TRANSMISSION AND ECOLOGY OF *Thrips palmi* Karny, THE VECTOR OF PEANUT BUD NECROSIS VIRUS

BY

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CERTIFICATE

Ms. K. Vijaya Lakshmi has satisfactorily prosecuted the course of research and that the thesis entitled **TRANSMISSION AND ECOLOGY OF *Thrips palmi* Karny, THE VECTOR OF PEANUT BUD NECROSIS VIRUS** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

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This is to certify that the thesis entitled **"Transmission and ecology of *Thrips palmi* Karny, the vector of Peanut Bud Necrosis Virus"** submittd in partial fulfillment of the requirements for the degree of **'DOCTOR OF PHILOSOPHY IN AGRICULTURE'** of the Andhra Pradesh Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Ms. K. Vijaya Lakshmi** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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CONTENTS

LIST OF ILLUSTRATIONS

LIST OF PLATES

LIST OF TABLES

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DATE: 18 July 1994

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DECLARATION

I, **K. Vijaya Lakshmi**, hereby declare that the thesis entitled "**TRANSMISSION AND ECOLOGY OF *Thrips palmi* Karny, THE VECTOR OF PEANUT BUD NECROSIS VIRUS**" submitted to ANDHRA PRADESH AGRICULTURAL UNIVERSITY for the degree of **Doctor of Philosophy in Agriculture** is a bonafide record of work done by me during the period of research at ICRISAT, Patancheru. This thesis has not formed in whole or in part, the basis for the award of any degree or diploma.

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Date: 18 July 1994

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Abstract

Investigations undertaken on the transmission of three thrips species of groundnut viz., *Thrips palmi, Frankliniella schultzei* and *Scirtothrips dorsalis* under laboratory conditions at ICRISAT Asia Center from 1990 to 92 showed that only *T. palmi* could transmit peanut bud necrosis virus (PBNV) of groundnut in India. *T. palmi* could acquire the virus in the larval stage, but only transmit it as adults. Male and female adults transmitted the virus with equal efficiency. Maximum transmission (100%) was recorded when 10 adults were released per plant. *T. palmi* failed to transmit an isolate of PBNV which was maintained continuously by mechanical inoculation for two years under glass house conditions. *T. palmi* larvae acquired the virus in 5 minutes acquisition access period. The adults needed a minimum of 1 hour inoculation feeding period to transmit the virus. The majority of the adults have transmitted the virus for more than half of their life period and the virus was found to be persistent in the vector. The optimum temperature for rearing the vector was 25°C. Cowpea was found to be the best host for rearing and multiplication of *T. palmi* under laboratory conditions.

Field studies conducted at Rajendranagar on PBND resistant/susceptible groundnut genotypes during the rainy seasons of 1991 and 92 indicated that PBND susceptible genotype (JL 24) had more number of *T. palmi* than field resistant genotypes i.e ICGV 86031, ICGV 86388 and ICGV 86430. Crop plants such as blackgram, greengram, cowpea and soybean, most of the cucurbitaceous vegetables and some of the most common weeds in groundnut fields (*Parthenium histerophorus, Cassia tora, Ageratum conyzoides and Commelina bengalensis*), harboured large numbers of *T. palmi*.

INTRODUCTION

CHAPTER I

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the principal crops of the world which is cultivated on 20.4 million hectares. In India groundnut is grown on 8.5 million hectares contributing to 55 per cent of the total oil seeds production. Though India ranks first in the world both in area and total production, it ranks eighth in productivity (700-800 kg ha^{-1}), which is below the global average production (1000 kg ha^{-1}) (FAO, 1992).

Several biotic and abiotic stress factors are known to be responsible for low productivity of groundnut in India. Insect pests contribute to the yield loss both directly as crop pests and indirectly as vectors of viral diseases.

Groundnut is attacked by many viral diseases in India. Of them peanut bud necrosis disease (PBND) is considered to be the most important one. PBND was first reported from India by Reddy *et al.* (1968) on groundnut. Causal agent of PBND was reported to be tomato spotted wilt virus (TSWV) (Ghanekar *et al.*, 1979). However recently it has been shown that the causal agent of PBND in India is a distinct tospovirus, named as peanut bud necrosis virus (PBNV) (Reddy *et al.*, 1992).

The losses caused by PBNV were estimated to be 89 million dollars (ICRISAT Medium Term Plan, 1994-1998). Losses in yield resulting from PBND infection in the groundnut crop depend mainly on the level of incidence and severity of symptoms. If the infection occurs on young plants (less than 2 months old), they do not produce any pods. Yield losses decrease with delayed infection, plants infected nearing to maturity do not suffer much loss.

In India until 1977, the vector of PBNV was unknown. Preliminary studies made by Amin *et al.* (1978) showed that the causal virus of PBND (regarded as TSWV at that time) was transmitted by *Scirtothrips dorsalis* Hood. In 1981 Amin *et al.*, have reported *Frankliniella schultzei* Tribom as the most efficient vector. However Palmer *et al.* (1990) discovered the presence of *Thrips palmi* Karny in groundnut and suspected its involvement in the transmission of PBNV on groundnut in India.

Although rapid progress was made in India in the identification of insect vectors belonging to families Jassidae (14 viruses/Mycoplasma-like organisms), Aphididae (48 viruses), Aleyrodidae (18 viruses), Tingidae and Psyllidae (one virus each) (Rayachaudhari, 1977), thrips transmission was not studied thoroughly. Undoubtedly the small size of the thrips was one of the main reasons for slow progress in research on thrips as vectors of plant viruses.

For an effective management of PBND in the field it is essential to know the principal thrips vector, its relative abundance, ecology and the role of alternate

host plants in perpetuating the inoculum (Mitchell and Smith, 1991). The present study was undertaken to

- identify the principal thrips vector(s) of PBNV in India.
- determine the efficiency of PBNV transmission by various thrips which occur on groundnut.
- determine PBNV virus-vector relationships utilizing the principal thrips vector(s) (acquisition access period, inoculation access period, latent period and virus retention in the vector).
- apply serological methods for PBNV detection in the principal thrips vector.
- study the biology of the principal thrips vector (identification of alternate host plants which support multiplication).

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 THRIPS VECTORS OF TSWV

Tospoviruses are known to be transmitted by thrips. Pittman (1927) was the first to establish that onion thrips, *Thrips tabaci*, transmitted TSWV in tomato. Seven other thrips species including: *Frankliniella fusca*, *F. occidentalis*, *F. schultzei*, *Scirtothrips dorsalis*, *T. palmi*, *T. setosus* and *F. tenuicornis* have been established as vectors of tospoviruses (Kormelink, 1994)). Recent work on groundnut in India (Palmer *et al.*, 1990) indicates that *Thrips palmi* can be a vector of PBNV.

The currently known thrips vectors of TSWV and thrips involved in the transmission are listed in Table 1.

2.2 THRIPS VECTORS OF PBNV OF GROUNDNUT IN INDIA

PBND is the most important viral diseases of groundnut in India. Until 1977, the vector involved was unknown. Amin *et al.*, (1978) reported *S. dorsalis* as the vector involved in the transmission of the causal virus of PBND. This was confirmed by Ghanekar *et al.* (1979). Later Amin *et al.* (1981) reported the transmission of TSWV by *S. dorsalis* and *F. schultzei* which were considered at that time as the causal agent of bud necrosis disease of groundnut. *S. dorsalis* found to

Table 1. Thrips vectors of tomato spotted wilt virus (TSWV) in different host plants

Host crops	Vector	Reference
Tomato	*Thrips tabaci*	Pittman (1927)
Truck crops and ornamentals	*Frankliniella occidentalis* *T. tabaci*	Gardner *et al.* (1935)
Tomato and lettuce	*F. occidentalis* *F. fusca*	Sakimura (1961)
Tobacco	*F. fusca*	Sakimura (1963)
Aster, emilia and tomato	*F. fusca*	Paliwal (1974)
Tobacco	*T. tabaci tabaci*	Zawirska (1981)
Greenhouse crops	*F. occidentalis*	Stenier and Elliott (1983)
Dahlia, *Lycopersicon*, *Sinnigia* and *Tagetis* spp	*F. occidentalis*	Allen and Broadbent (1986)
Greenhouse crops	*F. occidentalis*	Broadbent *et al.*(1987)
Watermelon	*Thrips palmi*	Iwaki *et al.* (1988)
Guinea impatiens, gloxinia, tomato, cineraria, cyclamen, begonia, marigold, ageratum, dahlia, calendula and primrose	*F. occidentalis*	Matteoni *et al.* (1988)
Groundnut	*F. occidentalis*	Stewart *et al.* (1989)
Cineraria, calceolaria, salvia, capsicum and *Galinsoga parviflora*	*F. occidentalis*	Gofflot and Verhoyen (1990)
Anemone coronaria, Ranunculus asiaticus, Eustoma grandiflorum, pepper and tomato	*F. occidentalis*	Lisa *et al.* (1990)

Host crops	Vector	Reference
Pepper, tomato, egg plant, broadbean, lettuce, basil, chrysanthemum, aster, New guinea, anemone and gloxinia	*F. occidentalis*	Marchoux *et al.* (1991)
Groundnut	*F. schultzei* *F. occidentalis*	Mulder *et al.* (1991)
Grass-pea	*F. occidentalis*	Rogijo and Habers (1992)
Watermelon	*T. palmi*	Shyi-dong Yeh *et al.* (1992)
Weeds and native plants of Canada	*F. occcidentalis*	Stobbs *et al.* (1992)
Tomato and capsicum	*F. occidentalis*	Verhoeven and Roenjohrst (1992)
Capsicum annum and *Sonchus asper*	*F. fusca*	Hobbs *et al.* (1993)

be a less efficient vector than *F. schultzei*. Palmer et al. (1990) discovered the occurrence of *T. palmi* in groundnut in India and suspected its involvement in the transmission of PBNV.

2.3 TECHNIQUES FOR HANDLING THRIPS IN TRANSMISSION EXPERIMENTS

2.3.1 Rearing Methods

It is often necessary to rear thrips under controlled environmental conditions in the laboratory to study life histories, confirm observations made on their behaviour in the field and for virus transmission experiments (Sakimura, 1961). Several authors have described different techniques to rear thrips under laboratory conditions.

In earlier experiments (Samuel *et al.*, 1930) a small tube-spring clip cage for restricted feeding and a lamp globe cage for mass rearing of *T. tabaci* were used. The insects were handled with a camel hair brush in a specially constructed small transfer room. Linford (1932) used a jar or celluloid-covered cage for rearing thrips. He maintained the stock colonies in a special insect cage constructed of galvanized iron, clear sheet pyraline and a heavy grade of white cotton broad cloth over pea seedlings grown in gallon canners tins of soil. The surface of the soil was then sealed around the plant stems with wax pouring. Under these conditions thrips reproduced rapidly on peas. A specially designed aspirator and small transfer

chambers were used for handling the insects.

Smith (1932) used a microcage for restricted feeding. Wright and Blodgett (1948) employed a sticky ring barrier and a micro sandwich cage made of foam rubber for feeding the thrips larvae on detached leaves.

Sakimura (1961) used a small plastic cylindrical cage made with plastic sheeting for rearing of thrips. A hole cut covered with dacron was provided at the top, rolled into a cylinder. To transfer the thrips into the cage, they were first collected in to a small vial and then placed in the cage which was held upside-down. The cage containing thrips was quickly put over the plant and pushed finally into the soil. Transfer may also be effected by placing the vial near the plant and covering it with the cage. For removing thrips from the cage, the plant was first cut off at ground level and placed over black or white paper on which live insects were collected with aspirator.

Modifications to the sandwich cage were made by Sakimura (1961). It consists of a felt cage made of two small pieces of plastic sheeting, a central cut out and a stationary clip. For introducing the insect in to the cage, the top plastic piece was slid partly aside and the insect was introduced in the cut-out opening with a moistened brush. For removing the thrips from the cage, an aspirator was inserted into the central opening before the cage was disassembled.

Thrips were successfully reared on detached plant parts (Sakimura, 1961). Erlenmeyer flasks (125 ml) and glass or lusteroid tubes were used for cages. Both ends of the tubes and mouths of the flasks were covered with dacron pieces. After the insects were dropped into the cage, a small piece of food and a piece of blotting paper were inserted and then opening was covered. Cabbage midribs were found suitable for rearing *T. tabaci*.

2.3.2 Methods for Acquisition Access Feeding (AAF)

Plant species used in the AAF experiments should provide required food and adequate amounts of virus to the larvae.

Wright and Blodgett (1948) reported a sticky ring barrier for AAF of larvae on floating detached leaves.

Sakimura (1961) developed three different techniques for AAF by larvae on the diseased plant. The first was mass rearing of an infective colony, the other two involve restricted feeding over a specific leaf area during a specific length of time. For mass rearing of viruliferous thrips, the youngest two or three leaves of a young emelia plant were twice sap inoculated with virus. One or two days after inoculation, 2 or 3 females were introduced in to the cage. The progeny larvae hatching 4 to 5 days later were forced to feed on the sap inoculated leaves. Viruliferous adults from this progeny were used for transmission studies.

The felt cage method used for rearing the thrips was found to be satisfactory for restricted AAF. Another useful technique developed by Sakimura (1961) was the sticky barrier method which confines the feeding within a sticky ring barrier on a floating leaf.

Amin (1980) developed a simple technique for AAF. Leaflets showing good symptoms were floated on water in a Petri dish and about 10-15 first instar larvae were released on each leaflet. After required AAF, the larvae were transferred to small glass vials containing healthy leaflets until they became adults. These adults were used in transmission tests.

2.3.3 Methods of Inoculation Access Feeding (IAF)

Both routine and restricted feeding methods were used for IAF by Sakimura (1940). For routine feeding, a young individual plant with five to six leaves covered with a cylinder cage was used. A felt cage was found to be suitable for restricted feeding.

Amin (1980) used two methods for inoculation feeding. In the first method, one or two virus exposed adults were transferred onto a seedling with camel hair brush and the plants were covered with lantern globes. A thick moist cloth was used for covering the mouth of the lantern globe. The felt cage method developed by Sakimura (1940) was not successful due to the small size of groundnut leaflets. Therefore, for restricted feeding, Amin developed a new technique in which the

whole leaflet was inserted into a small glass vial containing thrips. The open end, through which the leaf was inserted, was closed with a cotton plug. The glass vial was supported with sticks and fastened with rubber bands.

Serial transmission studies involving transfer of a single insect require complicated manipulations of the test insects. Consequently, loss of insects may sometimes occur. Regardless of the methods used, the escape of viruliferous insects into the laboratory or greenhouse may have serious consequences. To minimise such accidents, transfers should be made during the cooler periods of the day in a small compartment or separate room (Sakimura, 1961). A felt cage method was used for serial transfers of viruliferous insects by Sakimura (1940).

Amin (1980) used very young plants closed in test tubes (0.8 x 2.5 cm) for serial transfers of individual thrips. Inspite of the great care, working with whole plants created problems in locating thrips. Mortality of the thrips was also high.

2.4 VIRUS-VECTOR RELATIONSHIPS

To become viruliferous, thrips must feed on infected plants during their larval stages. But once they became viruliferous, both larvae and adults can transmit the virus (Bald and Samuel, 1931; Linford, 1932). Later, Black (1954) reported that virus transmission occurs only when thrips feed on infected plants in the larval stage and only such larvae can transmit the virus as adults.

