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Transmission of peanut yellow spot virus (PYSV) by Thrips, *Scirtothrips dorsalis* Hood in groundnut[†]

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Peanut yellow spot virus (PYSV) was efficiently transmitted by *Scirtothrips dorsalis* Hood in groundnut. Larvae could acquire the virus in 30 min and the maximum percentage transmission of 43.8% by individual insects resulted following two days AAP. Single adult Thrip transmitted the virus after minimum IAP of 30 minutes. The percentage transmission (33.3%) increased linearly with an increase in IAP up to 1.5 days and maximum up to 55 h of IAP (36.1%). PYSV persistently transmitted more than 75% of their life span.

Keywords: peanut yellow spot virus; groundnut; *Scirtothrips dorsalis*; vector transmission

Introduction

Peanut yellow spot virus (PYSV) was reported first from India (Anon. 1978) followed by Thailand (Wongkaew et al. 1985). Recently PYSV was separated into a distinct species and proposed to be included in a newly established serogroup of the genus Tospovirus based on serological cross-reactivity and nucleic acid hybridisation with Tomato spotted wilt virus (TSWV), Impations spot necrosis virus (INSV) and Peanut bud necrosis virus (PBNV) (Satyanarayana et al. 1998). Though Amin and Mohammed (1980) from India and Keerati-Kasri Korn and Moalthong (1989) from Thailand reported the transmission of PYSV by *S. dorsalis* they have not reported the experimental evidence on transmission and virus–vector relationships of PYSV. There were no published reports on virus–vector relationship of PYSV and *S. dorsalis* at the time of experimentation.

Though the PYSV was reported to be transmitted by *Scirtothrips dorsalis*, systematic experimental evidence of transmission and virus–vector relationships were not reported. Therefore, detailed thrips transmission studies including virus–vector relationships were conducted and reported in this paper.

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Materials and methods

Thrips culture

Scirtothrips dorsalis, *Thrips palmi* and *Franklimiella schultzei* adult thrips were collected from chilli plants. Initially, young branches of chilli were tapped gently on to a white paper and then the adult thrips sucked into aspirator, and brought to the laboratory at ICRISAT. Different species were separated under a stereoscopic microscope. Other species were eliminated at the time of transferring into vials for egg laying.

Rearing thrips on groundnut leaflet

Thrips were reared on groundnut leaflets using the method developed by Amin et al. (1981). *S. dorsalis* was successfully reared on detached groundnut leaflets under controlled conditions in glass vials (3×1 cm) closed with corks. Leaflets remained in good condition for at least 10 days, during which egg and larval instars were completed.

The vials before use were washed with water and sterilized at 160°C for 1 h. Immobilised five females and one or two males of each thrips species were released into a glass vial which was held in an inverted position. The thrips moved upward and gathered in the upper portion of the inverted vial. Immediately a young leaflet of groundnut cv. TMV 2 was introduced into the vial and then closed with a cork. The vials with thrips were kept in an incubator adjusted to 12 h 1: d cycles of 25°C light period and 22°C dark period. After allowing one day oviposition access, the thrips were dislodged from the leaflet onto a paper by tapping the inverted vial and dislodged thrips were collected into a separate vial. Later fresh leaflets were introduced into the vial for further egg laying by thrips. This process was continued for about seven days during which 90% of the total egg laying was completed. The leaflets with eggs were transferred to a new vial for the incubation of eggs. This method of culturing thrips on detached leaflets, though laborious, helped in maintaining pure culture of thrips and facilitated collection of larvae of the required age for transmission.

Virus source

Peanut leaflets showing small chlorotic yellow spots (initial symptoms) were collected from the groundnut fields. ICRISAT, Patancheru, confirmed PYSV by DAC-ELISA (Hobbs et al. 1987) used as virus source for the acquisition of virus by first instar larvae of *S. dorsalis*.

Test plants

Seven-day-old groundnut cv. JL 24 seedlings growing in plastic pots were pulled out, washed under tap water to remove soil particles. Later three-quarters of the tap root system, two cotyledons and two primary (true leaf) leaves were also cut with a sharp razor by leaving the growing (unopened) bud. Green leaf-like bracts were also removed up to the base. The above said seedling parts were removed to facilitate the (viruliferous) virus acquired *S. dorsalis* adult feeding only on a growing unopened leaf bud. This is because the PYSV causes localised infection/spots on the leaflet wherever it feeds.

