

# **CROP PROTECTION PROGRAMME**

## **FINAL TECHNICAL REPORT**

### **PROJECT R 6811:**

### **Groundnut rosette disease epidemiology**

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**Production System: Semi-Arid**  
**GEOGRAPHIC FOCUS: sub-Saharan Africa**  
**START DATE: Dec 1996**  
**FINISH DATE: March 1999**

## **EXECUTIVE SUMMARY**

The project's purpose was to improve the productivity of smallholder, groundnut production in sub-Saharan Africa through an understanding of the groundnut rosette disease epidemiology and the development of appropriate integrated management strategies based on host plant resistance to the virus and/or vector.

Groundnut cultivation has a direct bearing on the nutritional and economic status of smallholder farmers in sub-Saharan Africa because it is an important source of protein and oil and also provides cash income, contributing significantly to food security and poverty elimination.

Rosette disease, caused by the groundnut rosette virus complex, is the most destructive virus disease of groundnut in sub-Saharan Africa. The International Centre for Research in the Semi-Arid Tropics (ICRISAT) has estimated that rosette disease causes greater yield loss to farmers in the semi-arid tropics than any other virus disease affecting groundnut. Although chemical control and cultural practices are known to reduce risk of rosette, they are seldom adopted by smallholder farmers because of labour and capital constraints. Improved high yielding lines with resistance to the disease and/or the vector, may, however, contribute to low input rosette disease management strategies.

In 1995, the ICRISAT groundnut improvement programme in Malawi identified several high yielding, agronomically acceptable short-, medium- and long-duration varieties with good levels of resistance to rosette. This project has interacted directly with an ICRISAT initiative to understand the epidemiology of groundnut rosette virus disease with particular reference to the potential impact of selected rosette resistant lines. The work has been of a strategic nature because little is known about the interactions between the disease agents, vector and host plants.

Specific outputs include

- the development of a new method to detect the three agents of the rosette disease complex in the plants and aphid vectors by Reverse Transcription-Polymerase Chain Reaction (RT-PCR).
- an improved understanding of the transmission efficiency of the three causal agents of rosette disease by the aphid vector on a range of groundnut genotypes including rosette-resistant and vector resistant lines under field and laboratory conditions.
- the identification of vector resistance in the short duration line ICG 12991.
- An assessment of yield loss due to the aphid transmissible, groundnut rosette assistor virus (GRAV) in a range of groundnut genotypes.

The outputs are being and will be used by breeders at ICRISAT to develop improved groundnut genotypes with a range of resistance mechanisms to either the causal agents of rosette disease and/or the vector. It is envisaged that a second phase involving a farmer participatory project using the promising rosette disease - and vector resistance will be developed with ICRISAT in Uganda at the Serere Agricultural and Animal Production Research Institute based at Soroti.

## **BACKGROUND**

Groundnut rosette disease is endemic to sub-Saharan Africa and its off-shore islands. The Working Group on Groundnut Virus Diseases in Africa, held in Pretoria in March 1996, confirmed that rosette disease continues to be a severe constraint to groundnut production in sub-Saharan Africa. The Working Group also recommended that host-plant resistance to the virus complex and/or vector should be improved because it is regarded by many African national programme and Non Governmental Organisations to be the most likely effective management for smallholder farming systems. The Southern African Development Community (SADC)/ICRISAT Groundnut Project places major emphasis on the development of rosette-resistant lines, particularly short duration varieties which are frequently preferred by farmers, but which have not hitherto been available. ICRISAT economists have estimated that the value of the potential yield gain through rosette disease management is 121 million US dollars per annum.

Earlier work (funded by DFID) at the Scottish Crop Research Institute (SCRI) and

ICRISAT has shown that rosette disease has a complex etiology involving three agents: groundnut rosette assistor luteovirus (GRAV), groundnut rosette umbravirus (GRV) and a satellite RNA (sat RNA) of GRV. The three agents are intricately dependent on each other and all play a crucial role in the biology and perpetuation of the disease. Variants of sat RNA cause different forms of the disease, whereas GRAV or GRV alone cause asymptomatic infections. GRAV acts as a helper virus in aphid transmission in that GRV RNA, which does not code for a coat protein, and sat RNA are packaged in the coat protein of GRAV to form virus particles that are aphid transmissible. The sat RNA depends upon GRV for replication and GRV, although autonomous, depends upon sat RNA for aphid transmission since it has been shown that the presence of sat RNA in the source plant is essential for the packaging of the GRAV-dependant transmission of GRV.

