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Epidemiology and management of the *Indian peanut clump virus*

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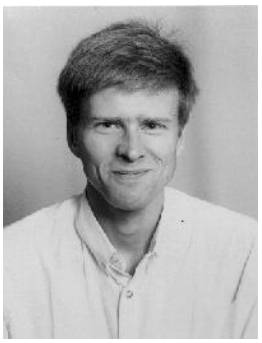
ABSTRACT

Epidemiology and management of the Indian peanut clump virus

Groundnut or peanut (*Arachis hypogaea* L.) is an important legume cultivated in several developing countries in the tropics and subtropics. It plays a significant role as a food crop in regions with alarming population growth rates. The disease "peanut clump", which is caused by viruses in the genus *Pecluvirus*, has been reported from India and from several countries of West Africa. In India, the causal agent is the *Indian peanut clump virus* (IPCV), which is transmitted by a soil-borne root parasite, *Polymyxa graminis*. The virus is also transmitted by infected seed and so far no economical method of control has been found. Therefore efforts have been concentrated on understanding the epidemiology of peanut clump disease with the aim of devising cultural methods of control. The work addressed in this thesis describes how investigation in various aspects of clump disease epidemiology, including identification of alternative hosts of the virus and the vector, and of factors that contribute to survival and spread of inoculum, has led to formulation of simple cultural practices that could reduce disease incidence.



Philippe Delfosse
January 2000

The work reported in this manuscript resulted from the research project on "Integrated control of *Polymyxa graminis*, vector of peanut clump virus" a collaborative program between the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Patancheru, India), and the Unité de Phytopathologie, Université catholique de Louvain, (UCL, Louvain-la-Neuve, Belgium). The project also included the collaboration of progressive farmers in Andhra Pradesh and Rajasthan in India as well as National agricultural research systems (NARS) such as the Rajasthan Agricultural University, Jaipur and the National Agricultural Research Center (NARC) Islamabad, Pakistan. The program initiated in 1993, was funded by the Belgian Administration for Development Cooperation (BADC, Brussels, Belgium). It ended officially in December 1999.

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This work was conducted in India, a vast multi-cultural country. On arrival travellers to India usually suffer from a cultural shock and Barbara and myself were not spared. India is rich in population, culture, sun, erratic-heavy rains and also rich in honesty. We always felt safe walking in villages and cities eventhough numerous people surrounded us, touching our hair, hands, giving us their smile and kindness as a testimony of their hospitality. More aggressive and dangerous was the driving on Indian roads where trucks, the mighty "King of Road – Blow horn please!", cohabit with cars, cycles, auto-rikshaw, pedestrians, buffalo and other cattle, chickens, dogs and cats. Burning was the food for our delicate European palates and irritating was the phlegmatic bureaucracy (heritage from the British?). It took us time to adjust to this new environment, far from our families and friends. Time also to understand and appreciate India. In these few words I want to thank all our Indian friends who in the initial months and all along our stay, led us and opened up our eyes on their country. I also thank Jim G Ryan and Shawki M Barghouti former Directors General of ICRISAT, and many members of the staff, for welcoming Barbara and myself to ICRISAT – Patancheru.

At the initiation of this project there were three major actors who held the role of scientific fathers of either the virus or its vector. The work conducted on peanut clump virus diseases by DVR Reddy's team at ICRISAT, triggered the interest of a *Polymyxa* addicted scientist, Henri Maraite (UCL), who's laboratory has a long experience with this soil-inhabiting organism, vector of plant viruses. Dear Henri, how could you trust a young nutritionist to initiate research in phytopathology ? Many thanks for offering me my first real job and pushing me to finalise this thesis. Charles Renard, former Regional Director at ICRISAT-Patancheru, played the important role of connecting the two partners. Without these three protagonists this work would not have been possible. Many thanks to T Avella, President of the jury and to JF Ledent and Mike J Adams, members of the jury, for their judicious advise to improve the manuscript.

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Far from them but so close by e-mail, they knew me as the one who makes potatoes rotting and smelly. For their friendship and the unconditional backing they gave me, I thank all the members of the Unité de Phytopathologie, UCL. Among them, Claude Bragard, Françoise Munaut, Brigitte Vanpee, André Goffin, Jeannine Weyns, Isabelle Vanacker, Hugue Minet, Tonio di Zinno, Luigi Sandri, Jean-Marc Moreau, Eric Ewbank, Jean-Marie Gérard, Sabine Pelsner, Marie-Chritine Flamand, Jean-Baptiste Bahama, Henri Batoko, Jean-Claude Vanschingen, and last but not least, Bruno Vandersmissen and Frederic Aerts, my collaborators of the years "potato". Many thanks to Jean Risopoulos and Diane Doucet, who added valuable contributions to this research as part of their MSc degree. In this group, Anne Legrève, corresponded with me almost every day. She was a continuous and comforting support during this expatriated work. We exchanged ideas and discussed results in an open and trusty manner. Her work was an essential complement to the studies conducted in India. Anne, I sincerely thank you for your straighforwardness, your advises and friendship.

