Systemic Infection of Solanaceous Hosts by Peanut Chlorotic Streak Caulimovirus Is Temperature Dependent and Can Be Complemented by Coinfection with Figwort Mosaic Caulimovirus

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ABSTRACT

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Peanut chlorotic streak caulimovirus (PClSV) developed systemically in tobacco (*Nicotiana tabacum*) and *Datura inoxia* at 33 C but not at 25 C even though the virus multiplied and was highly productive in the inoculated leaves of these plants at 25 C. In contrast to behavior in these hosts, the virus caused productive systemic infections at either temperature in *Datura stramonium*. PClSV was complemented for systemic infection in *D. inoxia* at 25 C by coinfection with a second caulimovirus, figwort mosaic virus. Gene I (tentative movement function) mutants of PClSV were not complemented for systemic development in *D. inoxia* at 25 C, suggesting that some other virus function was lacking for systemic development in tobacco and *D. inoxia* at 25 C.

Additional keywords: virus resistance, thermally repressed, virus movement.

Peanut chlorotic streak virus (PClSV), a recently described caulimovirus from peanuts (*Arachis hypogaea*) in India, has a wider host range than other caulimoviruses and occurs under very high temperatures of the semi-arid tropics (25). Interestingly, the virus is not transmitted by aphids, the common vector of other caulimoviruses. In fact DNA sequencing has shown that the gene corresponding to conserved gene II, which encodes the aphid transmission factor of other caulimoviruses (2,5,38), is missing from the PClSV genome (26).

In further work with PClSV we have found that the virus's invasiveness and productivity in plants is greatly stimulated by higher temperatures. In several plants we have found that PClSV develops systemically only if the plants are grown at elevated temperatures. In tobacco (*Nicotiana tabacum* L.) and *Datura inoxia* Mill., for example, PClSV was confined largely to the inoculated leaves unless the plants were grown above 28 C. In contrast, PClSV inoculated to *Datura stramonium* L. induced a prominent systemic disease at ordinary greenhouse temperatures (20–24 C). In this investigation we explored the effect of temperature on PClSV infections of tobacco and *D. inoxia* and found that its invasiveness to these plants at 25 C can be complemented by another caulimovirus. However, its cell-to-cell movement function (gene I) does not account for this phenomenon.

MATERIALS AND METHODS

Inoculation and immunological assay of PClSV. Two weeks after transplanting, seedlings of *D. stramonium, D. inoxia,* and *N. tabacum* (cv. Samsum NN) were mechanically inoculated with sap from PClSV-infected *D. stramonium*. At least 20 plants were inoculated in each case and the experiment was repeated five times. The inoculated plants were maintained under natural lighting conditions in a greenhouse (25 C) for 2 days, and then in growth chambers at either 33 C or 25 C with 16 h light/8 h darkness. Each species was divided into three equal groups. The first group was placed in the 33 C chamber during the entire period of each temperature experiment (30 days). The second group was maintained at 33 C for two days and then at 25 C for 28 days. The third group was held at a constant 25 C for 30 days.

The development of PClSV infection, concentration, and spread in the different plant groups were followed by capsid protein accumulation as determined by enzyme-linked immunosorbent assay (ELISA) (4). Assay samples were taken by removing 4–5 1-cm-diameter leaf disks from each leaf of comparable age at 15 and 25 days post-inoculation (p.i.). For the first samples, symptomatic inoculated leaves, leaves with or without symptoms immediately above the inoculated leaves from the same plants, and fully expanded top leaves were evaluated separately at 15 days p.i. Only fully expanded top leaves were assayed at 25 days p.i. Leaf disks were homogenized with a Polytron homogenizer using 10 ml of grinding buffer per gram of infected tissue.

ELISA tests were performed by the double antibody sandwich method (4). PClSV antiserum against purified virus was used as a source of IgG. Purified IgG was conjugated to horseradish peroxidase following described procedures (37).

The substrate for the horseradish peroxidase reactions was o-phenylene diamine (Sigma), 0.7 mg/ml in phosphate-citrate buffer, pH 5.0 (7.3 g Na_2HPO_4, 5.11 g citric acid, 0.4 ml 30% H_2O_2 per liter of solution). After suitable time (30–45 min) the absorbances were measured at 450 nm in a Titertek Multiskan colorimeter (Flow Laboratories, McLean, VA).