*Aphis craccivora* Koch. is the principal aphid vector of rosette disease agents. It is widely distributed throughout the world but in the tropics only females have been recorded and these reproduce parthenogenetically throughout the year. Since the viral agents of the disease are not seed borne they must be introduced into a groundnut crop by viruliferous aphids. The source of viruliferous aphids that initiate rosette disease (primary infection) is unknown and the biotic and abiotic factors associated with the development of disease epidemics are poorly understood. In this project, however, the detailed study of the transmission characteristics of the three causal agents by the aphid vector in laboratory and field conditions, has assisted in improving our understanding of rosette disease spread. A detection method based on RT-PCR to detect GRV and sat RNA, in both the aphid vector and groundnut was developed to assist this study.

The Working Group on Groundnut Virus Diseases also recommended that the epidemiology of the disease, particularly the identity and role of dry-season hosts in the disease cycle, should be examined using diagnostic tests for the three causal agents of rosette disease and that the management of the disease should be improved through the development of high-yielding, short-duration types with durable resistance to the virus agents, vector and/or both. Work on the identity of dry-season hosts of the disease agents was initiated at ICRISAT-Lilongwe but staff were not replaced during the course of the project and this objective could not be met. The virus-vector interactions on a range of genotypes and genotypes, however, was completed in collaboration with ICRISAT staff Dr R.A.Naidu, Dr P Subrahmanyam and the groundnut breeder, Dr P van der Merwe based at Chitedze, Malawi.

Sources of resistance to rosette disease were first identified in groundnut landraces of the late maturing Virginia type (*Arachis hypogaea* ssp. *hypogaea*, collected from Burkina Faso and Cote D'Ivoire, but not from the early maturing Spanish group (*A.hypogaea* spp *vulgaris*). These sources of resistance have been used in breeding programmes and resulted in the development of late maturing disease resistant genotypes (e.g. RMP12, RMP91 etc.). Resistance among these genotypes was found to be effective against both green and chlorotic rosette and governed by two independent recessive genes. The major disadvantage of these long maturing lines is that they require a long growing season (150-180 days) to attain maturity making them susceptible to drought at the end of the season. The challenge was to combine rosette resistance with early maturing (90-110 days), Spanish types which would be suitable for smallholder farmers in sub-Saharan Africa. A breeding and screening programme by the SADC/ICRISAT Groundnut Project, using an infector row technique, has resulted in the identification of several high yielding, agronomically acceptable short-, medium-, and long duration Virginia types with good levels of resistance to chlorotic rosette. However in all rosette-resistant genotypes and germplasm lines which have been analysed to date, resistance has been found to groundnut rosette virus (GRV). Three short duration lines (ICG-12991, ICG-12998 and ICG-2) which showed field resistance to rosette disease were evaluated using the new diagnostic tools.

## **PROJECT PURPOSE**

**Indicative output for the CPP Semi-Arid Production System Purpose 2:** Variability of economically significant viruses in cereal-based cropping systems and their interaction with

vector species identified and incorporated into improved disease control strategies.

The CPP component of the groundnut rosette disease epidemiological study focused on understanding the interaction between the transmission of the three causal agents of rosette disease by the aphid vector in a range of virus and vector resistant genotypes, particularly short duration lines which are less susceptible to drought and which can contribute to food security for smallholder farmers.

## **RESEARCH ACTIVITIES**

### **Output 1. Correlation between the infectivity of the aphid vector and the detection of the components of the rosette virus complex determined.**

Activity 1.1 Development of RT-PCR methodology to detect all three agents of rosette

This activity was carried out at NRI and with Dr D Robinson at the Scottish Crops Research Institute. The facilities were not available in Malawi, although a new facility is being developed at Chitedze. Techniques are described in:

Naidu R.A., D. J. Robinson and F.M. Kimmins 1998. Detection of each of the causal agents of groundnut rosette disease in plants and vector aphids by RT-PCR. *Journal of Virological Methods* 76 9-18. Further details provided under Output section.

