IDENTIFICATION AND IMPORTANCE OF VIRUSES OF SOYBEANS AND PEANUTS IN SOUTH AFRICA. FINAL REPORT. March, 1998.

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EXECUTIVE SUMMARY.

A survey for the viruses of soybeans was conducted during 1994 and 1995 in the major soybean production areas of South Africa. Commercial farms as well as experimental sites were surveyed. A total of 695 soybean samples were collected and analyzed for viruses previously reported on soybeans in South Africa by ELISA. These are, alfalfa mosaic virus (AMV), cowpea aphid-borne mosaic virus (CAMV), peanut mottle virus (PeMotV), and soybean mosaic virus (SMV). Plants were also mechanically inoculated onto a limited host range and analyzed by electron microscopy. In the later stages of the survey an immuno-electron microscopy (IEM) technique was used to detect a rhabdovirus of soybeans. SMV was detected in 19.3% of the samples collected, throughout the production areas. It occurred in low incidences in commercial farms, with two exceptions where it occurred at incidences between 10 and 15%. It was prevalent in experimental trails however. PeMotV was identified in 1.9% of the samples and was present in both commercial fields and experimental trails. CAMV was only detected in 1994, occurring in 1.2% of the samples collected. AMV was detected in 0.7% of samples. The soybean rhabdovirus associated disease was prevalent (10-50% of plants in a field with symptoms) at all commercial fields and experimental trails in the Brits/Thabazimbi, Loskopdam Irrigation Scheme, and Badplaas areas. The disease was diagnosed in 20.9% of samples collected, and in the later stages of the survey the virus was regularly detected in these plants. Tospoviruses were identified in two

samples from geographically separated sites, and an unidentified flexuous rod-shaped virus in a single sample.

During 1996 and 1997 viruses obtained during the survey were characterized, and in some instances further sources of the viruses collected.

SMV occurs as a number of strains, of which only one, G1, has been reported in South Africa. It was therefore important to identify the virus to strain level. SMV was isolated from 11 sources containing the virus by two serial single aphid (*Myzus persicae*) transfers. Sources were chosen to represent a wide variety of symptoms and geographical locations. The strain identity of the SMV isolates were determined by bioassay using soybean differential hosts with known SMV strains included as controls. As shown in the past, SMV strain G1 is prevalent, and eight of the isolates were identified as being of this strain. One isolate was identified as strain G2 , and is the first report of this strain in South Africa. Unusual differential responses were obtained with two isolates which require further studies. Polymerase chain reaction (PCR) amplified products of c. 700bp were obtained to a 3' portion of the coat protein gene and 3' untranslated region of the known strains of SMV in order to obtain the nucleotide sequence of these strains. Attempts to clone the PCR products were not successful.

The etiology of the rhabdovirus-associated mosaic disease of soybean was studied. A number of methods were employed to establish the disease in the laboratory and to transmit and isolate the virus. Transmission of the disease and virus particles by the *Igerna* sp. leafhopper was confirmed. An ELISA technique based on a crude antiserum produced to pooled, field-collected infected material yielded unacceptably high non-specific reactions, even when absorbed with healthy plant sap, and could not be used in further studies. An IEM technique based on the same antiserum was developed. This was successfully used in tests to monitor virus presence in infected plants. Virus was established and maintained by mechanical inoculation once soybean cultivar Forrest was employed as host. Virus was isolated by serial limiting dilution transfers, and referred to as soybean rhabdovirus Isolate 95/0131. The disease symptoms and virus particle transmission could be demonstrated following isolation and the rhabdovirus can be considered the the causal agent of the disease. Mechanical inoculation between soybean cultivars yielded

low rates of virus transmission, and studies were conducted to increase the rate of virus transmission. Virions were shown, based on the response of a single soybean plant, to occur consistently at the highest relative concentrations in the youngest leaves of the plant, but that virus levels differed considerably between leaves. *N. benthamiana* was found to be susceptible to the rhabdovirus, with transmission rates to *N. benthamiana* being high. It did not produce symptoms, however. Test various conditions for virus propagation, relative virus concentrations were shown to reach a peak followed by a drastic decline in virion numbers. The time of peak concentration was affected by the ambient temperatures following inoculation, occurring more rapidly at higher temperatures. Virus particles were shown to be enveloped, bullet-shaped particles between 272nm and 390nm X c. 94nm. Virions appeared to accumulate in the cell cytoplasm and no evidence for perinuclear accumulation was found suggesting the virus belongs to the genus Cytorhabovirus.

