Strategies to control virus and virus-like diseases: state of the art and perspectives for stone fruits

Dunez J.

in

Di Terlizzi B. (ed.), Myrta A. (ed.), Savino V. (ed.).
Stone fruit viruses and certification in the Mediterranean countries: problems and prospects

Bari : CIHEAM
Options Méditerranéennes : Série B. Etudes et Recherches; n. 19

1998
pages 73-79

Strategies to control virus and virus-like diseases: state of the art and perspectives for stone fruits

Jean DUNEZ
INRA - Station de Pathologie Végétale
Villenave d'Ornon Cédex (France)

SUMMARY - The application of sanitary selection procedures has contributed to a considerable improvement of the quality of fruit crops, mainly in the case of pathogens that are not naturally spread. In the Mediterranean region, two diseases cannot be easily controlled by the sanitary selection i.e. sharka, transmitted by aphids, and the apricot chlorotic leaf roll whose vector is still obscure. Breeding programmes for resistance to these two pathogens have been set up. The low number of natural resistance sources has led to approaches like interspecific hybridization. Remarkable results have been obtained through transgenosis: some plum and apricot lines resistant to sharka have been obtained. Transgenic plants, jointly with conventional plant breeding methods, may contribute to limit the impact of sharka on fruit production.

Key words: fruit trees, virus, phytoplasma, sanitary selection, resistance.

RESUME - *Là où elles ont été appliquées, les procédures de sélection sanitaire ont apporté une amélioration considérable et durable da la qualité de la production fruitière, notamment dans le cas de pathogènes non disséminés naturellement. En région méditerranéenne, deux maladies sont difficilement contrôlables par la simple sélection sanitaire, la sharka qui est transmise par les pucerons et l'enroulement chlorotique de l'abricotier dont le vecteur n'est pas encore identifié, mais qui se répand rapidement. Des programmes d'amélioration pour la résistance à ces deux pathogènes ont donc été mis en place. Ils sont limités par le très faible nombre de sources de résistance naturelle ce qui amène parfois à des approches lourdes comme l'hybridation interspécifique. Des résultats remarquables ont été récemment notés grâce à la transgénèse: des premières lignées de Prunier et d'Abricotier résistantes à la sharka ont été obtenues. La transgénèse pourrait ainsi contribuer, en complément des approches conventionelles d'amélioration des plantes, à limiter l'impact de la sharka sur la production fruitière.*

Mots-clés: *arbres fruitiers, virus, phytoplasmes, sélection sanitaire, résistance.*

I - Introduction

Fruit trees are susceptible to many viruses and virus-like agents (phytoplasmas, viroids and unidentified graft transmissible agents). In some cases, infection remains more or less symptomless but in others, very severe symptoms develop upon infection which can cause yield losses, make the crop unmarketable, or may have social and economical consequences resulting from the exclusion of certain fruit productions from traditional fruit tree growing areas.

In the years 1950-1960 when the first certification procedures were established, plant materials were generally infected with several viruses and virus-like agents. As a result of the vegetative propagation of infected fruit tree varieties and of several rootstocks, the levels of infection were very high and different disease agents distributed in most fruit tree growing areas. In addition, transportation on a long distance of infected fruit tree material had also contributed to the wide distribution of virus and virus-like agents.

The impact of the diseases results from the severity of the symptoms, the number of affected trees and of host plants and depends on the possibilities of natural spread of the causal agent. Strategies of control will depend on the spreading characteristics of the causal agent.

II - Viruses and virus-like agents which can be controlled by sanitation

During the last 30-40 years, virus and virus-like diseases of stone fruits have been studied intensively, many causal agents have been identified and their properties investigated. In particular, the vectors and the characteristics of natural transmission of several infectious agents have been described.

Some viruses, widespread in fruit trees 20-30 years ago, do not have any natural transmission or do not show any significant natural spread in the field. For instance, apple chlorotic leaf spot virus, the type member of the Trichovirus group is responsible for different symptoms (false plum pox, prune bark split, pear ring mosaic, graft incompatibilities in apricot, etc.), but no natural transmission has been described. Therefore the impact of the virus can be reduced, on the mid term, simply by sanitation, e.g. by the replacement of infected plants with virus-free plants.

