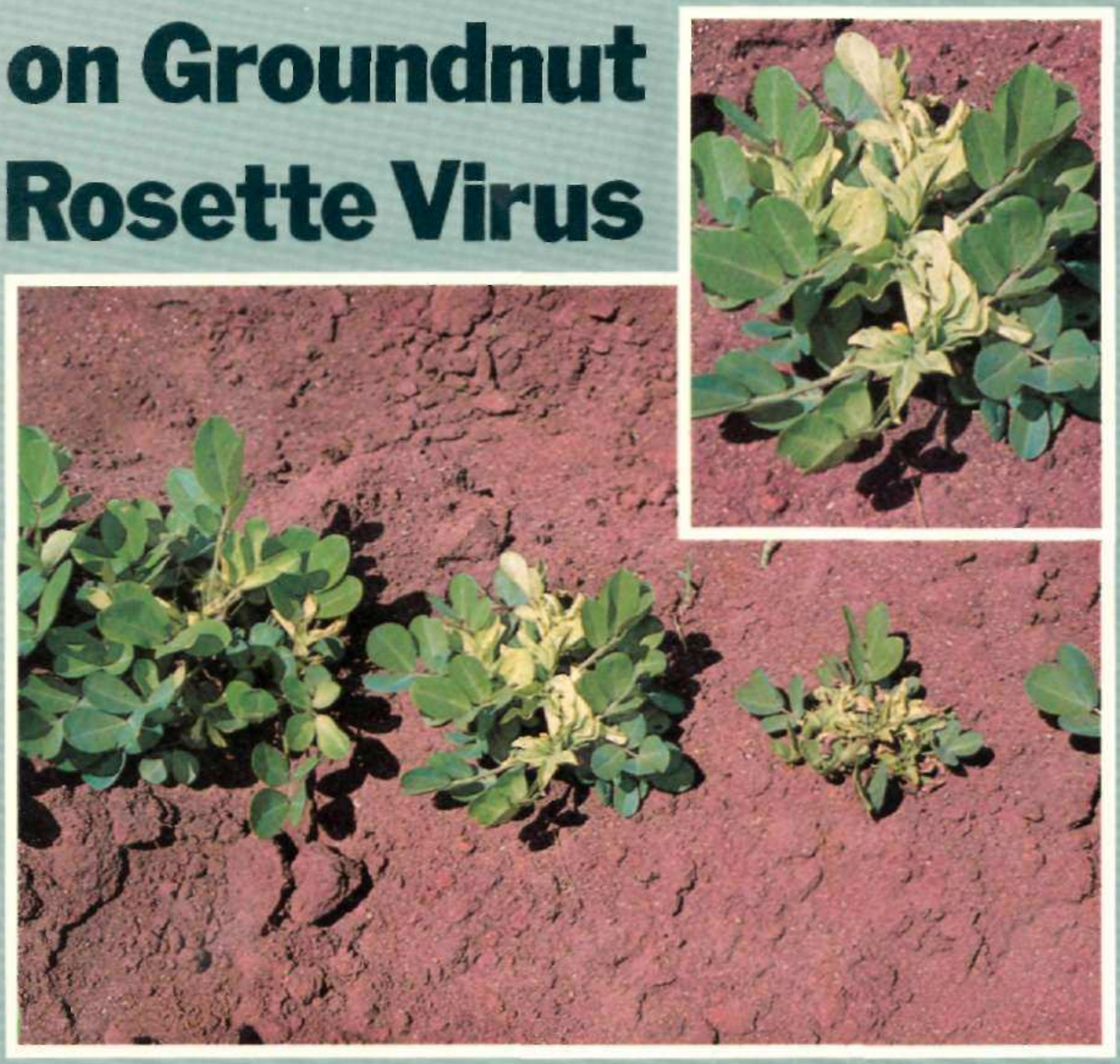
Collaborative Research on Groundnut Rosette Virus



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Cover: A row of groundnut plants infected by chlorotic rosette (Inset: a detailed close-up).

Collaborative Research on Groundnut Rosette Virus

Summary Proceedings
of the Consultative Group Meeting
to Discuss Collaborative Research
on Groundnut Rosette Virus Disease
held at Cambridge, England, UK
13-14 April 1985

ICRISAT
International Crops Research Institute for the Semi-Arid Tropics
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Opening Address and Objectives of the Meeting

R.W. Gibbons[1]

I welcome you all to this meeting on groundnut rosette virus disease. Several of you participated in the May 1983 rosette meeting organized in the USA by Peanut Collaborative Research Support Program (Peanut CRSP), and the usefulness of that meeting will be emphasized when we consider the very considerable advances made by the different research groups over the past 2 years.