Adults apparently cannot acquire the virus. This was observed in *T. tabaci* in Hawaii, England and Russia (Linford, 1932; Smith, 1932, Razvyazkina, 1953), *F. schultzei* in Australia, South Africa and India (Bald and Samuel, 1931; More, 1933, Reddy *et al.* 1983); and *F. occidentalis* in California (Bailey, 1935).

Data on AAF are limited. A minimum feeding of 30 minutes (min) was observed for *T. tabaci* (Razvyazkina, 1953), whereas Sakimura (1961) observed a minimum acquisition access period of 15 min for nymphs of *T. tabaci*. The larvae of *F. schultzei* required a minimum AAF of 30 min but the frequency of transmission increased with longer AAF, up to 48 hours (h) (Reddy *et al.*, 1983). Cho *et al.* (1991) reported that TSWV could only be acquired by larvae of thrips after a minimum AAF of few min. However, transmission efficiency increased with concomitant increase in the AAF.

The minimum inoculation access feeding (IAF) determined with *T. tabaci* was 5 min (Razvyazkina, 1953). In Hawaii it was 15 min with *T. tabaci* (Sakimura, 1962).

In general a latent period of several days was observed between the time of virus acquisition and its transmission to healthy plants. Bald and Samuel (1931) observed a minimum latent period of 5 days for *F. schultzei*, 5 and 10 days for *T. tabaci* (Smith, 1932; and Linford, 1932), and about 10 days for *F. occidentalis* (Bailey, 1935). Sakimura (1960, unpublished data) found a minimum latent period of 4 days and 18 days maximum with an average of 11 days for *T. tabaci* in Hawaii

and a minimum of 4 days and a maximum of 12 days with an average of 11 days latent period was observed for *F. fusca* in New Jersey. In Russia a minimum of 3 days was recorded for *T. tabaci* (Razvyazkina, 1953). In all cases where the latent period was minimum, successful inoculation was made by larvae before pupation. All the longer durations registered in these reports occurred when the latent period was completed after the emergence into the adult stage.

The virus retention period was apparently erratic in its pattern. In some cases it extended over the entire life time, in other cases, it lasted for a short period, ending long before the insect's death. In some cases, infectivity continued for a long period and in some insects, the insects transmitted the virus for shorter periods with a long non-transmission period in between. (Sakimura, 1962). He suggested that this inconsistency of the retention period was due to the variation in the initial amount of the virus acquired by different insects. Ananthakrishnan (1973) and Cho *et al.* (1991) reported that the virus was retained by the vector throughout the life period and found the erratic transmission of the virus by the vector.

Smith (1932) and Linford (1932) recorded a 5 and 10 days minimum retention period respectively for *Thrips tabaci* whereas Bailey (1935) observed a period of about 10 days for *F. occidentalis.* A maximum latent period of 22 to 24 days for *F. schultzei* in Australia (Samuel *et al.*, 1930; Bald and Samuel, 1931), 30 days for *F. occidentalis* in California (Bailey, 1935) was observed. In Russia the retention period for *T. tabaci* was stated as whole life (Razvyazkina, 1953), whereas

it was 30 days for *T. tabaci* in Hawaii and 43 days for *F. fusca* in New Jersey (Sakimura, 1960 unpublished data). Though TSWV could persist in the insect vector for extended periods, little was known about how it survived or whether the virus was transmitted in a circulative or in a propagative manner (Reddy and Wightman, 1988). As the retention by the thrips vector was inconsistent, it has been assumed that TSWV was circulative (Sakimura, 1962, 1963). Nevertheless Best (1968) considered this aspect to be unresolved.

Paliwal (1976) indicate that the TSWV titer decreased after adult thrips have been fed for two weeks on a susceptible host. Cho *et al.* (1987) reported that the virus may multiply in the vector.

Recently, enzyme-linked immunosorbent assay (ELISA) of individual thrips, hybridization of cDNA probes specific to viral and viral complementary strands to thrips RNA extracts, and electron microscopic observations of thrip's organs have provided indirect evidence that tospoviruses may replicate in thrips (Cho *et al.* 1991, German *et al.* 1991 and 1992). More recently Ullman *et al.* (1993) detected the presence of non-structural proteins encoded by the SRNA of TSWV in *F. occidentalis*. TSWV non-structural protein can only be observed when virus replication occurs. Thus the evidence presented shows that TSWV replicates in the cells of the thrips vector species.

2.5 EPIDEMIOLOGICAL STUDIES

2.5.1 Epidemiology of Tospoviruses relative to Thrips Populations

For an effective management of the diseases caused by tospoviruses in different crops, it is essential to understand the interrelationships between crop, virus and the principal thrips vector involved in disease spread. The population density of thrips and their transmission efficiency are expected to contribute to the incidence of the diseases caused by tospoviruses (Reddy and Wightman, 1988). The incidence of peanut bud necrosis disease in India has been correlated with the population density of the principal thrips vector, assumed to be *F. schultzei*, in the rainy (June-September) and postrainy (January-March) seasons in Hyderabad by Reddy *et al.* (1983). The *F. schultzei* population density was low until the second week of July but increased rapidly to reach a maximum in the last week of August and early September. By mid-September populations on the crop declined sharply. The maximum disease incidence, which ranged from 50 to 100%, occurred in the rainy season, 2-3 weeks after the maximum number of *F. schultzei* was recorded. In the postrainy season crop in the Hyderabad region (18°N 78°E) disease incidence was only 20-30%. In the postrainy season crop, the maximum number of *F. schultzei* occurred in January and February and most of the new PBNV infections were observed in February. *F. schultzei* populations declined sharply from March to July.

Cho *et al.* (1984) found a high correlation between *F. occidentalis* population density and TSWV incidence in lettuce. Kobatake (1984) also found a high correlation between thrips densities and TSWV incidence in lettuce. However, a high incidence of TSWV in Louisiana was not associated with the occurrence or abundance of any particular thrips species (Black *et al.* 1986).

Tospovirus diseases and thrips species surveys were conducted on lettuce farms at Maui, Hawaii during 1981-84 (Cho *et al.*, 1987). Three known insect vectors of TSWV were found: *F. occidentalis, F. schultzei,* and *T. tabaci. F. occidentalis* was the predominant species at both low and middle elevation farms. There were significant correlations between the mean number of *F. occidentalis* trapped per week and TSWV incidence in lettuce.

Joi and Summanwar (1991) studied the effect of seasonal variation on thrips population and its subsequent effect on TSWV incidence in tomato and groundnut. The thrips were found to be more or less similar in all seasons. However, the incidence of TSWV in groundnut and tomatoes was the highest in rabi (20.8 and 22.3%) followed by summer (17.6 and 20.3%) and kharif (4.8 and 18.6%). The thrips population density was 26 per groundnut plant and 14 per tomato plant. Early season abundance of tobacco thrips (*Frankliniella fusca*), incidence and disease progress of TSWV were compared in florunner and southern runner groundnut cultivars in Georgia, USA. In replicated plot experiments and large field quadrate studies, population densities of tobacco thrips adults and larvae were

similar for the two cultivars. In all experiments, the incidence of spotted wilt progressed linearly in both cultivars. Linear regression of disease incidence over-time indicated higher disease levels in florunner than in southern runner. The final apparent incidence of spotted wilt in florunner was approximately twice as high as that in southern runner (Culbreath *et al.*, 1992).

2.5.2 Thrips Species Composition and Relative Abundance

Doutt (1940) made field observations on the seasonal development of thrips populations in tomato fields. The predominant species were *T. tabaci* and *F. occidentalis*. *T. tabaci* reached high populations earlier in the spring and persisted till summer and fall. *F. occidentalis* maintained high population density from May to August. Sakimura (1961) studied the population status of thrips species on tomato and lettuce. On tomato, 199 *F. occidentalis* and 550 *T. tabaci* were counted in nine different samples, the former predominating on flowers, the latter on leaves. On lettuce leaves 11 *F. occidentalis* and 169 *T. tabaci* were counted in three samples, showing the predominance of the latter species.

Studies on seasonal population densities of tobacco thrips, *F. fusca* (Hinds) in terminal buds and flowers of florunner peanut, were carried out in 1977-78 (Tappan and Gorbet, 1979). Populations in buds and flowers comprised of 90% larvae and 92% adults respectively. The ratio of larvae to adults indicated no mass movement of immatures from buds to flowers after flowering began 23-31 days after planting. The density increased during the first 31 days of plant growth and

steadily declined thereafter. The mean number of thrips per bud in the initial counts ranged from 0.7-1.1 and 1.0-1.2 for 1977 and 1978 respectively.

Johnson (1986) studied the population trends of *T. palmi* on commercial watermelon plantings. Eight plantings were surveyed weekly for thrips on foliage and vine tips. Generally larvae composed of the greater proportion of the population and the adults were lower than four per leaf. In the spring plantings surveyed, peak thrips densities varied from 2.5 thrips per leaf on May to 53.6 thrips per leaf on June. In the summer plantings, peak thrips densities varied from 2.7 individuals per leaf in September to 27.0 individuals per leaf in August.

Su *et al.* (1985) found an increase in the population density of *T. palmi* on egg plant in late April-May and early in October-November. Su and Chen (1986) reported that on peppers in Hualien, Taiwan, *T. palmi* was dominant (92.01%) over the other thrips species, *F. intonsa, T. hawaiiensis, S. dorsalis, Haplothrips chinensis.* The density of *T. palmi* was highest during July to September on vegetable sponge (*Luffa cylindrica*) and in mid january in cucumber.

Citrus groves in Florida were sampled during 1986-1990 to determine the distribution and abundance of thrips species infesting closed buds and open flowers of citrus. *F. bispinosa* was the dominant species accounting for 92% of collected specimens. *F. kelliae* was the second most abundant species comprising 7% of specimens collected (Childers *et al.*, 1990).

Two tobacco beds and two tobacco fields were sampled every 7-10 days for the presence of thrips (McPherson and Beshear, 1990). Thrips were found on all seven sampling dates and *F. fusca* and *F. occidentalis* were the most common species collected. Most of the *F. occidentalis* were collected later in the season from tobacco blooms. *F. fusca* was commonly encountered on tobacco foliage.

Mitchell and Smith (1991) made weekly sampling in South Texas peanut fields during 1987 and 1988. *F. fusca* was the most common one representing over 80% of the total adult population. *F. occidentalis* was the next most abundant, representing about 18% of the population. Both are known vectors of TSWV. *Microcephalothrips abdominalis* and *F. tritici* constituted <0.5% and <0.1% of the population, respectively.

Mulder *et al.* (1991) identified 23 species of Thysanoptera in a field survey conducted from 9 May to 22 August 1989 in the major peanut growing areas of Oklahoma, USA. More thrips were collected from groundnut flowers than from the terminals. Over 75% were *F. fusca*. *F. occidentalis* accounted for 10% of the total population. Both species were confirmed as vectors of TSWV on groundnut. The largest numbers were collected in early July, which coincided with early flowering and the lowest numbers in late August which coincided with the post flowering period. Sukamto *et al.* (1992) carried out a survey of the thrips vectors of TSWV on groundnut in northwest Mississippi during spring and summer of 1991. The most common species was *F. fusca* followed by *F. tritici* and *F.*

occidentalis. On groundnut *F. occidentalis* was not found until 13 August.

Chamberlin *et al.* (1992) intensively sampled the groundnut plants for thrips during the autumn and spring in Georgia, USA. *F. fusca* adults constituted 60-95% of the samples, with *F. occidentalis* comprising the remainder.

In Alabama, thrips populations on peanuts increased 2 to 4 weeks after seedling emergence and declined sharply after 6 weeks. Feeding damage on peanut seedlings is more obvious at this stage. Once peanuts begin rapid growth and start to bloom (about 40 days after planting on florunner), they usually outgrow thrips damage. The population at this point in the growing season remained low, indicating little reproduction on fast-growing peanuts (Hagan *et al.*, 1992).

Ranga Rao and Wightman (1993) sampled the peanut crop during 1990-1991 at ICRISAT to determine the abundance and distribution of the thrips species complex inhabiting terminals and open flowers. *S. dorsalis* was the dominant species accounting for 72% of foliar thrips. Before flowering *T. palmi* was the second most abundant species (23%) followed by *F. schultzei* (5%). After flower production the foliar samples comprised of 93% *S. dorsalis*, 6% *T. palmi* and 1% *F. schultzei*. In flowers *F. schultzei* was the predominant species (68%), and *S. dorsalis* and *T. palmi* constituted 16% of each of the samples. The population densities of *S. dorsalis* and *T. palmi* were higher during the postrainy season than in the rainy season. Observations made over a two year period clearly indicated that *F. schultzei* was a flower feeder.

2.6 BIOLOGY OF *Thrips palmi*

Palacio (1978) studied the biology of *T. palmi* on watermelon. This species reproduces parthenogenetically and completes development in 13 days on an average. Adults lived for 2-18 days and the fecundity ranged from 6 to 38 eggs. It was found that in southern Taiwan *T. palmi* took 20-30 days to complete one cycle (egg to adult) and at least 10 generations were produced per year on cucurbits (Wen, 1984). Wang and Chu (1986) reported that *T. palmi* needed about 15 to 24 days to complete the immature and adult stage. *T. palmi* was reared under laboratory conditions on pieces of pumpkin leaf (Wang and Chu, 1986). It was found that *T. palmi* needed an average of 3.73 days to develop from egg to adult. The survival of the egg and larval stages was 95% and in the pupal stage it was 67%. About 61% of the eggs developed into adults.

Wang *et al.* (1989) studied the oviposition behaviour of *T. palmi*. The preoviposition period for virgin females was 1-3 days and for mated ones it was 1-5 days. Virgin females laid 1.0-7.9 eggs/day, while mated ones laid 0.8-7.3 eggs/day. Virgin females laid 3-164 eggs during their life span, while mated ones laid 3-204 eggs.

Studies on the reproductive mechanisms of *T. palmi* were also carried out under laboratory conditions (Wang and Chu., 1990). It was found that *T. palmi* reproduced both parthenogenetically and sexually. Under laboratory conditions it required 11.4-11.9 days to develop from egg to adult. Females mating immediately

after emergence produced males during the first 2 days of oviposition. Thereafter females and males were produced at various rates throughout the oviposition period.

2.7 HOST RANGE OF *Thrips palmi*

Palacio (1978) from Taiwan reported *T. palmi* on amaranthus, castor, corn, egg plant, potato, okra, rice, sweet pepper, soybean, winged bean and tomato. *T. palmi* was also found on onion, cotton, avocado, citrus, cowpea, peach, plum, squash, muskmelon, carnation and chrysanthemum. A wide variety of host plants was also reported by many authors from Taiwan (Wen and Lee, 1982; Chen, 1984; Su *et al.*, 1985; Su and Chen, 1986; Wang and Chen, 1987; Huang, 1989; Wang, 1990; Bhatti, 1980; Bournier, 1983). *T. palmi* was found to infest cotton, cucurbits, aubergines, onion, pulses and fruit trees in Philippines (Bournier, 1983).

Nakahara *et al.* (1984) collected *T. palmi* from watermelon, cucumber, cantaloupe, egg plant, chinese spinach, long beans, bush beans, lettuce and cheese weed in Oahu, Hawaii, USA. Bronzing of foliage and total destruction of vine tips in watermelon was reported to be due to the infestation of this crop with large populations of *T. palmi* (Johnson, 1986). *T. palmi* was recorded in Martineque and was found to cause damage to aubergines, beans, sweet pepper, tomato, and cucurbits (Denoyes *et al.*, 1988). Yudin *et al.* (1988) found *T. palmi* on lettuce and five common weeds viz., *Amaranthus hybridus, Bidens pilosa, Malva parviflora, Melilotus officinalis* and *Verbesina encelioides.*

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Huang (1989) recorded peak populations of *T. palmi* in late April and May on waxgourd in Taiwan. Morishita and Azuma (1988) reported peak populations in May on sweet pepper in Japan.

