Recent Studies on Peanut Bud Necrosis Disease:

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Edited by

A A M Buiel,
J E Parlevliet,
and
J M Lenné

Landbouwuniversiteit Wageningen
Department of Plant Breeding
Agricultural University of Wageningen

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Opening Remarks

Y L Nene[1]

Mr Chairman, ladies and gentlemen,

When I was requested last week by Hanneke Buiel to speak a few words at this meeting, I was initially reluctant because I have been out of touch with this subject for a long time. Later, I had second thoughts and agreed. I figured that if nothing else, I could contribute a historical perspective on the subject. Therefore, I accepted her request, for which I am grateful to her.

As I began reflecting on this disease–peanut bud necrosis– my thoughts went back some 28 years, i.e., to 1967, when I was a Professor at Pantnagar at one of the agricultural universities in India. With the help of a special grant called PL-480 from the United States Agency for International Development (USAID), I was able to initiate work on describing various viruses that affect legumes in the state of Uttar Pradesh in northern India, particularly mung bean, and the closely related black gram or urad bean.

During the course of that study, in addition to the already well-known disease called mung bean yellow mosaic caused by a distinct gemini virus, we could see three distinct diseases: mosaic mottle, leaf crinkle, and leaf curl, each identified by their distinct symptoms. Yellow mosaic was found to be transmitted by *Bemisia tabaci* (white fly). Mosaic mottle was found to be transmitted mechanically, and by aphids as well, and we related it to the Bean Common Mosaic Virus. Leaf crinkle was also found, with great difficulty, to be mechanically transmitted. Later on, after I left the University, it was found to be a distinct virus transmitted by beetles. However, we could make no progress with the leaf curl. We called it leaf curl because of necrosis at the top and the trifoliolates which showed distinct downward curling of margins. We did not succeed in effecting mechanical transmission

When I was about to leave Pantnagar to join ICRISAT in 1974, I invited Dr A M Ghanekar, who is now with ICRISAT, to work as a Visiting Scientist. He was on a special assignment with the Council of Scientific Research in India. I requested him to look at the leaf curl disease and see if we could get a breakthrough. About 6 months after I left Pantnagar, Dr Ghanekar wrote to me stating that he had succeeded in mechanically transmitting that virus, and that he could get excellent local lesions on cowpea leaves. In 1975, Dr S N Nigam joined ICRISAT as the first Groundnut Breeder. Dr Nigam began his work on groundnut but he needed the help of a pathologist. I was the only Principal Pathologist at that time. I was overseeing research on all the five crops until Dr R J Williams joined as the Cereals Pathologist. I noted that the symptoms of bud necrosis disease considerably resembled those of the leaf curl of mung bean. It was then the most dominant disease in Dr Nigam's groundnut plots.

Meanwhile, Dr Ghanekar expressed interest in joining ICRISAT as a Visiting Scientist with the same funding support. Since I was impressed with what he had accomplished with the mung bean leaf curl, I invited him to come here and have a go at the bud necrosis disease, because earlier attempts by researchers in Andhra Pradesh and Punjab had not resulted in successful mechanical transmission. I hoped that Dr Ghanekar might succeed. He did not disappoint me, because he was soon able to successfully demonstrate mechanical transmission of bud necrosis virus. I consider that a major breakthrough. It appeared to me that bud necrosis disease was almost similar to leaf curl, although I had no concrete evidence at that time.

Then Dr D V R Reddy, an eminent Virologist, joined ICRISAT. He seriously took up work on this disease. He went into details based on symptoms, thrips transmission, and electron microscopy, and came to the conclusion that the tomato spotted wilt virus was most likely to be the cause of the disease. However, it became clear by 1991 that the bud necrosis disease was not caused by the

1. Deputy Director General, ICRISAT Corporate Office, Patancheru 502 324, Andhra Pradesh, India.

tomato spotted wilt virus, but by a distinct tospovirus which at present exists only in Asia. It took time to establish the transmission of the virus by thrips.

Dr P W Amin, who worked at ICRISAT as an Entomologist, had excellent training in vector biology at the University of California, USA, and made significant contributions along with Dr D V R Reddy in establishing the transmission of the virus by thrips. Sometime during 1987-88, there was some debate about the various species of thrips involved. Finally by 1992, it seemed that we had probably settled the question on the thrips species involved in the transmission of the disease. *Thrips palmi* seems to be the most dominant species, although that was not the case earlier. In addition, *Frankliniella schultzei* but not *Scirtothrips dorsalis* was shown to be the vector of this virus.

Somehow, in subsequent years, I got the impression that bud necrosis disease receded into the background and more importance was given to the peanut stripe virus (PStV). I can understand why this was so. The PStV is seed transmitted, whereas bud necrosis disease is not; and as an international center we have to be more cautious about viruses that are seed transmitted. I can well understand Dr Reddy's anxiety to focus attention on PStV.

Work on PStV is well in hand now, and the time has again come for us to really put a major thrust in understanding the bud necrosis disease. As regards host resistance, a lot has been achieved, but a lot more needs to be done. I have seen reports of availability of field resistance, which I would term as "less susceptible" material because the disease is conspicuous in these field-resistant types. In addition, useful information exists on timely sowing and on adjusting the density of the plants to reduce the disease.

A few minutes before addressing you, I checked with Dr R A Naidu whether good electron micrographs of the bud necrosis virus now exist. As an ex-Virologist, I was not impressed with the photographs that I had seen earlier, and those I saw in ICRISAT publications. Dr Naidu assured me that good photographs are now available, though they have not been published yet. This indicates that we have made progress in purification and in getting proper electron micrographs.

I must also mention the work done on monoclonal antibodies. I am not sure of the status of the work. It is for this group to discuss and see how this work could be further augmented and utilized in field identification. The host range needs to be checked. Quite a few hosts are already known. Mung bean is known to be a host, but I also remember having seen the disease on soybean at Pantnagar. The disease was then confused with bud blight; it was not bud blight, but it was similar to leaf curl in mung bean. I do not yet see soybean on the lists of the host range of bud necrosis disease. However, I suspect that soybean in Central and northern India does have the same tospovirus. We need to monitor the changes that might be taking place in the vectors. Today *Thrips palmi* may be the dominant vector, but a few years hence this may not be so.

I am sure this one-day meeting will be extremely useful. My only regret is that I will not be present throughout the meeting as I have to catch a flight around noon. I am certainly delighted to be with you this morning and share my thoughts with you; at least to give you a historical perspective.

Let me record that we greatly appreciate the help we are receiving from the governments and scientists of the Netherlands, USA, Thailand, and India in this important research activity.

Thank you very much.

Peanut Bud Necrosis Disease : An Overview

D V R Reddy[1], A A M Buiel[1,3], T Satyanarayana[1], S L Dwivedi[2], A S Reddy[1], A S Ratna[1], K Vijaya Lakshmi[1], G V Ranga Rao[1], R A Naidu[1], and J A Wightman[1]

Abstract

Peanut bud necrosis disease (PBND) was first recorded in India in 1949. The economic importance of the disease was realized during the late 1960s when incidences up to 100% were recorded in many groundnut-growing regions in India. The disease has been described under different names. It was shown to be economically important in parts of Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, and Uttar Pradesh. Although it was earlier reported to be caused by tomato spotted wilt virus (TSWV), recently, the causal virus of PBND in India was shown to be a serologically distinct tospovirus, now referred to as peanut bud necrosis virus (PBNV), transmitted by Thrips palmi. *Surveys in many groundnut-growing countries indicate that PBNV is restricted to South and Southeast Asia. Several cultural practices are available to control the disease. Excellent progress has been made in the identification of sources of field resistance.*

The PBNV genome contains three RNA species and the sRNA has recently been sequenced and the two genes it codes for have been identified. Progress achieved will lead to the production of high quality diagnostic aids and for the development of transgenic resistance. Future research will focus on epidemiology, development of early-maturing resistant cultivars, sequencing of the entire viral genome, the production of high quality diagnostic aids, and assessment of biodiversity among PBNV isolates.

Introduction

The occurrence of a disease with symptoms similar to those of peanut bud necrosis disease (PBND) was mentioned in the Annual Report of the Indian Agricultural Research Institute in 1949. This appears to be the first record of occurrence of PBND in India. The name "Bud Necrosis" was given in 1968 and the disease was considered to be distinct at that time because none of the other groundnut viruses reported until 1968 were known to produce the bud necrosis symptom (Reddy et al. 1968). To our knowledge, PBND has been described in India since 1962 under at least seven different names: groundnut mosaic, groundnut rosette, bunchy top, chlorosis, ring mottle, bud blight, and ring mosaic (Reddy 1988). ICRISAT has conducted regular surveys in the major groundnut-growing areas of India from 1976 to 1982, and occasional surveys till 1992. The disease was

1. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
2. Genetic Enhancement Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India
3. Department of Plant Breeding, Agricultural University of Wageningen, P O Box 386, 6700 AJ Wageningen, The Netherlands
4. Present address: Agricultural College, Bapatla, Andhra Pradesh, India.

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apparently economically important in parts of Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, and Uttar Pradesh. Peanut bud necrosis disease is also currently recognized as economically important in parts of China, Nepal, Sri Lanka, and Thailand. Its distribution appears to be restricted to Asia. Losses due to PBND have been estimated at over 89 million US $ per annum (ICRISAT 1992).

Symptoms

Symptoms produced by peanut bud necrosis virus (PBNV) in groundnut are difficult to distinguish, if at all, from those caused by tomato spotted wilt virus (TSWV). Initial symptoms appear on young quadrifoliolates as mild chlorotic mottle or spots, which develop into necrotic and chlorotic rings and streaks. Necrosis of the terminal bud, a characteristic symptom, occurs on crops grown in the rainy and postrainy seasons, when ambient temperatures are relatively high. Secondary symptoms are stunting, axillary shoot proliferation, and malformation of leaflets. If plants are infected early, they are stunted and bushy. If plants older than 1 month are infected, the symptoms may be restricted to a few branches or to the apical parts of the plants.

Due to the severity of the symptoms, the virus causes severe losses to the groundnut crop, especially when plants are infected before they are a month old. Seeds from such plants are small, shriveled, mottled, and discolored. Late-infected plants may produce seed of normal size. However, the testae on such seed are often mottled and cracked.

Causal Virus

Until 1990, PBND in India was reported to be caused by TSWV (Reddy et al. 1991). High-quality antisera became available for the detection of tospoviruses, to which the group TSWV belongs, only during the late 1980s. Data from serological comparisons and subsequently from sequencing of nucleic acids revealed the existence of several distinct tospoviruses (German et al. 1992, de Avila et al. 1993).

In 1992, the virus causing PBND was identified as a distinct tospovirus and named PBNV. With ELISA as well as Western blots, PBNV was shown to be serologically distinct from TSWV and Impatiens necrotic spot virus (INSV) (Reddy et al. 1992).

PBNV contains three RNA species of about 9.0 kb (lRNA), 5.0 kb (mRNA), and 3.0 kb (sRNA) (D.V.R. Reddy and S. Gowda, unpublished). Recently sRNA has been sequenced and the details will be provided elsewhere in these proceedings (Satyanarayana et al. 1995).

Transmission

Sap transmission. Peanut bud necrosis virus can be transmitted by mechanical sap inoculations if care is taken to extract the virus only from young infected leaflets with primary symptoms. Extracts should be prepared in neutral phosphate buffer containing an antioxidant such as merceptoethanol, and must be kept cold throughout the inoculation process.

Thrips transmission. Amin et al. (1981) reported that the virus causing PBND in India is transmitted by *Frankliniella schultzei* and *Scirtothrips dorsalis*. Subsequent investigations, which involved accurate identification of thrips, showed that in fact *Thrips palmi* transmits PBNV, and not *F. schultzei* or *S. dorsalis*, which are also present on the plants. Further experiments showed that *T. palmi* could acquire PBNV as larvae and transmit it as adults. Maximum transmission (100%) was obtained when there were 10 adults per plant. The majority of individual adult thrips transmitted the virus for more than half of their life period, indicating the degree of erratic transmission. Cowpea was found to be the best host for rearing and multiplying *T. palmi* under laboratory conditions (Vijaya Lakshmi 1994, Wightman et al. 1995, these proceedings).

Diagnosis

Several methods can be used for the diagnosis of PBNV. The following are recommended, especially for developing countries.

- Sap inoculations on to cowpea (cv C-152) and *Petunia hybrida*. Cowpea produces concentric chlorotic and necrotic lesions; *Petunia* produces necrotic lesions (Reddy et al. 1991).
- ELISA using polyclonal antibodies. They clearly distinguish PBNV from TSWV and INSV (Reddy et al. 1992).
- Presence of typical tospovirus particles in leaf extracts. Even in leaf dip preparations, if young tissues showing initial symptoms are used, PBNV particles can be observed. They are 80–100 nm in diameter, and are surrounded by a double membrane of protein and lipid.

Management of PBND

Several cultural practices such as adjustments to sowing dates, sowing at the recommended rate, adopting measures to maintain plant population, intercropping with fast-growing cereal crops such as maize and pearl millet can reduce the incidence of PBND. These practices have been shown to reduce infestation by *T. palmi*.

Roguing of infected plants, especially during early stages of plant growth, should be avoided because this practice creates gaps in the field and can increase PBND incidence.

Excellent progress has been made in the identification of sources of field resistance to PBND. Since this aspect will be covered in two presentations (see Buiel et al. 1995, Dwivedi et al. 1995, these proceedings), we do not wish to deal with it here. Although many high-yielding PBNV-resistant varieties have been developed, they are medium-maturing types. Some of the field-resistant genotypes such as ICGV 86388, show resistance to PBNV and less colonization by vector thrips compared with susceptible genotypes (Buiel et al. 1995, Dwivedi et al. 1995, these proceedings). Cultivars such as ICGS 11, Kadiri 3, and ICGS 44 are field resistant to PBND.

Future Research

Peanut bud necrosis virus and *T. palmi* have extremely wide host ranges. Therefore, the virus is a potential threat to cropping systems which include legumes, vegetables, and ornamentals. Some of these crops are grown under irrigation and protected with insecticides. These conditions are likely to result in a gradual buildup of PBNV inoculum, leading to disease epidemics. Therefore, it is essential to closely monitor the incidence of PBNV in various cropping systems, which include highly susceptible hosts of the virus and the vector.

Since the field-resistant groundnut varieties are of medium duration, attempts should be made to breed early-maturing cultivars for environments where they are needed. To achieve this rapidly, transgenic groundnuts expressing PBNV genes could be developed. sRNA of PBNV has been fully sequenced and the coat protein gene located (Satyanarayana et al. 1995, these proceedings) for utilization in the transformation and regeneration of groundnut.

Thrips are known to have several parasites and predators. They have not been tested for their effectiveness to reduce populations of *T. palmi*. It would also be useful to study the effect of a range of synthetic and natural insecticides on the parasites and predators of *T. palmi*, once they are identified.

Peanut bud necrosis virus is currently known to cause economic losses to many commercial crops other than groundnut. These include chilli, potato, tomato, tobacco, and early-maturing legumes such as mung bean and urd bean. Data generated for the management of PBNV on groundnut are likely to be applicable to these crops. Due to the specialized skills required for the detection of PBNV, the economic importance of PBNV in many other high-value crops has not been realized.

Variation among isolates of PBNV has not yet been fully investigated. This should be carried out because of its implication for the durability of host-plant resistance. Limited tests conducted in the case of groundnut (Buiel et al. 1995, these proceedings) indicate that the resistance is effective in India in different ecoregions. These tests should be extended to PBNV hot spots in other South Asian countries.

Thrips palmi has recently been detected in large populations in many southeastern states of USA. In these states, currently, TSWV is considered to be economically important. To date, we have no evidence of the occurrence of PBNV in the USA or in other countries where *T. palmi* may be occurring in large populations. The potential threat from PBNV therefore exists in such places, indicating the need to conduct surveys for the occurrence of PBNV.

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Nucleotide Sequence of the Nucleocapsid Gene of Peanut Bud Necrosis Virus

T Satyanarayana[1], S E Mitchell[2], S Brown[2], S Kresowich[2], D V R Reddy[3], R A Naidu[3], R Jarret[2], and J W Demski[1]

Abstract

Peanut bud necrosis virus (PBNV) is widely distributed in Asia, infecting various economically important crops. Peanut bud necrosis virus was shown to be serologically distinct from tomato spotted wilt virus (serogroup I), groundnut ring spot virus (serogroup II), and impatiens necrotic spot virus (serogroup III). Peanut bud necrosis virus is included in serogroup IV along with watermelon silver mottle virus (WSMV) and the tomato isolate of groundnut bud necrosis virus (GBNV-To). As in the case of other tospoviruses, PBNV contains three RNA segments associated with nucleoprotein (N), enclosed in a lipid membrane containing two glycoproteins G1 and G2.

The nucleocapsid of PBNV was separated in sucrose gradients and the fraction containing the smallest of the three RNAs (RNA 3 or sRNA) was used for RNA isolation. The entire sequence of the RNA 3 was determined. The N gene is 831 nucleotides long, located on the complementary strand that encodes for a 30.7 KDa protein. The N gene was amplified by RT-PCR and expressed in vitro in Escherichia coli *(BL 21) after cloning into pET-15B. Immunoblot analysis of expressed protein (30 KDa) with polyclonal antisera against the purified virus confirmed that the 30.7 KDa protein is the N protein of PBNV. Amino acid sequence comparison of the N protein revealed identities of 32–35%, with members of serogroup I, II, and III, whereas it had 85–86% identity with members of serogroup IV. GBNV-To showed 99% identity with the WSMV N protein sequence. The data obtained confirm earlier reports that PBNV should be considered as a distinct species belonging to serogroup IV.*

1. Department of Plant Pathology, University of Georgia, Experiment Station, Griffin, GA 30223, USA.
2. Plant Genetic Resources Conservation Unit, USDA, Georgia Experiment Station, 1109 Experiment Street, Griffin, GA 30223, USA.
3. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.

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Thrips palmi, General Pest and Vector of Some Tospoviruses in Asia

J A Wightman[1], G V Ranga Rao[1], and K Vijaya Lakshmi[1,2]

Abstract

Thrips palmi *Karny was initially considered to be a pest of tobacco and cotton in Indonesia during the first 30 years of this century. A population explosion apparently occurred during the 1970s, which resulted in it attaining pest status, mainly on cotton, cucurbits, and solanaceous vegetables around the Pacific rim. This probably happened because natural control processes were disrupted by insecticide abuse. There has been little concern about this species as a vector of tospoviruses, except on groundnut crops. It has been identified as the vector of peanut bud necrosis virus in India. It acquires the virus as a larva and transmits it as an adult. The virus-vector relationship is persistent. Management of the thrips and the virus are clearly linked. Vector resistance is available and is the ideal solution within the context of integrated pest management, if the genes in question can be incorporated in a variety adapted to an endemic environment. The polyphagous nature of the thrips is considered to be an advantage as far as the maintenance of natural control processes is concerned.*

Introduction

There have been several advances in knowledge since the review by Reddy and Wightman (1988), of the transmission of the tomato spotted wilt virus (TSWV) by thrips—once listed as the causative agent of peanut bud necrosis disease in South Asia. In particular, we record the realization that the vector of the peanut bud necrosis virus (PBNV) is *Thrips palmi* Karny, and not *Frankliniella schultzei* Trybom (Palmer et al. 1990, Vijaya Lakshmi 1994). As *T. palmi* is relatively unknown in this context, general information about its distribution, host range, and applied ecology is provided, together with details of the host-vector relationship.

Distribution and Host Plants

Thrips palmi became conspicuous as an insect pest in the first decade of this century, as a result of the damage it caused to tobacco in Java (Karny 1925) where it subsequently also became a pest of cotton. The same report indicates that it may also have been feeding in tobacco flowers in India. It was officially described by Karny in 1925 from material collected from tobacco growing in Java. The specific name probably honors the entomologist Dr B T Palm who was prominent in Indonesia in the 1920s. There is no record of this insect being associated with palm trees.

1. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
2. Present address: Agricultural College, Bapatla, Andhra Pradesh, India.

