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Short communication

Effects of temperature on infection in *Capsicum* sp. and *Nicotiana benthamiana* by impatiens necrotic spot tospovirus

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Abstract

Infection by two isolates of impatiens necrotic spot tospovirus (INSV) under temperature regimes of 25/18 °C (day/night) or 33 °C (continuous) was studied in *Capsicum annuum* (systemically susceptible to tomato spotted wilt tospovirus, TSWV), *C. chinense* PI 152225 and PI 159236 (reacting hypersensitively to TSWV) and *Nicotiana benthamiana* (systemically susceptible to both tospoviruses). At 25/18 °C infection was systemic in all hosts tested. At 33 °C infection in *N. benthamiana* was systemic whereas in *C. annuum* and *C. chinense* it was restricted to the inoculated leaves. The result differed from that reported for TSWV, where high temperature made plants more susceptible, or caused no difference. Exchanging temperature regimes 6 h to 4 days after inoculation did not affect the final results one month later, with plants being only locally infected at 33 °C continuous regime, or systemically infected at 25/18 °C alternate regime. The two INSV isolates were biologically and serologically stable for 5 passages in *N. benthamiana* held continuously at 33 °C.

The genus *Tospovirus (Bunyaviridae)* includes tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV) (Murphy et al., 1995). TSWV, present worldwide, is among the most damaging viruses and has a very large natural host range (Goldbach and Peters, 1996; Peters, 1998). INSV has been reported only in temperate areas such as North America and Europe, mainly infecting ornamentals (Daughtrey et al., 1997; Vaira et al., 1993).

The effects of temperature regimes on tospovirus infection have been studied mainly for TSWV, and high temperatures generally favour infection. Llamas-Llamas et al. (1998) compared infection of *Datura stramonium*, *Nicotiana tabacum* and *Physalis ixocarpa* at 29/24 °C (day/night) or 23/18 °C and found that the former regime favoured infection and resulted in more severe symptoms. Roggero et al. (1996b) found that at a continuous 33 °C the hypersensitive reaction present at lower temperatures was broken in two *Capsicum chinense* accessions, PI 152225 and PI 159236 (Black et al., 1991), resulting in systemic infection in a high proportion of plants. Others later reported similar effects on the same *C. chinense* accessions and crosses with *C. annuum* (Moury et al., 1998; Soler et al., 1998). TSWV symptoms are severe on glasshouse-grown *C. annuum* in summer with maximum temperatures around 35–40 °C (P. Roggero, unpublished).

Temperatures of 27/24 °C day/night favoured the multiplication of INSV in *Nicotiana benthamiana* compared to 21/18 °C (Lawson et al., 1993), but after the third passage at the higher temperature regime, INSV disappeared and a new virus was found, later identified as similar to watermelon silver mottle tospovirus (Ueng et al., 1998) probably present in the original stock culture. Increased INSV accumulation in *N. benthamiana* at 33/26 °C day/night compared to 25/18 °C was also reported by Roggero et al. (1996a), leading to successful virion purification, not possible with plants grown at low temperatures.

INSV rarely infects pepper (Daughtrey et al., 1997; Verhoeven and Roenhorst, 1998). However the experimental host range of a field isolate from systemically infected peppers (INSV-P125) was reported to be similar to that of other isolates from ornamentals but it also systemically infected the two *C. chinense* accessions PI 152225 and PI 159236, causing severe necrosis (Vicchi et al., 1998).

In this paper we report the effects of different temperature regimes on infection of *C. annuum* and *C. chinense* PI 152225 and PI 159236 with INSV-P125 and another isolate from *Sinningia speciosa* (INSV-Glox1). Glox1 is serologically a typical INSV when tested with a panel of monoclonal antibodies (mabs) against nucleocapsids (Adam et al., 1996). We now also report the reaction of some mabs with P125 in parallel with Glox1. The stability of P125 and Glox1 after serial mechanical transmission in *N. benthamiana* grown at continuous 33 °C is reported.

P125 and Glox1 were maintained by mechanical transmission in *N. benthamiana* kept in a glasshouse at 25/18 °C day/night. To avoid generation of defective components they were stored under liquid nitrogen and passaged only a few times.