Acquisition access and inoculation access period

Both AAP and IAP were similar to those employed for *T. palmi* as described by Vijayalakshmi (1994) except that young groundnut leaflets showing PYSV symptoms were

used for AAP as a virus source for first instar larvae of the three thrips species. In IAP, young groundnut seedlings were transferred individually into transparent plastic centrifuge tubes (6×2.5 cm). All plants were tested using PYSV polyclonal antiserum in penicillinase DAC-ELISA.

Virus–vector relationships of S. dorsalis

Virus–vector relationships included AAP, IAP and virus retention in *S. dorsalis*, i.e. the persistence or non-persistence nature of PYSV in the vector.

Effect of AAP

Scirtothrips dorsalis larvae were tested for 15, 30 min, 1, 2, 4 and 24 h in one experiment; 0.5, 3, 24, 48 h in a second experiment; and 0.5, 1, 4, 16, and 24 h AAP in PYSV infected groundnut leaflet in a third experiment. Exposed larvae were transferred and allowed to become adults on healthy groundnut leaflets. Transmission efficiency was tested after two days IAP. Three replications were maintained for each AAP period. Thirty-five to 45 adult thrips were used in each replication.

Effect of IAP

Single *S. dorsalis* adults exposed to PYSV (AAP for one day) were given IAP of 7, 24, 31, 48 and 55 h on five-day-old groundnut seedlings recovered after the expiry of respective IAP. The seedlings were planted in plastic pots and the symptomatic plants were recorded (after five days). All symptomatic plants were tested by DAC-ELISA. In preliminary studies it was observed that single thrips could transmit PYSV and further it was also transmitted at an IAP of 7 h. Therefore, another set of IAPs with short time periods were set and carried out as detailed below.

Scirtothrips dorsalis larvae after an AAP of one day developed to adults and were given an IAP of 0.5,3,6,24 h on groundnut seedlings. Transmission efficiency was worked as described earlier (IAP section). Thirty-five to 40 virus acquired adult thrips were used in each replication. Such three replications were maintained for each IAP period. The experiment was repeated twice.

Virus assay

All symptomatic seedlings in the transmission tests were again confirmed by penicillinase DAC-ELSIA (Sudarshana and Reddy 1989). PYSV polyclonal antiserum was used at 1:5000 dilution.

Retention of PYSV in S. dorsalis

Serial transmission tests were conducted to find out how long the viruliferous *S. dorsalis* adults were able to retain the ability to transmit PYSV. Newly emerged first instar larvae were given an AAP of two days and were then transferred to healthy leaflets until they became adults. A single adult was transferred until its death serially to each of the groundnut seedlings at one day intervals. The total number of days the thrips had transmitted PYSV were divided into three classes, i.e. the insects that had transmitted for up to 50%, 51–75% and 76–100% of their life period. Transmission of PYSV by

S. dorsalis, i.e. whether the insect was transmitting the virus continuously or not, was recorded. The number of exposed/viruliferous *S. dorsalis* adults were 20.

Results

Transmission studies were conducted utilising *S. dorsalis*, *T. palmi* and *F. schultzei* colonies raised under laboratory conditions. Only *S. dorsalis* could transmit PYSV (Table 1). Transmission frequency was ascertained by the symptoms observed on young groundnut seedlings and also by ELISA tests.

Transmission studies

Transmission tests conducted with laboratory reared cultures of *S. dorsalis* indicated that single PYSV acquired adult can transmit the PYSV to groundnut seedlings (Table 1).

Symptomatology

Most of the groundnut seedlings which were positive in *S. dorsalis* transmission tests produced characteristic PYSV symptoms within three days. The symptoms include yellow chlorotic spots and yellow chlorotic patches on partially/fully opened growing buds. The infected seedlings and leaflets were reduced in size and the seedlings remained stunted for up to 15–20 days. In about 40% of positive seedlings, the next emerging leaflets i.e. 1–2 leaflets, showed symptoms. From the third leaf onwards, the symptoms were masked; the plants produced leaflets similar to healthy plants, but still there was difference in height of the plant. All the leaflets showing typical chlorotic yellow spots/patches fall-off after the transfer of chlorotic yellow area into necrotic patches.

Virus–vector transmission characteristics

Effect of AAP

Initial observation showed that the larvae were capable of acquiring the virus within 30 min AAP and resulted in 4.71% transmission. An increase in AAP from 3 h to 1 day resulted in an increase of 20% transmission (Table 2). Studies conducted with lower AAP indicate that the larvae were not capable of acquiring the virus if AAP was less than 30 min (Table 2). As observed in both the experiments (Table 2) at 1 day AAP, the rate of

Table 1. Transmission utilising three thrips species for their ability to transmit PYSV.