Activity 1.2 Surveys of rosette disease incidence in central and northern Malawi and collection of rosetted plants and aphids for transmission studies.

CPP staff were guests of ICRISAT-Malawi and Chitedze Research Station during field surveys and visits to Chitedze. RT-PCR facilities at NRI were used to detect all three components in field collected material. Field screening facilities were also provided by ICRISAT-MALAWI and Chitedze Agricultural Research Station for activities 4 and 5 in 1998/99.

### **Output 2. The transmission efficiencies of the green and chlorotic forms of rosette disease by the aphid vector in selected lines described.**

Activity 2.1 Evaluation of virus and vector resistance in groundnut genotypes with desirable agronomic characteristics.

The detailed studies of aphid performance and virus transmission were carried out at ICRISAT-MALAWI, Chitedze. Serological methods used to detect GRAV alone was also carried out at Chitedze.

*Output 3. The feeding behaviour and transmission efficiencies of aphid clones on at least one alternative host plant species described. This output was not achieved as the ICRISAT post doctoral fellow recruited to investigate alternative host plant species was not replaced.*

#### **Add-on outputs**

### **Output 4 Quantitative resistance to GRAV identified among short duration rosette disease resistant cultivars**

Activity 4 Disease progress and multiplication of GRAV measured over time in a range of groundnut cultivars with acceptable agronomic traits and field resistance to rosette disease.

This was completed at ICRISAT-MALAWI together with activity 5.

### **Output 5. The effect of GRAV infection on yield estimated on a range of rosette resistance genotypes**

Activity 5 Field trials established to obtain accurate estimates of yield loss to GRAV infection for at

least four groundnut genotypes with acceptable agronomic traits and rosette disease resistance.

## **OUTPUTS**

### **OUTPUT 1. CORRELATION BETWEEN THE INFECTIVITY OF THE APHID VECTOR AND THE DETECTION OF THE COMPONENTS OF THE ROSETTE VIRUS COMPLEX DETERMINED**

#### **Activity 1.1 Development of RT-PCR methodology to detect all three agents of rosette**

Paper available. Naidu R.A., D. J. Robinson and F.M. Kimmins 1998. Detection of each of the causal agents of groundnut rosette disease in plants and vector aphids by RT-PCR. *Journal of Virological Methods* 76 9-18.

Three procedures for extraction of total RNA from groundnut were tested, of which two were found to be useful in giving RNA of sufficient quality for RT-PCR. Of these two, the total RNA extraction kit supplied by Qiagen was found to be the most versatile for extraction of all three agents from individual vector aphids (*Aphis craccivora*).

Both groundnut rosette assistor virus and groundnut rosette virus could be detected from total RNA extracted from a single aphid that had been exposed to either green or chlorotic rosette-infected groundnut plants. They could be detected in aphids stored in 70% ethanol for up to 30 days at room temperature. However, satellite RNA could be amplified only when total RNA extracted from two or more aphids was used. The inability to amplify sat RNA from a single aphid might be due to its being present in low concentration. Alternatively, some properties of the sat RNA, such as secondary structure, may make reverse transcription inefficient. However, this scarcely limits the usefulness of the RT-PCR test. Indeed, our tests suggest that aphids that contain GRV RNA also contain sat RNA; this would not be unexpected, because the presence of sat RNA is required for aphid transmission of GRV.

Groundnut rosette assistor virus, groundnut rosette virus and its satellite RNA were detected by RT-PCR in aphids that had been exposed only to groundnut rosette diseased plants containing all three agents.

The results also provided unequivocal evidence that not all plants in the farmers fields that show rosette symptoms contain GRAV. *A. craccivora* exposed to rosette diseased plants lacking GRAV did not acquire GRV RNA and sat RNA. Consequently, such plants can not serve as sources of inoculum and thus remain “dead ends” of the disease with no epidemiological significance. These observations also stress the need to use a specific test, such as RT-PCR, when assessing the relationship between disease incidence, proportion of infective aphids in a given population at a given time, and field spread of the disease.

