QUANTITATIVE RESISTANCE TO PEANUT BUD NECROSIS TOSPOVIRUS IN GROUNDNUT

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QUANTITATIVE RESISTANCE TO PEANUT BUD NECROSIS TOSPOVIRUS IN GROUNDNUT

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The research described in this thesis was carried out at the Crop Protection Division, International Crops Research Institute for the Semi-Arid Tropics Asia Center (ICRISAT-IAC), India. The research was a collaborative project between Wageningen Agricultural University and ICRISAT-IAC, as part of the Durable Resistance Program funded by the Directorate General for International Cooperation of the Dutch Ministry of Foreign Affairs.

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STELLINGEN

1. De kwantitatieve vorm van resistentie in de aardnoot tegen het 'peanut bud necrosis virus' kan als model dienen voor de resistentie tegen andere tospovirussen.

2. In India komen geen differentiërende vormen van het 'peanut bud necrosis virus' voor. *Dit proefschrift.*

3. Kwantitatieve virusresistentie kan gemaskeerd worden wanneer monsters voor de analyse samengevoegd worden.

4. Veel virussen houden zich niet aan de mandaatgewassen van de diverse internationale instituten, de laatsten zouden de regels voor het toegepast onderzoek dan ook moeten versoepelen.

5. De Nederlandse term 'aardnootknopnecrosevirus' voor het 'peanut bud necrosis virus' heeft een hoog hottentottententententoonstelling-gehalte, en is bovendien volledig overbodig.
   Gewasbescherming 26: Supplement 1, 1995.

6. Gelukkig is vloeken aangeleerd.

7. Tospovirussen zijn de vegetariërs binnen de niet-vegetarische familie *Bunyaviridae*.

8. De slogan 'Let's make things better' waarmee Philips zijn producten tracht te verkopen suggereert dat er thuis ook nog aan deze producten gesleuteld moet worden.

9. Het aantal passagiers per 2-wielig voertuig in India, is gemiddeld hoger dan het aantal passagiers per 4-wielig voertuig in Nederland.

10. De stappen die in 1994 genomen werden om de verspreiding van de pest (*Yersinia pestis*) in India in te dammen, stonden in geen enkele relatie tot de epidemie van deze ziekte.
    The Lancet 1994, 344 (8933): 1033-1035.

11. Het gewicht van dit proefschrift dat bij verschijnen 0,260 kg was, zal toenemen.

Stellingen behorende bij het proefschrift van A.A.M. Buiel, getiteld 'Quantitative resistance to peanut bud necrosis tospovirus in groundnut', te verdedigen op 2 december 1996 in de Aula van de Landbouwuniversiteit te Wageningen.

ABSTRACT

Quantitative resistance to peanut bud necrosis virus (PBNV) is expressed as a reduced disease incidence (percentage of infected plants) in the groundnut crop. An increased plant density reduced this incidence, but the number of infected plants per unit area increased, maintaining high levels of PBNV.

No significant inter-plot interference was observed. It appeared that the quantitative resistance, assessed in small plots of the plant breeder, is representative of the farmers' situation.

The PBNV infection was higher in the center of the field as compared to the border zones of the field. This effect was probably caused by the dispersal pattern of the vector.

Selection for resistance to the virus was most effective in environments with average or high levels of natural infection. However, selection in environments with low levels of infection yielded similar results when the data of several years were combined.

At least three resistance factors were found in seven groundnut genotypes. Two different factors were present in the resistant ICGV genotypes, while another factor was present in groundnut cultivar TMV 2. The latter has a slight resistance compared to the most susceptible check, but it has been effective for several decades and can be considered durable.

In addition, mature plant and tissue resistance caused a reduction in incidence and increased the incubation period. This effect was shown to occur in the field, and caused a slowing down and a termination of the epidemic in the course of the growing season.

Virus resistance can be explained by an inhibition of virus multiplication and/or virus movement. These mechanisms result in a reduction of the disease incidence in resistant genotypes.

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Besides a working place, Hyderabad turned out to be a great 'Hash' city. Many Sunday afternoons were lightened up by the perfect mix of exercise and socializing. Digit, Diamond Lil, Big Bird, Madame Tall, Snookers, and Cleopatra are only a few (Hash) names of this active mob.

Going to India was a major change, not only for me but also for my family, especially my parents. I thank them for the unconditional backing they gave me.

Martin, as husband and best friend, you are the last on this list of thanks. I find it impossible to express my deepest gratitude here with mere words, when you have given me your whole-hearted support from the beginning to the end. Five years of a mainly long distance relationship was worth it in the end, as I finish this thesis. During these years India has taken a special place in our hearts, not in the least because our daughter, Imke Tara, began her earliest life in India, and she turned out to be of 'export quality'.

Contents

GENERAL INTRODUCTION

Peanut bud necrosis tospovirus (PBNV) causes bud necrosis disease, and is one of the most devastating viruses of groundnut (*Arachis hypogaea* L.). The disease has been reported from China, India, Nepal, Sri Lanka, and Thailand, and seems to be restricted to South Asia (Reddy et al., 1991, 1995). Besides groundnut, PBNV infects chili, potato, tomato, tobacco, mung bean and urd bean (Reddy et al., 1995), and probably many other crops. Knowledge of the occurrence of PBNV is limited because virus detection in the South Asian countries where PBNV presumably occurs is often hampered by a lack of equipment, skills etc. The occurrence of PBNV, based only on symptoms, has been reported from Bangladesh (Khatun et al., 1996), Indonesia (Baliadi and Saleh, 1996), Pakistan (Bashir et al., 1996), and Vietnam (Thuan and Trung, 1996). Extensive studies in India and Thailand have shown that PBNV occurs recurrently on groundnut in these countries. Sites with more than 50% infection are not uncommon (Reddy et al., 1983; Wongkaew, 1995). The International Crops Research Center for the Semi-Arid Tropics (ICRISAT) estimated the losses caused by this virus at more than 89 million US dollars per year (Anonymous, 1992).

PBNV is currently classified as a species within the genus *Tospovirus* of the *Bunyaviridae* (Reddy et al., 1992; Adam et al., 1993; Satyanarayana et al., 1995). Tomato spotted wilt virus (TSWV), the type member of the genus, has a host range of more than 700 different plant species, including many weeds and economically important crops (Best, 1968; Goldbach and Peters, 1994; Peters, personal communication). PBNV also appears to have a wide host range (Reddy et al., 1995).

Tospoviruses are transmitted by thrips (Thysanoptera: Tripidae), and PBNV has been shown to be transmitted by *Thrips palmi* Karny (Vijaya Lakshmi et al., 1995). The virus is not seed-transmitted (Reddy et al., 1983).

CAUSAL VIRUS

Reddy et al. (1968) first reported bud necrosis symptoms on groundnut, but the causal virus was not yet known. Ghanekar et al. (1979) concluded that the disease was caused by TSWV, as positive results were obtained in a haemagglutination test with TSWV-antiserum from South Africa and the USA. Sreenivasulu et al. (1991) described that the isolate causing peanut bud necrosis from India failed to react with antisera developed to TSWV

isolates from Texas (USA), Australia, Greece and The Netherlands. The antiserum developed to the Indian isolate only reacted with the isolates from India. These results strongly indicated that the Indian isolates were serologically distinct from TSWV.
Using serological techniques, Reddy et al. (1992) showed that the virus was indeed distinct from TSWV and it was named peanut bud necrosis virus. The name *groundnut* bud necrosis virus is preferred by several other authors as the disease was earlier described as 'groundnut bud necrosis' (Reddy et al., 1983), or as 'bud necrosis of groundnut' (Ghanekar et al., 1979). Recently, data on the nucleotide sequence of the nucleocapsid protein gene confirmed that PBNV should be considered as a distinct species in serogroup IV of the *Tospoviruses* of the *Bunyaviridae* (Heinze et al., 1995; Satyanarayana et al., 1995). TSWV, a serogroup I virus, causes a similar disease on groundnut in the USA. Groundnut ringspot virus, one of the two viruses forming serogroup II, has been found on groundnut in South Africa. Impatiens necrotic spot virus is the only species forming serogroup III and has not been found on any Leguminosae (De Ávila et al., 1993a, b). Serogroup IV contains, apart from PBNV, watermelon silver mottle virus (WSMV) and PBNV-To, an isolate similar to PBNV collected from tomato in Taiwan (Yeh and Chang, 1995; Yeh et al., 1992; Heinze et al., 1995). The N protein of PBNV showed a 85% sequence identity to WSMV and PBNV-To but 30-34% identity with the members of other serogroups, indicating that PBNV is closely related to WSMV and PBNV-To (Satyanarayana et al., 1995).

VECTOR

Initially, Ghanekar et al. (1979) produced some evidence that the virus causing bud necrosis disease was transmitted by *Scirtothrips dorsalis* Hood. Subsequently, it was shown that *Frankliniella schultzei* Trybom could also transmit this virus, and that it transmitted the virus more efficiently than *S. dorsalis* (Amin et al., 1981). However, Palmer et al. (1990) showed that the thrips, earlier identified as *F. schultzei,* were in fact *Thrips palmi* Karny. In subsequent laboratory studies it was shown that 38% of *T. palmi* individuals transmitted PBNV, while a rate of 2% was found for *F. schultzei,* and *S. dorsalis* failed to transmit PBNV. Only *T. palmi* adults, collected from infected plants in the field, were able to infect healthy groundnut test seedlings at a rate of 60%. Other thrips species failed to transmit,

indicating that *T. palmi* is the major vector of PBNV (Vijaya Lakshmi et al., 1995). Interestingly, *T. palmi* also transmits WSMV in Taiwan (Yeh et al., 1992). PBNV is acquired only by larval stages of *T. palmi*, while transmission is exclusively due to adult thrips (Vijaya Lakshmi, 1994). *F. occidentalis* Pergande, the main vector of TSWV and the only vector of INSV, was able to transmit in the second larval stage as well as in the adult stage (Wijkamp an Peters, 1993; Wijkamp et al., 1995). Some sources of vector resistance have been reported (Amin et al., 1985).

SYMPTOMS

The first symptoms usually appear on the newly formed leaves as chlorotic spots that may develop into chlorotic and necrotic rings (Reddy et al., 1991). These leaflets become flaccid and droop, resulting in the typical necrosis of the terminal bud. The virus spreads systemically and induces in most cases necrosis of all buds. Stunting and proliferation of axillary shoots are common symptoms of PBNV after systemic spread. These symptoms usually occur on early infected plants, i.e. less than one month old, giving them a stunted and bushy appearance. However, early infected plants often die, and it becomes impossible to determine visually or serologically, whether these plants were infected by PBNV. Because of the large variation in the type of symptoms, produced on differnt groundnut plants of the same cultivar, the disease has previously been described as groundnut mosaic, groundnut rosette, bunchy top, chlorosis, ring mottle, bud blight, and ring mosaic (Reddy, 1988a).

RESISTANCE

Complete resistance (immunity), i.e. absence of systemic infections on genotype level, has not been found among cultivated groundnut (Reddy et al., 1991). However, several groundnut genotypes with resistance to PBNV (Amin, 1985; Dwivedi et al., 1993, 1995), and with resistance to TSWV (Culbreath et al., 1992b, 1993, 1994), have been identified. This resistance is of a quantitative nature and reduced the disease incidence in the crop. The disease incidence is determined as the percentage of plants showing systemic symptoms. Symptom expression and virus detection were always associated with the presence of

PBNV in the plants (Buiel, unpublished), and of TSWV (Prasada Rao et al., 1993). In rare cases, virus could be detected in some TSWV-infected plants without symptoms (Culbreath et al., 1992a). The symptoms caused by PBNV are highly variable, but they are not genotype specific (Reddy, 1988a).

SCOPE OF INVESTIGATION

Information on the quantitative resistance to PBNV was limited. The purpose of this thesis was to develop methods to assess resistance, to determine the inheritance, and to study the mechanisms of resistance. The effect of plant density and inter-plot interference on the disease incidence was reported in Chapter 1 and 2. Chapter 3 describes the distribution of PBNV in groundnut fields. In Chapter 4 the epidemiology of PBNV is studied in field-resistant and -susceptible groundnut genotypes. PBNV resistance was tested in multi-environments to determine whether resistance operates across environments, to define the optimal location for selection, and to investigate the occurrence of pathogenetic differences between PBNV populations (Chapter 5). Five resistant genotypes were crossed with two susceptible genotypes to study the inheritance of the quantitative resistance (Chapter 6). The occurrence of a mature plant and tissue resistance is presented in Chapter 7. Chapter 8 describes the mechanisms that cause the quantitative resistance in resistant groundnut genotypes. Findings on virus- and vector resistance are compared in Chapter 9. The results presented here are collectively addressed in the general discussion.

CHAPTER 1

FACTORS AFFECTING THE INCIDENCE OF PEANUT BUD NECROSIS VIRUS IN GROUNDNUT FIELDS

I. PLANT DENSITY

SUMMARY

The effect of plant density on the disease incidence of peanut bud necrosis virus (PBNV) in groundnut was investigated using nine plant density levels ranging from 6.7 to 33.5 plant/m^2. The disease incidence (percentage of infected plants) was reduced in high density plots of both a susceptible and a resistant genotype. However, when expressed as the number of infected plants per unit area, disease levels increased with plant density. Thus, eventhough an increased plant density appeared to reduce the disease incidence, it actually caused higher levels of the disease.

The use of a resistant genotype reduced the disease incidence to a much larger extent than the effect of an increased plant density. The cultivation of a resistant genotype would be recommended as a cultural practice to control the disease.

CHAPTER 1

INTRODUCTION

Peanut bud necrosis tospovirus (PBNV) is of great economic importance to the groundnut growing countries of South Asia. Losses due to PBNV were estimated at over 89 million US $ per year (Anonymous, 1992). Several cultural practices have been recommended to reduce the incidence of the disease caused by PBNV, e.g. increasing plant density, sowing at early dates to prevent vector invasion in the crop, and using resistant cultivars (Reddy et al., 1983, 1991). Insecticide application to control the vector failed to control the disease (Reddy et al., 1983).

Several groundnut genotypes with resistance to PBNV or to tomato spotted wilt tospovirus (TSWV) causing a similar disease on groundnut in the USA, have been found (Amin, 1985; Culbreath et al., 1992b, 1993, 1994; Dwivedi et al., 1993, 1995). The resistance present in these genotypes did not change the severity of symptoms in the plants but reduced the disease incidence levels in the crop.

In Asia, the resistance has hardly been combined with other desirable characteristics such as higher yield, seed size and seed shape. As a result, most of the cultivars grown by farmers in Asia are still highly susceptible to PBNV. Therefore, it is important to determine if other practices such as an increased plant density can indeed reduce the PBNV infection, and to what extent. Information on the relation between plant density and the disease infection could lead to specific recommendations to farmers. The aim of this study was to determine the effect of plant density on the disease incidence, using nine plant density levels.

MATERIALS AND METHODS

The effect of plant density was tested in two field experiments at ICRISAT Asia Center, in the rainy seasons of 1993 and 1994, each comprising 3 replicates. Three levels of inter-row distance (R) of 30, 60, and 75 cm, and three levels of inter-plant distance (P) of 10, 15, and 20 cm were used. The nine treatments (R*P) were randomized over nine

blocks within each replicate. Each block was divided into four plots each with two 4-m rows with JL 24 (susceptible), or ICGV 86031 (resistant) plants.

The percentage of infected plants (disease incidence) was determined visually per row, every two or three weeks during the growing season, until 63 days after sowing in the 1993 experiment, and 90 days after sowing in the 1994 experiment. The disease incidence at the start of the epidemic and the final disease incidence were used in the computations. The data were transformed to arcsine values and analyzed using Statistical Analysis Systems (SAS, 1989).

RESULTS

The effect of plant density was fairly significant (P=0.06, Table 1). Plant density could be divided in three components: the plant distance within the row, the row distance, and the interaction between plant distance and row distance. The disease incidence was clearly affected by the plant distance (P=0.03), while row distance had a smaller effect (P=0.07), hence, the effect of plant distance was apparently more important than row distance. The interaction between plant and row distance was of no importance (P=0.45). The effect of environment and genotype were both highly significant. The environment effect accounted for 10% of the total sum of squares, and the genotype effect for 64%, compared to 1.7% for the plant density effect. The effect of plant density was small compared to the effect of environment and genotype, yet is was quite substantial with an important plant distance component. The interaction between environment and plant density was not significant, nor was an interaction found between genotype and row distance, genotype and plant distance, or the composite interaction.

The average disease incidence was about 12 times lower in plots with ICGV 86031, than in plots with JL 24 (Table 2A). It increased from 2.8% in plots with ICGV 86031 (highest plant density) to 5.1% (lowest plant density), and from 41.7% to 58.2% in plots with JL 24. These results show, conclusively, that by increasing plant and row distance, the disease incidence in both genotypes was reduced.

Table 1. Analysis of variance of the arcsine transformed disease incidence tested in two environments (years), using three replicates within environments, three levels of plant distance (within the row), three levels of row distance, and two genotypes JL 24 (susceptible) and ICGV 86031 (resistant).

Source of variation	DF	Sum of squares	Mean squares	F value	Pr > F
Environment (E)	1	18400.5	18400.5	102.67	0.0001
Replication within environment (Rep(E))	4	6180.3	1545.1	8.62	0.0001
Plant density (D)	8	3067.6	383.5	2.14	0.0607
Row distance (R)	2	1027.0	513.5	2.87	0.0717
Plant distance (P)	2	1365.3	682.7	3.81	0.0328
R * P	4	675.3	168.8	0.94	0.4524
E * D	8	2541.2	317.7	1.77	0.1197
D * Rep(E)	32	5735.3	179.2		
Genotype (G)	1	114813.4	114813.4	1516.01	0.0001
R * G	2	51.3	25.6	0.34	0.7131
P * G	2	168.0	84.0	1.11	0.3310
R * P * G	4	250.5	62.6	0.83	0.5086
Error	369	27945.9	75.7		

The disease incidence (Table 2A) was transformed to the mean number of infected plants/m^2 using the plant density levels (Table 2B). This transformation resulted in a reversal of the effect of plant density on the number of infected plants for both genotypes, hence, the number of infected plants/m^2 increased with the plant density.

Only 0.3 plants/m^2 were infected in plots with the lowest density of ICGV 86031 plants, and 0.9 plants/m^2 in plots with the highest plant density. A more substantial effect was observed in plots with JL 24, in which 3.9 plants/m^2 were infected at the lowest plant density, and 13.9 plants/m^2 at the highest plant density. A linear relationship (Y=1.3+0.4X) was found between the infected plants and the total number of plants per m^2 (Figure 1).

The natural infection pressure was considerably higher in 1994 than in 1993 (Table 3). In the 1993 experiment four infected JL 24 plants and no infected ICGV 86031 plants

Table 2. The mean PBNV infection in ICGV 86031 and JL 24 for nine levels of plant density, using the final scoring date of two experiments. **A.** Percentage of infected plants/total plants. **B.** Number of infected plants per m^2.

A.

Plant distance (cm)	ICGV 86031 (mean=4.0) Row distance (cm)			JL 24 (mean=48.6) Row distance (cm)		
	30	60	75	30	60	75
10	2.8	2.9	3.6	41.7	46.7	40.6
15	2.9	3.3	6.7	44.1	48.8	57.0
20	3.4	6.8	5.1	46.7	54.0	58.2

B.

Plant distance (cm)	ICGV 86031 Row distance (cm)			JL 24 Row distance (cm)		
	30	60	75	30	60	75
10	0.9	0.5	0.5	13.9	7.8	5.4
15	0.6	0.4	0.6	9.8	5.4	5.1
20	0.6	0.6	0.3	7.8	4.5	3.9

Table 3. The number of infected plants in JL 24 and ICGV 86031 plots, 34 and 47 days after sowing (DAS) in 1993, and 28 and 49 DAS in 1994.