In other cases, the situation is less clear because the characteristics of the virus or of the causal agent are not completely determined. Nevertheless, the impact of the several diseases (little cherry, cherry rusty mottle...) has been eliminated or, at least considerably reduced, by the use of virus-free material.

Similar results can be obtained with naturally transmitted pathogens as long as their vectors are absent. Tomato ring spot virus causes major diseases in North America on fruit trees (peach yellow bud mosaic, peach-plum stem pitting, apple union necrosis, etc.). It is naturally transmitted by a nematode, *Xiphinema americanum*, which is very common in North American soils but which is absent from European soils. Should virus infected material be introduced and planted in European conditions, it could be controlled by simple sanitation and elimination of virus infected plants once infection was detected.

Regarding nematode-borne viruses, important populations of nematodes are often observed in European and Mediterranean soils but very few of them are potential vectors of stone fruit viruses: the only significant exception is strawberry latent ring spot virus. Therefore most of the nematode-borne virus diseases (Nepovirus group) can be controlled only by sanitation.

Viruses of the Ilarvirus group (especially *Prunus* necrotic ring spot virus and prune dwarf virus) are responsible for severe diseases (decline of trees, reduction of yield and of fruit quality, reduction of bud uptake in the nursery). Those viruses are pollen-borne and the control of their natural transmission is impossible. Infection occurs during pollination e.g. when the trees are 4-6 years old and start flowering. Comparisons between plants initially infected at the time of plantation and pollen-infected plants showed a significantly reduced impact in the case of pollen infections on a 4-6 year-old tree. In most cases, the use of virus-free plants is an efficient tool to control the impact of Ilarviruses (Bernhard *et al.*, 1975). Those viruses are also seed borne. The percentage of seed infection varies with the species, it is usually low or very low in peach, higher in cherry (*Prunus avium*) and very high in *Prunus cerasus*. It means that sanitation should be extended to seed production.

III - Virus and virus-like agents which are naturally spread

Some major diseases of fruit trees are efficiently transmitted in nature and cannot be controlled by sanitation. This is the case of plum pox virus (PPV), a major aphid-borne virus disease and of apricot chlorotic leaf roll (ACLR) (European stone fruit yellows) (Diekmann and Putter, 1996), a phytoplasma-disease of increasing importance in the Mediterranean areas which is probably transmitted by leafhoppers.

For countries where such transmissible diseases have not been introduced, control can still be based on sanitation with a very severe control of plant introductions.

In areas where the disease has been detected but where it remains at a low level, it is sometimes possible to rely on prophylaxis with a rapid and severe eradication of the infected trees or orchards and use of virus-free plants.

When a high level of infection is observed, virus-free material planted in an infested environment can be infected within a few months and develop symptoms rapidly. Here sanitation also may not be sufficient to control virus spread.

Control of vectors has a limited effect on the spread of viruses such as PPV which are transmitted in the non-persistent manner with a cycle of acquisition and transmission completed in a few seconds or minutes. Usually the effect of the insecticide is not rapid enough to interrupt the cycle and prevent the aphid from transmitting the disease. Nevertheless, insecticide treatments should be recommended: as they reduce aphid population, they contribute to reduce the number of potential vectors, and do slow down virus spread.

In the case of ACLR, insecticide treatments during the growing season seem to slow down disease spread. Nevertheless, it is difficult to optimise the insecticide treatments as long as the vector is not identified.

The only possibility to control diseases which are naturally and efficiently spread is to use resistant varieties. In the absence of such varieties, different countries have used tolerant (e.g. symptomless infected) varieties. This strategy reduces the impact of the disease but contributes to increase the inoculum.

IV - Breeding for resistance

The impact of different viruses in several crops (cereals, potato, beet, vegetables, legumes) has been reduced by the use of resistant cultivars which have been obtained by crossing cultivars of agronomical interest with cultivars or species containing resistant genes.

In the field of stone fruit, the two major diseases concerned by a breeding program for resistance are PPV and ACLR. A common feature is the very limited number of resistant sources identified presently.

All known cultivars of peach and plum are susceptible to PPV and only 3 of the known cultivars of apricot are resistant (Syrgiannidis, 1980; Dosba *et al.*, 1992).