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Wightman, J.A., Ranga Rao, G.V., and **Vijaya Lakshmi, K.** 1995. *Thrips palmi*, general pest and vector of some tospoviruses in Asia. Pages 11–15 *in* Recent studies on peanut bud necrosis disease: proceedings of a Meeting, 20 Mar 1995, ICRISAT Asia Center, India (Buiel, A.A.M., Parlevliet, J.E., and Lenné, J.M., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and P O Box 386, 6700 AJ Wageningen, The Netherlands: Department of Plant Breeding, Agricultural University of Wageningen.

Little more was heard of this species until the late 1970s and early 1980s when reports appeared from many countries in the Pacific rim (Table 1). Cucurbits were mentioned as host plants in many of these reports. However, the experience of Bernardo (1991) in the Philippines indicates that this may be because *T. palmi* was previously thought to be *T. tabaci*. A misidentification also occurred among groundnut thrips in India (Palmer et al. 1990). Collection records at ICRISAT Asia Center indicated that this species was widely distributed on groundnut crops in India by 1980. The proceedings of a workshop on thrips in southeast Asia (Talekar 1991) leaves little doubt about the economic importance of this species as a pest of vegetables.

Houston et al. (1991) included the Sudan among the countries where infestations have been detected, but provided no citation. Palmer (1990) did not include *T. palmi* among the 45 genera and 42 species of common African thrips for which she provided a key. *Thrips palmi* has also been found in Georgia, USA (D V R Reddy, ICRISAT, personal communication). The host list in Table 1 is undoubtedly incomplete, as indicated by Bernardo (1991) and information collected by Vijaya Lakshmi (1994) from two sites near Hyderabad in southern India. She detected *T. palmi* on 44 of 64 cultivated plant species and on 27 of 45 wild plant species.

Table 1. Distribution and hosts of *Thrips palmi*.

Country	Host plants	Year[1]	Reference
Indonesia	Tobacco Cotton Soybean	From 1908 1982	Karny (1925) Miyazaki et al. (1984)
Japan	Cucurbits Egg plant Ornamentals	From 1978	Bournier (1987)
Philippines	Cotton Watermelon	1978	Schmutterer (1978)
Thailand	Cotton	Before 1981	Wangboonkong (1981)
India	Groundnut Mango	Before 1980 Before 1987	Palmer et al. (1990) Verghese et al. (1988)
Mauritius[2]			Verghese et al. (1988)
Hong Kong[2]			Verghese et al. (1988)
New Caledonia	Cucurbits Egg plant	From 1979	Bournier (1987)
Wallis Islands	Egg plant	1981	Bournier (1987)
Reunion Island	Onion	1980	Bournier (1987)
Australia	Watermelon	1989	Houston et al. (1991)
Sudan			Houston et al. (1991)
West Indies			Houston et al. (1991)
Hawaii	Cucumbers Orchids	Before 1985	Hata et al. (1993)

1. Indication of earliest record.
2. Reference cited from indirect sources.

Thus, *T. palmi* is a significant pest in its own right. It is common throughout Asia and the Pacific, and has been detected in Australia. It also came into prominence relatively suddenly. Speculation exists that the 'explosion' since the late 1970s was because of prior confusion with other species. An alternative suggestion is that biotypes adapted to a number of hosts developed during the 1970s (Bournier 1987). However, the over-riding factor is likely to be the intensification of insecticide application to cash crops during the 1970s and 80s. Hirose (1991) presents evidence from Thailand showing that, where insecticides were applied to vegetable crops, *T. palmi* was abundant and there was no parasitism. The converse was true in home gardens which were insecticide-free and where the predators and parasites of this species could easily be detected. This parallels experience with TSWV reported in Wightman and Amin (1988), indicating a positive relationship between insecticide application and the incidence of the virus in groundnut crops. It is possible that this species has always been widely distributed and has always had the ability to colonize a wide range of hosts. However, the potential did not manifest itself until the 1970s, when pesticide application became more and more intense throughout Asia and released this species from the regulation of natural enemies.

Thrips palmi as a Virus Vector

Thrips palmi has been linked with the transmission of watermelon silver mottle virus (WSMV) to water melon in Japan (Kameya-Iwaka et al. 1988) and Taiwan (Yeh et al. 1995). There was no mention of this species being a virus vector in Talekar (1991), even though tospoviruses exist in several crops infested by this insect. This may indicate either that virus diseases of the cash crops concerned have probably not been studied in detail or that it is a further example of the currently inexplicable irregularities governing the distribution of this family of viruses by thrips (Reddy and Wightman 1988).

We do know that *T. palmi* is at least the main vector of PBNV to groundnut in India. Vijaya Lakshmi (1994) showed that *F. schultzei* and *Scirtothrips dorsalis* Hood did not transmit the virus under confined, experimental conditions, whereas *T. palmi* did.

These three are the predominant thrips species living on groundnut in South Asia. Observations at ICRISAT Asia Center during 1990/91 (Wightman and Ranga Rao 1994) indicated that before flowering, *S. dorsalis* was the dominant species (72%) living in folded leaflets. After flowering, *F. schultzei* showed a clear preference for living in flowers: *T. palmi* was found in folded leaflets and in flowers.

Laboratory experiments carried out to quantify the transmission process (Vijaya Lakshmi 1994) showed that only *T. palmi* adults transmit the virus. Presumably, larvae cannot do this because, under the experimental conditions, the larval stage lasted for 5 days and there is an 8-day latent period. It was possible for larvae to acquire the virus within 5 min of commencing feeding, although 24 h was needed for the maximum recorded rate of acquisition (67%) to occur. Longer periods of exposure did not increase this rate. A minimum of 1 day access to a host was required for adults to acquire and transmit the virus. Two days was considered to be the optimum inoculation access period in terms of the conduct of laboratory experiments. Serial transmission studies showed that *T. palmi* adults were able to transmit the virus until they died, a period extending up to 20 days from eclosion. However, the pattern of transmission was erratic. These findings indicate a persistent virus-vector relationship.

All stages were exposed to constant temperatures between 15 and 35°C. The optimum temperature for rearing was 25°C. The highest and lowest temperatures tested were outside the normal range of this species under the experimental conditions.

Integrated Management of *T. palmi* and PBNV

Comments above direct us away from recommending to farmers that they apply insecticides for the management of thrips and of PBNV. The natural enemies appear capable of maintaining

populations at subeconomic levels. However, information about these natural enemies is sparse, and would certainly be a good subject for further study.

Available evidence indicates that this species if not polyphagous, is certainly oligophagous. This has several implications. The value of removing alternative host plants growing near the crop is in question. In fact, the alternative host plants almost certainly act as hosts for the predators and parasites needed to initiate the natural control process of the vector in a newly sown crop.

There are several groundnut genotypes and advanced lines with resistance to thrips (Wightman et al. 1990). Vijaya Lakshmi (1994) demonstrated that, compared with ±30% PBNV incidence in a thrips-susceptible variety, PBNV incidence was low (±1%), in thrips-resistant material growing in open field conditions during the seedling stage when this virus is likely to have the most effect on yield. As the vector is a 'pest' in its own right, clearly the thrips resistance genes should be considered for material bred for environments where the thrips and this disease are endemic, provided yield gap analysis indicates the need in economic terms. The 'genetic' approach combined with the conservation of natural control processes is clearly called for, because no other(s) appear to be available.

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Dynamics of the Spread of Tospoviruses by their Vectors

D Peters, I Wijkamp, F van de Wetering, and R W Goldbach[1]

Abstract

Studies of the relationships between vectors and tospoviruses have revealed previously unrecognized aspects, which lead to a better understanding of the spread of these viruses. Acquisition of the virus occurs during a rather small period, and seems restricted to the first days after emergence from eggs. The larvae which acquire the virus early in their development, transmit it at a high percentage before they pupate. Acquisition of the virus later in larval development does not result in infectious thrips. The development of infectivity is apparently inversely related to the amount of virus ingested, as determined by the enzyme-linked immunosorbent assay (ELISA). The number of infectious punctures made by different viruliferous thrips varies considerably per unit of time. The infectivity of the thrips is not only a function of the virus load, but may also depend on the probing or feeding activity of the thrips.

Frankliniella occidentalis *appeared to be the most efficient vector of four different tospovirus species tested. Three populations of* Thrips tabaci *did not transmit any of these viruses, whereas one population of this species inefficiently transmitted tomato spotted wilt virus.* F. intonsa *appeared to be a new vector of tospoviruses.*

Although the virus replicates in its vector, pathogenic effects on the thrips by the virus could not be demonstrated. On the contrary, as the food quality diminishes, infected plants may have a harmful effect on the development of the larvae.

Introduction

Tospoviruses, which cause devastating diseases of many economically important crops worldwide, are exclusively spread by some thrips species in a persistent way. So far, eight thrips species (*Frankliniella fusca, F. intonsa, F. occidentalis, F. schultzei, Scirtothrips dorsalis, Thrips palmi* Karny, *T. setosis*, and *T. tabaci*) have been recorded as vectors. The spread of tospoviruses depends on specific interactions between the host plant, the thrips, and the virus. The female adult selects the host plant on which the eggs are deposited, and the larval offspring develop. From them, a new generation of adults ultimately disperses. The viruses are acquired and transmitted in these close relationships between plant host and thrips. In the past, the transmission has simply been described as a process in which the acquisition was thought to occur by the larvae, and the transmission was mainly by viruliferous adults. Globally, inspired research on tospoviruses during the last decade has led to further unraveling of the relations between the tospoviruses and their vectors.

Increased knowledge on, e.g., the mean length of the latent period, the length of the acquisition and inoculation periods, the involvement of larval stages in acquisition and transmission, and the effect of the infected host on the thrips has thrown new light on the dynamics of the diseases

1. Department of Virology, Agricultural University, Wageningen, The Netherlands.

Peters, D., Wijkamp, I., van de Wetering, F., and **Goldbach, R.W.** 1995. Dynamics of the spread of tospoviruses by their vectors. Pages 17–23 *in* Recent studies on peanut bud necrosis disease: proceedings of a Meeting, 20 Mar 1995, ICRISAT Asia Center, India (Buiel, A.A.M., Parlevliet, J.E., and Lenné, J.M., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and P O Box 386, 6700 AJ Wageningen, The Netherlands: Department of Plant Breeding, Agricultural University of Wageningen.

caused by tospoviruses. This paper aims to present an integrated view of the relationship between the virus and its vector.

Acquisition of the Virus

It is well demonstrated that thrips become viruliferous when larvae ingest virus from infected plants, whereas repeated observations leave no doubt that the adults do not become viruliferous when they acquire the virus (Sakimura 1962). Although acquisition can take as little as a few minutes, the chance of larvae becoming viruliferous increases with the time of feeding on infected plants. Ranga Rao and Vijayalakshmi (1992) showed that this period may be shorter than 30 min in studies on groundnut bud necrosis virus (GBNV) using *Thrips palmi* as a vector. Mean values for the acquisition access period (AAP_{50}) have thus far not been reported. Recent studies with *Frankliniella occidentalis* have shown that when given AAPs of 24 h on tomato spotted wilt virus (TSWV)-infected plants, significantly more adults became viruliferous when the virus was acquired by first larval instars compared with virus acquisition by second stage larvae (Fig. 1). This effect occurred even though the amount of virus ingested increased proportionally with the age of the larvae (Fig. 2; van de Wetering, unpublished). This finding has some consequences for infection in the field, and will be discussed later.

Latent Period

The latent period varied from a few days to at least 18 days (Sakimura 1962). Data on the median latent period (LP_{50}) were lacking until recently. This parameter was established for two tospoviruses in a series of daily transfers with larvae which were given an AAP of 24 h to first larval instars which were 0–4 h old (Wijkamp and Peters 1993). This study revealed that approximately 80% of the *F. occidentalis* individuals which finally became viruliferous, transmitted TSWV and Impatiens necrotic spot virus (INSV) before they pupate. These results indicate that all processes leading to viruliferous thrips can be completed before pupation. The LP_{50} found for the transmitting larvae ranged between 80 and 170 h depending on the temperatures applied during the experiment (Wijkamp and Peters 1993). Although the LP_{50} for TSWV and INSV do not differ, the efficiency by which these viruses are transmitted differs considerably. Tomato spotted wilt virus was transmitted by 55% of the thrips used and INSV by 92%. The observation that larvae can transmit tospoviruses may enhance the spread of these viruses when the plant canopy is closed or in other situations, where plants are touching one another.

Inoculation Access Period

Inoculation access periods (IAPs) as short as 5 min have been reported. However, as for the AAP, no median values are known. Using the data from experiments in which *Thrips palmi*, viruliferous for GNBV, was tested on groundnut (Ranga Rao and Vijayalakshmi 1992), a median IAP of almost 8 h could be calculated by probit analysis. Most inoculation studies are performed with test plants. In inoculation experiments, Wijkamp and Peters (1993) used leaf disks of Petunia, a host which responds by the production of readily recordable necrotic lesions. The necrotic lesions appear at the sites where infectious piercings are made. The number of infectious piercings made by each individual larva or adult varies between one and more than 30 in IAPs of 24 h (Wijkamp and Peters 1993). This variation in the number of local lesions may be explained by the number of piercings made by each individual thrips, or the amount of virus introduced. The latter possibility can be excluded as no strong correlation was found between the number of lesions and the amount of virus detected by ELISA in the individuals transmitting the virus (Wijkamp and Peters 1993).

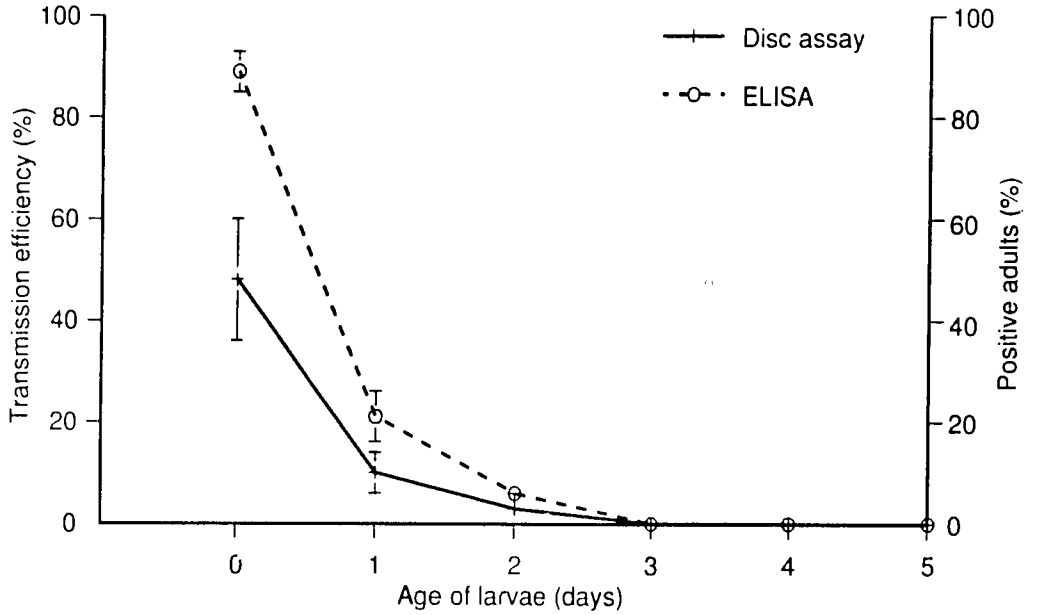

Figure 1. Transmission of tomato spotted wilt virus by *Frankliniella occidentalis* adults, which were given an acquisition access period of 24 h when the larvae were 0–5 days old.

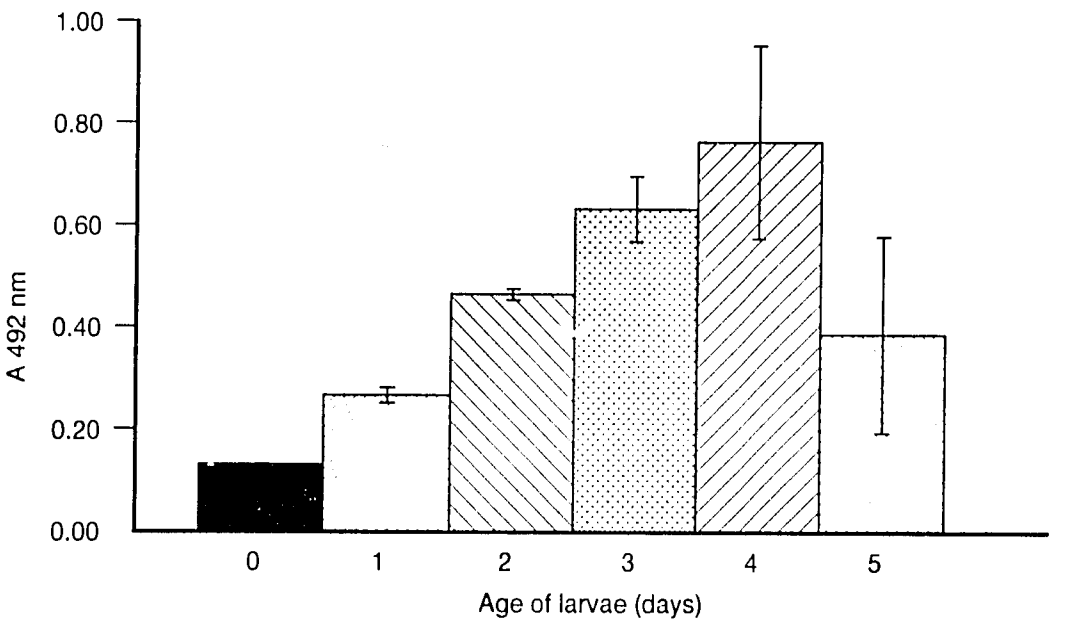

Figure 2. Virus content of thrips, 8 h after an acquisition access period of 24 h, as 0–5 day-old larvae.

The number of local lesions is thus a function of the number of piercings made, which may reflect the probing or feeding activity of the individual thrips.

Efficiency by which Tospoviruses are Transmitted

The spread of tospoviruses is a function of several factors, e.g., the efficiency by which they are transmitted by a particular thrips species, the number of thrips transmitting them, the mobility of the vectors, the host plant species, and the number of infected plants. Evidence in support for differences in vector efficiency and specificity for TSWV isolates have been provided by Amin et al. (1981), Mau et al. (1991), and Paliwal (1976). The efficiency by which a few thrips populations transmitted four tospovirus species (Table 1) was determined by Wijkamp et al. (in press) using the petunia leaf disk system. *Frankliniella occidentalis* appeared to be an efficient vector for all four viruses tested, followed by a dark form of *F. schultzei*, which did not transmit INSV. A light form of the latter species transmitted only TSWV and tomato chlorotic spot virus (TCSV) at low rates. The species *F. intonsa* appeared to be a rather efficient vector of TSWV. Of particular interest is the poor transmission found for four different populations by *Thrips tabaci*. Three *T. tabaci* populations, consisting of only females, did not transmit any tospovirus at all. A population producing males and females transmitted TSWV at a low rate, whereas the other tospoviruses were not transmitted. *Thrips tabaci* has been reported on several occasions as a vector of TSWV (Linford 1932, Sakimura 1963, Fusijawa et al. 1988, and Lemmetty and Lindquist 1993), but there have also been instances when this species did not transmit TSWV (Jones 1959, Paliwal 1976, and Mau et al. 1991).

Table 1. Efficiency by which four tospovirus species are transmitted by several thrips species.

	Tospovirus species[1]			
Thrips species	TSWV	TCSV	GRSV	INSV
Frankliniella occidentalis	66	28	10	85
F. schultzei (dark)	14	38	16	0
F. schultzei (light)	2	6	0	0
F. intonsa	32	1	0	0
Thrips tabaci (arrhenotokous)	10	0	0	0
Thrips tabaci (thelokotous)	0	0	0	0

1. TSWV = tomato spotted wilt virus, TCSV = tomato chlorotic spot virus, GRSV = groundnut ringspot virus, INSV = impatiens necrotic spot virus.

Such conflicting results were thus also obtained in our experiments in which one population was able to transmit TSWV and three others did not. Failure to transmit TSWV has been explained by the existence of a long latent period in thrips (Sakimura 1963), incompatibility between the TSWV isolate and thrips species (Paliwal 1976), or the existence of subspecies that do not transmit TSWV (Zawirska 1976). Two subspecies were distinguished by the last author; one, which transmitted TSWV, consisted of males and females, and the other of only females. The latter subspecies did not transmit TSWV. The observation that only populations with males and females are able to transmit TSWV seems consistent with our results.

