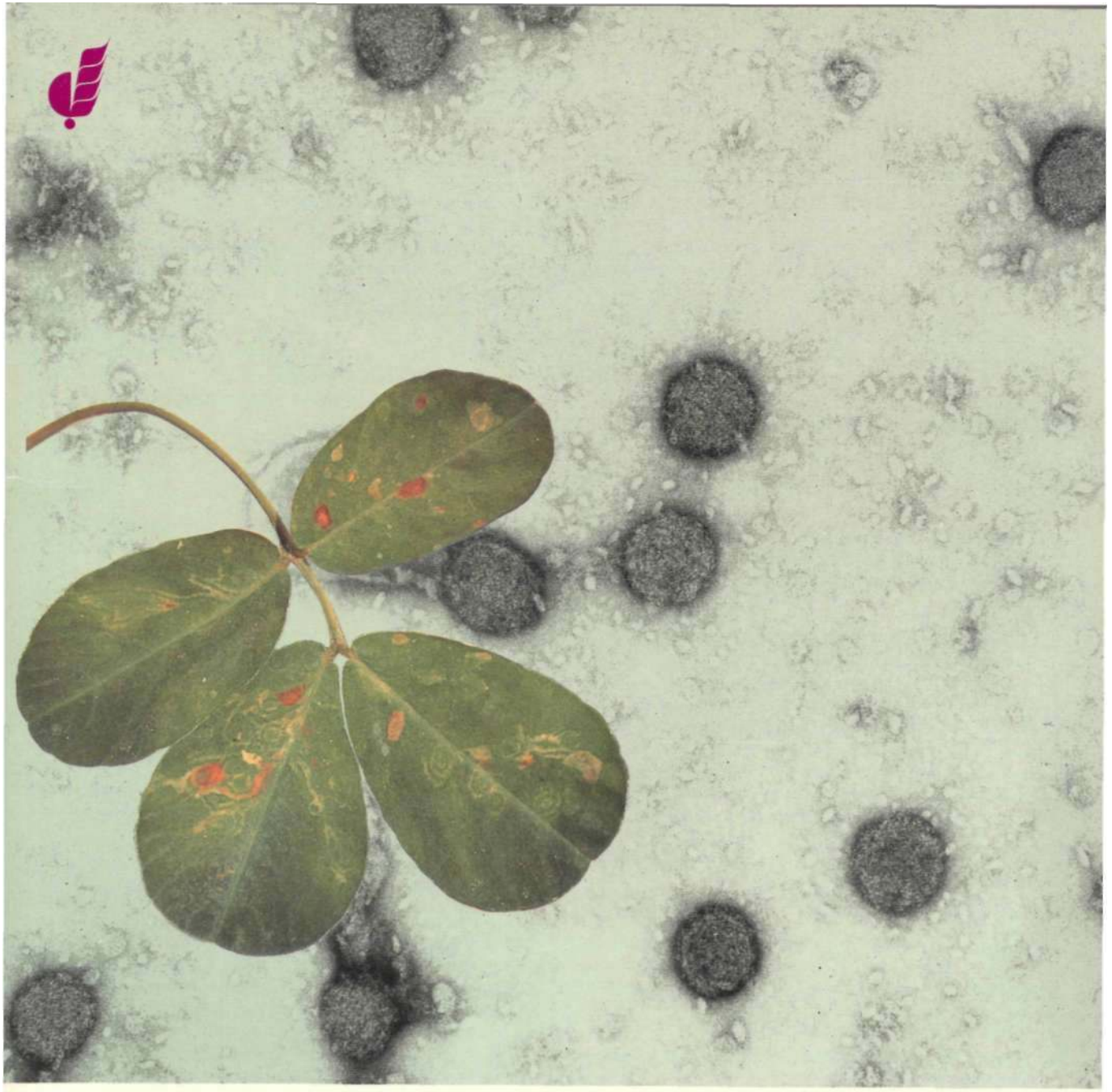

Bud Necrosis: A Disease of Groundnut Caused by Tomato Spotted Wilt Virus

Information Bulletin no. 31

International Crops Research Institute for the Semi-Arid Tropics

Abstract

Reddy, D.V.R., Wightman, J.A.. Beshear, R.J., Highland, B., Black, M., Sreenivasulu, P., Dwivedi, S.L., Demski, J.W., McDonald, D., Smith Jr J.W., and Smith, D.H. 1990. Bud necrosis: a disease of groundnut caused by tomato spotted wilt virus. Information Bulletin no. 31. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

This is the first ICRISAT Information Bulletin that deals with a virus disease of groundnut. Attention is focused on bud necrosis disease, caused by tomato spotted wilt virus, because of its economic significance on three continents. Epidemics build up rapidly with little warning and cause serious losses to growers. Protocols for purification and identification of the virus are given in detail. The symptoms of the disease in groundnut are illustrated. Procedures for a simple enzyme-linked immunosorbent assay for the detection of the virus are given. The identification of the vector insects—species of Thysanoptera (thrips)—is difficult, and is still to be fully resolved. But a key is provided as an aid in identifying seven thrips species that have been implicated as vectors of tomato spotted wilt virus on groundnut. The current situation concerning management of bud necrosis disease is outlined. Suitable insecticides, cultural practices, biological control, and host-plant resistance are discussed to assist crop protection and extension workers in formulating integrated management systems appropriate to their particular situations.

Résumé

Reddy, D.V.R., Wightman, J.A., Beshear, R.J., Highland, B., Black, M., Sreenivasulu, P., Dwivedi, S.L., Demski, J.W., McDonald, D., Smith Jr J.W.et Smith, D.H. 1991. **Nécrose des bourgeons : maladie de l'arachide causée par le virus de la maladie bronzée de la tomate (TSWV).** Bulletin d'information n° 31. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

C'est le premier bulletin d'information de l'ICRISAT consacré à une virose de l'arachide, la nécrose des bourgeons due au TSWV. Cette maladie a une incidence économique importante sur trois continents. Les épidemies se développent rapidement et presque sans avertissement, occasionnant des pertes considérables aux cultivateurs. Les méthodes de purification et d'identification du virus sont précisées. Les symptômes décrits pour le TSWV sur l'arachide sont illustrés. Sont exposés également, les procédés d'un test ELISA simple pour la détection du virus. L'identification des vecteurs—des espèces de Thysanoptera (thrips)—est difficile et reste à résoudre. Cependant, une clé de détermination est présentée afin de permettre aux utilisateurs de déceler sept espèces de thrips qui sont soupçonnées d'être les vecteurs du TSWV sur l'arachide. L'ouvrage fait le point sur la lutte actuelle contre le TSWV. Les mesures sanitaires et agronomiques, l'utilisation d'insecticides, la lutte biologique ainsi que la résistance variétale sont examinées en vue de permettre aux agents de vulgarisation et de défense des cultures d'établir des systèmes de lutte intégrée appropriés aux situations particulières.

Extracto

Reddy, D.V.R., Wightman, J.A., Beshear. R.J., Highland, B., Black. M.. Sreenivasulu. P.. Dwivedi. S.L.. Demski, J.W., McDonald. D., Smith Jr J.W. y Smith. D.H. 1991. Necrosis del brote : una enfermedad del mani causada por el virus del marchitamiento manchado del tomate. Boletin informativo no. 31. Patancheru. A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Este es el primer Boletin Informativo de ICRISAT que versa sobre una virosis del mani. En virtud de su significado economico en tres continentes, enfoca la enfermedad necrosis del brote causada por el virus del marchitamiento manchado del tomate. Las epidemias aumentan rapidamente con pocos indicios previos y provocan graves perdidas a los cultivadores. Se pormenorizan las normas para el aislamiento e identification del virus. Se ilustran los sintomas de la enfermedad en el mani. Se facilita el procedimiento para un enzimoinmunoanalisis directo para detectar la presencia del virus. La identificacion del insecto vector — una especie de Tisanopteros (thrips) — es dificil y todavia esta por resolver. No obstante se proporciona una clave como ayuda a la identificacion de las siete especies de thrips enredados como vectores del virus del marchitamiento manchado del tomate en el mani. Se explica en terminos generales la situation actual con respecto al tratamiento de la enfermedad necrosis del brote. Se habla de los insecticidas mas indicados, las practicas de cultivo, el control biol6gico, y resistencia de la planta hospedante, con el fin de ayudar al personal de protection de cosechas y tecnicos auxiliares a formular sistemas integrados de tratamiento apropiados para sus circunstancias particulares.