As well as producing a robust test for the detection of all three agents, these results also suggest that GRAV can be separated from GRV and its sat RNA in time and space. How this happens required further study and also raises the fundamental question of whether a single aphid can transmit both particles containing GRAV RNA and those containing GRV RNA and sat RNA. Serological methods, which detects only GRAV coat protein, cannot answer such questions. By using RT-PCR, it is now possible to address these issues and understand their implications in rosette disease epidemics.

#### **Activity 1.2 Surveys of rosette disease incidence in central, northern and southern Malawi and collection of rosetted plants and aphids for transmission studies.**

Published paper available Naidu, R.A., H Bottenburg, F.M.Kimmins, D.J.Robinson and J.M.Thresh. 1998. Epidemiology of groundnut rosette virus disease: current status and future research needs. *Annals of Applied Biology* 132: 525-548.

Paper in press. Naidu, R.A., Kimmins, F.M., Holt, J., Robinson, D.J., Deom, C.M., and Subrahmanyam, P. 1999. Spatio-temporal separation of groundnut rosette virus disease agents. *Phytopathology*. Accepted March 18 1999.

During each field visit to ICRISAT-Lilongwe, surveys were undertaken in either the central and southern or northern regions of Malawi. Groundnut plants showing chlorotic rosette symptoms were found in the three regions, but plants showing green rosette symptoms were only located in the northern region beyond the Nyika Plateau. This suggested that aphid vectors do not move with the green form of the disease from the north to the central and southern regions of Malawi, although movement from the south to the north could occur. Attempts to test this proposal are underway by using research funds from the University of Greenwich. Some of the plants showing clear rosette symptoms (both green and chlorotic forms) did not give a positive reaction with the triple-antibody sandwich (TAS) enzyme linked immunosorbent assay (ELISA) test indicating that they did not contain GRAV. Also some of the apparently symptomless plants in the field gave positive results with TAS-ELISA showing that they contained GRAV but not GRV or sat RNA (see activities 1.1 and 2.1).

Analysis by TAS-ELISA of groundnut samples from farmers' fields in two seasons from different regions of Malawi showed the absence of groundnut rosette virus (GRAV) from a certain percentage of plants showing rosette symptoms and the presence of GRAV in some symptomless plants. Viruliferous *Aphis craccivora* collected from farmers' fields transmitted GRAV alone, groundnut rosette virus (GRV) and its satellite RNA (sat RNA), or all three agents together, in different proportions. More plants became infected with all three agents when increased numbers of viruliferous aphids were used per plant, suggesting a dosage response for infection. Electrical Penetration Graph (EPG) studies showed successful transmission of GRV and its sat RNA during both the stylet pathway phase and salivation into sieve elements, whereas GRAV was transmitted only during the latter phase. However, aphids transmitted GRAV alone, GRV and its sat RNA, or all three agents together during the salivation phase. RT-PCR testing of viruliferous aphids and of inoculated plants revealed no correlation between the presence of rosette disease agents in vector aphids and their transmission to groundnut plants. These results show that separation of the groundnut rosette disease agents occurs over time and space.

Groundnut rosette is regarded as a polycyclic disease because it spreads from primary sources of inoculum whose number increases during the growing season as progressively increasing numbers of plants become sources. Thus the number of groundnut plants in the field with primary infections containing all three agents, the conditions which lead to development of vector progeny on these primarily infected plants, the density and transmission efficiency of infective vector populations in a given field, and the number and frequency of inoculation events all influence whether all three agents are inoculated into each plant subsequently infested by these aphids.

**OUTPUT 2. THE TRANSMISSION EFFICIENCIES OF THE GREEN AND CHLOROTIC FORMS OF ROSETTE DISEASE BY THE APHID VECTOR IN SELECTED LINES DESCRIBED.**

**Activity 2.1 Evaluation of virus and vector resistance in groundnut genotypes with desirable agronomic characteristics.**

Paper in press. R.A. Naidu, Kimmins F.M., Deom C.M., Subrahmanyam P., Chiyembekeza A.J., and van der Merwe P.J.A. Groundnut Rosette: A virus disease affecting the sustainability of groundnut production in sub-Saharan Africa. Plant Disease. Accepted Dec 1998. To be published in August 1999.