Pathogenic Effects of Tospoviruses in Thrips

Multiplication of TSWV in thrips has been demonstrated by Wijkamp et al. (1993) and Ullman et al. (1993), showing that an intimate relationship exists between the virus and the vector. As viruses are potential pathogens, the question has been raised whether tospoviruses are pathogenic to viruliferous thrips. A high mortality among larvae and adults was found when the thrips were reared on infected *N. rustica* plants (Robb 1989). Cytopathogenic aberrations in adults have also been explained as evidence for pathogenic effects caused by TSWV in thrips (Ullman et al. 1993). The thrips used in these studies were reared for the total larval lifespan on infected plants. A deleterious effect on the thrips by a poor quality of the food cannot be ruled out in these experiments.

A study in which the larvae were given a short AAP and kept on healthy plants afterwards showed that viruliferous and nonviruliferous thrips, and thrips not exposed to the virus had similar mortality rates (Fig. 3). Also, the egg production of the females did not differ for these groups (Fig. 4), indicating apparent absence of pathogenic effects.

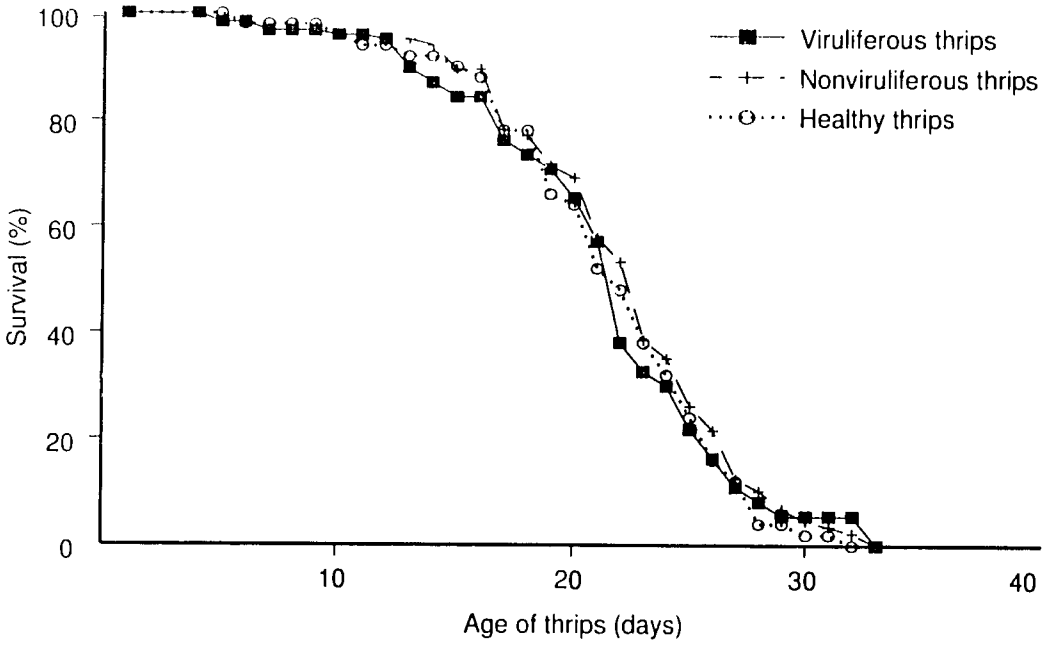

Figure 3. Survival of viruliferous, nonviruliferous, and control thrips after an acquisition access period of 6 h, on tomato spotted wilt virus (BR-01)-infected leaves as 0–4 h-old larvae.

Comparing the results obtained by other authors and ourselves, it can be concluded that a long exposure of larvae to infected plants may have serious pathogenic effects on their development (Wijkamp et al. 1995).

Discussion

Some unique properties can be discerned in the relationships of tospoviruses with their vectors which are not observed among other persistently transmitted plant viruses and their vectors. Firstly, thrips cannot transmit viruses when adults acquire the virus from infected plants.

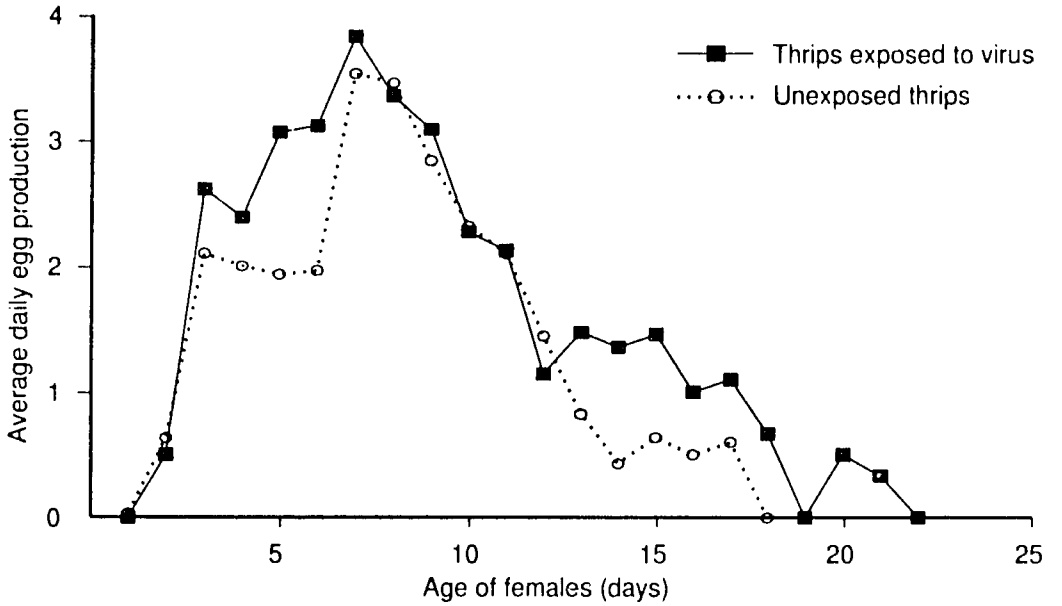

Figure 4. The mean number of larvae per female per day emerging from petunia leaf disks.

Secondly, the finding that a high percentage of thrips are converted into efficient transmitters when first instar, and not the second instar larvae, ingest the virus, is also a new phenomenon. Further, the possible pathogenic effect of infected leaf tissue on the vector is also different from that known for other persistently transmitted viruses.

These three phenomena affect the spread of the virus and determine the infection pressure. The first two phenomena result in the presence of thrips on infected plants which do not become transmitters. Healthy adults alighting on infected plants do not become viruliferous. However, the offspring emerging from the eggs deposited by these healthy thrips transmit the virus after some time. Secondly, viruliferous thrips infecting and infesting healthy plants give rise to a population consisting of nonviruliferous and viruliferous thrips. The first larvae which hatch, do not ingest the virus as the incubation period of the virus in the plant exceeds the period required for the development of the egg and the time in which the larvae are able to develop into viruliferous thrips. The length of the incubation period of the virus in the plant and the moment at which the eggs are oviposited after infection of the plant, determine the ratio of viruliferous and non-viruliferous thrips on an infected plant.

The effect of the deteriorating food quality of infected plants on the infection pressure is difficult to evaluate. A decrease in food quality may result in a higher mobility of the adult and thus in an increase of the infection pressure. On the contrary, it negatively affects the egg production, the development of the larvae, and the longevity of the larvae and adults.

This discussion shows that the infection pressure is not only a function of the number of thrips and the number of TSWV-carrying thrips. The presence of a viruliferous and nonviruliferous thrips population on a plant and the declining food quality are also factors which play a role as parameters in the measurement of the infection pressure.

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Utility of Antibodies to Explore and Control Tomato Spotted Wilt Virus

J L Sherwood[1], M D Bandla[1], K D Chenault[1], D E Ullman[2], D M Westcot[3], and T L German[4]

Abstract

Methods to develop and utilize antibodies to explore and possibly control plant viruses are beginning to come of age. Success in obtaining quality antibodies to tomato spotted wilt virus (TSWV), which has been recalcitrant to routine separation from host tissue, has been expedited by techniques to express foreign proteins in bacteria and then isolating them. Polyclonal antibodies or monoclonal antibodies (Mabs) have been made to the nonstructural and virion-associated proteins of TSWV for localization of TSWV in plants or thrips. Electron microscopy of TSWV-infected thrips cells, immunolabeled with polyclonal antibodies to TSWV N protein, the glycoproteins, and NSs indicated that these proteins are compartmentalized within several types of inclusions which appear to be similar to structures involved in intracellular transport of proteins. Viral proteins were also localized in the golgi complex and at intercellular membranes. Observation of virion maturation in thrips was limited to the salivary glands. Mabs made to NSs were used to identify thrips that could potentially transmit TSWV. Assay by enzyme-linked immunosorbent assay (ELISA) and transmission of TSWV by thrips to Petunia grandiflora *gave similar results. Cloning of sequences for antibodies has permitted expression of immunoglobulin genes in organisms which do not naturally express antibodies. Expression of Mabs or the antigenic binding site of an antibody in plants has been proposed as a way to study cellular processes and modulate host-pathogen interaction. A single chain antibody with affinity to the TSWV N protein has been produced for expression in plants. Results obtained by the authors in utilizing antibodies to investigate the biology of TSWV are presented.*

Introduction

The impact of tospoviruses on agriculture is well established. Tomato spotted wilt tospovirus (TSWV) remains a problem in many field crops in the USA, and Impatiens necrotic spot tospovirus (INSV) is commonly found in horticultural crops, particularly in greenhouses. TSWV and INSV are transmitted in a persistent manner, by a number of thrips species, of which the western flower thrips (*Frankliniella occidentalis* Perg.) is considered to be the most important (German et al. 1992). Although much progress has been made in determining the genome organization of TSWV and other tospoviruses (reviewed in German et al. 1992), advances in the control of diseases caused by tospoviruses remain limited. The strategy for the use of the pathogen-derived

1. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078, USA.
2. Department of Entomology, University of California, Davis, CA 95616, USA.
3. Department of Entomology, University of Hawaii, Honolulu, HI 96822, USA.
4. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706, USA.

Sherwood, J.L., Bandla, M.D., Chenault, K.D., Ullman, D.E., Westcot, D.M., and **German, T.L.** 1995. Utility of antibodies to explore and control tomato spotted wilt virus. Pages 25–33 *in* Recent studies on peanut bud necrosis disease: proceedings of a Meeting, 20 Mar 1995, ICRISAT Asia Center, India (Buiel, A.A.M., Parlevliet, J.E., and Lenné, J.M., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and P O Box 386, 6700 AJ Wageningen, The Netherlands: Department of Plant Breeding, Agricultural University of Wageningen.

resistance (PDR) is well documented, and several laboratories have reported that plants expressing the nucleocapsid (N) protein of TSWV exhibit resistance to TSWV. Efforts are also underway to utilize other TSWV-derived sequences to modulate the effects of TSWV. In addition, new cultivars of groundnut, e.g., Georgia Browne from the University of Georgia, that are less affected by TSWV, are being produced through traditional breeding programs, and cloning and expression of natural resistance genes is being pursued. Routine transformation of the crop of interest is needed for the expedient expression of any new value-added trait in that crop. The lack of much-reported success in the development of TSWV-resistant groundnut by exploiting PDR, could be due to the nonavailability of a widely adaptable efficient transformation system for groundnut.

With a better understanding of the relationship of thrips and TSWV, data on the epidemiology of TSWV, which may be useful to develop control strategies, are being obtained. The host range of both TSWV and its vector are quite wide, but the relationship between weeds, vector, and crop has not been well defined. Thrips have been found associated with newly emerging groundnut plants in southern USA, but it has not been reported whether the thrips are viruliferous. Identifying primary and secondary sources of the virus is essential.

Serological reagents have long been utilized to study the biology of plant viruses. This has been primarily for the detection of a virus by the use of polyclonal antiserum. Success in obtaining quality antiserum depends partially on success in separating the plant virus to be used as an immunogen from host plant components. This was generally such a problem with TSWV that it may have lead to the conclusion by Francki and Hatta in 1981 that "serology has not been used to any extent in TSWV identification but holds obvious potential for the future." As the technique for the production of monoclonal antibodies (Mabs) became widely mastered, antibodies that were useful for the detection of TSWV became more available. Techniques to express foreign proteins in bacteria and then isolating them has afforded the opportunity to produce antibodies to proteins that are produced in low amounts during virus infection or are difficult to separate from other proteins.

Techniques to express immunoglobulin genes in organisms that do not naturally express antibodies, has offered new ways to develop and utilize antibodies to explore and possibly control plant viruses. Progress to this goal was facilitated by demonstrating that the variable region fragments (Fv), consisting of the light chain variable region (VL) and the heavy chain variable region (VH), linked in tandem to form a single chain antibody (ScFv) binds to antigen. Taviadoraki et al. (1993) expressed a ScFv to the coat protein of artichoke mottled crinkle tombusvirus (ACMV) in *Nicotiana benthamiana* Domin. When ScFv-expressing plants or protoplasts from ScFv-expressing plants were inoculated with ACMV, symptom development was delayed in the plants and the amount of ACMV produced in protoplasts was reduced. Similar work with a ScFv to the TSWV N protein and other results obtained by the authors in utilizing antibodies to investigate the biology of TSWV are presented.

Methods

Virus isolate, host plants, and thrips

The TSWV isolate was collected on the Hawaiian island of Maui and maintained in *Emilia sonchifolia* L. by thrips transmission as previously described [Bandla et al. 1994, Ullman et al. in press (a), Ullman et al. in press (b)].

Electron microscopy and immunocytochemistry

Methods for electron microscopy observation and immunocytochemical analyses have been described [Ullman et al. in press (b), Westcot et al. 1993]. Immunolabeling was done on insects embedded in LR-White as Spurrs embedding destroys antigenicity of TSWV.

Cloning and expression of TSWV genes

The open reading frame (ORF) of several virion-associated and nonstructural proteins were cloned and subsequently expressed using the pET expression vector system (Novagen, Madison, WI). This resulted in the production of antibodies to the N [Ullman et al. in press (b)], NSs (Ullman et al. 1993), NSm (Choi et al. 1993), or L (Adkins et al. 1993) proteins. The NSs and L proteins were isolated from PAGE fragments, and N and NSm were isolated using a His-tag system as described by Novagen. The G1 and G2 proteins were gel isolated from electrophoresed TSWV preparations prepared from infected *Datura stramonium* L.

Production of polyclonal antibodies

Rabbit polyclonal antibodies were produced to N, NSs, NSm, and L by independently immunizing New Zealand white rabbits with each protein.

Production of monoclonal antibodies

In addition to Mabs to N (Sherwood et al. 1989) and NSs (Bandla et al. 1994), Mabs to G1 or G2 were produced essentially as previously described for N and NSs. Protein in PBS was emulsified in Freund's complete adjuvant (Sigma Chemicals, St. Louis, MO) and used to immunize BALB/c mice. Three subsequent immunizations were given at 10-day intervals using Fruend's incomplete adjuvant. After 20 days, a booster dose of protein without adjuvant was injected. The spleen cells were fused with P3X63Ag8.653 myeloma cell line, 48 h after the booster dose. Cell lines were initially selected based on results from ELISA or western blot. Selected cell lines were grown in RPMI1640 (Mediatech, Inc., Herndon, VA) with 10% horse serum (HyClone Laboratories, Inc., Logan, UT).

Serological analysis with peanut bud necrosis virus (PBNV)

For double antibody sandwich (DAS), ELISA rabbit polyclonal antiserum to either PBNV or TSWV were used to coat the plate. Mabs (to NSs, G2, or N), mouse polyclonal serum (to G2 or NSs), or Mabs to PBNV (F63A11, F63A6, F63A7) were used as secondary antibodies. In addition to the above, in antigen-coated plate (ACP), ELISA rabbit polyclonal serum to PBNV or TSWV were used. Samples of lyophilized PBNV-infected leaf tissue were prepared in carbonate coating buffer for ACP-ELISA or in PBS-Tween with 2% PVP for DAS-ELISA. Western blots were also conducted with the Mabs and polyclonal sera listed above, in addition to a Mab to G1 of TSWV. For western blots, PBNV in lyophilized infected leaf tissue was prepared in SDS-PAGE sample buffer.

Cloning and expression of immunoglobulin genes

cDNAs coding for the heavy chain (HC) and light chain (LC) of Mab to the N protein were produced from mRNA isolated from a hybridoma cell line by first strand cDNA synthesis followed by PCR (Hiatt et al. 1989, Hein et al. 1991). DNAs coding for the HC and LC were inserted into several vectors which included pKYLX71(HC) (Berger et al. 1989) and pMON530(LC) (Rogers et al. 1987), for the transformation of *Agrobacterium tumefaciens* (E. F. Sm. & Towns.) Conn. strain LBA4404. *Agrobacterium tumefaciens* was directly transformed (Chen et al. 1994) and then used for plant transformation of *Nicotiana tabacum* L. cv. Xanthi. Plants were recovered after kanamycin selection from leaf discs incubated with *A. tumefaciens* containing either HC or LC constructs (Chenault et al. 1993).

A construct for expression of a single chain variable fragment (ScFv) which includes the variable heavy (VH) and variable light (VL) regions of a Mab to the N protein of TSWV was produced using the procedure outlined by Sassano et al. (1994). The mRNA from the hybridoma line was isolated and double-stranded (ds) cDNA produced. The ds cDNA was blunt-ended and ligated to produce a circular molecule. PCR products were produced containing the VH region of the HC and the VL region of the LC. For the HV region, primers specific for constant regions of the HC were used (Sassano et al. 1994). The PCR fragment was further subcloned to obtain the HV. The VL fragment was obtained by PCR using primers based on microsequencing of the LC and the Kabot databases of immunoglobulin sequences. The cloned VH and VL fragments were subcloned for addition of the peptide linker (Chaudhary et al. 1990, Brinkmann et al. 1991) and expressed in a bacterial expression system using the pET14b vector. The ScFv was isolated from *Escherichia coli* and renatured by dialysis against PBS.

Results

Immunolabeling of structural and nonstructural proteins

Observations by electron microscopy of TSWV-infected thrips cells immunolabeled with polyclonal antibodies to TSWV N protein, the glycoproteins, and NSs indicated that these proteins are compartmentalized within several types of inclusions. These inclusions appeared to be similar to structures involved in intracellular transport of proteins, e.g., vesicles, autophagic vacuoles, and residual bodies (Figures 1A and B). Viral proteins were also localized in the golgi complex and at intercellular membranes. Observation of virion maturation in thrips was limited to the salivary glands (Figure 1F). The intensity of labeling with Mabs was generally less than that with polyclonal antibodies. However, polyclonal antibodies and Mabs made to the same protein labeled the same structures (Figures 1A-E).

Serological analysis with peanut bud necrosis virus

In both DAS-ELISA and ACP-ELISA, with the various sera, there was no indication of cross reactivity between PBNV and TSWV. Similar results were obtained from western blots. We have not investigated if PBNV is reactive to antibodies to L, G1, or NSm.