In Trinidad, *T. palmi* was recorded and collected from several crops including cucumber, pumpkin, squash, watermelon, sweet pepper, chillies, tomato, egg plant, cabbage, 'Dak choi', chinese cabbage, cauliflower and Bhagi (a leaf vegetable) (Cooper, 1991). In Malaysia. *T. palmi* was reported on vegetables (Fauziah and Saharan, 1991). Mau *et al.* (1991) reported that *T. palmi* has a wide host range and has become a major pest of cucurbits and certain solanaceous crops. Talekar (1991) gave the following list of host plants attacked by *T. palmi* in Southeast Asia: Mulberrry *(Morus latifolia* Poir), carnation *(Dianthus caryophyllus* L.), spinach *(Spinacia oleracea* L.), pepper *(Piper nigrum* L.), betel *(Piper betle* L.), tea (*Camellia sinensis* (L.) Kuntze), *Brassica* sp., radish (*Raphanus sativus* L.), peach (*Amygdalus persica* L.), apple (*Pyrus* sp.), plum (*Prunus salicina* Lindl.), apple (*Malus sylvestris* Mill.), soybean (*Glycine max* (L.) Merrill), snapbean (*Phaseolus vulgaris* (L.)), broadbean (*Vicia faba* L.), adzuki bean (*Vigna angularis* Wight), mungbean (*Vigna radiata* (l.) Wilezek), white clover (*Trifolium repens* L.), peas (*Pisum sativum* L.), *Oxalis* spp., citrus (*Citrus* spp.), grapes (*Vitis* spp.), cotton (*Gossypium* sp.), okra (*Hibiscus esculentus* L.), cucumber (*Cucumis sativus* L.), watermelon (*Citrullus vulgaris* Schrad.), melon (*Cucumis melo* L.), cucurbits (*Cucurbita* sp.), wax gourd (*Benincasa cerifera* Savi.), balsam pear (*Momordica charantia* L.), gourd (*Luffa* spp.), cucurbit (*Lagenaria siceraria* Stand.), carrot

(*Daucus carota* L.), sweet potato (*Ipomoea batatas* (L.) Lam.), *Perilla frutescens* (L.), eggplant (*Solanum melongena* L.), potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), sesame (*Sesamum indicum* L.), garland chrysanthemum (*Chrysanthemum morifolium* Ram.), dahlia (*Dahlia pinnata* Cav.), lettuce (*Lactuca sativa* L.), sunflower (*Helianthus annus* L.), corn (*Zea mays* L.).

2.8 HOST SUITABILITY STUDIES OF THRIPS VECTORS

Brodsgaard (1989) reported that the developmental time of *F. occidentalis* was greatly influenced by temperature and with different host plants, e.g., at 15°C development time from egg to adult was 44 days on radish leaves and 34 days on bean pods.

Mau *et al.* (1991) reared *F. occidentalis* in no choice test on healthy leaves of several host plants viz., *Amaranthus hybridus* (green amaranth), *Brassica compestris* (cabbage), *Emilia sonchifolia* (Flora's paint brush), *Fagopyrum esculentum* (buck wheat), *Galinsoga quadrimaculata* (peruvian daisy), *Limonium latifolium* (statice), *Lycopersicon esculentum* (tomato), *Nicandra physalodes* (apple of Peru), *Nicotiana tabacum* (tobacco), *Sonchus oleraceus* (sow thistle), *Tropaeolum majus* (nasturtium), and *Verbena litoralis* (verbena). First instar larvae were placed in groups of 5 to 10 on the leaves of each host. Each leaf was enclosed in acrylic sandwich type cages. Insect development was observed at 25°C daily, and leaves were changed at 2- to 3-day intervals. In evaluating the hosts, they found a considerable range in host suitability for *F. occidentalis* larval development.

Between plant species, variation in the larva to adult developmental times was low. Larva to adult duration ranged from about 9 to 12 days. Buckwheat was suitable for larval development. Larval development was poor on other hosts. About 76 per cent of the larvae developed into adults on Buckwheat. Sowthistle, green amaranth, and cabbage comprised the second group with 22.7, 22.5, and 17.7% respectively of the thrips completing their development on these hosts. Nasturtium, peruvian daisy and verbena were unsuitable for *F. occidentalis* development. Only 9.2, 3.3 and 2.5% of the larvae developed into adults respectively on the above hosts. Flora's paint brush, and statice leaves were completely unsuitable for *F. occidentalis* development. No choice tests were conducted at three temperatures (15, 20 and 26°C) on leaves of *Amaranthus lappa* (burdock), *Datura stramonium* ((Jimson weed), *Lactuca sativa* (romaine lettuce), *Malva parviflora* (cheese weed), and *Verbesina encelioides* (golden crown beard) to further evaluate the suitability of the hosts. Lettuce, jimson weed and cheese weed leaves were the most suitable for *F. occidentalis* development at 26°C. Burdock and golden crown-beard leaves were the least suitable hosts. The same relative order of host suitability was observed at 20 and 15°C. They have also partially completed an evaluation of *F. occidentalis* oviposition preference on the above five hosts. Their results suggest that the order of preference for oviposition was Lettuce>burdock> cheese weed>jimson weed>golden crown-beard.

Calilung (1990) reared *T. palmi* on potato, cucumber, egg plant, watermelon, cotton and pepper. She observed the longest average developmental period (15

days) on pepper and shortest (11.2 days) on watermelon. The highest fecundity (15.6 eggs) and longest adult life (17.4 days) were recorded on watermelon. Adult thrips lived for the shortest period on pepper (3.9 days) and did not reproduce. When the thrips were offered a choice among the six crops, they showed a lower preference for cotton and pepper compared with the other four hosts. Potato, egg plant, cucumber, and watermelon were equally preferred.

2.9 DETECTION OF TSWV IN PLANT TISSUES AND THRIPS VECTORS

TSWV has several properties by which it can be readily distinguished from other viruses. Serology has not often been used to identify TSWV in the past. A strong evidence for its identity could be obtained by sap transmission tests to several selected host plants. The use of specific antiserum offers one of the most reliable criterion to identify plant viruses. The conspicuous lack of availability of high quality antisera until recently in the immuno diagnosis of TSWV has been attributed to the difficulties in obtaining sufficient amounts of pure viral antigen (Francki and Hatta, 1981). Thus far three techniques have been used in the serology of TSWV, i.e., gel diffusion test (titers between 1/8 and 1/128) reported by Tsakirides and Gooding, (1972), Joubert *et al.* (1974) and Tas *et al.* (1977b), tube precipitin and ring test (titers between 1/256 and 1/512) reported by Best and Hariharasubramanian, (1967) and Paliwal, (1976). These techniques were not very sensitive for the detection of TSWV. The development of enzyme linked

immunosorbant assay (ELISA) has dramatically increased the potential for the detection of TSWV, both in extracts from infected plants and in thrips. Since ELISA is more sensitive than any other serological techniques it can be readily applied than for TSWV detection (Hobbs *et al.*, 1987).

Gonsalves and Trujillo (1986) purified the lettuce isolate of TSWV and produced an antiserum. The antiserum was found to be effective for detecting TSWV in leaf tissues by sodium dodecyl sulfate agar gel immuno diffusion tests and by direct and indirect ELISA. Hobbs *et al.* (1987) standardized direct antigen coating (DAC) and protein A coating (PAC) forms of indirect ELISA and compared them with the double-antibody sandwich (DAS) form of ELISA for detection of PBNV (it was referred to be TSWV). PBNV was detectable in peanut leaves at a 1:1000 dilution with the DAC method and 1:100 dilution with the PAC and DAS methods.

Cho *et al.* (1988) used ELISA to detect TSWV in individual thrips. TSWV was readily detected in 210 of 391 *F. occidentalis* and 24 of 120 *F. schultzei*. These were laboratory-grown adult thrips and were provided with an acquisition access on infected host plants as larvae. With three insects, six positive transmissions occurred out of seven (86%) ELISA positive *F. occidentalis* groups, and seven occurred out of eight (88%) ELISA positive *F. schultzei* groups. In single insect transmission tests 14 positive transmissions occurred out of 186 (7%) ELISA positive *F. occidentalis* and none occurred out of two ELISA positive *F.*

schultzei. TSWV was detected in 32 of 275 (12%) adults and 233 of 527 (44%) larval thrips removed from TSWV infected lettuce plants.

Sherwood *et al.* (1989) produced a stable hybridoma cell line secreting IgG2b subclass monoclonal antibodies (MAB) for TSWV. It reacted to five isolates of TSWV in various forms of ELISA. Isolated nucleocapsid reacted with the MAB in ELISA but the envelope associated proteins did not. This was the first report of the production and utilization of a MAB to detect TSWV.

Antibodies to electrophoretically isolated 26,000 MW nucleoprotein (26K NP) and 78,000 MW membrane protein (78K MP) of the lettuce isolate of TSWV and for the whole virion were produced by Wang and Gonsalves (1990). They were tested in different ELISA assays. Thirty TSWV isolates were compared by ELISA. All 30 isolates reacted positively in DAS ELISA with antibodies to the whole virion. Nineteen isolates were consistently detected in different ELISA tests using antibodies prepared to the whole virion or to specific structural proteins, while the other 11 isolates were either not detected or were inconsistently detected. Of the 11 isolates that gave inconsistent results, eight were purified and compared by western blots. All the eight isolates reacted similarly to antibodies to the virion and to the anti 26K NP and anti 78K MP.

Marchoux *et al.* (1991) and Cho *et al.* (1991) detected the TSWV in naturally or artificially infected cultivated and weed plants and also in individual

F. occidentalis thrips by using DAS ELISA.

Ronco *et al.* (1989) used complementary DNA (cDNA) probe prepared for viral RNA for TSWV detection. Dot blot assay allowed detection of as little as 2 ng of RNA from purified virions as well as TSWV RNA present in total RNA extracts of infected leaves of *Nicotiana rustica* and in crude sap. Slight modification to the homogenization procedure facilitated TSWV RNA detection in crude extracts of infected tomato plants by the dot blot assay.

Rice *et al.* (1990) used two clones containing 460 and 870 bp derived from RNA for detection. It was possible to detect 16 ng of total RNA from tobacco, 80 ng from tomato and lettuce and 400 ng from chrysanthemum and pepper.

German *et al.* (1991) developed a diagnostic dot blot for TSWV using cDNA probes. This assay was useful for detecting the presence of the virus in individual thrips. Probes were generated and used to determine the time course of appearance of positive and negative strands in plants and to demonstrate the presence of both strands in thrips.

Ullman *et al.* (1993) observed the presence of non-structural small proteins (NSs) encoded by the SRNA of TSWV in thrips vector of *F. occidentalis*. TSWV NSs can only be present following virus replication. A specific and sensitive antibody to the NSs has been produced and used to localize NSs in the cells of *F. occidentalis*. The direct immuno cytochemical evidence presented showed that NSs

were present in thrips cells. This result indicated that TSWV replicates in the cells of the vector.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The present investigations were carried out on the transmission of PBNV by thrips in groundnut. Laboratory studies were conducted at the ICRISAT Asia Center, Patancheru, Andhra Pradesh, and field studies at the Directorate of Oilseeds Research (DOR), Rajendranagar, Hyderabad. They were undertaken during April 1990 to January 1993.

3.1 DEVELOPMENT OF VIRUS FREE COLONIES OF THRIPS

3.1.1 Collection and Identification of Thrips

The cultures of three thrips species viz., *Thrips palmi* (Plate 1), *Frankliniella schultzei*, and *Scirtothrips dorsalis* were initiated with the thrips collected from apparently healthy groundnut plants in the fields at the ICRISAT Asia Center. Large number of terminal leaves or flowers were collected in the early morning hours. They were placed in a plastic jar and covered with a glass funnel attached with a small homeopathic glass vial (3 x 1 cm). The adult thrips crawled along the walls of the funnel and collected in the glass vial. The glass vial was changed at 2 h interval. An aspirator was used for collecting small number of thrips.

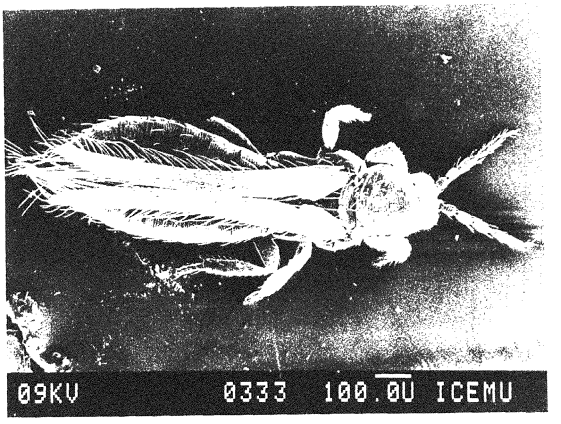


Plate 1: *Thrips palmi*, the vector of Peanut bud necrosis virus

Thrips were immobilized in an aspirator or vial by placing them in a refrigerator for 15 min and were dislodged on to an ice tray. After immobilization by the cold treatment, thrips were sorted to different species (within 15 min) using a stereoscopic binocular microscope. Thrips were identified into different species based on the following key characters (Palmer *et al.*, 1989 and Reddy, *et al.*, 1991) (Plate 2).

Identification Features of Thrips

Frankliniella schultzei	*Thrips palmi*	*Scirtothrips dorsalis*
Adult female pale in colour, 1 mm long	Adult female straw yellow to pale brown, 0.9 mm long	Adult female relatively small, yellow in colour, 0.7 mm long
Pronotum with 2 pairs of setae on the anteriolateral margin and 2 pairs on the posteriolateral margin	Pronotum having 2 pairs of setae on the posteriolateral margin; no setae on the anteriolateral margin	No setae on the pronotum. Dark patches on the dorsal side of abdominal tergites
Forewings with two complete rows of wing-vein setae	Forewings with broken rows of wing-vein setae	Forewings with few small setae on the veins, hind wings with 2 setae
Larvae pale yellow move slowly and bend their abdomen while changing the direction	Larvae whitish	Larvae whitish. Both larvae and adults active, moving in a darted fashion

Thrips identification was confirmed by M/s. R. Bashear of University of Georgia, GA, ETP State, Griffin, USA and Ms. Jenny Palmer of British Museum,

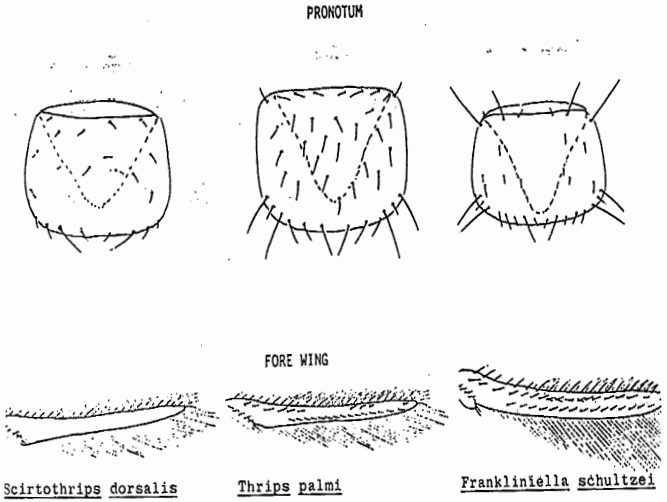


Plate 2: Identification features of different thrips species

UK. Thrips were cultured and maintained in the laboratory separately on the detached groundnut leaves. The identity of each thrips species was checked from time to time to ascertain the purity of the colonies.