Thrips[a]	No. of infected[b]/inoculated	Percent transmission
Scirtothrips dorsalis	12/39	30.8
Thrips palmi	0/43	0
Frankliniella schultzei	0/37	0

[a]First instar larvae were given 1 day AAP. Individual adults were transferred to each groundnut seedling (7-days-old) and allowed two days IAP.
[b]Virus presence was confirmed in DAC-ELISA.

Table 2. Effect of different acquisition access periods on the transmission of PYSV by *S. dorsalis*.[a]

AAP[b]	No. infected/inoculated[c]	Transmission (%)
Experiment 1		
30 min	2/43	4.7
3 h	6/36	16.7
1 day	12/34	35.3
2 days	14/32	43.8
Experiment 2		
15 min	0/38	0.0
30 min	1/38	2.6
1 h	2/31	6.5
2 h	5/32	15.6
4 h	8/32	25.0
2 days	13/33	39.4
Experiment 3		
30 min	1/28	3.57
1 h	3/28	10.7
4 h	5/30	16.7
16 h	6/25	24.0
1 day	10/31	32.3

[a]One-day-old larvae were used in all experiments for AAP.
[b]All exposed insects were given two day AAP after they became adults.
[c]Single insect was used. All symptomatic plants were confirmed in DAC-ELISA for PYSV presence.

transmission was more than the other periods tested and further increases in AAP after 1 day did not show a great increase in transmission.

Effect of IAP

Results presented in Table 3 indicate that single exposed adult thrips can transmit PYSV with a minimum IAP of 7 h (11.11%). The percentage transmission increased linearly with increasing IAP (24 h, 25%; 31 h, 33.33%; 48 h, 32.35%; 55 h, 36.11%). Further, the apparent rate of transmission decreased after IAP of 31 h. The results presented in Table 4 indicate that single PYSV acquired *S. dorsalis* adult could not transmit the virus with 30 min IAP, whereas it could be transmitted within a minimum of 3 h. The percentage transmission increased linearly with an increase in IAP (3 h, 4.69%; 6 h, 10.48%; 24 h, 26.18%).

PYSV retention in S. dorsalis

It is clear from the data of 20 adult *S. dorsalis* tested with two day AAP and one day IAP, one thrip transmitted the virus throughout its life period (Table 3). Two adults transmitted the virus up to 50% of their life period, six from 51 to 75% of their life period, 12 from 76 to 100% of their life period. Of the 20 adult thrips only one could transmit the virus up to 53.8% frequency. The range of transmission frequency was 25–53.8%. Ten adults could transmit the virus from 40 to 46% frequency. Further, it is evident from the table that most of the PYSV acquired *S. dorsalis* adults retained the virus from 76 to 100% of the life period with a transmission frequency of 40–46.7%.

Table 3. Effect of different acquisition access periods on the transmission of PYSV by *S. dorsalis*.[a]

IAP	No. infected/inoculated[b]	Transmission (%)
Experiment 1		
30 min	0/64	0
3.0 h	3/64	4.7
6.0 h	5/65	7.69
24 h	16/62	25.8
Experiment 2		
7 h	4/36	11.1
24 h	9/36	25.0
31 h	12/36	33.3
48 h	11/36	30.5
55 h	13/36	36.1

[a]One-day-old larvae were given an AAP of one day in both the experiments and single insects were used in transmission tests.
[b]All symptomatic seedlings were confirmed by DAC-ELISA for PYSV presence.

Discussion

It is interesting to note that some degree of specificity exists with regard to transmission of various serogroups of tospoviruses. TSWV and INSV groups are more or less transmitted by *F. fusca* (Gardner et al. 1935; Sakimura 1961; Moulder et al. 1991). PBNV and WSMV group is transmitted only by *T. palmi* and INSV by *F. schultzei* and *T. tabaci*. On the contrary, the TSWV group not be transmitted by *T. palmi* (Cho et al. 1991; Mau et al. 1991). PYSV is only transmitted by *S. dorsalis* (Amin and Mohammad 1980).

Yellow chlorotic spots and yellow patches were produced on fully opened leaflets within three days after IAP. Most of the seedlings with yellow patches symptoms were reduced in height with reduced leaflet size compared to seedlings with yellow chlorotic spots and healthy seedlings. These observations are in agreement with the report of Reddy et al. (1991).

In most of the seedlings the virus could produce symptoms in 1–2 leaves in addition to the first symptomatic leaf. In infected leaves, yellow chlorotic patches/spot turned necrotic and leaflets dried and fell off. Symptoms of PYSV indicate that the virus is localised or partly systemic in nature. All infected plants under field conditions also shows that the upper/top leaves will be free from symptoms although 2–3 middle leaves had symptoms.