Cloning viral genomes and plasmid constructs. Cloning of the full-length infectious genome of PClSV has been described (25). This clone, pPClSV-K1, consists of insertion of the viral genome, after cleavage at a unique *Kpn* I site, into the *Kpn* I site of bacterial plasmid pUC119 (36). The viral genome of this plasmid is infectious only after cleavage with *Kpn* I. To obviate the need for excision of the viral genome from the cloning vector, a partially redundant clone of PClSV was constructed by the steps outlined in Fig. 1.

pPClSV-K1 was digested with *Pst* I and *Nsi* I followed by religation to itself to create pUC-R, which contains successively the 3′ end of ORF V, a complete ORF VI, the promoter for the viral polycistronic mRNA, the polyadenylation signal, and the 5′ end of ORF VII (Fig. 1). To generate a partially redundant clone of PClSV, a full-length PClSV genome (with kanamycin

resistance gene [*Kn'*] inserted at the 4648 position) was cloned in the proper orientation at the unique *Kpn* I site of pUC-R. *Sal* I cleavage to remove the *Kn'* gene was used to create pPClSV-KIR, which is infectious to plants without restriction cleavage. The redundancy of the viral genome, illustrated in Fig. 1, was created in a manner to allow transcription of a full-length RNA without interruption by the cloning vector. The DNA sequence of PClSV has been described by Richins et al (26).

In experiments involving complementation between PClSV and a second caulimovirus, figwort mosaic virus (FMV), a plasmid containing genomes of both viruses in a partially redundant state was constructed. Inoculation with this plasmid without restriction cleavage was used to insure that DNA of both viruses was delivered to individual cells of the inoculated plant. The FMV genome used for this redundant end construct was derived from pH85 (33). This plasmid consists of a partially redundant clone of wild-type FMV with a chloramphenicol acetyl transferase (CAT) gene at the unique *Stu* I site (nucleotide 5577) of the FMV genome (27). The redundant portion of the genome consisted of a second copy of the distal end of gene VI, the large intergenic region and gene VII inserted near the end of gene VI in the wild-type genome. It is referred to hereafter as the 1.2 mer of the FMV genome. pH85 was linearized at its unique *Sph* I site (in the multiple cloning site of its cloning vector). The 3′ overhang left by the *Sph* I digestion was removed using T4 polymerase (18) and converted to a *Sma* I site by the addition of *Sma* I linker (New England BioLabs Inc., Beverly, MA). This new clone was named pDuc82. The same strategy was followed to introduce *Sma* I linkers at the unique *Sph* I site of the multiple cloning site of pPClSV-KIR. This pPClSV-KIR derivative containing a *Sma* I site in place of the *Sph* I site of its cloning vector was designated pDuc23. After digesting pDuc82 to completion with *Sma* I, the 1.2-mer fragment of the FMV genome was cloned at the *Sma* I site of pDuc23 to create pDuc39 (Fig. 2). Finally, the CAT gene was removed from pDuc39 by digesting it with *Stu* I to generate the clone pDuc40 that contains both the PClSV and FMV genomes in a partially redundant state. These steps and the redundant portions of each viral genome are illustrated in Fig. 2. Infectivity assays showed that pDuc39 DNA without restriction gave rise to systemic PClSV infections on tobacco at 33 C and systemic FMV infections on *Datura stramonium* at 25 C.

Double redundant length plasmids containing PClSV gene I mutants (see below) coupled to the wild type of FMV were constructed following this same procedure.

