EFFECT OF TEMPERATURE ON PLUM POX VIRUS INFECTION

M. GLASA[1,*], G. LABONNE[2], J.-B. QUIOT[2]

[1]Department of Plant Viruses, Institute of Virology, Slovak Academy of Sciences,
Dúbravská cesta 9, 845 05 Bratislava, Slovak Republic;
[2]UMR Biologie et Génétique des Interactions Plante-Parasite pour la Protection Intégrée,
INRA-ENSAM, Montpellier, France

Received February 10, 2003; accepted March 3, 2003

Summary. – One of the key factors of progress of an epidemic is the duration of virus availability for a vector in plants, which could be influenced by temperature. Using five epidemiologically different isolates of Plum pox virus (PPV) we studied the effect of temperature on the virus infectivity, intensity of disease symptoms and virus accumulation in *Nicotiana benthamiana* plants as determined by a double-antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA). No differences in infectivity and intensity of disease symptoms between the five isolates were observed at 17°C. However, they differed in their capacity to infect and multiply in the plant at higher temperatures. The temperature of 32°C was inhibitory to the multiplication of all the five PPV isolates studied. Fewer plants were infected and a significantly decreased amount of virus antigen was detected at 30°C. The natural PPV recombinant BOR-3 isolate showed a greater temperature tolerance compared to other PPV isolates tested. We conclude that adaptation to higher temperatures may favour the epidemiological impact of PPV.

Key words: sharka; potyvirus; effect of temperature; ELISA; epidemiology

Introduction

Insect-borne virus disease epidemiology depends upon interactions among viruses, vectors, plants and environment. Temperature is a significant factor capable of influencing the disease process in virus-infected plants. A temperature increase can enhance the amount of available virus in the plant (e.g. Potato virus Y (de Bokx *et al*., 1978)), as well as diminish it (e.g. Red clover mottle virus (Musil and Lešková, 1969), thermosensitive strains of Cucumber mosaic virus (Walkey, 1976), and Bean yellow mosaic virus (Tu, 1989)).

PPV is the agent responsible for sharka, the world-wide detrimental disease of stone fruits (*Prunus* spp.). The virus is naturally transmitted by aphids in a non-persistent manner (Kunze and Krczal, 1971; Labonne *et al*., 1994). Two main subgroups of PPV isolates, namely PPV-M and PPV-D, were differentiated on the basis of distinct serological and molecular properties (Candresse *et al*., 1998; Lopez-Moya *et al*., 2000; Glasa *et al*., 2002). The effect of temperature on PPV infection is still less documented. Under field conditions, the symptoms on *Prunus* plants infected with PPV are often masked late in the season (Németh, 1986). A possible explanation of this observation could be that high summer temperatures decrease the virus amount in the plant and that the latter correlates with symptoms disappearance.

The objective of this study was to determine whether the infection with geographically and epidemiologically distinct PPV isolates differs at different temperatures.

Materials and Methods

Virus isolates. Five natural PPV isolates differing in their geographical origin, original host and subgroup affiliation were studied (Table 1). The isolates were pre-multiplied in *N. benthamiana,* the systemically infected leaves were stored at -80°C in order to use a similar inoculum in the experiments.

*E-mail: virumig@savba.sk; fax: +4212-54774284.

Abbreviations: PPV = Plum pox virus; DAS-ELISA = double-antibody sandwich-enzyme-linked immunosorbent assay; PBS = phosphate-buffered saline; p.i. = post inoculation

Table 1. PPV isolates tested

Isolate	Country of origin	Original host	PPV subgroup
BOR-1	Slovakia	Plum	D
BOR-3	Slovakia	Apricot	natural D/M recombinant
VAR-2	Slovakia	Peach	M
92.011	France	Peach	D
91.003	France	Nectarine	M

Mechanical inoculation and growth conditions. Three fully expanded leaves of *N. benthamiana* were mechanically inoculated with a virus-containing sap (1/15 dilution in 0.03 mol/l Na_2HPO_4 supplemented with 0.2% Na-diethyldithiocarbamate. For each experiment, a homogeneous group of 20 plants grown singly in pots was inoculated per isolate. Immediately after inoculation the groups were each divided into two lots of 10 plants. The lots were then placed in two growth chambers operating at constant temperature. Experiment I was carried out at 17°C and 32 ± 1°C, respectively, while Experiments II and III were performed at 17°C and 30°C, respectively. Mock- and non-inoculated plants were used as negative controls. Plants were grown under a 16 hrs day/8 hrs night photoperiod, 13,000 lux luminosity and 80 ± 10% humidity.