It is fortunate that so many of you traveled to Cambridge to participate in the Association of Applied Biologists' meeting on 'New Developments in Techniques for Plant Virus Detection', held during 10-12 April 1985 as this enabled us to obtain the maximum number of participants for our rosette meeting on a restricted budget. We are fortunate that Dr A.J. Gibbs and Mrs K.F. Boswell have been able to attend, and are willing to explain their Virus Identification and Data Exchange (VIDE) project for the benefit of those of us who did not attend the Association of Applied Biologists' meeting. We are also pleased to have Dr K.H. Garren attending the meeting as our observer for Peanut CRSP Review.

The importance of groundnut rosette virus disease in West, East, and southern Africa has long been recognized and, from the start of our ICRISAT Groundnut Improvement Program in 1976, we have given high priority to the problem. It was soon found that many of the reports of rosette from outside Africa were based on incorrect identifications and we have not been able to find groundnut rosette virus disease outside the African continent. Plant quarantine regulations prevent our working on the disease in ICRISAT Center, but we have been able to do useful research on the identification and characterization of the causal viruses, through collaborative projects in the Federal Republic of Germany and the United Kingdom. Several of the cooperating scientists are fortunately present at this meeting. Our collaboration with the Peanut CRSP group, and the Institute for Agricultural Research of Ahmadu Bello University, Samaru, in Nigeria, has also proved to be valuable. In 1982, we established the ICRISAT Regional Groundnut Program for Southern Africa based in Malawi, and Drs K.R. Bock, as pathologist, and S.N. Nigam, as breeder, are devoting a considerable portion of their time to research on the epidemiology of groundnut rosette virus disease, and to breeding rosette-resistant groundnut cultivars.

The objectives of our meeting are to bring together representatives of the various research groups to discuss their recent findings on rosette, and their plans for future research. It is important to avoid expensive duplication of research, and even more important to develop a coordinated research approach to fully identify and characterize the components of the virus complex, and to develop diagnostic aids to assist with epidemiological investigations and with resistance breeding. The existence of a coordinated international collaborative plan for research on the rosette problem may well assist the individual research groups in obtaining funds to continue their work. It will also help to organize collection and transportation of diseased materials. There may also be possibilities to exchange staff, and train staff with mutual benefits. I am sure that over the 2 days of this meeting we can achieve these objectives.

Thank you.

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Summaries
of
Papers

Groundnut Rosette Virus Disease: The Present Situation and Research Needs

D.V.R. Reddy[1]

Background and Present Situation

Groundnut rosette virus disease, first reported by Zimmermann in 1907, is recognized as the most important virus disease of groundnut (Arachis hypogaea L.) in Africa south of the Sahara, including Madagascar. Several reports have appeared on the various types of rosette disease, and on management of the disease by cultural practices, spraying with aphicides, and utilizing host-plant resistance. By 1983, only three reports had been published on the causal viruses of groundnut rosette.

In 1975, rosette appeared on over 1 million ha of groundnuts in Nigeria, the overall loss in yield being estimated at around 560 000 tonnes. It is currently recognized as the most destructive of all groundnut viruses in Africa.

Groundnut rosette is transmitted by Aphis craccivora Koch., and the virus/vector relationship, first investigated nearly 30 years ago, has been shown to be of the persistent type. Although some workers achieved successful sap transmission of groundnut rosette virus, others were unable to transmit groundnut rosette virus by mechanical sap inoculation. Groundnut rosette virus can be readily transmitted by graft inoculation.

Prior to 1983, very little was known about the causal viruses of groundnut rosette disease. Rosette-infected plants were presumed to contain two viruses, one of which, groundnut rosette virus (GRV), was the major or only cause of symptoms in groundnut but was dependent on the other, groundnut rosette assistor virus (GRAV), for transmission by aphids.

ICRISAT's interest in research on rosette started with surveys on groundnut virus diseases in African countries in 1981. Since most countries where rosette disease occurred did not have fully equipped plant virus laboratories, it was considered essential for effective research on this problem to establish cooperative links with plant virus research units in technically advanced countries, where groundnuts were not grown.

In 1981, rosette-infected groundnut materials were processed in the virus unit of the Institute for Virus Research in Braunschweig, Federal Republic of Germany, by Dr R. Casper and his colleagues. In 1982, an ICRISAT Research Associate was sent to work in Braunschweig for 3 months to assist in characterizing the causal viruses. A luteovirus, serologically related to beet western yellows virus (BWYV) and to potato leaf roll virus (PLRV), was detected in both chlorotic and green rosette-infected groundnuts and was shown to be GRAV. Since the GRAV failed to produce typical groundnut rosette virus disease symptoms, it was evident that additional work would be necessary to isolate and characterize the symptom-inducing GRV.

