Transmission of Tomato Spotted Wilt Virus, the Causal Agent of Bud Necrosis of Peanut, by *Scirtothrips dorsalis* and *Frankliniella schultzei*

P. W. AMIN, D. V. R. REDDY, and A. M. GHANEKAR, Groundnut Improvement Programme, International Crops Research Institute for the Semi-Arid Tropics, Hyderabad 500 016, India; and M. S. REDDY, Department of Plant Pathology, Andhra Pradesh Agricultural University, Hyderabad 500 030, India

ABSTRACT

Amin, P. W., Reddy, D. V. R., Ghanekar, A. M., and Reddy, M. S. 1981. Transmission of tomato spotted wilt virus, the causal agent of bud necrosis of peanut, by *Scirtothrips dorsalis* and *Frankliniella schultzei.* Plant Disease 65:663-665.

Tomato spotted wilt virus, the cause of bud necrosis of peanut, was transmitted by *Scirtothrips dorsalis* and *Frankliniella schultzei. S. dorsalis* is a new vector of the virus, but it is a less efficient vector than *F. schultzei.* Hemagglutination tests detected viral antigens in extracts from both thrips species exposed to infected leaves.

Bud necrosis is probably the most important viral disease of peanut (*Arachis hypogaea* L.) in India (3,6). Until 1977, the vector was unknown, although several insects including *Aphis craccivora* Koch, *Empoasca devastans* Stal, *Orosius* spp., *Bemisia tabaci* Genn., and *Tetranychus* spp. had been tested (8,11,12,16, Amin *unpublished*).

Initial investigations at the International Crops Research Institute for the Semi-Arid Tropics research center showed that thrips were the most common insect pests at the time of disease development, both during and after rainy seasons (Amin, *unpublished*). Recently, the disease was shown to be caused by tomato spotted wilt virus (TSWV), which is transmitted by thrips (6,14). The most abundant thrips on peanuts in Hyderabad are *Scirtothrips dorsalis* Hood and *Frankliniella schultzei* (Trybom), and preliminary tests showed that the former transmits the causal virus of bud necrosis (1).

Approved as ICRISAT journal article 142.

Accepted for publication 25 November 1980.

0191-2917/81/08066303/$03.00/0
©1981 American Phytopathological Society

This paper describes further research on the transmission of TSWV by *S. dorsalis*, on the role of *F. schultzei* as another vector, and on the application of the hemagglutination technique to detect viral antigen in thrips.

MATERIALS AND METHODS

Peanut (cv. TMV-2) plants infected by sap inoculation (5) were used for acquisition feeding tests. In preliminary experiments, terminal leaves of infected plants were the best source of inoculum. Peanut seedlings in the second quadrifoliate stage were used as test plants in early experiments, but urd bean (*Vigna mungo* L. cv. UPU-1) was later substituted for peanut since it was more susceptible and showed symptoms in 15–20 days compared with 30–40 days for peanut.

Virus-free colonies were initiated from 5–10 adult thrips collected in the field and raised on peanut plants. A peanut seedling, with the roots removed, was placed in a screw-capped 500-ml plastic jar containing water and then enclosed in a lantern globe. Five to ten thrips were released into each cage and allowed to oviposit for 24 hr. The newly hatched nymphs were transferred to a new set of healthy peanuts enclosed in lantern globes. A glass funnel with a small glass vial (3 × 1 cm) inserted in its stem was attached to the open end of the lantern globe (Fig. 1). The adult thrips were collected in the glass vial and used for starting virus-free colonies.

Alternatively, the insects were raised on peanut leaves enclosed in 3 × 1 cm glass vials (Fig. 1B) kept at 28 C. Thrips from healthy colonies were frequently released onto test plants to ascertain that they were virus-free.

Transmission tests. Fine-tipped camel's hair brushes were used to transfer nymphs and adults. Test plants were covered by lantern globes.