DAS-ELISA. The plants were tested individually for PPV antigen by DAS-ELISA (Clark and Adams, 1977) 21 days post inoculation (p.i.) using the polyclonal antibody V.196 produced in INRA-ENSAM, Montpelier, France. Hereby, virus antigen accumulation in systemically infected leaves of each plant was estimated by DAS-ELISA and the data were compared for each isolate. Five leaf discs of 0.15 g were taken from 5 upper leaves of each of the inoculated plants and uninfected control plants. The discs were homogenized in PBS (1/25 w/v) containing 0.05% Tween-20 and 2% polyvinylpyrrolidone 40. All the samples corresponding to a given isolate were applied in quadruplicate to a single plate (NUNC Polysorp), so that absorbance values measured at 405 nm (A) for single isolate and several temperatures and plants could be compared. As each isolate was analyzed in this way independently, it was not possible to compare directly A values for different isolates.

Statistical analysis. The mean A value for the 4 wells corresponding to one sample was calculated and used as the value for the sample. Then the Student's t test was used to assess significance of differences in the effects of different temperatures on the amount of virus antigen for each PPV isolate. Namely, the effects of 17°C and 30°C were compared (experiments II and III); only plants considered as infected were evaluated. A weighted measure (D) was used to obtain a representation of the difference between the mean A values at 17°C (A_{17}) and at 30°C (A_{30}) and to compare different isolates on the same scale. We used the difference between A_{17} and A_{30} for the infected plants weighted by the mean A value at both temperatures:

$$D = (A_{17} - A_{30})/[(A_{17} + A_{30})/2]$$

As the A values were measured on a scale from 0 to 2, D could range between the limits of –2 (if A_{30} is 2 and A_{17} close to 0) and +2 (in the inverse case).

Results

Experiment I

Whatever the isolate, the symptoms on inoculated *N. benthamiana* plants kept at 17°C appeared first on about day 9 p.i. The totals of plants were considered infected for all the isolates based on visual observation and DAS-ELISA on day 21 p.i. The infection was pronounced through intensive mosaic and leaf deformations. On the other hand, no symptoms were observed on virus-inoculated tobacco plants kept at 32°C. Only 1 of 10 plants inoculated with the BOR-3 isolate was detected by DAS-ELISA as infected (Table 2). However, the A value was about 15 times lower than that for the infected plants kept at 17°C (data not shown). To reveal a possible subliminal infection, the plants giving negative results in DAS-ELISA were cut, shifted to a growth chamber with a temperature of 25/18°C (day/night) for 14 days and then retested by DAS-ELISA. No additional infection was detected.

Table 2. Evaluation of the infectivity of PPV isolates at different constant temperatures

Isolate	Experiment I		Experiment II		Experiment III	
	17°C	32°C	17°C	30°C	17°C	30°C
BOR-1	10/10	0/10	10/10	8/10	10/10	5/10
BOR-3	10/10	1/10	10/10	10/10	10/10	10/10
VAR-2	10/10	0/10	10/10	2/10	10/10	1/10
92.011	10/10	0/10	10/10	6/10	10/10	5/10
91.003	10/10	0/10	10/10	9/10	10/10	4/10

Experiments II and III

Different rates of infection were observed on the infected plants held at 30°C depending on the isolate (Table 2). In both experiments, only the BOR-3 isolate infected all the 10 plants at 30°C. The VAR-2 isolate infected only 1 and 2 of 10 plants, respectively. Fairly variable results were obtained with the 91.003 isolate (4 and 9 plants infected, respectively).

The symptoms on infected tobacco plants grown at 30°C developed on about days 10–11 p.i. The symptoms were milder in comparison with those on plants maintained at 17°C (only light chlorosis without leaf deformations appeared). There were no differences in symptom expression and intensity among the isolates BOR-1, VAR-2, 91.003 and 92.011. An exception was the BOR-3 isolate inducing only weak chlorosis or even no symptoms.

To estimate the effect of temperature on virus accumulation in systemically infected leaves of *N. benthamiana* plants maintained at two different constant temperatures, the plants were tested by DAS-ELISA and the data were compared for each isolate. In both experiments a significant differences between A values could be detected for the isolates BOR-1,

BOR-3, VAR-2 and 92.011. The results revealed a lower amount of virus antigen in systemically infected leaves of the plants maintained at 30°C than at 17°C (P <0.001). The only exception was the 91.003 isolate in experiment III for which this difference was not significant (Fig. 1).

