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The host range of *Tobacco streak virus* in India and transmission by thrips

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Summary

Tobacco streak virus (TSV) recently caused an epidemic in peanut (= groundnut, *Arachis hypogaea*) crops in Andhra Pradesh, India. In the epidemic area TSV occurred in many widely distributed weeds of which *Parthenium hysterophorus* probably plays a major role in its spread by thrips. Three thrips species, *Megalurothrips usitatus, Frankliniella schultzei* and *Scirtothrips dorsalis* were vectors in the presence of infected pollen. Of crop species, *Helianthus annuus* (sunflower) and *Tagetes patula* (marigold) could act as sources of inoculum. In limited tests, the virus was not seed-transmitted in the peanut cultivar JL-24 or in the sunflower hybrids KBSH-41, -42, -44, and -50, MSFH-17 and ZSH-976. Strategies adopted to reduce the incidence of TSV are discussed.

Key words: Peanut stem necrosis, *Tobacco streak virus*, parthenium, thrips transmission, pollen

Introduction

Nine viruses infect peanuts (groundnut = *Arachis hypogaea* L.) in India under field conditions. An epidemic of *Tobacco streak virus* (TSV) that causes peanut stem necrosis disease (PSND) has recently been reported in peanut crops in the state of Andhra Pradesh, India (Reddy *et al*., 2002). Losses in peanut crops in the Ananthapur district of Andhra Pradesh, India, alone were estimated to exceed 42 million pounds. TSV was also shown to cause sunflower (*Helianthus annuus*) necrosis disease (SND) (Prasada Rao *et al*., 2000; Ravi *et al*., 2001). This investigation was undertaken to investigate the sources of inoculum, transmission of the virus by thrips that colonise flowers of TSV hosts and its seed transmission in peanut and sunflower.

Materials and Methods

Virus culture

Leaves from peanut plants showing characteristic symptoms of TSV were field-collected in Ananthapur. Extracts from the infected leaves were mechanically inoculated to cowpea, *Vigna unguiculata* cv. C-152. Following three successive single lesion transfers by mechanical sap inoculations, virus from a single lesion was maintained in cowpea.

Experimental host range

Ten plants of each species were inoculated mechanically utilising extracts from young infected leaves of cowpea. Irrespective of symptoms produced, all inoculated and newly emerged leaves were tested for virus presence by sap inoculations to cowpea and by enzyme-linked immunosorbent assay (ELISA).

Natural incidence in weeds and crop plants

At least 50 plants of each weed species and selected crop plants were collected randomly at each location in Ananthapur district from fields in which TSV incidence exceeded 20% in peanut or sunflower crops during the year 2000 and 2000-01 rainy and post-rainy seasons, respectively.

Thrips transmission

Thrips from flowers of uninfected sunflower, parthenium (*Parthenium hysterophorus*) and marigold (*Tagetes patula*) were collected from the field and separated into *Frankliniella schultzei, Scirtothrips dorsalis* and *Megalurothrips usitatus*. The thrips were examined under a binocular microscope and only individuals not carrying pollen grains were used in transmission tests. Pollen collected from TSV-infected (confirmed by ELISA) sunflower, marigold or parthenium plants was dusted on to leaves of healthy cowpea, sunflower and peanut

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seedlings. At least 10 adult thrips of each of the three species were released on to each seedling and then covered with a polystyrene cage. In another set of experiments adult thrips which carried pollen grains were separated under a binocular microscope and 10 of each species were transferred to individually caged sunflower or cowpea seedlings. After one day of exposure, thrips were killed by spraying with dimethoate. Exposed plants were maintained in a glasshouse at temperatures ranging from 28-32ºC. Virus presence was assessed by symptoms induced on cowpea and by ELISA tests. Pollen and thrips from uninfected plants were used as controls.

Seed transmission

Tests were done on field-collected and laboratory-inoculated plants. Sunflower plants that showed symptoms within 6 wk of sowing were tagged. Two-week old seedlings of peanut (cv. JL 24) and all the major sunflower hybrids (KBSH-41, KBSH-42, KBSH-44, KBSH-50, MSFH-17 and ZSH-976) were included. All inoculated plants were maintained under glasshouse conditions. Prior to a grow-out test, a small portion of cotyledons of peanut seeds were tested by ELISA, as described by Bharathan *et al*. (1984).

For the grow-out test, seedlings were raised in trays filled with a sterilised potting mixture. After six weeks all seedlings were tested for virus presence by ELISA.

Enzyme-linked immunosorbent assay (ELISA)

Direct antigen coating ELISA, as described by Hobbs *et al.* (1987), was used. Crude antiserum (Reddy *et al*., 2002) diluted to 1:10 000 and cross adsorbed with healthy leaf extracts was used. Goat anti-rabbit IgG conjugated to alkaline phosphatase (Sigma Chemicals, St Louis, USA) was used at 1:5000 dilution. The substrate p-nitrophenyl phosphate was used at 1 mg ml^{-1}. Absorbance was recorded at 405 nm after 1 h of incubation with substrate at room temperature (28-30°C).