	1993		1994	
	Days after sowing		Days after sowing	
Genotype	34	47	28	49
JL 24	4	158	23	1602
ICGV 86031	0	0	3	125
Sum	4	158	26	1727

Table 4. The mean PBNV infection in JL 24 for nine levels of plant density, 49 DAS, in the 1994 experiment. **A.** Percentage of infected plants/total plants. **B.** Number of infected plants per m^2.

A. Percentage of infected plants/total plants				**B.** Number of infected plants/m^2		
	Row distance (cm)			Row distance (cm)		
Plant distance (cm)	30	60	75	30	60	75
10	29.7	33.4	19.2	9.9	5.6	2.6
15	30.5	23.8	30.8	6.8	2.6	2.7
20	25.5	28.4	27.6	4.3	2.4	1.8

were found 34 days after sowing (DAS). The number of infected plants increased to 158 JL 24 plants, 47 DAS. In the 1994 experiment, 26 infected plants were observed 28 DAS, of which three were ICGV 86031 plants. Three weeks later (49 DAS), this number had increased to 1727 infected plants, and most of the infected plants were JL 24 plants. These data were used to determine the effect of plant density at the start of the epidemic.

At the start of the epidemic, the disease incidence in plots of ICGV 86031 varied between 0 and 1.4% (data not shown). In JL 24 plots, it ranged between 19.2 and 33.4%, and did not differ significantly between different plant density levels (Table 4A). Forty-nine days after sowing 1.8 plants/m^2 were infected at the lowest plant density level, whereas 9.9 plants/m^2 were infected at the highest plant density level for JL 24 (Table 4B). The relation between the infected JL 24 plants and the total number of plants per m^2 was also linear 49 DAS in the 1994 experiment (Figure 1).

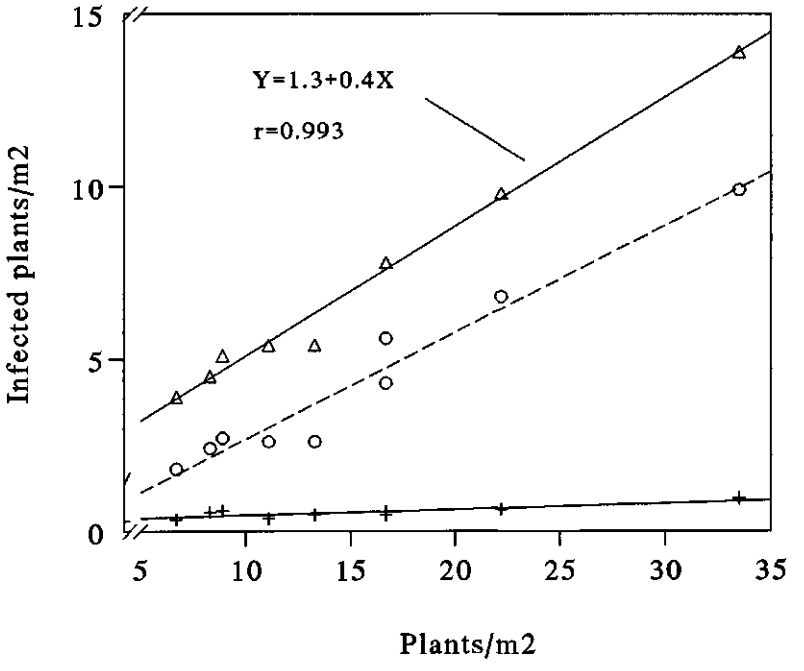

Figure 1. The relation between plant density and the number of PBNV infected plants per m^2 for JL 24 (▲) and ICGV 86031 (+) on the final scoring date (mean of two experiments). The same relation is also shown for JL 24 (○), 49 days after sowing in 1994.

DISCUSSION

The perception that increasing plant density reduces the disease incidence (Reddy et al., 1983; Culbreath et al., 1994) was confirmed here. Plant distance within the row was of more importance to the disease incidence than row distance.

Increasing the plant density resulted at the same time in an increased number of infected plants per unit area. The number of infected plants/m^2 in the resistant genotype increased with a factor 3, when increasing the plant density with a factor 5. In the susceptible genotype, the number of infected plants/m^2 increased with a factor 3.6 when increasing the plant density with a factor 5. Thus, increasing the plant density resulted in a larger increase of infected plants per unit area in the susceptible genotype than in the resistant genotype. The linear regression of the data of JL 24 showed that raising the plant density with ten plants/m^2 resulted in an average increase of four infected plants/m^2.

Increasing the plant density leads (to a certain extent) to an increase in yield (Reddy et al., 1983). Firstly, this is due to the increased plant stand, and secondly, to the increased percentage of healthy plants. Therefore, an increased plant density would be an advantage to farmers. On the other hand, the larger number of infected plants per unit area would maintain a higher number of PBNV reservoirs. Consequently, the number of viruliferous thrips would increase, and enlarge the number of infected plants, and thus also the virus sources in the field. However, thrips may prefer diseased plants over healthy plants (Bautista et al., 1995). Thus, if few thrips on the diseased plants would migrate to other (healthy) plants because of their preference, the number of infected plants would probably not increase much.

In early and advanced phases of the epidemic a similar linear relationship was shown between the number of infected plants and the plant density (Figure 1). In the early phase of the epidemics the majority of infections are expected to originate from primary infections, i.e. from viruliferous thrips coming from external sources. Here, differences in disease incidence due to plant density are probably related to the number of immigrating vectors. Thrips are possibly more attracted by high plant densities than by low densities, or alternatively, thrips landing between plants in a low plant density plot,

failed to reach a host plant. In advanced phases of the epidemic both primary infections and secondary infections (spread within the field) could play a role. Similar factors related to vector preferences are probably involved because a comparable relationship in early and advanced phases of the epidemic was observed. However, little is known on the movement and settling of thrips. Lewis (1973) suggested that thrips have minimal control of the track during flight, but they may have some control over landing and settling. Other authors suggested that several climatic factors would influence the dispersal of the vector (Reddy and Wightman, 1988).

The plant density varied in this study between 6.7 and 33.5 plants/m^2. Reddy et al. (1983) varied the plant density in a similar experiment from 4.4 to 53.3 plants/m^2, and observed a decrease in disease incidence from 40% to 10% when increasing the plant density. Re-evaluating their data with respect to the number of infected plants per unit area, an increase from 1.9 infected plants/m^2 (low plant density) to 5.3 infected plants/m^2 (high plant density) was detected (data of field experiments from 1978/79). These data confirm our results.

The application of a high plant density has been recommended earlier (Reddy et al., 1983) to suppress PBNV infection. Eventhough a high plant density may lead to a decrease in disease incidence, considerable levels of PBNV infection are being maintained in this way, possibly leading to increasing numbers of viruliferous thrips. Rather than increasing the plant density, a larger impact would be achieved to reduce PBNV infections when highly susceptible genotypes such as JL 24 would be replaced by resistant cultivars. The resistant genotype ICGV 86031 (Dwivedi et al., 1993) used in this study, proved to be an excellent candidate.

CHAPTER 2

FACTORS AFFECTING THE INCIDENCE OF PEANUT BUD NECROSIS VIRUS IN GROUNDNUT FIELDS

II. INTER-ROW INTERFERENCE

SUMMARY

The presence of an inter-row interference, possibly affecting the evaluation of peanut bud necrosis virus (PBNV) resistance in groundnut, was analyzed in this chapter. The disease incidence was determined in a series of rows, comprising rows with plants of a susceptible genotype, and rows with plants of a resistant genotype. Inter-row interference could not be discovered in these experiments. The experiments were fairly representative of PBNV selection fields, thus, inter-row interference was not expected to be of importance in the breeder's situation. The absence of inter-plot interference leads us to believe that either secondary spread was not important, or the dispersal of thrips affected both primary and secondary infections.

CHAPTER 2

INTRODUCTION

Peanut bud necrosis disease affects groundnut crops in most parts of Asia (Reddy et al., 1991). The disease is caused by peanut bud necrosis virus (PBNV), a distinct member of the *Tospovirus* genus of the *Bunyaviridae* (De Haan et al., 1989; Reddy et al., 1992; Murphy et al., 1995). Tomato spotted wilt virus (TSWV) causes a similar disease on groundnut in the USA (Reddy et al., 1991). The natural disease incidence varied, depending on the location and season (Buiel et al., 1995; Chapter 5). PBNV is transmitted by *Thrips palmi* Karny (Vijaya Lakshmi et al., 1995). Initially, viruliferous thrips carry virus from external sources to the crop (primary infection). The primary infected plants will probably serve as virus sources for secondary infection within the crop (Reddy et al., 1983).

Breeding for resistance is one of the most promising solutions to prevent yield losses caused by peanut bud necrosis disease. Field resistance to PBNV is usually assessed in small plots and entries are often placed adjacent to each other. Van der Plank (1963) suggested that errors due to inter-plot interference would arise when assessing resistance in small plots. Resistant entries would receive many more infectious units from their susceptible neighbours, whereas susceptible entries would receive fewer units from their resistant neighbours. The level of (partial) resistance would be underestimated for resistant entries, but would be overestimated for susceptible entries. Inter-plot interference could also affect the ranking order of the resistance of the entries in small plots (Parlevliet and Danial, 1992).

A similar inter-plot interference could also occur for insect transmitted viruses such as PBNV, when the disease spreads over short distances in the case of secondary infections. A susceptible (JL 24) and a field resistant groundnut genotype (ICGV 86598) were chosen to study the effect of inter-plot interference on the disease incidence. Mechanical inoculation of JL 24 and ICGV 86598 with the virus (Chapters 8 and 9) showed that ICGV 86598 was susceptible to the virus. Therefore, it was assumed that the field resistance of this genotype was caused by thrips resistance, whereas JL 24 was highly susceptible to virus and vector.

MATERIALS AND METHODS

FIELD

Two field trials were conducted at ICRISAT, Hyderabad, India, and one at the Directorate of Oilseeds Research, Rajendranagar, India. Tetramethyl thiuram disulphide-treated seed (3 g/kg) was sown in 1993 on July 21 (July trial) and December 9 (December trial) at ICRISAT, and on August 6 at Rajendranagar. Row distance was 60 cm at ICRISAT and 45 cm at Rajendranagar. Plant distance within the row was 20 cm at both locations.

Each trial comprised six blocks with 4-m rows. Each block contained 100 rows in the July trial, 70 rows in the December trial, and 62 rows at Rajendranagar. The blocks comprised a number of sub-blocks with rows of JL 24 (S), and ICGV 86598 (R). Each sub-block contained two central rows of R plants, flanked at both sides with four rows of S plants. These were again flanked by ten rows of R plants in the ICRISAT trials, and with eight rows of R plants in the Rajendranagar trial. Two sub-blocks were present in each block of the July trial, and one sub-block in each block of the December trial and the trial at Rajendranagar. These sub-block(s) were flanked by a set of rows at the beginning and end of each block. This set consisted of: ten rows with R plants flanking the sub-block (eight rows at Rajendranagar), followed by four rows with S plants, then two rows of R plants, and finally four rows of S plants at the beginning or end of the block.

DISEASE ASSESSMENT

The percentage of infected plants (disease incidence) was determined visually per row, every two or three weeks during the growing season, until 82 days after sowing (DAS) for the July trial, 126 DAS for the December trial, and 81 DAS at Rajendranagar. The final disease incidence was arcsine transformed.

THRIPS

To determine the number of *Thrips palmi* adults, leaf terminals were collected regularly

from R and S plants in one block of each experiment, and stored in 30% ethanol. Ten terminals were collected from each two center rows of R plants, twenty terminals from each of the four flanking rows with S plants, ten terminals from the two rows with R plants flanking the rows with S plants, and ten terminals were taken from the last row of R plants of each sub-block. The July trial was sampled on three dates, eight dates in the December trial, and on six dates at Rajendranagar. The thrips counts were cumulated over sampling dates and standardized to the average thrips numbers per ten terminals.

RESULTS

The natural disease incidence varied considerably between the trials (Figure 1A). Highest disease incidence was observed in the trial at Rajendranagar, where an average of 95% (nontransformed) of S plants were infected. Lowest disease incidence was found in the July trial (15%, nontransformed). The disease incidence of JL 24 was significantly higher than ICGV 86598, irrespective of the location. The disease incidence in the rows with R plants did not differ significantly with their position, indicating that the disease incidence was independent of the row position of the resistant genotype in relation to the row position of the susceptible genotype.

The highest cumulative number of *Thrips palmi* were found in the December trial, whereas the lowest number of thrips were observed in the July trial (Figure 1B). The cumulative number of thrips at Rajendranagar was intermediate between the other two trials. The number of thrips was cumulated over collection dates, and although dependent on, not correlated with the number of collection dates. The number of thrips was usually high in the first part of the growing season but was considerably lower later in the season (Table 1). Consistently fewer thrips were found on R plants than on S plants. The highest level of disease incidence was not necessarily correlated with the highest cumulative number of thrips and vice versa (Figure 1A and B).

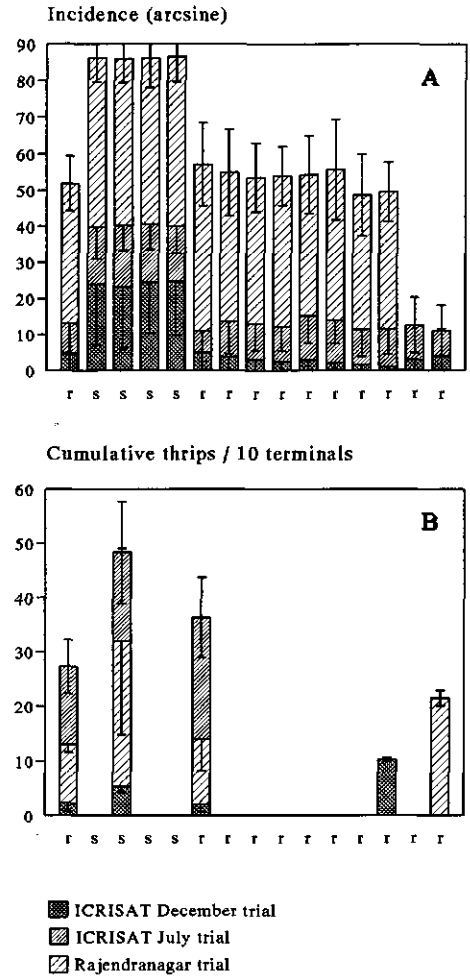

Figure 1. The mean disease incidence (arcsine transformed) (**A**), and the mean number of *Thrips palmi* on 10 terminals cumulated over sampling dates (**B**), on JL 24 (S) and ICGV 86598 (R) in half a sub-block at Rajendranagar and ICRISAT. Vertical bars indicate the standard deviation of the means.

Table 1. The cumulative number of adult *Trips palmi* collected from leaf terminals of JL 24 (susceptible) and ICGV 86598 (resistant), during the course of the season (days after sowing) in the December trial at ICRISAT.

Genotype	Location	Days after sowing							
		29	40	55	67	77	92	104	116
ICGV 86598 JL 24	*two center rows flanking the center rows*	8.0 13.3	12.7 25.4	19.4 36.1	25.7 42.6	26.4 43.9	26.7 44.4	27.0 47.2	27.3 48.3
ICGV 86598	*flanking JL 24*	14.3	18.6	28.4	34.2	36.2	36.2	36.2	36.2
ICGV 86598	*separated by 8 resistant rows from JL 24*	8.0	10.3	15.1	20.1	21.6	21.6	21.6	21.6

DISCUSSION

The disease incidence of the R genotype was independent of the row position in relation to the row position of the S genotype. Similarly, the disease incidence of S plants was consistent and independent of their position. These results show that no inter-row interference effect, as was clearly demonstrated in the case of leaf rust on barley (Parlevliet and van Ommeren, 1984) could be discovered in these experiments, using ICGV 86598 and JL 24. Similar results were also observed in small plots and large area studies on the TSWV infection of both a resistant and a susceptible cultivar (Culbreath et al., 1992b). The plot size of 4-m single rows is fairly representative of the breeder's situation in early generation selections, thus inter-row interference is not expected to be of importance in breeders' plots.

The absence of a correlation between the number of thrips and the disease incidence indicates that the proportion of viruliferous thrips must have been different for the different locations and seasons. These field observations clearly show that the proportion of viruliferous thrips is highly variable. Cho et al. (1987) compared tomato spotted wilt disease incidence and thrips numbers in lettuce crops at four farms in Hawaii and also

observed no association between these parameters.

The results in this chapter indicate the absence of an inter-row interference effect. An inter-row interference effect is expected when the spread of the disease within a field occurs over short distances (secondary spread). Thus, a plausible explanation for the absence of an inter-row effect could be that the majority of virus infections were the result of primary virus infections, i.e. from external sources. Alternatively, the secondary virus infections between rows over a short distance was of little importance. Also, a preference of the thrips for JL 24 during both primary and secondary infection could have overruled any inter-row interference. Experiments using a virus resistant, but thrips susceptible genotype in stead of ICGV 86598, could elucidate these points. In such experiments the vector activity would not be restricted in any way. However, genotypes with virus resistance but lacking thrips resistance have not been found yet (Buiel and Kendre, unpublished).

CHAPTER 3

DISTRIBUTION OF PEANUT BUD NECROSIS DISEASE IN GROUNDNUT FIELDS

SUMMARY

The distribution of diseased plants, infected by peanut bud necrosis virus (PBNV) was monitored in three groundnut fields. A lower disease incidence was found in the border areas than in the central area of these fields. This border effect appeared early and could be distinguished during the entire season. In one field, a lower disease incidence was observed in a border area next to fallow land, but not in the other border areas adjacent to early sown groundnut crops. The incorporation of an extra border zone around PBNV-selection fields, promotes a homogeneously distributed infection. Possible explanations for the occurrence of a border effect are discussed in relation to the spread by the vector.

CHAPTER 3

INTRODUCTION

Peanut bud necrosis disease (PBND) is a major virus disease of groundnut in South Asia (Reddy et al., 1991). The disease is caused by peanut bud necrosis tospovirus (PBNV)(Reddy et al., 1992), which is transmitted by *Thrips palmi* Karny (Vijaya Lakshmi et al., 1995). The infection is quantified as the percentage of plants with PBNV symptoms (disease incidence). Natural infection of PBNV varied considerably between environments and cultivars (Buiel et al., 1995). An average disease incidence of 60% across four locations was shown for a susceptible cultivar (Buiel et al., 1995, Chapter 5). Analysis of the results from earlier experiments showed that PBNV infected plants occurred in a scattered pattern in the field. However, some areas in the field were more affected than others. A non-uniform dispersal pattern was also suggested for tomato spotted wilt virus (TSWV), causing a similar disease on groundnut in the USA (Culbreath et al., 1990). Presumably, these deviations from a uniform distribution are related to the vector. The aim of this study was to analyze the distribution of PBND in the field.

MATERIALS AND METHODS

Three field trials were performed in the rainy season of 1992. One trial was sown on 15 July at ICRISAT Asia Center, the second on 16 July at Rajendranagar (located approximately 25 km South-East of ICRISAT), and the third trial at Raichur, Karnataka State (approximately 250 km South of ICRISAT) on 5 August. Each trial comprised 81 groundnut entries in plots of two 4-m rows, in a 9x9 lattice design with four replicates. Each two plots were separated by one 4-m row of the susceptible control JL 24. The row distance was 60 cm, plant distance 20 cm, and the total size of each trial was approximately 50 x 60 m.

