies, polyclonal antisera to several different strains may be mixed (polyvalent antisera) (Koenig *et al.*, 1979; Uyemoto, 1980).

Thus, several viruses will detected simultaneously by using polyvalent antisera (Cambra *et al.*, 1983), and its sensitivity was not compromised, if similar conditions were required for the reliable detection of the different viruses (James, 1997).

2. Other serological techniques alternative to ELISA

2.1. Dot Immunobinding Assay (DIBA)

DIBA has the same sensitivity of ELISA and need very little equipment. It is based on the use of membranes (nitrocellulose or other) in place of plates used for ELISA, and eliminate the need for a plate reader (Makkouk *et al.*, 1993; Poggi Pollini *et al.*, 1993).

2.2. Tissue blot immunoassay

While it may not always reach the same sensitivity as ELISA and DIBA, tissue imprinting is remarkably rapid (sample grinding and preparation used virtually eliminated) and, as DIBA, it can be performed with little equipment. In addition, tissue imprinting can provide data on virus localisation within plant organs (Makkouk *et al.*, 1993; Knapp *et al.*, 1995).

III - Stone fruit viruses

1. Trichoviruses

1.1. Apple chlorotic leafspot virus (ACLSV)

This virus is of medium immunogenic power and detectable by serological tests, mainly ELISA or immuno-tissue imprinting (Knapp *et al.*, 1995). Immuno electronmicroscopy has been used for ACLSV detection also (Kerlan *et al.*, 1981; Kalashjan and Lipartia, 1986).

In detecting ACLSV by ELISA with the diversity of its isolates, difficulties were encountered stemming from virus low particle stability and low concentrations. To overcome this

difficulty, Flegg and Clark (1979) modified the DAS technique by incubating the enzyme conjugate with the sap instead of adding it sequentially. The modified version of the ELISA technique for detecting ACLSV, (Flegg and Clark, 1979), was used successfully by other researchers (Fuchs *et al.*, 1979; Fuchs, 1980, 1982, 1983). However, Detienne *et al.* (1980) failed to detect ACLSV in apricot, using the same modified procedure. Positive reactions were later obtained with apricot extracts in the presence of stabilising agents and nicotine to neutralise plant tannins.

According to Fuchs (1980, 1982), the concentration of ACLSV in apple trees increase beginning in March and reach its maximum titers by May and June. ACLSV was detected by ELISA in September using extracts prepared from forced buds also. Detienne *et al.* (1980) detected ACLSV under field conditions in September using special buffers.

There are considerable differences in the virus concentration between tissues of different plant parts. Fuchs (1980, 1982) recommended to test flower petals for the detection of ACLSV in ELISA, whereas Llácer *et al.* (1985) suggested fruit tissue. Barba and Clark (1986) reported an erratic distribution of ACLSV, with leaves at the base of the branches containing more virus than apical ones. They found that extracts from one or two year-old wood was the most reliable tissue for assay, particularly later in the season.

Although ACLSV has a high degree of symptomatological variability, its antigenic properties appear to be very stable and polyclonal reagents detect all known sources of the virus. Any differences in antigenic properties of various strains had not posed any problem and does not require polyvalence of polyclonal reagents. Therefore, the serological assays for ACLSV called for the production and use of monoclonal antibodies (Poul and Dunez, 1990; Malinowski *et al.*, 1997).

2. Potyvirus

2.1. Plum pox virus (PPV)

PPV (causal agent of Sharka) infects many cultivated and wild *Prunus* species and is one of the most serious virus disease of Prunoideae. Severe symptoms develop in plum, apricot and peach. Recently, PPV was found in sweet and sour cherry (Crescenzi *et al.*, 1994; Kalashjan *et al.*, 1994). Currently, four distinct strains of PPV have been characterised, with differences in their biological, serological, molecular and epidemiological properties. The strains include Marcus (PPV-M), Dideron (PPV-D), El Amar (PPV-EA) and Cherry (PPV-C) (Pasquini and Barba, 1997; Candresse *et al.*, 1998; Boscia *et al.*, 1998a). PPV-M, endemic in South-Eastern Europe, spread rapidly, particularly in peach orchards. Virus isolates of this strain lack the site Rsa I, present in PPV-D (Candresse *et al.*, 1994). Candresse *et al.* (1995) had designed serotype-specific primers for M and D strain. PPV-M was also differentiated from

PPV-D by differences in coat protein subunits mobilities (38 KDa) in SDS-PAGE (Adamolle, 1993; Pasquini and Barba, 1994). PPV-D was common in apricot and plum orchards, and rare in peach. Candresse *et al.* (1994, 1995) identified the strain by enzymatic digestion and by serotype-specific primers. PPV-EA strain was isolated from apricot in Egypt. This isolate showed high level of heterogenity in the nucleotide and amino acid sequences compared to other isolates. Hammond *et al.* (1998) designed primers based on Nib (replicase) sequences and differentiated PPV-M from PPV-EA. The PPV-C isolates were identified by RFLP of PCR products (Nemchinov and Hadidi, 1996; Hammond *et al.*, 1998), and with RNA probes specific to PPV-C (Nemchinov *et al.*, 1996), and strain-specific primers (Nemchinov and Hadidi, 1997).

PPV has good immunogenic power and detection by ELISA (using polyclonal or, more recently, specific monoclonal antibodies) had been used since 1977 (Dunez, 1977; Polàk, 1988; Pasquini *et al.*, 1995).

By tests for PPV, Nyujtó *et al.* (1985) obtained amplified sensitivity by ELISA using avidin-biotin reagents. The method was successfully used for testing apricot, peach, and plum seeds for the presence of PPV (Németh and Kölber, 1983).

In addition to polyclonal antibodies, MAbs are now being used for rapid, reliable identification of PPV strains. In fact, one antibody source (MAb5B) was specific for a common antigenic determinant present on all isolates of PPV (Cambra *et al.*, 1994). To identify isolates to a PPV group, four Mabs, which are specific for four strain-specific antigenic determinants: e.g. MAb4DG5 for PPV-Dideron (Cambra *et al.*, 1994), MAbAL for PPV-Marcus (Boscia *et al.*, 1997), MAbAC for PPV-Cherry (Boscia *et al.*, 1998b), and MAbEA24 for PPV-El Amar (Myrta *et al.*, 1998) are available.

