CONTROL OF PLANT VIRUS DISEASE BY PREIMMUNIZATION

ABSTRACT

Preimmunization is the technique used to control the damage caused by most severe strains of virus or complex of the same virus in the susceptible host. The technique has been widely used and demonstrated as an alternative for management of virus diseases in various crops. This review focuses on the requisite conditions for using this technique, mandatory steps involved in employing the preimmunization program, examples of preimmunization in various crops/viruses and the experiences in various countries in employing this technique. The success stories of preimmunization program adopted in various countries or its failure have also been discussed, besides the risks and advantages of this technology.

INTRODUCTION

Preimmunization (cross protection), the activity of a virus in a plant preventing the expression of a subsequent challenge virus was first reported by Wingard (1928), for Tobacco ringspot virus and McKinney (1929), for Tobacco mosaic virus. Although cross protection is the universal name, this phenomenon has also been called acquired immunity, acquired tolerance, cross immunization, mutual antagonism and premunity (Bennet 1953). In Brazil, the term preimmunization has been widely adopted by several investigators, as well as in Australia, South Africa and Japan.

Utilization of preimmunization as an alternative for virus disease control was first proposed by Salaman (1937) and Jonhson (1937), on two independent works. Yet, more than sixty years later, preimmunization has been experimentally demonstrated for several viruses, but it has been used for controlling only a few plant virus diseases in the field.

Preimmunization has not only been observed to occur between strains of the same virus. Koizume and Sasaki (1980) reported protection against Citrus tristeza virus (CTV) from Citrus vein enation virus. It was also reported to occur between viroids (Nibblett et al. 1978). Satellite RNA Cucumber mosaic virus (CMV), that ameliorate symptoms caused by the virus, can also be used for preimmunization (Tien et al. 1987).

This review will briefly focus on methods used for searching protective mild strains, examples in which preimmunization has proved effective or failure for virus disease control and comments on risks and advantages of this technology. As any sig-
nificant progress has not been achieved in the last 10 years on the mechanisms of preimmununization, information on this subject can be obtained in the reviews by Fulton (1986), Sherwood (1987), Urban et al. (1989) and Rezende & Müller (1995).

Pre-requisites for the employment of preimmunization.

According to Posnette & Todd (1955), the utilization of mild strains to protect the plants against the normal complex shall only be implemented when the following pre-requisites can be fulfilled: (a) the disease is endemic and, due to any reason, political or economically, impossible to be eradicated; (b) the disease disseminates rapidly, putting in risk the new plantings, even when the older plantings that are infected have already been eliminated; (c) the losses with the disease are so large that even a small reduction caused by the infection with the mild strains is the preferable alternative and (d) as sine qua non condition, enough evidence exists that the mild strain will efficiently protect the preimmunized plants, without inducing great losses itself. Besides these, Fletcher (1978) adds other pre-requisites that shall be considered in a later step of implementation of the commercial use of the method. They are: a) the mild strain shall be easily transmitted, in such a manner that large scale inoculations are efficient and of low cost; b) there shall be availability of enough amount of the mild strain inoculum; c) the mild strain shall be easily purified and stored in the case in which great number of plants of annual crops shall be inoculated; d) if the crop is vegetatively propagated, a great number of mother plants already infected with the mild strain shall be available to the growers; e) in case in which the preimmunization inoculation is carried out by means of aerosol under pressure, this should not offer risk to the workman. Furthermore, there are some traits which a mild strain should have to be a candidate for preimmunization: 1) the strain has to be mild in all cultivar combinations it is thought for; 2) it should be stable and not prone to change or mutation; 3) the strain must be well distributed in all parts of the plant and must move quickly through new growth; 4) the mild strain should be easily graft transmissible for plants propagated on this way; and 5) subinoculations from the original mild strain shall remain stable.

Steps for a preimmunization program.

A research program that aims the establishment of preimmunization as control measure of phytoviruses may be divided into five linked steps: a) selection of mild strains; b) assay of the protective value of the mild strains under greenhouse conditions; c) assay of the protective value through field pilot experiments; d) evaluation of the stability of the mild strains and its effect upon the development of the plants, yield and quality of the product; and e) integration of the preimmunization technique into the system of crop management. All these steps obey logical sequence and each one shall be carefully evaluated before the investigation of the following step.

The selection of stable mild strains is the crucial step, and apparently the most difficult, in such a program. It can be carried out by means of different methods, being that some of them may be used single or in combinations with the aim of increasing the chance of obtaining the desired strains. Some of these methods are: a) selection of outstanding plants in severely affected crop (Müller & Costa 1987, Posnette & Todd 1955); b) selection of mild strains from atypical areas on leaves with severe systemic symptoms (Rezende et al. 1982); c) selection of mild or attenuated strains from plants submitted to thermal treatments (Desjardins 1959, Müller & Costa 1973, Kosaka & Fukunishi 1993); d) selection of strains by filtration (passage) through alternative hosts (Johnson 1947, Yeh & Cheng 1989); e) selection of mild strains by means of vectors (Müller & Costa 1973); f) selection of mild strains through the use of mutagenic agents (Rast 1972, Yeh & Gonsalves 1984); and g) construction of attenuated strains through the technique of DNA recombination (Holt et al. 1990).