Identification of viruliferous thrips by ELISA

Replication of TSWV in thrips results in production of the nonstructural proteins of TSWV. Using immunoelectron microscopy, NSs was readily detected in thrips, but NSm was less frequently

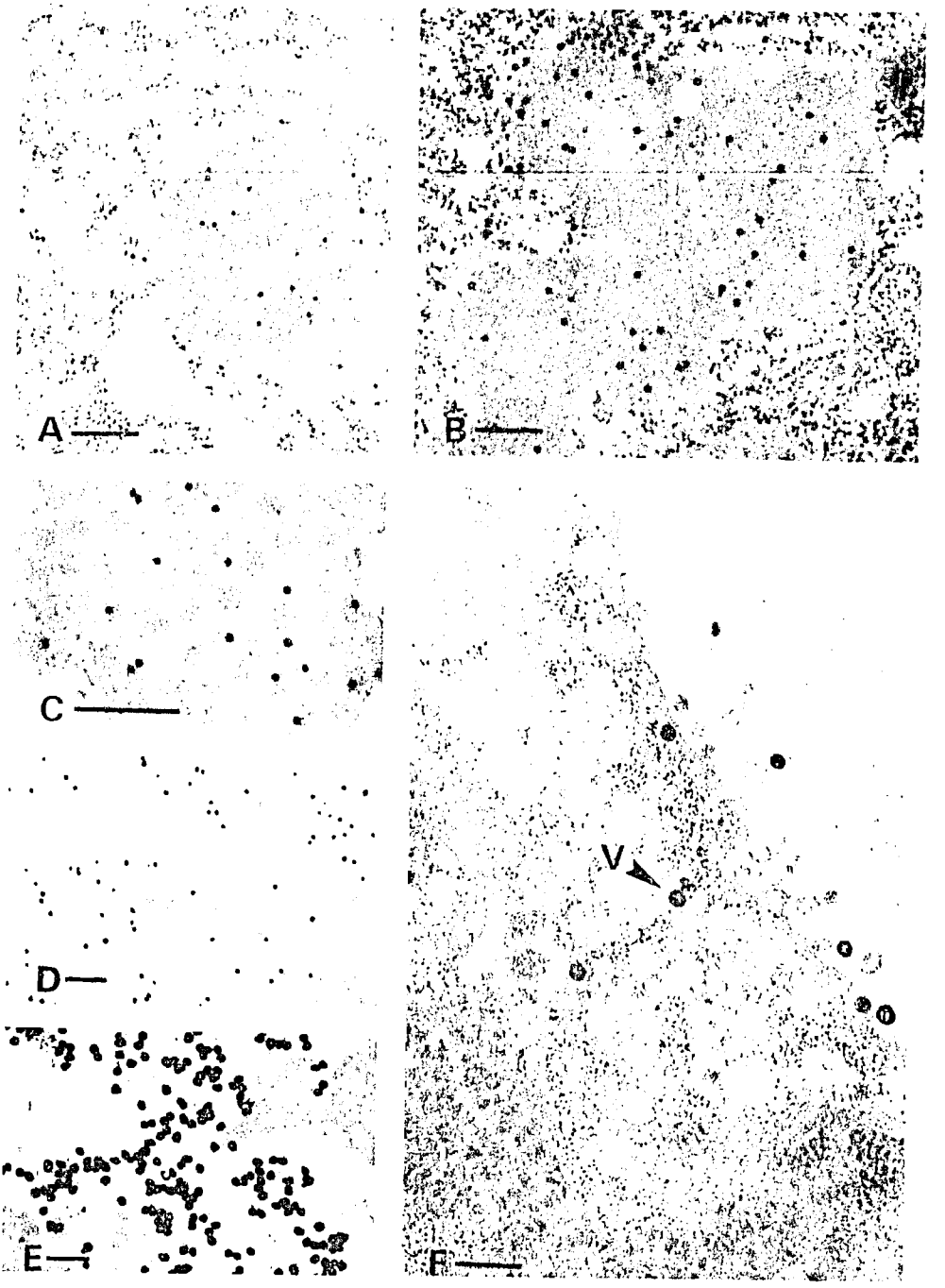

Figure 1. Comparative immunolabeling of multivesicular bodies in viruliferous thrips with polyclonal antibody to a glycoprotein fraction of TSWV (Panel A) and a monoclonal antibody to TSWV G2 (Panel B). Comparative immunolabeling of TSWV virions in *Emilia sonchifolia* with monoclonal antibody to G1 (Panel C), monoclonal antibody to G2 (Panel D), or a polyclonal antibody to a glycoprotein fraction of TSWV (Panel E). Maturation of TSWV virions (V) in salivary glands of viruliferous thrips (Panel F) (bar = 360 nm).

detected. In ELISA, with polyclonal antisera against NSs, viruliferous thrips can be identified, but absorbance values may be low and antiserum may react with nonviruliferous thrips. Mabs made to NSs were used in ACP-ELISA with the Zwitterionic detergent, Empigen-BB (E-BB) at 0.1% (a.i.) in the antibody dilution buffer to reduce nonspecific binding which results in high absorbance readings of control samples commonly observed with insects in ACP-ELISA. With E-BB, a 10-fold difference in absorbance values was observed between adult thrips fed on healthy plants and adult thrips fed on virus-infected plants as larvae, compared with ACP-ELISA with Tween-20 in which there was only a three-fold difference in absorbance values between the same samples of thrips. The lower limit of detection of gel-isolated NSs was about 0.244 ng mL^{-1} NSs.

The utility of the ACP-ELISA in identifying viruliferous thrips was compared with transmission of TSWV by thrips to *Petunia grandiflora* (L.) DC. ex Wright (Table 1). Based on the results of four replicates using 25, 50, 90, or 100 thrips, the ACP-ELISA and the plant transmission assay were similar in identifying viruliferous thrips. In a *G* test (SAS 1994) for independence, the two different assays showed close agreement. The *G* test indicated that the results of the two tests were not independent (G=97.72, 1 df, P <0.0001). The two assays were in agreement 92% of the time. The errors were divided with 6% occurring when ACP-ELISA identified thrips as potential transmitters which were not identified as transmitters in the plant transmission assay, and 2% of the error occurring when ACP-ELISA did not detect individuals which transmitted TSWV in the plant transmission assay.

Table 1. Identification of tomato spotted wilt tospovirus (TSWV) viruliferous thrips by antigen-coated plate (ACP)-ELISA (absorbance at 405 nm), using monoclonal antibody to TSWV NSs and by thrips transmission to *Petunia grandiflora*.

ELISA		Transmission	
Positive (≥0.100)	Negative (<0.100)	Positive (lesion present)	Negative (lesion absent)
47	188	35	200

Expression of a ScFv to the N protein of TSWV

Dot blots of genomic DNA from plants transformed to produce either HC or LC of a Mab to N protein of TSWV and probed with ^{32}P-labeled plasmid of either the HC or LC construct indicated transformation (Table 2). Analysis of plants transformed for the production of LC were positive by PCR analysis. Northern blots of R_0 plants showed transcripts from the HC and LC constructs of the predicted size. Analysis for NPT-II by ELISA was positive for plants transformed with the HC and LC constructs. Plants transformed with the LC construct produced LC protein at 1–42 µg mg^{-1} plant protein. Northern analysis indicated plants transformed to express HC produced the correct size transcript, but HC protein was not detected from either R_0 plants or progeny of R_0 plants that were crossed with plants expressing LC. The crosses were done to try to stabilize any HC which might have been expressed. Because of differences in the morphology of the petiole of the cultivars of *N. tabacum* used, we are certain that successful crosses were made. Subsequent sequencing of 30 cDNA clones from mRNA from three different hybridoma cell lines for full-length HC, indicated that multi-mRNAs were produced by the hybridomas, and that many of the mRNAs code for a HC that is not functional. Single or multiple stop codons, or frameshifts were found outside the VH region in the clones. Immunoglobulin subclass switching, which is documented in hybridoma cells (Spira et al. 1994), could result in the production of the variant mRNA.

Table 2. Analysis of some plants transformed to express either heavy chain (HC) or light chain (LC) of Mab to N protein of tomato spotted wilt tospovirus (TSWV). Blanks indicate analysis not conducted.

						ELISA for Immunoglobulin[3]			
				NPT-II		Whole IgG molecule			
R_0 plant[1]	Vector	PCR signal[2]	Northern signal[2]	R_0	R_1	R_0	R_1	HC	LC
71HN 8-0-1	pKYLX71	+	+	+		-/+			-
71HN 5-0-1	pKYLX71	+	+	+		-/+			-
LLn 2-0-1	pMON530			+	+(3:1)[4]	++			
LLn 5-0-1	pMON530			+		+	+		
LLn 9-0-1	pMON530	+	+	+		++	+(3:1)	-	+
Vector control						-			
nontransformed		-	-	-		-			

1. 71H, pKYLX71 binary vector containing the heavy chain construct; N, *N. tabacum* cv. Xanthi-NN; n, *N. tabacum* cv. Xanthi-nn; LL, pMON530 binary vector containing the light chain construct; F, *N. tabacum* cv. Xanthi-'nc'; vector control, plant transformed with vector not containing heavy or light chain sequences.
2. +, presence of DNA (via PCR), RNA (via northern blot).
3. Values from ELISA of R_0 for immunoglobulin (probed with antibody for whole immunoglobulin molecule or probed with antibody specific for either light chain or heavy chain; -, ELISA value = background, -/+, ELISA value approximately 2X background; ++, ELISA value ≥10X background.
4. Values in parentheses are the segregation ratios for the transgene in the R_1 generation.

Because of the variability encountered in the clones to the HC obtained, the strategy to produce a ScFv was pursued. A ScFv produced as outlined above, was isolated from *E. coli*, renatured by dialysis against PBS, and tested in ELISA to determine if TSWV could be detected. The isolated ScFv was successfully used to detect TSWV in infected *D. stramonium* by ELISA. The construct is now being placed in plasmids for plant transformation and transient expression.

Discussion

The utility of antibodies to investigate plant viruses has rapidly progressed. This is particularly true in the case of TSWV. Polyclonal antibodies and Mabs to TSWV will be used to facilitate the development of control tactics for TSWV and other tospoviruses. This may range from the diagnosis of infected plants, to the detection of viruliferous thrips, to the production of transgenic plants expressing some form of an antibody to one or more virion-associated or nonstructural proteins of TSWV. As efforts are made to understand PBNV to the extent that TSWV has been investigated, progress towards understanding the biology of PBNV will be made.

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Rabbit polyclonal antiserum prepared to a glycoprotein fraction of TSWV was provided by D Gonsalves (Cornell University) and to PBNV by D V R Reddy (ICRISAT). Mabs to PBNV were provided by D V R Reddy. M Hein (The Scripps Research Institute) cloned the cDNAs for HC and LC for transformation of plants. The analysis of plants for HC and LC analysis was done in cooperation with R Nelson (The Samuel Roberts Noble Foundation).

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Progress in Breeding Groundnut Varieties Resistant to Peanut Bud Necrosis Virus and its Vector

S L Dwivedi[1], S N Nigam[1], D V R Reddy[2], A S Reddy[2], and G V Ranga Rao[2]

Abstract

Peanut bud necrosis disease (PBND), caused by peanut bud necrosis virus (PBNV), and transmitted by Thrips palmi *is an important disease of groundnut in South and Southeast Asia. Several cultivated groundnut germplasm lines showed consistently low disease incidence under field conditions (field resistance). Eight accessions of wild* Arachis *species did not show disease under field conditions. Field resistance could be due to vector and/or to virus resistance. The current breeding strategy includes improving the level of resistance to thrips and PBNV, and combining them into superior agronomic backgrounds. Several high-yielding varieties with high levels of resistance to PBND have been developed. These varieties possess moderate resistance to the vector. Two of these, ICGV 86031 and ICGV 86388, show resistance to PBNV when mechanically sap-inoculated with low virus concentration (10^{-2}). Considering the level of resistance to the vector and PBNV, it appears that further improvement in the level of resistance through conventional breeding may be difficult to achieve.*

Introduction

Peanut bud necrosis disease (PBND) is an economically important virus disease of groundnut (*Arachis hypogaea* L.) in South and southeast Asia. It is caused by peanut bud necrosis virus (PBNV) and transmitted by *Thrips palmi* Karny. The disease can cause yield losses of over 50% and its incidence ranges from 5 to 80% in all the major groundnut-growing areas of India (Ghanekar et al. 1979, Amin and Mohammad 1980, Amin and Reddy 1983, Reddy et al. 1991, and Patil 1993).

In the field, genotypes can differ considerably in the incidence of PBND due to the collective effects of resistance to the virus and resistance to the vector. Reduced incidences are indicated as field resistance.

Genotypic differences in field resistance are reported among the 8000 groundnut germplasm accessions screened for this resistance at ICRISAT Asia Center (IAC), Patancheru, India. Compared with subsp *hypogaea*, the genotypes belonging to subsp *fastigiata* are, in general, more susceptible. In most cases, field resistance is associated with nonpreference of the vector. In a few genotypes, slower multiplication of the virus in the plant is also responsible for a lower disease incidence in the field.

We report here, the progress made in identification of sources of field resistance, and the development of breeding populations with an improved level of resistance.

1. Genetic Enhancement Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
2. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.

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Field Resistance

Resistance in cultivated groundnut

Several germplasm lines with consistently low disease incidence under field conditions have been identified at IAC. These are: ICG numbers 848, 851, 852, 862, 869, 885, 2271, 2306, 2307, 2323, 2741, 3042, 3806, 3873, 5030, 5024, 5043, 5044, 6135, 6317, 6323, 7676, and 7892, and belong to subsp *hypogaea*. These lines showed less than 20% disease incidence compared with over 80% in the susceptible control JL 24 (ICRISAT unpublished data).

Resistance in wild *Arachis* species

Five accessions of *A. duranensis* (30064, 30065, 36002, 36002-2, and 36005) and one accession each of *A. volida* (30011), *A. correntina* (9530), and *A. monticola* (30063) showed no disease symptoms under field conditions. Of these, *A. duranensis*, *A. correntina*, and *A. monticola* are cross-compatible with cultivated groundnut.

Resistance to Vector and Virus

Field resistance is a result of resistance to the vector, the virus, or a combination of both.

One-hundred-and-forty varieties and interspecific derivatives of groundnut with field resistance were screened in the field for resistance to the vector, on the basis of thrips injury on a 1–9 scale, where 1 = highly resistant, 2–3 = resistant, 4–5 = moderately resistant, 6–7 = susceptible, and 8–9 = highly susceptible. The vector-resistant genotypes were then screened for PBNV resistance by mechanical inoculation (using a 10^{-1} and 10^{-2} dilution of infected plant extract) under controlled greenhouse conditions. The thrips injury score and PBND incidence of the selected genotypes are presented in Table 1. The thrips injury score of ICGV numbers 86029, 86031, 86388, 89281, 90046, 91177, 91180, 91220, 91223, 91239, 91241, 91245, 91246, 91249, and an interspecific derivative 346-2 ranged from 2.5 to 5.0, compared with 7.5 of the susceptible control ICGV 87123. They also showed field resistance with a disease incidence ranging from 4.8 to 20.0%, compared with 54.4% in JL 24. Forty-two genotypes were screened for resistance to PBNV. All the genotypes were susceptible to PBNV at higher virus concentration (10^{-1} dilution). However, at the lower virus concentration (10^{-2} dilution), three genotypes, ICGV 86388, ICGV 91239, and ICGV 91245 showed resistance to the virus while the others were highly susceptible. The disease incidence in ICGV 86388, ICGV 91239, and ICGV 91245 ranged from 23 to 42%, compared with 40% in ICGV 86031 (resistant control) and 80% in JL 24 (susceptible control). Of these, ICGV 86388 was further tested in three additional inoculation tests (Table 2). The disease incidence in ICGV 86388 averaged 31% compared with 45% in ICGV 86031 and 87% in JL 24. The mean yield of ICGV 86388 over three seasons and eight locations was 2.04 t ha^{-1}, compared with 1.68 t ha^{-1} of JL 24, the susceptible control (Table 3). The mean PBND incidence in these fields was 17.8% in ICGV 86388 and 60.7% in JL 24. ICGV 86388, a selection from the cross (Dh 3-20 × USA 20) × NC Ac 2232, is a sequentially branched variety with dark green elliptic leaves, mostly 2-seeded small pods, with a shelling turnover of 70%, and a 100-seed mass of 37 g. Its tan-colored seeds contain 53% oil. It has higher resistance to PBNV than the earlier reported resistant variety ICGV 86031 (Dwivedi et al. 1993).

Table 1. Thrips injury score and peanut bud necrosis disease (PBND) incidence (%) in 15 groundnut genotypes at Rajendranagar and ICRISAT Asia Center.

Genotype	Thrips injury score[1]	PBND incidence (%)		
		Field[2]	Mechanical inoculation[3]	
			10^{-1}	10^{-2}
ICGV 86029	4.0	20.0	100.0	69.0
ICGV 86388	5.0	15.0	90.0	37.0
ICGV 91177	4.0	4.8	80.0	85.0
ICGV 91180	4.0	10.8	83.0	83.0
ICGV 91220	3.5	15.8	100.0	70.0
ICGV 91223	3.5	14.8	95.0	52.0
ICGV 91239	2.5	10.0	81.0	23.0
ICGV 91241	4.0	7.5	62.0	65.0
ICGV 91245	4.0	7.7	100.0	42.0
ICGV 91246	4.0	8.0	54.0	48.0
ICGV 91249	4.0	8.9	94.0	56.0
346-2	2.5	12.5	-[4]	-
Controls				
JL 24	-	54.4	93.0	79.5
ICGV 86031	4.5	11.1	100.0	40.2
ICGV 87123	7.5	20.5	-	-

1. Mean of nonreplicated data reported from two locations (Rajendranagar and Patancheru) during the 1992/93 postrainy season.
2. Nonreplicated data from the 1992 rainy season.
3. Plants were mechanically inoculated with 10^{-1} and 10^{-2} dilution of infected plant extract during the 1993 rainy season under controlled greenhouse conditions.
4. - = data not available.

Table 2. Cumulative peanut bud necrosis disease (PBND) incidence (%) of ICGV 86388 and controls by mechanical inoculation under controlled greenhouse conditions, ICRISAT Asia Center, 1993–95.

Genotype	Cumulative PBND incidence (%) at 10^{-2} dilution of infected plant extract			Mean
	1993/94	1994	1994/95	
ICGV 86388	17.7 (24.4)[1]	52.7 (46.6)	21.0 (27.4)	30.5
Controls				
ICGV 86031	26.2 (17.4)	71.7 (58.0)	37.0 (37.6)	45.0
JL 24	78.2 (62.8)	93.7 (76.9)	90.0 (72.1)	87.3
SE	(±4.27)	(±2.64)	(±1.86)	-[2]
CV (%)	(23.0)	(11.0)	(9.0)	-

1. Figures in parentheses are angular transformed values.
2. - = data not available.

Table 3. Pod yield and peanut bud necrosis disease (PBND) incidence (%) of ICGV 86388 and JL 24.

Genotype	Pod yield (t ha^{-1}) 1988[1]	1989[2]	1993[3]	Mean	Mean PBND incidence[4] (%)
ICGV 86388	2.10	2.38	1.35	2.04	17.8
JL 24 (control)	1.65	2.24	0.95	1.68	60.7

1. Mean of six locations.
2. Mean of three locations.
3. Mean of two locations.
4. PBND incidence averaged over three rainy seasons under field conditions.

Breeding Strategy

The breeding strategy to improve the level of field resistance includes improving the resistance to thrips and to PBNV, and combining them in superior agronomic backgrounds. The segregating populations (F_2 and subsequent generations) derived from crosses made with these objectives are sown late in the season at wider spacing. The wider spacing and late sowing encourage thrips infestation. These populations are advanced by the bulk pedigree method under mild selection pressure for yield. Each population is divided into different bulks, based on plant type and pod and seed characteristics at the time of harvest. The advanced generation bulks (F_5) are initially screened for field resistance in a nonreplicated, one-row plot disease nursery at Narkoda, Andhra Pradesh. The Narkoda location achieves high disease incidence in most years. The resistant (ICGV 86031) and susceptible (JL 24) controls are sown after every 10 rows of test materials. The PBND incidence is recorded from 30 days after sowing (DAS) at a 15-day interval until 1 week before harvest. The promising uniform bulks are then assigned ICGV numbers and are further screened in replicated trials at Narkoda and at Mainpuri in Uttar Pradesh. The field-resistant varieties, selected on the basis of two seasons of screening, are evaluated for their yield potential under high- and low-input conditions at IAC. They are also screened for resistance to the vector under field conditions, and for resistance to PBNV by mechanical inoculation (using 10^{-1} and 10^{-2} dilutions of infected plant extract) under greenhouse conditions. The varieties with combined resistance to the vector and PBNV are again used in the crossing program at IAC and are also supplied to national programs for further agronomic evaluation.