The reaction of mabs against INSV nucleocapsids was tested in TAS ELISA with P125 and Glox1. Plates were coated with polyclonal antibodies against INSV virions (Roggero et al., 1996a) and the antigen consisted of serially diluted sap from systemically infected *N. benthamiana*. Mabs 5E4, 5G11, 5E8, 1E11 and 1E12 against INSV nucleocapsids were from the *Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH* (DSMZ), Germany, and were detected with anti-mouse IgG antibodies from rabbit conjugated with alkaline phosphatase. Taking into account small differences in antigen concentration in the crude plant sap, the pattern of reaction with all the mabs was very similar for the two isolates. Since Glox1 was found similar to other INSV isolates by direct comparison (Adam et al., 1996) it was concluded that the nucleocapsids of P125 do not differ from other INSV isolates.

The effects of temperature regimes were studied in *C. annuum* cv Quadrato d'Asti (systemically susceptible to TSWV), in *C. chinense* PI 152225 and PI 159236 and in *N. benthamiana*. Plants were grown in a glasshouse at about 25/18 °C. The cotyledons and first two true leaves were inoculated with plants having 8 leaves. After inoculation, plants were immediately transferred to the following temperature regimes:

(a) glasshouse at around 25/18 °C day/night, with a photoperiod of about 14 h, light supplemented with Philips SON-T 400 sodium lamps and a light intensity of 120 µm m^{-2} sec^{-1} PAR;
(b) growth chamber at a continuous 33 °C with a photoperiod of 14 h, provided by Philips TL95 fluorescent lamps and a light intensity of 90 µm m^{-2} sec^{-1} PAR.

Symptoms were recorded weekly, and one month after inoculation INSV was assayed by TAS ELISA on inoculated and uninoculated leaves.

The results shown in Table 1 were similar for the two isolates. Infection in *N. benthamiana* was not influenced by the different temperature regimes, and all plants developed local and systemic symptoms in the glasshouse and at 33 °C, and tested positive by ELISA on both inoculated and uninoculated leaves. At 25/18 °C almost all the *Capsicum* plants tested positive by ELISA on the inoculated leaves and a high proportion were systemically infected,

Table 1. Effect of temperature regimes on local and systemic infection of INSV isolates P125 and Glox1 on *Capsicum* sp. and *Nicotiana benthamiana*. The first four leaves were inoculated, on plants having eight leaves. Virus was assayed by TAS ELISA one month after inoculation

Host	P125				Glox1			
	25/18 °C		33 °C		25/18 °C		33 °C	
	Local	Systemic	Local	Systemic	Local	Systemic	Local	Systemic
C. annuum	12/13[a]	11/13	12/14	0/14	10/10[a]	8/10	7/7	0/7
C. chinense PI 152225	9/9	5/9	7/10	0/9	10/10	5/10	9/10	0/10
C. chinense PI 159236	10/10	5/10	10/10	0/12	8/10	5/10	8/10	0/10
N. benthamiana	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10

[a]Figures are number of plants testing positive by ELISA over plants inoculated.

especially with *C. annuum*. Only plants showing symptoms gave positive ELISA results. At a constant 33 °C, a high proportion of *Capsicum* plants again tested positive on inoculated leaves but systemic infection for both *C. annuum* and the two *C. chinense* accessions was completely blocked. No systemic symptoms were recorded and all plants tested negative by ELISA. Some samples were back inoculated to *N. benthamiana* but there was no infection. Some samples from inoculated leaves of *C. annuum* plants held at 25/18 °C or 33 °C continuous were tested at the same dilution in the same ELISA plate. Absorbance values were similar, suggesting that viral antigen accumulation was not materially affected. This result is the opposite of that found with TSWV and *C. chinense*, in which a continuous 33 °C regime increased systemic infection (Roggero et al., 1996b).