In 1982, the U.S. Peanut CRSP initiated a project on the identification of groundnut viruses in Nigeria, with cooperation from scientists at the Institute for Agricultural Research (IAR) of Ahmadu Bello University, Samaru, Zaria, Nigeria. Characterization and diagnosis of the causal viruses of groundnut rosette were

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given high priority. Consistent mechanical transmission of the symptom-inducing GRV component was achieved for the first time. Absence of luteovirus particles from plants manually inoculated with the virus and their presence in aphid-transmitted and field-infected, rosetted groundnut plants confirmed earlier suggestions that GRV depends on GRAV for transmission by A. craccivora.

Although the role of GRV in causing groundnut rosette virus disease was thus firmly established, its nature had still to be determined, and methods for its diagnosis had yet to be worked out. Because of the expertise and excellent facilities available at the Scottish Crop Research Institute (SCRI), Invergowrie, Scotland, further characterization of GRV and of GRAV was attempted there. Plants infected in the field with groundnut rosette, and plants infected artificially with GRAV, contained isometric particles approximately 25 nm in diameter serologically related to the luteoviruses of bean leaf roll, potato leaf roll, and beet western yellows. The host range of mechanically transmissible GRV was determined. Chenopodium amaranticolor was found to be a good local lesion host, and Nicotiana clevelandii and Nicotiana benthamiana to be excellent systemic hosts. A method for partial purification of GRV was devised. GRV was shown to be of single-stranded ribonucleic acid (ssRNA) of molecular weight of approximately 1.55×10^6. Evidence was also obtained indicating that GRV did not form true virus particles; it appeared to be present in the membrane-fraction from infected leaves.

These findings have paved the way for more extensive epidemiological studies and more meaningful screening of germplasm accessions for resistance to the viruses.

Further Research Needs

It will be necessary to produce a specific antiserum for GRAV to facilitate its detection in plant hosts and aphid vectors. Although A. craccivora can transmit groundnut rosette virus disease very efficiently, possible transmission by other aphids that feed on groundnut foliage should be investigated. Serological methods such as Enzyme-Linked Immunosorbent Assay (ELISA) and Immunosorbent Electron Microscopy (ISEM) should be standardized to enable detection of GRAV in individual aphids. Since the protein specifically coded by GRV has not yet been identified, detection of GRV by serological methods alone is not possible. Infectivity tests have several limitations, and the most sensitive method for detecting GRV is likely to be the application of nucleic acid hybridization tests. Biotin probes have recently been used to detect nucleic acids and they obviate the need to use radioisotopes. It will be necessary to prepare diagnostic kits that can be used by trained technicians to detect GRV in Africa.

Evidence obtained so far indicates that chlorotic and green rosette are caused by strains of the same virus. Nucleic acid hybridization tests employing complementary DNA, and determination of host ranges, would be valuable in determining the relationship between chlorotic and green rosette viruses.

Epidemiological studies on groundnut rosette virus disease, which can be done only in Africa, must be intensified. The aphid vector is known to have many hosts. A search should be made for annual and perennial hosts of the rosette viruses. It is essential to determine the environmental factors that favor long-distance dissemination of the aphids. In addition to monitoring aphid populations, it is essential to know how many aphids are carrying the rosette viruses.

Growing rosette-resistant groundnut cultivars is an important way to combat the groundnut rosette virus disease. The majority of rosette-resistant cultivars have long growing seasons, and for use in many regions of Africa, it will be essential to breed shorter-duration, rosette-resistant cultivars for both oil and confectionary uses. Since rosette is caused by two viruses, it is essential to determine the reactions of resistant breeding lines to both components. It is also necessary to study how resistance to

both the viruses is inherited.

Data obtained from epidemiological research, current knowledge of the effects of cultural practices in controlling rosette disease, and resistant cultivars could all be utilized in the integrated management of this very important disease.

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The Peanut Collaborative Research Support Program (CRSP) Project on Rosette Virus Disease

J.W. Demski[1] and C.W. Kuhn[2]

The three major objectives of the Peanut CRSP virus project are:

- to determine the etiology of groundnut rosette disease;
- to determine the epidemiological factors of groundnut rosette disease; and
- to select and determine the nature of resistance in groundnut to groundnut rosette viruses.

Two components of groundnut chlorotic rosette have been identified : a mechanically transmissible component that induces typical rosette symptoms in groundnut that we call the symptom-inducing agent (SIA); and a virus that reacts to antisera of potato leaf roll and beet western yellows viruses (PLRV; BWYV), but causes no symptoms in groundnut.