Even though the data on acquisition thresholds are limited in thrips, reports on TSWV indicate increased transmission efficiency with a concomitant increase in AAP. Sakimura (1962) reported an increase in the percentage of infection with increased feeding periods; 4% with 15 min feeding, 33% with 1 h feeding, 50% with 24 h feeding and 77% with 4 day feeding periods. Increased transmission rates of TSWV by *F. occidentalis* with increased AAP were observed by Cho et al. (1991). However, in the present study increased AAP did not result in a corresponding increase in transmission rate of PYSV by *S. dorsalis*. Similar kinds of transmission were observed in PBNV by *T. palmi* (Vijayalakshmi 1994).

In other studies on IAP by thrips, 5–30 min (Razvyazkina 1953; Sakimura 1961, 1963; Amin et al. 1981; Allen and Broadbent 1986) were found to be adequate. However, *S. dorsalis* failed to transmit PYSV in 30 min IAP. The maximum transmission rate was observed at 31 h IAP (Table 2).

Table 4. Serial transmission of PYSV by *S. dorsalis*[a] after 1 day AAP and 2 day IAP.

Thrips No.	Transmission of individual *S. dorsalis* (in days[b])																			%Transmission	% life period transmission
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		
1	+	−	−	+	−	+	−	+	−	+	−	M								41.7	83.3
2	+	+	−	−	−	+	−	−	−	−	+	+	−	+	D					46.7	100
3	+	−	+	−	+	−	+	−	−	+	−	−	+	D						42.9	100
4	+	−	−	−	+	+	−	+	+	−	+	−	−	D						42.9	78.6
5	+	−	+	−	+	−	+	−	+	−	D									45.5	81.8
6	+	+	+	+	−	−	+	−	+	−	−	−	−	−	−	−	−	D		33.3	50.0
7	+	+	−	−	+	−	−	+	+	−	+	−	−	D						42.9	78.6
8	+	−	−	+	+	M														40.0	83.3
9	+	−	−	+	−	−	E													28.6	57.1
10	+	+	+	−	−	+	−	+	+	+	−	−	D							53.8	76.9
11	+	−	−	+	−	+	−	−	−	D										30.0	60.0
12	+	+	+	−	−	−	+	−	−	−	−	−	−	E						28.6	50.0
13	+	−	−	+	−	−	+	−	−	−	−	D								25.0	58.3
14	+	−	+	−	+	−	+	−	−	+	−	+	−	D						42.9	85.7
15	+	−	−	+	−	+	−	−	+	−	−	−	−	D						28.6	64.3
16	+	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	D		16.7	50.0
17	+	−	+	+	−	−	−	−	−	+	−	+	−	−	−	D				35.3	75.0
18	+	−	+	−	−	+	−	+	−	+	+	−	−	M						30.8	78.6
19	+	+	−	−	−	−	−	+	−	+	+	−	D							41.7	84.6
20	+	−	+	+	−	−	+	−	+	−	−	+	−	−	D					42.9	80.0

[a]After they became adults individual insects were transferred to series of groundnut seedlings.
[b]All symptomatic seedlings were confirmed by DAC-ELISA for PYSV presence.
− = Insect not transmitted the virus. + = Insect transmitted the virus. M = Insect missing. D = Insect dead. E = Insect escaped.

Study of serial transmission tests to provide evidence for the persistency of PYSV in *S. dorsalis* indicated that $>50\%$ of viruliferous thrips transmitted the virus for $>75\%$ of the life period, although transmission by individual thrips was erratic (Table 3). Retention of the virus throughout the lifespan of adult thrips has been reported by Sakimuara (1962) and Reddy and McDonald (1983) in *F. schultzei*. The erratic transmission was not totally unexpected because of the long IAP required for *S. dorsalis* to transmit PYSV. Additionally Sakimura (1962, 1963) and Vijayalakshmi (1994) reported erratic transmission of TSWV and PBNV with *T. tabaci* and *T. palmi*, respectively.

Recently several lines of evidence suggest that tospoviruses replicate in the cells of the thrip vectors. These include increasing of virus titers in *F. occidentalis* adults as determined by ELISA (Cho et al. 1991) and cDNA probes that could specifically detect genomic and complementary TSWV RNA strands in larval thrips (German et al. 1991). Ullman et al. (1995) detected the presence of nonstructural protein encoded by the RNA of TSWV in *F. occidentalis*. Therefore, the evidence is unequivocal for the replication of tospoviruses in thrip vectors. Murali et al. (1994) identified viruliferous thrips by (antigen coated plate) CP-ELISA with Empigen-BB.

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