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Short communication

Mechanism of transmission of tobacco streak virus by *Scirtothrips dorsalis*, *Frankliniella schultzei* and *Megalurothrips usitatus* in groundnut, *Arachis hypogaea* L.

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Peanut stem necrosis disease (PSND) was first reported in groundnut in the 2000 rainy season in Anantapur district of Andhra Pradesh. The disease affected nearly 225,000 ha and the crop losses were estimated to exceed Rs. 3 billion (Reddy *et al.*, 2002). Tobacco streak virus (TSV) was identified as the causal agent of PSND. The pollen-assisted TSV transmission process has been studied in detail in other host plants, *Nicotiana clevelandii, Chenopodium amaranticolor, Lycopersicon esculentum* and *Cucumis sativus* by Sdoodee and Teakle, 1987 and Greber *et al.*, 1991. The TSV transmission occurs when thrips carrying pollen from TSV infected plants on their bodies, land on host-plants and cause them to dislodge on leaves and while feeding on host plants wound both leaf tissue and infected pollen to facilitate virus infection of the plants. After identification of the causal agent of PSND in India, TSV transmission studies were initiated. Adults of all the three thrips species [(*Frankliniella schultzei* (Trybom), *Scirtothrips dorsalis* Hood and *Megalurothrips usitatus* (Bagnall)] were experimentally proved to transmit TSV in groundnut, sunflower and cowpea in the presence of pollen from TSV infected parthenium, sunflower or marigold plants (Prasada Rao *et al.*, 2003). Further studies were needed to study the role of nymphs of thrips in virus transmission and possibility of disease spread from the infected plants in the field. This paper summarises the results obtained from experiments designed to further study virus-vector-pollen-host relationships in groundnut.

Thrips from sunflower heads (*F. schultzei*), groundnut flowers (*M. usitatus*), and young folded leaflets of groundnut (*S. dorsalis*) were collected in homeopathic vials and were identified as per Amin and Palmer, 1985. After identification, *S. dorsalis* and *F. schultzei* were reared separately on groundnut plants (cv. JL 24) in the glasshouse at 23-25°C. Thrips from healthy colonies were frequently released onto test plants (cowpea) to confirm their virus free status. *M. usitatus* did not survive on young groundnut plants in the absence of flowers for more than a week, so they were used directly in the transmission tests. The sunflower plants were sap inoculated with TSV and tested by ELISA for the presence of the virus. Pollen from these TSV infected sunflower plants was dislodged by gently brushing the flower heads over an open petridish. Pollens were then picked up on a camel's hair brush and dusted onto the test plant leaves. Cowpea cv. C 152 and groundnut cv. JL 4 plants were raised in 10 cm plastic pots in the glasshouse at temperatures ranging from 25 to 30°C. All the plants used in the experiments were one week old.

Transmission tests

Virus acquisition by nymphs and adults: For acquisition feeding tests with nymphs, groundnut plants were sap inoculated with TSV. After 5-6 days of sap inoculation, young groundnut leaflets showing clear PSND symptoms were detached and placed on water in petridishes. Twelve petridishes were used and each petridish contained three leaflets. Usually 10-15 nymphs of *S. dorsalis* and *F. schultzei* were released separately on each leaflet with the help of a fine brush and allowed to feed for 24 h. These nymphs were then transferred separately onto nine healthy plants each of groundnut and cowpea covered by polystyrene cylindrical cages (75 mm high x 33 mm diameter) and allowed to feed for 2 days. A similar procedure was followed for the control except that the nymphs fed on healthy groundnut leaves. After the inoculation access period of 2 days, the groundnut and cowpea plants were sprayed with 0.4% dimethoate to kill the feeding nymphs. Nymphs of *M. usitatus* could not be included in the study, as they were not easily found. For acquisition feeding tests with adults, adults of *S. dorsalis*, *F. schultzei* and *M. usitatus* were released separately for 24 h on TSV infected groundnut plants covered individually by polystyrene cylindrical cages and maintained in the glass house. After 24 h, the thrips were collected from these infected plants and 10-15 adults of each thrips species were released separately onto nine healthy plants each of groundnut and cowpea covered by polystyrene cylindrical cages and allowed to feed for 2 days. A similar procedure was followed for the control except that the adult thrips were exposed to healthy groundnut plants. After the inoculation access period of 2 days, the groundnut and cowpea plants were sprayed with 0.4% dimethoate to kill the feeding thrips. The exposed plants in

both the cases were maintained in a glasshouse at 28 to 32°C for two weeks and later tested for TSV presence by ELISA.

The results of the virus acquisition and transmission tests are presented in Table 1. In virus acquisition experiments with nymphs of *S. dorsalis* and *F. schultzei* and adults of *S. dorsalis*, *F. schultzei* and *M. usitatus*, none of the plants showed positive reaction in ELISA indicating that these failed to directly acquire and transmit the virus. Similar observations on the failure of nymphs and adults of thrips to directly acquire TSV were reported in *C. amaranticolor* (Sdoodee and Teakle, 1987).