Initial studies were directed to mechanical manipulation of groundnut rosette virus disease from and to groundnuts. Mechanical transmission of chlorotic rosette from groundnut to groundnut in Africa has been increased to over 80% efficiency. Phosphate, borate, and citrate buffers were used at different molarities for triturating infected tissue in initial studies. Phosphate buffer gave the most consistent and highest percentage infection. Therefore, a standard buffer was used that consisted of 0.1 M phosphate, pH 7.4,0.02% mercaptoethanol, and 1.0% Mg bentonite.

Results of individual tests were: using the standard procedure—6/10, 8/8,4/7, 7/8,7/8, 7/8, 7/8, 6/8, 7/8, and 6/8 (infected/number inoculated); the standard procedure minus Mg bentonite—0/10 and 0/8; the standard procedure with 5% Mg bentonite instead of 1% Mg bentonite—9/10 and 8/8; high pH buffer (9.5) consisting of 0.1 M glycine, 0.05 M K2HPO4, and 0.3 M NaCl—1/10 and 1/8; high pH buffer plus 1% Mg bentonite—6/8; standard procedure comparing plants held in the dark overnight or plants in the greenhouse without special treatment—dark 7/8, greenhouse 7/8; standard procedure comparing plants dusted with corundum powder or using 1% celite in the inoculum—corundum 7/8, celite 7/8; and standard procedure comparing method of inoculation—finger 6/8, cheesecloth pad 7/8, and cotton tip 6/8. Initial mechanical transmission percentages from field-infected plants (presumably aphid-inoculated) were lower (25 to 60%) compared to transmission percentages from mechanically inoculated groundnut plants. Serological assays (ELISA—Enzyme-Linked Immunosorbent Assay) using PLRV/BWYV r-globulins were negative when mechanically-infected groundnut plants were tested.

Both green and chlorotic rosette "strains" could be acquired by Aphis craccivora within 30 min of feeding on source plants (presumably aphid-inoculated) from the field. After this, a latent period of at least 24-48 h was required before the aphids could transmit the virus. Although our trials showed some inconsistency in vector efficiency with an increasing inoculation-access period up to 6 h, we were able to establish that viruliferous aphids were able to transmit chlorotic rosette virus within 10 min of feeding access on healthy groundnut seedlings. As much as 18% transmission success was obtained after 10 min inoculation-access period.

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Green rosette had transmission patterns similar to chlorotic rosette, but the degree of success was lower. Percentage transmission was higher for chlorotic rosette than for green rosette, using similar feeding access and inoculation-access periods. An incubation period of 6-9 days in groundnut was necessary for symptom expression after aphid-inoculation. With simultaneous inoculations, of the two strains, chlorotic rosette generally predominated. When one strain was challenged by the other, the first to be inoculated predominated, and symptom expression in the challenged strain was delayed. Serological assays (ELISA) using PLRV/BWYV r-globulins were positive when aphid-inoculated groundnut plants were tested.

Throughout the dry season, successive overlapping populations of A. craccivora were found on irrigated legumes, including isolated cowpeas and groundnuts, and other wild hosts, especially Gliricidia sepium in northern Nigeria. During the growing season, irrespective of planting date, plant density, or pesticide treatment, peak populations of A. craccivora (in situ count or yellow pan traps) are attained between the last week of July and first week of August. Groundnuts were found to be colonized as early as seedling emergence, particularly in late-planted crops or in epidemic years.

Application of insecticides generally depressed and delayed aphid population buildup. Furadan ® 3G(carbofuran) and Croneton ® 500 E.C. (ethiofencarb) significantly ($P < 0.001$) lowered the aphid populations more than Pirimor ® (pirimicarb) E.D. or Mocap ® 10G (ethoprophos).

Natural field occurrence of groundnut rosette virus disease has been monitored by surveying growers' plantings of groundnut and marking infected plants. Weekly inspection and recording of new infections, as the season progresses, reveals that a few primary infections occur early in the season, but that most new infections occur next to the primary-infected plants, indicating a local dissemination. Spread of groundnut rosette virus disease is greater within a row than between rows and this may be the result of walking apterae rather than alates. This results in many infected plants in certain areas of the plantings only, indicating that secondary spread leads to the development of epidemics.

Six cultivars MK 374, Samaru 38, Ex-Dakar, Spanish 205, M 25.68, and 69-101 were tested for differential resistance to rosette and the vector, in the field. Both green and chlorotic rosette strains were observed with varying degrees of incidence on all the cultivars. Aphid population levels were generally similar on cultivars of similar growth habits. Although 69-101 and M 25.68 proved to be rosette-resistant, all 6 cultivars tested were similarly heavily attacked by the aphid vectors.

Greenhouse transmissions, using aphids or mechanical inoculation, showed that the cultivars RMP 12 and RMP 91 (Burkina Faso), 69-101 (Senegal), and M 25.68, M 516.78, M 562.79, and REB (Nigeria) have excellent resistance to both chlorotic and green rosette. The cultivar 1204.781, that was supposed to be rosette-resistant, proved to be susceptible both in greenhouse and field tests. All other cultivars tested were susceptible and had higher percentage infection with chlorotic rosette than with green rosette.