3.1.2 Rearing Thrips on Detached Groundnut Leaflet

Thrips were reared on groundnut leaflets using the method developed by Amin *et al.* (1981) (Plate 3). All the three species viz., *F. schultzei*, *S. dorsalis*, and *T. palmi* were successfully reared separately on detached groundnut leaflets under controlled conditions in glass vials (3 x 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. Immobilized 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 l:d cycles of 25°C light period and 22°C dark period. After allowing 1 day oviposition access, the thrips were dislodged from the leaflet on to the paper by tapping the inverted vial and the dislodged ones 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 10 days during which 90% of the total egg laying was completed.

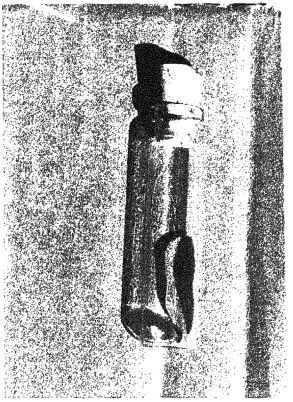


Plate 3: Cultures of thrips species maintained on detached groundnut leaflets

The leaflet with eggs was transferred to a new vial for the incubation of eggs. *F. schultzei* and *T. palmi* were reared on leaflets with pollen whereas *S. dorsalis* was reared without pollen.

The culture of each thrips species was maintained separately. Thrips from these cultures were frequently released on to susceptible test plant (black gram) to ascertain that they were virus free. This method of culturing thrips on detached leaflets though laborious helped in maintaining pure thrips cultures and facilitated collection of larvae of required age for transmission studies.

3.1.3 Biology of *T. palmi* at Different Temperatures

To find out the most suitable temperature for rearing *T. palmi* under laboratory conditions, the duration of different developmental stages, adult emergence, fecundity and hatching period were studied at five different temperatures i.e., 15, 20, 25, 30 and 35°C on detached groundnut leaflet. Percival incubators with 12:12 (light:dark) photoperiods maintained at the temperature indicated above with an RH of 75 ± 10% was used.

For larval, pupal development and adult emergence studies twenty glass vials (each containing five first instar larvae) were used at each temperature. Only individuals that survived to adult stage were included in the analysis.

For fecundity, hatching period and adult longevity studies, a female and a male were released in to the glass vial. Fifteen such vials were maintained at

different temperatures. The adults were transferred daily on to healthy groundnut leaflets until their death.

For hatching period studies, leaflets carrying eggs were kept in an incubator and observed daily for larval emergence. For fecundity studies, after allowing 1 day oviposition period, the leaflet was stored in 70% ethyl alcohol, later processed in different series of ethyl alcohol (30 min in 80 % alcohol, 10 min in 90% and 5 min in absolute alcohol), cleared in clove oil for 3 min and eggs were counted under stereoscopic binocular microscope. The experiment was repeated three times and the data were analyzed by "t" test.

3.1.4 Host Suitability Studies of *T. palmi* Under Laboratory Conditions

To find out the suitable host for the multiplication of *T. palmi*, common hosts of economic importance (blackgram, greengram, cowpea, soybean, groundnut, and sun hemp) were used by the detached leaflet technique at the most suitable temperature (25°C) and relative humidity (70 ± 10%).

The total developmental period (larval and pupal) and rate of adult emergence were estimated by releasing five first instar larvae (1 day old) into glass vials containing a fresh leaflet (5 days after emergence (DAE)) of each host and closing the vial with cork. Twenty of such vials were maintained for each host. Observations were taken on larval and pupal developmental period and adult emergence.

Since it was laborious and difficult to count the number of eggs laid in different hosts, the number of larvae hatched from the eggs of each host was studied. Larval counts and adult longevity studies were carried out using single insect pairs. Single female along with a male were released in to the glass vial containing a fresh leaf of each host. Fifteen of such vials were maintained for each host. The adults were transferred daily onto a healthy leaf till their death. The leaflets containing the eggs were kept in the incubator and observations were taken on the number of larvae from each host and the longevity of the adults on each host. The experiment was repeated three times and the data were analyzed by "t" test.

3.2 TRANSMISSION STUDIES

3.2.1 Virus Source

Leaves showing typical PBND symptoms were collected from naturally infected groundnut plants from the ICRISAT farm and sap inoculated to one week old groundnut plants (cv. TMV2). The virus source was maintained on groundnut by periodical mechanical sap inoculation. Cowpea (cv. 152) was used as a test plant. All plants were kept in a glasshouse at 25-30°C. Young infected leaflets of groundnut plants showing faint chlorotic ring spots were found to be the best source of inoculum for transmission studies.

3.2.2 Test Plants

Initially groundnut and blackgram seedlings (5 days after germination), raised in Petri dishes, were used in transmission studies. Since no difference was found in the transmission rates between the two test plants, subsequent transmission studies were carried out with groundnut.

3.2.3 Acquisition Access Period (AAP)

Young groundnut leaflets showing faint ring spots of PBND (symptomless portion removed) were floated on water in a Petri dish and 15-20 first instar larvae of each thrips species (*T. palmi*, *F. schultzei* and *S. dorsalis*) were released on a leaflet with the help of a fine, moist camel hair brush (Plate 4). The larvae were allowed an AAP of 1 day at 22-26°C. Larvae fed on healthy leaflets were used as controls. After the AAP, 10 larvae were transferred to each glass vial containing healthy groundnut leaflet and were kept in an incubator for 6-8 days. Adults which emerged were used for transmission studies.

3.2.4 Inoculation Access Period (IAP)

Young groundnut seedlings were used for IAP. Seedlings raised in Petri dishes (5 days old) were transferred individually to a glass vial (Plate 5). A single adult thrips (*T. palmi*, *F. schultzei* and *S. dorsalis*, exposed to the virus source as larvae) was released on to each seedling with a camel hair brush. The vial was closed with cork and kept in an incubator (22-26°C). The adult insects after the

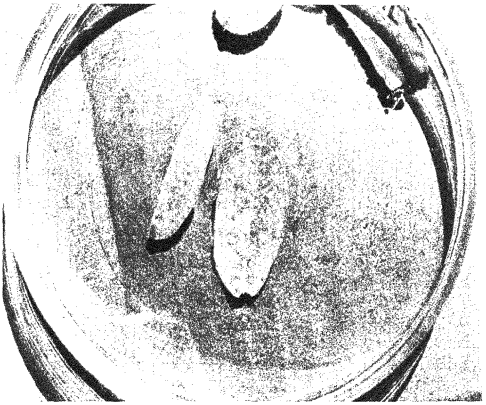


Plate 4: Leaflets infected with PBNV, floated on water in a Petri dish were used for acquisition of virus by *T. palmi*

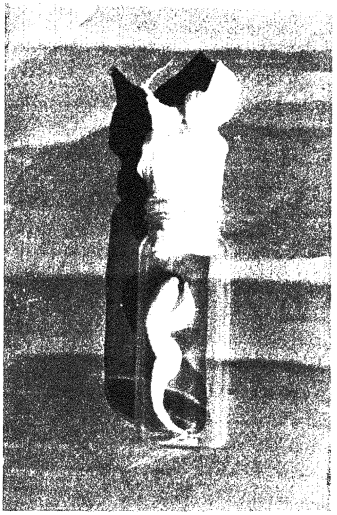


Plate 5: Groundnut seed germinated in Petri dishes were transferred to a vial for feeding with *T. palmi* exposed to PBNV. After allowing the necessary inoculation access period seedlings were transferred to wooden trays.

required IAP were removed with a camel hair brush and the seedlings were transplanted to a wooden tray (60 x 40 cm) with sterile soil. One hundred seedlings were held in such a tray. The trays were kept in a glasshouse for a maximum period of 20 days to observe for symptoms. They were sprayed with 0.025% metasystox at weekly intervals. Transmission tests were also carried out with thrips populations (*T. palmi, F. schultzei and S. dorsalis*) collected from Raichur, Karnataka, a hot spot for PBND.

3.2.5 Transmission Tests with Thrips Collected from Fields Showing PBNV Infected Plants

Three species of thrips viz., *T. palmi*, *F. schultzei*, and *S. dorsalis* were collected from PBNV infected plants of groundnut (Plate 6) on the IAC farm. Ten adults of each species were released separately onto young groundnut seedlings. The procedure described in 3.2.4 was followed for recording the frequency of transmission. The IAP allowed was 2 days. The experiment was repeated 3 times.

3.2.6 Effect of Number of *T. palmi* on Transmission Rate

Transmission tests with the three thrips species indicated that *T. palmi* only could transmit PBNV. After an AAP of 1 day as larvae, adults of *T. palmi* were allowed 2 days IAP singly as well as in groups of 2, 5, and 10 per plant. Experimental details for AAP and IAP were described in sections 3.2.3 and 3.2.4. This experiment was repeated 4 times.

Plate 6: Groundnut plants infected when they were young with peanut bud necrosis virus. The plant on left was totally necrosed. The plant on right shows sterility, auxillary shoot proliferation and leaflet deformity.

3.2.7 Relationships of Sex of *T. palmi* to Virus Transmission

To test the relative efficiency of male and female *T. palmi* in transmitting PBNV, adults (which were given an AAP of 1 day as larvae) were divided into two lots according to sex and allowed an AAP of 2 days onto the young groundnut seedlings. The difference between the transmission rates of the sexes was recorded and expressed as percentage.

3.2.8 Transmission of PBNV Maintained in Groundnut by Continuous Mechanical Sap Inoculations for Two Years

PBNV maintained by continuous mechanical inoculation for 2 years in the glasshouse was tested for transmission by *T. palmi*. Larvae were allowed 2 days AAP. Adults were given 2 days IAP and 10 adults were transferred to each of the groundnut seedling and observed for symptoms.

3.2.9 Transmission of PBNV by Larvae of *T. palmi*

Virus transmission by larvae of *T. palmi* was tested by allowing first instar larvae 1 day AAP. They were then transferred to groundnut seedlings and allowed 5 days IAP. Then the larvae were collected and allowed to become adults. They were utilized in transmission tests by allowing 2 days IAP.

3.3 VIRUS-VECTOR RELATIONSHIPS OF *T. palmi*

Virus-vector relationships included AAP, IAP, and virus retention in *T.*

palmi i.e., the persistent or non persistent nature of PBNV in the vector.

3.3.1 Effect of AAP

T. palmi larvae were tested for 5, 10, 15, 20 min, 0.5, 1, 3, 6, 12, 24, and 48 h AAP on PBNV infected groundnut leaflets (3.2.3). Exposed larvae were transferred and allowed to become adults on healthy groundnut leaflets. Transmission efficiency was tested after 2 days IAP (3.2.4). The experiment was repeated four times.

3.3.2 Effect of IAP

T. palmi larvae after an AAP of 1 day (which was found to be the optimum in the studies conducted) were developed to adults and were given IAP of 0.5, 1, 2, 4 h, 1, 2 and 3 days on groundnut seedlings. Transmission efficiency was worked as described (3.2.4). The experiment was repeated four times.

3.3.3 Retention of PBNV in *T. palmi*

Serial transmission studies were conducted to find out how long the viruliferous *T. palmi* adults were able to retain the ability to transmit PBNV. Newly emerged first instar larvae were given a range of AAP (0.5, 3, 6, 12, 24 and 72 h) (3.2.3) and were then transferred to healthy groundnut leaflets until they became adults (3.2.4). A single adult was transferred until it's death serially to each of the groundnut seedling at 1 day interval. The total number of days the

thrips had transmitted PBNV 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 pattern of PBNV by *T. palmi* i.e., whether the insect was transmitting the virus continuously or not was recorded. The experiment was repeated three times with at least 20 viruliferous adults for each experiment.

3.3.3.1. Technique used for handling single *T. palmi* adults during serial transmission studies

Different procedures of caging and transferring of single adult have been used in the investigations on serial transmission of individual insects. However, the procedure for handling single adult thrips during serial transmission studies was not described clearly in the literature. Therefore, the following technique has been developed during this study. A single, virus exposed *T. palmi* adult (larvae allowed an AAP of 1 day) was introduced in to a 6 x 2.5 cm glass vial with a fine brush and was allowed to move upwards after inverting the vial. A 2-day old groundnut seedling (kept for 2 days for germination in a Petri dish) was introduced into the vial and the vial was closed with a cork wrapped with tissue paper (kimwipes). The adult thrips was transferred to a fresh vial containing 2-day old groundnut seedling after 1 day IAP and this procedure was continued until the death of the insect. The exposed groundnut seedlings were transplanted to a wooden tray filled with sterile soil (3.2.4) and observed for the symptom development. The exposed seedlings were transplanted in row(s) to facilitate identification of each seedling.

The method adopted was found to be safe and did not cause any injury to the adult thrips. Thrips could be removed more easily from 2-day old seedling than from 5-day old seedlings employed in the transmission tests.

3.4 POPULATION ASSESSMENT OF *T.palmi*

T. palmi counts were made under field conditions to relate the incidence of PBND to thrips population in different groundnut genotypes. Counts were also taken in several host plants to determine the host range of *T. palmi*. Field studies were conducted at DOR, Rajendranagar, Hyderabad, during rainy 1991 season on three groundnut genotypes viz., ICGV 86388, (F 334 A-B-14 X NCAC 2214) 86031 [(Dh 3-20 X uSA 20) X NCAC 2232] (resistant to PBND) and JL 24 (selection from EC 94943) (susceptible check) and in rainy 1992 season on ICGV 86388, 86031, 86430 (ICGS 20 X NCAC 2214) (resistant to PBND) and JL 24 (susceptible check). The experiment was planted in a rectangular lattice design. Each genotype was planted in two rows with at least twenty plants per row. One row of susceptible genotype, JL 24, was planted between the genotypes. PBND incidence and *T. palmi* counts were assessed at 10 days intervals during the crop growth period. PBND incidence was assessed based on symptoms and infected plants were tagged at 10 days intervals. Per cent PBND incidence was recorded periodically to assess progressive incidence of PBND starting from germination to harvest. *T. palmi* density was assessed by collecting them from four replications of 25

terminals each for each variety. Leaf terminals were collected in 70% ethanol in the field, thoroughly washed and stored in 70% ethanol. They were checked under stereoscopic binocular microscope for the presence of *T. palmi*. The data were analyzed by using split plot design.

3.4.1. HOST RANGE OF *T. palmi*

Leaf terminals and flowers from weeds, ornamentals and other crop plants growing in and around the groundnut fields at the ICRISAT Asia Center, DOR fields at Rajendranagar, were collected in 70% ethanol. The samples were checked under the stereoscopic binocular microscope for *T. palmi* presence.

3.5. ASSAY FOR VIRUS

After noting symptoms on test plants, further confirmation of PBNV presence was done by mechanical inoculations and by enzyme-linked immuno sorbent assay (ELISA).

3.5.1 Mechanical Inoculations

Symptomatic leaflets were ground in a cold mortar containing 0.05 M potassium phosphate buffer (pH 7.0) with 0.02 M 2-mercapto-ethanol. Cowpea was used as an assay host. The primary leaves of cowpea were lightly dusted with 600-mesh carborundum. A pestle was used for rubbing the extracts on leaves. Soon after inoculation the plants were washed and kept for symptom development in a

screen house for 5-10 days.