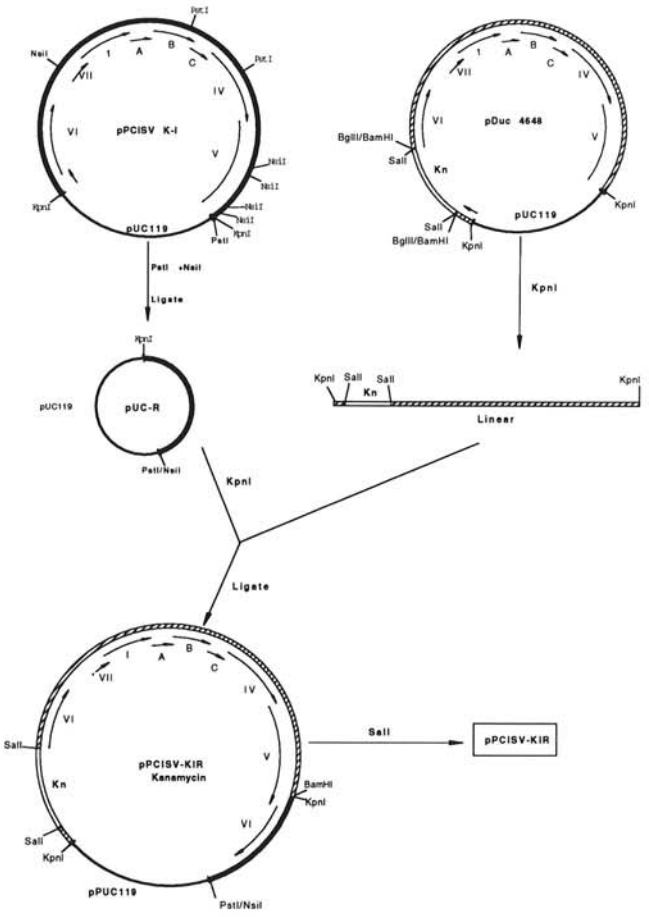

Fig. 1. Construction of a redundant clone of peanut chlorotic streak virus (pPClSV-KIR) that is infectious without restriction enzyme cleavage. pPClSV K-1 is the original clone of PClSV inserted into *Kpn* I site of a pUC119 cloning vector. Arrows show positions of viral genes. pDuc 4648 consists of pPClSV K-1 clone with a kanamycin resistant gene inserted into the viral genome near the beginning of gene VI (position 4648 of viral genome). After preparation, both clones, pUC-R and pDuc 4648, were digested to completion with *Kpn* I. The 9-6 kb fragment from pDuc 4648 was gel isolated and cloned into the *Kpn* I site of pUC-R. The kanamycin gene was then removed after *Sal* I digestion, leaving an insert of 12 bp containing a unique *Sal* I site. This plasmid was then ligated to create pPClSV-KIR. Gene VI and a portion of the large intergenic region are redundant.

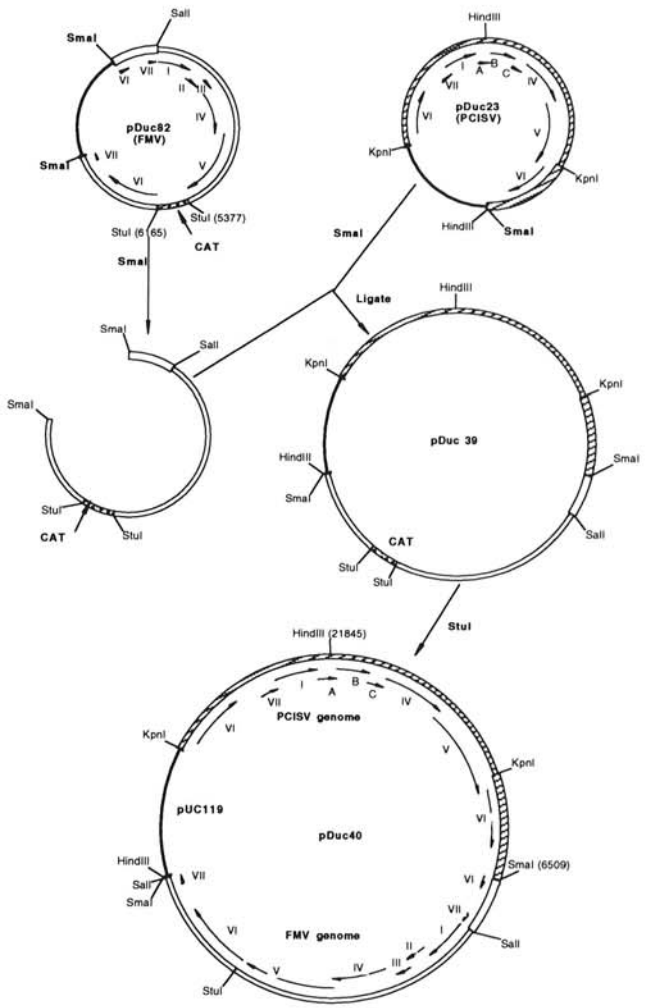

Fig. 2. Construction of double redundant length plasmids containing genomes of both peanut chlorotic streak caulimovirus (PClSV) and figwort mosaic caulimovirus (FMV). pDuc82, a partially redundant FMV clone containing the chloramphenicol acetyl transferase gene at the unique *Stu* I site (position 5577 of viral genome) was excised from its cloning vector by digesting with *Sma* I. The fragment containing the FMV partially redundant genome was cloned into the unique *Sma* I site at the multiplecloning region of the plasmid vector of pDuc23 (an infectious partially redundant PClSV clone) generating pDuc39. pDuc39 was digested with *Stu* I (to remove the CAT gene) and religated, generating the clone pDuc40, that contains both the PClSV and FMV genomes in a partially redundant state.