From the foregoing observations, it would appear that the use of resistant cultivars with some timely application of systemic insecticides shows promise of providing rosette control.

More than 20 years ago green rosette was dominant in West Africa, but currently chlorotic rosette is common and may now be the dominant type of rosette in the region.

In Nigeria, Miss S. Meyer (Braunschweig, Federal Republic of Germany) used ELISA (PLRV/BWYV antisera) to test groundnuts with different types of symptoms and different weed hosts. All reactions were weak but seemed to indicate the presence of a luteovirus component in most rosetted plants (both chlorotic and green). The luteovirus could not be detected in all rosetted plants. The luteovirus was detected in some groundnuts that did not have visual symptoms. Additionally, positive luteovirus

serological reactions were obtained from some unidentified weed hosts.

In 1984, antisera to PLRV and bean leaf roll virus (BLRV) were obtained from Dr R.O. Hampton (Washington, USA). The antisera were conjugated and used to test groundnuts in Nigeria. Although preserved homologous antigens gave positive reactions in ELISA plates, a positive reaction could not be obtained in rosetted groundnuts using the US antisera.

A search for alternate experimental hosts for groundnut rosette virus disease has shown Glycine max L. (cv CNS) and Nicotiana benthamiana are hosts of the SIA. Back inoculations from both hosts to groundnuts have produced characteristic symptoms.

When total nucleic acid (TNA) was extracted from groundnuts with chlorotic rosette, the protein-free preparation was infectious to groundnuts and to soybeans. Furthermore, infectivity appeared to be sensitive to ribonuclease but not to deoxyribonuclease. Fractionation of the TNA by lithium chloride (LiCl) precipitation showed infectivity to be associated with single-stranded RNA. Electrophoresis of a portion of the LiCl preparation demonstrated the presence of one or more molecules of double-stranded ribonucleic acid (dsRNA) in both groundnuts with rosette virus disease symptoms and soybean with chlorotic rosette symptoms.

In Nigeria, work on purification has not been so successful as mechanical transmission. Numerous efforts have produced negative results. However, recent efforts using diethyl ether to remove polyphenolic compounds from whole leaf tissues have allowed us to obtain a band towards the bottom of the 20% region of sucrose gradients. This band appears only when infected tissue is used, and is absent when healthy tissue is processed. Initial attempts to mechanically inoculate seedlings with material from this band have proved negative.

Progress on Groundnut Rosette Virus Disease Research at the Institute for Agricultural Research (IAR), Samaru, Zaria, Nigeria

S.M. Misari[1] and O.A. Ansa[2]

Purification of Groundnut Rosette Assistor Virus

A major objective of virus research at the IAR Samaru is purification of the GRAV component of the groundnut rosette virus disease. Past attempts at purification have failed to yield any virus particles. Current results are, however, most encouraging and it is hoped to obtain electron micrographs of the GRAV preparations. Experiments are currently being conducted to improve methodology and to work out a standard procedure for GRAV purification.

Rosette Transmission

Comparative transmission mechanisms of both green and chlorotic rosette have been worked out using Aphis craccivora as the vector. Cross-transmission studies on the isolates suggest that they may be strains of the same virus and work is continuing along this line.

Rosette Resistance

Resistance studies initially involving the screening of several groundnut lines in both greenhouse and field have been undertaken to determine the mechanisms of resistance to the rosette complex. These studies are yielding encouraging results.

Epidemiology

The occurrence and incidence of rosette in relation to vector population dynamics in groundnut ecosystems is being studied with a view to achieving integrated control of the disease, using resistant or tolerant cultivars, and inexpensive systemic chemicals. Based on this work, definite statements can now be made on integrated control of the disease.

Alternate hosts of the aphid and the virus are also being investigated.

Epidemiological studies to determine the spread of green and chlorotic rosette viruses in several groundnut cultivars are being vigorously pursued with interesting results.

Further Research Needs

Future lines of research will depend upon the characterization and purification of GRAV. They are:

- To determine relationships between the GRAVs of green and of chlorotic rosette, following the GRAV characterization.
- To produce antiserum for GRAV for use in resistance screening and epidemiological investigations.
- To identify the occurrence and sources of resistance to other groundnut viruses, and determine mechanisms and inheritance of resistance.
- To further clarify the biological relationships and epidemiology of rosette and its vector.

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2. Virologist in the same program.

Research on Groundnut Rosette Virus Disease in Southern Africa

K.R. Bock[1]

Research is in progress on three major aspects of the groundnut rosette virus disease problem:

- the seasonal origin(s) of rosette;
- varietal resistance; and
- identification of a diagnostic host plant.