After selection and perfect characterization of the mild strains of a virus, all other steps should be carefully evaluated. Once the practical and economical viability of preimmunization is established as adequate and safe for control of the virus disease, adequate forms of multiplication and maintenance of the mild strains inocula shall be studied, as well as efficient and economical methods for mass inoculation of plants, in such a way that the demand for this technology can be satisfactorily attended.

Examples of preimmunization.

The pioneer example on the efficiency of preimmunization with mild strains for the control of
virus disease was reported by Crowdy & Posnette (1947) and Posnette & Todd (1955), in Africa, in experiments for the control of cocoa swollen shoot. In spite of this experimental success, the program for cocoa preimmunization was interrupted for several years, for unknown reasons. Presently, no preimmunized cocoa plant is commercially grown in Ghana (LAA Ollenu, personal information).

The second pioneer work on the efficiency of preimmunization for plant virus disease control was developed by Simonds (1959), in Australia, for the control of *Passion fruit woodiness virus* (PWV).

Preimmunization was very efficient and popular for the control of *Tomato mosaic virus* (ToMV) on tomato in several European countries in the 1970s (Rast 1975, Fletcher 1978), but it was discontinued due to development of resistant cultivars of good agronomic quality. However, as stated by Channon et al. (1978), mild strain protection may still be useful for the tomato industry in the future, if strains able to overcome resistant genes evolve from the virus population.

The largest commercial application of preimmunization was achieved in Brazil for the control of *Citrus tristeza virus* (CTV), especially on the ‘Pera’ sweet orange (*C. sinensis* Osb.) growing on tolerant rootstocks. Previous work carried out had established the existence of mild CTV strains with protective effect. A cooperative research project funded by US Public Law 4-80 started in 1961, aiming to control CTV injury to commercial citrus types by preimmunization (Costa & Müller 1980). Release of the best preimmunized Pera sweet orange clone, started 35 years ago, and led to its rapid increase by growers. Experiments carried out along these more than 30 years in the State of São Paulo and other states of Brazil, as well as comparisons carried out by growers, showed that the preimmunized ‘Pera’ clone has been superior to other existing clones. Large-scale propagation of preimmunized ‘Pera’ has revealed almost no breakdown in protection in successive clone generations. Presently, some 80 millions trees descend from the original preimmunized ‘Pera’ material attest their satisfactorily behavior. More recently however, in a few cases, orchards formed with budwood from preimmunized trees have a great number of plants (30%) showing severe CTV symptoms. This could be due to an exhaustion of the protective strain after all this years or due to the arising of new severe strains able to supplant it. Studies are now under-way with the help of molecular biology, to investigate the reasons of the breakdown in protection and the possibility to find new mild strains that may continue to protect efficiently the ‘Pera’ sweet orange orchards, mainly in the Southern part of the State of São Paulo where the Capão Bonito complex occurs. Considering the good results obtained along these last three decades, this is perfectly feasible (Müller et al. 2000, Müller & Carvalho 2001).

Preimmunization has also been used to protect grapefruit trees in the Australian Citrus Budwood Scheme against stem pitting in different climatic conditions for the last 30 years (Broadbent et al. 1991, 1995).

In South Africa, in the mid 1950’s, Joubert, an extension officer, discovered a 30 year old planting of grapefruit trees on trifoliate orange (*Poncirus trifoliata* Raf), on the ’Nartia’ farm in the Western Cape, showing only mild stem pitting (Lee et al. 1992). Good results are still being obtained with this selection. The mild CTV source infecting the ’Nartia’ was named GFMS-12, and was used extensively to inoculate all commercial citrus propagation for many years (von Broembsen 1988). Presently, the GFMS-12 is kept as the protective strain for white grapefruit and pummelos, GFMS- 35 replaced GFMS-12 for all red grapefruit and, LMS- 6, a mild strain selected from acid lime replaced GFMS- 12 in all sweet oranges and mandarin types (clementines and satsumas) and limes (Roistacher 2001).

In Peru, native budlines of ‘Washington navel’ and Mexican (Key) lime preimmunized with protective mild strains introduced from California have been planted with commercial success since the early 1990’s (Bederski & Roistacher 2001). Washington Navel and Mexican lime production in coastal areas of Peru has increased significantly (Bederski, personal communication).

Papaya ringspot, caused by *Papaya ringspot virus* – type P (PRSV-P), is the most destructive disease for papaya crops in several tropical and subtropical countries, for which preimmunization was studied as an alternative for control. Investigations for the use of this technique for the control of PRSV-P initiated in the 1980’s, through independent studied in Taiwan, Brazil and USA (Lin 1980, Rezende et al. 1981, Yeh & Gonsalves 1984). Besides many efforts toward the finding of very protective and stable mild strains of the virus, the results obtained were not consistent; mainly due to problems associated with the occurrence of severe
revertants from the mild strains and breakdown of protection under severe disease pressure (Rezende & Costa 1987, Yeh & Gonsalves 1994). Development of transgenic resistant lines of papaya has apparently replaced mild strain protection in Hawaii (Manshardt 1998). Field evaluation of transgenic resistant papaya is also underway in Brazil (Souza & Gonsalves 1999).