Construction of PClSV gene I mutants. Two mutations were introduced within the gene I coding region of PClSV using pPClSV-K1. One of these consisted of an in-frame insertional mutant. This mutant was created by the transposon strategy described by Daubert et al (5). An insert of 12 base pairs (GATCCGTCGACG) containing the *Sal* I palindrome (underlined) was inserted at position 7315, in a *Bgl* II site, within the coding region of gene I of the PClSV genome. The authenticity of this insert was confirmed by sequencing.

The second type of modification of gene I of PClSV consisted of a point mutation introduced into the genome using the oligonucleotide directed mutagenesis technique of Kunkel (15). The oligonucleotide (3′-GTA CAA TTG CAA CAA TTC TAT CAT TAA TC-5′) was designed in such a way that it would allow a codon change to produce the desired amino acid modification in the gene I product (residue Gln^{119}→Lys), and to create an *Hpa* I site immediately upstream of the point mutation. The latter change did not further affect the amino acid sequence encoded by the DNA. This *Hpa* I site provided an easy way to screen for DNA of the mutant.

Both the in-frame insertional mutant and the point mutation were constructed in a double redundant length plasmid containing the PClSV genome (with one of the two gene I mutations) and the FMV wild-type genome. The construction was done following the same procedure described above for pDuc 40. The new constructs were designated pDuc 91 and pDuc 95, respectively.

Viral DNA isolation and Southern blot analysis. The method described by Gardner and Shepherd (7) was used to isolate viral DNA from infected plant tissue. The viral DNA was digested with *Bgl* II restriction endonuclease. After electrophoresis of the DNA through a 1% agarose gel, it was analyzed by Southern blotting as described by Sambrook et al (28).

Experimental design of complementation experiment. The complementation experiments were done by mechanical inoculation of cloned DNA of pDuc 40, pDuc 91, and pDuc 95 onto *D. inoxia* seedlings kept at 25 C and *D. stramonium* seedlings kept at either 25 or 33 C. Twenty plants were used for each replication. The inoculated plants were tested by ELISA every 2 wk p.i. and samples were taken for Southern blot hybridization analysis toward the end of the 3-mo period used for these experiments.

RESULTS

Effect of temperature on systemic development of PClSV. In preliminary tests based solely on symptom development, tobacco and *D. inoxia* were selected for further study because both plants showed no systemic symptoms at 25 C but developed prominent symptoms at 33 C. Neither plant developed systemic symptoms of PClSV below 30 C. Table 1 summarizes symptoms in these three solanaceous plants at each temperature. PClSV could not be transmitted from the top leaves of either plant held at 25 C. However, the virus was readily transferred from either the inoculated leaves, which showed chlorotic local lesions 7–8 days after infection, or from tip leaves at 33 C. In contrast, *D. stramonium* developed both local and systemic symptoms at 25 C and the virus was readily transmitted from either inoculated or tip leaves at 25 C. At 33 C, tobacco showed vein clearing in the tip leaves followed by slight crinkling and a faint mottle. At 33 C *D. inoxia* developed a prominent chlorotic mottle of the tip leaves that was accompanied by necrotic spots but very little distortion, while *D. stramonium* showed a prominent vein clearing and blistering.

Measurement of virus levels in these three solanaceous hosts at 25 and 33 C showed that the virus productivity is greater at the elevated temperature (Fig. 3). *Datura stramonium* was the only host in which substantial amounts of virus developed in top leaves at 25 C (Fig. 3). The virus was not detectable by ELISA in the top leaves of either tobacco or *D. inoxia* at 25 C when samples were taken for assay 15 days p.i. Essentially the same results were obtained when samples from these various treatments were taken 25 days p.i. (Fig. 4). However, PClSV was detectable at low levels in some samples of leaves taken immediately above the inoculated leaves of tobacco (Fig. 3).

Whenever tobacco or *D. inoxia* held at 33 C showed well developed symptoms of PClSV infection and were moved to 25 C, the new growth of these plants did not show any signs of infection. Moreover, after 1 or 2 wk PClSV could not be detected in the top leaves of these recovered plants by either ELISA or infectivity assays (mechanical inoculation to *D. stramonium*). Consequently, it appears that PClSV is unable to move upward from the site of inoculation into the terminal leaves of tobacco or *D. inoxia* at 25 C to establish systemic infections. Although the virus appears to multiply well in the inoculated leaves and to move for short distances (Fig. 3), it is largely confined to sites of inoculation even though these tissues show little hypersensitivity. At 33 C the virus moved rapidly upward to invade the terminal portions of tobacco and *D. inoxia* with the first symptoms in the tip leaves appearing in 6–7 days.