Paper in prep.

In rosette screening field trials, groundnut line ICG-12991 consistently showed lower rosette disease incidence and GRAV infection compared to the genotypes JL-24 and CG-7. This lines also possessed good agronomic qualities, such as high yield, tan seed colour, two seeded etc., as well as resistance to rosette (van der Merwe and Subrahmanyam 1997). Under low disease pressure it significantly outyielded the other varieties including the widely adapted JL24. The higher yield obtained under high disease pressure was ascribed to resistance of ICG 12991 to rosette virus disease. Only 1% rosette disease was observed on this line compared with 45% incidence on JL 24.

Grafting of ICG-12991 (with infected scion from a susceptible genotype), however, resulted in clear rosette symptoms and presence of GRAV, so it was totally susceptible to the disease agents (GRAV, GRV and sat RNA). This was a surprising finding as it had been assumed that ICG-12991 was resistant to the rosette viruses including GRAV. An alternative explanation for the field results was that aphid resistance rather than virus resistance may play a role in preventing the inoculation of the disease agents into ICG 12991. To test this hypothesis, aphid survival and reproduction was measured on plants of the four genotypes (CG-7, ICGV-SM 90704, JL-24 and ICG-12991) over a 15 day period starting with plants aged 7 days after sowing (das), 11 das and 28 das. The results showed that aphid survival and fecundity was markedly lower on ICG-12991 than on the other three genotypes at all ages (Table 1).

**Table 1 Fecundity of *Aphis craccivora* on four groundnut genotypes with agronomically acceptable characteristics.**

Cultivar	Age	Mean no of days to reproduction	se	n	Total no of offspring in 1st five days of reproduction	se	n
JL24	7-22	6.00	0.09	23	49.20	1.41	22
	11-26	6.27	0.12	22	34.43	1.42	21
	28-43	6.62	0.16	21	37.80	0.98	20
CG7	7-22	6.47	0.10	21	44.43	1.60	21
	11-26	6.22	0.11	23	35.86	1.87	21
	28-43	6.45	0.18	20	36.33	1.98	21
90704	7-22	5.91	0.09	22	43.36	1.93	22
	11-26	6.24	0.10	21	35.45	1.34	21
	28-43	6.42	0.18	19	28.42	2.5	19
<b>12991</b>	<b>7-22</b>	<b>6.47</b>	<b>0.14</b>	<b>19</b>	<b>25.20</b>	<b>1.87</b>	<b>10</b>
	<b>11-26</b>	<b>7.67</b>	<b>0.17</b>	<b>9</b>	<b>14.70</b>	<b>1.35</b>	<b>6</b>
	<b>28-43</b>	<b>8.55</b>	<b>0.16</b>	<b>11</b>	<b>15.00</b>	<b>0.00</b>	<b>2</b>

The feeding behaviour of the aphid on three ages (7-8, 11-14 and 28-33 das) of JL-24 and ICG-12991 (Spanish types) was also studied in detail using the Electrical Penetration Graph (EPG). Significantly fewer aphids on all ages of ICG-12991 located phloem ( $\chi^2_{(5)} = 30.0$ ;  $P < 0.001$ ), as represented by the E1 pattern, during the 2 hr test period compared with aphids on all ages of JL-24 (Figure 1). The few aphids that initiated phloem penetration did not maintain sustained ingestion and only lasted for 1-2 minutes. On JL-24, however, 80% of the aphids on plants aged 7 and 14 das located the phloem within 52 mins of access to the plant.

This interval was similar to that previously recorded for aphids on JL-24, CG-7, ICGV-SM-90704, EC 36892 and ICGV-SM-93535 (field visit Nov 1997). The aphids also maintained phloem penetration and ingestion for the duration of the test period (range 19-101 mins). A resistance mechanism in ICG-12991 which hinders phloem location in leaf tissue and therefore inoculation of the virus complex has been proposed. Whilst phloem location in leaf tissue of ICG-12991 was hindered, aphids were able to locate the phloem in flower stems of the same plant. This suggests a non-phloem based mechanism and chemical analyses of these tissues would provide valuable information on the mechanism of resistance.