A scientific work is usually achieved in combination with the help of the people you love, who extend their support and encouragement and tolerate your absence during late works, travels and meetings. For her patience, and true love, I thank and dedicate this thesis to Barbara, my wife and best friend.

I thank all you, my parents, sisters and brothers, their spouses, and my dearest nieces, for being such a nice and true family.

Philippe

Table of Content

Terminology[1]

Ecology: The totality or pattern of relations between organisms and their environment.

Epidemiology: The sum of the factors controlling the absence or the presence of a disease.

Immune: Plant exempt from virus after exposure to inoculum.

Inoculum potential: Number of independent infections that are likely to occur in a given situation in a population of susceptible host plants.

Integrated control: The complementary use of biological and chemical methods to control pests and pathogens.

Isolate: Culture or population of IPCV or *P. graminis* in their host plant made by direct separation from host or substratum and subcultures from them.

Pecluvirus: Virus genus containing two virus species responsible for peanut clump disease in groundnut crops in the Indian sub-continent and West Africa, and characterised by rod-shaped particles of two predominant lengths (former members of the Furovirus group).

Plasmodiophoromycetes: Class of obligate endoparasites of vascular plants with a life cycle characterised by: (1) a mobile stage, the zoospore with two, anterior, whiplash flagella of unequal length, (2) a vegetative phase under the form of plasmodia, (3) environmentally resistant resting spores giving rise to a single zoospore.

Plasmodium: Multinucleated protoplast. Two types exist: sporangiogenous plasmodia give rise to zoosporangia with zoospores, and sporogenous plasmodia develop into resting spores.

Propagule: Infective unit of *Polymyxa. graminis* (see inoculum potential).

Resistant: Infection with virus may occur but the host will not support virus multiplication. Often overt symptoms are not produced.

Resting spore: Survival stage of *P. graminis*, unicellular structure protected in a thick and highly resistant wall and giving rise to a single zoospore.

Seed-borne: Virus present in seed but not necessarily transmitted to the progeny.

Seed-transmitted: Virus transmitted to the progeny through seed-borne inoculum

[1] Adapted from (*i*) A guide to the use of terms in plant pathology (1973), prepared by the Terminology Sub-Committee of the Federation of British Plant Pathologists, *Phytopathological Papers*, No 17, Commonwealth Mycological Institute, Kew, Surrey, England. (*ii*) Dictionary of Science and Technology (1992), Academic Press, $$$$, USA.

Serotype:	Group of viruses sharing none or only few of its antigens in common with another group. In the case of IPCV, 3 serotypes have been recognised (IPCV-D, IPCV-H, IPCV-L).
Sporosorus:	Resting spore clusters also called cystosorus.
Transient:	Host in which infection with virus did occur at an early stage but subsequently the virus could not be detected.
Zoosporangium:	A multinucleate stage of the life cycle of *P. graminis* surrounded by a thin wall and discharging numerous secondary zoospores through small cells structured as exit tubes.
Zoospore:	The only mobile stage of *P. graminis*, equipped with two flagella of two different lengths. Primary zoospores germinate from resting spores. Secondary zoospores are produced by zoosporangia.

Abbreviations

A_{405}: Optical absorbance at 405 nanometres
AIS: automatic immersion system
ANOVA: analysis of variance
°C: degree Celsius
cv.: cultivar
d.f.: degree of freedom
DAS-ELISA: double antibody sandwich form of enzyme linked immunosorbent assay
ELISA: enzyme linked immunosorbent assay
g: gram
ha: hectare
ICRISAT: International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh, India.
IP: irrigation pattern
IPCV: Indian peanut clump virus
IPCV-B: Indian peanut clump virus, Bapatla isolate (sea shore areas of Andhra Pradesh), belongs to the serotype IPCV-D.
IPCV-C: Indian peanut clump virus, Chinnaganjam isolate (Andhra Pradesh), belongs to the serotype IPCV-D
IPCV-D: Indian peanut clump virus, Durgapura isolate (Rajasthan), representative of the serotype IPCV-D.
IPCV-H: Indian peanut clump virus, Hyderabad isolate (in-land area of Andhra Pradesh), representative of the serotype IPCV-H
IPCV-L: Indian peanut clump virus, Ludhiana isolate (Punjab), representative of the serotype IPCV-L
IPCV-T Indian peanut clump virus, Talod isolate (Gujarat), belongs to the serotype IPCV-D.
IR: Indian Rupee (equivalent to 1.2 Belgian franc)
ISEM: immunosorbent electron microscopy
IU: infective unit
kDa: 10^3 Dalton
kg: kilogram
l: litre
m: meter
mm: millimetre rainfall
n: size of a category
ni/N: number of plants infected / number of plants tested
OD: optical density
***P*:** probability