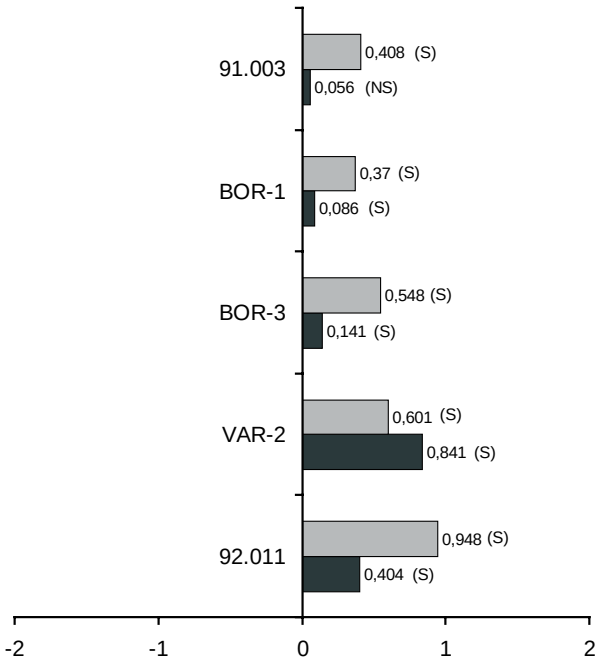

Fig. 1
Weighted mean differences (D) between A values for the plants kept at 17°C and 30°C in the experiments II and III for the five PPV isolates tested

S/NS = significant/non-significant difference between A values for the infected plants maintained at 17°C and 30°C. Abscissa: the scale of D values. Ordinate: D values (right side) for individual PPV isolates (left side). Experiments II (grey columns) and III (black columns).

Discussion

One of the key factors of progress of an epidemic is the duration of virus availability for a vector in plants. The temperature could modify the evolution of the attainability of virus throughout the vegetation period (Kassanis, 1957). In this work the effect of temperature on PPV infectivity and accumulation in an artificial host was studied using DAS-ELISA. The latter is a reliable method for a semiquantitative measurement of the amount of virus antigen in a plant. A correlation between the virus titer in plants and the success of acquisition by aphids is well known for non-persistently transmitted viruses (de Bokx *et al*., 1978; Labonne *et al*., 1997).

The present analysis included five PPV isolates from two geographical areas, France and Slovakia, characterized by different climate – Mediterranean and continental, respectively. The tested isolates belonged to the two main PPV subgroups, M and D. A recently discovered natural PPV-D/M homologous recombinant (Glasa *et al*., 2001) was included too.

This study clearly demonstrated that although no distinct differences in infectivity and intensity of symptoms could be detected in the five isolates tested at 17°C, they differed in the infectivity and multiplication in a herbaceous host at higher temperatures. The temperature of 32°C (experiment I) was inhibitory to the multiplication of all the five PPV isolates. Fewer plants were infected and lower amounts of virus antigen were detected at 30°C. This observation suggests two different mechanisms affecting virus infection. The first mechanism could affect first steps of viral RNA replication in the cells and/or cell-to-cell movement just after inoculation. Based on the presented experiments, the BOR-3 isolate seemed less sensitive than the others to this mechanism. By the second mechanism, virus accumulation in the plant could be affected, either by increasing its degradation rate or by decreasing its replication rate. All the isolates seemed sensitive to this mechanism, as the estimated amount of virus antigen at 30°C was lower than that at 17°C for all the five isolates and experiments (Fig. 1).

The different behavior of the PPV isolates in *N. benthamiana* at 30°C, however, had no correlation with their geographical origin. The absence of clear-cut geographical groups within PPV, ascertained also by the phylogenetic studies (Glasa *et al*., 2002), could be explained by an intensive exchange of biological PPV propagation material within the European countries in the past and easy transmission of PPV by vegetative material. As well, no differences between PPV-M and PPV-D isolates could be noted in our experiments. Reactions were rather isolate-specific than subgroup-specific.

Virus concentration did not appear to correlate directly with symptom severity. For instance, weak symptoms or latent reaction was observed in plants infected with BOR-3 and maintained at 30°C despite a high virus antigen level detected by DAS-ELISA.

Recombination is considered to play an important role in RNA virus evolution and it is generally thought to provide to a recombinant virus some selective advantage (Aaziz and Tepfer, 1999). The determined temperature-tolerant behavior of the BOR-3 isolate compared to other isolates tested cannot be, however, definitively linked to its recombinant character.