All the fruit tree species are susceptible to ACLR but the more severely affected species include all apricot and Japanese plum cultivars. Nevertheless, there are some differences in the susceptibility of the apricot cultivars but, since a precise quantification technique of the pathogen in the infected trees is not available, their degree of resistance is difficult to be evaluated. Breeding programmes are carried out with a few cvs. of apricot trees which do not express symptoms with ACLR infection.

Regarding PPV, breeding programmes are in progress. They are based on intraspecific crossings for apricot, on interspecific crossings with *P. davidiana* for peach and with clones of *P. cerasifera* for plum (Dosba *et al.*, 1994). Apricot hybrids resulting from these crossings are now under evaluation: some seem to show interesting characteristics.

Because of the paucity of natural resistance sources, pathogen-derived resistance strategies have been developed to produce artificial genes of resistance to PPV. They have been carried with plum and apricot. Several viral sequences have been cloned and several constructs have been transferred into plants. But the majority of transgenic plants which have been evaluated so far were transformed with the coat protein gene of PPV. The technique was first applied to herbaceous hosts of PPV such as *Nicotiana benthamiana* (Ravelonandro *et al.*, 1993), then extended to plum and apricot. Several lines of transformed plants have been tested: some of them recover after infection and a few appear to be immune after artificial inoculation, regardless of the type of inoculation used, chip budding or aphid transmission (Ravelonandro and Dunez, 1995; Ravelonandro *et al.*, 1997). Further evaluation of some of those lines, especially plum lines, is now carried out under natural conditions in European highly infested areas. Such field experiments will also contribute to evaluate the stability of that resistance.

Several questions have been asked about the safety of those transgenic plants. They concern the spread of resistant genes to other plants, and the possible effect of the transgene on the virus-vector specificity, and on consumer behaviour.

It has been experimentally demonstrated that the expression by a transgenic plant of the coat protein of a naturally transmissible PPV isolate can interfere with the recognition of an infecting virus by aphids because of the heteroencapsidation of the infecting viral RNA with the coat protein expressed from the viral genome (Lecoq *et al.*, 1993). Such risk can be eliminated by using viral sequences deleted or modified which cannot be encapsidated or recognised by aphids. In addition, in most of the resistant plants, especially those which appear to be immune, virus coat protein cannot be detected in the plant, thus reducing the risk of heteroencapsidation (Jacquet *et al.*, 1998).

Another risk is the spread of resistant genes by pollen. This raises the question of the possible evolution of PPV strains under the pressure of an increasing number of resistant plants and of their capacity to overcome resistant genes. Such problem is not specific to transgenic plants but can also occur with natural plant genes of resistance. The stability of resistant sources is questioned and programmes based on the use of a combination of resistant sources are required.

The last question will deal with the acceptability of transgenic fruits by the consumers. No clear reply can be made except that, in highly infested countries, infected fruits are already used or eaten by consumers.

For phytoplasmas, especially ACLR, such pathogen-derived resistant strategies have not been developed. Research is now being done to evaluate the potentialities of a plantibody strategy. The technique has been already developed for a few viruses and is now under investigation for the phytoplasma associated with tomato stolbur disease. The technique consists of producing monoclonal antibodies against the pathogen, then in constructing a synthetic gene encoding the variable regions of one monoclonal antibody selected for a clear interference with the pathogen. As the pathogen is phloem limited, the synthetic gene should be under the control of a specific promoter before its transfer into the plant genome. Encouraging results have been reported with a few viruses and very preliminary results obtained with stolbur. However, no significant conclusions can be drawn regarding the general potential of this technique for protection against phytoplasmas.

V - Conclusions and perspectives

Strategies to be developed for the control of virus and virus-like agents depend on the existence and type of their natural transmission. Many virus and virus-like agents are not naturally transmissible or do not have efficient vectors and therefore can be eliminated by sanitation. Sanitation procedures have been applied to various crops and have shown to be very effective in the control of virus diseases including some viruses which spread naturally.

Because of their rapid and natural spread under the European and Mediterranean conditions, PPV and ACLR can not be efficiently controlled by sanitation.

PPV is transmitted by several aphid species common in all fruit growing areas. As it is transmitted in a not persistent manner, aphid control by insecticide application has a limited efficiency. Nevertheless, it can contribute to slowing down disease spread and therefore should not be neglected.