Studies on the Seasonal Origin(s) of Rosette

The seasonal origins of the groundnut rosette virus disease in southern Africa are not known. The question is perhaps best addressed as—'where does Aphis craccivora spend the dry season (May to November)?' There are three possibilities:

1. A. craccivora is present throughout the area on dry-season hosts which may (or may not) also be reservoirs of rosette virus(es). Growth flushes immediately before, or subsequent to the onset of the rains may result in rapid buildup in numbers and short-distance dispersal.
2. A. craccivora is more or less restricted during the dry season to special ecological areas such as moist coastal lowlands, mist-valley grasslands, etc. Dispersal subsequent to the onset of the rains would be mainly by upper-level winds and over long distances, in the manner of the armyworm moth, Spodoptera exempta.
3. Both vector and virus survive the dry season on groundnut volunteers. The subsequent dispersal at the onset of the rains may be over long or short distances.

A great deal of the previous work on rosette (Adams 1967; Adams and Farrell 1967) has been on the 1st possibility, none on the 2nd, and only a few observational studies have been made on the 3rd in South Africa (Storey and Bottomley 1928) and Tanzania (Evans 1954).

Current Research on the First Possibility

Previous studies on dry-season hosts of the vectors were confined to herbaceous or woody herbaceous species. None of the several vector hosts contained rosette in the field, or proved susceptible to rosette on inoculation. Our search has shifted to recorded tree hosts of A. craccivora and also to shrub or tree species which occur widely over the groundnut-growing regions of Africa. Such species are being tested for their possible role as hosts of A. craccivora and also as possible hosts of rosette virus(es).

Current Research on the Second Possibility

This involves regional cooperation. Two sites where first arrival of vectors can be monitored have been selected in Malawi (Chitedze), and in Zimbabwe (Tobacco Research Station, Kutsaga, Harare). Detailed climatic data including directions and speeds of local surface and upper winds, and synoptic charts are available for these sites. Such studies may afford clues to whether the vector arrival is dependent on local climatic patterns or whether the arrival is directionally sequential, which possibly indicates long-distance dispersal.

Current Research on the Third Possibility

This also includes the initiation of a regional

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cooperative program to quantitatively assess dry-season survival of groundnut volunteers across the region. This program also hopes to establish, within reasonably narrow limits, the arrival of vectors in the newly-emerged groundnut crops across the region.

Studies on Resistance of Groundnut Cultivars to Rosette

Large Scale Screening of Plants for Resistance in the Field

Hitherto, reliance has been placed on late planting to induce high levels of rosette disease. This has two major disadvantages. For any given season heavy rosette incidence can never be assured. Also the selection based on desirable characters other than rosette resistance can only be attempted in less than optimal conditions. The solution lies in establishing rosette virus disease nurseries in which heavy selection pressure for rosette resistance can be maintained and guaranteed. The evolution of methodology to this end is in progress, and success is likely to be achieved.

Screening for Resistance of Important Breeding Lines, Progenies, or Cultivars

Work is in progress involving standardized methods of inoculation under greenhouse conditions. Using nonviruliferous aphids, it also involves backtests from both rosetted and apparently symptomless plants to find whether or not rosette disease is transmissible from these. Such tests are crucial and have obvious practical implications in regard to epidemiology.

Inheritance of Resistance

Studies are being made of plant progenies derived from resistant x susceptible crosses. Although resistance is held to be governed by two recessive genes and is expressed in an F2 ratio of about 15:1, no critical studies have been made on whether such resistance is to GRV or GRAV, or to both. Current work is attempting to confirm inheritance ratios and, if possible, to determine whether resistance is to one or both the viruses. Success is largely, but not entirely, dependent on finding a suitable diagnostic host for GRAV.

Search for a Diagnostic Test Plant Host of the GRAV

It is of obvious importance to know whether inoculated test groundnut plants that remain symptomless are immune or are tolerant to infection by one or both viruses. It has now been shown that symptomless, inoculated plants may contain GRV (Reddy, D.V.R., Murant, A.F., 1985, personal communications). GRV may itself be detected by sap inoculation to Nicotiana benthamiana or Chenopodium amaranticolor, but high temperatures in greenhouses in many areas of southern Africa preclude the culture of good test plants, at least for many months of the year. Detection of GRV and GRAV by means of aphid inoculation to easily cultured test plants is thus of great importance. The search for such possible diagnostic hosts is in progress.