PClSV in *D. stramonium* developed systemic infections at 25 C almost as rapidly as plants at 33 C, in contrast to tobacco and *D. inoxia*. Virus concentration in the top leaves of plants at 25 C was similar to that of the top leaves of plants at 33 C (Fig. 3). We concluded from these observations that some virus function required for systemic movement is not expressed as well in tobacco or *D. inoxia* as it is expressed in *D. stramonium*. In an effort to associate this function with a particular portion of the virus genome we carried out complementation tests with a second caulimovirus that infects solanaceous plants.

FMV complements PClSV for systemic infection of *D. inoxia* at 25 C. FMV causes productive infections in *D. inoxia* at 25 C (more than 1 μg virus per gram of infected tissue) (34). Although FMV infects tobacco systemically at 25 C, it is scarcely detectable by ELISA (less than about 20 ng/g tissue). Joint infections were tested to determine if FMV would complement PClSV for systemic development in *D. inoxia* at 25 C. If successful these tests could be used with mutants of PClSV to determine whether a virus-specified function was lacking at 25 C. Since we assumed that transport (movement) was the phenomenon being tested, it was important to deliver both viral genomes, PClSV and its tentative helper FMV, to the same cells during inoculation. Toward this aim, a plasmid containing infectious genomes of both PClSV and FMV in a partially redundant manner was constructed (pDuc40) as described in Materials and Methods (Fig. 2). DNA of this clone is infectious for both viruses without releasing either genome by restriction cleavage.

Datura inoxia seedlings inoculated with pDuc40 and maintained at 25 C showed necrotic local lesions 8–10 days p.i. Fifteen to 20 days p.i., a mild vein clearing and mottling was evident in the top leaves. When these plants were analyzed by

TABLE 1. Symptom development following PClSV inoculation of *Datura stramonium*, *D. inoxia*, and *Nicotiana tabacum* (cv. Samsun NN) under different temperature regimens

	Leaves[a]								
	D. stramonium			*D. inoxia*			*N. tabacum*		
Temperature	1	2	3	1	2	3	1	2	3
Constantly at 33 C	NLL[b]	VC	VC;B	NLL	VC	VC;M	CLL	VC	VC;B
Two days at 33 C and then 25 C	NLL	VC	VC;B	NLL	NS	NS	CLL	NS	NS
Constantly at 25 C	NLL	VC	VC;B	NLL	NS	NS	CLL	NS	NS

[a]Leaf position 1 = inoculated leaves; position 2 = leaves immediately above inoculated leaves; position 3 = top leaves.
[b]NLL, necrotic local lesions; VC, vein clearing; B, blistering; M, mottling; CLL, chlorotic local lesions; NS, no symptoms.