The presence of a phytoplasma has been consistently associated with ACLR but, so far, despite intensive investigations the vector has not been identified. Therefore, as the vector and alternate host plants (except apricot and some other *Prunus* species) are not known, insecticide application can not be adapted to the biology of vector, as done with other phytoplasma diseases (such as flavescence dorée of grapevine). Insecticide usage remains of limited effect.

Breeding for resistance appears to be the only way to control both diseases efficiently. Unfortunately, natural sources of resistance are rare. So in the case of ACLR, breeding programmes are only aimed at the production of tolerant plants developing faint or expressing no symptoms upon infection. In the case of PPV, some breeding programmes need interspecific crosses to introduce natural resistant genes.

In the last twenty years, pathogen-derived resistance has been effective in the control of different virus diseases. Several transgenic Plum and Apricot lines expressing PPV genome sequences especially the coat protein gene have been obtained. Some show a high degree of resistance or immunity after artificial inoculation by grafting or by aphids. They are now being evaluated under field conditions.

Even if those strategies appear to be efficient and acceptable in terms of safety it is clear they will not replace more conventional strategies. Therefore, the control of virus and virus-like diseases of fruit trees will be based, in the future, on a combination of sanitation, conventional breeding techniques and genetic engineering. Development of such programmes need not only to make these technologies available in the stations in charge of virus control but also to build and to maintain carefully a virus-free stock of cultivars and plant origins usable for sanitation and as genetic resources in breeding programmes.

References

- BERNHARD, R., SAUNIER, R. and C. MARENAUD (1975). Evolution et performance d'un clone sanitaire de pêcher issu de thermothérapie après huit années d'observation. *Acta Horticulturae*, 44: 69-79.
- DIEKMANN, M. and C.A.J. PUTTER (1996). European stone fruit yellows. *In*: Stone Fruits. FAO/IPGRI Technical Guidelines for the Safe Movement of Germoplasm. N° 16. FAO Publication: 51-53.
- DOSBA, F., LANSAC, M., EYQUARD, J.P., BONNET, A. and G. SALESSES (1994). Behaviour towards PPV of *Prunus* interspecific hybrids and plum varieties. *Acta Horticulturae*, 309: 136-144.
- DOSBA, F., ORLIAC, S., DUTRANNOY, F., MAISON, P., MASSONIE, G. and J.M. AUDERGON (1992). Evaluation of resistance to plum pox virus in apricot trees. *Acta Horticulturae*, 309: 211-220.
- JACQUET, C., DELECOLLE, B., LECOQ, H., DUNEZ, J. and M. RAVELONANDRO (1998). Use of modified plum pox virus coat protein genes developed to limit heteroencapsidation-associated risks in transgenic plants. *Journal of General Virology*, 79: 1509-1517.
- LECOQ, H., RAVELONANDRO, M., WIPF-SCHEIBEL, MONSION, M., RACCAH, B. and J. DUNEZ (1993). Aphid transmission of a non transmissible strain of zucchini yellow mosaic Potyvirus from transgenic plants expressing the capsid protein of Plum Pox Potyvirus. *Molecular Plant-Microbe Interactions*, 6: 403-406.
- RAVELONANDRO, M., MONSION, M., DELBOS, R.P. and J. DUNEZ (1993). Variabile resistance to plum pox virus and potato virus Y infection in transgenic Nicotiana plants expressing plum pox virus coat protein. *Plant Science*, 91: 157-169.
- RAVELONANDRO, M. and J. DUNEZ (1995). Récents progrès dans la lutte contre la sharka des arbres fruitiers à noyau. *Cahiers Agriculture*, 4: 153-161.
- RAVELONANDRO, M., SCORZA, R., BACHELIER, J.C., LABONNE, G., LEVY, L., DAMSTEEGT, V., CALLAHAN, A.M. and J. DUNEZ (1997). Resistance of transgenic plums (*Prunus domestica* L.) to plum pox virus infection. *Plant Disease*, 81: 1231-1235.
- SYRGIANNIDIS, G. (1980). Selection of two apricot varieties resistant to sharka virus. *Acta Phytopathologica*, 15: 85-88.