3.5.2 ELISA test

The direct antigen coating (DAC) procedure reported by Hobbs *et al.* (1987) was used to detect the virus in the test plants. The test plant samples were ground in carbonate buffer, pH 9.6, containing diethyl dithiocarbomate (carbonate buffer is prepared by adding Na_2CO_3, 1.5 g; $NaHCO_3$ in 1lt distilled water. After dissolving the above two compounds diethyl dithiocarbomate 1.71 g for 1 lt was added). Samples were (200 µl/ well) were dispensed into ELISA plate. The plate was incubated at 37°C for 1 h. The plant extracts were poured off and the plate was rinsed three times (allowing three minutes for each wash) with PBS Tween ($Na_2HPO_4.2H_2O$ 2.88 g or Na_2HPO_4 2.38 g; $KH_2PO_4$0.4 g; KCl 0.4 g; NaCl 16.0 g; Distilled water 2 liters; Tween-20 0.5ml/l). Cross adsorbtion of antisera with healthy plant extracts was done by grinding healthy leaves in a antibody buffer (PBS-tween 100 ml; polyvinyl pyrollidone 2.0 g (2% final concentration), ovalbumin 0.2 g, 0.2% final concentration) to give 1:20 dilution, filtered through two thickness of cheese cloth, and then diluted to 1:1000 in antibody buffer. The plates were incubated at 37°C for 1 h and washed three times in PBS-tween as described above. To each well 200 µl of goat rabbit IgG or Fc labelled with penicillinase and (Sudharshana and Reddy, 1989) diluted to 1:5000 in antibody

buffer was added and the plates were incubated at 37°C for 1 h. After the plate was washed three times with distilled water tween (0.05% tween-20 in distilled water) 200 µl of substrate mixture (20 mg bromo thymol blue, (BTB) 0.2M NaoH and sodium pencillin-G 0.5 mg/ml, pH.7.2) was added to each well. Results were recorded at room temperature for 30 to 120 minutes. The blue colour of the substrate mixture turning to light green indicated weak positive reaction and orange colour indicated strong positive reaction.

RESULTS

CHAPTER IV

RESULTS

4.1 TRANSMISSION STUDIES

4.1.1 Transmission Studies with Three Thrips Species Under Laboratory Conditions

Transmission tests conducted under laboratory conditions with laboratory reared cultures of *Thrips palmi*, *Frankliniella schultzei* and *Scirtothrips dorsalis* indicated that only *T. palmi* could transmit PBNV to both groundnut and blackgram (Table 2). The per cent transmission was 37.8 in groundnut and 36.8 in blackgram. *F. schultzei* and *S. dorsalis* (from IAC and from Raichur) did not transmit the virus either to groundnut or to blackgram.

4.1.2 Transmission Studies with Thrips Collected from the Field

The three species of thrips, *T. palmi*, *F. schultzei*, and *S. dorsalis* collected from the PBND infected groundnut plants in the field at ICRISAT Asia Center and tested for the virus transmission, only *T. palmi* transmitted PBNV to groundnut to the extent of 60 per cent. Both *F. schultzei* and *S. dorsalis* did not transmit PBNV (Table 3).

Table 2. Peanut bud necrosis virus (PBNV) transmission by different thrips species under laboratory conditions at ICRISAT Asia Center.

Thrips species[1]	Test plant	Plants infected/ tested	Transmission (%)
Thrips palmi[2]	Groundnut[4]	59/156	37.8
	Blackgram[5]	42/114	36.8
Frankliniella schultzei[2]	Groundnut	0/203	0.0
	Blackgram	0/100	0.0
Scirtothrips dorsalis[3]	Groundnut	0/110	0.0
	Blackgram	0/100	0.0

1. Larvae were allowed 1 day acquisition access period (AAP) and adults 1 day inoculation access period (IAP).

2. 1 exposed adult plant^{-1}.

3. 5 exposed adults plant^{-1}.

4. *Arachis hypogaea* cv.TMV2

5. *Vigna mungo* cv Local

4.1.3 Effect of Number of *T. palmi* on Transmission Rate

A single adult *T. palmi* was capable of transmitting PBNV with a frequency of 30 per cent (Table 4). The frequency of transmission increased with the increase in the number of adults per seedling. Transmission was 40 per cent with 2 adults and 70 per cent with 5 adults/seedling. As high as 100 per cent transmission was recorded with 10 adults per seedling.

4.1.4 Effect of Sex of *T. palmi* on PBNV Transmission

Both male and female *T. palmi* transmitted the virus with equal efficiency and there was no significant difference in the transmission percentage. Males showed a transmission frequency of 55.0 per cent and females 54.9 per cent transmission (Table 5).

4.1.5 Vector Transmissibility of PBNV Maintained Continuously by Mechanical Sap Inoculations for Two Years

PBNV maintained under glasshouse conditions by continuous mechanical inoculation for two years was not transmitted by *T. palmi* even when 10 adults were used for transmission per seedling (Table 6).

4.1.6 Transmission of PBNV by *T. palmi* larvae

None of the 178 plants exposed to *T. palmi* larvae following 1 day AAP and 5 days IAP transmitted PBNV in the independent experiments (Table 7).

Table 3. Transmission of PBNV with different adult thrips species collected from peanut bud necrosis disease (PBND) infected groundnut fields.

Thrips species[1]	Plants infected/tested[2]	Transmission (%)
Thrips palmi	15/25	60
Frankliniella schultzei	0/30	0
Scirtothrips dorsalis	0/30	0

[1] Inoculation access period: 5 days.
[2] No. of adults released plant^{-1}: 10

Table 4. Influence of number of adults of *T. palmi* on the transmission of PBNV.

T. palmi adults plant^{-1}	Plants infected/tested	Transmission (%)
1	9/30	30
2	12/30	40
5	21/30	70
10	30/30	100

[1] Larvae were given one day acquisition access period and adults 2 days inoculation access period.

Table 5. Efficiency of transmission of PBNV by adult male and female *T. palmi*.

Experiment	Females[1]		Males[1]	
	Plants infected/ tested	Transmission (%)	Plants infected/ tested	Transmission (%)
1	22/40	55.0	21/39	53.9
2	27/47	57.5	24/41	58.5
3	23/44	52.3	21/40	52.5
Mean		54.9		55.0
SEm ±	1.0			
CD at 5%	3.8			

[1] Larvae were given one day acquisition access period and adults 2 days inoculation access period.

Table 6. Transmissibility of PBNV maintained continuously by mechanical sap inoculation for 2 years under laboratory conditions by *T. palmi*.

Experiment[1,2]	PBNV isolates	
	Wild type[3]	Maintained for 2 years
1	29/30[4]	0/30[4]
2	30/30	0/25
3	30/30	0/22

1. Larvae were given 2 days acquisition access period and adults 2 days inoculation access period.
2. 10 adults were released seedling^{-1}.
3. Virus inoculum derived from thrips transmitted plants.
4. Infected/tested.

Table 7. Tests on *T. palmi* larvae for transmission of PBNV.

Experiment[1,2]	Plants infected/tested	
	Larvae	Adults[3]
1	0/100(0)[4]	20/52 (38.5)
2	0/78 (0)	25/55 (48.5)

1. First instar larvae were allowed one day acquisition access period and five days inoculation access period.

2. Five larvae were released on to each groundnut seedling and were retained till they pupated.

3. Adults emerged from larvae were used in transmission.

4. Figures in parenthesis represents transmission percentage.

4.2 VIRUS-VECTOR RELATIONSHIPS

4.2.1 Effect of AAP

Initial observations (Table 8) showed that the larvae were capable of acquiring the virus within 0.5 h AAP and resulted in 50 per cent transmission. Increase in AAP up to 12 h had no considerable effect on transmission efficiency whereas increase from 12 h to 24 h resulted in 50 to 64.6% transmission frequency. However extending AAP from 24 h to 48 h had no effect on transmission frequency.

Studies conducted with lower AAP (5 min - 48 h) (II experiment) indicate that the larvae were capable of acquiring the virus even at 5 min AAP (Table 8). As observed in both the experiments (Table 8) at 24 h AAP the rate of transmission was more than the other periods tested and further increase in AAP after 24 h did not show any increase in the transmission.

4.2.2 Effect of IAP

The results (Table 9) indicate that 0.5 h IAP was not enough to transmit sufficient virus to induce PBND symptoms. With 1, 2 and 4 h IAP 6.3, 8.9 and 11.4 per cent of the plants were infected respectively. There was an increase (25%) in the transmission frequency from 1 day IAP. The maximum transmission frequency (40.4 to 41.7)) was observed after 2 days IAP.

Table 8. Effect of different acquisition access periods on the transmission of PBNV by *T. palmi*.

Acquisition access period[1] (h)	Plants infected/tested[2]	Transmission (%)
Experiment 1.		
0.5	21/42	50.0
1	16/30	53.3
3	22/40	55.0
6	21/39	53.9
12	20/40	50.0
24	31/48	64.6
48	25/40	62.5
Experiment 2.		
5 min	12/23	52.2
10 min	11/22	50.0
15 min	11/21	52.4
20 min	10/20	50.0
30 min	14/29	48.3
1 h	11/22	50.0
6 h	18/35	51.4
24 h	24/36	66.7
48 h	22/35	62.9

[1] One day old larvae were used.
[2] Adults were allowed an inoculation access period of 2 days.

Table 9. Effect of different inoculation access periods on the transmission of PBNV by *T. palmi*.

Inoculation access period[1,2]	Plants infected/tested	Transmission (%)
0.5 h	0/40	0.0
1 h	3/48	6.3
2 h	4/45	8.9
4 h	5/44	11.4
1 day	10/40	25.0
2 days	19/47	40.4
3 days	25/60	41.7

[1] First instar larvae were allowed 1 day acquisiton access period.
[2] Newly emerged adults were used for inoculation access period.

4.2.3 Virus Retention in the Vector

It is evident from the data that of the twenty two adults tested with 0.5 h AAP, two insects transmitted the virus throughout their life period (Table 10). Seven adults transmitted the virus up to 50% of their life period, eight from 51-75% of the life period and six from 76-100% of the life period.

With 3 h AAP, of the twenty five adults tested, seven insects have transmitted the virus up to 50% of the life period where as nine insects have transmitted the virus from 51-75% of the life period (Table 11). The remaining eight insects have transmitted the virus from 76-100% of their total life period.

With 12 h AAP, of the twenty adults, four have transmitted the virus up to 50% of the life period, eight insects from 51-75% the life period and seven from 76-100% of the life period (Table 12).

Of the twenty five adults tested following 24 h AAP, five insects have transmitted the virus up to 50% their life period, eleven with 51-75% and eight insects from 76-100% of the life period (Table 13).

Twenty adult thrips tested following 72 h AAP indicate that six have transmitted the virus up to 50% of the life period, five insects from 51-75% of life period and eight insects from 76-100% of their life period (Table 14).

Table 10. Serial transmission of PBNV by *T. palmi* adults after 0.5 h AAP and 1 day IAP.

Individual insect	Transmission by individual T. palmi (Days)[1]																				%[2]
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1	+	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	ID		36
2	+	-	-	+	+	+	-	-	-	+	+	+	-	-	-	ID					75
3	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ID	35
4	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ID		14
5	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	ID	62
6	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	ID			39
7	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	ID			89
8	+	+	+	-	-	-	-	IM													33
9	+	+	+		+	+	+	+	-	+	+	+	+	-	-	+	-	-	ID		86
10	-	-	-	-	-	-	-	-	-	-	-	-	-	ID							0
11	-	-	+	+	+	-	IM														71
12	-	+	+	-	-	-	-	+	+	-	-	-	ID								69
13	+	-	+	-	+	-	-	-	+	+	+	+	+	+	+	+	-	-	-	ID	75
14	+	+	+	-	+	-	-	-	+	+	-	-	+	+	-	-	+	-	ID		89
15	+	+	+	-	-	+	+	-	-	-	-	-	-	IM							44
16	+	+	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	IM			67
17	+	-	+	-	+	-	+	-	-	-	-	-	+	+	-	-	+	+	ID		89
18	+	+	+	-	+	-	IM														71
19	-	-	+	-	+	-	-	+	-	+	-	-	+	+	+	-	+	+	ID		100
20	+	+	+	+	+	+	-	-	-	-	-	+	+	-	-	ID					61
21	-	-	+	+	-	-	+	+	-	-	+	+	+	-	-	-	-	ID			72
22	+	+	+	+	-	-	+	+	-	-	-	-	ID								62

[1] After the adults have emerged they were transferred individually to each of groundnut seedling.

[2] Frequency of transmission

+ Insect transmitted the virus; - Insect not transmitted the virus;

ID = Insect died; IM = Insect missing;

Table 11. Serial transmission of PBNV by *T. palmi* adults after 3 h AAP and 1 day IAP.

Individual insect	Transmission by individual F. palmi (Days)[1]																			W[2]
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
1	-	-	+	+	+	-	-	-	-	-	-	-	-	-	ID					33
2	+	+	+	-	-	-	-	+	-	-	-	-	IM							58
3	+	+	+	+	+	+	+	+	+	+	-	+		ID						86
4	+	+	+	-	+	-	-	-	+	-	-	-	+	ID						100
5	+	+	+	+	+	+	-	+	IM											100
6	+	+	-	+	+	-	-	-	-	-	IM									100
7	+	-	-	-	-	-	IM													66
8	+	-	+	-	-	+	+	-	ID											14
9	+	+	+	-	-	+	+	-	-	-	+	-	-	-	-	ID				63
10	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	ID			82
11	+	-	-	-	-	IM														20
12	+	+	-	+	-	-	IM													57
13	-	+	+	+	+	-	-	ID												63
14	-	+	+	+	-	+	+	-	-	ID										78
15	+	+	-	+	+	+	-	+	+	+	+	+	-	-	-	ID				75
16	+	+	-	-	+	-	-	+	-	+	-	ID								83
17	+	-	+	-	+	-	-	ID												63
18	+	-	+	-	+	-	-	-	-	IM										50
19	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	ID				36
20	-	-	+	+	+	+	+	-	+	+	+	-	-	-	-	ID				69
21	-	-	+	+	+	+	-	-	-	IM										60
22	-	+	+	-	+	+	+	-	+	-	-	-	-	ID						64
23	-	-	-	-	-	-	-	-	-	-	-	IM								0
24	+	-	+	-	+	+	+	-	+	-	+	-	IM							66
25	+	-	-	-	-	-	-	-	-	-	ID									9

1 After the adults have emerged they were transferred individually to each of groundnut seedling.

2 Frequency of transmission

+ Insect transmitted the virus; - Insect not transmitted the virus;

ID = Insect died; IM = Insect missing;

Table 12. Serial transmission of PBNV by *T. palmi* adults after 12 h AAP and 1 day IAP.

Individual insect	Transmission by individual T. palmi (Days)[1]																			%[2]
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
1	+	+	+	-	+	+	+	-	-	-	ID									44
2	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	ID				93
3	+	-	+	-	-	-	-	-	-	+	+	+	-	IM						86
4	+	+	-	+	+	-	ID													71
5	+	+	+	-	+	-	-	+	+	-	ID									82
6	-	-	-	-	-	-	-	-	-	-	-	-	-	ID						0
7	+	-	+	-	-	-	-	-	-	-	-	-	-	ID						21
8	+	+	+	-	-	-	-	-	-	-	IM									27
9	+	+	+	-	+	+	+	+	+	-	ID									82
10	+	+	-	+	+	+	IM													100
11	-	-	+	-	+	+	-	-	ID											47
12	-	-	+	+	+	+	-	+	-	ID										40
13	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	ID				6
14	-	-	+	+	+	-	IM													71
15	+	+	+	-	-	-	+	-	-	-	-	-	ID							58
16	+	+	+	-	+	+	+	+	-	+	-	ID								83
17	+	-	+	-	+	+	+	+	+	+	+	+	-	ID						86
18	+	+	+	-	+	+	-	IM												75
19	+	-	-	+	-	-	-	-	-	-	-	-	-	ID						21
20	+	-	-	-	+	+	+	+	+	+	-	-	-	-	-	ID				63

1 After the adults have emerged they were transferred individually to each of groundnut seedling.

2 Frequency of transmission

+ Insect transmitted the virus; - Insect not transmitted the virus;

ID = Insect died; IM = Insect missing;

Table 13. Serial transmission of PBNV by *T. palmi* adults after 24 h AAP and 1 day IAP.