PPV concentration in leaves was highest in May and June. Later, virus concentration was higher in lower leaves than in terminal ones (Németh and Kölber, 1980). PPV was detected in leaves, flowers, fruits, bark and roots (Clark *et al.*, 1976; Adams, 1978; Dosba *et al.*, 1986; Llácer *et al.*, 1986; Adams *et al.*, 1998). This assessment was largely influenced by the host species and the buffers used (Dunez, 1977). Dosba *et al.* (1986) found that assays of peach trees were most reliable using bark of one or two-year-old twigs and in young shoot leaves. On apricot trees, PPV was detected in flowers and very young shoots, and in late summer in leaves.

In PPV testing it must be remembered that virus is unevenly distributed in infected tree and so infected and virus-free areas can be found not only within the same leaf but also within the tree because only some branches may be infected (Dunez, 1977; Casper, 1979). Hamdorf (1983) detected PPV in leaves after appearance of symptoms in 9 to 12-year-old, originally virus-free mother trees. A thorough analysis of an adult tree with PPV demonstrated the uneven distribution of the virus: over 55% of 700 samples from the tree resulted ELISA

negatives. In contrast, 15% were negative when the same collections were tested by molecular hybridisation, suggesting of low virus titer i.e. below detection threshold of ELISA (Varveri *et al.*, 1988). To minimise sampling errors we suggest collecting three samples per quadrant: this is obviously a rather cumbersome procedure as it means taking and analysing different samples per tree. These observations indicate that even a very sensitive method like ELISA can not guarantee freedom from PPV.

Immuno tissue imprinting was also used in host resistance studies (Dicenta and Audergon, 1994; Knapp *et al.*, 1995). Immunoelectron microscopy can be utilised also (Noel *et al.*, 1978; Kerlan *et al.*, 1981; Himler *et al.*, 1988).

3. Ilarviruses

Ilarviruses can be detected by ELISA. However, it has been noted (Boari *et al.*, 1998b; Fulton, 1968) that there is a great serological variability, especially with PNRSV and PDV.

3.1. Apple mosaic virus (ApMV)

ApMV was routinely detected by ELISA (Clark *et al.*, 1976; Voller *et al.*, 1976; Thresh *et al.*, 1977; Barbara *et al.*, 1979; Korpraditskul *et al.*, 1979; Hardcastle and Gotlieb, 1980; Torrance and Dolby, 1984).

ELISA detection can be done throughout the growing season in individual samples of young leaves or twigs with newly formed buds, and less readily in mature leaves after June (Torrance and Dolby, 1984). However, virus detection was easiest from mid-April to mid-June (Fuchs, 1980).

There are considerable differences in virus concentration between tissues of several plant parts. As with ACLSV, Fuchs (1980, 1982) recommended the flower petals for direct ELISA in assaying for ApMV.

Studies made with monoclonal antibodies in two different laboratories (Pasquini and Barba, 1991; Boari *et al.*, 1998b) indicated that the serological properties of the ApMV population were highly stable and, consequently, single MAbs were suitable for routine detection.

3.2. Prune dwarf virus (PDV)

PDV was also detected by serological tests, i.e. ELISA (Casper, 1977; Torrance and Dolby, 1984; Mink and Aichele, 1984a). ELISA detection of PDV was reported in germinated *Prunus avium* seeds (Casper, 1977), and is used in routine testing of seedlings for PDV and PNRSV of *P. avium*, *P. mahaleb*, *P. cerasifera*, and *P. persica* (Mink and Aichele, 1984a).

Protein A-ELISA (PAS ELISA) was developed by Cooper *et al.* (1986) for CLRV and PDV diagnosis in cherry seeds. The test provide a quick assessment of seed lots, requiring issue of phytosanitary certificates. DASI-ELISA with monoclonal antibodies was applied to study seed transmission of PDV in *Prunus mahaleb* also (Boari *et al.*, 1998a).

In plum and sweet cherry, PDV was detected during the whole vegetation period in young leaves or in newly formed buds (Torrance and Dolby, 1984).

PDV isolates were characterised by great serological variability. Boari *et al.* (1998b) identified 36 different serogroups in 128 isolates originated by different *Prunus* species. Consequently, if monoclonal antibodies were used for PDV assays, would be advisable to prepare appropriate cocktails (mixtures of MAbs) to reduce risks of false negatives.

3.3. *Prunus necrotic ringspot virus (PNRSV)*

ELISA was widely used for the detection of PNRSV in tissues collected early in the vegetation period (Thresh *et al.*, 1977; Barbara *et al.*, 1978, 1979; Barbara, 1980; Thomas, 1980; Mink and Aichele, 1984 a, b; Torrance and Dolby, 1984). The method was also used in assays of seedlings of *P. avium, P. mahaleb, P. cerasifera,* and *P. persica* (Mink and Aichele, 1984a). A specific study carried out by Torrance and Dolby (1984) ascertained that PNRSV can be detected in plum during the whole vegetation period in young leaves or in newly formed buds. The sensitivity of DAS-ELISA was ten-fold increased when an amplification of the enzyme reaction was applied (Varveri, 1994). Immuno tissue imprinting effectively detected PNRSV as well (Knapp *et al.*, 1995).

Different serological strains of PNRSV occur (Casper, 1973; Mink *et al.*, 1987), and care must be given when using MAbs. Because of its high specificity, false negative results may be realised. Boari *et al.* (1998b) had identified 17 serological variants among 38 isolates tested. Consequently, as done for PDV, and when using MAbs, the adoption of cocktails must be considered. In the past, high specificity of polyclonal reagents was also reported. But, it led to erroneous identification of one isolate, initially classified as PNRSV, but later as ApMV.

4. Nepoviruses

Nepoviruses, in general, are good immunogenics and can be readily detected by ELISA (Clark *et al.*, 1976; Korpraditskul *et al.*, 1979; Voller *et al.*, 1976; György, 1979; Thomas, 1980, Dunez, 1977, Gonsalves, 1979; Lister *et al.*, 1980; Parish and Converse, 1981; Bitterlin *et al.*, 1984; Hoy and Mircetich, 1984; Hoy *et al.*, 1984; Powell, 1984; Powell *et al.*, 1984; Bitterlin and Gonsalves, 1986).