Cover: Purified preparation of tomato spotted wilt virus which causes bud necrosis disease (in the background) and in the foreground, groundnut leaflets showing primary symptoms of necrotic lesions with chlorotic borders, produced by a TSWV isolate in India.

Bud Necrosis: A Disease of Groundnut Caused by Tomato Spotted Wilt Virus

D.V.R. Reddy, J.A. Wightman, R.J. Beshear, B. Highland, M. Black, P. Sreenivasulu, S.L. Dwivedi, J.W. Demski, D. McDonald, J.W. Smith Jr, and D.H. Smith

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Contents

Introduction

The disease of groundnut (*Arachis hypogaea* L.) caused by tomato spotted wilt virus (TSWV) is widely distributed and has caused serious losses in the yield of this and many other crops in Australia, India, Nepal, the People's Republic of China, Thailand, and the USA. Early infections cause the most severe damage and can lead to total crop loss.

TSWV produces a wide range of symptoms in groundnut. This has complicated diagnosis and led to the disease being given several names. To avoid confusion, the disease in groundnut will be referred to as bud necrosis disease (BND) in this Bulletin because necrosis of the terminal buds is one of the most commonly observed symptoms. This symptom is not generally produced by any other virus occurring on groundnut. Since TSWV is transmitted by thrips. this Bulletin also deals with identification of the principal thrips vectors and BND control by means of their management.

The information provided is by no means complete because much research remains to be done, particularly in the areas of thrips transmission and resistance breeding. But we publish at this time in the hope that research and extension workers will be encouraged to concentrate their attention on this complex disease problem and provide a basis for its management.

Distribution

The geographical distribution of BND covers all major groundnut-growing areas. BND was a minor groundnut disease in India until the mid-1960s; since then it has occurred in epidemic proportions. Similarly, BND became severe in Australia in the mid-1970s and was then considered to be economically important. In the mid-1980s the disease assumed economic importance in the southern groundnut-growing states of the USA. Factors that contributed to these sudden and dramatic increases in incidence and severity of TSWV are currently unknown. Because BND has changed from being a minor to a major disease over wide areas in three pans of the world, it is now considered to be a potential threat to other groundnut-growing countries. There is thus a need to increase the awareness of all groundnut workers and to encourage them to collect information on the distribution and intensity of BND outbreaks wherever they appear. This Information Bulletin will, in fact, facilitate this desirable activity.

Symptoms

TSWV in groundnut produces a wide variation of symptoms. As a result, in India alone different workers have called it rosette, mosaic, bunchy top, chlorosis, ring mottle, ring mosaic, and bud blight. This caused much confusion among specialists and general readers. We describe all known variations of the symptoms of TSWV on groundnut plants. Although bud necrosis and the characteristic ring spots on leaflets are commonly produced by TSWV, they should not be used exclusively for diagnosis of the disease.

Primary symptoms

Symptoms first appear on young leaflets as faint chlorotic spots or mottling that may develop into chlorotic and necrotic rings and streaks (Figs. 1, 2, 3,4). Occasionally, the leaflets may show a general chlorosis with green islands. Petioles bearing fully expanded leaflets with initial symptoms usually become flaccid and droop. Necrosis of the terminal bud soon follows (Fig. 5).

The bud necrosis symptom is common on crops grown in the dry (summer) and rainy seasons in India, indicating that this symptom is probably associated with high temperatures. If bud necrosis occurs on plants less than 1 month old. total necrosis of the plant may follow.

Figure 1. Groundnut leaflets showing primary symptoms of chlorotic spots and necrosis produced by a TSWV isolate occurring in India.

Figure 2. Groundnut leaflets showing primary symptoms of chlorotic spots produced by a TSWV isolate occurring in India.

Figure 4. Groundnut leaflets showing primary symptoms of necrotic lesions with chlorotic borders produced by a TSWV isolate in India.

Figure 3. Groundnut leaflets showing primary symptoms of chlorotic and necrotic rings produced by a TSWV isolate occurring in Texas, USA.

Figure 5. Groundnut plants showing characteristic terminal bud necrosis produced by a TSWV isolate in India.

Necrosis on older plants usually spreads only to the petiole, or to the portion of the stem immediately below the necrotic terminal bud. In late-infected runner type groundnuts, a few branches may show mild ring spots or necrosis of the bud and then the whole plant turns yellow, wilts, and sometimes dies.

Secondary symptoms

The stunting (Fig. 6) and proliferation of axillary shoots are common secondary symptoms of BND (Fig. 7). Leaflets formed on these axillary shoots show a wide range of symptoms including reduction in size, distortion of the lamina, mosaic mottling, and general chlorosis. Rarely, the lamina is reduced to the midrib, giving the leaflet a "shoe string" appearance. These secondary symptoms are most common on early-infected plants, giving them a stunted and bushy appearance. Oniy a few branches on late-infected plants may show these symptoms.

Symptoms on seeds

Seeds from early-infected plants are small and shriveled, and their testae show red, brown, or purple mottling (Fig. 8). Late-infected plants may produce seed of normal size, but the testae on such seeds are often mottled.

Figure 6. An early TSWV (Indian isolate)-infected (on the right) and apparently healthy groundnut plant. Note severe stunting and axillary shoot proliferation.

Figure 7. Secondary symptoms produced by a TSWV isolate in India. Note terminal bud necrosis (on the left) and axillary shoot proliferation and deformed leaflets (on the right).

Figure 8. Seed from early-infected plants. Note shriveling and purple or red testa.

Causal virus

The structure of TSWV is unique among plant viruses. The particles are 70-90 nm in diameter and are surrounded by a double membrane of protein and lipid (Fig. 9). They sediment at 520-530 s. The virus protein consists of four major polypeptides of molecular weights 27, 52, 58, and 78 x 10^3 daltons. TSWV nucleic acid is comprised of three single-stranded RNA molecules of 8300, 5200, and 3400 nucleotides (Verkleij and Peters 1983).

Examination of thin sections of infected leaf tissue with the electron microscope reveals that the virus particles are associated with endoplasmic reticulum (Fig. 10). Several virus particles are often seen to be clustered in the cisternae of the endoplasmic reticulum. Crude plant extracts and purified preparations should be fixed in 1.5% glutaraldehyde. Negative staining with 1% uranyl acetate is preferable to the use of phosphotungstate or ammonium molybdate.

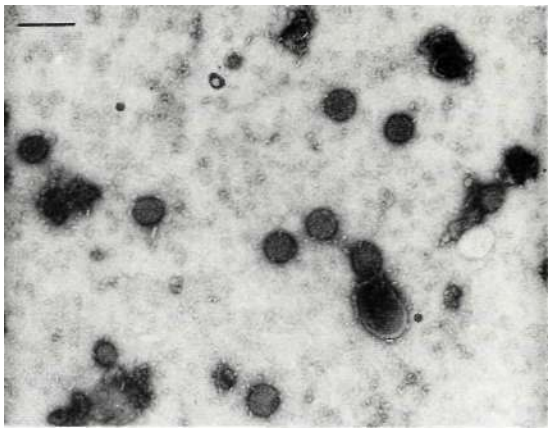


Figure 9. Purified TSWV particles stained with uranyl acetate. Bar represents 145 nm.