The plants were visually scored for PBNV symptoms, every two or three weeks until 91 days after sowing (DAS) at ICRISAT, 99 DAS at Rajendranagar, and 96 DAS at

Raichur. Infected plants were labelled to facilitate recurrent scoring. The disease incidence was calculated as the percentage of infected plants (showing symptoms)/total number of plants per row. Only the data of JL 24 were used in the analysis of the spatial distribution of infected plants. The arcsine transformed data were used in SAS analyses (SAS, 1989).

RESULTS

In the three trials the disease incidence appeared to be lower in the border areas than in the central area of the field. Figure 1 shows the disease distribution in the field at Rajendranagar, 36 DAS, averaged per block of seven adjacent JL 24 rows. The average incidence varied between 11 and 41%. The average incidence was lower in the blocks of the border areas (15.6, 14.3, 20.3, and 18.1%) than in the central area (28.7%).

The field was imaginary divided into nine parts: a central area of 38 x 50 m, four corners each of 4 x 5.4 m, and the remaining border area (consisting of two parts of 5.4 x 50 m, and two parts of 4 x 37 m). The average disease incidence of the central area was higher than the disease incidence in the four corner areas and the remaining border areas, during the season at Rajendranagar (Table 1). No significant border effect was found 22 DAS, when the level of infection was less than 3%. Two weeks later the level of infection had increased to about 30%, and a border effect was significant. This difference persisted during the entire season.

The disease incidence at ICRISAT developed similarly but it increased towards the South side of the field (Table 2). The incidence in the northern part of the field (29%), was significantly lower than in the middle part (44%), and lower than the southern part of the field (50%). The North side of the field was bordered by fallow land, whereas the other sides of the field were bordered by early sown groundnut. No significant differences were found between East-West orientated parts of the field. A significant higher disease was observed in the central area of the field (46.7%), despite the high disease incidence at the South side of the field.

The disease incidence on the final scoring dates of the three trials (Table 3) shows that

the central area of the fields at ICRISAT and Rajendranagar had a significantly higher disease level than the border areas of these fields. At Raichur, the disease incidence at the central area of the field was higher but not significant. This is probably due to the low natural infection pressure at Raichur.

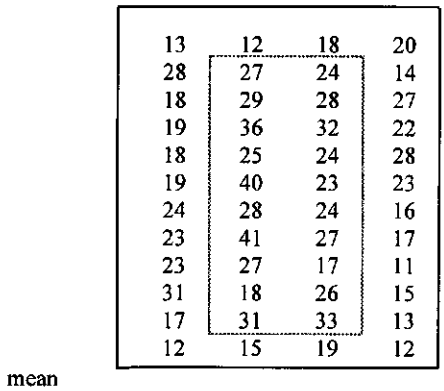

Figure 1. Schematic representation of the distribution of disease incidence in blocks of seven JL 24 rows, in a field 36 days after sowing at Rajendranagar.

Table 1. Average (arcsine transformed) disease incidence of n number of JL 24 rows in the corners, central area, and the remaining border area of the field at Rajendranagar in 1992. The central part of the field represented an area of about 1890 m^2, the corners an area of about 88 m^2, and the remaining border an area about 842 m^2.

		days after sowing					
	n	22	36	50	64	83	99
Central area	200	2.8 a	30.9 b	48.2 b	67.3 b	79.1 b	81.5 b
Corners	16	0.9 a	22.8 a	40.6 a	61.1 ab	71.6 a	73.9 a
Remaining border	120	1.3 a	20.7 a	40.7 a	61.0 a	71.0 a	73.7 a

1. Different letters indicate significant differences (Tukey-Kramer, $P<0.01$) within observation dates.

Table 2. Disease incidence (arcsine transformed) of JL 24 in different parts of the ICRISAT field, 91 days after sowing.

	West	middle	East	mean
North	25.6[1]	36.1[2]	25.2[1]	29.0 a[5]
middle	44.0[3]	46.7[4]	41.7[3]	44.1 b
South	46.9[1]	48.8[2]	54.3[1]	50.0 c
mean	38.8 A[5]	43.9 A	40.4 A	

1. Mean disease incidence of 4 rows in the corner areas of the field.
2. Mean disease incidence of 40 rows in the North or South border area.
3. Mean disease incidence of 20 rows in the East or West border area.
4. Mean disease incidence of 200 rows in the central area.
5. Different letters indicate significant differences (Tukey-Kramer, $P<0.01$) between North-South direction means (lowercase), or between East-West means (uppercase).

Table 3. Average (arcsine transformed) disease incidence of n number of JL 24 rows on the final scoring date in the central area, the corners, and the remaining border areas of the fields at Raichur, ICRISAT (ICR), and Rajendranagar (RN) in 1992. The central part of the field represented an area of about 1890 m^2, the corners an area of about 88 m^2, and the remaining border an area about 842 m^2.

		Location		
	n	Raichur	ICR	RN
Central area	200	21.6 a[1]	46.7 b	81.5 b
Corners	16	19.3 a	38.0 a	73.9 a
Remaining border areas	120	19.5 a	42.6 a	73.7 a

1. Different letters indicate significant differences (Tukey-Kramer, $P<0.01$) within locations.

DISCUSSION

The border areas of these groundnut field analyzed, clearly had a lower disease incidence compared to the central area of the field. This border effect was significant at ICRISAT and Rajendranagar where the level of infection was high (about 40% and 80% respectively). At Raichur the effect could not be distinguished, probably because the infection level was low (20%).

This non-uniform distribution was presumably a result of a preference of the vector for the central area of the field. The border effect was observed throughout the epidemic, and implies that either one source of infection was involved (only primary infections from external sources), or both primary and secondary sources (within the field) were involved, but with similar patterns of virus spread (Chapters 1 and 2). Reddy et al. (1983) suggested that primary infection is probably more important than secondary infection. Secondary spread of TSWV in groundnut in the USA has been supposed (Culbreath et al., 1990), based on clustered patterns of infected plants. However, Camann et al. (1995) concluded that their data were consistent with the hypothesis that most infections arise as a result of primary transmissions.

A lower disease incidence was observed in the border area of the ICRISAT field adjacent to fallow land. Apparently, less thrips had moved into this part of the field. Either thrips had entered the trial from adjacent early-sown groundnut fields, or thrips immigrating from external sources did not favour the area of the field adjacent to the fallow land. The adjacent fields at Rajendranagar and Raichur included non-host crops or weeds, and no such effect as at ICRISAT was observed here.

The occurrence of a border effect on the distribution of PBND had not been shown before. For PBNV-selection fields, a homogeneous distribution of the infection is required. The border effect as described here, causes a non-uniform distribution in the field, and interferes with the selection of resistant material. Therefore, it is advisable to surround PBNV-selection fields with an extra border with groundnut plants that are excluded from the selection. Alternatively, susceptible checks could be included frequently to correct for environmental differences within the field.

CHAPTER 4

EPIDEMIOLOGY OF PEANUT BUD NECROSIS DISEASE IN GROUNDNUT

SUMMARY

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, in which these tests were done, 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 terminations of epidemic development is mature plant resistance of the groundnut to PBNV.

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CHAPTER 4

INTRODUCTION

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

PBNV 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 unfavourable weather conditions such as low night temperatures, high day temperatures, and 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 ten environments (location x year combinations), each comprising four replicates. Plots consisted of two 4-meter rows, with 20 cm plant-to-plant distance, and 50 or 60 cm inter-row distance. Data used in this study were from seven of these environments: ICRISAT Asia Center (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. PBND 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 ICRISAT Asia Center in 1991 (data not shown). The final PBND incidence of the susceptible cultivar JL 24 was high at Rajendranagar and Narkoda (more than 85%), moderate at ICRISAT (around 55%), and low at Raichur (around 25%).

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). The onset of the plateau phase for seven environments ranges approximately between 60 and 75 days (Table 2), and occurs thus 35-50 days before harvest, suggesting that factors other than crop maturity caused 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 the increase in incidence after the onset of the plateau phase (about 76 DAE) in six groundnut genotypes at Rajendranagar during the 1992 rainy season.

		Days after emergence (DAE)						
Genotype	Group[1]	15	29	43	57	76	92	Increase after 76 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 (OPP) of the peanut bud necrosis disease (PBND) epidemic as days after emergence (DAE), and average increase in incidence of PBND during the plateau phase per group of groundnut genotypes at Rajendranagar (RN), Narkoda (NAR), ICRISAT Asia Center (IAC), and Raichur (RAI).

Location	RN	RN	NAR	IAC	IAC	RAI	RAI	
Year	1991	1992	1993	1991	1992	1991	1992	
OPP (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. 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.

Table 3 shows the disease incidence at the onset of the plateau phase for seven environments. The incidence at this onset ranges 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 in dimensions; 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 crop 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 a 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.

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 (disease pressure). In all environments, the epidemic build-up 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 mature tissue, and plant resistance, or a combination of these factors.

Weather data of 3 years at ICRISAT Asia Center, 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 early in 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 behaviour was affected.

Consequently, we assume that it is the mature plant resistance which causes the decline in disease progress. Mature plants and mature plant tissue are highly resistant to the virus.

Only the young tissues of the relatively young plants are highly susceptible to PBNV (Buiel and Parlevliet, 1996; Chapter 7). Mature (or adult) 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 occurs particularly often in perennial crops (Smit and Parlevliet, 1990). We, therefore, consider mature plant resistance to be the cause for the low PBND incidence when groundnut is sown early (June) in South 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 mature plant resistance.

In North India, late sowing (July, August) results in low infection levels whereas high infection levels are found when the crop is sown early. This situation is different from that in South 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 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.

CHAPTER 5

MULTI-ENVIRONMENT TESTING FOR REDUCED INCIDENCE OF PEANUT BUD NECROSIS DISEASE

SUMMARY

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 x 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 favourable for disease development.

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CHAPTER 5

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 x environment (G x E) interactions are present. At the initiation of this study, no information was available on the extent of G x E interaction. Similarly, we did not have information on whether selection would yield corresponding results across environments. Substantial G x 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 x 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, Karnataka, and Andhra Pradesh - and trials were carried out in the 1991-1993 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 inter-row distance.

PBND occurred in the field as a result of natural infection. The incidence (the percentage of plants showing symptoms) was recorded, and infected plants were labelled 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 labelling of infected plants was done regularly because often infected plants die, and the PBND symptoms can no longer be identified on these dead plants.

Table 1. Mean peanut bud necrosis disease incidence across 40 groundnut genotypes at 10 environments in India.

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

DATA ANALYSIS

Analysis of the response of 40 genotypes in 10 environments was done by clustering the genotypes. The final data of incidence were arcsine transformed and standardized (to mean = 0 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, 1989). 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 x E interaction was performed on the arcsine transformed data in GENSTAT (GENSTAT, 1994).

RESULTS

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

The mean 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%.

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, rainy seasons 1991-93.

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

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 ICRISAT Asia Center, 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 form 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.

Main effects (environment, genotype, and genotype clusters) were highly significant in the ANOVA of the arcsine transformed incidence (Table 3). The G x 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 arcsine transformed incidence for different environments. The differences in incidence among clusters increased with increasing infection level and is shown in as the lines of the clusters diverge (Figure 2). It implies that the small G x 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

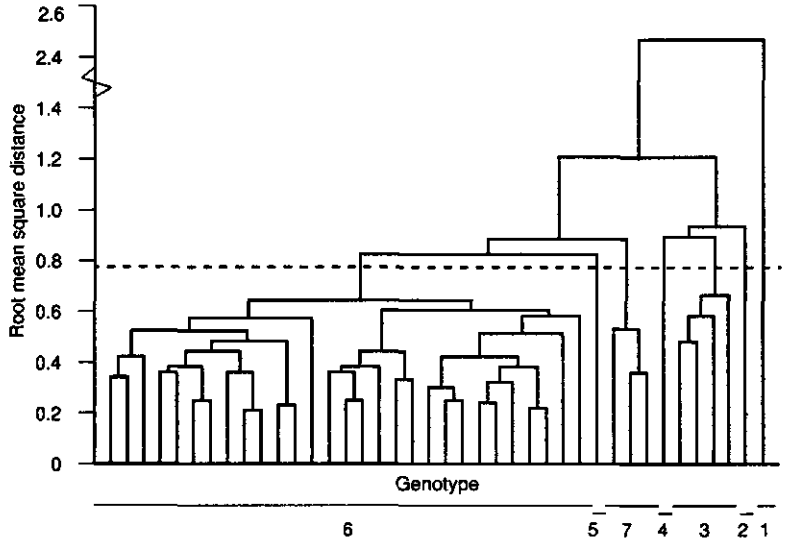

Figure 1. Dendrogram of cluster analysis of 40 groundnut genotypes tested for peanut bud necrosis disease incidence in 10 environments in India.

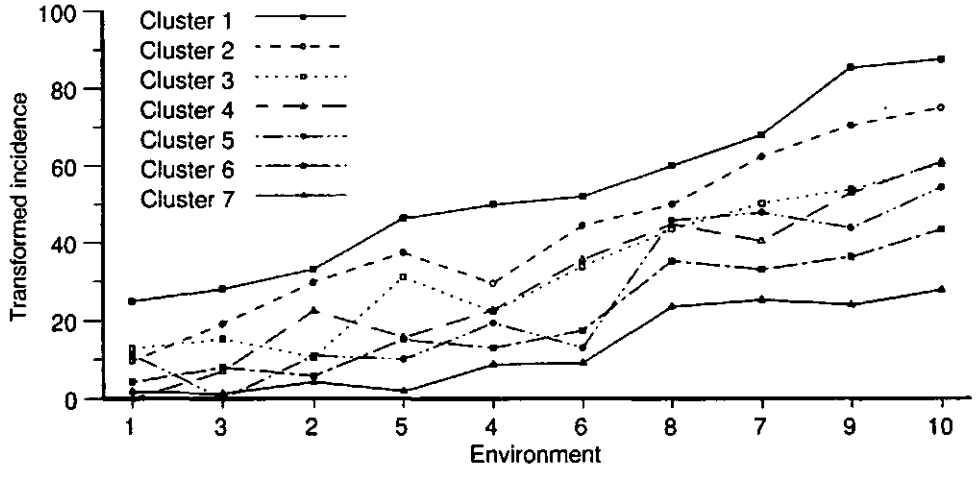

Figure 2. Peanut bud necrosis disease incidence of seven genotypes clusters in 10 environments.

Table 3. Analysis of variance for arcsine 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 x E	351	41575.35	118.45	1.95 ***
Residual	1162	70701.59	60.84	
Total	1591	546136.56	343.27	

*** P <0.001.

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 $r_s > 0.893$.

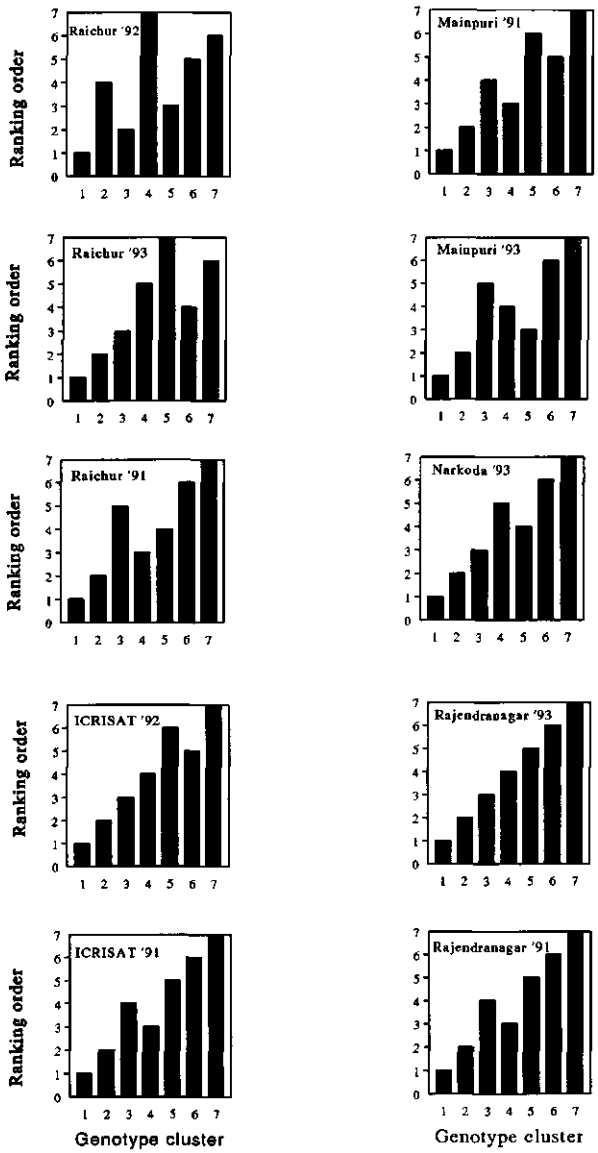

Figure 3. Ranking order of the mean peanut bud necrosis disease incidence of seven genotype clusters in 10 environments.

were consistent across environments. Secondly, Figure 3 shows that the results were rather erratic at Raichur in 1992 (with the lowest infection level).

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

G x E 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.

PBND 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

peanut bud necrosis virus (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.

CHAPTER 6

INHERITANCE OF QUANTITATIVE RESISTANCE IN GROUNDNUT TO PEANUT BUD NECROSIS VIRUS

SUMMARY

Five groundnut genotypes with quantitative resistance to peanut bud necrosis tospovirus (PBNV), were crossed with two susceptible cultivars in a half diallel to study the inheritance of the quantitative inheritance. This type of resistance is expressed as a reduced percentage of infected plants (disease incidence). Four levels of resistance were identified, which can be explained by at least three resistance factors. The mean disease incidence of the F_1 to F6 progenies were consistently close to the mid-parent values. Dominance and epistatic factors seemed to be absent, and the resistance factors are probably additively inherited. The susceptible groundnut cultivar TMV 2 had a consistently lower disease incidence as compared to the susceptible cultivar JL 24. TMV 2 has been grown for several decades in India, hence, this slight resistance level of TMV 2 appears to be durable. Two distinct levels of resistance were found in the five resistance genotypes, reducing the average disease incidence with a factor 5 tot 10, compared to JL 24. This fair level of quantitative resistance was stable across environments.

CHAPTER 6

INTRODUCTION

The resistance to peanut bud necrosis tospovirus (PBNV) in groundnut (*Arachis hypogaea* L.) is quantitatively expressed as the percentage of infected plants (disease incidence). Because of the large variation in the type of symptoms, the disease has previously been described as groundnut mosaic, groundnut rosette, bunchy top, chlorosis, ring mottle, bud blight, and ring mosaic (Reddy, 1988a). Mechanical inoculations with the virus revealed that groundnut plants of the same cultivar can produce different symptoms (Reddy et al., 1991). As the severity of symptoms is not genotype specific, it can not be used as an appropriate assessment for resistance. However, disease incidence could be used successfully to determine resistance levels. Initial studies with over 900 genotypes demonstrated a large variation in disease incidence. Part of this variation was explained by environmental variation, yet a substantial part could be contributed to genetic variation (Buiel et al., 1995; Chapter 5).

The use of disease incidence to assess incomplete or quantitative resistance, implies that the resistance cannot be determined from individual plants, but from a population of plants. Preferably, the population is genetically homogeneous with respect to PBNV resistance. Consequently, heterogenic populations, e.g. the F_2, would yield only an average incidence (infected F_2 plants/total F_2 plants), and thus genetic analysis of the resistance is not possible within the F_2. From the F_3 onwards, the level of heterozygosity decreases and thus the accuracy to determine the disease incidence of a line increases.