Protein A-ELISA (PAS-ELISA) was developed by Cooper *et al.* (1986) for CLRV assays of cherry seeds. This test provide a quick assessment of seed lots requiring the issue of phytosanitary certificates.

Different serological strains of TBRV have been reported (Kerlan *et al.*, 1982). Antigenic differences may lead to a strong specificity of polyclonal reagents.

Many authors reported ELISA detection of tomato ringspot virus (TomRSV). However, the virus was irregularly distributed in some hosts. Lister *et al.* (1980) reported that TomRSV was detected by ELISA in apple trees most easily in leaf- and bark extracts of root suckers and peach trees in root extracts. Bitterlin *et al.* (1984) showed, in apple trees that this virus was detected more consistently in leaves, slightly less in the bark, and erratically in the roots. Towards the end of the growing season, the reliability of the assays decrease. Bitterlin and Gonsalves (1986) found the distribution of TomRSV in peach trees to be irregular but mostly concentrated at and below the soil line.

5. Closteroviruses

Initially defined by transmission on sensitive cherry varieties (Van or Sam), a closterovirus was recently identified in trees affected with "little cherry" disease and serological or molecular assays were developed for the associated closterovirus (Eastwell *et al.*,1996). However, for now, the association of this closterovirus to the "little cherry" disease is basically related to the strict correlation between the presence of the virus and the presence of symptoms.

6. Foveaviruses

A RT-PCR system for the detection of cherry green ring mottle virus (CGRMV) was developed (Zhang *et al.*, 1998). No serological assays are currently available.

7. Tombusviruses

Petunia asteroid mosaic virus (PeAMV) was unevenly distributed within diseased trees. The virus was restricted to the symptom-bearing tissues in the different plant parts tested (leaves, fruits, young twig-tips, bark). Trees showing few or no symptoms of the disease were often negative in ELISA test. Therefore, reliable indexing for latent infections with PeAMV by means of serology is not possible at the moment (Diekmann and Putter, 1996).

IV- Diagnostic problems

1. Sampling

The nature of the sampled tissues has great influence on the results. Old leaves, often rich in tannins and oxydative substances, should be avoided. For imported samples, the materials often consists of dormant budwood (Babovic and Bulajic, 1995). Sanitary controls may be done in three ways: i) bioassays by bud and graftings onto rootstocks for further testing; ii) force dormant buds to sprout and assay fresh tender tissues; and iii) direct analysis of the bark. Although virus concentrations in dormant material may be low, laboratory based assays can be done with a certain amount of success.

2. Uneven distribution of the pathogen in the infected plant

This phenomenon was frequently encountered with many viruses infecting stone fruits. Not only when virus was localised around a point of infection, but also in classical systemic infections, when the virus was unevenly distributed and/or present in variable concentrations in the plant. This happens frequently in woody plants and true for PPV, but apply to other viruses as well.

3. Grouping of samples

Currently, ELISA is the method for routine testing of large numbers of samples or group test of different samples. According to Torrance and Dolby (1984) the sampling date influences also the efficiency of group-test results. For example, PDV was detected in 1/40 (infected/total leaves) cherry leaves in April and May and plum leaves up to July, and PNRSV was detected in 1/20 cherry leaves until July and in apple and plum leaves to May. Also, ApMV was detected in 1/20 apple and plum leaves to July.

4. The variability of pathogens

The direct DAS ELISA may be highly strain specific, whereby conjugate prepared for a given strain are unable to detect other, serologically related strains. So, with distantly related serological relationships, some viruses also intermediary ones, go undetected (Koenig, 1978; Lister *et al.*, 1980). The high degree of selectivity may be an advantage in epidemiological studies where specific virus strains must be distinguished. However, in diagnostic work and extensive routine testing where all the strains of a virus, both known and unknown, must be detected, it is a major drawback.

As reported by van Regenmortel and Burckard (1980) and Lommel *et al.* (1982), strain specificity does not present a problem with the indirect ELISA (TAS) systems in which, instead of antiviral antibodies, anti-IgG antibodies were labelled with the enzyme.

Although ACLSV has a high degree of variability at the symptom level, its antigenic properties were stable and polyclonal reagents detected all known virus strains. Barba and Clark (1986) reported on the existence of different serotypes of ACLSV. However, a study involving eight MAbs and 29 virus isolates, by Poul and Dunez (1989), demonstrated that the antigenic properties were highly conserved. These results were confirmed by Candresse *et al.* (1995) in the comparative analysis of coat protein genes of several ACLSV isolates.

5. Serological cross-reactions

Serological cross-reactions have been reported for isometric and filamentous viruses of stone fruits. The cross-reaction occurred between virus members of the same taxon or even different ones. James *et al.* (1994; 1996) reported positive reactions with a PPV polyclonal antibody and Prunus virus isolates, and Jordan and Hammond (1991) described several potyvirus-specific MAbs reacting with PPV isolates.

Serological relationships between ApMV and PNRSV have been reported (Fulton, 1968; Barbara, 1988a,b; Halk *et al.*, 1984). However, recent investigations using MAbs have not shown any cross reaction reactivity, at least not with our Italian isolates (Pasquini and Barba, 1991; Boari *et al.*, 1998b).

V - Conclusions

Serological tests remain very useful as detection methods in general, and for certification purposes in particular, due to their sensitivity, easily adaptability, relatively low cost and excellent large-scale use. However, we must avoid false negative reactions due to the wrong choice in the time of year and method of sampling, quality of antisera and correct conduction of ELISA. Serological methods may not satisfy all requirements for sanitary assessment of the plants (biological assay still remain priority), however they play an important role in a range of detection and analytical techniques, for their adaptability and rapid assays of large sample numbers. Molecular techniques as PCR, for their tremendous sensitivity, should now be taken in consideration, where it involve small number of trees.

References

- ADAMOLLE, C. (1993). Le virus de la sharka: Obtention et charactérisation partielle d'anticorps polyclonaux spécifiques de protéines non struturales. Approche de la bioécologie de deux sérotypes épidémiques en verger. Doctorat de l'Université de Bordeaux II. 153 pp.