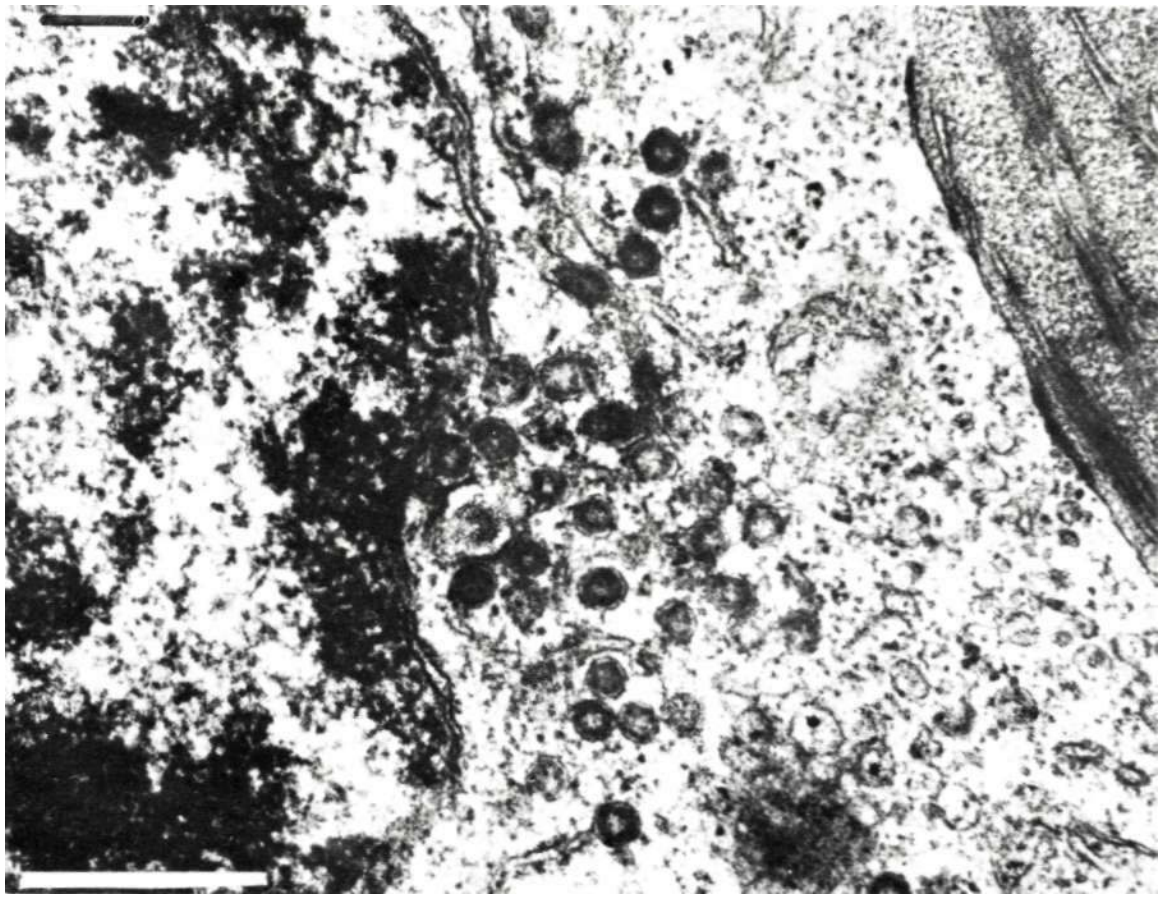
Virus purification and antiserum production

Since the virus is unstable, it is necessary that all purification steps should be performed at 4-6°C.

Purification procedure

Since the methods adopted for Indian and U.S. isolates of TSWV are slightly different, both are described. The procedure for the Indian isolate was developed in ICRISAT and that for the U.S. isolate at the University of Georgia at Griffin.

Figure 10. Electron micrograph of a thin section of groundnut leaflet showing TSWV particles. Bar represents 180 nm.



Purification procedure for Indian TSWV isolate

1. Collect young quadrifoliates (groundnut leaves) showing primary symptoms.
2. Triturate in chilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.01 M Na_2SO_3 at the rate of 3 or 4 mL buffer for each gram of tissue.
3. Filter through two thicknesses of cheese-cloth.
4. Clarify at 5000 rev min^{-1} (rpm) for 5 min in a refrigerated centrifuge at 4°C.
5. To the supernatant, add NaCl to give 0.2 molarity and polyethylene glycol [(PEG) mol. wt. 6000 - 8000] to give 4%.
6. After dissolving NaCl and PEG, leave the mixture for 1.5 - 2 h at 4°C.
7. Collect the precipitate by centrifuging at 10 000 rev min^{-1} for 10 min in a refrigerated centrifuge at 4°C.
8. Resuspend the precipitate in 0.01 M potassium phosphate buffer, pH 7.5, containing 0.01 M sodium sulfite (0.01 PPBS).
9. Clarify at 5000 rev min^{-1} for 10 min in a refrigerated centrifuge at 4°C.
10. Prepare sucrose columns containing 8 mL of 20%, 8 mL of 30%, and 12 mL of 60% sucrose (W/V) in 0.01 PPBS, in a Beckman SW 27 rotor tube, and layer 10 mL of supernatant obtained from step 9. Centrifuge for 45 min at 23 000 rev min^{-1}.
11. Remove a diffused light-scattering zone between 2.6 and 3.1 cm from the bottom of the tube. Resuspend pellets in 0.01 PPBS at a rate of 2.0 mL for each pellet. Mix sucrose zones and resuspended pellets and stir at 4°C for 1 h.
12. Prepare sucrose gradients by layering 7 mL of each of 30%, 40%, 50%, and 60% sucrose in 0.01 PPBS, in a Beckman SW 27 rotor tube. Allow sucrose solutions to form a gradient by leaving them in a refrigerator at 4-6°C for 15-18 h.
13. Layer 10 mL of supernatant from step 11 on each sucrose gradient and centrifuge at 4°C at 23 000 rev min^{-1} for 2.5 h.
14. Draw zones (usually 6 mL from each tube) at a depth of 2.5-3.1 cm from the bottom of the tube.
15. Perform another cycle of sucrose gradient centrifugation as described in steps 12 and 13.
16. Draw a single clear light-scattering zone at a depth of 2.6-3.0 cm from the bottom of the tube.
17. Dilute the zone in 0.01 PPBS and centrifuge in a Beckman R 40 rotor at 30 000 rev min^{-1} for 2 h to pellet the virus.

Purification for a U.S. (Texas) isolate of TSWV

1. Harvest leaves from a systemically infected tobacco plant (*Nicotiana tabacum* cv Burley 21), derib the leaves, and weigh them.
2. Grind the leaves in a Waring blender using cold 0.1 M potassium phosphate buffer, pH 7.0, containing 0.01 M Na_2SO_3 (0.1 PPBS) at 1 g : 3 mL ratio. This, and all subsequent purification steps, should be carried out in a cold room (4°C).
3. Squeeze the extract through four layers of cheese-cloth and discard the debris.
4. Centrifuge the extract at 4500 rev min^{-1} for 15 min at 4°C.
5. To the supernatant, add polyethylene glycol (mol. wt 6000, Fisher Product) to 4% and NaCl to 0.2 M while stirring. Dissolve the chemicals and leave the preparation at 4°C for 1.5-2.0 h.
6. Centrifuge at 10 000 rev min^{-1} for 20 min at 4°C.
7. Resuspend the pellets in cold 0.1 PPBS. Use a minimal volume depending on the number of sucrose gradient tubes to be used in step 9.
8. Centrifuge at 8000 rev min^{-1} for 20 min at 4°C.
9. Layer the supernatant on preformed 10-40% (8 mL each concentration) linear sucrose gradients prepared in 0.1 PPBS, and centrifuge at 25 000 rev min^{-1} for 1 h at 5°C (SW 28 rotor).
10. Observe the tubes in a dark room for light-scattering zones.
11. Collect the zones (use a syringe with a bent needle) at a height of 3.8-4.2 cm, dilute with 0.1 PPBS, pH 7.0, and pellet the virus at 35 000 rev min^{-1} for 1 h at 5°C. Resuspend pellets and layer onto 25-50% preformed linear sucrose gradients (8 mL each concentration) prepared in 0.1 PPBS, and centrifuge at 25 000 rev min^{-1} for 6 h at 5°C (SW 28 rotor).
12. Collect the virus zone at a height of 4.6-5.0 cm.
13. Dilute in 0.1 PPBS, and pellet the virus at 30 000 rev min^{-1} for 2 h in a R 40 rotor.
14. Use the final preparation for infectivity assay, serology, electron microscopy, and for biochemical studies.

Buffer Composition

0.1 M potassium phosphate buffer, pH 7.0, containing 0.01 M Na_2SO_3 (0.1 PPBS)

KH_2PO_4 (M.W. 136.1) - 10.61 g

K_2HPO_4 (M.W. 174.2) - 21.23 g

Na_2SO_3 (M.W. 126.0) - 2.25 g

Dissolve in 1.8 L distilled water, adjust the pH to 7.0 (if necessary) and make up to 2.0 L with distilled water.

Production of antiserum

Use a purified virus preparation from 100 g infected tissue for each injection. Resuspend purified virus in 0.5 mL of 0.01 M potassium phosphate buffer, pH 7.5, and mix with 0.5 mL of Freund's incomplete adjuvant. Make a thick emulsion by repeatedly drawing into a syringe and ejecting with force. Inject a New Zealand White inbred rabbit intramuscularly in the hind leg at two to three sites. Give five injections at weekly intervals followed by a booster injection 2 weeks after the fifth injection. Bleed the rabbit 2 weeks after the booster injection and subsequently at weekly intervals. Test the titre of the antiserum using the enzyme-linked immunosorbent assay (ELISA). Six bleedings from a single rabbit can yield more than 80 mL of antiserum.