Results

Experimental host range

In laboratory tests, TSV infected 24 species in nine families (Table 1). The virus symptomlessly infected *Amaranthus viridis,Commelina benghalensis, Parthenium hysterophorus* and *Trianthema portulacastrum.* The following species were not infected: *Brassica oleracea capitata* and *botrytis, Capsicum annuum, Cardiospermum helicacabum, Cucumis sativus, Cyamopsis tetragonolobus, Datura stramonium, D. muricata, Euphorbia geniculata, E. hirta, Hyptis brevipes, Lagenaria cineraria, Lycopersicon esculentum, Nicotiana glutinosa, N. rustica, N. tabacum* cv. *Samsun, Physalis minima,*

Table 1. *Experimental host range of* Tobacco streak virus

	Symptoms*	
Host	Local	Systemic
Abelmoschus esculentum	NLL	SN, D
Ageratum conyzoides	NLL	LN
Amaranthus viridis	None	-
Arachis hypogaea cv. JL 24	NLL	SN, D
Cajanus cajan	NLL	TN, M
Chenopodium quinoa	CLL	NLL, TN, St
Chenopodium amaranticolor	CLL	LD, St
Cicer arietinum	None	SN, D
Commelina benghalensis	None	SI
Dahlia rosea	CLL, NLL	SN, D
Gomphrena globosa	RLL	-
Glycine max cv. Bragg	NLL	SN, D
Helianthus annuus cv. PAC 36	NLL	M, LD
Lagasca mollis	None	LN, M
Luffa acutangula	CLL, NLL	-
Momordica charantia	CLL, NLL	-
Parthenium hysterophorus	None	SI
Phaseolus vulgaris cv. Top Crop	NLL	VN
Trianthema portulacastrum	None	SI
Trigonella foenum-graecum	None	W, D
Vigna mungo cv. LBG-20	None	VN, D
Vigna radiata	None	VN, D
Vigna unguiculata cv. C-152	NRSp	LD
Zinnia elegans	CLL, NLL	-

* All species were tested by ELISA and by sap inoculation to cowpea for TSV presence
CLL – Chlorotic local lesions; D – Death; LD – Leaf distortion; LN – Leaf necrosis; M – Mosaic; NLL – Necrotic local lesions; NRSp – Necrotic ring spots; RLL – Red bordered local lesions; SI – Symptomless infection; SN – Systemic necrosis; St – Stunting; TN – Top necrosis; VN – Veinal necrosis; W – Wilting; – Viral antigens not detected by ELISA

Sida acuta, S. spinosa, Tridax procumbens and *Zea mays.*

Weed and crop plants infected under natural conditions

TSV was detected in nine of 26 crop plant species and naturally occurring weeds. *Abutilon indicum, Acanthospermum hispidum, Ageratum conyzoides, C. benghalensis, Corchorus trilocularis, Lagasca mollis,* and *P. hysterophorus* (parthenium), were infected. The highest incidence (up to 60%) occurred in *A. conyzoides, C. trilocularis* and parthenium. The most widely distributed host was parthenium. Among the crop species, the highest incidence was detected in *T. patula* (marigolds) (15-37%) and *V. unguiculata* (cv. C-152) (29%). The virus was detected in pollen from infected cowpea, parthenium, marigold and sunflower by ELISA and infectivity assays. No infection was detected in the

following weed species, *A. viridis, Argemone mexicana, Cassia auriculata, Cleome viscosa, Croton sparsiflorus, Eclipta alba, E. hirta, E. heterophylla, Lantana camara, Physalis floridana*, *Tridax procumbens, Trichodesma zeylanicum,* and *Tribulus terrestris* or the crop species, *Capsicum annuum, C. sativus*, *Luffa acutangula,* and *Lycopersicon esculentum.*

Thrips transmission

The number of thrips recovered from a single sunflower flower head varied from 37 to 312 and from a single parthenium flower from 8 to 13. Flowers of sunflower and parthenium were colonised by three thrips species, *F. schultzei, S. dorsalis,* and *M. usitatus*. Pollen from infected sunflower or parthenium, dusted on to seedlings of caged cowpea, peanut and sunflower, facilitated TSV transmission by all three thrips species (Table 2). *F. schultzei* and *M. usitatus* collected from the flowers of field-infected parthenium, sunflower and marigolds and carrying pollen, transmitted TSV to sunflower and cowpea.

Seed transmission

Five hundred seeds from six hybrids of sunflower collected from laboratory inoculated plants were tested. More than 60% of the seeds germinated and none transmitted the virus. Of 473 seeds collected from field-infected sunflower cv. Morden, 353 germinated and none of them transmitted the virus. Of 1325 seeds collected from field-infected peanut cv. JL 24, none of the cotyledons contained the viral antigens. In grow-out tests, none of 1110 seedlings were found to be infected by the virus. Of 95 seeds collected from laboratory-inoculated peanut cv. JL 24, 75 germinated and no seed transmission was observed. Additionally cotyledons of all the 95 seedlings did not contain the viral antigens.