Progress in Resistance Breeding

Several high-yielding cultivars released in India such as ICGVs 87123 (ICGS 11), 87128 (ICGS 44), 87187 (ICGS 37), and 87141 (ICGS 76), which were developed primarily for high yield potential, were found to have field resistance. Following the above approach, several new high-yielding varieties have been developed with higher levels of field resistance (Table 4). The average PBND incidence in these varieties ranged from 13.6 to 23.7% compared with 16.7% in ICGV 86031 and 58.4% in JL 24. ICGVs 91228 and 90013 produced high mean pod yield (3 t ha^{-1}). While ICGV 91228 is better adapted to the rainy season, ICGV 90013 is adapted to both rainy and postrainy seasons. The mean pod yield of ICGV 86031 and JL 24 in these trials was 2.67 t ha^{-1} and that of JL 24 was 1.98 t ha^{-1}. JL 24, an early-maturing cultivar, is widely adapted to rainfed conditions in India. It has also been released in Myanmar and the Philippines under different names. Whereas

Table 4. Performance of selected peanut bud necrosis disease (PBND) field-resistant groundnut varieties, ICRISAT Asia Center, rainy and postrainy seasons, 1993 and 1994.

	Pod yield (t ha^{-1})						
	Rainy 1994	Postrainy 1993/94		Rainy 1993			
Variety	EBDRGVT[1] (SB/VB)	ABDRGVT (SB)	ABDRGVT (VB)	ABDRGVT (SB)	ABDRGVT (VB)	Mean	PBND (%)[2]
ICGV 91228	2.14	-[3]	4.01	-	2.85	3.00	21.0
ICGV 90266	2.08	-	3.56	-	2.45	2.70	20.8
ICGV 91229	2.08	-	3.95	-	2.78	2.94	20.3
ICGV 91190	2.06	4.29	-	2.44	-	2.93	16.4
886 × 2741	2.01	-	4.18	-	2.73	2.97	15.6
ICGV 90009	1.77	3.59	-	2.60	-	2.65	21.2
ICGV 90013	1.77	4.45	-	2.81	-	3.01	20.3
ICGV 91192	1.73	4.36	-	2.35	-	2.81	15.1
ICGV 91071	1.62	-	3.89	-	2.65	2.72	23.7
ICGV 90056	1.62	-	4.18	-	2.39	2.73	22.6
ICGV 91249	1.60	-	3.64	-	2.34	2.53	17.2
ICGV 86598	1.54	3.24	-	2.46	-	2.41	16.4
ICGV 91053	1.52	3.96	-	2.66	-	2.71	19.7
ICGV 91177	1.42	4.16	-	2.07	-	2.55	13.6
ICGV 88248	1.35	3.13	-	1.81	-	2.10	14.9
Controls							
ICGV 86031	1.39	4.37	4.28	1.23	2.09	2.67	16.7
JL 24	1.08	2.52	2.70	1.42	2.17	1.98	58.4
SE	±0.118	±0.247	±0.226	±0.200	±0.175		
CV (%)	12	11	10	19	13		

1. EBDRGVT = Elite Peanut Bud Necroses Disease Resistant Groundnut Varietal Trial, ABDRGVT = Advanced Peanut Bud Necroses Disease Resistant Groundnut Varietal Trial, VB = Virginia Bunch, SB = Spanish Bunch.
2. Mean of six locations.
3. - = data not available.

the newly developed varieties show better field resistance and have a greater yield potential than JL 24, they have 5–8% lower shelling percentage and are late-maturing. However, some of them, e.g., ICGV 90013, 90056, and 88248, contain more oil (50%) than JL 24 (45%).

Of the several interspecific derivatives evaluated for field resistance and yield, only 886 × 2741 showed stable resistance (mean PBND incidence 15.6%) and high pod yield (2.97 t ha^{-1}). It is derived from a cross between *A. hypogaea* × *A. cardenasii*.

The field-resistant varieties reported here are not immune to the disease but have reduced disease incidence under field conditions. The resistance in these varieties is mainly due to their moderate resistance to the vector. Most lack resistance to the virus. ICGV 86031 and ICGV 86388 also have, in addition to vector resistance, PBNV resistance at lower virus concentration. Considering the level of resistance to the vector and to PBNV in newly developed varieties, it seems that further improvement in the level of resistance through conventional breeding may be difficult to achieve.

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Epidemiology of Peanut Bud Necrosis Disease in Groundnut in India

A A M Buiel,[1,2] and J E Parlevliet[2]

Abstract

Peanut bud necrosis disease is caused by peanut bud necrosis virus (PBNV) and is transmitted by Thrips palmi *Karny. The rate of epidemic development of this disease was strongly affected by the resistance level of the host genotype and by the conduciveness of the environment for the disease (disease pressure). In all the environments tested, epidemic development reached a plateau before the crop became fully mature. This termination of the epidemic development appeared independent of disease pressure, phase of the epidemic, rate of the epidemic development, and resistance level of the host genotype. The most probable factor causing termination of epidemic development is adult plant resistance of groundnut to PBNV.*

Introduction

Peanut bud necrosis disease (PBND) is the most important virus disease of groundnut (*Arachis hypogaea* L.) in Asia, where it causes severe yield losses every year. Peanut bud necrosis disease is caused by peanut bud necrosis virus (PBNV), a member of the tospovirus group. The virus is well characterized, and many of its properties have been described (Reddy et al. 1992).

Peanut bud necrosis virus is transmitted by *Thrips palmi* Karny in a persistent manner (Palmer et al. 1990, Wightman and Ranga Rao 1994, Ranga Rao and Vijaya Lakshmi 1993). Under laboratory conditions, larvae acquired the virus but were not able to transmit it. After a larval period of 5 days and after pupating for 3 days, about 60% of the adults transmitted the virus throughout most of their life period of approximately 20 days. From thrips collected from groundnut terminals it was found that *Thrips palmi* is present throughout the year in Hyderabad, India. Yet, thrips populations declined in some periods because of such unfavorable weather conditions as low night temperatures, high day temperatures, and after heavy rains (Reddy et al. 1983).

The aim of this study was to investigate the epidemiology of PBND under field conditions in India, in field-resistant and -susceptible genotypes. Understanding of the epidemiology of PBND will provide information on the plant-virus interaction, the role of thrips, and the effect of plant resistance.

Materials and Methods

Forty-two groundnut genotypes were grown in 10 environments (location × year combinations), each comprising four replicates. Plots consisted of two 4-meter rows, with 20 cm interplant

1. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
2. Department of Plant Breeding, Agricultural University, P O Box 386, 6700 AJ Wageningen, The Netherlands.

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distance, and 50 or 60 cm interrow distance. Data used in this study were from seven of these environments: ICRISAT Asia Center (IAC) (Andhra Pradesh), Rajendranagar (Andhra Pradesh), and Raichur (Karnataka), in 1991 and 1992, and from Narkoda (Andhra Pradesh) in 1993. The trials were sown in the third or fourth week of July, except the trial at Raichur in 1992, which was sown in the first week of August.

Peanut bud necrosis disease occurred in the field as a result of natural infection. The incidence (the number of plants showing disease symptoms) was recorded every 2 weeks, from approximately 2 weeks after emergence until 3 weeks before harvest, except the trial at Raichur, where the PBND incidence was recorded monthly. For this study, we chose two susceptible genotypes (S), two moderately resistant (M), and two resistant (R) genotypes. The time to maturity varied among the genotypes, the range being approximately 2 weeks.

Results

Plants with PBND symptoms were observed as early as 13 days after emergence (DAE) at IAC in 1991 (data not shown). The final PBND incidence was high at Rajendranagar and Narkoda (more than 85%), moderate at IAC (around 55%), and low at Raichur (around 25%), on the susceptible cultivar JL 24.

The effect of resistance on the rate of epidemic development was large (Tables 1 and 3). The effect of the environment was equally large (Table 3).

At all locations and over all years (all environments), the disease incidence reached an apparent plateau. The onset of this plateau phase of the epidemic was estimated as the number of days between emergence and the moment the increase in incidence became almost zero. For instance, the epidemic at Rajendranagar in 1992 showed an initiation of the plateau phase just before or at 76 DAE (Table 1). Table 2 presents the onset of the plateau phase for seven environments, and it ranges approximately between 60 and 75 days. Thus, the onset of the plateau phase occurs 35–50 days before harvest, suggesting that factors other than crop maturity cause the decline of the disease progress.

Table 2 further presents the increase in incidence after the plateau has been reached per genotype group (S, M, R) for each environment. The mean increase of incidence was low, between 1.5 for the R group, 2.0 for the M group, and 2.8 for the S group. The onset of the plateau phase occurred for all groups, independent of the level of resistance and earliness of maturation at about the same time in a given environment.

Table 1. Incidence (%) of peanut bud necrosis disease at six dates after emergence, and increase in incidence after the onset of the plateau phase of the epidemic (about 76 DAE) in six groundnut genotypes, Rajendranagar, rainy season 1992.

		Days after emergence (DAE)						Increase after 76
Genotype	Group[1]	15	29	43	57	76	92	DAE
JL 24	S	1	30	60	83	95	99	4
TMV 2	S	1	14	45	69	85	86	1
85/202-1	M	2	18	31	46	58	60	2
ICGV 89283	M	1	7	14	23	34	36	2
ICGV 86029	R	0	4	6	11	16	18	2
2169-5(9)	R	0	3	6	11	15	15	0

1. S = susceptible, M = moderately resistant, R = resistant.

Table 2. Onset of plateau phase (PP) of the peanut bud necrosis disease (PBND) epidemic in days after emergence (DAE), and average increase in incidence during the PP per group of groundnut genotypes at Rajendranagar (RN), Narkoda (NAR), ICRISAT Asia Center (IAC), and Raichur (RAI), 1991–93.

Location	RN	RN	NAR	IAC	IAC	RAI	RAI
Year	1991	1992	1993	1991	1992	1991	1992
PP (DAE)	<76	<76	69	<75	71	<70	<62
Group[1]	Incidence (%)						Mean
S	4.5	2.5	5.0	2.0	5.0	0.0	0.5	2.8
M	6.5	2.0	1.5	1.5	2.0	0.0	0.5	2.0
R	4.5	1.0	2.5	0.5	2.0	0.0	0.0	1.5

1. S = susceptible, M = moderately resistant, R = resistant.

Table 3 shows the disease incidence at the onset of the plateau phase for seven environments. The incidence at this onset ranged from 19% at the location with the lowest infection, to 95% at the location with highest infection for JL 24. The epidemics in these environments apparently varied widely; yet all epidemics reached a plateau at about the same time per environment and independently of the infection level. The fact that the plateau phase was reached at the same time for all genotypes in each environment indicates that the termination of the epidemic was independent of the rate of epidemic development and of the earliness of maturation.

To compare the rate of disease development for the six genotypes, the time to reach 50% of the maximum disease level was determined. Table 4 presents the results of the three environments with the highest infection. The more susceptible the genotype, the earlier this 50% point was reached. This is expected in the case of logistic development of the epidemic. The higher the disease level, the greater the chance that viruliferous thrips visit already-infected plants. The rate of epidemic development, therefore, is reduced more at higher disease levels. This in turn, results in a slightly earlier 50% point for the more-susceptible genotypes.

Table 3. Incidence (%) of peanut bud necrosis disease in six groundnut genotypes at the onset of the plateau phase of the epidemic in seven environments at four locations—Rajendranagar (RN), Narkoda (NAR), ICRISAT Asia Center (IAC), and Raichur (RAI), 1991–93.

	Environment							
Genotype	RN 1991	RN 1992	NAR 1993	IAC 1991	IAC 1992	RAI 1991	RAI 1992	Mean
JL 24	95	95	81	55	49	29	19	60.4
TMV 2	86	85	71	24	30	25	4	46.4
85/202-1	71	58	59	19	36	9	6	36.9
ICGV 89283	54	34	36	3	6	1	1	19.3
ICGV 86029	23	16	18	5	4	2	1	9.9
2169-5(9)	14	15	20	5	2	1	0	8.1

Table 4. Number of days after emergence to 50% of the maximum disease level of six groundnut genotypes in three conducive environments at two locations, Rajendranagar (RN) and Narkoda (NAR), 1991–93.

		Environment			
Genotype	Group[1]	RN 1991	RN 1992	NAR 1993	Mean
JL 24	S	33	38	52	41.0
TMV 2	S	38	42	52	44.0
85/202-1	M	51	42	50	47.7
ICGV 89283	M	53	49	56	52.7
ICGV 86029	R	66	51	62	59.7
2169-5(9)	R	54	47	59	53.3

1. S = susceptible, M = moderately resistant, R = resistant.

Discussion

As expected, the rate of epidemic development depended strongly on both the resistance level of the host genotype and on the conduciveness of the environment for disease development (disease pressure). In all environments, the epidemic buildup ended independently of the disease pressure, phase of the epidemic, rate of the epidemic development, time of maturation, and degree of resistance. This termination of epidemic development could be caused by changes in weather conditions, thrips numbers, amount of adult tissue, and plant resistance, or a combination of these factors.

Weather data of 3 years from IAC showed no major variation between years in minimum and maximum temperatures, wind speed, and relative humidity during each growing season. Therefore, weather does not seem an important factor in reaching the plateau phase. Thrips numbers declined after reaching a maximum in the early phase of the crop-growing period (Ranga Rao and Vijaya Lakshmi 1993), but this decline (data not shown) could not be related to the termination of the epidemic. Since weather conditions did not change drastically, it is also unlikely that thrips behavior was affected.

Consequently, we assume that it is the adult plant resistance which causes the decline in disease progress. Adult plants and adult plant tissues are highly resistant to the virus. Only the young tissues of the relatively young plants are highly susceptible to PBNV (Buiel, unpublished). Adult (or mature) plant resistance to viruses has been repeatedly reported for potato (Beemster 1987, Venekamp and Beemster 1980, Wislocka 1984, Sigvald 1985, Gibson 1991). Mature plant and/or mature tissue resistance has been reported from other host-pathogen combinations also, such as the rice-blast pathosystem (Roumen 1992). It is common in perennial crops (Smit and Parlevliet 1990).

We therefore consider adult plant resistance to be the reason for low PBND incidence when groundnut is sown early (June) in southern India. In June, the thrips population is just building up after the hot season in March-May. The thrips population (and number of viruliferous thrips) is small during the first 60–75 days after emergence, when the crop is still susceptible, thus escaping most of the infection. When the thrips population has become large, the crop has acquired adult plant resistance.

In northern India, late sowing (Jul, Aug) results in low infection compared with a high infection when sown early. This situation is different from that in southern India because many vegetable crops (e.g., cucumber, watermelon, and sweet melon), which are known hosts of PBNV and *Thrips palmi* (Reddy and Wightman 1990), are cultivated from April to June. Early sowing exposes the

young, susceptible, groundnut crop to PBNV infection, carried over from these alternative hosts. By sowing late (Jul, Aug), the groundnut crop escapes high infection pressure.

This study also showed that resistant genotypes reduce the rate of epidemic development and considerably reduce the incidence of PBND. Similar results were found for spotted wilt disease, caused by tomato spotted wilt virus, on groundnut in the USA (Culbreath et al. 1993). Using resistant cultivars and timely sowing is of great importance in the control of peanut bud necrosis disease.

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Multi-environment Testing for Reduced Incidence of Peanut Bud Necrosis Disease in India

A A M Buiel[1,5], S L Dwivedi[1], M V R Prasad[2], A B Singh[3], P S Dharmaraj[4], and J E Parlevliet[5]

Abstract

Forty groundnut genotypes were tested for field resistance (reduced incidence) to peanut bud necrosis disease during 3 years at four locations in India. The 40 genotypes were grouped into seven clusters using the average linkage cluster analysis. Clusters 1 and 2 contained highly susceptible genotypes (JL 24 and TMV 2). Susceptible to moderately susceptible genotypes formed clusters 3,4, and 5. Cluster 6 represented 29 fairly resistant genotypes, and cluster 7 had the most resistant genotypes [ICGV 86430, 2192- 8(50), and 2169-5(9)]. Genotype × environment interaction variance was significant but small. The field resistance of the genotypes studied was equally effective in all environments. Selection in any of these environments is possible, but is more effective in environments which are favorable for disease development.

Introduction

Groundnut (*Arachis hypogaea* L.) genotypes show a remarkable variation in peanut bud necrosis disease (PBND) incidence. Reduced incidence (field resistance) is the collective result of resistance to peanut bud necrosis virus (PBNV) and of resistance to the vector, *Thrips palmi* Karny. Amin (1985) reported considerable field resistance in cultivar Robut 33-1, and Dwivedi et al. (1993) reported resistance in the ICRISAT germplasm line ICGV 86031. In earlier field studies, in which approximately 900 groundnut genotypes were tested, a wide range of PBND incidence was observed. These differences in disease incidence indicated various degrees of resistance. Therefore, it seemed possible to select among genotypes in a crossing program to improve the level of field resistance. Natural PBND incidence varied between locations. This could result from differences in resistance to the virus and/or the vector, as well as from differences in resistance of the genotypes grown at different locations.

The performance of a genotype depends on both its resistance and the environmental factors. To select efficiently for field resistance, we need to know whether environment and genotype are independent factors or to what extent genotype × environment (G × E) interactions are present. At the initiation of this study, no information was available on the extent of G × E interaction. Similarly, we did not have information on whether selection would yield corresponding results

1. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
2. Directorate of Oilseeds Research, Rajendranagar 500 030, Andhra Pradesh, India.
3. Groundnut Research Station, Mainpuri 205 001, Uttar Pradesh, India.
4. Agricultural University of Agricultural Science, Regional Research Station, Raichur 584 101, Karnataka, India.
5. Department of Plant Breeding, Agricultural University, P O Box 386, 6700 AJ Wageningen, The Netherlands.

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across environments. Substantial G × E interaction or dissimilar results across environments are not only important in determining selection methods in a breeding program, but they may also reveal the occurrence of different virus strains.

The objectives of this multi-environment study were to determine:

- if field resistance operates across environments,
- the optimal location(s) for selection, and
- whether the field resistance is equally effective to the various virus populations to which it is exposed.

The results will lead to the development of effective selection methods for field resistance.

Materials and Methods

Field trials

Forty groundnut genotypes were grown in 12 environments (4 locations × 3 year combinations, Table 1). A large proportion of these 40 genotypes were chosen for their putative field resistance. Seven genotypes, ranging from a low incidence to a high incidence are shown in Table 2. The four locations were spread over three states in India—Uttar Pradesh (Mainpuri), Karnataka (Raichur), and Andhra Pradesh [Rajendranagar and ICRISAT Asia Center (IAC)]—and trials were carried out in the 1991–93 rainy seasons. Each trial comprised four replicates in a randomized complete block design. Plots consisted of two 4-m rows, with 20-cm interplant distance and 50- or 60-cm interrow distance.

Peanut bud necrosis disease occurred in the field as a result of natural infection. The incidence (the percentage of plants showing symptoms) was recorded, and infected plants were labeled every 2 weeks, from approximately 2 weeks after emergence until 3 weeks before harvest. At Mainpuri and Raichur, the PBND incidence was recorded monthly. Scoring and labeling of infected plants was done regularly because often infected plants die, and the PBND symptoms can no longer be identified on these dead plants.

Data analysis

Analysis of the response of 40 genotypes in 10 environments was done by cluster analysis of the genotypes. The final data of incidence were arc sine transformed and standardized (to mean = 0

Table 1. Mean peanut bud necrosis disease incidence (%) across 40 groundnut genotypes at 10 environments in India, 1991–93.

Location	Year	State	Incidence (%)
Raichur	1992	Karnataka	2.5
Raichur	1991	Karnataka	4.4
Raichur	1993	Karnataka	4.5
ICRISAT Asia Center	1991	Andhra Pradesh	9.4
ICRISAT Asia Center	1992	Andhra Pradesh	11.5
Mainpuri	1991	Uttar Pradesh	15.7
Narkoda (Rajendranagar)	1993	Andhra Pradesh	36.5
Mainpuri	1993	Uttar Pradesh	36.7
Rajendranagar	1992	Andhra Pradesh	41.1
Rajendranagar	1991	Andhra Pradesh	51.8

Table 2. Peanut bud necrosis disease incidence (%) at four locations, mean incidence over 10 locations, and the classification in the cluster analysis of seven groundnut genotypes tested in 10 environments in India, 1991–93 rainy seasons.