- ADAMS, A.N. (1978). The detection of plum pox virus in *Prunus* species by enzyme-linked immunosorbent assay (ELISA). *Annales of Applied Biology*, 90: 215-221.
- ADAMS, A.N., GUISE, C.M. and S.J. CROSSLEY (1998). Detection of plum pox virus in root and bark samples from dormant plum trees. *Acta Horticulturae*, 472 (2): 469-475.
- ANONIMOUS (1992). Certification scheme. Virus-free or virus-tested fruit trees and rootstocks. Part III. Testing methods for viruses present in the EPPO region. *Bulletin OEPP/EPPO Bulletin*, 22: 265-275.
- BABOVIC, M. and A. BULAJIC (1995). Possibility of sharka (plum pox) virus isolation from plum and apricot branches actived during winter. *Acta Horticulturae*, 386: 404-408.
- BARBA, M. and M.F. CLARK (1986). Detection of strains of apple chlorotic leafspot virus by $F(ab)_2$-based indirect ELISA. *Acta Horticulturae*, 193: 297-304.
- BARBARA, D.J. (1980). Detecting *Prunus* necrotic ringspot virus in *Rosaceous* hosts by enzyme-linked immunosorbent assay. *Acta Phytopathologica Academiae Scientiarum Hungaricae*, 15: 329-332.
- BARBARA, D.J. (1988a). Apple mosaic virus (ApMV). *In*: European Handbook of Plant Diseases (Smith J.M. *et al.*, Eds.) Blackwell Scientific Publications. 13-14.
- BARBARA, D.J. (1988b). Prunus necrotic ringspot virus (PNRSV). *In*: European Handbook of Plant Diseases (Smith J.M. *et al.*, Eds.) Blackwell Scientific Publications. 17-19.
- BARBARA, D.J., CLARK, M.F. and C.L. FLEGG (1979). Virus detection and characterization. *Annual Report East Malling Research Station* 1978, A 62: 100-102.
- BARBARA, D.J., CLARK, M.F., THRESH, J.M. and R. CASPER (1978). Rapid detection and serotyping of Prunus necrotic ringspot virus in perennial crops by enzyme-linked immunosorbent assay. *Annales of Applied Biology*, 90: 395.
- BITTERLIN, M.W., GONSALVES, D. and J.N. CUMMINS (1984). Irregular distribution of tomato ringspot virus in apple trees. *Plant Disease*, 68: 567-571.
- BITTERLIN, M.W. and D. GONSALVES (1986). Serological and sampling techniques for detecting tomato ringspot virus in peach trees. *Acta Horticulturae*, 193: 291-296.
- BOARI, A., BOSCIA, D., DI TERLIZZI, B. and V. SAVINO (1998a). Study on seed transmission of prune dwarf virus (PDV) in *Prunus mahaleb* L. *Advances of Horticultural Sciences*, 12: 89-92.
- BOARI, A., POTERE, O., BOSCIA, D., TURTURO, C. and V. SAVINO (1998b). Uso di anticorpi monoclonali per la diagnosi di ilarvirus del ciliegio. *In*: Atti del Convegno Nazionale del Ciliegio. Valenzano (BA) 19-21 giugno 1997. 577-582.
- BOSCIA, D., MYRTA, A., POTERE, O., CAMBRA, M., CRESCENZI, A., DI TERLIZZI, B., CANDRESSE, T. and V. SAVINO (1998a). Impiego di anticorpi monoclonali per la identificazione dei diversi ceppi del virus della vaiolatura delle drupacee (PPV). *Notiziario sulla Protezione delle Piante*, 9: 207-212.
- BOSCIA, D., MYRTA, A., POTERE, O., CRESCENZI, A. and M. NUZZACI (1998b). Produzione di anticorpi monoclonali al ceppo del virus della vaiolatura del susino isolato dal ciliegio dolce (PPV-SwC). *In*: Atti del Convegno Nazionale del Ciliegio. Valenzano (BA) 19-21 giugno 1997. 141-146.
- BOSCIA, D., ZERAMDINI, H., CAMBRA, M., POTERE, O., GORRIS, M.T., MYRTA, A., DI TERLIZZI, B. and V. SAVINO (1997). Production and characterization of a monoclonal antibodiy specific to the M serotype of plum pox potyvirus. *European Journal of Plant Pathology*, 103: 477-480.
- CAMBRA, M. and M.T. GORRIS (1996). Biotinylation of immunoglobulins. *In:* Serology and electrophoretic plant virus diagnosis techniques, IAM-Bari, Italy. 91-93.