Individual insect	Transmission by individual *T. palmi* (Days)[1]																					%[2]
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
1	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	-	+	+	ID	100
2	+	-	+	+	-	-	IM															43
3	+	+	+	-	+	+	-	-	-	-	ID											55
4	+	+	+	-	+	+	+	+	+	+	+	-	+	-	-	ID						85
5	+	+	+	-	+	+	+	-	IM													70
6	+	-	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	ID			79
7	+	+	+	-	+	-	-	-	-	-	-		-	-	-	-	ID					29
8	+	-	+	+	+	-	IM															71
9	+	+	+	-	+	-	IM															71
10	+	+	+	+	+	+	-	ID														75
11	+	-	+	+	+	-	-	-	IM													56
12	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	ID						38
13	+	+	+	-	-	-	IM															62
14	-	+	+	-	+	+	+	+	+	-	+	-	-	+	ID							100
15	-	-	+	+	+	+	+	-	+	-	-	-	-	-	-	ID						56
16	-	+	+	+	+	-	+	-	-	-	-	-	-	-	ID							47
17	+	-	+	-	+	+	+	+	+	-	-	-	-	-	ID							60
18	+	+	+	-	+	+	+	+	+	-	-	-	-	-	IM							60
19	+	-	+	+	+	+	+	ID														100
20	+	+	+	-	+	-	+	IM														100
21	-	+	+	-	+	+	+	-	IM													78
22	+	+	+	-	+	+	+	+	ID													100
23	+	-	+	+	+	+	+	-	-	ID												70
24	+	-	+	-	+	-	IM															71
25	+	+	+	-	ID																	60

After the adults have emerged they were transferred individually to each of groundnut seedling.

[1] Frequency of transmission

[2] + Insect transmitted the virus; - Insect not transmitted the virus;

ID = Insect died; IM = Insect missing;

Table 14. Serial transmission of PBNV by *T. palmi* adults after 72 h AAP and and 1 day IAP.

Individual insect	Transmission by individual T. palmi (Days)¹																					%
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
1	-	+	+	-	+	-	-	+	+	+	-	-	-	ID									72
2	-	-	-	-	-	-	-	-	-	ID													0
3	+	+	+	+	+	+	+	-	+	+	IM												100
4	+	+	+	+	+	+	+	+	-	-	+	-	-	ID									79
5	-	-	-	-	+	+	+	-	-	-	IM												67
6	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	ID						58
7	+	-	-	-	+	+		-	-	-	-	-	-	-	-	-	-	-	-	-	-	ID	27
8	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	ID						65
9	+	-	+	+	+	+	+	+	+	+	+	+	-	ID									84
10	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ID	27
11	-	+	+	+	-	+	+	+	+	-	-	+	+	ID									100
12	+	+	-	-	-	-	-	ID															28
13	+	+	+	+	+	+	IM																100
14	+	-	-	+	-	-	ID																57
15	+	+	+	+	+	+	+	-	IM														78
16	+	+	+	-	-	-	+	+	+	+	+	-	-	-	+	+	ID						100
17	-	+	+	-	-	-	-	-	-	IM													30
18	+	-	-	-	-	-	-	-	-	-	-	ID											0
19	+	+	+	-	-	+	+	+	-	-	-	-	-	IM									57
20	-	+	-	+	-	+	-	+	+	+	-	-	ID										65

After the adults have emerged they were transferred individually to each of groundnut seedling.

Frequency of transmission

Insect transmitted the virus; - Insect not transmitted the virus;

D = Insect died; IM = Insect missing;

The serial transmission studies conducted after different AAP indicate (Table 15) that this factor had no influence on the serial transmission of the virus. In all cases, irrespective of AAP more than 60% of adults transmitted the virus for more than 50% of their life period. It was also apparent that the serial transmission was not continuous and that the erratic nature of the transmission was not influenced by the length of the AAP.

4.3 EFFECT OF TEMPERATURE ON THE DEVELOPMENTAL BIOLOGY OF *T. palmi*

Increase in temperature from 15-35°C showed a gradual decrease in egg, larval and pupal developmental periods (Table 16). Significant and negative correlation was found between temperature and developmental periods of egg, larval and pupal periods (Fig. 1). Eggs laid at 15°C did not hatch into larvae. The egg period was longest (6.3 days) at 20°C and decreased gradually with increase in temperatures i.e., 5.1 days at 25°C, 4.0 days at 30°C, and 3.6 days at 35°C. Similarly the larval period also decreased with increased temperatures. The larval period was significantly longest (7.7 days) at 15°C followed by 6.5 days at 20°C, 4.7 days at 25°C, 3.8 days at 30°C and 3.4 days at 35°C. Similar trend of decrease in pupal period was observed with increase in temperatures. the pupal periods were 6.0, 5.4, 3.7, 3.0 and 2.9 days at 15, 20, 25, 30 and 35°C respectively.

Table 15. Summary of results of serial transmission of PBNV by *T. palmi*.

Acquisition access period (h)	Percentage of insects transmitting the virus		
	50% of life span	51-75% of life span	76-100% of life span
0.5	25	37	38
3	27	40	33
12	27	40	33
24	20	40	40
72	43	21	36

Experimental details as in Tables 10 to 14.

Fig. 1 : Effect of temperature on the developmental period T. palmi under laboratory conditions.

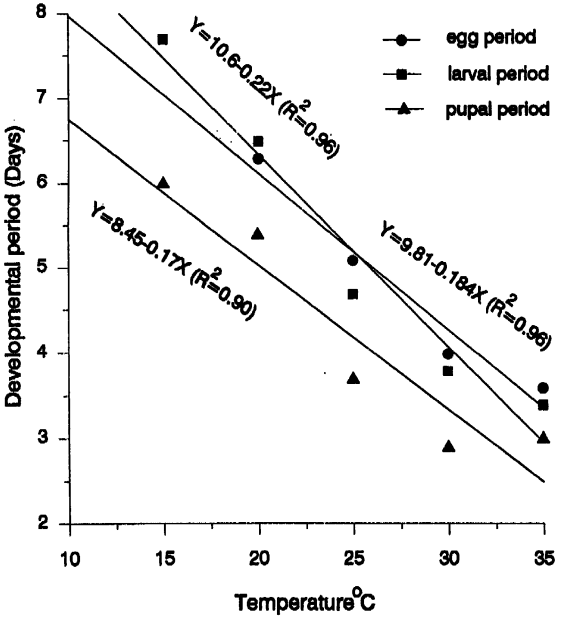


Table 16. Effect of temperature on the developmental biology of *T. palmi*.

Temperature (°C)	Mean developmental period (days)@			Fecundity (total No. of eggs/ female)	Adult emergence (%)	Adult longivity (days)	Total No. of larvae produced/ female
	Egg	Larva	Pupa				
15	*	7.7	6.0	22.6	3	28.6	*
20	6.3	6.5	5.4	75.7	15	25.4	66.6
25	5.1	4.7	3.7	78.2	30	24.1	68.2
30	4.0	3.8	2.9	55.6	13	12.9	54.5
35	3.6	3.4	3.0	6.8	5	8.4	6.2
SE (m)	0.5	0.2	0.2	8.2	-	1.9	8.8
CV (%)	36	10	16	54	-	30	57
CD at 5%	1.6	0.5	0.7	23.2	-	5.4	23.5

* No egg hatching.

@ Experimental details in Materials and Methods 3.1.3.

The fecundity also varied with the temperature. Very few eggs (6.8) were laid by the female at 35°C and the fecundity was more at 25°C (78.2) and at 20°C (75.7)and decreased at 30°C (55.6). However, at 15°C, the female laid only 22.6 eggs during the life period.

The adult emergence was found to be the highest at 25°C (30%) followed by at 20°C (15%), and 30°C (13%). The lowest number of adults were emerged at lowest (3% at 15°C) and highest (5% at 35°C) temperatures.

The adult longevity decreased with the increase in temperature. Lowest adult longevity of 8.4 days was observed at 35°C which differed significantly from the adult longevity periods of 15, 20 and 25°C. The female adult lived longer at 15°C (28.6 days).

Observations recorded on the total number of larvae produced per female during its life span indicated that significantly low number of larvae (6.2 larvae/female) were observed at 35°C, where as 66.6, 68.2 and 54.5 larvae/female were recorded 20, 25 and 30°C respectively.

4.4 HOST SUITABILITY STUDIES

The larval period on the 6 legumes tested ranged from 4.0 to 5.1 days depending on the host (Table 17). Significantly shortest larval developmental period of 4.0 days on cowpea and longest developmental period (5.1 days) on sun hemp was observed. In soybean the larval period was 4.3 days followed by greengram (4.4 days), groundnut (4.5 days) and blackgram (4.7 days).

Table 17. Effect of various legume hosts on the growth and development of *T. palmi* under laboratory conditions.

Hosts[1]	Larval period (Days)	Pupal period (Days)	No. of larvae produced/ female	Adult longivity (Days)	Adult emergence (%)
Cowpea	4.0	3.8	138.3	21.0	40.0
Blackgram	4.7	4.5	117.3	22.0	50.0
Greengram	4.4	4.8	150.1	23.0	48.0
Soybean	4.3	3.9	63.5	15.7	47.0
Groundnut	4.5	4.0	68.9	19.9	20.0
Sun hemp	5.1	4.1	74.9	14.7	50.0
SE (m)	0.1	0.1	10.4	1.4	-
CV (%)	9.6	11.5	32.1	22.6	-
CD at 5%	0.2	0.2	29.4	3.9	-

1 Experimental details in Materials and Methods 3.1.4.

The pupal period was 3.8 days on cowpea followed by soybean (3.9 days), groundnut (4 days), sun hemp (4.1 days), blackgram (4.5 days), and greengram (4.8 days).

The total number of larvae produced per female was the lowest on soybean (63.5) and highest on green gram (150.1), followed by cowpea (138.3), blackgram (117.3), sun hemp (74.9) and groundnut (68.9).

The female adult longevity varied between 14.7 and 23 days. The lowest adult longevity (14.7 days) was observed on sun hemp followed by soybean (15.7 days), groundnut (19.9 days), cowpea (21 days), blackgram (22 days) and green gram (23 days). There was no difference in the per cent adult emergence (40-50%) between the hosts except groundnut (28%).

4.5 NUMBER OF *T. palmi* ADULTS COLONIZED ON PBND RESISTANT AND SUSCEPTIBLE GROUNDNUT GENOTYPES

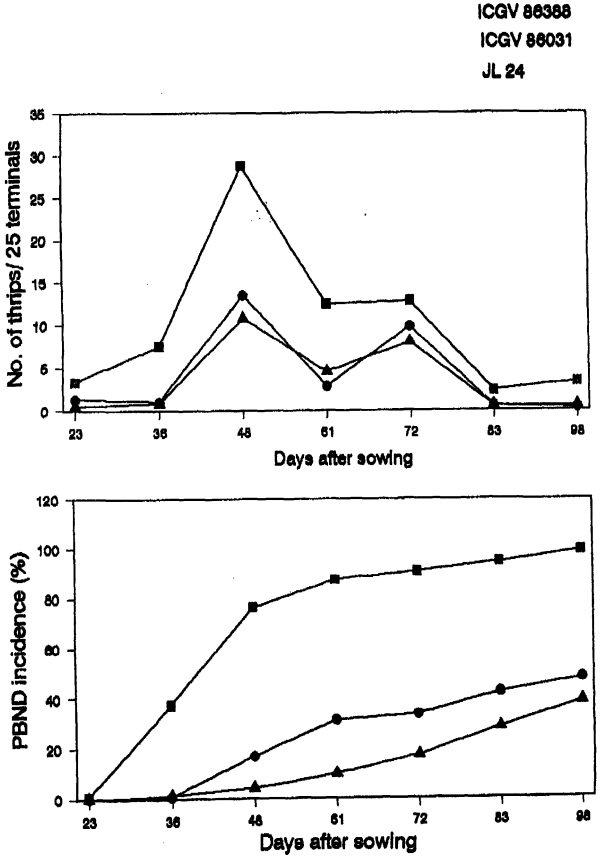
Field studies conducted at Rajendranagar during 1991 rainy season showed significantly more number of *T. palmi* on JL 24 (10 thrips/25 terminals) than the PBND resistant genotypes, ICGV 86031 (3.6 thrips/25 terminals) and ICGC 86388 (4.1 thrips/25terminals) (Table 18). At the beginning of the crop growth period (23 days after sowing (DAS)) *T. palmi* population was very low, reached a peak at 48 DAS, fell down sharply there after and reached to minimum numbers by the end of the crop growth period (98 DAS) in all the genotypes.

Table 18. Incidence of PBND and population of *T. palmi* on different groundnut genotypes at different stages of crop growth at DOR, Rajendranagar, Hyderabad during the 1991 rainy season (Jul 16 to Oct 20).

Days after sowing (DAS)	No. of Thrips/ 25 terminals[1]				% PBND incidence[2]			
	ICGV 86388	ICGV 86031	JL 24	Mean	ICGV 86388	ICGV 86031	JL 24	Mean
23	1.3	0.5	3.3	1.7	0.0	0.0	0.6	0.2
36	1.0	0.8	7.5	3.1	0.7	1.1	36.9	12.9
48	13.5	10.8	28.8	17.7	16.1	2.9	39.1	19.4
61	2.8	4.5	12.5	6.6	14.5	5.6	10.8	10.3
72	9.8	8.0	12.8	10.2	2.3	7.6	3.4	4.4
83	0.5	0.5	2.3	1.1	8.8	11.3	3.9	8.0
98	0.3	0.5	3.3	1.3	5.7	10.4	4.5	6.9
Mean	4.1	3.6	10.0	5.9	5.5	6.8	14.2	8.9
SE (m)		1.3				2.0		
CD at 5% (days)		3.9				5.9		
SE(m)		0.6				1.4		
CD at 5% (cultivar)		1.7				4.0		
SE(m)		1.8				3.6		
CD at 5% (days x cultivar)		5.2				10.3		

Terminals were collected at random from 40 plants.
Pegs were used to mark the infected plants.

Fig. 2 : Thrips palmi population and progress of PBND incidence in different groundnut genotypes at Rajendranagar, Hyderabad, rainy season 1991.



The mean PBND incidence was significantly higher on JL 24 (14.2%) than ICGV 86031 (6.8%) and ICGV 86388 (5.5%) in the 1991 rainy season (Table 18). The PBND incidence was observed in the field at 23 DAS. The maximum PBND incidence was observed at 48 DAS in JL 24 (39.1%) and ICGV 86388 (16.1%), where as in ICGV 86031 the maximum incidence (11.3%) was observed at 83 DAS. The cumulative PBND incidence (Fig. 2) has reached to a maximum of 99% in JL 24, 39% in ICGV 86031 and 48% in ICGV 86388 at the end of crop season (98 DAS).