Preimmunization has also proved very effective for the control of the two most important virus diseases affecting cucumber crops worldwide, which are caused by Papaya ringspot virus – type W (PRSV-W) and Zucchini yellow mosaic virus (ZYMV). Two mild strains of PRSV-W, selected by Rezende et al. (1994) in Brazil, effectively protected plants of zucchini squash (Cucurbita pepo L.), long neck squash (C. moschata Duch. ex Poir. cv Menina Brasileira), and hybrid squash type Tetsukabuto (C. maxima Duch. ex Lam x C. moschata ‘Takayama’) against the effects of the severe strains present in the field and allowed the yield of good quality fruits (Rezende & Pacheco 1998, Rezende et al. 1999, Dias & Rezende 2000).

Studies for the control of ZYMV by preimmunization was apparently first proposed by Lecoq et al. (1991) in France, after selection of a protective mild strain named ZYMV-WK. This strain was later introduced to Taiwan (Wang et al. 1991) and UK (Walkey et al. 1992) and provided a very effective protection on field tests with zucchini squash. Preimmunization for the control of zucchini yellow mosaic has also been experimentally tested in California, USA, with promising results (Perring et al. 1995). Commercial use of preimmunization for the control of zucchini yellow mosaic has been reported in Hawaii (Cho et al. 1992) and Israel (Yarden et al. 2000). In Israel, this technology has been applied in the last 5 years for protecting zucchini squash, squash, melon and watermelon crops. In 1999, almost 800 ha were planted with plants of these species protected with a mild strain of ZYMV.

In Brazil, double inoculation with the mild strain PRSV-W-1, and a recently selected mild strain of ZYMV, named ZYMV-M was successfully tested in zucchini squash plants under greenhouse and field conditions (Rabelo & Rezende 2001, 2004). Due to the good performance and the stability of these mild strains, commercial practical application of double preimmunization for the control of PRSV-W and ZYMV on zucchini squash and longneck squash has been evaluated in a cooperative project between the ESALQ/USP, Piracicaba, SP and SAKATA Seed Sudamerica Ltda, Brazil.

Control of Cucumber mosaic virus (CMV) using satellite-mediated protection is another example which has been investigated extensively in China (Tien et al. 1987, Wu et al. 1989, Tien & Wu 1991); Japan (Yoshida et al. 1985, Sayama et al. 1993), the United States (Montasser et al. 1991) and Italy (Galitelli et al. 1991), and it has proved useful in protecting tomato and pepper plants from CMV infection. In spite of the efficiency of this method, it has not been widely put to practical use, except in China where satellite-mediated protection has been applied successfully in pepper crop (Tien & Wu 1991) and in Japan, where 1.5 to 2.0 million of protected pepper seedlings were sold in 1998 by NDM company (Dr. JM Kaper, personal communication).

Concluding remarks.

Even though the phenomenon of preimmunization has been known for more than 70 years, there are still very few examples in which this technology has been commercially applied for the control of virus diseases. Part of the reduced use of preimmunization may be due to the fact that, for many years, this method was only thought as viable for perennial crops or annual crops propagated by means of tubers, bulbs, etc. This method was considered time consuming and expensive for annual crops propagated by seeds, in which inoculation of seedlings would be repeated every crop season. However, the last two decades have experienced several changes on agricultural practices for several species of annual crops, including the mass production of seedlings. This practice has facilitated the use of preimmunization for the control of virus diseases on cucurbit crops, for which seedlings were not produced in the past years. Mass inoculation of seedlings can be easily achieved with a paint spray gun attached to an air compressor (Rezende & Pacheco 1998, Dias & Rezende 2000) or with automatic inoculation equipment as proposed by Yarden et al. (2000) in Israel.

Other reasons for the reduced use of preimmunization may be related to the risks that deliberate distribution of the mild strain of a virus may cause to the protected crop and, specially, to non-target crops. These include the stability of the mild strain in the target crop, the longevity of the protection offered by the mild strain in the target crop, the damage that a mild strain may cause to
nontarget crop and the occurrence of synergistic interaction of the mild strain with unrelated viruses or other pathogens.

Although all these risks should be considered in any program of preimmunization, they should not be used as arguments to prevent studies and practical application of this technology. Preimmunization has several advantages that may overcome the risks. First, it can be included in any integrated pest management program. Second, can be applied to cultivars resistant to other diseases caused by virus or other pathogen, which were obtained by classical plant breeding or genetic transformation (transgenic). Third, preimmunization does not pollute, apparently does not represent any risk to the growers and consumers, does not interfere with cultural practices, it is simple to apply and may have a reasonable cost/benefit. Finally, as mentioned by Dodds (1999), preimmunization can fill a technological gap, while waiting for transgenic plants to arrive, and can be deployed again should new technologies fail.

REFERENCES


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