The nymphs were allowed to acquire the virus by feeding on detached infected leaves floating on water in a petri dish. Usually 20–25 nymphs were released on each leaflet and allowed to feed for 2–3 days. A group of 10–15 nymphs was then

Table 1. Transmission of tomato spotted wilt virus by thrips[a]

Thrips	Test plant	Acquisition access: Infected leaves[b]	Acquisition access: Healthy leaves[c]
Scirtothrips dorsalis	Peanut	2/206	0/200[d]
	Urd bean	7/131	0/55
Frankliniella schultzei	Urd bean	26/60	0/60
	Urd bean[e]	25/102	0/60

[a] Nymphs were given 2–3 days of acquisition access and 10–12 days of inoculation access feeding. Ten to 15 nymphs were fed on each test plant.
[b] Nymphs exposed to infected leaves.
[c] Nymphs exposed to healthy leaves served as a control.
[d] Number of plants infected/exposed.
[e] Single adult thrips fed on each plant.

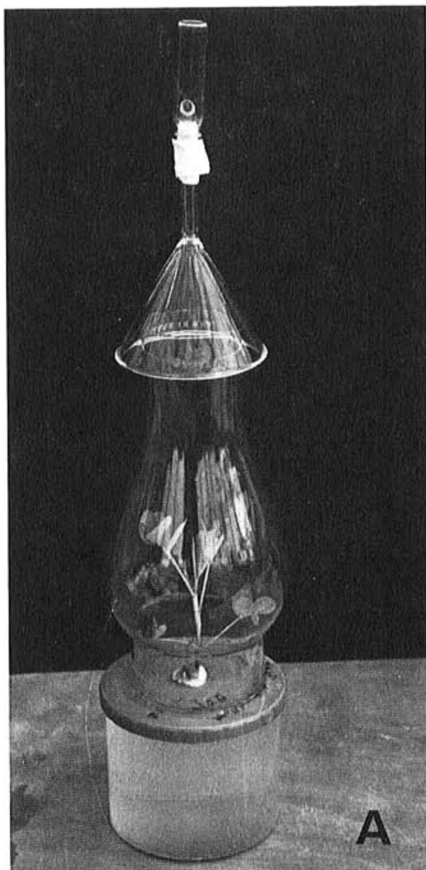
transferred to a test plant and allowed to feed for 10–15 days.

For transmission tests with single thrips, nymphs were allowed an acquisition access feeding of 6–10 days, and single adults that emerged were used in transmission tests. The inoculation access feeding period was 4–5 days. A similar procedure was followed for the controls except that the thrips were exposed to healthy peanut leaves.

After the inoculation access period, the exposed plants were sprayed with 0.025% demeton methyl (Bayer, India) and maintained in a screenhouse. Peanut test plants were maintained for 45 days and urd bean plants for 20 days. During this period, plants were sprayed with insecticide at weekly intervals. After symptoms appeared, the presence of virus was confirmed by assays on cowpea, *Vigna unguiculata* (L. cv. C-152), as described by Ghanekar et al (6).

Hemagglutination test. Samples of thrips were collected and held at 13 C for about 1 hr. Insects were then counted and triturated in 500 times their weight of 0.05 M phosphate buffer, pH 7.0, containing 0.02 M 2-mercaptoethanol. The weight of an individual insect was assumed to be 2.5 $\times$ 10^{-2}mg (4), and the extract was considered to be 1:500 dilution. Each extract was clarified at 2,000 *g* for 5 min in a refrigerated Remi K-24 centrifuge.

The hemagglutination test was performed as described by Rajeshwari et al (10). Glutaraldehyde-treated tanned red blood cells were suspended in 0.15 M phosphate buffered saline, pH 7.0, at a 3.0% concentration. One volume of TSWV antibody suspension was added to nine volumes of tanned red blood cells and incubated at 37 C in a water bath for 30 min. The coated cells were resuspended in phosphate buffered saline, pH 7.2, containing 0.5% bovine serum albumin, washed three times, and suspended in the same solution to give a concentration of 3.0% packed cells. Serial twofold dilutions of thrips extract were prepared in the phosphate buffered saline with bovine serum albumin, and 0.5 ml was placed in each well of a lucite plate. Later, 0.08 ml of sensitized cells (3.0%) was added to each well, mixed gently, incubated for 2 hr at room temperature and then overnight at 4 C. In a positive reaction, red cells formed a smooth mat (with serrated margin) on the bottom of the well; a negative reaction resulted in a discrete red ring at the periphery of the well (Fig. 2).