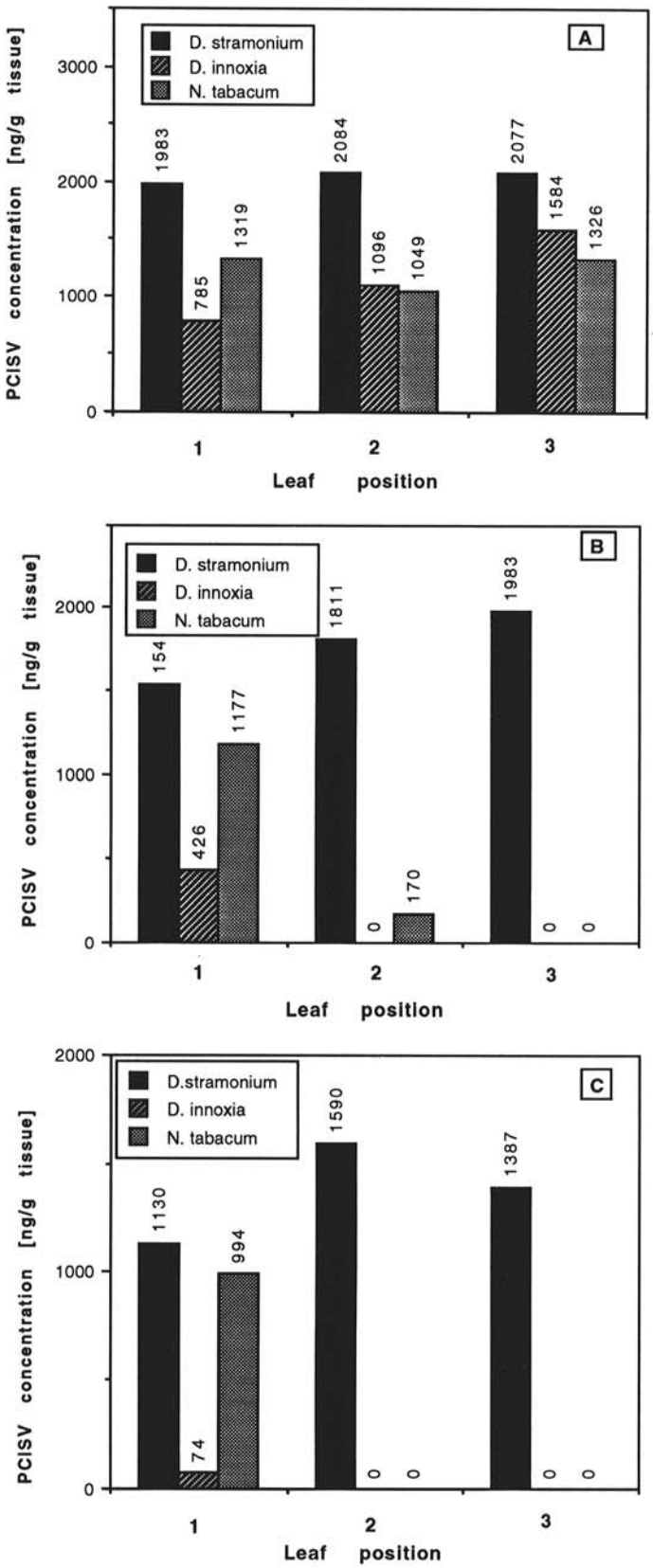

Fig. 3. Peanut chlorotic streak caulimovirus (PCISV) concentration (ng/g tissue) detected by enzyme-linked immunosorbent assay (ELISA) from *Datura stramonium, D. inoxia,* and *N. tabacum* cv. Samsun NN maintained at different temperature regimen. **A,** Plants kept constantly at 33 C. **B,** Plants maintained 2 days at 33 C and later constantly at 25 C. **C,** Plants kept constantly at 25 C. Leaf positions are: 1 = inoculated leaves at the base of the plants; 2 = leaves immediately above the inoculated leaves; 3 = top leaves. All ELISA values were adjusted by subtracting value of the negative control (healthy plant extract). Samples for assay collected 15 days after inoculation.

ELISA 2 wk p.i., positive readings were obtained for FMV but not for PCISV. However, samples taken 30 days p.i. were positive for both viruses. The levels of virus based on the immunosorbent tests were equivalent to singly infected plants, i.e., FMV-infected plants at 25 C and PCISV-infected plants at 33 C.

Viral DNA was extracted from the top leaves of *D. inoxia* plants showing systemic symptoms 30 days p.i. with pDuc 40. After purification and digestion with *Bgl* II restriction endonuclease and electrophoresis in 1% agarose gels, it was subjected to Southern hybridization analysis. *Bgl* II restriction was used in these experiments because it gives a characteristic array of fragments from each viral DNA. Cloned DNA of FMV or PCISV was used as the probe in two independent hybridization experiments that demonstrated both viral DNAs were present in systemic infections at 25 C (Fig. 5).

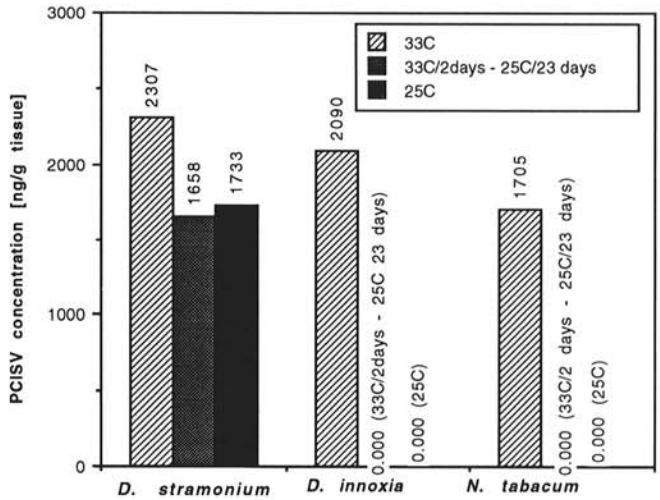

Fig. 4. Peanut chlorotic streak caulimovirus (PCISV) concentration (ng/g tissue) detected by enzyme-linked immunosorbent assay from *Datura stramonium, D. inoxia,* and *N. tabacum* cv. Samsun NN maintained at three different temperature regimens 25 days after inoculation.