In general, adaptation to higher temperatures may favor the epidemiological impact of a PPV isolate in the presence of aphids and sensitive hosts during the warm period of the growing season. In Mediterranean areas, a peak of the aphid flight activity can be observed in the spring (Labonne *et al*., 1994). At that time, temperature values do not seem high

enough to result in a significant effect on virus availability to aphids. On the contrary, the flight activity of aphids in Central European countries could appear later in the season (Weismann, 1966; Kuroli, 1999), when temperatures frequently reaching 30°C or more are common. We conclude that the PPV isolates, whose infectivity and accumulation in host plants are less sensitive to a temperature increase, may have an advantage over the other isolates in their dissemination by aphids.

Acknowledgement. The authors are grateful to Mr M. Boeglin, INRA-ENSAM, Montpelier, France for technical assistance. The work was supported by the INRA-DADP/Région Languedoc-Roussillon grants and in part by the grant APVT-51-011802 from the Slovak Science and Technology Assistance Agency (APVT).

References

Aaziz R, Tepfer M (1999): Recombination in RNA viruses and in virus-resistant transgenic plants. *J. Gen. Virol*. **80**, 1339–1346.

Candresse T, Cambra M, Dallot S, Lanneau M, Asensio M, Gorris MT, Revers F, G. Macquaire G, Olmos A, Boscia D, Quiot JB, Dunez J (1998): Comparison of monoclonal antibodies and polymerase chain reaction assays for the typing of isolates belonging to the M and D serotypes of plum pox potyvirus. *Phytopathology* **88**, 198–204.

Clark MF, Adams AN (1977): Characteristics of the microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. *J. Gen. Virol*. **34**, 475–483.

de Bokx JA, van Hoof HA, Piron PGM (1978): Relation between concentration of potato virus Y and its availability to Myzus persicae. *Neth. J. Pl. Pathol*. **84**, 95–100.

Glasa M, Kúdela O, Marie-Jeanne V, Quiot JB (2001): Evidence of a naturally occurring recombinant isolate of Plum pox virus from Slovakia. *Plant Dis*. **85**, 920.

Glasa M, Marie-Jeanne V, Moury B, Kúdela O, Quiot JB (2002): Molecular variability of the P3-6K1 genomic region among geographically and biologically distinct isolates of Plum pox virus. *Arch. Virol*. **147**, 563–575.

Kassanis B (1957): Effect of changing temperature on plant virus diseases. *Adv. Virus Res*. **IV**, 221–241.

Kunze L, Krczal H (1971): Transmission of sharka virus by aphids. *Ann. Phytopathol*. HS, 255–260.

Kuroli G (1999): Swarming of aphids and changes in the number found on potato. *Novenytermeles* **48**, 153–166 (in Hungarian).

Labonne G, Yvon M, Quiot JB (1994): Dissemination du plum pox potyvirus par les pucerons: analyse des vecteurs potentiels du virus dans un verger d'abricotiers. *EPPO Bull*. **24**, 681–685.

Labonne G, Quiot JB, Yvon M, Quiot-Douine L (1997): Immunoprinting: a quick and reliable method to measure the efficiency of plants as virus sources for aphids in field epidemiology. *J. Plant Pathol*. **79**, 135–141.

Lopez-Moya JJ, Fernandez-Fernandez MR, Cambra M, Garcia JA (2000): Biotechnological aspects of plum pox virus. *J. Biotechnol*. **76**, 121–136.

Musil M, Lešková O (1969): Studies on the effect of temperature on the red clover mottle virus in vivo and in vitro. *Biologia* **24**, 535–544.

Németh M (1986): *Virus, Mycoplasma and Rickettsia Disease of Fruit Trees*. Akadémia Kiadó, Budapest, pp. 840.

Tu JC (1989): The role of temperature and inoculum concentration in the development of tip necrosis and seedling death of beans infected with Bean yellow mosaic virus. *Plant Dis*. **73**, 405–407.

Walkey DGA (1976): High temperature inactivation of cucumber and alfalfa mosaic viruses in Nicotiana rustica cultures. *Ann. Appl. Biol*. **84**, 183–192.

Weismann L (1966): Aphids from the family Aphididae participating in the transmission of potato viral diseases in Slovakia. *Biologické práce*, Vydavateľstvo SAV Bratislava, pp. 7–25 (in Slovak).