The inheritance of PBNV resistance in groundnut has not been studied earlier. In tomato, the inheritance of resistance to tomato spotted wilt virus (TSWV, related to PBNV and causing a similar disease on groundnut in the USA) was based on a single dominant gene, which was not isolate-specific (Stevens et al., 1992). In another study, Kumar and Irulappan (1992) reported the presence of a few recessive genes controlling the resistance of *Lycopersicon* germ plasm to TSWV in India.

The present study was carried out to gain information on the genetic basis of the resistance to PBNV. Seven groundnut genotypes with different levels of resistance were crossed in a half-diallel (no reciprocal crosses) to study the inheritance of PBNV resistance. A single

seed descent (SSD) approach was used to study the inheritance up to the F_6. The resistance to PBNV was used here as a general term for the complex of virus and vector resistance that may exists in the field. Virus resistance was studied specifically through mechanical inoculation of plants.

MATERIALS AND METHODS

PLANTS

Seven groundnut genotypes were crossed in a half-diallel at ICRISAT Asia Center, Hyderabad, India. Twenty-one crosses were made in a glasshouse between December 1991 and March 1992. Two parents were highly susceptible to PBNV (JL 24 and TMV 2), and five parents were partially resistant (ICRISAT groundnut variety (ICGV) numbers 86029, 86031, 86363, 86388, and 86430). The cultivars JL 24 and TMV 2 are grown in India for several decennia (Reddy, 1988b). The ICGV numbers are selected lines from crosses and were at least 10 generations selfed.

Plants of the parents used in the crossing block, and the consecutive F_1's were grown in 30 cm diameter round pots in the glasshouse. The F_1's were also grown in the field in the rainy season of 1994. From the F_1 onwards each generation was advanced to the next generation through SSD. The F_2 and subsequent generations were grown in the field.

The 200 to 600 F_2 plants per cross were planted in the field in the post rainy season of 1992-93. Five seeds from 100-200 F_2 plants were planted as F_3 SSD lines in the next rainy season. From each F_3 SSD line, one plant was randomly selected and advanced to the F_4. Twenty-one F_4 plants of each line were grown in the post-rainy season of 1993-94. One plant was randomly taken from each F_4 SSD line and 21 seeds were planted in the rainy season of 1994, to produce the F_5 SSD lines. From the six crosses with JL 24, the remainder of the F_4 SSD lines was harvested in bulk per F_4 SSD line, and planted in three replicates (F_5 bulked lines). Thus, the F_5 SSD lines were derived from single F_4 plants, whereas the 'F_5 bulked lines' were derived from the bulked F_4 SSD line, and thus derived from single F_3 plants. From six other crosses the F_5 SSD lines were harvested in bulk per

F_5 SSD line to produce F_6 lines, and 25 seeds of 60 F_6 lines were mechanically inoculated in the glasshouse. The inoculation procedure is described elsewhere (Buiel et al., 1996, Chapter 7 and 8).

As four crosses yielded too few F_1 seed, their parents were crossed again between June and September of 1992. The F_1 up to F_4 of these crosses were planted in the field and although they were one season behind that of the other 17 crosses, they were otherwise treated similarly.

FIELD

At ICRISAT inter-row distance was 60 cm and plant spacing was 15 cm. Seeds were treated with tetramethyl thiuram disulphide (3 g/kg). Calcium was applied at the pegging phase (400 kg/ha). In a few cases the plants were sprayed with monocrotophos (1.5 ml/l) to suppress the leafminer *Aproaerema modicella* Deventer.

Each season, the 21 crosses were randomized in the field, and lines were randomized within the cross. Plots of the parents were included frequently in each generation. With each cross two, four, twelve, and ten plots of the two parents were included in the F_2, F_3, F_4, and F_5 generation respectively.

The F_5 bulked lines were planted in 3 rows x 3 m plots in a randomized complete block design. Two replicates were located at ICRISAT and a third replication was located at Rajendranagar, approximately 25 km from ICRISAT, with recurrent high natural PBNV infections. The inter-row distance at Rajendranagar was 50 cm.

DISEASE ASSESSMENT

In each generation, plants were visually scored for PBNV symptoms every 2-3 weeks. Often, early PBNV inf cted plants died, and it proved to be impossible to determine visually or serologically, whether these plants had been infected by PBNV. Therefore, infected plants were labelled to facilitate the recurrent scoring. The disease incidence was determined as the percentage of infected plants.

Randomly chosen plants were harvested and progressed to the next generation. The seeds were differently labelled depending on the fact whether the parental plant was healthy or

infected. In some cases when the randomly chosen plant was infected and had no seed, a subsequent plant was chosen. However, when this plant was healthy, the seed was labelled as selected indirectly. Here, infected plants without seeds would fail to pass on their genes to the next generation. If susceptible plants are lost more frequently than resistant plants, we defined this as a 'selection by substitution'. This selection by substitution would probably cause a small shift towards to a higher level of resistance.

DATA ANALYSIS

The data of the percentage of infected plants (inf) were arcsine transformed, using arcsin($\sqrt{}$(inf/100)), to minimize variance differences related to the magnitude of incidence. The data of the parents were analyzed using generalized linear models (GLM) in SAS (SAS, 1995). Because of the considerable differences in infection pressure in which these progenies were grown, a transformation was applied using the disease incidence of the parents. Therefore, the arcsine transformed data of the progenies, and the parents, were adjusted using the ratio of the average mid-parent value (from field data) and the mid-parent values per generation for each cross. A mean incidence of zero was converted to 1.0, to avoid large scaling differences.

RESULTS

PARENTS

Natural PBNV infection differed greatly among the years and seasons between 1992 and 1994, as seen from the parental data (Table 1). The season mean incidence was highest in the rainy season of 1994 (38%), and lowest in the rainy season of 1993 (3%).

In most environments the two susceptible parents (JL 24 and TMV 2) had a significantly higher PBNV incidence than the resistant parents. Only in the post-rainy season of 1992-93, the incidence of TMV 2 was not significantly higher than the resistant parent. In general, the incidence in TMV 2 appeared to be approximately 25% lower than JL 24, showing that the former is less susceptible. Within the group of resistant parents, ICGV 86388 with the

Table 1. Mean incidence and overall means of seven groundnut genotypes that were used as parents in a half-diallel and tested in four environments.

Genotype	Environment				
	Post-Rainy 1992-93	Rainy 1993	Post-Rainy 1993-94	Rainy 1994	Overall Mean
ICGV 86388	5.4[1] ab[2]	0.0 a	2.3 a	18.1 a	4.1 A[2]
ICGV 86031	4.1 a	1.7 a	2.8 a	16.5 a	5.2 AB
ICGV 86029	5.7 ab	0.6 a	2.4 a	20.0 ab	5.4 AB
ICGV 86363	4.5 a	1.9 a	3.3 a	24.7 ab	6.8 AB
ICGV 86430	11.1 ab	1.0 a	4.5 a	33.7 b	9.8 B
TMV 2	28.4 bc	16.2 b	21.6 b	73.2 c	33.9 C
JL 24	50.5 c	19.4 b	28.9 b	84.7 c	46.0 D
Overall Mean	13.1 C[2]	3.4 A	7.3 B	38.1 D	

1. Back transformed mean incidence of the arcsine transformed incidence. The number of plots per parent, per cross was 2, 4, 12 and 10 in the seasons of 1992-93, 1993, 1993-94, and 1994 respectively.
2. Different characters indicate significant differences (Tukey-Kramer, $P<0.05$) between parents within one season (lowercase), or significant differences (Tukey-Kramer, $P<0.01$) between parental means, or season means (uppercase).

Table 2. Analysis of variance of the arcsine transformed disease incidence of seven groundnut genotypes used as parents in a half-diallel (21 crosses) and tested in four environments. A block contained the parents of one cross.

Source of variation	df	sum of squares	mean squares	F value	Pr > F
Environment (E)	3	148408.2	49469.4	105.94	0.0001
Block (B)	20	117280.6	5864.0	12.56	0.0001
B*E	60	28016.9	466.9		
Genotype (G)	6	89082.3	14847.0	168.90	0.0001
G*E	18	7951.1	441.7	5.03	0.0001
Error	1051	92388.8	87.9		
Total	1158	483127.9			

lowest incidence, could be distinguished from ICGV 86430 with the highest incidence, based on the overall means. No significant differences between resistant parents were observed within season, when the level of the disease was low (e.g. rainy season 1993 and post-rainy 1993-94).

The disease incidence of the parents was used in an analysis of variance (Table 2) to test the effect of environment, block (containing the parents of one cross), and genotype. A significant environment effect (E) was observed for the incidence in the four growing seasons tested. Apparently the disease levels varied considerably between years and seasons, even though all trials were performed at ICRISAT. The block effect (B) was significant but of a smaller size when compared to the environment effect. The effect of genotype (G) was highly significant as was expected. The interaction G*E was significant but much smaller than the effect of genotype of the parent. The G*E for disease incidence accounted for less than 2% of the total sum of squares. Thus, even under these variable conditions, the genotype x environment interactions were not very important. The G*B and G*E*B interactions were not significant and were therefore included in the error term.

PROGENIES

The environments of Table 1 correspond with the environments in which the F_2, F_3, F_4 and F_5/F_1 generations were tested. The progeny and parental means were grouped on the basis of the type of cross: susceptible x susceptible (S x S), resistant x susceptible (R x S), and resistant x resistant (R x R), and discussed per group (Table 3). The data presented in Table 3 are adjusted using the average mid-parent value, whereas the data in Table 1 were calculated based on GLM, resulting in a discrepancy between the incidence values presented in these Tables.

CROSS BETWEEN SUSCEPTIBLE PARENTS

The F_1 of the cross between JL 24 and TMV 2 was close to the mid-parent value. The mean generation disease incidence for the F_2 and F_3 appeared to be higher than the mid-parent value, and would indicate a recessive inheritance. This was not confirmed in the F_4 and F_5.

Table 3. Mean field incidence (%) of parents (P_1, P_2), F_1, F_2, F_3, F_4, and F_5 of 21 crosses between 7 groundnut genotypes, and mean incidence (%) after mechanical inoculation of the F_6 and parents of 6 crosses between 4 groundnut genotypes. Data of incidence are arcsin transformed and adjusted using the ratio of the average mid-parent (MP) value and the mid-parent values per generation, per cross.

Parents of cross no	P_1	P_2	Field Mean incidence (%) P_1	P_2	MP	F_1	F_2	F_3	F_4	F_5	Mechanical inoculation Mean incidence (%) F_6	P_1	P_2
S x S													
1	JL 24 x TMV 2		41.5	33.1	37.3	38.3	41.0	48.3	35.6	36.7			
R x S													
3	86029 x JL 24		13.2	45.2	29.2	30.4	29.2	43.4	23.7	24.5	26.5	15.3	43.1
5	86430 x JL 24		16.7	47.5	32.1	33.9	33.4	37.1	31.9	24.7	31.7	16.8	47.3
8	86363 x JL 24		16.2	43.9	30.1	31.6	28.7	35.1	31.6	29.6			
12	86388 x JL 24		11.7	45.2	28.5	21.5	27.6	30.5	28.2	29.5	25.2	26.5	30.4
17	86031 x JL 24		14.8	51.4	33.1	29.5	30.1	32.0	29.9	-			
		mean	14.5	46.6	30.6	29.4	29.8	35.6	29.1	27.1	27.8	19.5	40.3
		std	0.83	1.18	0.78	1.88	0.88	2.02	1.34	1.24	1.62	2.87	4.15
R x S													
2	86029 x TMV 2		13.4	37.1	25.3	-	32.7	28.2	25.6	22.0			
4	86430 x TMV 2		17.6	40.1	28.9	19.8	31.8	31.4	26.0	24.2			
7	86363 x TMV 2		14.1	39.2	26.7	31.2	28.0	30.9	24.9	24.5			
11	86388 x TMV 2		11.1	35.4	23.3	23.8	27.2	18.6	22.6	20.2			
16	86031 x TMV 2		13.9	37.6	25.8	30.4	43.3	21.8	24.6	24.6			
		mean	14.0	37.9	26.0	26.3	32.6	26.2	24.7	23.1			
		std	0.93	0.74	0.82	2.36	2.57	2.28	0.53	0.78			

Table 3 contd. Mean field incidence (%) of parents (P_1, P_2), F_1, F_2, F_3, F_4, and F_5 of 21 crosses between 7 groundnut genotypes, and mean incidence (%) after mechanical inoculation of the F_6 and parents of 6 crosses between 4 groundnut genotypes. Data of incidence are arcsin transformed and adjusted using the ratio of the average mid-parent (MP) value and the mid-parent values per generation, per cross.

Parents of cross			Field Mean incidence (%)								Mechanical inoculation Mean incidence (%)		
no	P_1	P_2	P_1	P_2	MP	F_1	F_2	F_3	F_4	F_5	F_6	P_1	P_2
R x R													
6	86430 x	86029	16.6	15.3	16.0	25.4	11.3	29.6	14.2	15.2	11.5	16.1	15.9
9	86363 x	86029	12.4	8.8	10.6	-	12.2	23.2	12.0	11.1			
10	86363 x	86430	13.4	19.6	16.5	5.0	17.7	17.4	15.7	-			
13	86388 x	86029	12.5	13.2	12.9	12.3	13.6	13.4	8.7	8.0	12.7	16.5	9.3
14	86388 x	86430	11.2	20.6	16.0	12.5	18.7	21.7	18.7	17.2	18.2	18.3	13.5
15	86388 x	86363	7.7	8.2	8.0	-	22.7	12.9	12.0	6.0			
18	86031 x	86029	10.8	12.0	11.4	18.2	15.0	10.8	16.3	9.1			
19	86031 x	86430	14.7	19.6	17.2	-	21.1	24.8	21.3	17.3			
20	86031 x	86363	13.6	16.6	15.1	10.6	33.2	21.7	13.5	-			
21	86031 x	86388	14.5	12.1	13.3	11.2	20.5	15.3	16.6	-			
		mean	12.7	14.6	13.7	13.6	18.6	19.1	14.9	12.0	14.1	17.0	12.9
		std	0.74	1.34	0.90	2.27	1.93	1.82	1.09	1.61	1.68	0.55	1.57

CROSSES BETWEEN A RESISTANT AND SUSCEPTIBLE PARENT

JL 24 had a consistently higher incidence than TMV 2 (Table 1, 3). The progenies of R x JL 24 also had a higher incidence compared to the progenies of R x TMV 2, except for the incidence in the F_2. Clearly, the moderate resistance of TMV 2 is heritable. No consistent differences between crosses with different resistant parents were observed. The disease incidence in the F_1 to F_5 was close to the mid-parent value of 30.6% for the R x JL 24 crosses, and 26.0% for the R x TMV 2 crosses. The F_6 of three crosses with JL 24 was tested by mechanical inoculation, and the mean incidence values of the F_6 were also close to the mid-parent values. The reaction of the parents was similar to the incidence in the field, indicating that the resistant parents had virus resistance (they may also have vector resistance).

CROSSES BETWEEN RESISTANT PARENTS

The mean parental values (P_1, P_2) ranged from 7.7 to 20.6 (Table 3). Individual F_1 values ranged from 5.0 to 25.4 and could indicate different dominance factors (low values) or different recessive factors (high values), but these are not confirmed in the subsequent generations. These F_1 values are probably extreme values of a population with a mean around that of the mid-parent, like the other crosses. The same applied to some of the extreme values in the other generations. The mechanically inoculated F_6 of three crosses was close to the mid-parent value, and consistent with the field data.

The mid-parent values and the generation means of the 21 crosses were ranked and the correlations were determined using Spearman's ranking correlation (Table 4). Almost all correlations were significant and suggests that the disease incidence of the progeny is closely related to the disease incidence of the mid-parent. The F_4 and F_5 values were close to the mid-parent values in all crosses. This is confirmed by the strong positive correlation in the F_4 (0.934) and the F_5 (0.942) between the disease incidence of the mid-parent and the generation means, indicating that epistatic factors are probably of little or no importance. The significant correlation between the F_1 and the mid-parent value was 0.816, suggesting that additive factors rather than dominance factors play a role in the resistance.

Table 4. Correlation coefficients (Spearman) between the mid-parent values, from field (MP_{field}) or from mechanical inoculation (MP_{inoc}), and the generation means (F_1 to F_6) of the disease incidence based on n number of crosses between seven groundnut genotypes in a half diallel.

	F_1	F_2	F_3	F_4	F_5	F_6	MP_{field}
MP_{field}	0.816**	0.709**	0.882**	0.934**	0.942**	0.899*	
MP_{inoc}						0.829	0.986*
n	17	21	21	21	17	6	6

** Significant at $P < 0.01$
* Significant at $P < 0.05$

F5 BULK

Figure 1 shows the cumulative frequency distribution of the F_5 bulked lines of each of the six crosses with JL 24. The average LSD was 12.8. The mean incidence of a number of F_5 bulked lines was lower than the resistant parents (transgression) but all except one were not significantly different from the incidence of the resistant parent. Similarly, a few transgressing lines were found with a higher disease incidence compared to JL 24. One line of the cross JL 24 x TMV 2 had a significant lower disease incidence compared to TMV 2 and JL 24. This could indicate that JL 24 and TMV 2 both had resistance factors (with very small effects), and that these factors were not identical. However, the significance of the disease incidence from these lines are ambiguous, due to the relatively large experimental error.

SELECTION BY SUBSTITUTION

F_2 plants without seed could not be advanced to the next generation. Selection by substitution in the F_2 would be absent if the percentage of infected F_2 plants was reflected in the percentage of F_3 lines from infected F_2 plants. Table 5 shows that for most crosses the percentage of F_3 lines from infected F_2 plants was lower than the percentage of infected plants in the F_2. Thus some selection by substitution occurred in the F_2 of almost all crosses.

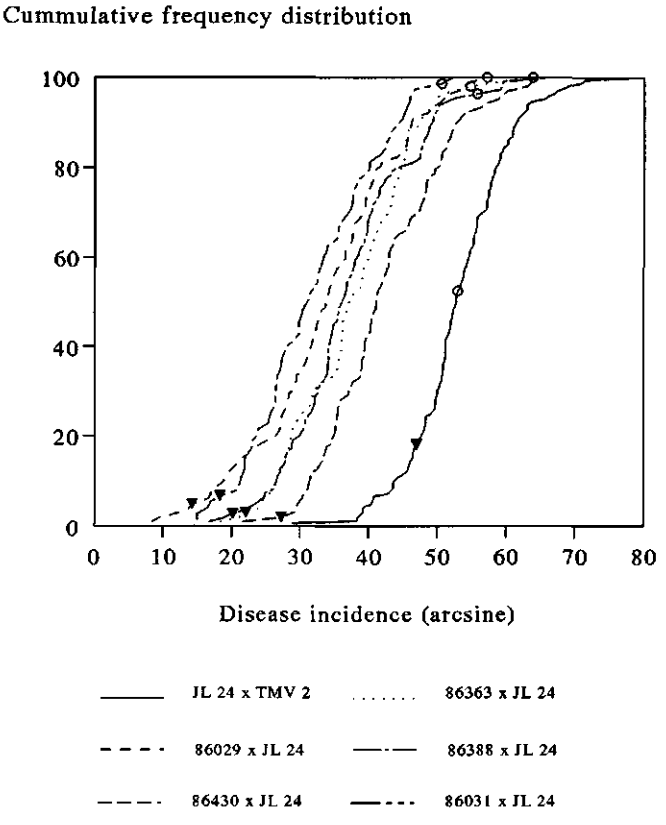

Figure 1. The cumulative frequency distribution of the disease incidence (arcsine transformed) of F_5 bulked lines of six crosses between the susceptible parent JL 24 (0), and six other genotypes (▼).