Two Tospoviruses, originally from soybeans, were isolated by serial local lesion transfers on *Chenopodium quinoa*. Typical tospovirus-like particles were present in ultrathin sections *of N. benthamiana* following mechanical inoculation of the isolates. Both isolates reacted in indirect ELISA systems to antisera to the nucleoprotein of tomato spotted wilt virus (TSWV), groundnut ringspot tospovirus (GRSV) and to tomato chlorotic spot tospovirus (TCSV), but failed to react against a monoclonal antibody to TSWV. Neither isolate reacted to antisera to Impatiens necrotic spot tospovirus (INSV) or to groundnut bud necrosis tospovirus (GBNV). Isolates therefore are members of the serogroup II tospoviruses, related to GRSV and TCSV.

Alfalfa mosaic virus (AMV), has previously been reported on soybeans in South Africa, based on symptomatology and host range studies. The original virus cultures are no longer available and the report could not be verified by serology or electron microscopy. Virus was isolated from plants infected with virus from soybean sample 94/2006 by two serial local lesion transfers on *Vigna unguiculata*. Typical AMV-like bacilliform particles of varying sizes were detected by electron microscopy. Virus was shown to react against AMV antiserum to a local clover isolate in both IEM and ELISA, verifying the previous report of this virus on soybeans in South Africa. The AMV-94/2006 isolate was used to determine the most useful antiserum bleed to AMV available at PPRI, and to develop and optimize a $F(ab')_2$ ELISA to the virus. This ELISA was

successfully employed in the detection of AMV in further field collected soybean plants.

A virus with flexuous rod-shaped particles was detected in a single soybean plants from Delmas, Mpumulanga, with blotchy mosaic symptoms similar to that caused by the soybean rhabdovirus. The virus did not react in ELISA to antisera to any of the viruses known to occur on soybeans in South Africa. Virus was isolated by two serial limiting dilution transfers using mechanical inoculation of soybean infected leaf sap, and was referred to as Isolate 95/0158. Virus particles showed no obvious substructure and were between 728-756nm long with a modal length of 728nm. Aphid transmission could not be demonstrated using *Myzus persicae* or *Aphis craccivora.* No obvious inclusion bodies could be detected in ultrathin sections of infected soybean leaf material, but bundles of virus-like particles could be detected. The virus did not react to antisera to a number of legume Potyviruses or to a monoclonal antibody directed to an epitope common to most potyviruses. No polymerase chain reaction (PCR) product was obtained using two sets of primers to conserved sequences within Potyviruses. Isolate 95/0158 also did not react with antiserum to cowpea mild mottle carlavirus (CMMV) or produce an amplification product in a PCR with Carlavirus genus specific primers. The virus therefore remains unidentified but does not appear to be a member of the Potyvirus genus.

Surveys on peanut viruses were initiated in 1990 by the virology division at PPRI. Surveys were ongoing and were continued through 1994 to 1997 as a project funded by the Protein Research Trust. This has ensured a thorough investigation of the virus status of the crop. Since the crop has been surveyed for a period of 7 years, seasonal variation in climatic conditions, which could influence the outcome of shorter surveys, has been taken into account.

The viruses that have been identified as occurring on peanuts in South Africa as a result of this survey are peanut mottle potyvirus (PeMotV), tomato spotted wilt tospovirus (TSWV), groundnut ringspot tospovirus (GRSV), the groundnut rosette disease complex and isolated occurrences of other viruses including tobacco streak ilarvirus (TSV), bean yellow mosaic potyvirus (BYMV), the newly characterized peanut chlorotic blotch potyvirus and an interception of peanut stripe potyvirus. The most commonly found viruses on the crop are TSWV, GRSV, PeMotV and the groundnut rosette disease viral complex.

Since the onset of the survey we have detected TSWV using an antiserum raised to the nucleocapsid protein. With a number of samples very weak reactions to TSWV were obtained and occasionally a positive reaction was only obtained after inoculation onto herbaceous indicator plants. While this could be ascribed to low virus concentrations in the plants we suspected that some of these plants may be infected with a related tospovirus, groundnut ringspot virus (GRSV), which is serologically related, but different from TSWV-L. We obtained a DAS ELISA kit specific to GRSV and antiserum to peanut bud necrosis tospovirus with which an ELISA system was developed just prior to the 1997 season. This was therefore the first season that we could differentiate between the different tospoviruses. Sixty-six percent of the tospovirus samples collected in 1997 were GRSV whereas 34% were TSWV. Peanut bud necrosis virus was not detected in any of the samples. All the samples collected in Barkly-West (Northern Cape) were TSWV. Of the samples collected around the Vaalharts irrigation scheme (Northern Cape) 90% were TSWV. GRSV was the predominant tospovirus detected in samples collected at all the other localities, although both viruses were detected. Since sample collection was not quantitative, definite deductions as to the relative incidence of these two viruses in specific areas cannot be made. There does seem however, that there may be a predominance of the one virus over the other in certain areas.