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**Figure 1 Percentages of aphids producing EPG patterns E1 and E2 during a 2hr recording period on two groundnut genotypes.**

The groundnut breeder at ICRISAT- Lilongwe, Dr. P. van der Merwe, is extremely interested in exploiting the aphid resistance in ICG-12991 by combining it with the known resistance to GRV and sat RNA in ICGV-SM 90704. ICRISAT would appreciate a simplified screening methodology to identify aphid resistance in segregating lines produced from crosses between aphid and virus resistance genotypes.

***OUTPUT 3 THE FEEDING BEHAVIOUR AND TRANSMISSION EFFICIENCIES OF APHID CLONES ON AT LEAST ONE ALTERNATIVE HOST PLANT SPECIES DESCRIBED.***

*NOT ACHIEVED AS ICRISAT DID NOT APPOINT POST-DOCTORAL FELLOW TO IDENTIFY ALTERNATIVE HOSTS.*

**ALTERNATIVE OUTPUT 4. QUANTITATIVE RESISTANCE TO GRAV IDENTIFIED AMONG SHORT-DURATION ROSETTE DISEASE RESISTANT GENOTYPES.**

Paper in preparation.

During routine screening of materials in rosette disease trials, the level of GRAV accumulation, as indicated by O.D. values in ELISA varied (data not shown). Studies were required to understand more precisely the relative susceptibility to GRAV and to find out whether quantitative resistance to GRAV multiplication exists among these accessions. The benefits of exploiting such quantitative resistance to GRAV are that plants with low levels of GRAV would be poor sources of virus for acquisition by the aphid vector and in the field the amount of virus spread from infected plants would be considerably lower than from plants susceptible to virus multiplication.

Culture plants containing only GRAV were established in the glasshouse at Chitedze and 'clean' aphids collected from the Chitedze culture were placed on these plants for 72h. After that period, aphids were removed and placed on seedlings (7 das) of four test cultivars (eight adults per plant) for a further 72hrs. The inoculated seedlings were sprayed with Actellic, planted in a field plot (4 rows, 3m long) and sprayed every 21days with Actellic to

kill insect pests and avoid infection from immigrant, viruliferous aphids.

60 days after aphid inoculations, leaf samples were taken from 20 individual plants at random and the presence of GRAV was tested by the triple sandwich antibody form of enzyme linked immunosorbent assay (TAS-ELISA) as described by Rajeshwari, Murant and Massalski 1987.

**Table2: Percent infection with GRAV (as tested by TAS-ELISA) among four cultivars.**

Replication #	CG-7	ICGV-SM-90704	JL-24	ICG-12991
1	17/20	11/20	18/20	3/20
2	17/20	14/20	15/20	5/20
3	16/20	17/20	17/19	1/20
4	18/20	13/20	19/20	2/20
Mean	17/20 <b>(85.00%)</b>	13.75/20 <b>(68.75%)</b>	17.25/19.75 <b>(87.34%)</b>	2.75/20 <b>(13.75%)</b>

The results in Table 2 show the incidence of GRAV was much lower in ICG12991 than in any of the other genotypes with desirable agronomic traits, but as the previous activity has demonstrated this is due to vector resistance which appears to play a valuable role in preventing the aphids from inoculating the plants with the rosette complex.