The level of selection by substitution in the F_3 and F_4 was calculated directly from the number of harvested plants labelled as substituted, divided by the total number of harvested plants. In the F_3 a selection by substitution occurred between 0 and 12.7% (Table 5), but was generally less than 7%. The average selection by substitution level in the F_4 was 9.2%

and ranged from 0-19.8%. The number of lines in which repeated selection by substitution occurred was very low. In only 7 lines of 4 different crosses a healthy plant of both the F_3 and the F_4 line was advanced to the next generation, when the randomly chosen plant was infected and had no seed. On average no apparent shift towards susceptibility was found in the F_5 obtained from infected F_4 plants.

Table 5. The level of selection by substitution in the F_2 deducted from difference between the % infected F_2 plants (first column) and the percentage F_3 lines from infected F_2 plants (second column) **(A)**, in the F_3 **(B)**, and in the F_4 **(C)**.

Cross		**A.** % infected plants in the F_2	% F_3 lines from infected F_2 plants	**B.** F_3	**C.** F_4
1	JL 24 x TMV 2	52.2	45.5	12.7	19.1
2	86029 x TMV 2	22.7	16.2	8.1	9.9
3	86029 x JL 24	31.5	26.2	3.7	5.5
4	86430 x TMV 2	23.4	13.0	9.3	12.1
5	86430 x JL 24	31.8	26.1	6.3	11.5
6	86430 x 86029	5.8	3.2	0.0	0.0
7	86363 x TMV 2	20.0	12.9	4.4	11.0
8	86363 x JL 24	25.1	18.1	6.3	15.6
9	86363 x 86029	6.9	1.9	1.0	7.0
10	86363 x 86430	7.5	3.5	5.9	-
11	86388 x TMV 2	15.5	12.5	0.9	7.8
12	86388 x JL 24	23.7	16.9	5.7	19.8
13	86388 x 86029	4.2	4.2	1.0	4.0
14	86388 x 86430	12.8	9.4	3.9	4.9
15	86388 x 86363	8.8	5.2	0.0	4.0
16	86031 x TMV 2	24.8	23.2	5.7	17.1
17	86031 x JL 24	18.6	9.7	12.2	-
18	86031 x 86029	6.6	2.7	0.0	4.8
19	86031 x 86430	11.6	10.8	1.1	2.2
20	86031 x 86363	7.5	3.6	11.5	-
21	86031 x 86388	7.5	3.8	4.4	-

CHAPTER 6

DISCUSSION

The parents chosen for this study clearly differed in field resistance to PBNV. The susceptible genotypes JL 24 and TMV 2 were significantly different from each other. TMV 2 has one or more resistance factors decreasing the level of infection by a quarter compared to JL 24. In another study (Buiel et al., 1995; Chapter 5), TMV 2 had a consistently lower disease incidence compared to JL 24 across 10 environments. The groundnut cultivar TMV 2 was first released in 1940 and has been grown on a large scale in India. Therefore, it can be assumed that the quantitative resistance of TMV 2 is a durable form of resistance.

The five resistant genotypes have been developed by ICRISAT in 1986. The resistance was stable across environments (this Chapter; Buiel & Parlevliet, 1995; Buiel et al., 1995; Chapter 4 and 5). However, a judgement on the durability of this resistance can not be made because of the relatively short life span and small-scale usage of these genotypes.

The mean disease incidence of the progenies of the 21 crosses were quite consistently close to the mid-parent value. This is confirmed by the strong correlation between the F_4's, F_5's and the mid-parent values. These observations indicate the absence of epistatic inheritance. The F_1 values were close to the mid-parent values and indicate that dominance factors are probably not present. The resistance factors are likely to inherit additively.

With a quantitative trait such as the resistance to PBNV it is difficult to estimate the number of genes because of the large variation of the trait. Furthermore, the resistance could only be determined on a population level. For example, the F_2 yielded only single values of the disease incidence without a variance . Therefore, the approaches being used to study the inheritance of quantitative traits (Mather and Jinks, 1971), based on the distributions in the progenies cannot be applied here.

Other approaches, such as developed by Jinks and Towey (1976), are based on the proportion of segregating lines (families). However, measuring disease incidence will yield only one value of incidence per line and the level of segregation cannot be determined. In our study, one way to determine the segregation within a line would be to evaluate the variation between the F_5 bulked plots (within lines), which were planted in three replicates.

However, the variation of disease incidence is rather large. As a result, these methods to estimate the number of genes cannot be applied here.

Four significantly different levels of resistance could be identified here, thus at least three resistance factors were present in the seven groundnut genotypes used here. The slight level of resistance of TMV 2 is caused by at least one resistance factor. The level of resistance of the ICGV genotypes was significantly higher than the resistance of TMV 2, and must be caused by at least one resistance factor other than the TMV 2 resistance factor. Within the ICGV numbers two levels of resistance were distinguished, ICGV 86388 was significantly more resistant than ICGV 86430. This can be explained by at least two resistant factors in the ICGV group of genotypes. Therefore, this study revealed that a minimum of three resistance factors causing quantitative resistance to PBNV were present in the groundnut genotypes tested here.

In the past, other authors have reported on the inheritance of virus resistance when the resistance was expressed as a reduced disease incidence. Two to three genes with additive gene action were postulated for the quantitative resistance in maize to maize chlorotic dwarf virus, which is expressed as the percentage of diseased plants (Rosenkranz and Scott, 1987). In perennial ryegrass, the resistance to ryegrass mosaic virus was reported to be polygenically inherited (Salehuzzaman and Wilkins, 1984; Wilkins, 1987). Yet, these authors applied conventional approaches using semi-arbitrair classes to investigate the resistance.

Stevens et al. (1992) concluded from the segregation in the F_2, BC_1, and BC_2, that the resistance to TSWV in tomato was based on a single dominant gene. In this case however, the reactions of the parental populations were either 100% healthy or 100% infected. In field studies, Kumar and Irulappan (1992) also studied crosses between a 100% infected susceptible parent vs. a 100% healthy resistant parent. However, some of this material was also tested in studies using mechanical inoculation and thrips transmission (Krishna Kumar et al., 1993). A clear quantitative reaction was shown in these tests.

In the present study, it was assumed that cross pollination between plants in the field was negligible. Reddy (1993) reported a natural outcrossing between 0 and 5.4% in India when the ratio between acceptor and donor was 1:4. Though some outcrossing may have

occurred, it is not expected to have a significant contribution to the error.

To some extent selection by substitution occurred in the F_2 to F_4 generations of all crosses. Selection by substitution was more important in crosses with one or two susceptible parents. Thus, some highly susceptible genotypes may have been lost. Therefore, selection by substitution imposes an extra complication on the genetical analysis of the resistance to PBNV.

CHAPTER 7

MATURE PLANT AND TISSUE RESISTANCE IN THE GROUNDNUT - PEANUT BUD NECROSIS VIRUS SYSTEM

SUMMARY

Leaves and plants of different ages of a susceptible and two resistant groundnut genotypes were mechanically inoculated with peanut bud necrosis virus, and the percentage of plants with systemic symptoms (incidence) and the incubation period were determined. The incidence decreased sharply in all three genotypes with the age of the inoculated leaves and plants. The incubation period increased with the age of leaves and plants. Apparently, only young tissue of young plants is susceptible, while mature tissue and plants are highly resistant. This mature tissue and plant resistance occurs irrespective of the susceptibility level of the genotype to peanut bud necrosis virus, however, it develops earlier in the resistant than in the susceptible genotypes.

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INTRODUCTION

Peanut bud necrosis virus (PBNV) causes a serious disease in groundnut (*Arachis hypogaea* L.) in Asia. The virus is presumably a distinct member in the genus *Tospovirus* of the *Bunyaviridae* (Reddy et al., 1992). Plants infected with PBNV have a strongly reduced yield, or do not yield at all. Natural infection can be very high, e.g. in India an average of 46% in seven environments was reported for TMV 2 (Buiel & Parlevliet, 1995; Chapter 4), the predominantly grown groundnut cultivar in India. Resistance is therefore extremely important to reduce yield losses caused by PBNV.

Complete resistance (immunity) has not been found in the cultivated groundnut (Reddy et al., 1991). However, resistance of a quantitative nature is present in groundnut and is expressed as a reduced percentage of systemically infected plants. This quantitative resistance is characterized by a wide variation (Buiel et al., 1995; Chapter 5), and occurs both when naturally infected by thrips in the field, and when mechanically inoculated.

In addition to complete resistance and quantitative resistance, a third type described as mature plant resistance, has been reported from many other host-pathogen systems, such as bean-tobacco mosaic virus (Schein, 1965), *Nicotiana glutinosa*-lettuce necrotic yellows virus (Crowley, 1967), potato-potato virus X (Venekamp and Beemster, 1980; Wislocka, 1984), potato-potato virus Y^0 (Sigvald, 1985), potato-potato leaf roll virus and potato-potato virus Y (Beemster, 1987), barley-barley leaf rust (Smit & Parlevliet, 1990), potato-potato virus Y^0 and potato-potato virus Y^N (Gibson, 1991), and rice-rice blast (Roumen, 1992; Roumen et al., 1992). Mature plant resistance is generally genotype independent, i.e. it occurs in all genotypes, even in the most susceptible ones (Smit & Parlevliet, 1990). It has not been described for groundnut-PBNV.

Bald (1937) studied *inter alia* the mature plant resistance of tomato to tomato spotted wilt virus (TSWV, the type member of the genus *Tospovirus*) in Australia. He observed a delay in the incubation period in mature plants compared to young plants. No further studies on mature plant resistance in the tomato-TSWV system were reported after Bald's publication, nor on any other host-*Tospovirus* system. Yet on groundnut, Savary (1987) described a clear effect of plant development and leaf age on the resistance to

rust (*Puccinia arachidis*).

In this study, the occurrence of mature plant resistance in the groundnut-PBNV system was investigated. Three groundnut genotypes and one PBNV isolate were used to determine the effect of leaf and plant age on the percentage of plants with systemic symptoms (incidence) and the incubation period.

MATERIALS AND METHODS

MECHANICAL INOCULATION

The PBNV isolate used was originally collected at ICRISAT, Asia Center, India. The virus was not more than six times mechanically transmitted to plants of the susceptible genotype TMV 2, to minimize the risk of generating defective interfering RNA mutants, as was shown to occur in TSWV (Resende et al., 1991). Inoculum was prepared by grinding systemically infected leaves of TMV 2 plants with clear chlorotic ring spots in 0.05 M phosphate buffer, pH 7.0 containing 0.01 M Na_2SO_3 (1:10, w/v). This extract was kept chilled during the inoculation of the test plants. The plants were grown in a greenhouse with minimum/maximum temperatures of 15-20°C/25-35°C.

The incidence of systemically infected plants was recorded daily. The incubation period (IP_{50}) was determined as the interval between inoculation and the appearance of the first systemic symptoms on 50% of the ultimately infected plants. In the absence of any systemically infected plants, it was assumed that the IP_{50} was at least longer than the last observation date (x). Here, x+1 was used in the computations.

LEAF AGE

The effect of leaf age was tested on three groundnut genotypes, JL 24 (susceptible), ICGV 86031 (resistant), and ICGV 86388 (resistant). To inoculate leaves of different ages at the same time, pre-germinated seeds were sown in 15 cm diameter pots at two-day intervals. The third leaf (numbered in order of appearance) of each plant was inoculated on 10, 12, and 14 days after sowing. The third leaves were unfolded (leaf age

1), expanded (leaf age 2), and expanded and matured (leaf age 3).

The experiments were repeated three times (series 1 to 3) and consisted of two or three replicates. Each treatment comprised five pots with five plants each. Plants were removed before inoculation when the third leaf did not develop uniformly with the others within the same treatment. The three series were mechanically sap-inoculated on 9 January 1991, 5 August 1994, and 4 October 1994.

PLANT AGE

To test the effect of plant age, leaves at different positions, but with identical age were inoculated. Pre-germinated seeds of JL 24, ICGV 86031, and ICGV 86388 were sown in 15 cm diameter pots at regular intervals to inoculate leaves at different positions at the same time. Leaves were numbered in order of their appearance: the first two quadrifoliate leaves, leaf 1 and 2 appear simultaneously (2-leaf stage), followed by leaf 3, (3-leaf stage), leaf 4 (4-leaf stage) etc. From plants in the 2- to 5-leaf stage, one unfolded quadrifoliate leaf was inoculated per plant.

Three tests (series 4 to 6) were performed, each comprising three or four replicates. Every treatment comprised five pots with five plants each. Plants were discarded before inoculation when the newly formed leaf layer was still folded or already expanded. Mechanical sap-inoculation of these three series was performed on 12 March 1991, 24 February 1993, and 10 January 1995.

RESULTS

INCIDENCE IN RELATION TO LEAF AGE

The percentage of systemically infected plants (incidence) was monitored up to 23 days after inoculation (DAI) for series 1, 21 DAI for series 2, and 20 DAI for series 3. The average incidence of infected plants of JL 24 for leaf age 1 (unfolded) was 100.0% in series 1, 91.4% in series 2, and 98.0% in series 3.

The genotype and treatment means of the incidence and the standard deviation of the

means over series 1 to 3 were calculated, and shown in Table 1. In all three genotypes a strong and significant reduction in the incidence of infected plants was observed when leaves with a higher age were inoculated. In JL 24 the incidence reduced from 96% for leaf age 1 (unfolded) to 67% for leaf age 2 (expanded). A further raise in maturity to leaf age 3 (expanded and matured) reduced the incidence to 12%. The incidence of infected plants decreased in ICGV 86031 from 67% (leaf age 1) to 27% (leaf age 2) and to 9% for leaf age 3. Similarly, the values of ICGV 86388 reduced from 52% to 21% (leaf age 2) and to 5% (leaf age 3).

The greatest reduction in incidence for JL 24 (55%) was found when plants with leaf age 2 and leaf age 3 were compared. On the other hand, the greatest reduction for the two resistant genotypes (36% on average) was found when the leaf age increased from leaf age 1 to leaf age 2, thus at an earlier stage than in JL 24.

Table 1. Mean incidence (%), standard deviation of the mean, and overall mean after inoculation of the third leaf at different leaf ages, of three groundnut genotypes.

Leaf age		Genotype JL 24	ICGV 86031	ICGV 86388	Overall mean
1 (unfolded)	mean[1]	96.0	67.3	52.4	71.9 a[3]
	s.d.[2]	2.2	7.8	6.8	
2 (expanded)	mean	66.7	27.5	21.0	38.4 b
	s.d.	10.0	10.6	6.3	
3 (expanded and matured)	mean	11.9	9.1	5.2	8.7 c
	s.d.	3.1	3.9	3.4	

1. Mean incidence (%).
2. Standard deviation of the mean incidence.
3. Different characters indicate significant differences (Tukey, $P<0.001$).

INCIDENCE IN RELATION TO PLANT AGE

The incidence was monitored up to 20 DAI for series 4, 16 DAI for series 5, and 10 DAI for series 6. JL 24, in the 3-leaf stage, had an average incidence of 86.9% in series 4, 97.1% in series 5, and 98.8% in series 6.

The genotype and treatment means of the incidence, and the standard deviation of the means over series 4 to 6 were calculated, and presented in Table 2. The incidence of infected plants in the three genotypes tested, decreased strongly and significantly with the plant age. In JL 24 the incidence of the 2-leaf stage (89%) and the 3-leaf stage (94%) did not differ significantly. Raising the plant age from the 3-leaf stage to the 4-leaf stage reduced the incidence to 71%. Increasing the plant age to the 5-leaf stage dropped the incidence subsequently to 20% (Table 2). In the resistant genotypes, the incidence of infected plants of the 3-leaf stage was significantly lower than the incidence of the 2-leaf stage. The values decreased further when plant age was increased to the 4- and 5-leaf stage.

Table 2. Mean incidence (%), standard deviation of the mean, and overall mean after inoculation of the unfolded leaf from plants at different plant ages, of three groundnut genotypes.

Plant age		Genotype			Overall mean
		JL 24	ICGV 86031	ICGV 86388	
2-leaf stage	mean[1]	89.0	57.1	46.9	64.3 a[3]
	s.d.[2]	6.7	12.0	10.5	
3-leaf stage	mean	94.4	37.6	27.0	53.0 b
	s.d.	2.2	7.6	6.1	
4-leaf stage	mean	71.2	4.9	4.5	26.9 c
	s.d.	7.5	1.8	1.7	
5-leaf stage	mean	20.0	3.3	12.0	11.8 c
	s.d.	7.8	3.3	1.5	

1. Mean incidence (%).
2. Standard deviation of the mean incidence.
3. Different characters indicate significant differences (Tukey, $P<0.001$).

The major reduction in incidence was observed between the 4- and 5-leaf stage in JL 24 (51%), whereas for the resistant genotypes this was observed between the 3- and 4-leaf stage (28% on average). The 3-leaf stage had a lower incidence than the 2-leaf stage in the resistant genotypes, but not in the susceptible genotype.

INCUBATION PERIOD

The incubation period (IP_{50}) clearly increased with leaf age (Table 3). The overall treatment means of IP_{50} increased with about 2.5 days between leaf age 1 (unfolded) and leaf age 2 (expanded). A further increase in IP_{50} of 4 days was observed when the leaf age was raised from leaf age 2 (expanded) to leaf age 3 (expanded and matured).

The IP_{50} also increased with plant age, except in young plants, i.e. younger than the 3-leaf stage (Table 4). The overall treatment means of IP_{50} were not significantly different between these plants. The IP_{50} raised with 3.5 days from plants in the 3-leaf stage to the 4-leaf stage. Increasing the plant age to the 5-leaf stage raised the IP_{50} with another 3.3 days.

The IP_{50}'s found for the genotypes used here did not differ much. The IP_{50} was generally short in JL 24, and longer in ICGV 86031 and ICGV 86388 (Tables 3 and 4).

Table 3. Mean incubation period (IP_{50}), standard deviation of the mean, and overall mean after inoculation of the third leaf at different leaf ages, of three groundnut genotypes.

Leaf age		Genotype JL 24	ICGV 86031	ICGV 86388	Overall mean
1 (unfolded)	mean[1]	8.0	9.0	9.4	8.8 a[3]
	s.d.[2]	0.57	0.65	0.96	
2 (expanded)	mean	9.6	12.5	12.0	11.4 b
	s.d.	0.48	1.71	1.64	
3 (expanded and matured)	mean	13.2	15.2	17.8	15.4 c
	s.d.	2.87	3.12	3.47	

1. Mean IP_{50} (days).
2. Standard deviation of the mean IP_{50}.
3. Different characters indicate significant differences (Tukey, $P<0.05$).

Table 4. Mean incubation period (IP_{50}), standard deviation of the mean, and overall mean after inoculation of the unfolded leaf from plants at different plant ages, of three groundnut genotypes.

Plant age		Genotype JL 24	ICGV 86031	ICGV 86388	Overall mean
2-leaf stage	mean[1]	8.6	10.0	9.9	9.5 a[3]
	s.d.[2]	0.43	1.89	1.96	
3-leaf stage	mean	8.9	9.0	10.2	9.4 a
	s.d.	0.39	0.56	0.44	
4-leaf stage	mean	10.3	14.1	14.2	12.9 b
	s.d.	0.47	1.27	1.47	
5-leaf stage	mean	14.3	19.7	14.7	16.2 c
	s.d.	1.33	1.33	0.66	

1. Mean IP_{50} (days).
2. Standard deviation of the mean IP_{50}.
3. Different characters indicate significant differences (Tukey, $P<0.01$).