- CAMBRA, M., ASENSIO, M., GORRIS, M. T., PÉREZ, E., CAMARASA, E., GARCIA, J.A., MOYA, J.J., LÒPEZ-ABELLA, D., VELA, C. and A. SANZ (1994). Detection of plum pox potyvirus using monoclonal antibodies to structural and non-structural proteins. *Bulletin OEPP/EPPO Bulletin,* 24: 569-579.
- CAMBRA, M., LLÁCER, G. and C. PÉREZ DE SANROMÁN (1983). Use of enzyme-linked immunosorbent assay (ELISA) for virus detection on stone fruit trees in Spain. *Acta Horticulturae,* 130: 145-150.
- CANDRESSE, T., CAMBRA, M., DALLOT, S., LANNEAU, M., ASENSIO, M., GORRIS, M.T., REVERS, F., MACQUAIRE, G., OLMOS, A., BOSCIA, D., QUIOT, J.B., and J. DUNEZ (1998). Comparison of monoclonal antibodies and PCR assays for the typing of isolates belonging to the D and M serotypes of plum pox virus. *Phytopathology,* 88 (3): 198-204.
- CANDRESSE, T., MACQUAIRE, G., LANNEAU, M., BOUSALEM, M., QUIOT-DOUINE, L., QUIOT, J. B. and J. DUNEZ (1995). Analysis of Plum pox virus variability and development of strain-specific PCR assay. *Acta Horticulturae,* 386: 357-370.
- CANDRESSE, T., MACQUAIRE, G., LANNEAU, M., BOUSALEM, M., WETZEL, T., QUIOT-DOUINE, L., QUIOT, J.B. and J. DUNEZ (1994). Detection of plum pox potyvirus and analysis of its molecular variability using immunocapture-PCR. *Bulletin OEPP/EPPO Bulletin,* 24: 585-595.
- CASPER, R. (1973). Serological properties of Prunus necrotic ringspot and apple mosaic virus isolates from rose. *Phytopathology,* 63: 238-240.
- CASPER, R. (1977). Testung von *Prunus avium*-Samen auf prune dwarf mit dem ELISA-Verfahren. *Phytopathologische Zeitschrift,* 90: 91-94.
- CASPER, R. (1979). Anwendung neuen serologischen Verfahrens (ELISA) zum Nachweis pflanzenpathogener Viren *Mitteilungen aus der Biologischen Bundesanstalt für Land und Forstwirtschaft in Berlin-Dahlem,* 191: 191-209.
- CLARK, M.F. ADAMS, A.N., THRESH, J.M. and R. CASPER (1976). The detection of plum pox and other viruses in woody plants by enzyme-linked immunosorbent assay (ELISA). *Acta Horticulturae,* 67: 51-57.
- CLARK, M.F. and M. BAR JOSEPH (1984). Enzyme immunosorbent assay in plant virology. *In*: Methods in Virology. Acc. Press. New York. Vol. III. 51-85.
- COOPER, J.I., EDWARDS, M.L. and J. BRADLEY (1986). Protein A-alkaline phosphatase in ELISA detection of cherry leaf roll and prune dwarf viruses in cherry seeds. *Acta Horticulturae,* 193: 187-191.
- CRESCENZI, A., NUZZACI, M., LEVY, L., PIAZZOLLA, P. and A. HADIDI (1994). Infezioni di sharka su ciliegio dolce in Italia meridionale. *L'informatore Agrario,* 34: 73-75.
- DETIENNE, G., DELBOS, R. and J. DUNEZ (1980). Use and versatility of the immunoenzymatic ELISA procedure in the detection of different strains of apple chlorotic leaf spot virus. *Acta Phytopathologica Academiae Scientiarum Hungaricae,* 15: 39-45.
- DICENTA, F. and J.M. AUDERGON (1994). Utilisation de la technique d'immunoempreinte pour l'étude de la résistance à la sharka chez les *Prunus. Bulletin OEPP/EPPO Bulletin,* 24: 749-755.
- DIEKMANN, M. and C.A.J. PUTTER (1996). Stone fruits. FAO/IPGRI Technical Guidelines for the safe Movements of Germplasm. N° 16. 54 pp.
- DOSBA, F., PIQUEMAL, J.P., PECHEUR, G., TEYSSIER, B., LANSAC, M. and M. MICHEL (1986). Plum pox virus detection by ELISA technique in peach and apricot infected trees at different growing stages. *Acta Horticulturae,* 193: 187-191.

- DUNEZ, J. (1977). Application des techniques enzymatiques à la détection de certain virus phytopathogènes des végétaux. La mèthode ELISA. *Annales de Phytopathologie,* 9: 219-221.
- EASTWELL, K.C., BERNARDY M.G. and T.S.C. LI (1996). Comparison between woody indexing and a rapid hybridisation assay for the diagnosis of little cherry disease in cherry trees. *Annales of Applied Biology,* 128: 269-277.
- FLEGG, C.L. and M.F. CLARK (1979). The detection of apple chlorotic leaf spot virus by a modified procedure of enzyme-linked immunosorbent assay. *Annales of Applied Biology,* 91: 61-65.
- FUCHS, E. (1980). Serological detection of apple chlorotic leaf spot virus (CLSV) and apple stem grooving virus (SGV) in apple trees. *Acta Phytopathologica Academiae Scientiarum Hungaricae,* 15: 69-73.
- FUCHS, E. (1982). Studies of the development of concentration of apple chlorotic leaf spot virus (CLSV) and apple stem grooving virus (SGV) in apple trees. *Acta Phytopathologica Academiae Scientiarum Hungaricae,* 17: 23-27.
- FUCHS, E. (1983). Vergleich verschiedener serologischer Methoden zum Nachweis des Stammfurchunges-Virus des Apfels (SGV) und des Chlorotischen Blattfleckungs-Virus des Apfels (CLSV). *Archiv für Gartenbau,* Berlin, 31: 237-245.
- FUCHS, E., MERKER, D. and H. KEGLER (1979). Der Nachweis des chlorotischen Blattfleckungs-Virus des Apfels (apple chlorotic leaf spot virus) des Stammfurchungs-Virus des Apfels (apple stem grooving virus) und des Tomatenzwergbush-Virus (tomato bushy stunt virus) mit dem ELISA. *Archiv für Phytopathologie Pflanzenschutz,* Berlin, 15: 419-422.
- FULTON, R.W. (1968). Serology of viruses causing cherry necrotic ringspot, plum line pattern, rose mosaic and apple mosaic. *Phytopathology,* 58: 635-638.
- GONSALVES, D. (1979). Detection of tomato ringspot virus in grapevines: a comparison of *Chenopodium quinoa* and enzyme-linked immunosorbent assay (ELISA). *Plant Disease Reporter,* 63: 962-965.
- GYÖRGY, B. (1979). Új lehetöség a fás szárú növények virusfertözöttségének közvetlen kimutatására az "ELISA"- teszt. *Agrártudomanyi Egyetem. Közlemenyei,* 38 (1-2): 101.
- HALK, E.L., HSU, H.T. and J. AEBIG (1982a). Properties of virus specific monoclonal antibodies to *Prunus* necrotic ringspot (NRSV) apple mosaic (ApMV), tobacco streak (TSV) and alfalfa mosaic (AMV) viruses. *Phytopathology,* (abstr.) N° 189, 72: 953.
- HALK, E.L., HSU, H.T., AEBIG, J. and J. FRANKE (1984). Production of monoclonal antibodies against three ilarviruses and alfalfa mosaic virus and their use in serotyping. *Phytopathology,* 74: 367-372.
- HALK, E.L., HSU, H.T., AEBIG, J. and K. CHANG (1982b). Production of hybrid cell lines secreting virus specific monoclonal antibodies to three *ilarviruses* and alfalfa mosaic virus. *Phytopathology,* 74: 367-372.
- HAMDORF, G. (1983). The detection of plum pox virus (PPV) by indicator plants and enzyme-linked immunosorbent assay (ELISA). *Acta Horticulturae,* 130: 151-159.
- HAMMOND, J., PÜHRINGER, H., DA CÂMARA MACHADO, A. and M. LAIMER DA CÂMARA MACHADO, (1998). A broad-spectrum PCR assay combined with RFLP analysis for detection and differentiation of plum pox virus isolates. *Acta Horticulturae,* 472 (2): 483-490.
- HARDCASTLE, T. and A.R. GOTLIEB (1980). An enzyme-linked immunosorbent assay for the detection of apple mosaic virus yellow birch. *Canadian Journal of Forest Research,* 10: 278-283.