Disease diagnosis

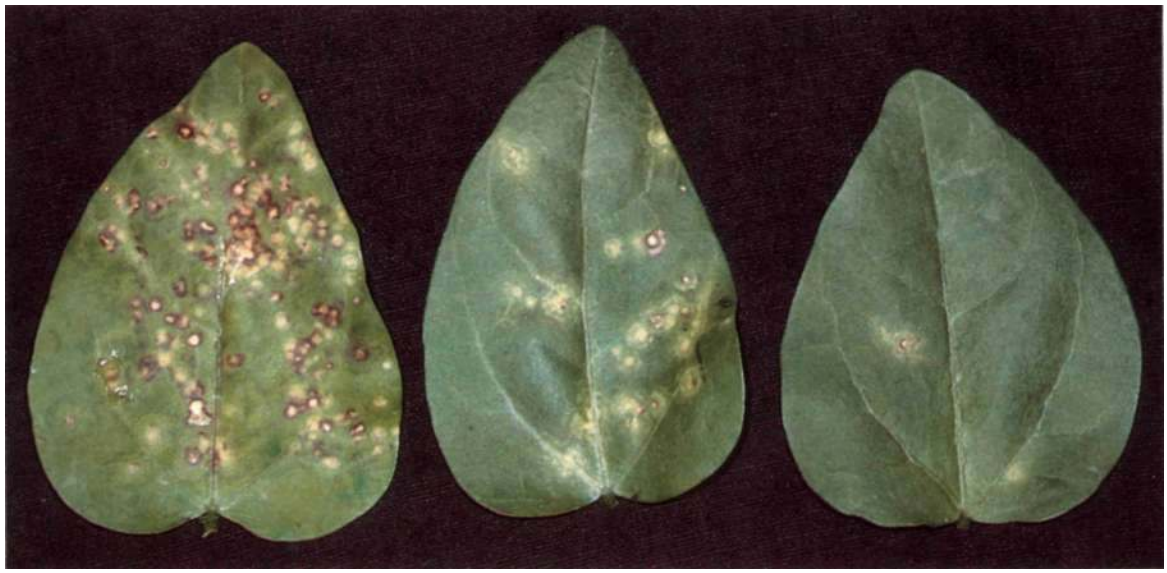
Diagnostic hosts

Several methods can be used for the detection of TSWV. Serological methods and electron microscopy are rapid and give precise results, but, if expertise and facilities are not available, reaction on diagnostic hosts, such as cowpea (*Vigna unguiculata*) and petunia (*Petunia hybrida*), can be used for TSWV identification because TSWV is mechanically transmissible. Both of these hosts produce characteristic local lesions as described below. The test needs only minimal laboratory facilities and expertise. It can be carried out as a partial or preliminary diagnostic test. The following precautions are essential to achieve mechanical transmission:

- Use only young infected leaflets showing primary disease symptoms for preparing extracts.
- Prepare extracts in 0.05 M phosphate buffer, pH 7.0, containing 0.2% mercaptoethanol or thioglycerol.
- Indicator plants, including cowpea, should be kept in the dark for a day prior to inoculation of fully expanded primary leaves.
- Keep inoculum chilled (at 4°C) throughout the inoculation procedure.
- Inoculate in the early morning when temperatures are likely to be low.

On cowpea (*Vigna unguiadata*) cv C-152 and cv California Black Eye, TSWV produces concentric necrotic and chlorotic lesions on leaves 4 or 5 days after inoculation (Fig. 11). On petunia, TSWV produces necrotic lesions on leaves 3 or 4 days after inoculation.

Figure 11. Concentric chlorotic or necrotic or both types of lesions produced by a TSWV isolate in India on inoculated leaves of *Vigna unguiculata* (cowpea, cv. C-152).



Serology

Gel diffusion has been used for the diagnosis of TSWV, but it is not very sensitive and requires large quantities of antiserum. The ELISA test is much more sensitive than gel diffusion, and is currently being used for routine TSWV detection. Several ELISA procedures for TSWV detection have been standardized at ICRISAT. The simplest is the direct antigen coating (DAC) procedure (Hobbs et al. 1987) and details of this procedure are given below.

Prepare the following solutions

1. 0.05 M sodium carbonate buffer (carbonate coating buffer), pH 9.6. Add 1.59 g Na_2CO_3 and 2.93 g $NaHCO_3$ to 1 L of distilled water. If it is necessary to store this buffer for a lengthy period, add 0.2 g of NaN_3 (sodium azide) L^{-1} of buffer to prevent microbial growth.
2. Phosphate buffer saline Tween (PBS-Tween), pH 7.4: mix 8.0 g NaCl, 0.2 g KH_2PO_4, 2.9 g Na_2HPO_4, and 0.2 g KCl in 800 mL of distilled water and, after dissolving all the salts, make up the volume to 1 L.
3. Conjugate buffer: To PBS-Tween add polyvinyl pyrrolidone (PVP) to give a 2% concentration (e.g., 2 g in 100 mL buffer), and albumin to give 0.2% concentration (e.g., 0.2 g in 100 mL buffer).
4. Substrate buffer: Dissolve 20 mg bromothymol blue (BTB) in 50 mL of 0.2 M NaOH. Neutralize the alkali by adding concentrated HC1 in drops. Make up the volume to 100 mL. Incorporate sodium penicillin-G (potassium penicillin-G and procaine penicillin can also be used) at 0.5 mg mL^{-1} and adjust the pH to 7.2 using either HC1 or NaOH (0.01-0.1 M). Store the mixture at 4°C. It is absolutely essential to adjust the pH to 7.2 before use.

Note: BTB solution alone is stable for several months at 4°C but, with penicillin added, it is stable for only 2-3 weeks.

Test procedure

1. Collect young leaflets showing primary symptoms of BND and grind them in a pestle and mortar with the sodium carbonate buffer, using a dilution of 1 part leaflets to 50 parts of buffer.
2. Follow the same procedure with leaflets from healthy plants.
3. Using a micropipette, add 0.2 mL of plant extract to each well of a microtitre ELISA plate. Leave at least two wells for adding healthy plant extracts and two wells to serve as buffer controls.
4. Incubate the ELISA plates containing the plant extracts for 1 h at 37°C.
5. Pour off the suspension and rinse the ELISA plates in PBS-Tween. Follow this by washing the plates in three changes of PBS-Tween, taking 3 min for each wash.
6. Grind healthy groundnut leaflets in conjugate buffer to give a 1:20 dilution (each gram of healthy leaflets requires 20 mL buffer). Then filter the dilution through two layers of cheese-cloth.
7. Prepare a 1:1000 dilution of TSWV antiserum in conjugate buffer containing healthy groundnut leaflet extract and incubate it for 45 min at 37°C. Add 0.2 mL to each well of the ELISA plate.
8. Incubate the ELISA plates for 1 h at 37°C.
9. Wash the plates in PBS-Tween as in step 5.
10. Dilute penicillinase-labeled antirabbit IgG or Fc to 1:5000 or 1:10 000 in antibody buffer. Dispense 200 µL into each well and incubate at 37°C for 1 h.
11. Wash the plate in 0.05% Tween-20 in distilled water.

Caution: If plates are washed in PBS-Tween as in steps 5 and 9, traces of buffer left in wells are adequate to buffer the reaction between penicillin and penicillinase and prevent the color change from occurring.

12. Dispense 200 µL of substrate mixture (penicillin + BTB) to each well and incubate at room temperature. Observe the reaction for 30 min to 2 h and record the results. It is not advisable to read results after overnight incubation (either at room temperature or in a refrigerator).
13. Results: The blue color of bromothymol blue (at pH 7.2) first turns to light green, and then light orange yellow to orange yellow. Green indicates a weak positive, and orange yellow a strong positive. Results can be quantified by measuring loss in absorbance of BTB at 620 nm. Normally 0.2 mg mL^{-1} BTB gives an optical density (O.D.) of over 2 units and a positive reaction (orange yellow) gives less than 0.1 O.D. unit.

Serological tests using direct antigen coating (DAC) ELISA have recently been conducted to compare a TSWV isolate from India with those from elsewhere. The Indian TSWV isolate cross-reacted with the homologous antiserum but failed to react with the antisera of two

U.S., one Japanese, one Netherlands and one Australian isolates. The U.S. isolates cross-reacted strongly with their homologous sera, with antisera to another U.S. and an Australian isolate but not with the Indian isolate. Thus it appears that the Indian isolate is serologically distinct.