Entry	Raichur 1993	ICRISAT Asia Center 1991	Mainpuri 1993	Rajendra-nagar 1992	Mean	Cluster
JL 24	22	59	75	99	60	1
TMV 2	11	24	59	89	46	2
89310	13	12	56	75	36	3
86522	1	15	50	64	31	4
89268	0	11	51	48	25	5
86031	3	5	46	23	17	6
2192-8(50)	0	0	13	11	8	7

and SD = 1/ per environment for clustering. Standardization of the data set was done because we were interested in the interaction effects. Clustering was performed using the average linkage cluster analysis in SAS (SAS 1988). The average incidence per cluster was used to examine correlations between environments.

The analysis of variance (ANOVA) with environments (E), genotypes (G), and genotype clusters as main effects, and G × E interaction was performed on the arc sine transformed data in GENSTAT (GENSTAT 1994).

Results

Germination was very poor in two environments, Mainpuri in 1992 and IAC in 1993. These environments were therefore omitted from the analysis.

The average nontransformed incidence of the 40 genotypes across 10 environments ranged from 8% [2192-8(50)] to 60% (JL 24) (Table 2). Most of the genotypes had an average incidence between 10% and 25%.

The average incidence of environments ranged from 2.5% at Raichur in 1992 to 51.8% at Rajendranagar in 1991 (Table 1). Raichur had a low level of PBND in all 3 years, with an average incidence below 5%. At IAC, the average incidence was around 10%. At Mainpuri, the average incidence was 16% in 1991, and 37% in 1993. The average incidence at Rajendranagar was 41% in 1991 and 52% in 1992. At Narkoda, which is located near Rajendranagar, the average incidence was 37%.

Results of the cluster analysis of genotypes are shown in Figure 1. Genotype clustering was truncated, resulting in seven clusters, explaining 87% of the genotype sum of squares (SS). Clusters 1 and 2 contained highly susceptible genotypes (JL 24 and TMV 2). Susceptible to moderately susceptible genotypes formed clusters 3, 4, and 5. Cluster 6 represented the largest group of 29 resistant genotypes, whereas the three most resistant genotypes [ICGV 86430, 2192-8(50), and 2169-5(9)] were grouped in cluster 7. The number of genotypes was not equally distributed over the clusters, as cluster 6 contained almost 75% of the genotypes. This was not surprising since we were interested in resistance, and had chosen many promising genotypes for this study. The unequal distribution emphasizes the need for clustering, because a large group of genotypes with a similar incidence will interfere with the comparison of incidence across environments.

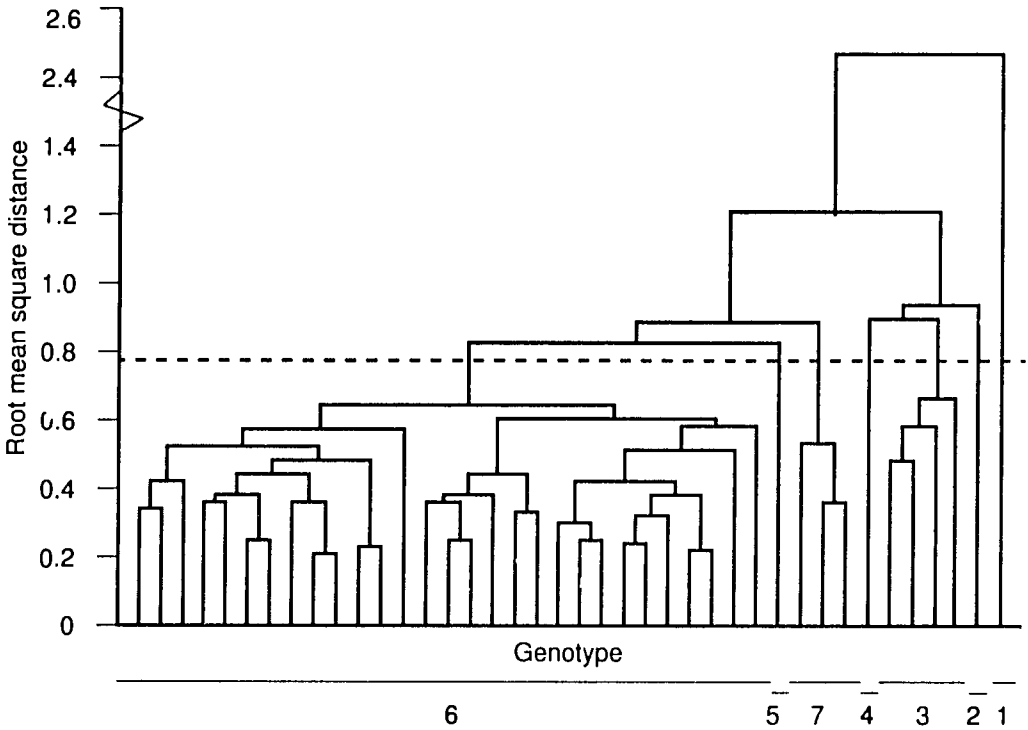


Figure 1. Dendrogram of cluster analysis of 40 groundnut genotypes tested for peanut bud necrosis disease incidence in 10 environments in India.

Table 3. Analysis of variance for arc sine transformed peanut bud necrosis disease incidence of 40 groundnut genotypes across 10 environments in India.

Source of variation	df	SS	MS	F
Replicates	3	369.68	123.23	
Environments (E)	9	326497.53	36277.50	214.01***
Residual	27	4576.85	169.51	
Genotypes (G)	39	102415.56	2626.04	43.16***
Among clusters	6	89048.95	14841.49	243.92***
Within clusters	33	13366.61	405.05	6.66***
G × E	351	41575.35	118.45	1.95***
Residual	1162	70701.59	60.84	
Total	1591	546136.56	343.27	

*** $P < 0.001$.

Main effects (environment, genotype, and genotype clusters) were highly significant in the ANOVA of the arc sine transformed incidence (Table 3). The G × E interaction was significant but small (Table 3) because the variance of the interaction (σ_{ge}=14.40) was small compared with the variance of the smallest main effect (genotype, σ_g=62.69).

Figure 2 shows the arc sine transformed incidence for different environments. The differences in incidence among clusters increased with increasing infection level and is shown as the lines of the clusters diverge (Figure 2). It implies that the small G × E interaction was primarily caused by this divergence in incidence between environments. Interactions caused by a reversed order (shown as crossover of lines in Figure 2) did occur but these were of minor importance.

In Figure 3, the interactions are shown in more detail. The clusters were ranked according to the average transformed incidence per environment. Figure 3 shows two main findings. Firstly, most of the interaction resulted from clusters 3, 4, and 5. Clusters 1, 2, 6, and 7 were consistent across environments. Secondly, Figure 3 shows that the results were rather erratic at Raichur in 1992 (with the lowest infection level).

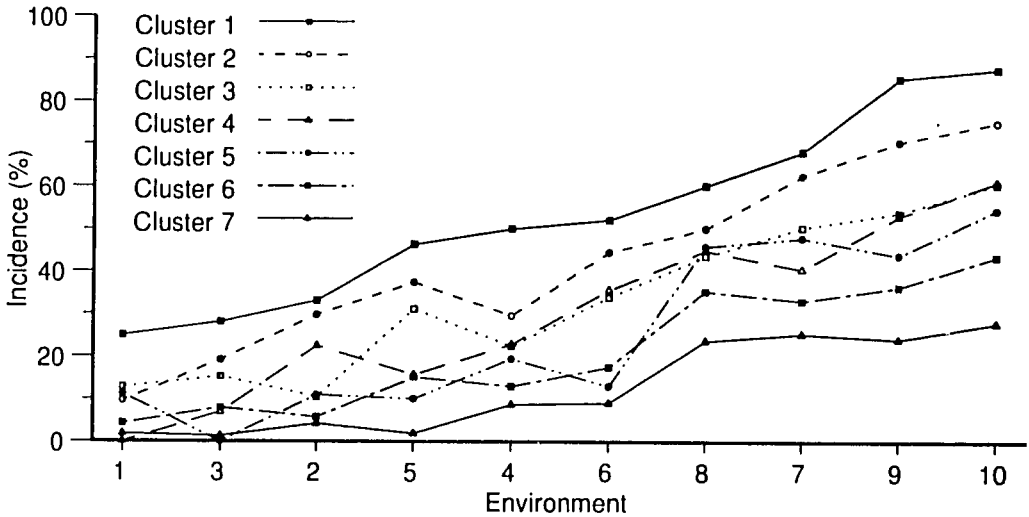


Figure 2. Peanut bud necrosis disease incidence of seven genotypes clusters in 10 environments.

Correlation coefficients (Spearman's r_s) were calculated from the ranking order of clusters among environments (Table 4). Most correlations between environments were significant at $P < 0.05$, except the correlations between Raichur in 1992 (environment 1) and other environments. The average correlation between environment 1 and other environments was 0.54. Furthermore, the average correlation among environments with a low infection (L) was poor (0.52), but a high average correlation was found among environments with an average (A) infection (0.95) and a high (H) infection (0.91).

Discussion

Genotype × Environment interaction was significant but small, and was shown to result largely from a divergent reaction of genotypes across environments and to a much lesser extent from crossover of genotypes. Thus, selection in any of the environments studied here yielded similar results. However, A and H environments discriminated considerably better among genotypes than L environments. Further, the small crossover interactions were relatively more important in L environments than in A and H environments. These interactions caused noise in the data of L environments. The infection level at Raichur (L) was low in three consecutive years; nevertheless, the most resistant genotypes of cluster 7 could be identified as highly resistant on the basis of the combined 3-year data at Raichur.

Table 4. Correlation matrix (Spearman's r_s) of 10 environments with low (L), average (A), and high (H) peanut bud necrosis disease incidence based on ranking of average incidence of seven genotype clusters.

		L 1	L 2	L 3	A 4	A 5	A 6	H 7	H 8	H 9	H 10
L	1	-									
L	2	0.43	-								
L	3	0.54	0.61	-							
A	4	0.46	0.96	0.75	-						
A	5	0.57	0.82	0.93	0.93	-					
A	6	0.39	0.89	0.86	0.96	0.96	-				
H	7	0.79	0.86	0.75	0.89	0.89	0.82	-			
H	8	0.57	0.96	0.54	0.89	0.75	0.79	0.89	-		
H	9	0.64	0.89	0.82	0.96	0.96	0.93	0.96	0.86	-	
H	10	0.46	0.96	0.75	1.00	0.93	0.96	0.89	0.89	0.96	-
Mean		0.54	0.82	0.73	0.87	0.86	0.84	0.86	0.79	0.89	0.87

Mean correlation among:

L environments	0.52 (n=3)
A environments	0.95 (n=3)
H environments	0.91 (n=6)

$P < 0.05$ if r_s 0.750.
$P < 0.01$ if r_s 0.893.

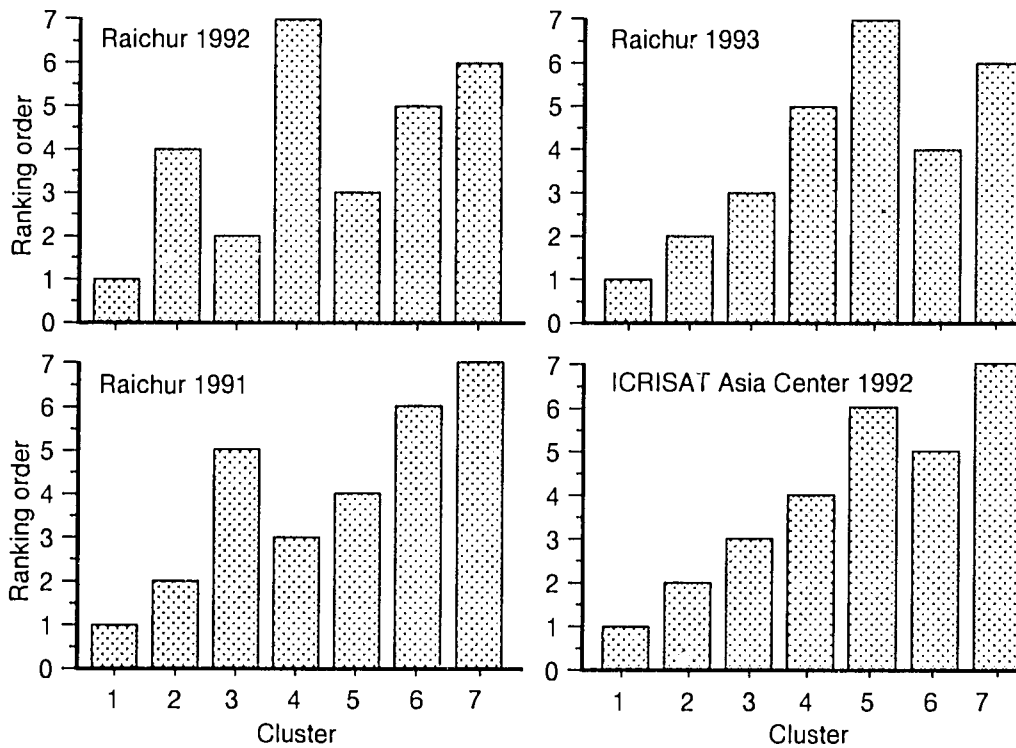
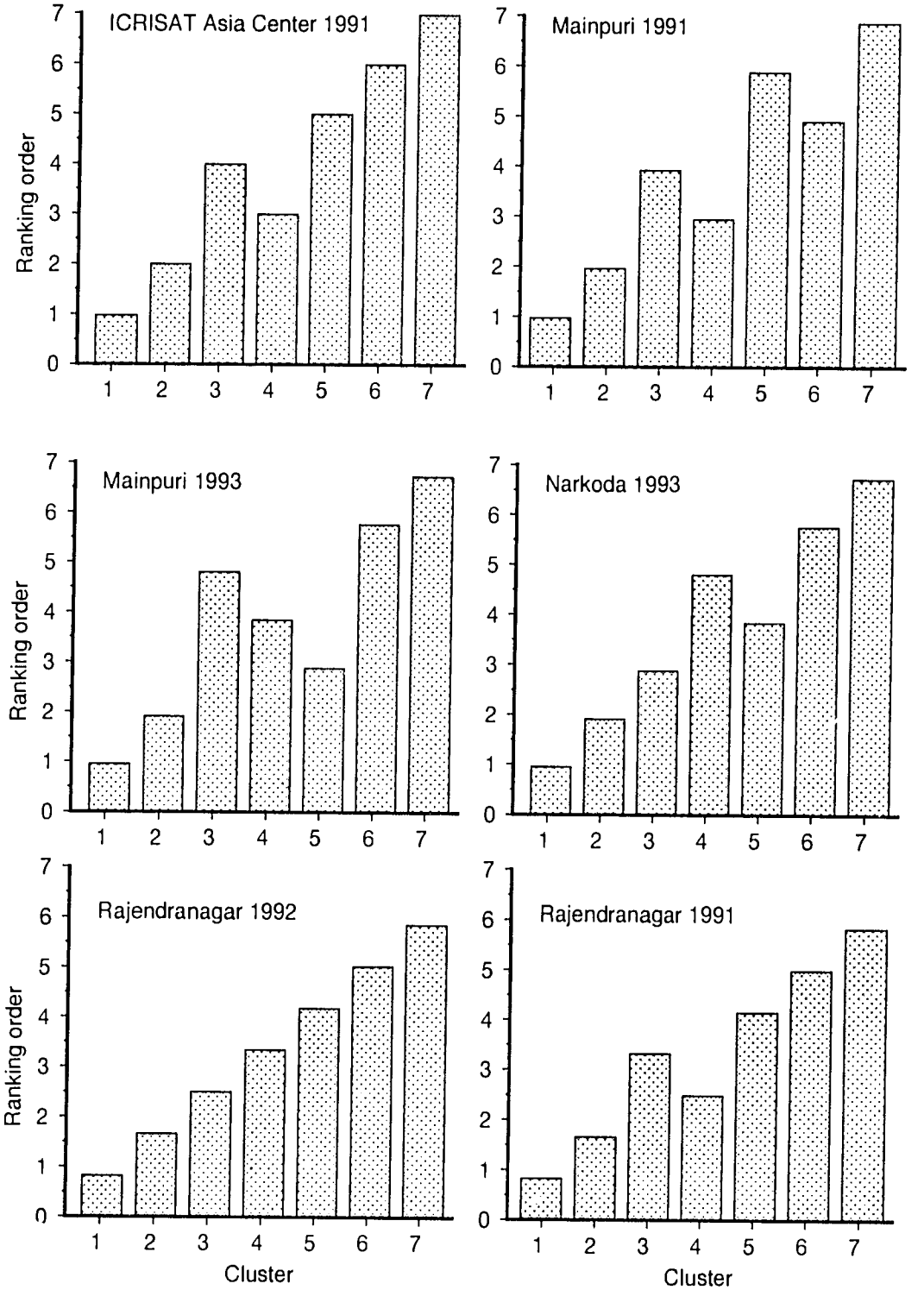


Figure 3. (Above, and opposite page) Ranking order of the mean peanut bud necrosis disease incidence of seven genotype clusters in 10 environments.


ICRISAT Asia Center 1991
Mainpuri 1991
Mainpuri 1993
Narkoda 1993
Rajendranagar 1992
Rajendranagar 1991
Ranking order
Cluster
0
1
2
3
4
5
6
7

Peanut bud necrosis disease resistance for the genotypes in this study operated in all environments. The ranking of clusters 1, 2, 6, and 7 was consistent. For clusters 3, 4, and 5, the ranking was somewhat irregular. This is probably due to the small differences in mean incidence levels for these clusters (i.e., 25.6%, 30.3%, and 33.4%).

The results showed that the PBND infection levels varied considerably among locations and to a lesser extent, among years within the same location. The interactions observed were very small compared with the main effects, and provided no evidence for virus differences among locations. In earlier studies, Reddy et al. (1992) and Poul et al. (1992) found that PBNV isolates from different locations in India (including those used in this study) reacted with PBNV polyclonal antiserum and with 10 monoclonal antibodies directed against the nucleocapsid protein. This finding, and the results presented here based on genotype reaction under field conditions, indicate that it is unlikely that the prevailing virus populations in these environments were pathogenically different.

The results presented here allow us to draw some general conclusions which will help in establishing a selection program for field resistance to PBND. Highly resistant and highly susceptible genotypes can easily be identified at locations with high or low disease levels. Results obtained at one location are also valuable to predict resistance at other locations. In locations with a low disease pressure, differences between genotypes are relatively small, and as a result, the data are noisier. This makes it more difficult to distinguish between moderately resistant genotypes, but the selection of highly resistant genotypes is not seriously impeded in these environments. We recommend selection at locations with an average or high disease pressure because selection in these discriminating environments yields more reliable results. Nevertheless, when the disease pressure is low (and it may be impossible to predict this beforehand), the combined data of repeated experiments can be used for selection.

Acknowledgements

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Peanut Bud Necrosis Disease in Thailand

Sopone Wongkaew[1]

Abstract

Peanut bud necrosis disease (PBND) was first reported in Thailand in 1985. Serological assays of diseased groundnut plants collected from 1992 to 1994 gave positive results only with peanut bud necrosis virus (PBNV) antiserum and not with tomato spotted wilt virus and impatiens necrotic spot virus antisera. PBND incidence of up to 20% was recorded during the dry season in many farmers' fields, and in eastern Thailand, incidence as high as 90% was occasionally observed. In the rainy season, the incidence in most locations was lower than 1%. Four species of thrips were found on the groundnut crop. Among them, Scirtothrips dorsalis *was found in large numbers in most plants while* Thrips palmi *was rarely detected. The number of* S. dorsalis *appeared to correlate with PBND incidence, and it could transmit the virus to healthy plants. PBNV was also found to severely affect tomato, sweet pepper, egg plant, and cucurbits. Five weed species:* Cleome viscosa, Physalis minima, Spilanthes paniculata, Synedrella nodiflora, *and* Catharanthus roseus *were identified as alternative hosts of PBNV. Disease control measures currently recommended are: close spacing, avoiding growing groundnut in the dry season, applying aldecarb at sowing time, and using plastic mulching in other cash crops. Future research will be directed towards genetic resistance and identification of PBNV strains.*

Introduction

Bud necrosis of groundnut was first reported in Sakon Nakorn Province in 1985 (Wongkaew 1987) when only a few plants were affected. At that time, the causal agent was identified as tomato spotted wilt virus, and thrips were suspected as possible vectors. It was not until 1991 that the true identity of the virus was recognized as peanut bud necrosis virus (PBNV). Most of the groundnut samples with typical bud necrosis symptoms collected in Thailand reacted negatively with tomato spotted wilt virus (TSWV)-antiserum [Wongkaew and Chuapong, in press(a)]. Therefore, it may be concluded that bud necrosis in Thailand was caused by PBNV only. At present, PBNV is ranked first in economic importance, because of its severity and widespread distribution.