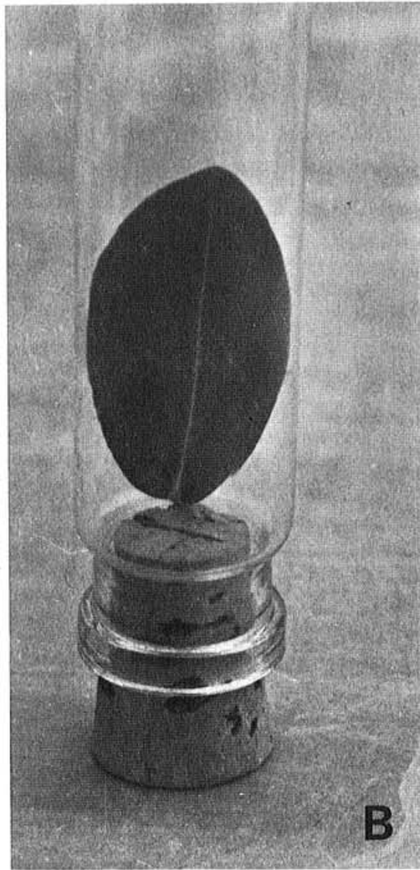


Fig. 1. Virus-free thrips colonies: **(A)** On detached shoot. **(B)** On a leaflet of peanut.

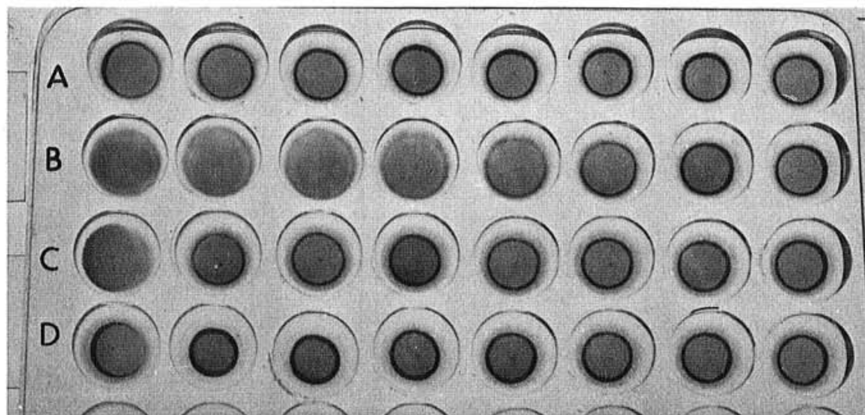


Fig. 2. Hemagglutination test showing positive reaction of: **(A)** Healthy leaf extract. **(B)** Extract from leaf infected with tomato spotted wilt virus. **(C)** Extracts from *Scirtothrips dorsalis* exposed to infected leaves. **(D)** Extract from thrips not exposed to infected leaves.

RESULTS AND DISCUSSION

Both *S. dorsalis* and *F. schultzei* transmitted TSWV, but *S. dorsalis* was a less efficient vector (Table 1). The virus was detected by the hemagglutination technique in *S. dorsalis* in three separate tests using 80, 40, and 20 insects and in *F. schultzei* in a test using 11 insects. Extracts from virus-free *S. dorsalis* and *F. schultzei* gave nonspecific agglutination at dilutions of 1:500 to 1:1,000. The extracts prepared from exposed *S. dorsalis* gave hemagglutination titers of 1:4,000 to 1:8,000 and that of *F. schultzei* gave titers of 1:4,000.

Our results show that *S. dorsalis* and *F. schultzei* can transmit TSWV, the causal agent of bud necrosis disease of peanut. *S. dorsalis* is a new vector of TSWV (13), but it appears to be less efficient than *F. schultzei*. Other *Scirtothrips* spp. (*S. longipennis* (Bagnall), *S. citri* Moulton, and *S. manihoti* (Bondar)) have been reported as crop pests from different parts of the world (7).

F. schultzei has not previously been reported on peanut although it occurs in India (2). Heavy infestations of *F. schultzei* were observed on groundnut at Hyderabad and other areas and may explain the widespread occurrence of TSWV in India. Peanuts are also infested by *Caliothrips* (*Heliothrips*) *indicus* (Bagnall), *F. dampfi* Priesner, *Retithrips syriacus* Mayet, *Megalurothrips distalis* Karny (2), *Thrips hawaiiensis* (Morgan) (15), *T. flavus Schrank* (Amin, *unpublished*), and *T. tabaci* (Deshmukh, *personal communication*). Their role as possible vectors of TSWV, except *T. tabaci* (13), needs to be assessed.