The information on O.D. values is still being calculated but initial analysis indicates that low values were consistently recorded from ICGV-SM-90704 (Table 3a and b)

**Table 3a and b: ELISA values (OD<sub>405</sub>) from GRAV yield gap trial**

**3a**

**Trial 1:**

Cultivar	Inoculated	Uninoculated
CG-7	1.300+1.276+1.294+1.116= <b>1.247</b>	0.008+0.008+0.008+0.005= <b>0.007</b>
ICGV-SM-90704	0.797+0.909+0.475+0.810= <b>0.748</b>	0.007+0.010+0.009+0.009= <b>0.010</b>
JL-24	1.690+1.531+1.890+1.385= <b>1.624</b>	0.007+0.012+0.009+0.008= <b>0.009</b>
ICG-12991	2.765+2.553+2.889+3.012= <b>2.804</b>	0.005+0.006+0.003+0.002= <b>0.006</b>

**3b**

**Trial 2:**

Cultivar	Inoculated	Uninoculated
CG-7	0.943+1.014+1.197= <b>1.051</b>	0.011+0.012+0.010= <b>0.011</b>
ICGV-SM-90704	0.666+0.542+0.559= <b>0.589</b>	0.009+0.011+0.005= <b>0.009</b>
JL-24	0.465+0.928+1.547= <b>0.980</b>	0.007+0.012= <b>0.010</b>
ICG-12991	2.303= <b>2.303</b> (only one sample)	0.009+0.008+0.010= <b>0.009</b>

The results suggest that ICGV-SM-90704 is not only a source of resistance to GRV and its sat RNA, but that it also supports a lower titre of GRAV. This moderate resistance could be extremely useful in future breeding trials as lower titres of GRAV could reduce field to field and with field spread of the disease by aphids. Lines developed from this cultivar will be monitored carefully in future trials.

**OUTPUT 5. THE EFFECT OF GRAV INFECTION ON YIELD ESTIMATED ON A RANGE OF ROSETTE RESISTANCE GENOTYPES**

Paper in preparation.

This experiment, using four genotypes with good agronomic characteristics (JL-24, CG-7,

ICG-12991 and ICGV-SM 90704), was completed at the end of March and data are being analysed.. Test plants only containing GRAV were prepared as described above in Output 4 before transfer to a field plot. After 90 days after inoculation measurements of plant height, GRAV detection by TAS-ELISA optical density measurements and yield were collected. The yield results are presented below.

**Table 4 : Effect of GRAV infection on yield of four groundnut cultivars (I=inoculated; H=healthy)**

Cultivar	Haulm wt (kg/plant)		Pod wt (kg/plant)		Seed wt (kg/plant)	
	I	H	I	H	I	H
CG-7	0.050	0.078	0.025	0.079	0.013	0.034
	0.064	0.064	0.037	0.082	0.017	0.037
	0.046	0.075	0.022	0.074	0.012	0.037
	0.044	0.068	0.030	0.063	0.013	0.026
	<b>0.051</b> <b>(-28.17%)</b>	<b>0.071</b>	<b>0.029</b> <b>(-61.33%)</b>	<b>0.075</b>	<b>0.014</b> <b>(-58.82%)</b>	<b>0.034</b>
ICGV-SM-90704	0.059	0.076	0.040	0.051	0.021	0.022
	0.069	0.073	0.044	0.069	0.020	0.030
	0.044	0.054	0.029	0.038	0.012	0.016
	0.041	0.050	0.037	0.047	0.013	0.018
	<b>0.053</b> <b>(-15.81%)</b>	<b>0.063</b>	<b>0.038</b> <b>(-26.47%)</b>	<b>0.051</b>	<b>0.017</b> <b>(-23.26%)</b>	<b>0.022</b>
JL-24	0.040	0.048	0.094	0.139	0.016	0.030
	0.035	0.050	0.104	0.142	0.020	0.030
	0.036	0.046	0.068	0.126	0.014	0.026
	0.036	0.045	0.076	0.136	0.028	0.029
	<b>0.037</b> <b>(-21.28%)</b>	<b>0.047</b>	<b>0.088</b> <b>(-35.29%)</b>	<b>0.136</b>	<b>0.020</b> <b>(-31.03%)</b>	<b>0.029</b>
ICG-12991	0.035	0.065	0.051	0.162	0.024	0.035
	0.032	0.042	0.072	0.155	0.016	0.034
	0.030	0.037	0.045	0.079	0.010	0.020
	0.027	0.053	0.088	0.120	0.011	0.026
	<b>0.031</b> <b>(-36.74%)</b>	<b>0.049</b>	<b>0.064</b> <b>(-50.39%)</b>	<b>0.129</b>	<b>0.015</b> <b>(-46.43%)</b>	<b>0.028</b>