Transmission

TSWV is unusual among plant viruses in that its vectors are thrips (Thysanoptera). The seven involved are *Thrips tabaci, Frankliniella schultzei, F. occidentalism F, fusca, Scirtothrips dorsalis, Thrips palmi,* and *T. setosus. F. schultzei* appears to be the principal vector for TSWV transmission in groundnut in Australia, and *F. occidentalis* and *F. fusca* are the primary suspect vectors of TSWV in the southern USA. The principal thrips vectors in other regions where BND is economically important are currently not known.

Since thrips transmission is important both for diagnosis and in devising control measures for BND, in the following section detailed instructions for handling thrips in transmission studies are described.

Only nymphs should be used in tests on acquisition access period. It is preferable to allow a 2-4 h acquisition access period. Since the latent period varies from 3 to 12 days, it is preferable to use adults in inoculation tests. An inoculation access period of 1-2 h is adequate. TSWV is transmitted in a persistent manner by the vector thrips which, under optimum conditions, retains the virus throughout its life.

Electron microscopy

Early- or late-infected tissues can be used for thin sectioning. However, tissue showing necrosis or extensive chlorosis will not give satisfactory results.

Cut leaflets into 2 x 3 mm portions. Immediately add 3% glutaraldehyde prepared in 0.1 M phosphate buffer of pH 7.2, and leave overnight for infiltration of glutaraldehyde. Wash the specimens in the same buffer several times and post-fix in 2% aqueous osmium tetroxide for 5 h. Wash the tissue in distilled water and dehydrate in a graded series of acetone. Infiltrate dehydrated tissue with a 1:1 mixture of Spurr® resin and acetone for 2 h, then leave in pure Spurr® resin for 1 day. Prepare tissue blocks by incubating Spurr®-soaked tissues in molds at 65-70°C for 18 h. Trim the blocks, prepare ultra-thin sections (60-90 nm thick) using an ultramicrotome, stain sections with uranyl acetate and lead citrate, wash thoroughly, dry, and observe under a transmission electron microscope.

Virus particles in thin sections are associated with endoplasmic reticulum and are enclosed in membranous bags in the cytoplasm.

Thermal inactivation

The low thermal inactivation point (TIP) (45°C) of TSWV is a diagnostic aid. It is emphasized that the determination of the TIP alone is insufficient for full diagnosis. Proceed as follows to determine the TIP:

1. Select young infected leaflets showing primary symptoms. Grind 1 g in 9 mL of 0.05 M phosphate buffer, pH 7.0, containing 0.2% mercaptoethanol or 0.2% thioglycerol.
2. Transfer a 1 mL aliquot of the extract to each of several 5 mL-capacity Pyrex glass tubes and heat in a water bath for 10 min at the selected test temperature (35, 40, 50 or 55°C) and then chill quickly in an ice bath.
3. Assay each aliquot on cowpea by mechanical sap inoculation. Aliquots exposed to temperatures above 45°C will rapidly lose their infectivity.

Identification and handling of vectors

Identification of adult vector thrips

(Descriptions are provided by R.J. Beshear and drawings by Tong-Xian Liu of the University of Georgia, Griffin, GA, USA). Several thrips genera, including *Caliothrips* and *Megalurothrips,* are found on groundnuts. However, the key applies only to adult thrips TSWV vectors or suspected vectors. It is modified from Amin and Palmer (1985)[1].

1. Head and pronotum with closely striate, transverse sculpture (Plate 1A); tergites and sternites each with a contrasting dark anterior margin covered with rows of microtrichia, tergite VIII with a complete comb on posterior margin; antennae eight segmented.....*Scirtothrips dorsalis* Hood.

   Head and pronotal sculpture not closely striate; abdominal tergites and sternites not densely covered with microtrichia; comb on tergite VIII com-

1. Key modified from identification of Groundnut Thysanoptera by P.W. Amin and J.M. Palmer, Tropical Pest Management, 1985,31 (4): 286-291.

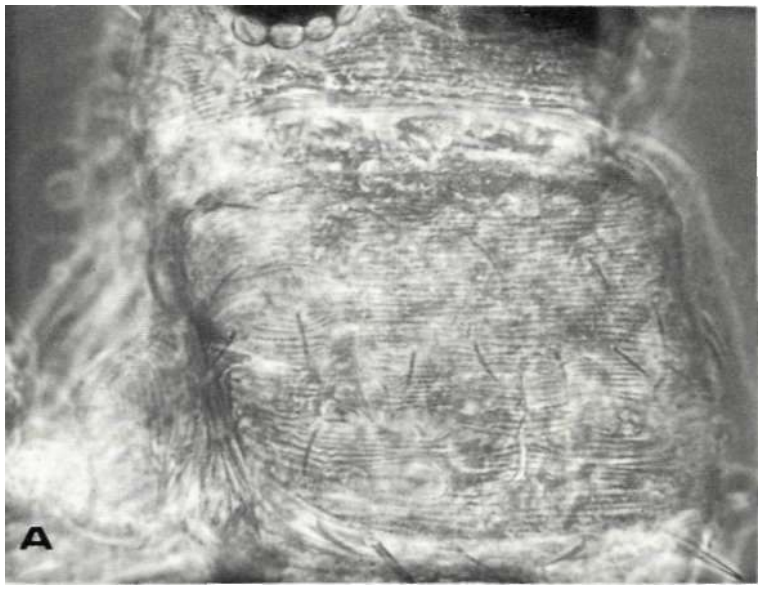
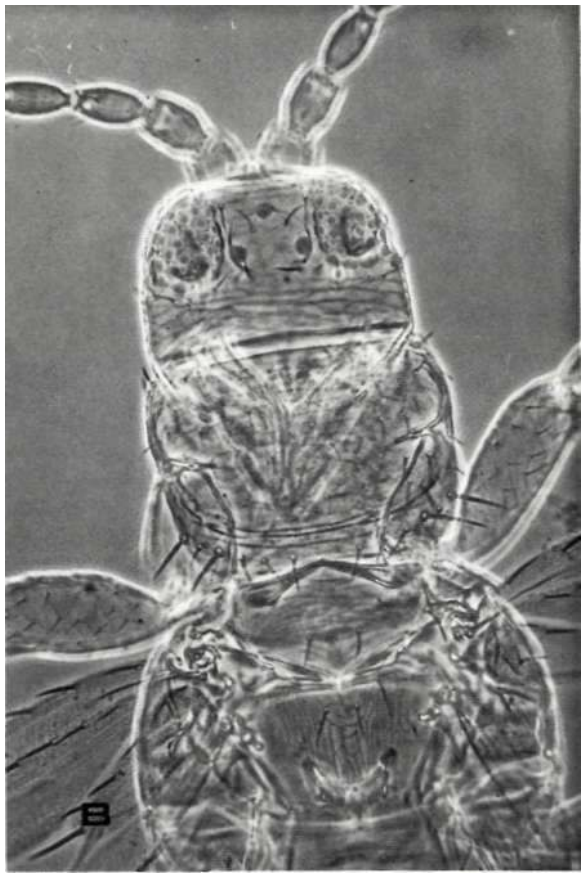


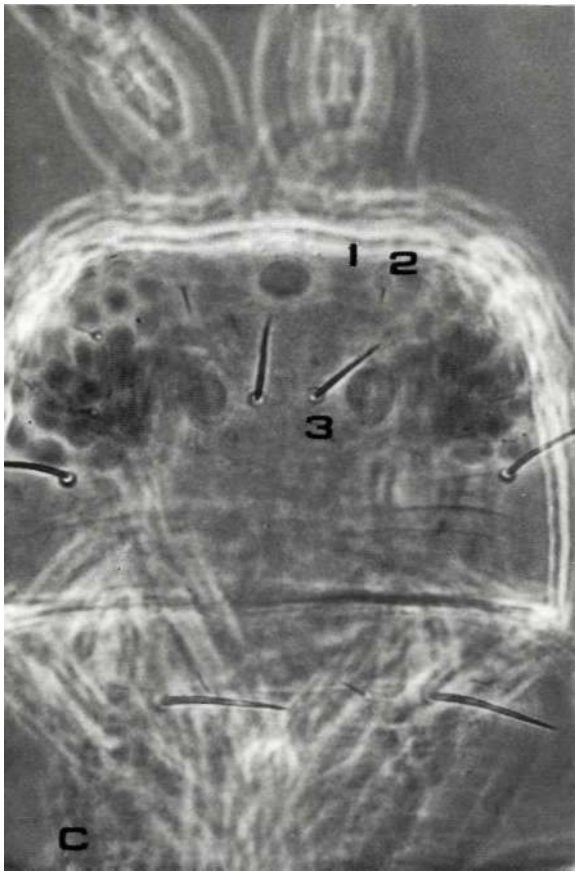
Plate I.