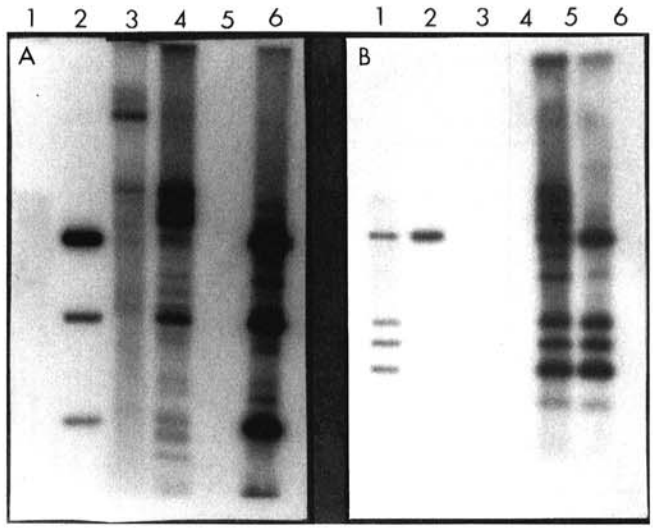

Fig. 5. Southern blot hybridization analysis of viral DNA. Viral DNA isolated from top leaves of *Datura inoxia* plants mixedly infected with figwort mosaic caulimovirus (FMV) and peanut chlorotic streak caulimovirus (PCISV) was digested with *Bgl* II restriction endonuclease and applied to 1% agarose gels. After electrophoresis, the DNA was transferred to nylon membranes and hybridized to FMV (left panel) or PCISV (right panel) probes. Lanes in both gels contain: 1 = PCISV cloned DNA; 2 = FMV cloned DNA; 3 = FMV viral DNA; incomplete digest, 4 = FMV viral DNA; 5 = PCISV viral DNA; and 6 = PCISV + FMV viral DNA from *D. inoxia* infected plants inoculated with both viruses. Band in lane 2 of right panel is due to presence of pUC119 sequences in cloned DNA of FMV that are recognized by the plasmid vector portion of the PCISV probe.

The hybridization experiments also indicate that each viral genome in these mixedly infected plants maintains its individual identity, with no recombination between the viruses.

These experiments demonstrate that at 25 C PClSV is assisted in its systemic development by FMV, suggesting the latter provides some complementing function that is not active in plants infected with PClSV alone.

Gene I mutants of PClSV are not complemented by FMV at 25 C. To test whether gene I of PClSV specified the function complemented by FMV in the joint infection experiments described above, new mixed genome clones were constructed. The PClSV gene I mutants described in Materials and Methods were used instead of wild-type PClSV. The clones pDuc91 (PClSV gene I insertional mutant plus wild-type FMV) and pDuc95 (PClSV gene I point mutation plus wild-type FMV) were constructed in a double genome state essentially in the same manner as pDuc40. These clones were inoculated to *D. inoxia* seedlings kept at 25 C and *D. stramonium* kept at either 25 C or 33 C. In every case, only FMV was detected by ELISA and Southern hybridization analysis of viral DNA isolated from the systemically infected leaves of the infected plants (data not shown). Four experiments with each mutant were negative for PClSV development in the terminal leaves of *D. inoxia* or *D. stramonium* at either temperature. However, these gene I mutants of PClSV were found to be competent for replication as shown by polymerase chain reaction experiments to be described elsewhere.

DISCUSSION

The development of PClSV in all three solanaceous hosts is favored by elevated temperatures. In contrast, most other caulimoviruses, such as cauliflower mosaic virus (CaMV), are favored by cooler temperatures. In *Brassica* sp. CaMV multiplies best at 16–20 C (23,41). A similar temperature response is exhibited in solanaceous plants by strains that infect and multiply in these noncruciferous hosts (30). Multiplication of CaMV ceases at 33 C but the matrix material of the viral inclusion bodies is more extensive at 30 C than at the optimum for virus multiplication (41).

In one solanaceous host, *D. stramonium,* PClSV multiplied almost as well at 25 as at 33 C, which suggests that the temperature response was host related. In this plant the inoculated leaves and tip (systemically invaded) leaves developed similar amounts of virus (Fig. 3). In tobacco, however, only the inoculated leaves developed about the same amount of virus at 25 C as at 33 C. Neither tobacco or *D. inoxia* developed any systemic disease or detectable virus in tip leaves except at the elevated temperature, although there was a very low level of virus that was eventually detectable in the proximal leaves adjacent to the inoculated leaves. This result suggests the virus moves well in a cell-to-cell manner in the inoculated leaves.