Occurrence of Peanut Bud Necrosis Disease

From 1992 to 1994, six surveys were made during the rainy season (Jul to Sep) and the dry season (Dec to Mar) to observe the epidemiological pattern of bud necrosis in groundnut and other crops. Altogether 154 locations in the north, northeast, east, and central regions were visited. Some of them were visited in both rainy and dry seasons. In each survey, both diseased samples and thrips were collected for further identification. Direct antigen coating ELISA was employed in the diagnosis using PBNV-, TSWV lettuce strain- or impatiens necrotic spot virus-antisera.

1. Plant Pathology Department, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand.

Wongkaew, S. 1995. Peanut bud necrosis disease in Thailand. Pages 55–59 *in* Recent studies on peanut bud necrosis disease: proceedings of a Meeting, 20 Mar 1995, ICRISAT Asia Center, India (Buiel, A.A.M., Parlevliet, J.E., and Lenné, J.M., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; and P O Box 386, 6700 AJ Wageningen, The Netherlands: Department of Plant Breeding, Agricultural University of Wageningen.

Bud necrosis was found in most locations in the surveys made during the 1993 dry season (Table 1). The incidences varied from zero to as high as 90% [Wongkaew and Chuapong, in press (a)]. It was noted that the areas where groundnuts were grown in close spacing had a consistently lower bud necrosis incidence. In the following rainy season, disease incidence was lower than 1% in most locations. High incidence was again observed in the dry season of 1994 [Wongkaew and Chuapong, in press (c)]. It may be concluded that in Thailand, bud necrosis is prevalent mainly during the dry season.

In both seasons, four species of thrips were found infesting groundnuts. Among them, *Scirtothrips dorsalis* Hood was consistently observed in large numbers and on most plants (Table 2) while *Thrips palmi* Karny, *Haplothrips gowdeyi* Franklin and *Caliothrips indicus* Bagn. were occasionally detected. Because *T. palmi* was rarely found on groundnut, it is unlikely that this species is a major vector of PBNV in Thailand. The large numbers of *S. dorsalis* found on groundnut indicated its possible role as the PBNV vector.

Table 1. Details of sites with groundnut crops and peanut bud necrosis disease (PBND) incidence (%) surveyed in Thailand, dry season 1992/93.

Site	Surveyed areas (ha)	Spacing (cm × cm)	Incidence (%)
Northeast			
Kalasin	2.5	20 × 40	15–30
Khon Kaen 1	1.3	20 × 30	5–15
Khon Kaen 2	2.4	20 × 40	4–40
Mahasarakam	1.9	20 × 40	5–15
Nong Kai	0.5	20 × 30	5
Roi et	2.9	10 × 40	30–80
Sakon Nakorn	1.9	20 × 40	30
Surin	2.5	20 × 30	0–15
Ubol Ratchathani	1.6	20 × 30	0
North			
Lampang 1	1.9	10 × 15	tr[1]
Lampang 2	1.6	10 × 15	tr
Lampoon	0.3	10 × 20	tr
Prae	0.8	10 × 15	0
Utradit 1	1.9	10 × 15	0
Utradit 2	1.3	10 × 15	1–6
East			
Chantraburi	1.3	20 × 30	2
Prachinburi	1.3	20 × 30	30
Rayong	0.8	20 × 30	5
Sra kaew	1.6	20 × 30	70–90
Central			
Singhaburi	1.6	20 × 30	tr

1. tr ≤ 1.0%.

Table 2. Peanut bud necrosis incidence (%) and thrips number collected from groundnuts from January to March 1994, Thailand.

Location	Peanut bud necrosis incidence (%)	Thrips number/terminals[1]	
		Scirtothrips dorsalis	*Thrips palmi*
Northeast			
Nong Kai 1	2	0.90	0
Nong Kai 2	0.6	2.60	0
Nong Kai 3	1	1.85	0
Soong Nuan 1	1	2.00	0
Soong Nuan 2	1	0.45	0
Soong Nuan 3	5	0.66	0
Sakarach 1	5	1.12	0
Sakarach 2	5	2.33	0
Toong-Sabang 1	13	0.33	0
Toong-Sabang 2	13	0.35	0
Kalasin 1	11	0.85	0
Kalasin 2	11	0.60	0
Bokum 1	13	2.54	0
Bokum 2	13	0.85	0
Dong singh 1	5	0.59	0
Dong singh 2	5	0.20	0
Prakonchai 1	0	0.71	0
Prakonchai 2	0	1.35	0
Surin 1	11	3.90	0
Surin 2	17	3.10	0
Varin	17	1.54	0.5
East			
Laem singh 1	12	0.85	0
Laem singh 2	12	0.40	0
Wang Namyen 1	60	0.70	0
Wang Namyen 2	50	2.16	0

1. Mean of 20 young terminals randomly picked per site.

Besides groundnut, tomato (*Lycopersicon esculentum* Mill.), sweet pepper (*Capsicum annuum* L.), egg plant (*Solanum melongena* L.), cucumber (*Cucumis sativus* L.), and watermelon (*Citrullus vulgaris* Schrad.) also seemed to be severely affected by PBNV. Symptoms appearing on these species are shown in Table 3. Although the virus isolates from these plants reacted positively with PBNV antiserum from ICRISAT Asia Center, they differed slightly from those infecting groundnut in symptomatology, host range, and some physical properties. Research is now underway to clarify whether they are strains of PBNV.

In addition to the above-mentioned cash crops, PBNV was detected in five weed species which could act as natural hosts in groundnut fields. These weed species were *Cleome viscosa*, L. *Physalis minima* L., *Spilanthes paniculata* L., *Synedrella nodiflora* Gaertn, and *Catharanthus roseus* G. Don [Wongkaew and Chuapong, in press (b)].

Table 3. Symptoms on crops naturally infected with peanut bud necrosis virus, dr season, Thailand.

Species	Symptoms
Capsicum annuum	Shoe-string like leaves with reduced laminar growth. Some isolates induce chlorotic or necrotic ringspots on leaves. Fruits are malformed with scars on surface.
Citrullus vulgaris	Malformed leaves with necrotic spots and tip dieback. Fruits are malformed with necrotic scars.
Cucumis sativus	Leaves are malformed and curl upward with silvery etching on the lamina.
Lycopersicon esculentum	Leaves are purplish with necrotic rings or specks. Etching on petioles and stems is common. Plants are stunted. Fruits are malformed and have scars at or near the blossom end.
Solanum melongena	Mottled leaves sometimes with oak-leaf pattern. Fruits are malformed with necrotic scars.

Management Strategies to Control Peanut Bud Necrosis Disease

At present, there is no recommendation for effective control of PBNV derived directly from thes experiments. But through observation, it was noted that close spacing could reduce disease inci dence. This practice is now recommended in areas where PBND is prevalent (Wongkaew 1993. Avoiding sowing groundnuts during the dry season is also effective because PBND confines itself t this season. In highly valued crops such as tomato, sweet pepper, and cucumber, plastic mulchin is effective in repelling the thrips, resulting in less PBNV-infected plants. This practice is no widely adopted but may not be practical or cost effective in groundnut. For chemical control aldecarb in granular form appears to be most effective when applied at the time of sowing.

Genetic Resistance

With collaboration from ICRISAT, two standard trials were attempted during the 1992 and 199 dry seasons. The test entries were those that have been reported to have field resistance to PBND However, the trial in 1992 was abandoned because of severe drought. In 1993, the trial was conducted too late in the season resulting in low disease incidence in most lines. During 1994, on trial was conducted at Wang Nam Yen, Sra Kaew Province. This location was selected because o the very high PBND incidence recorded in two consecutive years. Khon Kaen University has als initiated one trial composed of resistant lines from ICRISAT, lines with low thrips infestation from Khon Kaen University, and a newly released cultivar.

Future Research Plans

Experiments will continue to identify possible sources of resistance. The selected entries will be tested for resistance to different PBNV isolates. Research on the PBNV strains will also continue to

support the breeding program. Various disease control strategies will be tested experimentally under Thai cropping conditions.

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Peanut Bud Necrosis Disease: Activities in the Indian National Program

M S Basu[1]

Abstract

Peanut bud necrosis disease is a serious disease in groundnut in India. Within the National Coordinated Research Project on groundnut, 56 resistant lines were identified from 1380 germplasm accessions, and 47 resistant lines were obtained from breeding programs. Selection for thrips resistance produced 24 lines that were considered to be resistant.

India accounts for 40% of the total world groundnut area and contributes 35% of the total production, and is thus the world's largest producer. Depending upon the variation in edaphic and climatic factors, groundnut-producing areas in India have been divided into five zones. Each zone is represented by several research centers under the All India Coordinated Research Project on Groundnut (AICRPG), to develop region-specific agroproduction and protection technologies. Multidisciplinary research on biotic and abiotic stresses constitutes the major thrust in the Coordinated Research Project on groundnut.

Among the viral diseases, peanut bud necrosis virus (PBNV) is one of the most damaging viruses in groundnut, causing 30–90% yield losses. On the basis of the severity of PBNV incidence, a number of hot-spots such as Mainpuri (Uttar Pradesh), Tikamgarh (Madhya Pradesh), Latur (Maharashtra), Rajendranagar (Andhra Pradesh), Palem (Andhra Pradesh), and Raichur (Karnataka) have been identified. Currently, research on PBNV has been directed towards:

- Screening of germplasm and elite breeding lines against PBNV and identification of resistant sources.
- Utilization of resistant sources in crop improvement programs.
- Identification of lines resistant to thrips, the vector of the PBNV.
- Development of cultural practices to reduce the incidence of peanut bud necrosis disease (PBND).

In the national network, a total of 1380 germplasm accessions and elite breeding lines have been screened for PBND resistance in different hot spots during the last 5 years. Of the 1380 lines, 56 have been identified as being resistant to PBND and having less than 10% infection when JL 24, a susceptible control, had 60% infection (Table 1). The important resistant lines identified were: Spanish 5512, Spanish C7-5, ICGS 18, ICGV 86699, J 14, R 33- 1, R 8821, R 7015, R 9021, ICG 1703, ICG 2711, EC 2215, ICG 5042, ICGV 98304, and RSG 1.

Using the above resistant sources as one of the parents in a series of crosses, 1102 segregating populations were screened for field resistance at hot spots during the last 5 years in the national network system, and 47 elite lines with high yield potential and field resistance have been identified (Table 2). Some of those elite lines are presently being evaluated at the national level. In addition, the National Research Centre for Groundnut has evolved a number of elite interspecific

[1] National Research Centre for Groundnut, P B No. 5, Junagadh, 362 001, India.

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Table 1. Screening of groundnut germplasm and elite breeding lines for field resistance to peanut bud necrosis disease, 1989–93.

Year	Lines screened	Resistant lines
1989	167	2
1990	270	23
1991	336	19
1992	106	4
1993	501	8
Total	1380	56

Table 2. Screening of groundnut populations segregating for resistance to peanut bud necrosis disease, 1989–93.

Year	Segregating populations screened (F_2 onwards)	Selections made (in F_7 and F_8 generations)
1989	33	-
1990	401	10
1991	509	15
1992	74	14
1993	85	8
Total	1102	47

cross derivatives using *A. chacoense* and *A. cardenasii* as one of the parents. These derivatives are being taken to hot spots for their evaluation.

Thrips act as the vector in the transmission of the PBNV and it is well known that the virus is not seed transmitted. Hence an alternative approach of bud necrosis management could be the control of the vector either through cultural practices or by developing genotypes resistant to the vector. Screening for resistance to thrips is carried out in the field and supported by laboratory observations. Table 3 presents results of the screening efforts for thrips resistance. Twenty-four thrips-resistant lines have been identified from 480 germplasm accessions and elite breeding lines.

Table 3. Screening of groundnut germplasm and elite breeding lines for thrips resistance, 1989–93.

Year	Lines screened	Lines found resistant to thrips
1989	120	8
1990	152	4
1991	112	10
1992	44	2
1993	52	0
Total	480	24

Transfer of field resistance to elite lines or susceptible cultivars is in progress. After the release of ICGS 11 and ICGS 44, which possess field resistance, two more varieties with detectable resistance, R 8806 and R 8808, have been identified for release.

Early sowing and close spacing (20 cm × 10 cm) have been found effective in managing bud necrosis disease in Peninsular and Central India. However, late sowing with even closer spacing has been found effective in minimizing the incidence in the northern states. This might be due to differential population buildup, and to migration and/or movement of the thrips in different regions.

Spraying of coconut or sorghum leaf extracts has been found to be as effective as the application of systemic insecticides in reducing thrips attack and thereby, the incidence of PBND. This technology is in the process of being standardized in the national system.

Status and Control Strategy of Peanut Bud Necrosis Disease in Uttar Pradesh

A B Singh and S K Srivastava[1]

Abstract

Groundnut is an important oilseed crop in Uttar Pradesh. Peanut bud necrosis disease has become a major constraint. Incidence, management, strategies, and future research on this important virus disease are discussed.

Introduction

Groundnut has a special significance in Uttar Pradesh where it contributes about 25% of the total edible oil produced. In Uttar Pradesh, groundnut occupies an area of 127 000 ha, with a production of 148 000 t, thus standing ninth in both area and production in India. Low levels of production are mainly attributed to insect-pest manifestation, nonavailability of quality seed, and lack of information on improved production and protection technologies for the farmer.

Occurrence of Peanut Bud Necrosis Disease

In recent years, peanut bud necrosis disease (PBND) has become a serious threat to groundnut cultivation in Uttar Pradesh. This has mainly been due to the early sowing of the crop (between 10 and 20 Jun), in order to reduce the damage caused by white grub (Yadava 1985). Early sowing is no doubt very effective in minimizing the damage due to white grub. But, during 1993, this caused a 70–90% loss of groundnut at Mainpuri, due to the incidence of PBND in the early stages of the crop. Normally, the incidence of PBND ranges from 10 to 20%, but during 1970–71, it assumed epidemic proportions with incidences of over 70–80% at several places in the Mainpuri and Etah districts of Uttar Pradesh. The disease sometimes even caused 90–100% yield loss (Singh 1989). Considering the severity of the disease, Mainpuri, the central area of Uttar Pradesh, has been identified as a hot-spot area on an all-India basis (Basu 1993).

Management Strategies for the Control of PBND

Research conducted on different aspects of PBND management is described.

Screening of groundnut germplasm for field resistance

Out of 450 lines and varieties screened for field resistance (reduced incidence of plants with PBND) under early-sown conditions at Mainpuri, only 32 entries were found promising, showing less than

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10% incidence. The entries are TMV 4, CSMG-12, CSMG-15, CSMG-36, CSMG-84-1 (released as Amber), T-12-11, T-11-11, MC-14-38, MC- 11-1, MC-9-2, MC-4-1, MC-7, MC-70, MC-76, EC-20923, EC-21688, MA-19, C-335, C-433, C-471, ICG 170, ICG 869, ICG 5042, ICG 6317, ICG 7484, ICGV 8633, ICGV 86005, ICG 869, ICG 6317, C-12-5-81, 5702 and 5915.

ICG 869 and ICG 6317, screened at Mainpuri, have been identified as field-resistant sources, and have been recommended for use in breeding programs (Punjabrao Krishi Vidyapeeth 1987).

Effect of date of sowing on the incidence of PBND

Experiments conducted at the Groundnut Research Station, Mainpuri, during the 1985 and 1988 rainy seasons clearly revealed that under early-sown conditions, the disease pressure was much higher than when the crop was sown on later dates (Table 1). This situation is in contrast with the one in Karnataka State, where maximum PBND incidence was reported in late-sown crops (Patil 1993). In Uttar Pradesh, crops sown very late showed low incidences of PBND, but yields were low due to poor setting of the pods.

Table 1. Effect of sowing dates on the peanut bud necrosis disease incidence (%) and pod yields at Mainpuri, India, 1985 and 1988.

	Incidence (%)			Mean pod yield (kg ha^{-1})		
Sowing dates	1985	1988	Mean	1985	1988	Mean
15 Jun (early sowing)	9.6	11.1	10.4	1399	887	1143
1 Jul (normal sowing)	5.6	3.9	4.8	1813	1193	1503
15 Jul (late sowing)	5.2	1.6	3.4	468	913	691
30 Jul (very late sowing)	1.0	0.5	0.8	113	643	378
SE(m)				57.8	32.7	

Reaction of promising varieties that were released

Experiments conducted at the Groundnut Research Station, Mainpuri, during the 1989 and 1990 rainy seasons clearly indicated that T-64 followed by CSMG-83-1 were the most susceptible varieties with mean PBND incidence of 16.7% in T-64 and 14.5% in CSMG-83-1. CSMG-12 and CSMG-15 were found promising, showing less than 10% incidence and high yields, compared with other varieties (Table 2). These promising varieties are being used in resistance breeding programs.

Chemical control

Peanut bud necrosis disease is transmitted by thrips, and experiments were conducted using chemical and plant products for vector control during the 1992–94 rainy seasons. Results indicated that a maximum yield of 992 kg ha^{-1} was recorded in quinalphos-treated plots, followed by oak leaf extract-treated plots, with 945 kg ha^{-1}. These treatments increased the pod yield by 75% in the quinalphos-treated plots and 67% in the oak leaf extract-treated plots over the control (Table 3). When plants were affected in early stages, they were not able to produce a single pod, while plants infested in later stages were able to produce some pods.

Table 2. Reaction of some promising groundnut varieties at Mainpuri, India, rainy seasons 1989 and 1990.

Varieties	PBND[1] incidence (%)			Yield (kg ha^{-1})		
	1989[2]	1990[2]	Mean	1989	1990	Mean
T-28	15.8	6.2	11.0	522	450	486
G-201	8.7	11.5	10.1	568	589	578
T-64	21.2	12.2	16.7	536	514	525
CSMG-12	14.7	2.9	8.8	909	802	855
CSMG-83-1	15.4	13.7	14.5	650	359	504
Chitra	11.8	11.6	11.7	700	824	762
CSMG-15	11.7	2.8	7.3	950	854	902
CSMG-84-1	16.3	7.9	12.1	763	834	798
SE(m)				62.5	92.8	

1. Peanut bud necrosis disease.
2. Sowing dates: 15 Jun 1989 and 11 Jul 1990.

Table 3. Effect of different chemicals on the peanut bud necrosis disease incidence (%) and pod yield at Mainpuri, India, rainy seasons 1992–94.

Treatment	Incidence (%)				Pod yield (kg ha^{-1})				Increase over control (%)
	1992[1]	1993[1]	1994[1]	Mean	1992	1993	1994	Mean	
Monocrotophos 0.04%	15	77	21	37.8	738	314	1614	888	57
Endosulfan 0.07%	18	80	31	42.8	710	208	1306	741	31
Dichlorovas 0.02%	21	84	36	47.0	653	180	1232	688	22
Dimecron 0.02%	16	78	34	42.8	1006	239	1551	932	65
Quinalphos 0.02%	13	71	38	40.8	590	348	2029	992	75
Dimethoate 0.02%	24	81	37	47.3	682	198	1259	713	26
Water extract of oak leaf 1.00%	17	78	31	41.8	941	312	1584	945	67
Water extract of neem leaf 1.00%	14	80	40	44.5	923	223	1451	865	53
Control	28	89	55	57.4	405	125	1169	566	0
SE(m)					21.6	5.4	10.0	-	

1. Sowing dates: 16 Jun 1992, 9 Jun 1993, and 29 Jun 1994.

Integrated management

It is essential to undertake well-organized, integrated, sequential control measures, to safeguard the crop.