A. *Scirtothrips dorsalis:* pronotum.

B. *Thrips labaci:* head showing two pairs of ocellar setae and pronotum.

C. *Frankliniella schultzei:* head showing three pairs of ocellar setae.





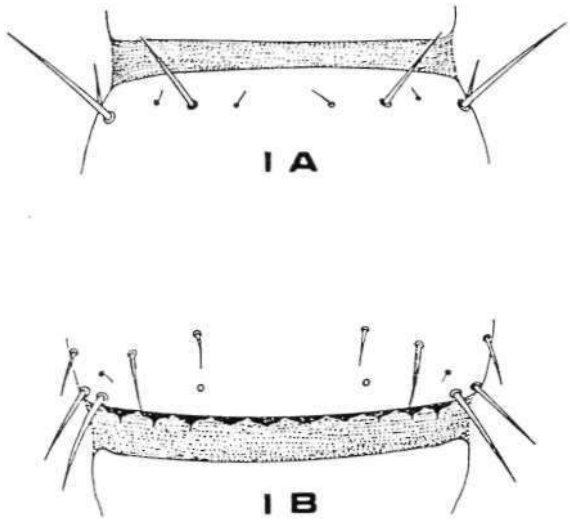


Diagram 1. ***Frankliniella fusca:*** **A. anterior margin of pronotum, and B. tergite VIII.**

plele (Diag. 3B) or incomplete (Diags. 1B and 2B); antennae seven or eight segmented.....2

2(1). Head with two pairs of ocellar setae (Plate 1B); antennae seven-segmented; pronotum with two pairs of well-developed posteroangular setae (Plate 1B); comb or tergite VIII complete.....3

Head with three pairs of ocellar setae (Plate 1C); antennae eight-segmented; pronotum with two pairs of well-developed anteroangular (Diag. 1A) as well as posteroangular setae (Plate 1D); comb on tergite VIII complete or incomplete.....4

3(2). Abdomen yellow except blotches on thorax and median portions of abdominal tergites; sometimes appearing entirely brown; ocellar crescent grey-brown; forewings pale, usually with four (5-6) setae on first vein in distal half (Plate 1E) .....*Thrips tabaci* Lindeman

Abdomen brown; ocellar crescent red; forewings dark, pale basally, usually with three setae on first vein in distal half.....(known only from Japan and Korea).....*Thrips setosus* Moulton.

Abdomen clear yellow without any greyish or brownish blotch, but with blackish and thick body setae; usually with three setae on first vein in distal half; abdominal tergite II with four lateral setae (Plate 1F) interocellar setae outside of ocellar triangle (Plate 1G).....*Thrips palmi* Karny *(T.flavus,* a yellow nonvector species that is often confused with *T.palmi* can be distinguished by the interocellar setae within the ocellar triangle).

4(2). Postocular setae shorter than and much more slender than the interocellar pair; comb incomplete (Diag. 1B); color generally dark brown to lighter brown especially on the thorax and head .....*Frankliniella fusca* (Hinds)

Postocular setae as long and as stout as the interocellar setae; comb complete or incomplete; color variable, almost entirely yellow (light form) or abdomen with dark blotching on the meson,

Diagram 2. ***Frankliniella schultzei:*** **A. anterior margin of pronotum, and B. tergite VIII.**

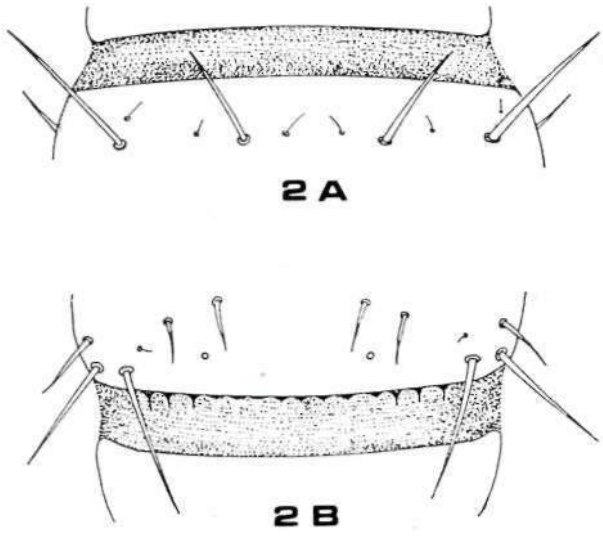
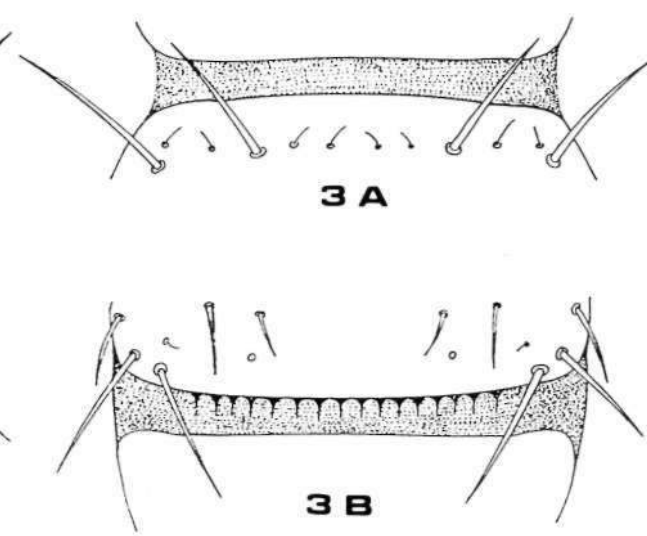


Diagram 3. ***Frankliniella occidentalis:*** **A. anterior margin of pronotum, and B. tergite VIII.**