The preliminary results indicate that yield is reduced by between 25-60% depending on the cultivar used. The lowest yield loss of -23% was recorded on ICGS-SM-90704, the genotype which is resistant to GRV and sat RNA. This result has important implications for the release of resistant groundnut genotypes such as ICGV-SM 90704, since in the event of high rosette disease pressure i.e during an epidemic, there will still be significant yield loss on this genotype because it is susceptible to GRAV. Only low numbers of plants of ICG 12991 were infected with GRAV presumably because it could not be inoculated into the plants by the aphids as a result of aphid resistance (see Output 2, Table 1, Output 2). In the absence of any genotypes with GRAV resistance, further work on the nature of resistance in ICG-12991 and its inheritance is needed because it is proposed to develop durable resistance by combining vector resistance with the GRV resistance.

## CONTRIBUTION OF OUTPUTS

The project to date has produced new tools for the detection of all three agents of rosette disease and has used them to describe the spread of chlorotic rosette in farmers fields. The results are also being used to identify and develop rosette and vector resistant genotypes with agronomically acceptable characteristics (short duration, high yielding etc.). The combination of virus and aphid resistance in the preferred 'Spanish' groundnut genotypes should lead to the development of groundnut cultivars with durable rosette resistance which are readily adopted by

farmers. The adoption of these lines by small holder farmers in sub-Saharan Africa will not only reduce crop losses caused by rosette disease but will also contribute significantly to household food security and poverty alleviation in this region.

An adaptive phase is needed to promote these new genotypes and demand for improved groundnut lines with rosette resistance has been identified in Uganda. In 1998, DFID financed a needs assessment exercise in the Teso farming system, Uganda. Rosette disease was identified as one of the most important crop pest/disease problems on groundnuts both by farmers and SAARI (Serere Agricultural and Animal Production Research Institute based at Soroti). It is proposed that a DFID-CPP funded collaborative project between NRI, SAARI and ICRISAT based on selection of appropriate planting materials, on-farm participatory trials, should be developed as a follow up project together with the development of a rapid screening test for aphid resistance. NGOs in the location (AT Uganda, Sokadido) have expressed interest in receiving information and improved groundnut lines for farmer distribution. They are perceived as a dissemination route for farmer preferred material.

## **PUBLICATIONS TO DATE**

During the project, one review article and one technical paper have been published; a second review article has been accepted for publication; a second technical paper has also been accepted and two more are in preparation.

Paper copies of these publications are appended to the original hard copy of the FTR, retained by the Programme Manager in NR International, from whom limited copies may be obtained. Electronic copies are not available.

### **Reviews**

R.A.Naidu, H.Bottenburg, P Subrahmanyam, F.M.Kimmins, M.J.Thresh and D.J.Robinson. **1998a**. "Epidemiology of groundnut rosette virus disease; review of current status and research needs." *Annals of Applied Biology* 132: 525-548. **Reprint attached.**

R.A. Naidu, F.M. Kimmins, C.M. Deom, P. Subrahmanyam, A.J. Chiyembekeza, and P.J.A. van der Merwe **1999a**. "Groundnut Rosette: A Virus Disease Affecting the Sustainability of Groundnut Production in Sub-Saharan Africa." accepted for publication in *Plant Disease* in November 1998. Publication date July 1999.

### **Papers**

R.A.Naidu, D.J.Robinson and F.M.Kimmins. **1998b** "Detection of the causal agents of groundnut rosette disease complex in plants and aphid vectors". *J.Virological Methods* 76:9-18. **Reprint attached.**

R.A. Naidu, F.M. Kimmins, J. Holt, D.J. Robinson, C.M. Deom, and P. Subrahmanyam. **1999b**. "Spatio-Temporal Separation of Groundnut Rosette Virus Disease Agents" Accepted following modifications by *Phytopathology*. April 1999.

### **In preparation**

Naidu, Kimmins, Subrahmanyam and van der Merwe "Aphid resistance in groundnut and its impact on rosette disease".

Naidu, Kimmins, Subrahmanyam and van der Merwe "Yield loss in rosette resistant lines".