- Late sowing (from the last week of June to the last week of July) results in less PBND incidence in Uttar Pradesh. This is probably due to the low incidence of migrant thrips, which is closely correlated to disease incidence.
- Groundnut cultivars such as CSMG-12, CSMG-15, ICG 869, and ICG 6317 were found resistant in Uttar Pradesh.
- Increasing the seed rate can compensate for the losses caused by the disease.
- Intercropping of groundnut with sesame and pearl millet can minimize the disease incidence.
- Two sprays of quinalphos 0.02% or water extract of oak leaf 1.00% within 40 days after germination are found effective in increasing pod yield.

Future research plans

There is no doubt that PBND is a limiting factor for the successful cultivation of groundnut in Uttar Pradesh. As long as varieties with complete resistance are not available, varieties with a reduced incidence should be used, supported by chemical control measures. Hence, chemicals are being screened, which are economical, effective, not very hazardous, easily available in the market, and easily handled by the farmer.

Because of the limited economic capacity of the farmer, emphasis is being given to develop resistant, commercially acceptable groundnut varieties.

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Peanut Bud Necrosis Disease in Karnataka

P S Dharmaraj, V B Naragund, and Somasekhar[1]

Abstract

Peanut Bud Necrosis Disease (PBND) is a major threat to groundnuts in the Tungabhadra and Upper Krishna areas of northern Karnataka. No definite trend in the severity of PBND incidence has been observed during the rainy and postrainy seasons. Preliminary studies on different insecticides on groundnut thrips revealed that spraying of Dichlorovos (DDVP) reduced the thrips population. Integrated approaches such as early sowing, close plant spacing, use of plant extracts, and growing disease-resistant varieties helped in improving the management of PBND in Karnataka. The Regional Research Station, Raichur, has released KRG-2, a high-yielding and resistant variety.

Introduction

Groundnut is one of the most important oilseed crops in Karnataka, with an area of 1.12 million ha and production of 0.88 million t (Anon 1991). This accounts for 58% of the total oilseeds produced in the State. It is grown in two major areas of northern Karnataka as an irrigated crop during the postrainy and summer seasons. It is also grown in the rainy season in various districts of Karnataka. Late sowing has become necessary because of irregularity in water supply from the Tungabhadra and Upper Krishna canals, and an unpredictable start of the rainy season. This results in heavily reduced production due to diseases. Among the major diseases, peanut bud necrosis disease (PBND), caused by peanut bud necrosis virus (PBNV), is most severe in both the rainy and postrainy/summer seasons, causing yield losses from 30 to 90% (Patil 1993). In the rainy season, under late-sown conditions, the PBND incidence can be as high as 90%, and in the postrainy/summer seasons, the incidence can reach 75%. Thus, PBND has become a major threat to groundnut cultivation, especially in the Thungabhadra Project (TBP) and Upper Krishna Project (UKP) areas.

Occurrence and Distribution

The start of the rainy season is relevant to the occurrence of PBND on groundnut crops in Karnataka. Farmers sow early in transitional tracts of Dharwad and Belgaum districts, where there is assured rainfall during the last week of May or beginning of June. PBND incidence is always lower in these areas than in Raichur, Bellary, Bijapur, and Gulbarga districts, where farmers usually sow late because of unpredictable rainfall. In most years, the disease pressure was relatively high when farmers sowed their crop during the second half of July or first week of August due to a delay in the start of the rainy season. In recent years, monitoring of groundnut diseases has shown higher incidence of PBND compared with other diseases in northeastern parts of Karnataka (Table 1). It has also been indicated that in certain years, postrainy/summer crops showed higher incidence than the rainy season crops. This could be due to differential immigratory flights of thrips to the crop (Reddy et al. 1983).

1. Regional Research Station, P B No 24, Raichur 584 101, Karnataka, India.

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Table 1. Approximated mean disease severities of four diseases from 1985 to 1994 in the northeastern parts of Karnataka.

Disease	Rainy season	Postrainy season
Incidence of peanut bud necrosis disease (%)	40	38
Leafspot (% affected)	33	24
Rust (% affected)	7	5
Collar rot[1] incidence (%)	5	4

1. *Sclerotium rolfsii*.

Preliminary studies conducted at the Regional Research Station, Raichur (RRSR), to evaluate different insecticides against groundnut thrips indicated that spraying of Dichlorovos (DDVP) at 1 mL L^{-1} on 30 and 45 days after emergence resulted in the highest mortality of thrips (89.2%), a lower PBND incidence at harvest (23.2%), and highest yield (1.5 t ha^{-1}) compared with the nontreated control, where instead of mortality of thrips, population increased by 5% and yield level was 1.2 t ha^{-1}. Low levels of PBND incidence were noticed in acephate, carbofuron + DDVP-treated and nontreated control plots; moderate levels in endosulfan- and carbaryl-treated plots, while a higher PBND incidence was observed in dimethoate- and monocrotophos-treated plots (Table 2).

Symptoms

In recent years, two distinct sets of symptoms have been noticed on various genotypes. One is chlorotic and necrotic ring spots which appears without necrosis of the bud. Only young quadrifoliolate leaves become chlorotic and necrotic spots appear on them. The other type is characterized by sudden necrosis of the bud with or without chlorotic or necrotic ring spots on leaves. In the latter case, bud necrosis is very rapid.

Table 2. Evaluation of insecticides against groundnut thrips in relation to peanut bud necrosis disease (PBND) incidence (%) at Regional Research Station, Raichur, rainy season 1994.

Treatment	Time of spraying (DAE)[1]	Thrips mortality (%)	PBND incidence (%) at 45 DAE[1,2]	Yield (t ha^{-1})[2]
Dichlorovos (DDVP) 0.5 mL L^{-1}	30 and 45	76	12	1.4
DDVP 1.0 mL L^{-1}	30 and 45	89	10	1.5
Acephate 1 g L^{-1}	30 and 45	82	12	1.3
Dimethoate 1 mL L^{-1}	30 and 45	81	15	1.0
Monocrotophos 1 mL L^{-1}	30 and 45	86	14	1.0
Phorate 25 kg ha^{-1} + DDVP 0.5 mL L^{-1}	45	83	12	1.0
Carbofuran 25 kg ha^{-1} + DDVP 0.5 mL L^{-1}	45	80	11	1.3
Endosulfan 2 mL L^{-1}	30 and 45	71	15	1.2
Carbaryl 4 g L^{-1}	30 and 45	73	14	1.1
Nontreated	30 and 45	+5	10	1.2

1. DAE = Days after emergence.
2. Differences not significant.

Management Strategies

Sowing time

Experimental results over eight seasons at the RRSR clearly indicated that in the rainy season, the early-sown crop, (sown in the first half of June) shows a lower incidence of PBND (1–10%) than the crop sown in late June (4–30%). As the sowing date advanced further to the first half of July, the PBND incidence ranged from 20 to 45%. In crops sown after 15 July, it increased further with each day's delay in sowing. The highest incidence, 90%, was noted in the crop sown around the first of August. Dry weather during the crop growth period promoted the disease to a great extent.

Spacing

Close spacing (20 × 10 cm) resulted in lower incidence of PBND compared with wide spacing. This was consistent over all eight seasons.

Host-plant resistance

Most spanish bunch varieties in Karnataka such as JL 24, KRG-1, and S-206 are highly susceptible to PBND, whereas R-8808, ICGS 11, and ICGS 44 have fair levels of resistance. Screening over several seasons in the hot-spot areas of Raichur indicated that entries such as R-8806, R-8970, R-8976, R-9021, R-9251, R-9214, R-9227, R-9204, ICGV numbers 86029, 86030, 86031, 89304, 86696, and ICG 2271 are promising, with less than 5% PBND incidence, while JL 24 had incidences of over 30%.

Integrated management

Early sowing in the first half of June, close plant spacing, and growing such resistant varieties as R-8808 and ICGS 11 have restricted PBND to very low levels. Results over several years clearly indicated that the use of natural pesticides such as sorghum or coconut leaf extracts proved to be more effective in reducing PBND incidence, thereby increasing groundnut yields (Table 3).

Resistant Varieties

To meet the demands of farmers of TBP and UKP areas, the RRSR released a new high-yielding, resistant variety KRG-2 (R-8808, Table 4) in 1994. This variety exhibited superiority over the prevailing control varieties JL 24 and KRG-1, with an increase in pod yield of 11.6% over JL 24 and 50.0% over KRG-1 during the rainy season, and 19.2% over ICGS 11 and 44.5% over KRG-1 during the postrainy/summer seasons.

To control PBND, the RRSR intensified the breeding activity by generating improved material from different resistance sources and identifying high-yielding varieties with resistance to both virus and vector. Due attention has also been given to the integrated management of PBND in Karnataka.

Table 3. Effect of natural products and chemicals on peanut bud necrosis disease incidence in groundnut, postrainy seasons 1991–94.

Treatment	Incidence (%) Postrainy season 1991/92	1992/93	1993/94	Weighted mean	Mean relative weighted pod yield
Coconut leaf extract	10	29	31	23.5	135
Sorghum leaf extract	13	31	32	25.1	136
Neem leaf extract	18	37	–	26.5	122
Neem seed	–[1]	–	33	36.0	100
Prosophis leaf extract	37	36	–	35.5	114
Monocrotophos	30	40	35	34.0	108
Coconut and monocrotophos	–	–	38	40.0	100
Sorghum and monocrotophos	–	–	32	35.0	126
Neem seed and monocrotophos	–	–	37	39.0	118
Monocrotophos regular	19	54	42	38.2	100
Control	39	39	35	37.5	100
CD at 5%	14.9	4.1	7.8		

– = data not available.

Table 4. Peanut bud necrosis disease incidence (%) on two resistant cultivars and two susceptible cultivars, JL 24 and KRG-1, rainy and postrainy seasons 1991–93.

Year	Rainy season KRG-2 (R-8808)	KRG-1	JL-24	Postrainy season KRG-2 (R-8808)	KRG-1	ICGS 11
1991	3.7	27	57	5.0	48	13
1992	0.0	24	52	2.2	39	13
1993	0.0	12	15	0.0	60	1.3
Mean	1.2	21	41	2.4	49	9

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Closing Remarks

J M Lenné[1]

This meeting has provided an important forum in which to discuss research findings, identify gaps in our knowledge of peanut bud necrosis virus (PBNV) and its vector, and plan future work. Over the past 3 years, considerable progress has been made in the understanding of PBNV, and other tospoviruses and their vectors, and in the development of tools to manage the disease. We have better knowledge of the virus itself and of its relationship with other tospoviruses. We have excellent tools to detect the virus in the plant and in the vector—and these are continuing to be refined.

We now know much more about the dynamics of the virus in the vector, and have a better understanding of its transmission and the factors that affect it. We know more about the nature of resistance to the virus and to the vector. Useful sources of resistance have been identified. We have a greater understanding of the epidemiology of the disease in India, and the best environments in which to select for resistance.

Resistances have been incorporated in suitable medium-duration backgrounds, and are beginning to be developed in early-maturing varieties. Cultural practices which reduce the peanut bud necrosis disease (PBND) have been identified. Some practices are site specific; others, such as maintenance of a dense plant stand, are global.

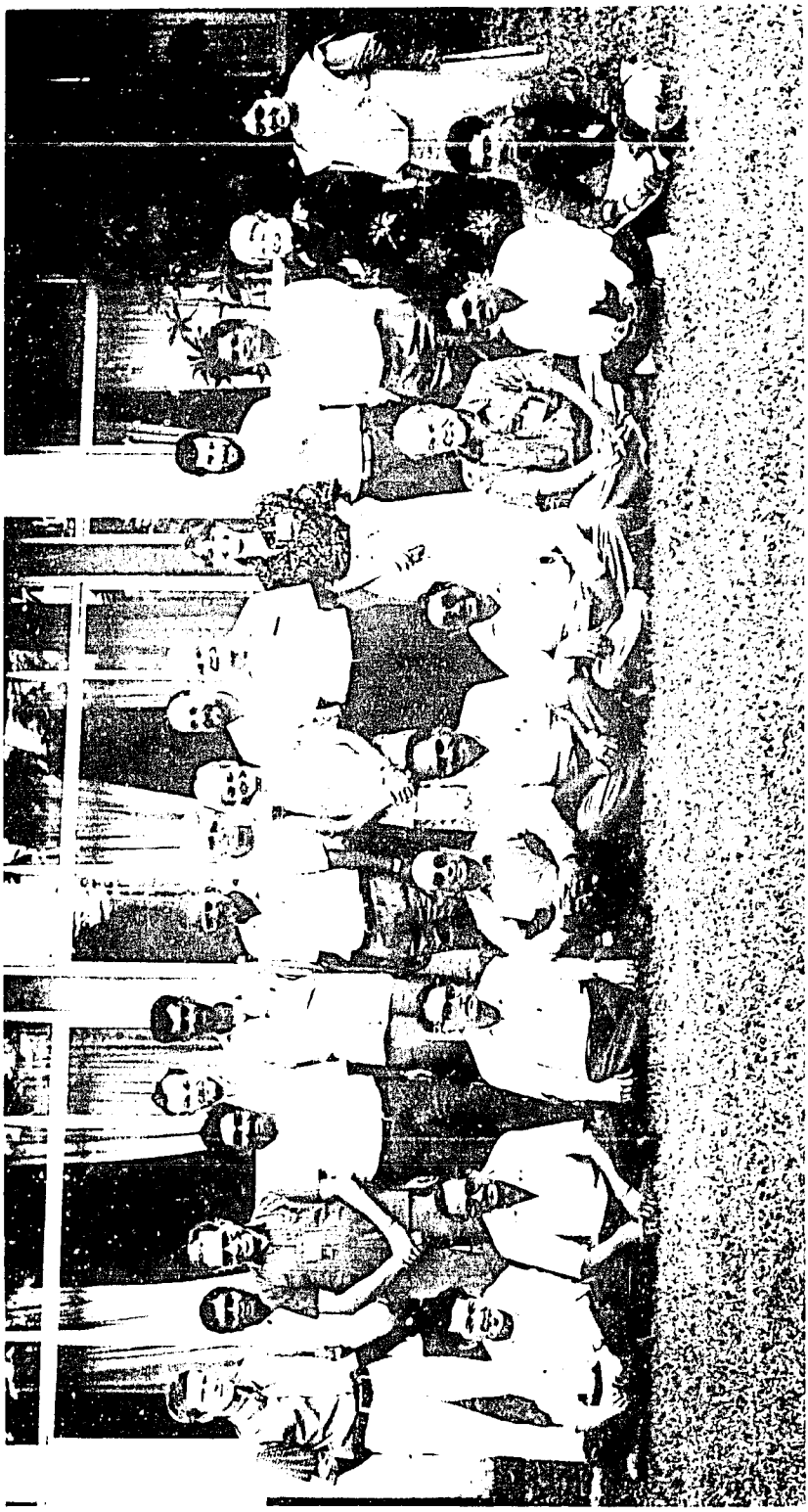
Future Needs

Although considerable progress has been made over the past few years, further work is needed, to develop stable management strategies for PBND. Of greatest importance, is a better understanding of the variability in the virus in Asia, of the distribution of the main vector/s (are there different thrips vectors in different countries/different regions?), and an increased knowledge of the host range of PBNV and *Thrips palmi*. Successful results from such studies will have significant implications for the development of management strategies for PBND. Besides, refinement of diagnostic tools would facilitate all of the above studies.

We need to have a greater understanding of the environmental conditions under which PBNV may be important, including the identification of hot-spots, and risks in new environments, e.g., irrigated groundnut production systems. More effort should be put into diversifying cultural control options in different production systems.

An understanding of the mechanisms of resistance to both the virus and the vector is necessary, and a continued effort should be directed at developing resistance in early-maturing types. As knowledge of the virus and the disease grows, we need to develop strategies to combine resistance with other control options to develop integrated virus disease management strategies. Development of transgenic plants with nonconventional sources of resistance will also be important.

1. Crop Protection Division, ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.



Participants

The Netherlands

J E Parlevliet
Professor in Plant Breeding
Department of Plant Breeding
Agricultural University of Wageningen
P O Box 386, 6700 AJ
Wageningen

Tel	+31 8370 83597
Fax	+31 8370 83457
E-mail	Jon.Parlevliet@USERS.PV.WAU.NL

D Peters
Department of Virology
Agricultural University of Wageningen
P O Box 8045, 6700 EM
Wageningen

Tel	+31 8370 83083
Fax	+31 8370 84820
E-mail	Dick.Peters@MEDEW.VIRO.WAU.NL

Thailand

Sopone Wongkaew
Head, Department of Plant Pathology
Faculty of Agriculture
Khon Kaen University
Khon Kaen 40002

Tel	+66 43 242331-44 Ext 2294
Telex	55 303 UNIKHON TH
Fax	+66 43 244474

United States of America

B G Cassidy
Samuel Roberts Noble Foundation
P O Box 2180
Ardmore, Oklahoma 73401

Tel	+1 405 223 5810
Fax	+1 405 221 7380
E-mail	BGCASSIDY@noble.org

J L Sherwood
Department of Plant Pathology
Oklahoma State University
Noble Foundation Building
Stillwater, Oklahoma 74078

Tel	+1 405 744 9950
Fax	+1 405 744 7373

India

M S Basu
Project Coordinator (Groundnut)
National Research Centre for Groundnut
P O Box No 5
Junagadh 362 001, Gujarat

Tel	+91 285 21550
Telex	0164-220 NRCG IN
Cable	GNUTSEARCH

P A Bhalani
Groundnut Entomologist
Oilseeds Research Station
Gujarat Agricultural University
Junagadh 362 001, Gujarat

Tel	+91 285 20218
Cable	AGRICAMP

P S Dharmaraj
Breeder (Oilseeds)
Regional Research Station
University of Agricultural Sciences, Dharwad
P O Box 24, Raichur 584 101
Karnataka

Tel	+91 8532 8378
Cable	AGRISEARCH

M P Ghewande
Senior Scientist (Plant Pathology) and
Principal Investigator (Crop Protection)
National Research Center for Groundnut
Ivnagar Road, P B No 5
Junagadh 362 001, Gujarat

Tel	+91 285 23041
Telex	0164-220 NRCG IN

M K Jana
Groundnut Breeder
Bidhan Chandra Krishi Viswavidyalaya
All India Co-ordinated Research Project
on Groundnut
Regional Research Station
P O Jhargram 721 507
Midnapore, West Bengal

M V R Prasad
7-1-282/C/46
Sree Ramnagar
S R Nagar Post
Hyderabad 500 038
Andhra Pradesh

Tel +91 40 274784

P N Reddy
Principal Scientist (Groundnut)
Andhra Pradesh Agricultural University
Agricultural Research Station
Kadiri 515 591, Andhra Pradesh

Tel +91 8494 2180

Sadhana S Ramchander
Consultant Editor
Flat 208, Mithila Apartments
3-5-906/1, Himayatnagar
Hyderabad 500 029, Andhra Pradesh

Tel +91 40 598465

Somasekhar
Junior Entomologist
Regional Research Station
University of Agricultural Sciences
Raichur 584 101, Karnataka

ICRISAT Asia Center
Patancheru 502 324
Andhra Pradesh
India

Tel +91 40 596161
Fax +91 40 241239
Telex 422203 ICRI IN
E-mail (Internet)
ICRISAT@CGNET.COM

A A M Buiel (Hanneke)
Research Scholar
Crop Protection Division

P Delfosse
Visiting Scientist
Crop Protection Division

B Diwakar
Program Leader (Acting)
Training and Fellowships Program

S D Hall
Manager, Editorial Unit
Information Management and
Exchange Program

M P Lakhe
Research Associate
Crop Protection Division

J M Lenné
Director
Crop Protection Division

Mohan Kendre
Research Associate
Crop Protection Division

R A Naidu
Scientist (Virology)
Crop Protection Division

K E Neering
Visiting Scientist (Entomology)
Crop Protection Division

Y L Nene
Deputy Director General

S N Nigam
Principal Scientist (Breeding)
Genetic Enhancement Division

A Ratna Surender
Senior Research Associate
Crop Protection Division

D V R Reddy
Principal Scientist (Virology)
Crop Protection Division

S V Reddy
Research Associate
Crop Protection Division

C Renard
Executive Director Asia Region

P Shobha Devi
Research Associate
Crop Protection Division

S V Wesley
Visiting Scientist
Crop Protection Division

J A Wightman
Principal Scientist (Entomology)
Crop Protection Division