- HIMMLER, G., BRIX, U., STEINKELLNER, H., LAIMER, M., MATTANOVICH, D., HERMAN W.D. and V. KATINGER (1988). Early screening for anti-plum pox virus monoclonal antibodies with different epitope specificities by means of gold-labelled immunosorbent electron microscopy. *Journal of Virological Methods,* 22: 351-358.
- HOY, J.W. and S.M. MIRCETICH (1984). Prune brownline disease: susceptibility of prune rootstocks and tomato ringspot virus detection virus detection. *Phytopathology,* 74: 272-276.
- HOY, J.W., MIRCETICH, S.M. and B.F. LOWNSBERY (1984). Differential transmission of *Prunus* tomato ringspot virus strains by *Xiphinema californicum. Phytopathology,* 74: 332-335.
- HSU, H.T. (1985). Development of monoclonal antibodies to plant viruses at the American Type Culture Collection: collaborative efforts among institutions. *In*: International Conference on New Developments in Techniques for Virus Detection. Cambridge, 10-12 April 1985, (Abstr.) 27.
- JAMES, D. (1997). Serological reactions of some filamentous viruses of temperate fruit trees. *In:* Filamentous viruses of woody plants. (Ed.P.L. Monette). 159-170.
- JAMES, D., GODKIN, S.E., EASTWELL, K.C. and D.J. MACKENZIE (1996). Identification and differentiation of Prunus virus isolates that cross-react with Plum Pox virus and Apple Stem pitting virus antisera. *Plant Disease* , 80 (5): 536-542.
- JAMES, D., THOMPSON, D.A. and S.E. GODKIN (1994). Cross reaction of an antiserum to plum pox potyvirus. *Bulletin OEPP/EPPO Bulletin,* 24: 605-615.
- JORDAN, R.L. and J. HAMMOND (1991). Comparison and differentiation of potyvirus isolates and identification of strain-, virus-, subgroup-specific and potyvirus group-common epitopes using monoclonal antibodies. *Journal of General Virology,* 72: 25-36.
- KALASHJAN, J.A. and Z.M. LIPARTIA (1986). Apple chlorotic leafspot virus (CLSV) in affected tissues and the possibility of its detection by immune electron microscopy (IEM). *Acta Horticulturae,* 193: 311-318.
- KALASHJAN, J.A., BILKEY, N.D., VERDEREVSKAYA, T.D. and E.V. RUBINA (1994). Plum pox potyvirus on sour cherry in Moldova. *Bulletin OEPP/EPPO Bulletin,* 24: 645-651.
- KERLAN, C., MILLE, B. and J. DUNEZ (1981). Immunosorbent electron microscopy for detecting apple chlorotic leaf spot and plum pox viruses. *Phytopathology,* 71 (4): 400-404.
- KERLAN, C., MILLE, B., DETIENNE, G. and J. DUNEZ (1982). Comparison of immunoelectronmicroscopy, immunoenzimology (ELISA) and gel diffusion for investigating virus strain relationships. *Annales de Virologie Institut Pasteur* 133E: 1-14.
- KNAPP, E., DA CÂMARA MACHADO, A., PÜHRINGER, H., WANG, Q., HANZER, V., WEISS, H., WEISS, B., KATINGER, H. and M. LAIMER DA CÂMARA MACHADO (1995). Localisation of fruit tree viruses by immuno tissue printing in infected shoots of *Malus* and *Prunus* sp. *Journal of Virological Methods,* 55: 157-173.
- KOËNIG, R. (1978). ELISA in the study of homologous and heterologous reactions of plant viruses. *Journal of General Virology,* 40: 309-318.
- KOENIG, R., FRIBOURG, C.E. and R.A.C. JONES (1979). Symptomatological, serological and electroforetic diversity of isolates of Andean potato latent virus from different regions of the Andes. *Phytopathology,* 69: 748-752.
- KORPRADITSKUL, P., CASPER, R. and D.E. LESEMANN (1979). Evaluation of short reaction times and some characteristics of the enzyme-conjugation in enzyme-linked immunosorbent assay (ELISA). *Phytopathologische Zeitschrift,* 96: 281-285.