Discussion

TSV occurred at a high incidence in a short period of 2 wk in nearly 250 000 ha of peanut crops grown during the 2000 rainy season in Ananthapur district (D V R Reddy, unpublished). Its incidence was relatively high near the field bunds and wastelands. This observation indicates that the virus occurred in various weeds. The most commonly occurring weeds were parthenium, *A. indicum, A. conyzoides,* and *C. benghalensis,* all of which were found to be infected by TSV. Parthenium is widely distributed and occurred at all the locations where PSND was recorded. No symptoms had been noticed in TSV-infected parthenium. All three thrips species (*F. schultzei, M. usitatus* and *S. dorsalis*) which colonized the flowers, served as vectors. Batches of 10 thrips of each of the species collected from flowers of infected plants carried enough inoculum in the form of pollen grains to give at least 40% transmission. Strong westerly winds occur during August and September and can cause lodging of

Table 2. *Transmission of TSV by thrips species in the presence of pollen from different plant species*

		Plants infected/Number inoculated		
Pollen Source	Thrips species	Peanut	Sunflower	Cowpea
From infected plants*				
Sunflower	Fs	6/8	3/7	9/15
	Sd	7/11	2/5	8/11
	Mu	9/12	7/17	13/18
Parthenium	Fs	4/10	13/13	14/15
	Sd	7/9	8/10	15/19
	Mu	4/8	6/7	12/15
Marigolds	Fs	8/9	2/4	9/12
From healthy sunflower	Mu	NT	NT	0/7
From infected flowers**				
Parthenium	Fs	NT	5/12	4/19
	Mu	NT	8/11	3/7
Sunflower	Fs	NT	2/5	3/10
	Mu	NT	7/9	12/15
Marigolds	Fs	NT	7/13	5/7
	Mu	NT	13/18	6/10

Fs – *Frankliniella schultzei*; Sd – *Scirtothrips dorsalis*; Mu – *Megalurothrips usitatus*; NT – not tested.
*Pollen from infected plants of the species listed were dusted onto leaves of healthy seedlings and then thrips, devoid of pollen, were released
**Thrips, carrying pollen, were collected from flowers of infected plants

pollen from infected parthenium and from such crop plants as sunflower and marigolds on leaves of other plants. These pollen grains can facilitate virus transmission in the presence of vector thrips. In laboratory tests, transmission was achieved with pollen from sunflower, marigolds and parthenium deposited on peanut leaves and colonised by any of the three vector thrips. These results confirm the findings by Greber et *al*. (1991) on the crucial role played by pollen in TSV transmission. Thrips free of pollen did not transmit the virus confirming the findings by Sdoodee & Teakle (1987) and Greber *et al*. (1991).

Surveys of TSV incidence during the post-rainy season (December 2000 to April 2001) also showed infection of peanut crops, until the end of March. TSV incidence in peanut could be correlated with the presence of infected parthenium plants near the peanut fields. Thus, parthenium is regarded as the principal source of virus spread and presumably played a crucial role in causing the virus epidemic. Therefore, removal of parthenium during the early stages of crop growth is expected to reduce the incidence of TSV. Experiments on the influence of removal of parthenium, especially from bunds and wastelands, on TSV incidence in peanut and sunflower crops are currently being conducted in farmers' fields.

Evidence that more than one thrips species can act as a vector of TSV has been provided. *M. usitatus* and *S. dorsalis* are new records and were found to colonise the TSV hosts.

Early infected peanut plants do not flower. As it is a self-pollinated crop, peanut is unlikely to contribute to virus spread. Sunflower is often grown adjacent to peanut crops. All the three thrips species carrying sunflower pollen could transmit TSV. Early infected sunflower plants usually produced malformed heads with few or no pollen grains. However, late-infected plants produced flowers that could serve as a source of inoculum. Peanut crops grown adjacent to TSV-infected sunflower crops invariably showed PSND. Therefore avoiding cultivation of peanut crops in the vicinity of sunflower crops should contribute to a reduction of TSV occurrence. Although marigolds can serve as an efficient source of inoculum, this crop is grown only under irrigation on a limited scale.

Early infected peanut plants often die following field infection, so seed could only be collected from late infected plants. Tests on dry seeds showed that viral antigens could not be detected in cotyledons by ELISA. Additionally, none of the seedlings contained the virus. However, the possibility of seed transmission in peanut requires further evaluation. Seed transmission was not observed in germinated seedlings of seven sunflower cultivars. To date TSV has not been reported to be seed-transmitted in any plants belonging to the Asteraceae (previously Compositae).

Since the presence of infective pollen on leaves is required by vector thrips for transmission, a tall border crop (sorghum, pearl millet or castor) might serve as a barrier. Farmers in the endemic area are currently following this practice. Measures to eliminate parthenium, especially from field bunds and waste lands, are also expected to be beneficial. Many peanut genotypes, especially those with resistance to thrips feeding, are being evaluated under field conditions for resistance to TSV.

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