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

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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 K_2HPO_4, 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 overnignt 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.