- LISTER, R.M., ALLEN, W.R., GONSALVES, D., GOTLIEB, A.R., POWELL, C.A. and R.F. STOUFFER (1980). Detection of tomato ringspot virus in apple and peach by ELISA. *Acta Phytopathologica Academiae Scientiarum Hungaricae,* 15: 47-55.
- LLÁCER, G., CAMBRA, M., LAVIÑA, A. and J. ARAMBURU (1985). Suitable conditions for detection for apple chlorotic leaf spot virus in apricot trees by enzyme-linked immunosorbent assay (ELISA). *Agronomie,* 5 (9): 809-812.
- LLÁCER, G., CAMBRA, M., LAVIÑA, A. and J. ARAMBURU (1986). Investigations on plum pox (Sharka) virus in Spain. *Acta Horticulturae,* 193: 155-161.
- LOMMEL, A.S., MCCAIN, A.H. and T.J. MORRIS (1982). Evaluation of indirect enzyme-linked immunosorbent assay for the detection of plant viruses. *Phytopathology,* 72: 1018-1022.
- MAKKOUK, K.M., HSU, H.T. and S.G. KUMARI (1993). Detection of three plant viruses by Dot-Blot and Tissue-Blot immunoassays using chemiluminescent and chromogenic substrates. *Journal of Phytopathology,* 139: 97-102.
- MALINOWSKI, T., CIELINSKA, M., ZAWADZKA, B., INTEREWICZ, B. and A. POREBSKA (1997). Characterisation of monoclonal antibodies against apple chlorotic leaf spot virus (ACLSV) and their application for detection of ACLSV and identification of its strains. *Phytopath. Polonica,* 14: 35-40.
- MINK, G.I. and M.D. AICHELE (1984a). Detection of Prunus necrotic ringspot and prune dwarf viruses in *Prunus* seed and seedlings by enzyme-linked immunosorbent assay. *Plant Disease,* 68: 378-381.
- MINK, G.I. and M.D. AICHELE (1984b). Use of enzyme- linked immunosorbent assay results in efforts to control orchard spread of cherry rugose disease in Washington. *Plant Disease,* 68: 207-210.
- MINK, G.I., HOWELL, W.E., COLE, A. and S. REGEV (1987). Three serotypes of Prunus necrotic ringspot virus isolated from mosaic-diseased sweet cherry trees in Washington. *Plant Disease,* 71: 91-93.
- MYRTA, A., POTERE, O., BOSCIA, D., CANDRESSE, T., CAMBRA, M. and V. SAVINO (1998). Production of a monoclonal antibody specific to El Amar strain of plum pox virus. *Acta Virologica,* 42: 248-250.
- NAVRÀTIL, M., SIMONOVA, V., PAPRŠTEIN, F. and R. KAREŠOVA (1998). Detection and serological identification of plum pox virus isolates in the Czech Republic. *Acta Horticulturae,* 472, Vol. 2: 373-379.
- NEMCHINOV, L. and A. HADIDI (1996). Characterization of the sour cherry strain of Plum Pox Virus. *Phytopathology,* 86 (6): 575-580.
- NEMCHINOV, L. and A. HADIDI (1997). Polymerase chain reaction detection of plum pox virus-cherry (PPV-C) subgroup using PPV-C specific primers. *In:* Abstracts of XVII International Symposium on Virus Diseases of Fruit Trees, June 23-27, 1997, Bethesda, MD, USA. p. 53.
- NEMCHINOV, L., HADIDI, A., MAISS, E., CAMBRA, M., CANDRESSE, T. and V. DAMSTEEG, (1996). Sour Cherry strain of Plum pox Potyvirus (PPV): Molecular and Serological Evidence for a New Subgroup of PPV Strains. *Phytopathology.* Vol.86 (11): 1215-1221.
- NÉMETH, M. and M. KÖLBER (1980). GF 31, a Reliable Myrobalan Field Indicator for Rapid Detection of Plum Pox Virus. *Acta Phytopathologica Academiae Scientiarum Hungaricae,* 15 (1-4): 207-212
- NÉMETH, M. and M. KÖLBER (1983). Additional evidence on seed transmission of plum pox virus in apricot, peach and plum proved by ELISA. *Acta Horticulturae,* 130: 293-299.

- NOEL, M., KERLAN, C., GARNIER, M. and J. DUNEZ (1978). Possible use of immune electron microscopy (IEM) for the detection of plum pox virus in fruit trees. *Annales de Phytopathologie,* 10: 381-386.
- NYUJTÓ, F., NÉMETH, M. and M. KÖLBER (1985). Investigations on the resistance to plum pox disease of the apricot variety Ceglédi Biborkajszi C 244. *Acta Horticulturae,* 192: 159-166.
- PARISH, C.L. and R.H. CONVERSE (1981). Tomato ringspot virus associated with apple union necrosis and decline in western United States. *Plant Disease,* 65: 261-263.
- PASQUINI, G. and M. BARBA (1991). Production and application of monoclonal antibodies against apple mosaic virus. *Petria,* 1: 31-36.
- PASQUINI, G. and M. BARBA (1994). Serological characterization of Italian isolates of plum pox potyvirus. *Bulletin OEPP/EPPO Bulletin,* 24: 615-625.
- PASQUINI, G. and M. BARBA (1997). Plum pox potyvirus strains: an overview. *In*: Proceedings of the Middle European Meeting '96 on Plum Pox. Budapest, 2nd – 4th October 1996, 168-171.
- PASQUINI, G., MAZZEI, M., PILOTTI, M. and M. BARBA (1995). Characterization of PPV isolates using monoclonal antibodies. *Acta Horticulturae,* 386: 346-354.
- POGGI POLLINI C., GIUNCHEDI, L. and R. CREDI (1993). A chemioluminescent immunoassay for the diagnosis of grapevine closteroviruses on nitrocellulose membrane. *Journal of Virological Methods,* 42: 107-116.
- POLÀK, J. (1988). Diagnosis of plum pox virus in infected symptomless trees of apricot peach and Prunus cerasifera spp. myrobalana by ELISA and ISEM. *Acta Horticulturae,* 235: 299-303.
- POUL, F. and J. DUNEZ (1989). Production and use of monoclonal antibodies for the detection of apple chlorotic leaf spot virus. *Journal of General Virology,* 25: 153-166.
- POUL, F. and J. DUNEZ (1990). Use of monoclonal antibodies for the identification of different antigenic domains in apple chlorotic leaf spot virus. *Archives of Virology,* 114: 191-202.
- POWELL, C.A. (1984). Comparison of enzyme-linked immunosorbent assay procedures for detection of tomato ringspot virus in woody and herbaceous hosts. *Fruitt.,* 70: 462-464.
- POWELL, C.A., FORER, R.F., STOUFFER, R.F., CUMMINS, J.N., GONSALVES, D., ROSENBERGER, D.A., HOFFMAN, J. and R.M. LISTER (1984). Orchard weeds as hosts of tomato ringspot and tobacco ringspot viruses. *Plant Disease,* 68: 242-244.
- THOMAS, B.J. (1980). The detection of serological methods of viruses infecting the rose. *Annales of Applied Biology,* 94: 91.
- THRESH, J.M., ADAMS, A.N., BARBARA, D.J. and M.F. CLARK (1977). The detection of three viruses of hop *(Humulus lupulus)* by enzyme-linked immunosorbent assay. *Annales of Applied Biology,* 87: 1-9.
- TORRANCE, L. and C.A. DOLBY (1984). Sampling conditions for reliable routine detection by enzyme-linked immunosorbent assay of three ilarviruses in fruit trees. *Annales of Applied Biology,* 104: 267-276.
- UYEMOTO, J.K. (1980). Detection of maize chlorotic mottle virus serotypes by enzyme-linked immunosorbent assay. *Phytopathology,* 70: 290-292.
- VAN REGENMORTEL, M.H.V. and J. BURCKARD (1980). Detection of a wide spectrum of tabacco mosaic virus strains by indirect enzyme-linked immunosorbent assays (ELISA). *Virology,* 106: 327-334.