The effect of length of treatment at the two temperature regimes on systemic infection was studied by inoculating *C. annuum* plants with isolate P125 and exchanging conditions at different times after inoculation, from 6 h to 4 days. One month after inoculation, symptom scores and ELISA tests were made on both inoculated and uninoculated leaves. The results are shown in Table 2. The two temperature regimes up to 4 days had no effects on the final results: with alternate temperature infection resulted systemic whereas with continuous 33 °C infection was only local. Thus the process of systemic invasion took place quite late after inoculation, since transfer to 33 °C 4 days after inoculation almost completely prevented systemic invasion. The potential for virus movement was not however disrupted by holding plants at 33 °C for 4 days immediately after inoculation, since after transfer to permissive conditions, systemic infection occurred. When INSV was transmitted from inoculated leaves of 33 °C plants to plants kept under glasshouse conditions, systemic infection occurred, confirming that the capacity of the virus to become systemic was conserved.

Table 2. Effects of alterning 33 °C continuous or 25/18 °C temperatures on local and systemic infection of INSV-P125 on *C. annuum*. The first four leaves were inoculated on plants having eight leaves. Virus was assayed by TAS ELISA one month after inoculation

Treatment and time after inoculation	Virus presence[a]	
	Local	Systemic
33 °C continuous followed by 25/18 °C		
0/30 days	7	5
6 h/30 days	10	6
1 day/29 days	8	7
2 day/28 days	10	10
4 days/26 days	10	8
30 days/0	4	0
25/18 °C followed by 33 °C continuous		
0/30 days	9	0
6 h/30 days	4	0
1 day/29 days	5	0
2 days/28 days	10	0
4 days/26 days	10	1
30 days/0	10	9

[a]Each experiment was done on 10 plants and figures are number of plants testing positive by ELISA.

Stability of P125 and Glox1 in *N. benthamiana* grown at a continuous 33 °C was tested by passaging the virus by sap inoculation five times from systemically infected leaves taken 10 days after inoculation. Symptoms appeared at the same time after all inoculation tests, with chlorotic spots on inoculated leaves about 3 days after inoculation and systemic mottling around 6 days after inoculation. Systemically infected leaves from the five passages were collected 10 days after inoculation and stored frozen. The sap was tested by TAS ELISA using the mab 5E4. No differences were found on the reactivity of the mab and the virus accumulated to a similar extent in all passages being detectable at a sap dilution up to 1/100,000. Thus apparently both isolates were biologically and serologically stable at 33 °C during at least five mechanical transmissions.

In conclusion, our experiments showed that:

(a) The effect of high but still physiological temperatures on systemic infection by the two tospoviruses INSV and TSWV in *C. chinense* and *C. annuum* was the opposite, with systemic invasion of INSV being prevented and that of TSWV favoured or unaltered.
(b) Inhibition of systemic infection by INSV at 33 °C was plant specific since no effects were found in *N. benthamiana*.
(c) There was no inactivation of INSV at 33 °C since after 4 days the virus could move systemically when plants were moved to a lower temperature. We note that TSWV was only inactivated *in vivo* in tobacco after 24 h at 46 °C (Roggero and Pennazio, 1997).
(d) The stability of P125 and Glox1 for 5 passages at 33 °C in *N. benthamiana* contrasts with the results of Lawson et al. (1993), but the discrepancy may be attributable to the mixed infection of INSV and another tospovirus in their experiments.

(e) High temperature did not alter INSV replication but only impeded systemic movement. Prevention of systemic infection cannot depend on a thermotherapeutic effect of high temperature on the establishment of infection because both isolates gave high proportion of plants infected on the inoculated leaves. The result was more likely due to blocking of long distance transport.

Little is known about the genes determining pathogenesis in TSWV and nothing in INSV. Qiu et al. (1998) using genome reassortment between TSWV isolates, mapped a genetic element responsible for local lesion morphology in cucumber to the M RNA (which encodes the glycoproteins and NS_M, the putative movement protein) and a second, responsible for systemic movement in tobacco was mapped to the L RNA (this segment encodes the replicase). The genomic organization of INSV, even if similar to that of TSWV, shows considerable differences in sequence for all the genes (Goldbach and Peters, 1996). High temperature in pepper may thus affect gene(s) function in different ways for TSWV (promoting or not altering systemic infection) and INSV (favouring local infection).

Our results stress the influence of temperature as a modulating factor acting on both TSWV and INSV diseases. Inhibition of long distance transport of INSV at high temperature in pepper may explain the rarity of natural systemic INSV infection in the crop.

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