In 1992 rainy season, the mean number of thrips were significantly higher (5.5) on PBND susceptible JL 24 than on the PBND resistant genotypes (Table 19). The mean number of thrips population did not differ significantly between ICGV 86031 (1.5 thrips) and ICGV 86388 (1.4 thrips). ICGV 86430 showed significantly higher number of thrips (2.9) than ICGV 86388 (1.4). The thrips population was found to be more at 36 DAS in all the 4 genotypes and declined sharply there after.

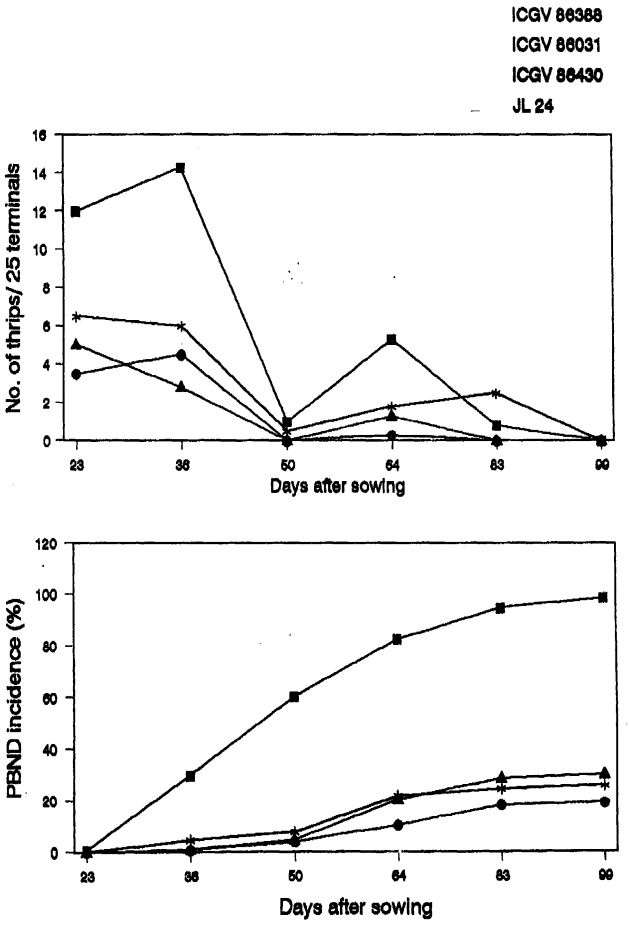
The mean PBND incidence was significantly higher on JL 24 (16.5%) than the other 3 genotypes (Table 19). There was no significant difference in the mean PBND incidence among ICGV 86031 (5%), ICGV 86388 (3.2%) and ICGV 86430 (4.3%). In JL 24, the maximum increase in PBND incidence was observed at 50 DAS (30.7%) whereas in ICGV 86031 and ICGV 86430, the increase in PBND incidence was significantly higher at 64 DAS (15.6% in ICGV 86031 and 14% in ICGV 86430). In ICGV 86388, the maximum increase was observed at 83

ible 19. Incidence of PBND and population of *T. palmi* on different groundnut genotypes at various growth stages at DOR, Rajendranagar, Hyderabad, during the 1992 rainy season (July 16-October 23).

Days after sowing (DAS)	No. of Thrips/ 25 terminals[1]					% PBND incidence[2]				
	ICGV 86388	ICGV 86031	ICGV 86430	JL 24	Mean	ICGV 86388	ICGV 86031	ICGV 86430	JL 24	Mean
23	3.5	5.0	6.5	12.0	6.8	0.0	0.0	0.0	0.6	0.2
36	4.5	2.8	6.0	14.3	6.9	0.7	0.9	4.7	29.1	8.8
50	0.0	0.0	0.5	1.0	0.4	3.3	3.7	3.0	30.7	10.2
64	0.3	1.3	1.8	5.3	2.1	6.4	15.6	14.1	22.2	14.6
83	0.0	0.0	2.5	0.8	0.8	7.8	8.3	2.6	12.2	7.7
99	0.0	0.0	0.0	0.0	0.0	1.0	1.6	1.4	4.1	2.0
Mean	1.4	1.5	2.9	5.5	2.8	3.2	5.0	4.3	16.5	7.3
SE(m)		1.2				1.4				
CD at 5% (days)		3.5				4.3				
SE(m)		0.4				0.9				
CD at 5% (cultivar)		1.2				2.7				
SE(m)		1.5				2.5				
CD at 5% (days x cultivar)		4.1				7.0				

erminals were collected at random from 40 plants.
egs were used to mark the infected plants.

Fig. 3 : Thrips palmi population and progress of PBND incidence in different groundnut genotypes at Rajendranagar, Hyderabad, rainy season, 1992.



DAS (7.8%). The cumulative PBND incidence has reached to 99% in JL 24, 30% in ICGV 86031, 19% in ICGV 86388 and 26% in ICGV 86430 at the end of the crop season (99 DAS) (Fig. 3)

4.6 HOST RANGE OF *T. palmi*

T. palmi adults were recorded on 27 weed species in 21 families, 12 of the 17 vegetable crops, 9 of the 15 crop plants, 6 of the 10 fruit crops and 17 of the 24 ornamental plants (Table 20). Among the weeds, *Ageratum conyzoides, Commelina bengalensis, Parthenium histerophorous* and *Cassia tora* were common in groundnut fields which harbored large number of *T. palmi*. Among the crop plants, blackgram, greengram, cowpea and soybean had the highest density of *T. palmi* during the early crop growth stage. All the cucurbitaceous vegetables checked harbored large number of *T. palmi*.

Table 20. Presence of *T. palmi* on various host plants in groundnut fields at ICRISAT Asia Center and at DOR, Rajendranagar.

Family	Common name	Botanical name	Presence of *T. palmi*
Crop plants			
Compositae	Niger	*Guizotia abyssinica*	+
	Sunflower	*Helianthus annuus*	+
Euphorbiaceae	Castor	*Ricinus communis*	-
Graminae	Maize	*Zea mays*	-
	Sorghum	*Sorghum bicolor*	-
	Pearl millet	*Pennisetum typhoideum*	-
Leguminosae	Cowpea	*Vigna sinensis*	+
	Soybean	*Glycine max*	++
	Pigeonpea	*Cajanus cajan*	-
	Chickpea	*Cicer arietinum*	-
	Urdbean	*Vigna mungo*	++
	Mungbean	*Vigna radiata*	++
	Sun hemp	*Crotalaria juncea*	+
Malvaceae	Cotton	*Gossypium arboreum*	+
Pedaliaceae	Sesame	*Sesamum indicum*	++
Fruit trees:			
Anacardiaceae	Mango	*Mangifera indica*	+
Anonaceae	Custard apple	*Anona squamosa*	-
Caricaceae	Papaya	*Carica papaya*	+
Euphorbiaceae	Phillanthus	*Phillanthus neruri*	-
Myrtaceae	Guava	*Psidium guajava*	+
	Pomogronate	*Punica granatum*	+
Rosaceae	Cherry	*Prunus cerasus*	+
Rutaceae	Large lemon	*Citrus limonum*	+

Family	Common name	Botanical name	Presence of *T. palmi*
Sapotaceae	Sapota	*Achras sapota*	-
Vegetables			
Chenopodiaceae	Spinach	*Spinacia oleraceae*	-
Cucurbitaceae	Ashgourd	*Benincasa lispida*	++
Cucurbitaceae	Cucumber	*Cucumis sativus*	++
-do-	Bittergourd	*Momordica charantica*	++
-do-	Pumpkin	*Cucurbita moschata*	++
-do-	Ridgegourd	*Luffa acutangula*	++
-do-	Watermelon	*Citrullus vulgaris*	++
Leguminosae	Broad beans	*Vicia faba*	+
-do-	Cluster beans	*Cyamopsis tetragonoloba*	+
-do-	Beans	*Dolichos lablab*	+
-do-	Drumsticks	*Moringa pterigosperma*	++
Malvaceae	Bhindi	*Abelmoschus esculentus*	+
Solanaceae	Brinjal	*Solanum melongena*	+
-do-	Celery	*Apium graveolens*	-
-do-	Chilli	*Capsicum annuum*	-
-do-	Tomato	*Lycopersicon esculentum*	-
Umbeliferae	Carrot	*Daccus carota*	-
Ornamental plants			
		Nerium oleander	+
Apocynaceae	Allamanda	*Allmonda cathartica*	-
Begnoniaceae		*Calymma alliaceum*	+
		Begnonia gracillis	++
		Techomaria capensis	+

Family	Common name	Botanical name	Presence of *T. palmi*
Cannaceae		*Canna indica*	++
Combretaceae		*Quisqualis indica*	+
Compositae	Chrysanthemum	*Chrysanthemum indicum*	+
		Arnica montana	+
	Zinnia	*Zinnia elegans*	+
Euphorbiaceae	Chenile plant	*Acalypha hispida*	+
Horsefalleaceae	Morning glory	*Ipomea carnea*	++
Leguminosae		*Albizia tulibrissin*	-
Magnoliaceae		*Michelia champaca*	+
Malvaceae	Chinese hibiscus	*Hibiscus rosasinensis*	-
Nyctaginaceae	Bougainvillea	*Bougainvillea sp.*	-
Portulacaceae		*Portulaca indica*	+
Punicaceae		*Plumeria alba*	-
	Dwarf pomogranate	*Punica granatum*	++
Solanaceae	Petunia	*Petunia hybrida*	+
		Solanum wendlandii	+
Verbenaceae		*Cleodendrum splendensis*	-
		C. thomsaii	-
	Verbena	*Verbena paciniata*	++
Weeds			
Amaranthaceae		*Alternanthera pungens*	-
	White cocks comb	*Celosia argentea*	+
		Gomphrena celosioides	-
		Digera arvensis	+
		Almania nodiflora	+
	Slender amaranth	*Amaranthus viridis*	+
Asclepiadaceae	Gigantic swallowort	*Calotropis gigantea*	+

Family	Common name	Botanical name	Presence of *T. palmi*
Asteraceae		*Flaveria australasica*	-
		Lagascea mollis	+
	Ragweed	*Parthenium histerophorus*	++
Compositae	Tropic ageratum	*Ageratum conyzoides*	++
		Tridax procumbens	-
Euphorbiaceae	Garden spurge	*Euphorbia hirta*	-
		Euphorbia heterophylla	-
	Copper leaf	*Acalypha indica*	+
		Phyllanthus maderaspatensis	+
Commelinaceae	Day flower	*Commelina bengalensis*	++
Convolulaceae	Morning glory	*Ipomoea* spp.	++
Capparaceae	Wilt mustard	*Cleome viscosa*	+
Labiatae		*Ocimum sanctum*	+
Leguminosae		*Alysicarpus longifolius*	+
		Alysicarpus monilifer	+
		Alysicarpus rugosus	+
	Begger weed	*Desmodium dicholomum*	+
		Indegofera glandulosa	-
	Sickle pod	*Cassia tora*	++
		Delonex regea	-
		Macroptilium atropurpureum	-
	Sesbania	*Sesbania spp*	++
Malvaceae		*Abelmoschus ficulneus*	-
Molluginaceae		*Mollugo cerviana*	-

Family	Common name	Botanical name	Presence of *T. palmi*
Monispermaceae		*Cocculus hirsutus*	-
Poaceae	Panic grasses	*Panicum* sp.	-
		Urochloa panicoides	-
	Crow foot grass	*Dactyloctenium aegyptium*	-
Portulacaceae	Common purslance	*Portulaca oleracea*	-
	Horse purselane	*Trianthema portulacastrum*	+
Rubiaceae		*Oldenlandea corymbosa*	++
Solanaceae	Cape gooseberry	*Physalis* spp.	+
	Jimson weed	*Datura stromonium*	+
Teleaceae	Jew's mallow	*Corchorus trilocularis*	-
Verbenaceae		*Cleodendron infortunatum*	+
	Lantana	*Lantana camera*	-
Zygophyllaceae	Puncture vine	*Tribulus terrestris*	++
		Trichoderma spp.	++

+ Thrips present: 1 to 10 thrips 25 terminals^{-1}.
++ Thrips present: more than 10 thrips 25 terminals^{-1}.
- No thrips

DISCUSSION

CHAPTER V

DISCUSSION

The main objectives of this study were

- to identify the principal thrips vectors of peanut bud necrosis virus (PBNV)
- to determine virus-vector relationships and
- to study the ecology of the principal thrips vector.

Peanut bud necrosis disease (PBND) was reported from India in 1968 (Reddy *et al.)* and initially the causal virus was identified as tomato spotted wilt virus (TSWV) (Ghanekar *et al.*, 1979). Subsequently several papers have been published on the transmission of the causal virus (considered to be TSWV) of PBND (Amin *et al.*, 1978, 1981). Evidence was presented to show that the virus was efficiently transmitted by *Frankliniella schultzei* and *Scirtothrips dorsalis* was identified as an inefficient vector (Amin *et al.*, 1981).

During the last decade many papers have appeared on the serological properties, chemical characterization and biodiversity among viruses earlier described as TSWV. As a result a new group called "tospoviruses" has been established to include the thrips transmitted, polymorphic viruses with a membrane (70-100 nm diameter) containing three RNA's (two ambisense and one negative stranded), all packaged in one particle. With improvements in purification and

production of antisera, high quality polyclonal and monoclonal antisera became available for various serotypes of tospoviruses. Following the determination of molecular mass of polypeptides of the causal virus of PBND and serological relationships, it was apparent that the virus was distinct from the known tospovirus serotypes, therefore it was named peanut bud necrosis virus (PBNV). All these developments added a new dimension to the problem of thrips transmission of PBNV and necessitated intensive study.

In 1988 *Thrips palmi* was reported to transmit the causal virus of watermelon silver mottle disease (WSMD) (Iwaki *et al.).* With the production of high quality antisera to PBNV, it was possible to determine the serological relationships of PBNV with other tospoviruses. It was apparent in 1990 (results published in 1992 by Reddy *et al.*) the causal virus of WSMD and PBNV were serologically related. Since the occurrence of *T. palmi* was reported from *India* in 1989 (Bhatti *et al.*) it was essential to test if *T. palmi* could transmit PBNV. Additionally, preserved thrips specimens, regarded as vectors of PBNV, when examined showed the presence of *T. palmi.* Palmer *et al.* (1990) examined the various thrips species collected on groundnut and critically identified the thrips species with potential to transmit tospoviruses. *T. palmi* was found to be widely occurring on groundnut. In this study unequivocal evidence has been presented to show that PBNV could be transmitted by *T. palmi.* Results of Amin *et al.* (1981) could not be repeated because both *F. schultzei* and *S. dorsalis* did not transmit PBNV. Since special skills are needed for the identification of thrips, the

possibility exists for the misidentification of *T. palmi* as *F. schultzei* in the studies conducted by Amin *et al.(1981)*. Chances for the hypothesis that TSWV was indeed the causal agent of PBND in early 1980's and subsequently PBNV became the dominant one are remote because attempts to locate TSWV on groundnut in extensive disease surveys since 1990 have failed (Reddy, D.V.R, personal communication).