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Breeding for Groundnut Rosette Virus Disease Resistance

R.W. Gibbons[1]

Despite many claims in the literature, genetic resistance to rosette has only been convincingly demonstrated in germplasm accessions collected from the border regions of the Ivory Coast and Burkina Faso. The resistant accessions consisted of phenotypically similar plants and it should be considered that the individual groundnut lines released in Senegal are very closely related. They are similar in respect of the following characteristics a compact spreading-bunch, growth habit, dark green foliage, medium-sized pods, and a long growing season. Berchoux (1960) reported that resistance to rosette is governed by two independent recessive genes (aabb).

He also found that resistance is apparently due to the production of an 'antivirus' substance by the plant, and this was confirmed by Daniel and Berchoux (1965). The resistant material was used in breeding programs in Senegal, Nigeria, and Malawi, and several higher-yielding, shorter-season hybrids have been released for cultivation. The original germplasm lines, and the hybrids derived from them, have maintained their resistance for over 20 years in both West Africa, where green rosette is common, and Central Africa, where chlorotic rosette is common. Grafting and vector feeding tests in Malawi have shown that the germplasm lines are resistant but not immune, and do not show overt symptoms or stunting, under normal disease pressure. There are later unconfirmed and unpublished reports that the resistance may not be as simply inherited as first reported by Berchoux (1960). Currently, further research on the genetics of resistance is being conducted in Zimbabwe and Malawi. Future research on resistance should take into account the recent findings on the nature of the viruses involved in the rosette complex.

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Molecular Cloning of a Double-Stranded RNA (dsRNA) Associated with Groundnut Rosette Virus Disease

E. Breyel[1], G. Gross[1], R. Casper[1], S. Meyer[1], C.W. Kuhn[2], J.W. Demski[2], O.A. Ansa[3], and S.M. Misari[3]

A double-stranded ribonucleic acid (ds RNA) of about 900 base pairs (bp), with 0.6 x 10^6 molecular weight has been isolated from groundnut plants *(Arachis hypogaea* L.) showing typical symptoms of groundnut rosette virus disease. The 900 bp dsRNA is associated with the mechanically-transmissible, symptom-inducing agent (SIA) of the disease, but not with the aphid-transmissible luteovirus that acts as a helper for the SIA in field transmission. The origin and function of 900 bp dsRNA remain unclear. As expected, it is not infectious. The infectious ribonucleic acid precipitates in 2 M LiCl from total RNA extracts and is indistinguishable from plant messenger RNA when separated by gel electrophoresis. No viral-coat protein has been found for the infectious RNA, making serological testing impossible. To develop a diagnostic test for groundnut rosette RNA, the 900 bp dsRNA has been cloned in an E. *coli* plasmid vector. 300 ng of in vitro polyadenylated dsRNA were reverse transcribed into complimentary DNA (cDNA) and cloned according to the method of Okayama and Berg (1982). About 6000 ampicillin-resistant clones were selected and will be screened for suitable hybrid clones.

Reference

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Studies on Viruses that Depend on Luteoviruses for their Transmission by Aphids

A.F. Murant[1] and J.H. Raschke[1]

There are several viruses that depend on unrelated viruses (most of them definitive or tentative members of the luteovirus group) for transmission by aphids in the persistent manner. The three studied in most detail—carrot mottle (CMotV), groundnut rosette (GRV), and lettuce speckles mottle (LSMV)—share many properties including extreme sensitivity to organic solvents, moderate stability in vitro, and, with CMotV and GRV at least, resistance to ribonuclease. Less is known about a fourth virus, tobacco mottle (TMotV), although it was the first dependent virus to be described; there is also some doubt that its helper virus is a luteovirus.

Purification of the infective particles of the CMotV, GRV, and LSMV has proved extremely difficult but the molecular weights of their infective ssRNA molecules are about 1.4-1.6 x 10^6. Leaf tissue infected with LSMV is known to contain dsRNA molecules with molecular weights of 2.7, 1.1 and 1.0 (x 10^6). Recent studies show that CMotV, GRV, and TMotV also produce large dsRNAs with approximate molecular weights (x 10^6) of 3.2, 3.0, and 3.3, respectively. These are presumably the replicative forms of the infective ssRNA. In addition, each of these three viruses produces one or more smaller dsRNA species, the most prominent of which have molecular weights (x 10^6) of o.9 (CMotV), 0.6 (GRV), and 0.9 (TMotV).

CMotV, GRV, LSMV, and TMotV are obviously very similar to each other in many ways and may constitute a new virus group.

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The Virus Identification and Data Exchange (VIDE) Project on Legume Viruses

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An increasingly important component of efficient plant virus diagnosis is collation and management of data.