DISCUSSION

The occurrence of mature plant resistance in groundnut to PBNV is shown here. Both increased leaf and plant age reduced the incidence strongly and increased the incubation period. This effect (a decreased incidence and an increased incubation period) can be explained by a decreased rate of virus multiplication at the entry site, and/or a decreased rate of virus transport from the entry site to other plant parts. In another study we found that older, systemically infected tissue, diminished virus multiplication (data not shown). The effect of mature leaves and mature plants on the incubation period of resistant genotypes is almost certainly underestimated. The incidence in resistant genotypes was low and therefore the assumption $IP_{50}=x+1$ was applied, while the actual incubation period could have been considerably higher.

It seems that only young tissue of young plants is susceptible. An increase in leaf or

plant age of a few days induces a mature plant resistance resulting in a longer incubation period and fewer infected plants. This mature plant resistance occurs irrespective of the level of susceptibility of the groundnut genotype.

The observations on mature plant resistance of groundnut to PBNV are in agreement with the results of Bald (1937) of TSWV on tomato, and with the results of Savary (1987) of rust on groundnut. Mature plant and tissue resistance in the groundnut - PBNV system is an effective and highly important feature in the epidemiology of PBNV. Under field conditions the groundnut crop is expected to become more resistant during the growing period as a result of mature plant resistance. Buiel & Parlevliet (1995; Chapter 4) showed that this effect did indeed occur in the field, in a study on six genotypes ranging from susceptible to resistant.

In this study it was shown that mature plant resistance occurred in susceptible as well as resistant genotypes. But, mature plant resistance developed earlier in resistant genotypes, and had a much larger effect on incidence than in the susceptible genotype. Furthermore, the IP_{50} was longer in the resistant genotypes than in the susceptible genotype and this directly and indirectly affects the development of the disease. Firstly, a longer IP_{50} directly slows down the rate of infection in a resistant crop. Secondly, it indirectly decreases the spread of the virus by thrips as fewer virus sources occur. The effect of mature plant resistance is altogether much larger in resistant genotypes, and the use of resistant genotypes can therefore be recommended to keep peanut bud necrosis disease at a low level.

CHAPTER 8

MECHANISMS OF RESISTANCE IN GROUNDNUT TO PEANUT BUD NECROSIS VIRUS

ABSTRACT

The mechanisms that cause a reduced incidence of peanut bud necrosis virus (PBNV) infected plants in resistant groundnut genotypes, were studied. The development of symptoms and of virus concentrations were analyzed in mechanically inoculated and systemically infected leaves. The rate of systemic virus spread, and the incidence of systemically infected plants were studied after removing the inoculated leaf at different intervals. The results indicate that virus multiplication was inhibited at the site of infection in resistant genotypes but not in systemically infected leaves of resistant genotypes. The rate of systemic spread was lower in the resistant genotypes than in the susceptible genotypes. The virus concentration in systemically infected leaves was positively correlated with the leaf area showing symptoms, but was genotype independent. The viral antigen concentration decreased with the age of the systemically infected leaves. The mechanisms resulting in the reduced incidence of PBNV in resistant groundnut are discussed.

CHAPTER 8

INTRODUCTION

Bud necrosis disease is one of the most serious virus diseases of groundnut *Arachis hypogaea* L. in South Asia. Groundnut production is severely affected by this disease, particularly when infection occurs early in the growing season (Reddy et al., 1991). The disease is caused by peanut bud necrosis tospovirus (PBNV), which is transmitted by *Thrips palmi* Karny (Palmer et al., 1990; Vijaya Lakshmi et al., 1995). PBNV has been shown to be a distinct member of the *Tospoviruses* in the *Bunyaviridae* (De Haan et al., 1989; Murphy et al., 1995; Reddy et al., 1992; Satyanarayana et al., 1996). Tomato spotted wilt virus (TSWV, type member of the genus) is the cause of a similar disease of groundnut in the USA (Reddy et al., 1991).

Efforts to control the virus are mainly geared towards breeding resistant genotypes. Control of the vector with insecticide sprays resulted in an increased spread of the disease (Wightman and Amin, 1988). Several groundnut genotypes with resistance to PBNV (Amin, 1985; Dwivedi et al., 1993; Buiel et al., 1995; Chapter 5), and resistance to TSWV (Black and Smith, 1987; Culbreath et al., 1994; Demski et al., 1991) have been identified. The type of resistance reported is in all cases a quantitative resistance, and expressed as a reduced incidence of infected plants, compared to that of susceptible genotypes.

The virus moves from the infection sites in the inoculated leaves to newly formed leaves and produces chlorotic spots that may develop into chlorotic and necrotic rings (Reddy et al., 1991). Older leaves which are full-grown and mature at the time of infection, do not become systemically infected, and virus can not be detected in these leaves. On the young newly formed leaves, symptoms usually appear within 6-10 days after systemic spread in the plants. Virus in these leaves can readily be detected by ELISA. Systemically infected plants of resistant and susceptible genotypes show similar symptoms, and the only visible difference between the genotypes is the contrast in disease incidence of infected plants. Complete resistance or immunity, i.e. absence of systemic infection on genotype level, has so far not been found in cultivated groundnut.

The mechanisms underlying the quantitative resistance observed in groundnut were not

known. Therefore this study was undertaken to investigate the development of infection in inoculated and systemically infected leaves of resistant and susceptible genotypes. The symptoms and viral antigen concentrations were examined in individual leaves. Virus movement in the plant was studied by removing the inoculated leaves at different intervals following inoculation.

MATERIALS AND METHODS

VIRUS

A PBNV isolate, collected at ICRISAT Asia Center, India, was maintained on the groundnut cultivar TMV 2 by mechanical sap inoculation. After six serial transfers the virus was thrips transmitted, to avoid the possible occurrence of defective interfering RNA mutants, reported to occur due to repeated mechanical transmissions (Resende et al., 1991). Inoculum was prepared by collecting systemically infected tissue from TMV 2 plants, and grinding it in 0.05 M phosphate buffer, pH 7.0, containing 0.01 M Na_2SO_3 (1:10, w/v). Leaves, to be inoculated, were dusted with carborundum powder and rubbed with a pestle dipped in inoculum, that was kept chilled during inoculation.

PLANTS

Seeds were treated with tetramethyl thiuram disulphide (3 g/kg), and pre-germinated in Petri dishes on moistened filter paper to promote uniform germination. Germinated seeds were planted after 2 days in pots (15 cm diameter, 5 plants per pot), and were placed in a randomized complete block design in a greenhouse with minimum/maximum temperatures of 15-20°C/25-35°C. In the experiments described below, the plants were in the 3-leaf stage and only the third leaf (in order of appearance) was inoculated when unfolded. This was done to avoid differences in resistance related to the age of the leaf (Buiel and Parlevliet, 1996; Chapter 7).

ELISA

A triple antibody sandwich (TAS) ELISA was used to determine the PBNV antigen concentration in individual groundnut leaves. ELISA plates were coated with a 1:2000 diluted PBNV polyclonal IgG (2 mg/ml) solution in 0.01 M carbonate buffer (pH 9.6), incubated for 2 h at 37°C and washed with PBS-Tween (the incubation time, temperature, and the washing conditions were the same throughout the whole ELISA procedure unless described otherwise). Individual leaves (four leaflets without the petiole) were weighed and ground in antigen buffer (PBS-Tween containing 2% polyvinyl pyrrolidone and 0.01 M diethyldithiocarbamate) in a 1:10 (w/v) dilution. Plant extracts were transferred into Eppendorf tubes and centrifuged for 5 min at 2000 rpm. The supernatant of each sample was added in duplicate to the wells of the coated ELISA plates. Plates were washed and a monoclonal antibody developed to the nucleocapsid protein of PBNV (Poul et al., 1992), diluted 1/10000 in PBS-tween, was added. After incubating overnight at 4°C, the plates were washed and goat anti-mouse alkaline phosphatase conjugate (Sigma), diluted 1:2000 in antibody buffer (PBS-Tween containing 2% polyvinyl pyrrolidone and 0.2% ovalbumin), was added. After incubation, p-nitrophenyl phosphate (Sigma) substrate (1 mg/ml) in 10% diethanolamine buffer (pH 9.8) was added to the wells, and the plates were left at room temperature for the development of the reaction. Absorption readings (405 nm) were taken up to 30 min. The ELISA values were considered positive when they were greater than three times the absorption values of the healthy controls.

ANALYSIS OF THE INFECTION IN INOCULATED LEAVES

Experiment 1 was a non- replicated experiment, whereas experiments 2 and 4 comprised three replicates, and experiment 3 two replicates. Each replication consisted of 15 to 25 plants per treatment. The genotypes ICGV 86598 (susceptible to PBNV), and ICGV 86388, ICGV 86031, and ICGV 86029 (resistant to PBNV) were used in these experiments. JL 24 was included as a susceptible control. The inoculated leaves were removed 1, 2, 3, 4, 6 or 8 days after inoculation (DAI). The inoculated leaves were not removed from the control plants, so that the infection could develop in an undisturbed

way. The virus content was determined by TAS-ELISA in seven or eight detached leaves of the plants of one replicate in each treatment. These leaves were also scored visually for the presence of symptoms. The incidence of systemically infected plants was recorded daily, and continued after the removal of the inoculated leaves, until 20 DAI.

ANALYSIS OF THE INFECTION IN SYSTEMICALLY INFECTED LEAVES

Plants were inoculated in January 1993 (experiment 5), August 1994 (experiment 6), and January 1995 (experiment 7). The genotypes JL 24 (susceptible to PBNV), and ICGV 86031 and ICGV 86388 (resistant), were used in these experiments. Systemically infected leaves were labeled when first symptoms appeared. These leaves were collected within 3 days after the appearance of symptoms, and the viral antigen concentration of each individual leaf was determined by TAS ELISA. When collecting the leaves, the number of leaves on the plant (plant age), leaf age, and the percentage of the area of each leaf with symptoms were recorded. The age of the systemically infected leaf was classified into four groups: leaf folded, unfolded, expanded, and expanded and full-grown. The relationships between these traits and the viral antigen concentration of systemically infected leaves were determined.

RESULTS

DEVELOPMENT OF THE INFECTION IN INOCULATED LEAVES

The first symptoms on the inoculated leaves became visible 3 DAI. The rate of symptom development is shown in Table 1. A significantly lower percentage of inoculated leaves of the resistant genotypes showed symptoms (3-10%), than the susceptible genotypes JL 24 and ICGV 86598 (23-36%) 3 DAI. All inoculated leaves of both susceptible and resistant genotypes showed symptoms 8 DAI. All three resistant genotypes had a delayed symptom development compared to the susceptible genotypes, ICGV 86029 being the most resistant genotype.

Table 1. The development of the symptoms on inoculated leaves (percentage) in five groundnut genotypes.

Date (DAI[1])	Genotype JL 24	ICGV 86598	ICGV 86388	ICGV 86031	ICGV 86029
1	0.0	-	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0
3	23.3	36.4	3.4	4.5	9.5
4	95.7	86.7	72.7	100.0	53.3
6	100.0	86.4	85.2	77.3	72.7
8	100.0	100.0	100.0	100.0	90.9

1. Inoculated leaves were collected 1, 2, 3, 4, 6 and 8 days after inoculation (DAI).

The viral antigen concentration in the inoculated leaves with symptoms is given in Figure 1. The ELISA values of JL 24 increased from 0.058 (antigen below the detection level) 1 DAI, to 0.218 (2 DAI) although no symptoms were visible on these leaves. The ELISA value further increased from 0.861, 3 DAI, to 1.207, 8 DAI. A maximum was found 6 DAI (1.303). The ELISA values of the susceptible genotype ICGV 86598 increased in a similar way. In the inoculated leaves of the resistant genotypes the virus concentration developed at a slower rate and reached a relatively lower level (around 60% of JL 24).

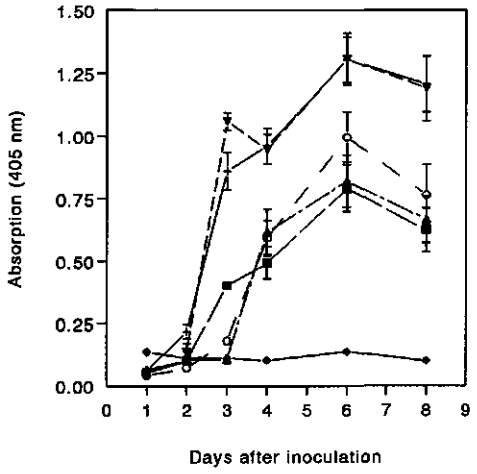

Figure 1. The development of the virus concentration in mechanically inoculated leaves with symptoms of susceptible genotypes JL 24 (+), ICGV 86598 (▼), and resistant genotypes ICGV 86388 (▲), ICGV 86031 (○), and ICGV 86029 (■). Vertical bars indicate the standard deviation. The detection level (◆) was set at three times the absorption value of the healthy control. Note: None of the leaves had symptoms one and two days after infection.

The percentage of inoculated leaves positive in ELISA, regardless of the presence of symptoms, is shown in Table 2. PBNV antigen could not be detected in any of the leaves, 1 DAI. Although none of the leaves showed symptoms 2 DAI, viral antigen could be detected in several inoculated leaves of all genotypes. However, the percentage of ELISA-positive leaves of the resistant genotypes was initially lower than that of the two susceptible genotypes. Six DAI, PBNV antigen could be detected in (nearly) all leaves. The number of ELISA-positive leaves was generally higher than the number of leaves with symptoms.

Table 2. The development of the infection in inoculated leaves (percentage of ELISA-positive leaves) in five groundnut genotypes.

Date (DAI[1])	Genotype JL 24	ICGV 86598	ICGV 86388	ICGV 86031	ICGV 86029
1	0.0	-	0.0	0.0	0.0
2	69.6	53.3	40.9	26.7	46.7
3	90.0	72.7	55.2	50.0	66.7
4	95.7	100.0	77.3	100.0	86.7
6	100.0	100.0	85.2	90.9	90.9
8	100.0	100.0	100.0	90.0	90.9

1. Inoculated leaves were collected 1, 2, 3, 4, 6 and 8 days after inoculation (DAI).

The PBNV antigen increase in the ELISA-positive leaves in the first eight days after inoculation is shown in Figure 2. The ELISA values of JL 24 increased from 0.058 (1 DAI) to 0.299 (2 DAI). The antigen concentration in ELISA-positive leaves of resistant genotypes was much lower than that of susceptible genotypes. In most cases, the virus concentration of leaves with detectable levels of viral antigen, regardless of symptoms, was lower than that of leaves with symptoms (Figure 1). Thus, leaves with symptoms usually had higher levels of viral antigen.

DEVELOPMENT OF SYSTEMIC INFECTION AFTER REMOVAL OF THE INOCULATED LEAF

The percentage of systemically infected plants (incidence) was recorded daily up to 21 DAI. The mean disease incidence was calculated for each genotype (Table 3). The

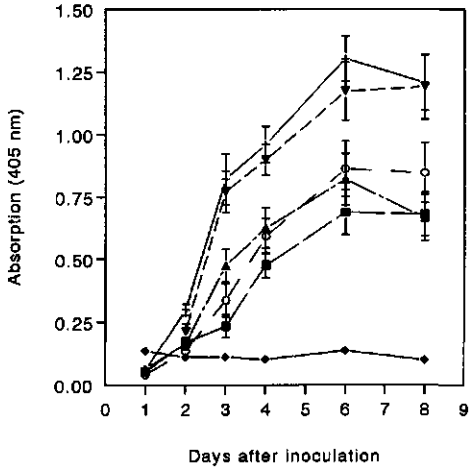

Figure 2. The development of the virus concentration in mechanically inoculated, ELISA-positive leaves of susceptible genotypes JL 24 (+), ICGV 86598 (▼), and resistant genotypes ICGV 86388 (▲), ICGV 86031 (○), and ICGV 86029 (■). Vertical bars indicate the standard deviation. The detection level (◆) was set at three times the absorption value of the healthy control. Note: PBNV could not be detected one day after infection.

incidence was lower in the resistant genotypes than in the susceptible genotypes, except for ICGV 86388 when the inoculated leaves were removed 1 DAI. More plants of this genotype became infected than of the susceptible genotype ICGV 86598. In resistant genotypes ICGV 86031 and ICGV 86029, the incidence was reduced to 0% when the inoculated leaves were removed 1 DAI. Twenty-seven percent of the JL 24 plants (susceptible check) became systemically infected when the inoculated leaf was removed one day after inoculation. The incidence increased to 78% in JL 24 (when removing the leaf 6 DAI), whereafter no significant difference was observed with the control (81%). The results show that the rate of infection was lower in resistant genotypes than in susceptible genotypes. The incidence of systemically infected plants was greatly reduced for all genotypes when the inoculated leaves were removed within 4 days after inoculation. Removing the inoculated leaves beyond 4 DAI had no effect on the incidence.

DEVELOPMENT OF THE INFECTION IN SYSTEMICALLY INFECTED LEAVES

The first symptoms on the systemically infected leaves were visible 6 DAI. The leaves which developed symptoms between 6 and 14 DAI were collected and tested by ELISA.

Table 3. The incidence (%) of systemically infected plants after removal of the inoculated leaf of five groundnut genotypes. The incidence shown in this table is the mean of three experiments and was recorded about 20 DAI.

Date of removal (DAI[1])	Genotype JL 24	ICGV 86598	ICGV 86388	ICGV 86031	ICGV 86029
1	27.4	15.8	19.5	1.1	0.0
2	25.7	18.8	10.4	9.1	3.8
3	44.9	42.2	29.6	20.6	8.1
4	57.1	66.2	31.6	36.5	14.8
6	78.0	86.6	38.8	39.2	22.4
8	79.7	75.6	42.1	43.6	17.9
Control[2]	80.8	80.5	39.5	41.1	22.1

1. Inoculated leaves were removed 1, 2, 3, 4, 6 and 8 days after inoculation (DAI).
2. Inoculated leaves were not removed from the control plants.

The relationship between the ELISA values and the leaf area with symptoms, was determined for the genotypes tested. A significant, positive correlation was found for all genotypes (Figure 3 A, B and C). Tests (Snedecor and Cochran, 1967) for homology of the regression and correlation coefficients (between symptoms and ELISA values) of the three genotypes showed that the right tailed significance levels were far greater than 5%, implying absence of differences between the regression and correlation coefficients. The computed common correlation coefficient between the leaf area with symptoms and the viral antigen concentration, was 0.856. Apparently, the viral antigen concentration was genotype independent but was linearly related with the leaf area with symptoms.

The mean ELISA values were calculated for the date of sampling and the date of appearance of symptoms on the systemically infected leaves (Table 4). A clear relationship was neither observed between the virus concentration and the sampling date, nor between the virus concentration and the date of appearance of symptoms. Obviously, independently both traits had no effect on the virus concentration in systemically infected leaves.