The temperature response of tobacco and *D. inoxia* to PClSV infection is similar to that of hypersensitive tobacco (10) or a subliminal host, barley (*Hordeum vulgare*), to tobacco mosaic virus (TMV). In both cases TMV develops systemically only when plants are held at elevated temperatures. TMV develops systemically in barley only when plants are held at 30 C (8,9). TMV could not be detected, even in the inoculated leaves of barley, at 20–25 C. Similarly, TMV moves systemically in tobacco possessing the N gene (of *N. glutinosa*) only when plants are subjected to temperatures above 30 C (13). The hypersensitive response, typical of plants at 16–25 C, is suppressed above 30 C and the virus develops systemically instead of remaining localized in the inoculated leaves. A similar behavior to temperature is exhibited by tomato (*Lycopersicum esculentum*) with Tm-2 resistance to TMV (21). TMV-inoculated plants at elevated temperatures become susceptible to systemic invasion by TMV.

Tm-2 resistance in tomato to TMV infection interferes with the normal transport process of the virus. Resistance-breaking strains of TMV were found to have mutations in the gene for the 30 kDa cell-to-cell movement protein (20). Other examples of resistance related to lack of systemic virus movement are recognized (39,40), suggesting that lack of virus transport might account for the lack of systemic development of PClSV in tobacco and *D. inoxia* at 25 C.

Gene I of the caulimoviruses is believed to function in cell-to-cell movement of these viruses. This gene at the amino acid level has relatedness to movement proteins of plus strand RNA viruses (11,14,19). Moreover, the gene I protein of caulimoviruses has single-stranded nucleic acid binding activity like that of the 30 kD transport protein of TMV (3). Of equal significance, the gene I product of CaMV is localized in cell walls (1) where it is associated with modified plasmodesmata (17). These observations strongly suggest that the gene I protein of caulimoviruses functions in intercellular movement. Further, complementation evidence suggests that gene I of CaMV is not involved in virus replication (35). For this reason we tested a second caulimovirus in mixed infections to determine if it would either complement wild-type PClSV for systemic movement in *D. inoxia* at 25 C or complement gene I mutants of PClSV at 25 or 33 C. These trials showed that FMV complemented wild-type PClSV for systemic invasion of *D. inoxia* but that gene I mutants of PClSV were not complemented for movement in the mixedly infected plants. Consequently, cell-to-cell movement is probably not the virus function that accounts for the failure of PClSV to move systemically in tobacco or *D. inoxia* at 25 C.

In previous investigations with CaMV, observations have shown that gene VI of the virus has a role in systemic infection by strains that infect solanaceous hosts (6,29–32). To a lesser extent gene IV also functions in development of systemic infections (24). Consequently, either of these regions could account for the complementation we have obtained between FMV and PClSV for systemic development of the latter in *D. inoxia* at 25 C. Alternatively, the effect of temperature on systemic disease may be accounted for by a plant response that differs in the various solanaceous hosts.

At temperatures above 30 C, which have a dramatic effect on the invasivemess of PClSV in tobacco and *D. inoxia,* the plant heat shock response becomes active. At these temperatures, patterns of gene expression are dramatically altered through changes in both transcription and translation (16). For example, many plant genes become thermally repressed above 30 C. Some of these repressed functions may be involved in host defense responses including some related to virus resistance. Expression of the N gene, derived from *Nicotiana glutinosa,* which specifies hypersensitivity to TMV infection in tobacco, is probably thermally repressed at temperatures above 30 C. As a consequence, normally hypersensitive plants become systemically susceptible to TMV (13). Conversely, gene expression by the virus is probably not markedly affected by temperature. Messenger RNA containing the untranslated leader sequences of TMV RNA, for example, is efficiently translated during heat shock, enabling viral RNAs to escape thermally induced translational repression (22). The same is probably true for PClSV since the virus is very active at elevated temperatures. Other plant viral RNAs are thermally repressed at elevated temperatures (12). Repression of host resistance responses that are active at 25 C may be thermally repressed at 33 C in tobacco and *D. inoxia,* thus permitting PClSV to systemically invade tissues that at 25 C would be resistant to infection. Gene VI of the caulimoviruses is known to control host hypersensitivity (29). This suggests that gene VI mediates the virus-host interaction and that it must function effectively in order for these viruses to develop systemically in their host plants. In mixedly infected plants, FMV may suppress this host reaction through a more effective function of its gene VI. Consequently, one can speculate that gene VI, rather than the movement function (gene I), may account for the complementation between FMV and PClSV for systemic development of the latter in tobacco and *D. inoxia* at 25 C.

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