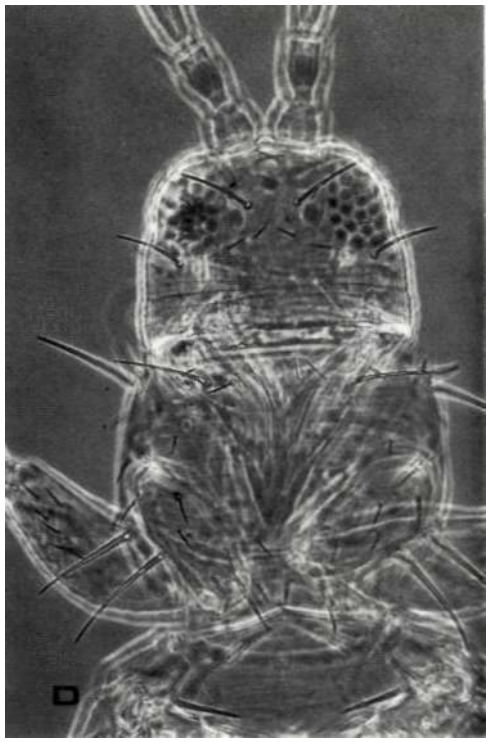
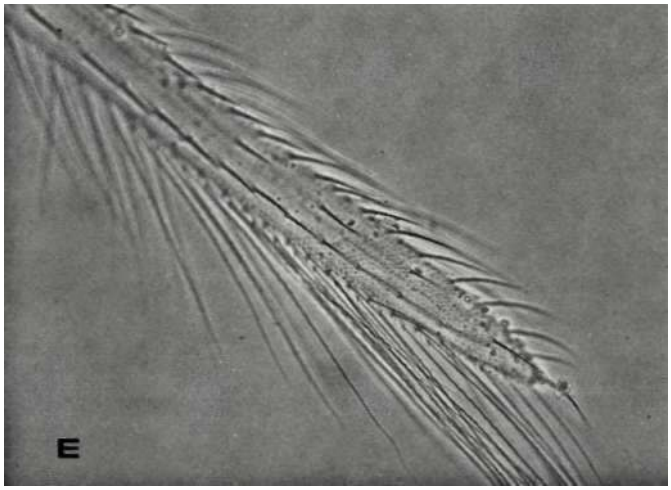
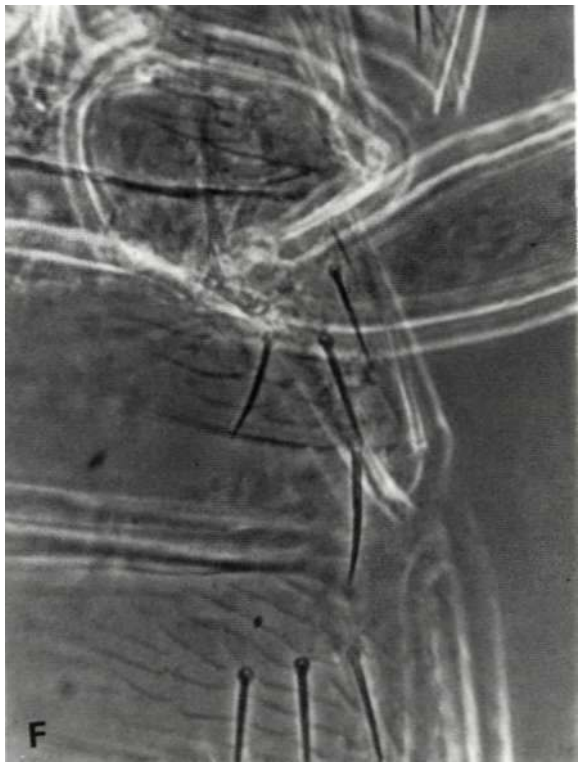
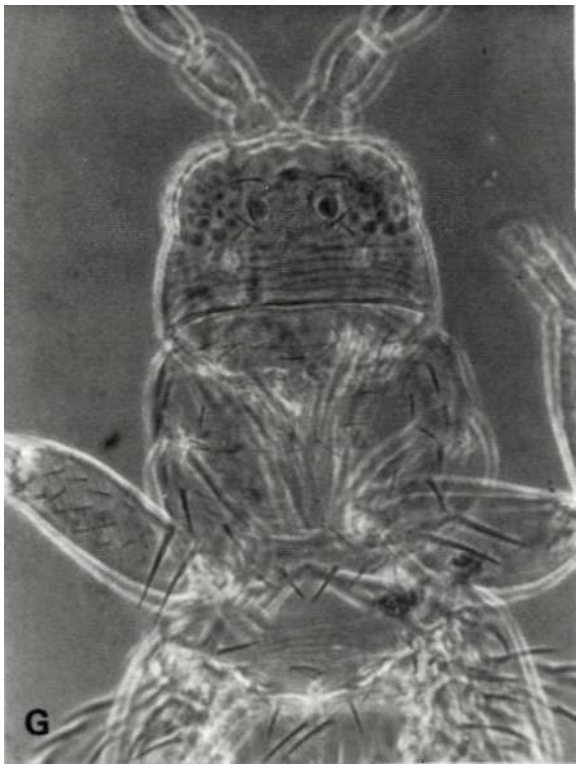


Plate I.

D. *Frankliniella occidentalis:* head and pronotum.
E. *Thrips tabaci:* forewing.
F. *Thrips palmi:* head and thorax.
G. *Thrips palmi:* abdominal tergite II showing four lateral setae.







sometimes appearing entirely brown (dark form) 5

5(4). Comb on tergite VIII incomplete, usually represented by only a few small teeth laterally (Diag. 2B); anteroangular setae usually longer than anteromarginal setae on pronotum (Diag. 2A) .....*Frankliniella schultzei* (Trybom)

Comb on abdominal tergite VIII complete (Diag. 3B); anteromarginal and anteroangular setae on pronotum of similar length (Diag. 3A).....*Frankliniella occidentalis* (Pergande)

Handling thrips in the laboratory

Thrips are relatively simple to maintain under laboratory conditions once the constraints imposed by their small size are overcome. Many authors refer to the need for using the moistened tip of a fine artist's brush for handling individual insects. They can be deactivated by chilling, exposure to carbon dioxide, or gentle pressure from a pad of cotton wool (Sakimura 1961). Sakimura (1961) found that *Emilia sonchifolia, Callistephus chinensis,* and *Stellaria media* were suitable hosts on which to rear *F.fusca* in the laboratory. Any container used for enclosing thrips on living plants must permit the exchange of water vapor (to prevent the accumulation of condensation), without allowing the insects to escape and without interfering unduly with the metabolism of the plant. Lamp glasses have been used to enclose colonies of virus-free thrips breeding on detached groundnut foliage (Amin et al. 1981). This and other techniques, including clip-on sandwich cages, are illustrated by Lewis (1973).

Disease cycle

Both TSWV and the vector thrips have wide host ranges that include crop plants, ornamentals, and weeds. The virus may survive in these hosts and so provide an inoculum source for vector thrips. In India, for example, tomato (*Lycopersicon esculentum*), egg plant (*Solanum melongenum*), mung bean *(Vigna radiata)* and urd bean (*Vigna mungo*) are grown under irrigated conditions during dry-season (summer) months; ornamentals such as zinnia and chrysanthemum are widely grown, and weeds such as *Ageratum conyzoides* and *Cassia tora* «re frequently present in and near groundnut fields. In southern Texas *Verbesina encelioides* (golden crown beard), *Ipomoea trichocarpa* (cotton morning glory), *Lactuca serriola* (prickly lettuce), and *Solanum americanus* (American black nightshade) are often found near groundnut fields. These plants are rarely infected with TSWV and no evidence presently exists to show that they serve as sources of inoculum for agricultural plants. Several other hosts that are either raised as crop plants (e.g., spinach — *Spinacia oleracea*) or survive during dry-season (summer) or postrainy-season (winter) months may act as TSWV reservoirs.

Vector thrips are mainly carried by the wind. The incidence of TSWV in groundnut crops at ICRISAT Center has been related to immigratory flights of thrips by means of suction traps and by counting thrips in young quadrifoliates before they unfold (Reddy et al. 1983). Plants that are reservoirs of TSWV and are the hosts of the thrips play a major part in the spread of the virus. In southern Texas, *F.fusca* reproduces freely on groundnut, and primary TSWV infections occur at varying levels throughout the season. It is believed that much of the incidence of this disease in Texas is due to subsequent spread (secondary infection) within crops.

Climatic factors that favor multiplication and spread of the vector thrips are likely to result in the spread of the disease. Reddy et al. (1983) found that a wind velocity of 10 km h^{-1} at 3 m above the crop canopy was more conducive to mass flights of thrips than higher wind velocities (10-15 km h^{-1}). Most migration occurred when air temperatures were in the range of 20-35°C, and there were no flights when the temperature exceeded 40°C. Relative humidity was thought to be of less importance than wind speed or air temperature in determining thrips migration because flights were detected when the relative humidity varied between 13 and 86%.

Evidence is overwhelming that TSWV is not seed-transmitted either in groundnut (Reddy et al. 1983; P. Sreenivasulu and J.W. Demski, University of Georgia, Griffin, GA, 30224, personal communication) or in other legumes (Reddy and Wightman 1988). Thus, seed is not likely to provide a primary source of inoculum, and the virus has little, if any, quarantine significance.

Disease management

BND can reduce yields of groundnut grown under high- or low-input conditions. There are methods of limiting the losses caused by BND that are suited to marginal farmers. The key lies in developing an understanding of the vector and in sound crop management.

Contemporary pest management depends on the integration of four areas of activity — insecticide application, crop management (cultural practices), biological control, and host-plant resistance. Since the disease is not seedborne, legislation in the form of quarantine regulations will not help in preventing its spread to BND-free areas.

The most economical way to control BND, as for most other virus diseases, is to provide the farmer with resistant cultivars.

Use of insecticides

As the feeding activities of the vector thrips cause conspicuous damage to the foliage of the host, they have had pest status designated to them independently of their ability to transmit TSWV. This means that they have received the attention of entomologists who have established chemical control procedures for them. This is especially so in the case of *F. fusca* on groundnut in the USA. Successful control using synthetic insecticides has been practiced for more than 40 years. Many, but not all, will kill thrips.

A common feature of the recommendations for the chemical control of these insects is that though thrips are small insects, the amount of pesticide applied per application is not greater than the amount applied for most other insects.This is presumably because their cryptic habit allows them to avoid direct contact with insecticides, unless they are moving between feeding sites at the time of application. Their high potential for mass immigration also implies that the insecticide treatment needs to have a strong residual effect to avoid reinfestation (Lewis 1973).