The mean ELISA values were calculated as a function of the time between the appearance of symptoms and sampling of the systemically infected leaves (Table 5). A mean ELISA value of 0.822 was found when the leaves were collected and analyzed by

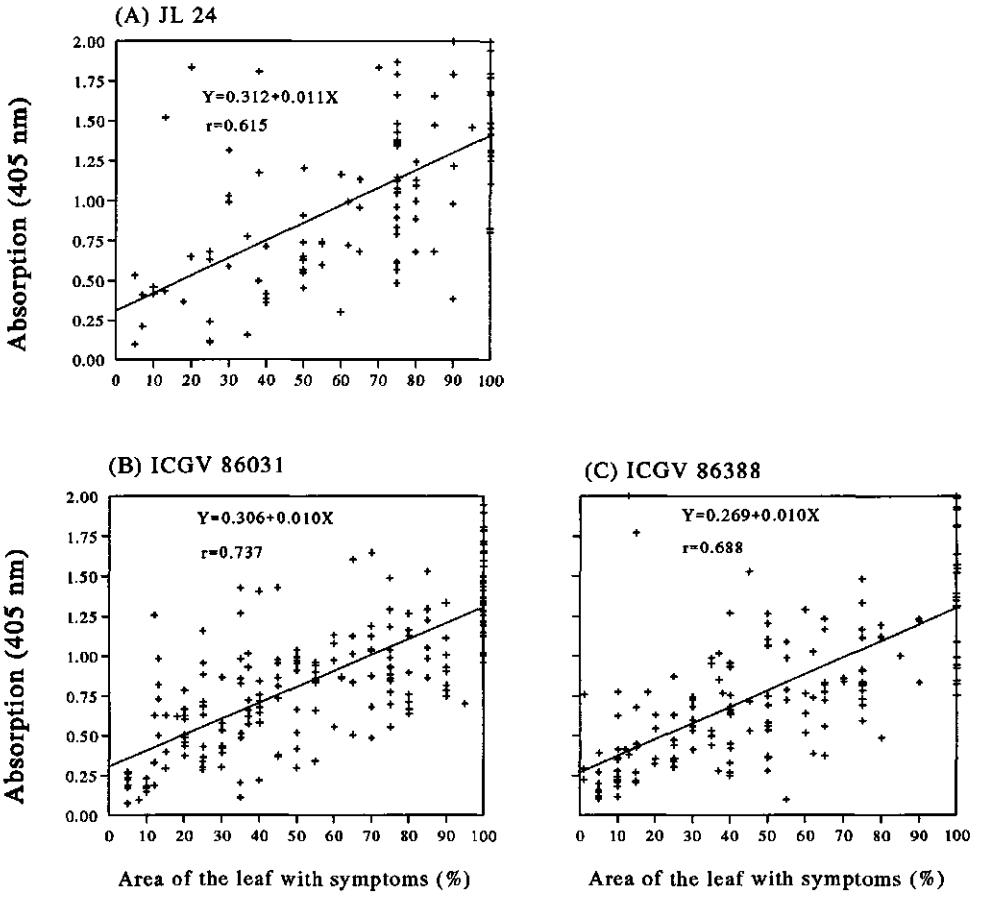

Figure 3. The relation between the percentage of the systemically infected leaf area with symptoms, and the ELISA values of these leaves from JL 24 (A), ICGV 86031 (B), and ICGV 86388 (C). The linear relationship and the correlation coefficient (r) is given for each genotype.

Table 4. The mean ELISA values (bold) and standard deviation (in parentheses below) of systemically infected leaves at six sampling days (days after inoculation, DAI), and eight dates on which symptoms appeared (DAI).

	Sampling date (DAI)					
	7	8	9	10	11	14
Appearance of symptoms (DAI)						
6			**1.382** (0.267)			
7	**1.026** (0.060)	**0.871** (0.202)	**1.158** (0.204)			
8		**0.765** (0.029)	**0.777** (0.103)		**1.079** (0.072)	
9			**0.760** (0.110)	**0.816** (0.057)	**0.881** (0.069)	
10				**0.739** (0.092)	**0.762** (0.056)	
11					**0.865** (0.079)	**1.520** (0.176)
13						**1.842** (0.044)
14						**0.783** (0.364)
mean		**0.769** (0.029)	**0.850** (0.081)	**0.752** (0.078)	**0.890** (0.035)	**1.437** (0.150)

ELISA on the day of appearance of symptoms. When leaves were collected and assayed three days after the appearance of symptoms, the mean ELISA value was 1.181. Thus, the viral antigen concentration in the systemically infected leaves increased between 0 to 3 days after the appearance of symptoms. Furthermore, the mean ELISA values in these experiments were calculated as a function of the maturity of the systemically infected leaf, and the age of the plant at the time of systemic infection (Table 6). The ELISA values clearly declined with increasing leaf maturity. The mean ELISA value of the folded leaf was 1.280, and reduced to 0.742 for the expanded and full-grown leaf. The ELISA values did not differ significantly when leaves of plants in the 5-, and 6-leaf stage were compared within leaf age group, nor for the overall means. Hence, it can be concluded that an increased leaf maturity, but not plant maturity, had a decreasing effect on the viral antigen concentration in the systemically infected leaf.

Table 5. The mean ELISA values of systemically infected leaves for the interval between the appearance of the symptoms and ELISA.

Interval (days)	mean	standard deviation	number of samples
0	0.822	0.025	295
1	0.831	0.054	77
2	0.906	0.067	44
3	1.181	0.072	55

Table 6. The mean ELISA values of systemically infected leaves at different levels of leaf maturity and plant age at the time of collection.

	Leaf maturity				overall	
Plant age	folded	unfolded	expanded	expanded & full-grown	mean	sd
4-leaf stage	-	-	-	0.827		
5-leaf stage	1.353	1.129	0.833	0.550	0.966	0.175
6-leaf stage	1.207	1.092	1.089	0.850	1.060	0.075
overall mean	1.280	1.111	0.961	0.742		
sd	0.073	0.019	0.128	0.167		

DISCUSSION

This study shows that the PBNV concentration increased rapidly in the inoculated leaves of susceptible genotypes. The virus could be detected 2 DAI, whereas the first symptoms were visible 3 DAI. The virus concentration increased slower in resistant genotypes, and attained only levels of about 60% of that in the susceptible genotypes. These observations suggest that the inhibition of virus replication is one of the mechanisms causing resistance in groundnut.

The virus movement from the inoculated leaf to other parts of the plant was measured indirectly by recording the incidence after removal of the inoculated leaf. By removing the inoculated leaf at different intervals we were able to show that the rate of systemic

infection was reduced in resistant genotypes (Table 3). The reduction could be solely caused by the inhibition of virus replication, or it may be due to restriction of virus movement. This is possibly a second mechanism of resistance.

No difference in the percentage of systemically infected plants was observed when the inoculated leaves were removed 6 or 8 DAI, or when they were not removed. Presumably, virus movement occurred mainly within 4-5 days after inoculation. In the systemically infected leaves, viral antigen levels reached the same levels in the resistar genotypes as those of the susceptible genotype. Evidently, the resistance was operating in inoculated leaves but not in systemically infected leaves of the resistant genotypes. This agrees with the observation that systemically infected leaves of resistant and susceptible genotypes show similar symptoms. Furthermore, a clear and positive association was found between viral antigen levels and symptoms of the systemically infected leaves of all three genotypes.

The delay from inoculation to systemic infection varied between plants and ranged from 6 to 14 days. Consequently, plants differed in age when systemic infection developed. Still, this difference in plant age did not affect the amount of viral antigen in the systemically infected leaves. However, differences in plant age in these experiments are expected to be too small to cause differences in the viral antigen concentration. Similarly, systemically infected leaves varied in maturity when infection developed. Fully expanded leaves did not support as much virus multiplication as young folded leaves (Table 6). Apparently, the age of the leaf influenced the virus multiplication in the leaf. This mature plant and tissue resistance has also been described elsewhere (Buiel and Parlevliet, 1996; Chapter 7).

Other investigations seem to confirm our results, e.g. Tu and Ford (1970) observed a reduced incidence of maize dwarf mosaic virus in resistant maize genotypes. The virus concentration in inoculated leaves of a resistant corn variety was lower than that of a susceptible variety. Later, Jones and Tolin (1972) described that the resistance of a corn hybrid they used in their studies was based on a decreased rate of virus multiplication or a limited virus spread. Barker and Harrison (1985) presented a possible explanation for the reduced incidence of systemically infected plants in resistant genotypes. They

showed that translocation of potato leaf roll virus (PLRV) from the infected tubers to the shoots was prevented in resistant potato lines. Therefore, it is apparent that the restriction of virus movement from the site of infection could lead to resistance to systemic invasion of the virus. Although PLRV is a phloem restricted virus, our findings illustrate that similar resistance mechanisms to PBNV exist in groundnut.

In the inoculated leaves of the resistant genotypes, virus movement was possibly also restricted. Inhibition of virus multiplication, and the restriction of virus movement, are the mechanisms presumably responsible for the reduction of incidence in resistant genotypes.

A third, passive mechanism identified as mature plant and tissue resistance (Buiel and Parlevliet, 1996; Chapter 7) may also be involved. This mechanism may have stronger effects in resistant genotypes than in susceptible genotypes. The virus replication is inhibited at the infection site in resistant genotypes and thus results in a delay between initial infection and systemic infection as compared to the susceptible genotypes. The leaves mature during this delay, and therefore do not permit systemic infection. This conclusion is supported by the data presented in Table 6 which show a decrease in virus concentration. The chances of a successful systemic infection in resistant genotypes are probably furthermore diminished because of mature plant resistance. Mature plant resistance may therefore enhance the genotypic resistance. Together, the mechanisms discussed presumably cause the reduced incidence of infected plants in resistant genotypes.

The practical implications of our results on the mechanisms of resistance to PBNV in groundnut are three-fold. Firstly, field selection for resistance should be done on the basis of incidence of systemically infected plants. Secondly, field selection for a reduced extent of the leaf area with systemic symptoms does not result in an increased resistance level, because this trait is genotype independent. Thirdly, serological tests of symptomatic plants are not required for field selection, but may still be necessary for the diagnosis of PBNV.

CHAPTER 9

VECTOR RESISTANCE

SUMMARY

Trips palmi Karny is the vector of peanut bud necrosis tospovirus (PBNV), which infects groundnuts in many parts of South Asia. Vector and virus resistance levels were evaluated in ten groundnut genotypes. Three groups with distinct resistance levels could be distinguished. The first group contained two cultivars JL 24 and TMV 2, highly susceptible to both vector and virus, and showing high disease incidence levels in the field. The second group was formed by three genotypes (ICGV 86985, ICGV 86030, and 2129-8[50]). These genotypes lacked virus resistance, and were colonized by fewer *T. palmi*. It is expected that the field resistance of the genotypes in this group was caused by vector resistance. The third group was represented by five field resistant genotypes, which had both virus and vector resistance. None of the groundnut genotypes studied here had field resistance which was entirely due to virus resistance, they all had some vector resistance.

INTRODUCTION

T. palmi Karny (Thysanoptera; Thripidae) is the vector of peanut bud necrosis tospovirus (PBNV), and it transmits PBNV in a persistent manner (Vijaya Lakshmi et al., 1995). PBNV is acquired only by larval stages of *T. palmi*, while transmission is exclusively due to adult thrips (Vijaya Lakshmi et al., 1995). Other thrips vectors of tospoviruses, e.g. *F. occidentalis* Pergande, the main vector of tomato spotted wilt virus and the only vector of impatiens necrotic spot virus, was able to transmit in the second larval stage as well as in the adult stage (Wijkamp and Peters, 1993; Wijkamp et al., 1995).

Independent or combined resistance to virus and vector are important tools to control PBNV infection. Resistance to the vector of PBNV has been reported (Amin et al., 1985). Furthermore, several sources of PBNV resistant groundnut were observed (Amin, 1985; Buiel et al., 1995; Chapter 5, Dwivedi et al., 1993, 1995). This resistance is quantitative and assessed using the percentage of infected plants.

The aim of this study was to determine levels of vector- and virus resistance. Therefore, vector counts were compared with data from virus resistance, and with data from field infection, which is the combined result of vector and virus resistance.

MATERIALS AND METHODS

A field trial at Narkoda, Andhra Pradesh, India, comprised ten groundnut genotypes in a randomized block design with four replicates. Each genotype was planted in a plot of two 4-m rows. A 4-m row of the susceptible cultivar JL 24 was included between all plots. The row distance was 60 cm, plant distance 20 cm. The disease incidence was determined regularly until the end of the season, as described earlier (Chapter 1). In the same trial, the number of *Thrips palmi* Karny were determined in 25 leaf terminals collected from each plot, as described in Chapter 2.

The groundnut genotypes were mechanically inoculated in several experiments using a standard mechanical inoculation procedure (Chapter 8). The number of systemically infected

plants were determined until approximately 25 days after inoculation.

RESULTS AND DISCUSSION

The *T. palmi* were collected on seven dates (Table 1). The number of *T. palmi* varied at each collection date. JL 24 had the highest mean number of 3.5 thrips per 25 terminals, whereas TMV 2 had 2.0 thrips. The other eight genotypes had lower mean number of thrips, ranging from 0.4 to 1.1.

Table 1. *T. palmi* population on 25 terminals (mean of 4 replicates), of ten groundnut genotypes during the growing season, at Narkoda.

	Collection date (days after sowing)							
Genotype	31	41	48	58	69	76	89	mean
JL 24	1.0	5.8	5.3	1.3	5.8	4.8	0.8	3.5
TMV 2	0.3	3.5	2.5	0.3	3.3	3.5	0.5	2.0
86031	0.0	0.8	1.8	0.5	2.5	2.0	0.0	1.1
86598	0.3	1.0	1.8	0.8	0.5	1.0	0.0	0.8
86030	0.0	1.8	1.3	0.3	0.0	0.8	0.0	0.6
86363	0.0	0.0	1.0	0.3	1.8	1.0	0.0	0.6
86029	0.5	0.8	0.5	0.3	0.5	0.5	0.3	0.5
86388	0.0	1.5	0.8	0.3	0.8	0.0	0.3	0.5
86430	0.5	1.3	1.0	0.3	0.3	0.3	0.0	0.5
2192-8(50)	0.0	0.3	0.8	0.5	1.5	0.0	0.0	0.4

The results of the final disease incidence in the field, are combined with *T. palmi* numbers occurring on the plants in the same trial, and with the results of mechanical inoculation from other experiments (Table 2). The ten genotypes tested were arranged into three groups. The first group (Table 2) contained groundnut cultivars JL 24 and TMV 2, with a high disease incidence in the field. TMV 2 had a lower incidence compared to JL 24, and this was consistent with the results found in other experiments (Buiel and Parlevliet, 1995; Buiel

Table 2. Peanut bud necrosis virus incidence (%) from field infection and mechanical inoculation (mean of several experiments), and *T. palmi* numbers (mean over seven scoring dates).

Genotype	Field infection (%)	Mechanical inoculation (%)	*T.palmi*
JL 24	85	79	3.5
TMV 2	77	69	2.0
ICGV 86598	29	73	0.8
2129-8(50)	24	61	0.4
ICGV 86030	23	68	0.6
ICGV 86031	24	28	1.1
ICGV 86029	21	20	0.5
ICGV 86388	20	26	0.5
ICGV 86363	16	16	0.6
ICGV 86430	13	9	0.5

et al., 1995; Chapter 4 and 5). In Chapter 6 it was shown that this slight resistance level in TMV 2 was heritable. Fewer TMV 2 plants were infected when mechanically inoculated, as compared to JL 24. Additionally, TMV 2 was colonized by fewer *T. palmi*, suggesting vector resistance. Therefore, the small field resistance level of TMV 2 could be the result of some resistance to virus and vector.

The second group represented ICGV 86598, 2129-8(50), and ICGV 86030 (Table 2), which are fairly resistant in the field (23-29% infection). However, mechanical inoculation of plants of these genotypes showed that the level of disease incidence was as high as in JL 24 or TMV 2. Thus, these genotypes lack virus resistance, or the effects are very small (as in TMV 2). Other results (Chapter 8) confirmed that ICGV 86598 did not have virus resistance. *T. palmi* numbers were lower in these genotypes, suggesting that the field resistance was caused by vector resistance.

The third group of genotypes (Table 2) comprised five genotypes with fair or good levels of field resistance (13-24% infection). These genotypes had virus resistance, as mechanical inoculations yielded low disease incidence levels. Furthermore, these genotypes all had low *T. palmi* numbers, suggesting that both virus and vector resistance were present.

None of these genotypes had similar thrips numbers as JL 24. The genotypes tested here,

with fair or good levels of field resistance, are representative of a larger set of genotypes that have been tested. In none of these genotypes the thrips counts were as low as in JL 24. *T. palmi* numbers were generally low throughout the season (Table 1), even in JL 24. It is remarkable that the disease attained such prominent levels in this cultivar. These results suggest that many thrips are viruliferous and that *T. palmi* is a very efficient vector. Vijaya Lakshmi et al. (1995) showed that 60% of the *T. palmi*, collected from PBNV infected plants in the field, were able to transmit the virus, hence, they must have been viruliferous.

GENERAL DISCUSSION

Resistance to peanut bud necrosis tospovirus (PBNV) and other tospoviruses has become increasingly important with the rapid spread of these viruses in many parts of the world (Best, 1968; de Ávila et al, 1990). In South Asia, PBNV is a serious problem to groundnut production. Breeding for resistance to the virus and to the vector is considered to be one of the most promising solution to prevent the occurrence of this virus. The results described in this thesis have elucidated several aspects of PBNV resistance. Information obtained on the assessment of resistance, the epidemiology, and the durability of the resistance, is discussed here.

ASSESSMENT OF RESISTANCE

I. NATURAL FIELD INFECTION

Quantitative resistance to PBNV is determined from the level of disease incidence, i.e. the percentage of plants with symptoms. To develop appropriate selection methods, the influence of several factors on the disease assessment was examined.

In this study an inter-row interference was not detected in plots in which rows with plants of a susceptible genotype were flanked with plants of a resistant genotype. The absence of any inter-row interference makes it highly likely that the resistance level of a groundnut genotype determined in small plots in breeders' selection trials is fairly representative for the resistance level when the genotype is grown on a large scale (Chapter 2). Other studies have shown that an inter-plot interference is clearly present in some pathosystems, whereas it is small or absent in others (Parlevliet and Danial, 1992).

The distribution of PBNV infected plants in the field was not uniform. A higher number of infected plants was observed in the central part of the field, as compared to the border area of the field (Chapter 3). This implies that to assess resistance, PBNV selection fields should be surrounded by a border zone of groundnut plants to avoid differences due to the location within the field.

The resistance to PBNV was consistent in ten environments (Buiel et al., 1995; Chapter 5). This indicated also that the prevailing virus populations in these environments did not differ

in virulence and pathogenicity. The results were supported by the finding that PBNV isolates from different locations in India reacted with a polyclonal antiserum (Reddy et al., 1992), and with 10 monoclonal antibodies developed against the nucleocapsid protein (Poul et al., 1992). Hence, it was concluded that selection for PBNV resistance can be performed at any location. However, discrimination between resistance levels was more effective in environments with a high level of PBNV infection (Buiel et al., 1995; Chapter 5).

II. MECHANICAL INOCULATION

Mechanical inoculation of plants with PBNV revealed virus resistance exclusively (Chapters 7, 8 and 9). Vector resistance explained field resistance in three genotypes, which lacked virus resistance (Chapter 9).

A distinct mature plant and tissue resistance was found (Buiel and Parlevliet, 1996; Chapter 7). This type of resistance was also detected in the field (Buiel and Parlevliet, 1995; Chapter 4). The percentage of infected plants decreased sharply with increasing age, whereas the incubation period increased. Therefore, differences in plant and leaf age have to be taken into consideration, when assessing virus resistance.

Studies on symptom development and virus concentration after mechanical inoculation provided insight in the mechanisms of virus resistance. Virus multiplication was inhibited at the site of infection in resistant genotypes, whereas in systemically infected leaves virus multiplication was not inhibited. The rate of systemic infection was also lower in resistant genotypes (Chapter 8). Likewise, the resistance to potato leaf roll luteovirus (PLRV) in potato is quantitative and expressed as a reduced incidence of infected plants. In potato this type of resistance is referred to as 'resistance to infection'. Virus multiplication and distribution was restricted in plants of resistant potato clones (Barker and Harrison, 1985, 1986). The expression of resistance in potato plants transformed with the PLRV coat protein, resembled the resistance found in some non-transgenic resistant potato clones. In both cases, virus multiplication (Barker et al., 1991) and virus distribution (Derrick and Barker, 1992) was restricted, resembling the results of this study (Chapter 8). Thus, the type of quantitative resistance of groundnut to PBNV, as well as the underlying mechanisms are similar to that of potato to PLRV.