Table 1 Results of TSV acquisition and transmission tests with *Scirtothrips dorsalis, Frankliniella schultzei* and *Megalurothrips usitatus* on groundnut (cv. JL 24) and cowpea (cv. C 152) at ICRISAT

Treatment*	Test plant	Test plants infected** (No.)	
		Symptoms	ELISA
Virus acquisition with nymphs of *S. dorsalis*	Groundnut	0/9	0/9
	Cowpea	0/9	0/9
Virus acquisition with adults of *S. dorsalis*	Groundnut	0/9	0/9
	Cowpea	0/9	0/9
Virus acquisition with nymphs of *F. schultzei*	Groundnut	0/9	0/9
	Cowpea	0/9	0/9
Virus acquisition with adults of *F. schultzei*	Groundnut	0/9	0/9
	Cowpea	0/9	0/9
Virus acquisition with adults of *M. usitatus*	Groundnut	0/9	0/9
	Cowpea	0/9	0/9
Infective pollen and adults of *S. dorsalis*	Groundnut	10/10	10/10
	Cowpea	10/10	10/10
Infective pollen and adults of *F. schultzei*	Groundnut	10/10	10/10
	Cowpea	10/10	10/10
Infective pollen and adults of *M. usitatus*	Groundnut	10/10	10/10
	Cowpea	10/10	10/10
Infective pollen and adults of *S. dorsalis*	Groundnut	10/10	10/10
	Cowpea	10/10	10/10
Infective pollen and adults of *F. schultzei*	Groundnut	10/10	10/10
	Cowpea	10/10	10/10
Infective pollen and *S. dorsalis* feeding wound	Groundnut	0/9	0/9
Carborundum and infective pollen	Groundnut	9/15	9/15

* In the corresponding control of the above treatments no infection was observed.
** Number of infected test plants over total number of test plants used.

Virus transmission using pollen from TSV infected sunflower plants: Ten adults of *S. dorsalis, F. schultzei* and *M. usitatus* and ten nymphs of *S. dorsalis* and *F. schultzei* were released separately onto ten healthy plants each of cowpea and groundnut prior to dusting with pollen from TSV infected sunflower plants. Thrips were retained on the plants by covering the plants individually with polystyrene cylindrical cages. Control plants were dusted with infected pollen only, or infected by adult thrips only or infested by nymphs only. After the inoculation access period of 2 days, the cowpea and groundnut plants were sprayed with 0.4% dimethoate to kill the feeding thrips. The exposed plants were maintained in a glasshouse at 28 to 32°C for two weeks and later tested for TSV presence by ELISA.

Cowpea plants showed clear vienal necrosis and groundnut plants showed stem necrosis symptoms. All plants were found positive for virus in ELISA test (Table 1). Sdoodee and Teakle (1987) also observed regular TSV transmission in *C. amaranticolor* when virus carrying pollen was placed on leaves of its test seedlings before introducing thrips.

Transmission of TSV by dusting infective sunflower pollen on thrips feeding wounds on groundnut leaves: Ten adults of *S. dorsalis* were allowed to feed on nine healthy groundnut plants for 24 h. Thrips were retained on the plants by covering the plants individually with polystyrene cylindrical cages. After 24 h, the thrips were removed with a soft brush and the plants were sprayed with 0.4% dimethoate to kill any escaped thrips. The wounds caused on groundnut leaves by thrips feeding were then dusted with pollen from TSV infected sunflower plants. For control, the feeding wounds were dusted with pollen from healthy sunflower plants. The plants were maintained in a glasshouse at 28 to 32°C for three weeks and later tested for TSV presence by ELISA.

Neither control nor the test plants showed positive reaction in ELISA. A possible reason for this could be that the virus is contained inside the pollen and it can not enter leaf cells by itself unless there is a mechanical damage to both pollen and leaf tissue. In this case, thrips were not present to damage the infected pollen, therefore, no TSV infection was observed. Klose *et al.* (1992) also reported that the success of TSV transmission depended on sufficient virus being released by pollen close to a thrips-induced wound that was susceptible to infection.

Transmission of TSV by rubbing infective sunflower pollen on carborundum dusted groundnut plants: Fifteen groundnut plants were dusted with carborundum and their young leaves were rubbed with pollen from TSV-infected sunflower plant using a camel hair brush. Similar procedure was followed for control except that the young leaves of the plants were rubbed with pollen from TSV infected sunflower plants without dusting carborundum on them. The plants were maintained in a glasshouse at 28 to 32°C for three weeks and later tested for the presence of TSV by ELISA.

Groundnut plants showed 60% infection, whereas in the control no infection was observed. The infection may be due to the mechanical damage caused to the leaf tissue as well as to the pollen by carborundum while rubbing.

Summary: The mechanism of TSV transmission in groundnut and other host crops is different from that of other viruses in plants. There is no leaf-to-leaf transmission of TSV by adults of all the three thrips-species, *S. dorsalis, F. schultzei* and *M. usitatus* and nymphs of *S. dorsali* and *F. schultzei* in groundnut and cowpea, as they do not acquire virus from infected plants. However, they assist in transmission of TSV in groundnut and cowpea in the presence of pollen from TSV infected sunflower plants by causing injury to both leaf tissue of host plants and pollen.

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