- VARVERI, C. (1994). Amelioration of the ELISA method for the detection of prunus necrotic ringspot virus. *Annales de l'Institut Phytopathologique Benaki,* (N.S.), 17: 89-95.
- VARVERI, C., CANDRESSE, T., CUGUSI, M., RAVELONANDRO, M. and J. DUNEZ (1988). Use of the 32P-labelled transcribed RNA probe for dot hybridization detection of plum pox virus. *Phytopathology,* 78: 1280-1283.
- VOLLER, A., BARLETT, A., BIDWELL, D.E., CLARK, D.E. and A.N. ADAMS (1976). The detection of the viruses by enzyme-linked immunosorbent assay (ELISA). *Journal of General Virology,* 33: 165-167.
- ZHANG, Y. P., KIRKPATRICK, B.C., SMART, C.D. and J.K. UYEMOTO (1998). CDNA cloning and molecular characterisation of cherry green ring mottle virus. *Journal of General Virology,* 79: 2279-2281.

Tab. 1. Viruses indicated in the certification protocols for EPPO countries to assess the sanitary status of stone fruit propogating material (Anonymous, 1992)

Acronym	Virus	Taxonomic Group	Availability of ELISA Kit
CGRMV	Cherry green ring mottle	*Foveavirus*	No
LChV	Little cherry	*Closterovirus*	No
ApMV	Apple mosaic	*Ilarvirus*	Yes
PDV	Prune dwarf	*Ilarvirus*	Yes
PNRSV	*Prunus* necrotic ringspot	*Ilarvirus*	Yes
ArMV	Arabis mosaic	*Nepovirus*	Yes
CLRV	Cherry leaf roll	*Nepovirus*	Yes
CRLV	Cherry rasp leaf	*Nepovirus*	Yes
MLRSV	Myrobalan latent ringspot	*Nepovirus*	Yes
RRSV	Raspberry ringspot	*Nepovirus*	Yes
SLRV	Strawberry latent ringspot	*Nepovirus*	Yes
TBRV	Tomato black ring	*Nepovirus*	Yes
TomRSV	Tomato ringspot	*Nepovirus*	Yes
PPV	Plum pox	*Potyvirus*	Yes
PeAMV	Petunia asteroid mosaic	*Tombusvirus*	No
ACLSV	Apple chlorotic leaf spot	*Trichovirus*	Yes

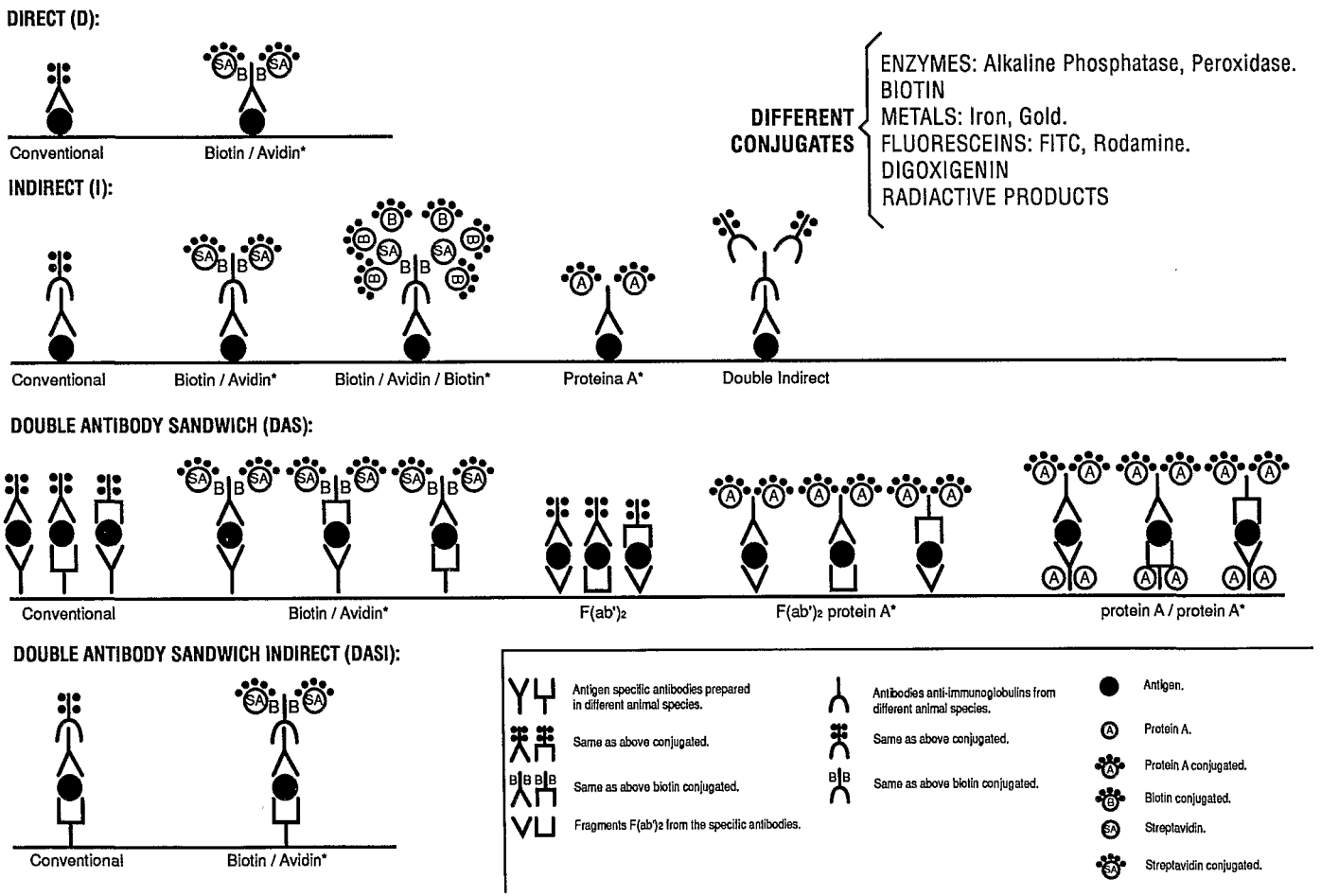

Fig. 1. Variants of ELISA mainly used for the diagnosis of stone fruit viruses (Cambra and Gorris, 1996).