Amin (in Wightman and Amin 1988) provided evidence that high and frequent doses of dimethoate have to be applied to groundnuts in India before there is any reduction in the incidence of BND. 'Low' rates (100 g a.i. ha^{-1} at 7- or 10-day intervals) induced higher levels of BND incidence than the control (no spray) or 400 g a.i. ha^{-1} at 3- or 5-day intervals.

However, preliminary observations from trials conducted in Texas indicate that the control of thrips using systemic insecticides reduced BND incidence. Granular formulations of thiofanox, aldicarb, acephate, and disulfoton gave significant thrips control; they are usually

Table 1. Insecticide recommendations to reduce BND in south Texas groundnuts.

Insecticide	Time to apply	Rate (kg ha^{-1})	Comments
Granulate Insecticide Soil Application			
Di-Syston 15G	At sowing, and at	10	Apply in a band and incorporate.
	21 days after sowing	10	Apply over top of row in band and irrigate lightly afterwards.
	Also at 42 days after sowing (if BND has been observed)	10	Apply over top of row in a band and irrigate lightly afterwards.
Temik 15G	At sowing, and at 21 days after sowing	8	Apply in a band and incorporate.
		8-11	Apply over top of row in a band and irrigate lightly afterwards.
Temik 15G	At sowing, and at 21 days after sowing	8	Apply in a band and incorporate.
Di-Syston 15G	42 days after sowing (if BND has been observed)	10	Apply over top of row in a band and irrigate lightly afterwards.
Foliar Insecticide Application			
Orthene	10-day intervals (being at 21 days after at-plant application of granular insecticide)	0.85	Three to four applications will be required.

applied at sowing. Thereafter, the application of systemic insecticides at fixed time-intervals is recommended (Table 1). It is worth noting that insecticides failed to avert severe losses to groundnut crops in Texas during the 1986 epidemic, presumably because the insecticides were not applied when required. Additionally, leaf-feeding caterpillars and spider mites became problems in some insecticide-sprayed fields.

Cultural practices

Adjustment of sowing dates

Thrips characteristically engage in mass migratory flights. These flights have more or less the same pattern every year in locations where the weather events are temporally consistent. This means that if a farmer can sow well before thrips are expected to invade, his crop will be well advanced when infestation by immigratory vectors occurs.

For instance, at ICRISAT Center, groundnut crops sown at the onset of the rains in mid- to late-June have a much lower rate and intensity of attack than crops sown 1 or 2 months later. The main vector flights usually occur in July and August in this part of Andhra Pradesh. Early-sown plants are sufficiently developed to withstand the infestation by migratory thrips (Reddy et al. 1983). Clearly, extension officers should be in a position to advise on the best time to sow with reference to vector flights in groundnut-growing areas that are prone to BND.

Plant density

Groundnut crops produce the best yields when they are sown at the correct density — one that gives canopy closure in the shortest time. This involves sowing good-quality seed that has been treated to avoid stand thinning by endemic pests and diseases. It was found at ICRISAT that the incidence of BND in crops that were sown at low densities was proportionately higher than in those sown at high densities.

Intercropping

Intercropping one row of a fast-growing cereal, such as sorghum or pearl millet, with three rows of groundnut can reduce the incidence of BND.

Elimination of alternative hosts

The elimination of weeds that are the primary source of TSWV inoculum from the vicinity of groundnut fields will reduce BND incidence. This was achieved in Australia where *Tagetes* sp and *Bidens pillosa* were the primary hosts concerned (Saint-Smith et al. 1972). Destroying alternative weed hosts in the context of a small-scale farm in the tropics is not a practical measure, because TSWV can infect several species of crop, and ornamental and weed plants (Reddy and Wightman 1988). Thus this option should be given low priority.

Roguing

The removal of infested plants from the field is not recommended as this would reduce the stand density and may lead to increase in percentage of infected plants. Furthermore, if infested plants are carried out of the field, viruliferous thrips are likely to drop onto healthy plants.

Host-plant resistance

If farmers in BND-prone areas could be supplied with agronomically acceptable cultivars that are resistant to TSWV, this disease would no longer be a problem. Unfortunately, despite intensive efforts over a number of years to detect resistance, none of the 7000 *Arachis hypogaea* genotypes tested has proved to be resistant. However, tolerance to the virus has been detected and is currently being evaluated in India and the USA.

The screening procedure at ICRISAT is to sow rows of the test material late and at low density to maximize disease incidence. This has led to the identification of a number of genotypes with consistently lower BND incidence (Amin 1985) than those of susceptible control cultivars. This would be termed 'field resistance' by Cooper and Jones (1983) and is a result of resistance to the vector (RW. Amin and K.N. Singh, ICRISAT, personal communication). Nevertheless some tolerance to TSWV might also be involved (E. Scholberg, A.S. Reddy, S.L. Dwivedi, D.V.R. Reddy, and S.N. Nigam, ICRISAT, per-

Table 2. ICRISAT groundnut cultivars with field-resistance to TSWV and good agronomic characteristics.

ICGV 86029	ICGV 86031	ICGV 86033
ICGV 86030	ICGV 86032	ICGV 86538

sonal communication). In 1990, this was ICRISAT's best offer to farmers of the semi-arid tropics whose groundnut plants were affected by BND. Our breeders have a number of varieties with field resistance to TSWV (Table 2).

Biological control

The slow-moving, soft-bodied thrips larvae are easy prey for many predators, including anthocorid bugs (e.g., *Orius* spp), mirids, wasps, ladybirds, syrphids, spiders, and predatory thrips. Thrips larvae are parasitized by minute wasps of the family Eulophidae, and the eggs by the even smaller Trichogrammatidae and Mymaridae (Lewis 1973).

At ICRISAT at least one vector species, *Scirtothrips dorsalis,* is parasitized. It is suspected that the apparent inefficiency of insecticides for the control of thrips and of BND may be due to the elimination of the natural enemies of the vector thrips by the insecticides. However this aspect requires further study.

Conclusions

Precise identification of the causal agent is essential prior to embarking on a program to control any virus disease. Fortunately, the precise and rapid methods that are available for detecting TSWV can be utilized by research workers who do not have access to advanced virology laboratories. Considerable progress has also been made in providing information for control packages that can be integrated with the needs of a wide range of farming 'styles'. Although the research is not complete, it provides the background needed for extension workers to help their clients and to act as a stimulus to national programs to solve the specific problems created by local conditions.

References

Amin, P.W. 1985. Apparent resistance of groundnut cultivar Robut 33-1 to bud necrosis disease. Plant Disease 69:718-719.

Amin, P.W., and Palmer 1985. Identification of groundnut thysanoptera. Tropical Pest Management, 31:286—291.

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 disease of peanut by *Scirtothrips dorsalis* and *Frankliniella schultzei.* Plant Disease 65:663-665.

Hobbs, H.A., Reddy, D.V.R., Rajeshwari, R., and Reddy, A.S. 1987. Use of direct antigen coating and protein A coating ELISA procedures for detection of three peanut viruses. Plant Disease 71:747-749.

Cooper, J.I., and Jones, A.T.I983. Letter to the editor. Phytopathology 73:127.

Lewis, T. 1973. Thrips: their biology, ecology and economic importance. London and New York: Academic Press. 349 pp.

Reddy, D.V.R., and Wightman, J.A. 1988. Tomato spotted wilt virus: thrips transmission and control. Pages 203-220 *in* Advances in disease vector research, Vol. 5 (Harris, K.F., ed.). New York, USA: Springer Verlag.

Reddy, D.V.R., Amin, P.W., McDonald, D., and Ghanekar, A.M. 1983. Epidemiology and control of groundnut bud necrosis and other diseases of legume crops in India caused by tomato spotted wilt virus. Pages 93-102 *in* Plant virus epidemiology (Plumb, R.T., and Thresh, J.M., eds.). Oxford, UK: Blackwell Scientific Publications.

Saint-Smith, J.H., McCarthy, G.J.P., Rawson, J.E., Langford, S., and, Colbran R.C. 1972. Peanut growing advisory leaflet no. 1178. Insecticide Division of Plant Industry, Department of Plant Industry, Department of Primary Industries, Australia. 18pp.

Sakimura, K. 1961. Techniques for handling thrips in transmission experiments with the tomato spotted wilt virus. Plant Disease Reporter 45:766-771.

Verkleij, F.N., and Peters, D. 1983. Characterization of defective form of tomato spotted wilt virus. Journal of General Virology 64:677-686.

Wightman, J.A., and Amin, P.W. 1988. Groundnut pests and their control in the semi-arid tropics. Tropical Pest Management 34:218-226.

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