Hemagglutination tests have been used to detect TSWV antigens in plant extracts (6), and now the test has been applied to detect viral antigen in thrips. Extracts from thrips fed on healthy leaves gave higher nonspecific hemagglutination titers than those from healthy peanut leaf extracts. Nevertheless, the titers obtained with exposed colonies were at least four to eight times higher than those of extracts from healthy thrips. Paliwal (9) demonstrated that viral antigen could be detected in thrips by the microprecipitin test, using 50 insects at a dilution 1:4.5. We used 11 *F. schultzei* adults and 20 *S. dorsalis* adults to detect viral antigens by the hemagglutination test. Experiments are under way to adapt the test to detect viral antigens in individual thrips.

ACKNOWLEDGMENTS

We are grateful to W. R. Richards, Biosystematic Research Institute, Ottawa K1A 0C6, Canada; T. A. Ananthakrishnan, Director, Zoological Survey of India, Calcutta 700 001; and L. A. Mound, British Museum (Natural History), for identifying the thrips. We also thank G. V. Gooding, Jr., for providing TSWV antisera.

LITERATURE CITED

1. Amin, P. W., Reddy, D. V. R., and Ghanekar, A. M. 1978. Transmission of bud necrosis virus (BNV) of groundnut by chilli thrips, *Scirtothrips dorsalis* Hood (*Thysanoptera: Thripidae*). (Abstr.) Indian Phytopathol. 31:118.
2. Ananthakrishnan, T. N. 1973. Thrips: Biology and Control. Macmillan. India. 120 pp.
3. Chohan, J. S. 1974. Recent advances in diseases of groundnut in India. Pages 171-184 in S. P. Rayachaudhari and J. P. Verma, eds. Current Trends in Plant Pathology. Lucknow University, Botany Department, Lucknow. 324 pp.
4. Day, M. F., and Irzykiewicz, H. 1954. Physiological studies on thrips in relation to transmission of tomato spotted wilt virus. Aust. J. Biol. Sci. 7:274-281.
5. Ghanekar, A. M., and Nene, Y. L. 1976. A method of assaying groundnut bud necrosis virus. Trop. Grain Legume Bull. 5:39.
6. Ghanekar, A. M., Reddy, D. V. R., Iizuka, N., Amin, P. W., and Gibbons, R. W. 1979. Bud necrosis of groundnut (*Arachis hypogaea*) in India caused by tomato spotted wilt virus. Ann. Appl. Biol. 93:173-177.
7. Lewis, T. R. 1973. Thrips: Their Biology, Ecology and Economic Importance. Academic Press, New York. 349 pp.
8. Nariani, T. K., and Dhingra, K. L. 1963. A mosaic disease of groundnut (*Arachis hypogaea* L.). Indian J. Agric. Sci. 33:25-27.
9. Paliwal, Y. C. 1974. Some properties and thrip transmission of tomato spotted wilt virus in Canada. Can. J. Bot. 52:1177-1182.
10. Rajeshwari, R., Reddy, D. V. R., and Iizuka, N. 1981. Improvements in the passive hemagglutination technique for serological detection of plant viruses. Phytopathology. 71:306-308.
11. Reddy, M., Reddy, D. V. R., and Appa Rao, A. 1968. A new record of virus disease of peanut. Plant Dis. Rep. 52:494-495.
12. Reddy, D. V. R., Hariprasad Rao, M., Padma, M. C., and Gopal Rao, V. 1969. Haemagglutination test to detect tobacco mosaic virus antigens. Indian J. Exp. Biol. 7:162-165.
13. Sakimura, K. 1963. *Frankliniella fusca*, an additional vector for the tomato spotted wilt virus, with notes on *Thrips tabaci*, another vector. Phytopathology 53:412-415.
14. Sakimura, K. 1969. A comment on the colour forms of *Frankliniella schultzei* (Thysanoptera: Thripidae) in relation to transmission of the tomato spotted wilt virus. Pac. Insects 11:761-762.
15. Sandhu, G. S., and Brar, K. S. 1977. Insect pests of groundnut and their control. Agric. and Agro-Ind. J. 10(11):7-10.
16. Sharma, D. C. 1976. Studies on bunchy top, chlorosis and ring mottle virus diseases of groundnut (*Arachis hypogaea* L.). Phytopathol. Z. 57:127-137.