Tospoviruses have now been recognised as a group consisting of several serologically distinct viruses viz., Tomato spotted wilt virus (formerly TSWV-L) (Francki *et al.*, 1991), Impatiens necrotic spot virus (INSV, formerly TSWV-I) (Law and Moyer, 1990 and Law *et al.*, 1992), Peanut bud necrosis virus (PBNV, formerly TSWV) (Reddy *et al.*, 1992), Watermelon silver mottle virus (TSWV-W) (Iwaki *et al.*, 1988) and Peanut yellow spot virus (PYSV) (Reddy *et al.*, 1990). Tospoviruses have been found to be transmitted by eight thrips species Viz., *T. tabaci, F. schultzei, F. occidentalis, F. fusca, S. dorsalis, T. palmi, T. setosus* and *F. tenuicornis* in different countries (Kormelink, 1994).

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 the same thrips species, *F. occidentalis, T. tabaci, F. schultzei* and *F. fusca* (Gardner *et al.*, 1935; Pittman, 1927; Mulder *et al.*, 1991; Sakimura, 1961). PBNV and WSMV group is transmitted only by *T. palmi* and INSV by *F. schultzei* and *T. tabaci*. *F. occidentalis* and *F. fusca* could not be

located on groundnut in India, to test their ability to transmit PBNV. On the contrary TSWV group could not be transmitted by T. palmi (Cho *et al.*, 1991; Mau *et al.*, 1991). PYSV is only transmitted by *S. dorsalis* and not by *T. palmi* and *F. schultzei.*

Virus transmission occurs only when thrips feed on infected plants in the larval stage and only such larvae can transmit the virus as adult. In the present investigation *T. palmi* could not transmit PBNV throughout the larval period (5 days) and only adults could transmit the virus. Bald and Samuel (1931) and Linford (1932) who worked with *F. schultzei* and *T. tabaci*, respectively, have shown that TSWV could only be transmitted by adult thrips that acquire the virus as larvae. However, the larvae sometimes also became viruliferous when the virus completed its latent period before pupation. Nevertheless several reports have subsequently been published on the ability of thrips larvae to transmit TSWV. Razvyazkina (1953) and Sakimura (1960, unpublished data) observed the transmission of TSWV by *F. fusca* and *T. tabaci* larvae, respectively. However, under natural field conditions, the stationary tendency of larval thrips, non feeding prepupal and pupal stages are not likely to play a role in the spread of viruses. Undoubtedly adults play the major role in the spread of tospoviruses. Large variation in latent period (i.e, the interval required by the vector , from the time it acquires the virus till it acquired the ability to transmit) was noted in different studies on virus vector relationships (for eg. a minimum of 4 days and a maximum of 18 days with an average of 11 days latent period for *T. tabaci* and 4 days

minimum and 12 days maximum with an average of 9 days for *F. fusca* (Sakimura, 1960 unpublished data); a minimum of 3 days for *T. tabaci* (Razvyazkina, 1953)). In addition to the vector species many factors which include virus source and environmental factors were likely to have contributed to this variation.

Transmission tests conducted with males and females of *T. palmi* did not show any significant difference in the transmission rate (Table 5). Similar observations were reported by Sakimura (1963) and Paliwal (1976) with *F. fusca*. Since thrips transmitted viruses have been shown to be propagative and only larvae could acquire the virus, it is not totally unexpected that the transmission rates between the sexes are similar.

Experiments on acquisition access period show that *T. palmi* larvae could acquire the virus in 5 min AAP and intervals up to 12 h only showed negligible differences in the transmission rates (Table 8). Eventhough the data on acquisition thresholds are limited in thrips, reports on TSWV indicate increased transmission efficiency with concomitant increase in AAP. Sakimura (1962) reported an increase in 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 was observed by Cho *et al.* (1991). However in the present study increased AAP did not result in corresponding increase in transmission rate of PBNV by *T. palmi*.

However 1 day AAP did show marginal increase in the transmission percentage.

The minimum inoculation access period (IAP) for the transmission of PBNV by *T. palmi* was found to be 1 h and with increased IAP up to 2 days there was a considerable increase in the transmission rate (Table 9). In other studies on IAP by thrips 5 to 30 min (Razvyazkina 1953; Sakimura 1960 and 1963; Amin *et al.*, 1981 and Allen and Broadbent, 1986) were found to be adequate. However *T. palmi* failed to transmit PBNV in 30 min IAP. The maximum transmission rate was observed after 2 days of IAP. This result indicate that insecticides with quick knock down effect or application of Dimethoate or Monocrotophos are likely to reduce PBND incidence.

One of the objectives of this investigation was to investigate if PBNV could multiply in its thrips vector, *T. palmi.* Initial attempts to determine virus increase by ELISA in groups of 10 have failed. Therefore the rather laborious serial transmission tests were undertaken to provide evidence for the persistency of PBNV in *T. palmi.* A procedure involving 2 day old groundnut seedlings, enclosed in a glass vial, facilitated serial transfer of individual thrips at 1 day intervals. Although the transmission by individual thrips was erratic, over 30% of viruliferous insects transmitted the virus for their entire life span. Retention of the virus throughout the life span of adult thrips has been reported by Sakimura (1962) and Ananthakrishnan (1973) in *T. tabaci* and Reddy *et al.* (1983) in *F. schultzei.* The erratic transmission was not totally unexpected because of the long IAP required by *T.*

palmi to transmit PBNV. Additionally Sakimura (1962, 1963) also reported erratic transmission of TSWV by *T. tabaci*. In the case of PBNV, AAP intervals did not influence the transmission pattern of individual thrips. Recently several lines of evidence suggest that tospoviruses replicates in the cells of the thrips vectors. These include increase 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.*, (1993) detected the presence of non structural protein encoded by the SRNA of TSWV in *F. occidentalis*. Therefore the evidence is unequivocal for the replication of tospoviruses in thrips vectors.

It is important to study the biology of the vector to facilitate transmission studies under laboratory conditions, to predict the disease incidence under field conditions and to take up timely control measures of the vector (Palacio, 1978). The present study on the biology of *T. palmi* using detached leaflet technique at different temperatures under laboratory conditions revealed 25°C as the optimum temperature for rearing and multiplication of the vector. The temperature at which the vector should have short developmental period, high fecundity and high per cent adult emergence is considered to be optimum. In the present study, 25°C satisfied all these requirements.This study confirms the reports on *T. palmi* under laboratory conditions by Palacio (1978) and by Wang and Chu (1990). However marginal differences in the number of eggs laid per day and the fecundity compared to the observations made by Wang *et al.* (1989) may be attributed to the different hosts

used.

In transmission experiments with a particular thrips species, a proper selection of plant species should be made for rearing and multiplication of the vector under controlled environmental conditions. The preferred plant species should have good tolerance for feeding and morphological fitness for easy recovery of thrips (Sakimura, 1961). Calilung (1990) observed the shortest developmental period and longest adult life period of *T. palmi* on watermelon under laboratory conditions. In the present study among the various hosts tested cowpea was found to be the best host for rearing *T. palmi*. Watermelon and cucumber, which harbored high number of *T. palmi* under field conditions proved to be unsuitable for laboratory rearing. High condensation of moisture in glass vials by these hosts resulted in rotting of leaves within 5 days, even before the hatching occurred. Among the other six hosts tried in the present study greengram was not preferred due to its hairy nature. Although the total number of larvae produced per female and adult emergence were higher on greengram than on cowpea, it was difficult to collect and transfer first instar larvae from greengram for the transmission studies. In addition the greengram leaflet was very thin and dried easily compared to cowpea. Groundnut was least preferred because of low fecundity, low adult emergence and relatively short life span of the adult. Hence cowpea was chosen to be the best host for rearing the vector.

Host range of *T. palmi* was studied in 45 weeds, 17 vegetables, 15 crop

plants and 24 ornamentals. Among the weeds *Ageratum conyzoides*, *Cassia tora*, *Parthenium histerophorus* and *Commelina bengalensis* harbored large number of *T. palmi*. These weeds were also recorded as the hosts for the virus (Reddy *et al.*, 1983). These weeds are usually abundant soon after the monsoon showers and are likely to serve as the reservoir hosts for PBNV and the vector. Crop plants such as blackgram, greengram, cowpea and soybean colonized large population of *T. palmi*. Therefore cropping with these short duration legumes, which are also hosts for the virus, either as a sole crop or as intercrops is expected to increase PBNV incidence. All the cucurbitaceous vegetables, cucumber, pumpkin, ridge gourd, bottle gourd and bitter gourd supported high population of *T. palmi*. Several workers (Bournier, 1983; Nakahara *et al.*, 1984; Johnson, 1986; Denoyes *et al.*, 1988; Cooper 1991; Fauziah and Saharan, 1991; Mau *et al.*, 1991 and Talekar, 1991) found cucurbits and other vegetables as the hosts of *T. palmi*. Growing of these vegetables around groundnut crop may also result in high incidence of PBND. In addition the annual and perennial ornamental hosts (Table 20) may also serve as sources for the multiplication and spread of *T. palmi*. The majority of these ornamentals are susceptible to PBNV. Removal of reservoir hosts unless practiced on a large scale, would not be of much use. Destroying weed reservoir hosts in small scale farms is not a practicle measure, as several crop plants also harbor PBNV and the vector and thus provide continuing alternative sources of infestation.

When *T. palmi* population was monitored under field conditions, JL 24 which is susceptible to PBND under field conditions colonized significantly more

number of *T. palmi* than the PBND resistant genotypes i.e. ICGV 86388, 86031 and 86430 in the both rainy seasons of 1991 and 1992 (Tables 17 and 18) indicating the resistance of the genotypes to the vector under field conditions. In both the rainy seasons *T. palmi* population and PBND incidence was very low at the beginning of the crop growth period (23 days after sowing), reached to maximum by 50 days after sowing and decreased thereafter indicating the primary spread of the disease by the vector. The results are in accordance with the findings of Amin (1985) who evaluated 3 groundnut genotypes, Robut 33-1, M 13 and TMV 2 for resistance to PBND and *F. schultzei* under field conditions. Robut 33-1 showed lower incidence of PBND than the other two genotypes, TMV 2 and M 13. Infestation of thrips was also lower on Robut 33-1 than the two cultivars which indicated resistance of the genotype to the vector under field conditions.

T. palmi failed to transmit an isolate of PBNV from a source maintained for 2 years continuously by mechanical inoculation. Similar findings were observed by Paliwal (1976) where *F. fusca* did not transmit TSWV, where the virus source was also maintained by mechanical inoculation for 2 years. In the case of two aphid transmitted viruses (bean yellow mosaic virus and pea enation mosaic virus) (Swenson *et al.*, 1964; and Tsai *et al.*, 1974) and wound tumor virus, a leaf hopper transmitted virus (Black, 1969), either a complete loss or considerable decline in vector transmissibility was observed when the viruses were maintained by means of transmission other than that by the insect vectors. Therefore the results obtained with PBNV were similar to those reported for several insect transmitted viruses.

Based on the experimental results obtained in the present study, the following conclusions can be drawn.

* Of the three thrips species tested, only *T. palmi* transmitted PBNV.
* Maximum transmission resulted with 1 day AAP and 2 days IAP.
* Serial transmission studies showed that the virus was persistent in *T. palmi*.
* Biology studies at different temperatures indicated 25°C as the optimum for rearing and multiplication of *T. palmi*.
* Host suitability studies revealed cowpea as the most suitable host for rearing under laboratory conditions.
* Host range studies of *T. palmi* showed a wide host range including leguminaceous, cucurbitaceous plants and some of the weeds found in groundnut fields.
* *T. palmi* populations monitored on selected PBND susceptible and resistant groundnut genotypes showed that the PBND susceptible genotype had significantly more number of *T. palmi* than PBND resistant genotypes.
* A PBNV isolate maintained for two years continuously by mechanical sap inoculation lost the ability to be transmitted by *T. palmi*.

SUMMARY

CHAPTER VI

SUMMARY

Studies were undertaken on the identification of thrips vector(s) of peanut bud necrosis virus (PBNV) and PBNV-thrips vector relationships under laboratory conditions at ICRISAT Asia Center (IAC), A.P., India from 1990 to 92. Field studies were carried out to monitor the populations of principal thrips vector (*Thrips palmi)* in some PBND resistant/susceptible genotypes at Directorate of Oilseeds Research (DOR), Rajendranagar, Hyderabad, A.P., India in rainy seasons of 1991 and 1992. Host range of *T. palmi* was studied on several crop plants, vegetables, ornamentals and weed plants at IAC and at DOR, Rajendranagar, Hyderabad from 1992 to 93.

Transmission tests conducted with laboratory reared and field collected thrips species of *Thrips palmi, Frankliniella schultzei* and *Scirtothrips dorsalis* indicated that *T. palmi* is the vector of PBNV of groundnut in India. *F. schultzei* and *S. dorsalis* are proved as non vectors.

T. palmi could not transmit PBNV throughout the larval period (5 days) and only freshly emerged adults transmitted the virus. *T. palmi* needed a minimum of 8 days latent period to transmit PBNV to groundnut. A single *T. palmi* adult was able to transmit PBNV and the maximum transmission (100%) was achieved with 10 adults/groundnut seedling. Both male and female *T. palmi* adults have

transmitted the virus with equal efficiency.

T. palmi failed to transmit an isolate of PBNV which was maintained continuously for 2 years by mechanical sap inoculations under glass house conditions.

T. palmi larvae could acquire the virus during 5 min acquisition access period (AAP). However to achieve the maximum transmission percentage (67) with individual thrips, 1 day AAP was required. AAP longer than 1 day did not result in increase in the transmission percentage. A minimum of 1 day inoculation access period (IAP) was required to transmit PBNV by *T. palmi* adults. The frequency of transmission increased considerably with increased IAP. Two days IAP was considered as the optimum for PBNV transmission.

A simple and easy method for handling single adult thrips during serial transmission studies was developed using 2 day old young groundnut seedling enclosed in a glass vial with cork. This method facilitated easy location and transfer of individual thrips in the seedling and transfer of individual thrips without any loss or mortality. Serial transmission studies indicated that most of the *T. palmi* adults can transmit the virus for more than half of their life period and increased AAP had no effect on the serial transmission of PBNV by *T. palmi*. Erratic transmission of PBNV by *T. palmi* confirmed observations on other thrips transmitted tospoviruses. Retention of PBNV throughout the life of *T. palmi* confirmed the persistent nature of the virus.

Studies on the biology of *T. palmi* at different temperatures indicated a significant and negative correlation between the temperatures and different developmental periods (egg, larva, pupa). The eggs did not hatch in to larvae at 15°C. Very few eggs were laid at 35°C and the fecundity was highest at 25°C. Adult emergence was also highest at 25°C. So 25°C was found to be the optimum temperature for rearing *T. palmi* under laboratory conditions.

Studies conducted on host preferences by *T. palmi* under laboratory conditions using detached leaflet technique indicated that it could complete its life cycle very fast (7.8 days) on cowpea. Watermelon and cucumber which harboured large number of *T. palmi* under field conditions were proved to be unsuitable for laboratory rearing. Groundnut was the least preferred host. Cowpea was identified to be the best host for rearing and multiplication of *T. palmi* under laboratory conditions.

Host range studies of *T. palmi* showed that crop plants such as cowpea, blackgram, greengram and soybean, most of the cucurbitaceous vegetables and some of the common weeds found in groundnut fields Viz., *Ageratum conyzoides*, *Commelina bengalensis*, *Cassia tora* and *Parthenium histerophorus* found to harbour large populations of *T. palmi* (more than 10 thrips/25 terminals).

When *T. palmi* populations were monitored on selected PBND susceptible and resistant groundnut genotypes, PBND susceptible groundnut genotype (JL 24) showed significantly more number of *T. palmi* than the PBND resistant genotypes

(ICGV 86031, ICGV 86388 and ICGV 86430) indicating the resistance/ susceptibility of the genotypes to the vector.

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