Versatile computer-based methods have recently been developed to collect, manipulate, and distribute data, and our Virus Identification Data Exchange (VIDE) project has been using such facilities to produce aids for plant virus diagnosis. We have initially concentrated on viruses of legumes with the aid of the International Legume Virus Working Group. Data have been handled using the DELTA (Description Language for Taxonomy) programs written by Dr M. Dallwitz of the Commonwealth Scientific and Industrial Research Organization (CSIRO) Division of Entomology. So far data have been distributed as two microfiche editions and an experimental book (Boswell and Gibbs 1983). Experience has convinced us of the value, both for diagnostic work and teaching, of using the database with an interactive diagnostic program in a personal computer, and this can be demonstrated.

Reference

Boswell, K.F., and **Gibbs, A.J.** 1983. Viruses of legumes, 1983. Description and keys from VIDE. Canberra, Australia: Research School of Biological Sciences, Australian National University.

Editors' note: The database on legume viruses is now on computers at The Scottish Crop Research Institute and the International Crops Research Institute for the Semi-Arid Tropics.

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Recommendations

Recommendations

After a thorough discussion of the different research findings the following recommendations were unanimously agreed:

- Each group should continue their current research and should cooperate fully with the other research groups.
- Research findings should be made available to all groups at the earliest possible opportunity.
- Duplication of work should, as far as possible, be avoided.
- Further meetings along similar lines to the present one should be arranged at convenient times to present and discuss new research data and coordinate research planning.
- Activities of the research groups should be on the following lines:

Peanut CRSP (USA) and IAR (Nigeria)

Drs J.W. Demski and C.W. Kuhn will be working closely with their cooperators in Nigeria and the Federal Republic of Germany. Greater emphasis will be laid on screening germplasm and breeding lines for resistance to rosette. Nigerian breeders will be encouraged to produce short-duration, rosette-resistant cultivars. Antisera for luteoviruses and cDNA probes produced in Germany will be used in epidemiological studies and used to identify sources of disease resistance.

Dr O.A. Ansa will continue his efforts to produce antisera for the luteovirus, and to develop simpler methods for dsRNA extraction from infected groundnut plants. Dr S.M. Misari, in addition to monitoring aphid populations for the presence of groundnut rosette virus, will investigate the biology of the vector and study factors contributing to disease spread.

Dr Kuhn, using the facilities in the Federal Republic of Germany, will investigate the possibilities of phenotypic mixing that may lead to isolation of the GRV-ssRNA. He will also participate in the development of cDNA probes for disease diagnosis.

Dr Demski, in addition to his coordinating role, will concentrate on resistance breeding and investigation of the disease epidemiology.

Drs Demski and Kuhn will also attempt to characterize the majority of economically important groundnut viruses in Nigeria.

Institut fur Viruskrankheiten (Federal Republic of Germany)

Dr R. Casper and his colleagues will assist Dr Ansa in purification and production of antisera for the GRAV. The dsRNAs isolated from rosette-infected plants will be cloned and used in the production of cDNA for probing GRV-RNA, in solution and in dot blots.

Both Peanut CRSP and ICRISAT will assist Dr Casper to obtain the necessary rosette-infected plant material.

ICRISAT Regional Program for Southern Africa (Malawi) and ICRISAT Center (India)

Drs K.R. Bock and S.N. Nigam will concentrate on resistance breeding, the identification of primary sources of infection, aphid monitoring (including the identification of viruliferous aphids), and on the identification of other hosts of vector and virus(es). Dr Bock will also investigate aphid migration and the climatic factors that contribute to aphid multiplication and spread. Dr Bock's experiments will be extended to other Southern African Development Coordination Conference (SADCC) countries as soon as possible.

ICRISAT Center Groundnut Improvement Program, together with Peanut CRSP, will help to coordinate research on groundnut rosette virus disease and assist the various research groups by arranging distribution of diseased materials, antisera, and other necessary requirements.

The Scottish Crop Research Institute (UK)

Drs B.D. Harrison and A.F. Murant are interested in the comparative aspects of dependant viruses and wish to determine the extent of nucleic acid homology within the group. In addition, they would like to investigate serological relationships among the assistor viruses in the group, to examine the role of satellite RNAs in symptom production, and to determine the mechanisms of aphid transmission of GRV encapsulated in the luteovirus coat protein.

To accomplish these goals Dr Murant would require a student, preferably a postdoctoral candidate. Dr Harrison intends to approach the Overseas Development Administration (UK) and other fund-granting agencies to secure the necessary financial help. ICRISAT will support SCRI in their effort to obtain funding and, if possible, may depute a member of ICRISAT staff to work at SCRI.

Coordination

Both ICRISAT and Peanut CRSP have agreed to coordinate research on groundnut rosette virus disease. They will assist the various groups in all possible ways to accomplish their research objectives.

ICRISAT is planning to establish a groundnut team in the West African region in 1986. Research on groundnut rosette virus disease will be given a high priority in this team's program.

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