EPIDEMIOLOGY OF PBNV INFECTION

Natural PBNV infections will develop after viruliferous thrips transmit the virus from external sources to healthy plants of groundnut crops (primary infection). Subsequently, further spread of the virus may occur from the infected plants by thrips to healthy plants in the same crop. This type of infection is referred to as secondary infection (Reddy et al., 1983). The magnitude of the two types involved in the spread of the epidemic are not known. The occurrence of tomato spotted wilt tospovirus (TSWV) in tomato crops has mainly been attributed to primary infections (Bald, 1937; Laviña et al., 1993). Analyzing the spatial and temporal pattern of TSWV disease in groundnut in the USA, Camann et al. (1995) observed that the data were consistent with the hypothesis that most infections arise from primary infections. A similar conclusion was drawn earlier in India, where it was observed that the majority of infections occurred shortly after the invasion of the vector in the crop (Reddy et al., 1983).

The data collected in this study either supported the hypothesis that the infection is chiefly caused by primary infections, or that both primary and secondary infections occur but that secondary infection is not restricted to plants in the immediate vicinity.

The experiments in which the effect of plant density on the spread of the disease was studied revealed that in both early and advanced phases of the epidemic a similar relation existed between the disease incidence and the plant density (Chapter 1). This observation suggests that the nature of the spread did not change during the epidemic. The absence of an inter-row interference effect (Chapter 2) is also indicative for a spread which results exclusively from primary infections. In Chapter 3, the non-uniform distribution in the field throughout the course of the epidemic was described. This non-uniform distribution was not a result from focal epidemics, but from a presumed preference of the vector for the central area of the field. The same effect was observed throughout the epidemic, implying that either one source of infection was involved (only external sources), or from both external and internal sources but with similar spreading patterns.

The level of the infection pressure varied considerably between locations (Chapter 5), and has to be explained by a difference in the number of viruliferous thrips and by the

occurrence of certain climatic conditions (Reddy and Wightman, 1988). Consistent differences between certain locations with comparable climatic conditions (e.g. ICRISAT and Rajendranagar) suggest that the extent of introduction of infections from external sources determined the disease level. At ICRISAT, the groundnut trials were surrounded by large areas of non-host crops (i.e. chickpea, pigeonpea, sorghum, and pearl millet), while at Rajendranagar the trials were located within a largely vegetable growing area, with many (suspected or identified) hosts of PBNV.

If PBNV infections primarily originated from external sources, one could also argue that the termination of the epidemic (Buiel and Parlevliet, 1995; Chapter 4), resulted from an abrupt cessation of the introduction of viruliferous thrips from the external PBNV sources. For example, when certain vegetable crops are the main external source of PBNV, the harvest of these crops would cease the migration of viruliferous vectors. However, weeds could also be sources of PBNV infection (Reddy et al., 1991), and their presence would not cease abruptly. Also, mature plant resistance to the virus was shown to exist (Buiel and Parlevliet, 1996; Chapter 7), and confirms the hypothesis that mature plant resistance plays a role in field infections. Furthermore, infected groundnut plants could also act as a secondary source.

DURABILITY OF PBNV RESISTANCE

The resistance to PBNV in groundnut appeared to be quantitative. At least three resistance factors could be distinguished in seven groundnut genotypes (Chapter 6). The durability of resistance of these groundnut genotypes can only be analyzed in retrospective, i.e. when grown for many years on a large scale. Cultivar TMV 2 has been grown all over India for more than 50 years, and has a factor resulting in a slight level of resistance. Thus it can be concluded that the quantitative resistance of TMV 2 is durable.

The multi-environment tests (Buiel et al., 1995; Chapter 5) showed that 42 groundnut genotypes could be divided into seven groups depending on their level of resistance. One group represented the highly susceptible cultivar, lacking resistance, and the other six

groups represented variable levels of resistance. These levels are probably due to different genetic factors, and can be directed towards virus- or vector resistance, or both. At least three factors, explaining the distinct virus resistance levels, were shown to be present in seven genotypes (Chapter 6).

SUMMARY

Tospoviruses cause world-wide economic losses in many agricultural, horticultural, and ornamental crops. In South Asia, peanut bud necrosis tospovirus (PBNV) is one of the most destructive viruses of groundnut (*Arachis hypogaea* L.). The development of resistant groundnut cultivars is an extremely important measure to reduce yield losses caused by this virus. Complete resistance (immunity) is absent in *A. hypogaea*, but quantitative resistant genotypes which reduced the percentage of infected plants (incidence) considerably, have been found. This type of resistance has been investigated in this thesis with an emphasis on the development of methods to assess resistance, and to analyze the inheritance and mechanisms of resistance.

Several factors influencing PBNV epidemics were analyzed to determine the optimal assessment of resistance. Increasing plant density reduced the disease incidence in a resistant and in a susceptible groundnut genotype. However, the number of infected plants per unit area increased, and caused higher levels of the disease. Therefore, an increased plant density is not recommended to reduce PBNV infection. The assessment of resistance in small plots was representative of the farmers situation, as no significant inter-plot interference was observed. The PBNV incidence appeared to be higher in the center of experimental trials, and was probably caused by vector preference.

A study of the quantitative resistance in field trials in ten environments in India, showed that selection in any of these environments yielded similar results. Environments with an average or high natural infection discriminated better among genotypes than environments with a low infection. At locations with a low PBNV infection, selection can be achieved based on the combined data of two or more years.

Five resistant and two susceptible groundnut genotypes were crossed in a half-diallel to study the inheritance of quantitative resistance to PBNV. Four different levels of resistance were identified and could be explained by at least three resistance factors. Epistatic interactions were absent and the resistance factors were likely to inherit additively. The disease incidence in the resistant genotypes was about five times reduced compared to that of JL 24 (susceptible check). From the two susceptible genotypes (JL 24 and TMV 2), TMV 2 had a consistently lower disease incidence than JL 24. This slight level of quantitative resistance in TMV 2 was effective for more than 50 years and appeared to be

durable.

A clear mature plant and tissue resistance was observed when groundnut plants were mechanically inoculated with a PBNV isolate. This type of resistance increased the incubation period and reduced the disease incidence drastically. The mature plant resistance also caused the termination of the PBNV epidemic in the field, and was independent of disease pressure, phase of the epidemic, rate of the epidemic growth, and resistance level of the host genotype.

A standard inoculation method was developed to study the mechanisms of resistance. The field resistant genotypes were resistant to the virus, except three genotypes, which were as susceptible to the virus as the susceptible control. It was shown that these field resistant genotypes were colonized by fewer thrips vectors, hence, the field resistance was caused by vector resistance.

Using the standard inoculation method, two different mechanisms of virus resistance could be distinguished. Firstly, virus multiplication was inhibited at the site of infection in resistant genotypes. In systemically infected leaves of the same genotypes, virus multiplication was not inhibited. Secondly, in resistant genotypes the rate of systemic infection was clearly lower. Thus, inhibition of virus multiplication, and restriction of virus movement, are the mechanisms presumably responsible for the reduction in disease incidence in resistant genotypes.

सारांश

टास्पो वायरस (Tospoviruses) कृषि, उध्यान एंव फूलों की अनेक फसलों को पूरे विश्व में आर्थिक क्षति पहुँचाती है । दक्षिण एशिया में बड नेक्रोसिस टास्पो वायरस पी. बी. एन. वी. (bud necrosis tospovirus P B N V) मूंगफली (*Arachis hypogaea* L.) की एक अत्यंत क्षति कारक वायरस है । इस रोग से होने वाली उत्पादन क्षति, कम करने के लिये, मूंगफली की रोग अवरोधी (disease resistant) जातियों का उपयोग, बहुत ही महत्वपूर्ण है । मूंगफली की संपूर्ण रोग रहित (immune) जातियाँ नहीं विकसित की गयी हैं लेकिन ऐसी जातियाँ जिसकी फसल में रोग ग्रसित पौधों की संरव्या (प्रतिशत) बहुत कम उपलब्ध हैं । इस प्रकार के गुणात्मक रोग अवरोधी (quantitative resistance) विषय पर अनुसंधान इस शोधलेख (thesis) मे किया गया है । जिसमें रोगप्रतिरोध (resistance) के मापन की विधि, इसके अनुवंशिकी (inheritance) और क्रिया विधि के विश्लेषण पर विशेष ध्यान दिया गया है ।

पी. बी. एन. वी. महामारी (epidemics) को प्रभवित करने वाले बहुत से कारणों का विश्लेषण किया गया है जिससे सर्वोतम प्रतिरोध क्रिया का सही मूल्यांकन किया जा सके । खेत में पौधों कि संरब्या (प्रति इकाई ज़मीन) बढ़ाने से रोग प्रतिरोधक एवं रोग ग्रसित (susceptible) दोनों प्रकार की मूंगफली किस्मों में रोग की कमी (incident) पायी गयी, फिर भी रोग ग्रसित पौधों की संरव्या (प्रति इकाई ज़मीन) में अधिकता हुयी जिससे रोग क्षति और अधिक बढ़ी । इस लिये पी. बी. एन. वी. रोग को कम करने के लिये अधिक घनी फसल उगाना सलाहकारक नहीं है । छोटी प्रयोगिक क्यारियों में रोग प्रतिरोध क्रिया का मूल्यांकन किसानों के खेतों में इसकी स्थिति का सही प्रतिनिधि पाया गया क्योंकि आपसी क्यारियों के बीच कोई विशेष बाधा (interference) नहीं पायी गयी । पी. बी. एन. वी. रोग प्रयोगिक क्यारियों के केन्द्र में अधिक पाया गया इसका कारण संभवतः वायरस संक्रमण करनेवाले थ्रिप्स (vector thrips) की प्राथमिक रुचि थी ।

गुणात्मक (quantitative) रोग अवरोध का भारतीय वातावरण (स्थानों) में प्रयोगिक खेती में अध्ययन करने से पता चला है कि रोग प्रतिरोध का चयन हर वातावरण में एक समान परिणाम देगा । लेकिन उन स्थानों पर मूंगफली कि जातियों में अधिक अंतर पाया गया जहाँ रोग औसत या औसत से अधिक था, उन स्थानों की तुलना मे जहाँ रोग कम था । जिस स्थान पर पी. बी. एन. वी. रोग कम लगता है, जातियों का चयन (selection) दो या अधिक वर्षों के औसत अंकों के आधार पर किया जा सकता है ।

पी. बी. एन. वी. के गुणात्मक रोग प्रतिरोध क्रिया का अध्ययन करने के लिये पांच रोग प्रतिरोधक तथा दो रोग ग्रसित (susceptible) मूंगफलि की जातियों को अर्ध डायलिल (half diallel) आधार पर संकरित (cross) किया गया और चार विभिन्न स्तर की प्रतिरोध क्रिया की पहचान की गयी जिसकी व्यारव्या से स्पष्ट है कि इस रोग की अनुवंशिकी कम से कम तीन प्रतिरोधक जीन (gene) पर निर्भर है ।

रोग प्रतिरोध संभवतः एडिटिव जीन (additive gene) पर आधारित है, क्योंकि इससे एपिस्टैटिक जीन इन्टरएक्सन (epistatic interaction) नहीं पाया गया, प्रतिरोधक जातियों में रोग कि घटना (incidence) रोग ग्रसित किस्म (JL 24 Susceptible check) कि तुलना में पांच गुना कम थी । दो रोग ग्रहित (susceptible) जातियाँ की परस्परिक तुलना करने पर पता चला कि TMV 2 में रोग घटना JL 24 तुलना में स्थायी रुप से कम थी । थोड़ा गुणात्मक (quantitative) प्रतिरोध TMV 2 में होने के कारण ही यह किस्म, पचास साल से भी अधिक अवधि तक कार्यसाधक रही और टिकाऊ साबित हुयी ।

मूंगफली के पौधों को पी. बी. एन. वी. की सूई देकर वयस्क पौद और तन्तु अवरोध (mature plant and tissue resistance) का स्पष्ट अवलोकन किया गया । इस तरह के अवरोधन (resistance) से वायरस की सुसुप्तावस्था (incubation) का समय बढ़ा और रोग घटना (incidence) में अत्यंत कमी हुयी । वयस्क पौद अवरोध (mature plant resistance) के कारण पी. बी. एन. वी. की महामारी खेत में समाप्त होती पायी गयी जो कि रोग दबाव, महामारी कि स्थिति, प्रगति की दर तथा फसल की प्रतिरोधी क्षमता पर नहीं निर्भर थी । प्रतिरोध क्रिया का अध्ययन करने के लिये एक मानक (standard) संचारी (inoculation) विधि का विकास किया गया । प्रक्षेत्र अवरोधी (field resistant) मूंगफली की जातियों के सभी वायरस का प्रतिरोध किया गया फिर भी इनमें तीन जातियाँ ऐसी थी जो रोगप्रभावी (susceptible) जातियों की तरह प्रभावित थी । प्रक्षेत्र अवरोधी (field resistant) जातियों में रोग वाहक-थ्रिप्स (vector-thrips) कम थे, अतः प्रक्षेत्र अवरोधन (field resistance) रोग वाहक थ्रिप्स की कमी के कारण था ।

मानक रोग संक्रमण (inoculation) विधि से दो तरह के वायरस प्रतिरोध क्रिया की पहचान की जा सकती है । प्रथम – प्रतिरोधक जातियों में वायरस का बढाव (infection) स्थान पर ही सीमित रहा । लेकिन उसी जाति के पौधों में जिसकी पत्तियाँ क्रमशः प्रभावित (systemically infected) थीं उनमें वायरस के बढ़ाव दर में कोई रुकावट नहीं आयी । दूसरा – वायरस प्रतिरोधक जातियों में वायरस बढ़ाव की दर में स्पष्ट रुप से कम थी । अतः वायरस बढ़ाव का अवरोधन और वायरस सीमित चलन (movement) संभवतः दो प्रति क्रियायें हैं जो प्रतिरोधक जातियों में रोग कम करने के उत्तरदायी हैं ।

SAMENVATTING

Tospovirussen veroorzaken wereldwijd grote economische verliezen in veel landbouw-, tuinbouw-, en siergewassen. In Zuid Azie is peanut bud necrosis tospovirus (PBNV) één van de schadelijkste virussen van de aardnoot (*Arachis hypogaea* L.). De ontwikkeling van resistente aardnootrassen is van groot belang om de opbrengstverliezen die PBNV veroorzaakt, terug te dringen. Volledige resistentie (immuniteit) is niet waargenomen in het genus *Arachis*. Wel zijn er genotypen gevonden met kwantitatieve vormen van resistentie, die tot uiting komt in een verlaagd percentage geïnfecteerde planten (incidentie). Deze kwantitatieve resistentie werd in dit proefschrift bestudeerd: er werden methoden ontwikkeld om deze resistentie te evalueren, de overerving werd bestudeerd, en de mechanismen van resistentie werden onderzocht.

Het verhogen van de plantdichtheid resulteerde in een verlaging van de incidentie in een resistent en in een vatbaar aardnootgenotype, maar het aantal zieke planten per oppervlakte eenheid nam toe. Een verhoging van de plantdichtheid kan derhalve niet aangeraden worden om de infectie met PBNV te verminderen. De evaluatie van resistentie in kleine veldjes was representatief voor de praktijksituatie, aangezien er geen significante inter-plot interferentie werd gevonden. Er werd wel een verhoogde PBNV infectie gevonden in het midden van het proefveld, en werd vermoedelijk veroorzaakt door een sterke voorkeur van de vector.

De kwantitatieve resistentie werd in tien milieu's in India getest. De volgorde van de rassen met betrekking tot de resistentie was ongeveer dezelfde in alle milieu's. Selectie in elk van deze milieu's zou hetzelfde resultaat opgeleverd hebben. Milieu's met een gemiddeld of hoog niveau van natuurlijke infectie onderscheiden de genotypen beter dan milieu's met een laag niveau van infectie. Op plaatsen met een lage PBNV infectie kan toch geselekteerd worden door de gegevens van twee of meerdere jaren te combineren.

Vijf resistente en twee vatbare aardnootgenotypen werden gekruist in een half-diallel om de overerving van de kwantitatieve resistentie tegen PBNV te kunnen bestuderen. Er konden vier resistentieniveau's onderscheiden worden die verklaard kunnen worden met minimaal drie resistentiefaktoren. Epistatische interacties waren niet aanwezig, en de resistentiefactoren erfden vermoedelijk additief over. Kwantitatieve resistentie verlaagde de incidentie in vijf resistente genotypen met ongeveer een factor vijf, vergeleken met JL 24 (vatbare controle). Van de twee vatbare rassen (JL 24 en TMV 2) had TMV 2 steeds een

lagere incidentie. Dit geringe niveau van kwantitatieve resistentie was effectief voor meer dan 50 jaar, en lijkt dus duurzaam te zijn.

Bij het mechanisch inoculeren van planten met een PBNV isolaat werd een duidelijke ouderdomsresistentie van de plant gevonden. Deze vorm van resistentie verlengde de incubatietijd en verlaagde de incidentie zeer sterk. De ouderdomsresistentie remde ook de PBNV epidemie in het veld af, en trad op onafhankelijk van de ziektedruk, stadium van de epidemie, snelheid van de epidemie, en het resistentieniveau van het waardplant genotype. Er werd een standaard inoculatie methode ontwikkeld, die gebruikt werd om de achterliggende mechanismen van resistentie te onderzoeken. De genotypen met veldresistentie waren allemaal resistent tegen het virus, behalve drie genotypen die net zo vatbaar waren als de vatbare controle. Er werd aangetoond dat deze veldresistente genotypen door minder thripsen gekolonialiseerd werden, dus kon aangenomen worden dat de veldresistentie van deze genotypen veroorzaakt werd door vectorresistentie.

Met behulp van deze methode kon worden aangetoond dat twee verschillende mechanismen een rol speelden bij de virusresistentie. Allereerst werd de virusvermeerdering geremd op de plaats van infectie in resistente genotypen. De virusvermeerdering werd niet geremd in systemisch geïnfecteerde bladeren van dezelfde genotypen. Tevens was de snelheid van systemische infectie lager in resistente genotypen. Hieruit volgt dat vermoedelijk virusvermeerdering en virusverspreiding de oorzaak zijn van de verlaagde ziekteincidentie in resistente genotypen.

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Hanneke (Anne Aloysia Maria) Buiel was born on the first day of 1963, in 's Gravenhage, The Netherlands. Between 1975 and 1981, she went to the Erasmus College, Zoetermeer, to obtain her VWO degree. She began her study on Plant Breeding at Wageningen Agricultural University in 1981. During this study she completed an 8-months research at the International Rice Research Institute, Los Baños, The Philippines, in 1986. After she received her 'ingenieur' degree in March 1988, she was employed by the 'Nederlands Graan Centrum' and stationed at the Institute of Plant Protection (IPO), Wageningen, for research on resistance in wheat to yellow stripe rust. In 1989 and 1990, she worked for another project of the 'Nederlands Graan Centrum', at the Center for Plant Breeding and Reproduction Research (CPRO) in Wageningen, on cross pollination of faba bean. From June 1990 to June 1995, she was employed by the Wageningen Agricultural University, for DGIS funded research on resistance to peanut bud necrosis virus in groundnut. The research, which resulted in this thesis, was carried out at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Asia Center at Hyderabad, India.