It was notable throughout the four year survey that PeMotV was substantially more prevalent in experimental plots of breeders than in commercial fields. Infected seed is the primary source of virus and by using the infected seed for the following season the virus is propagated. We would like to encourage a greater awareness with the breeders regarding the danger of spreading viruses in this manner, especially since many of their trials are conducted in plots within commercial fields.

Differences were also noted among the various PeMotV isolates collected and an attempt was made to differentiate strains of this virus. Differential host range studies were done and molecular characterization of different isolates was attempted, but was not successful. Amplification of a portion of the genomes of the different isolates using polymerase chain reaction (PCR) was not achieved despite using various universal potyvirus primers designed to amplify different regions

of the genome.

Groundnut rosette is essentially an African disease and can have devastating effects on the crop. The disease incidence seems to vary greatly from season to season, but the disease can be minimized and, to a large extent eliminated by adjusting cropping practices. Our observations were that the disease was only a problem when the crop was planted later than usual. Further only isolated plants or patches on the perimeter of certain fields had the disease. Early planting ensures that there is not an early infection in the crop and losses are minimized. The vector is probably only active later in the season. A dense canopy of plants where there are no open soil patches also minimizes infections. It appears that the vector, *Aphis craccivora,* is attracted to isolated plants surrounded by open soil patches. This control strategy however is very dependent on vector conditions. Should vector behavior change because of environmental conditions severe losses can still be incurred. We successfully established and propagated this mechanically non-transmissible disease complex by a top cleft grafting procedure. A nucleic acid probe that can detect the satellite component of the disease was obtained. We also obtained a limited amount of antiserum to the groundnut rosette assistor luteovirus (GRAV) and primers specific to the coat protein gene of GRAV, which enables immuno-capture polymerase chain reaction (IC-PCR) detection of this component. We therefore have detection systems in place should it be required that the aetiology of the disease be studied or should the breeders want to introduce resistance into their cultivars and need a monitoring system.

Amongst the viruses detected was peanut stripe potyvirus, a virus that causes severe yield reductions in peanuts and which is not known to be present in South Africa. PStV is one of the most widely distributed viruses of peanuts in South East Asia, but has not been reported from Africa or South America. This virus was intercepted on two separate occasions, once in a seed lot submitted by phytosanitary authorities and on a second occasion it was found in an experimental plot of imported germplasm material. These interceptions allowed immediate implementation of measures to prevent possible spreading of the virus into the industry. The accession, 95/0399, which was intercepted in the germplasm material was identified as PStV via host range studies and serology. Further studies were done on this isolate in collaboration with researchers at the Department of Queensland Industries (DQI), Australia. As there were

indications that this virus differed from previously characterized PStV isolates, this isolate was further characterized and the probable origin determined as the USA. An article submitted for publication to the journal *Plant Disease* on the identification and partial characterization of isolate 95/0399 is included in this report. We believe greater emphasis should be placed on quarantine measures to ensure that only virus-free seeds are used in germplasm exchange.

In the 1991/92 season a spherical virus (isolate 92/475) was detected in a peanut sample from Potchefstroom in the North West Province and was later identified as TSV. An ELISA detection system was developed to this virus. During the 1993/94 season a peanut sample (94/708) was collected near Christiana also in the North West Province with symptoms similar to those induced by isolate 92/475. A spherical virus was detected which reacted in the ELISA system developed to detect the first isolate. TSV was therefore detected in two plants at two different localities during different seasons in the North West Province. This suggests that the virus was not merely an incidental infection of peanuts, and that it may occur to a certain extent in peanuts in the North West Province.

In 1993 a potyvirus (isolate 93/632) causing chlorotic ringspots on peanuts was isolated from a peanut plant found on an experimental plot at Potchefstroom in the North West Province. This isolate did not react with any of the antisera to peanut viruses available and host range studies indicated that it was probably a new peanut potyvirus. This virus, named peanut chlorotic blotch potyvirus, was fully characterized as a new potyvirus and an article has been accepted for publication in the journal *Plant Pathology* relating to the above. This article is included within this report.