PCV: peanut clump virus, West African agent of peanut clump disease
PVC: polyvinyl-chloride
***R*:** correlation coefficient
SCRI: Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland.
Stdev: standard deviation
t: metric ton
UCL: Université catholique de Louvain, 1348 Louvain-la-Neuve, Belgium.
UK: United Kingdom
USA: United States of America
wk: week
WkAS: weeks after sowing
WR: weekly rainfall

General Introduction

Groundnut or peanut (*Arachis hypogaea* L.) is an important cultivated legume of South American origin (Hammons, 1994). Groundnut plays a significant role as a food crop in several countries in the tropics and subtropics with a large population. It is an excellent source of cooking oil (ca 48%) and protein (ca 30%) (Savage and Keenan, 1994). Developing countries contribute to about 90% of the world production of groundnut oil. Like other legumes, under most conditions it fixes nitrogen through symbiotic association with *Bradyrhizobium* spp. The crop requirement for nitrogen is thus very low and growing groundnut favours soil enrichment in nitrogen (Sprent, 1994). Groundnut haulms are also an important fodder source, especially for dairy cattle. For these reasons, groundnut is regarded as a key crop for the future of developing countries with alarming population growth rates.

In India, groundnut is grown on an estimated area of 8.1 million ha with a production of 7.45 million t of pods in shell (1998 production). This average productivity of 0.920 t ha^{-1} has increased this last decade but is still considerably lower than the world average of about 1.1 t ha^{-1} (Anonymous, 1999). One of the reasons for the low yield of groundnut, particularly in the semi-arid tropics, is the damage done to the crop by various abiotic stresses such as drought and low soil fertility, and by a number of diseases caused by fungi, bacteria and viruses. The disease "peanut clump", which is caused by a virus, has been reported from India and from several countries of West Africa. This virus disease can cause severe damage to the groundnut crop and the annual loss on a global scale has been estimated to be worth 38 million US dollars (Reddy *et al.*, 1999).

The disease

Peanut clump disease is one of the most damaging virus disease of groundnut, and is currently known to be widely distributed in West Africa, and in the Indian sub-continent (Reddy *et al.*, 1999). In India the disease was reported and described for the first time in the 1926 Year Book of Madras Agricultural Department (S. Sundararaman, 1927) from the then Madras Presidency (south Indian state). Sundararaman described the disease in these words "*…The disease appears in the rainfed crop and affected plants are characterized by a dense clump of tuffy dwarfed shoots with yellowed leaves and the plants stand erect instead of spreading on the ground. Such plants stop growing after a time and bear very sparingly. From the peculiar habit of the affected plants, the disease has been called*

"Clump disease". Only individual plants or a few in localized patches exhibit the disease, while others quite close to the affected ones are quite normal in their habit and bear well-developed pods…Observation made during succeeding seasons show that the disease is not confined to any particular variety, and has appeared in all the 20 varieties raised at the station." The description of Sundararaman is still accurate to characterise the major features of clump disease in the Indian sub-continent. Diseased plants are conspicuous because of their severe stunting and dark green appearance in most cases (Fig. 1). Young leaves show chlorotic symptoms such as faint mottling or marked yellow lines and rings (Fig. 2a and 2b). The disease occurs in patches and recurs in nearly the same position in the field in successive groundnut crops (Fig. 3). Stunting is most severe when relatively young plants are infected. Nevertheless these symptoms are not absolute. For instance, chlorotic lesions on leaves can be masked by leaf yellowing due to iron deficiency or zinc toxicity, or stunted infected plants can appear erect compared to healthy plants of especially spreading type varieties. Although infected plants can produce flowers, usually they do not yield and any pods formed are small and not marketable. Plants infected at later stage of their growth also become stunted, with shorter internodes. Yield losses up to 60% have been recorded in such late infected plants (Reddy *et al.*, 1988).

The first efforts reported from India to identify resistance to peanut clump disease were initiated in the early 70's in the northern state of Rajasthan (Mathur *et al.*, 1971). At that time the disease was identified as "rosette disease of groundnut". Recent discussions with one of the authors, LC Sharma, Rajasthan Agricultural University, Jaipur, and pictures taken in 1964 in fields (Fig. 4) of the Durgapura Agricultural Research Station near Jaipur, later identified as clump infested, allowed the unequivocal identification of the problem as clump. Additionally, the presence of groundnut rosette virus (GRV), which causes very similar symptoms (Germani *et al.*, 1975), was never confirmed in India. GRV seems to be restricted to Africa south of the Sahara (Reddy, 1991). In West Africa, the first observations of peanut clump were those of Trochain (1931), and Bouhot (1967) at Bambey, Senegal. Trochain and Bouhot adopted the terminology "clump" used by Sundararaman. The viral origin of the disease occurring in West Africa was confirmed by Thouvenel *et al.* (1974) and in India by Reddy *et al.* (1979). The virus in West Africa was named peanut clump virus (PCV) and the one occurring in the Indian sub-continent was referred to as Indian peanut clump virus (IPCV). The present work focuses on IPCV.

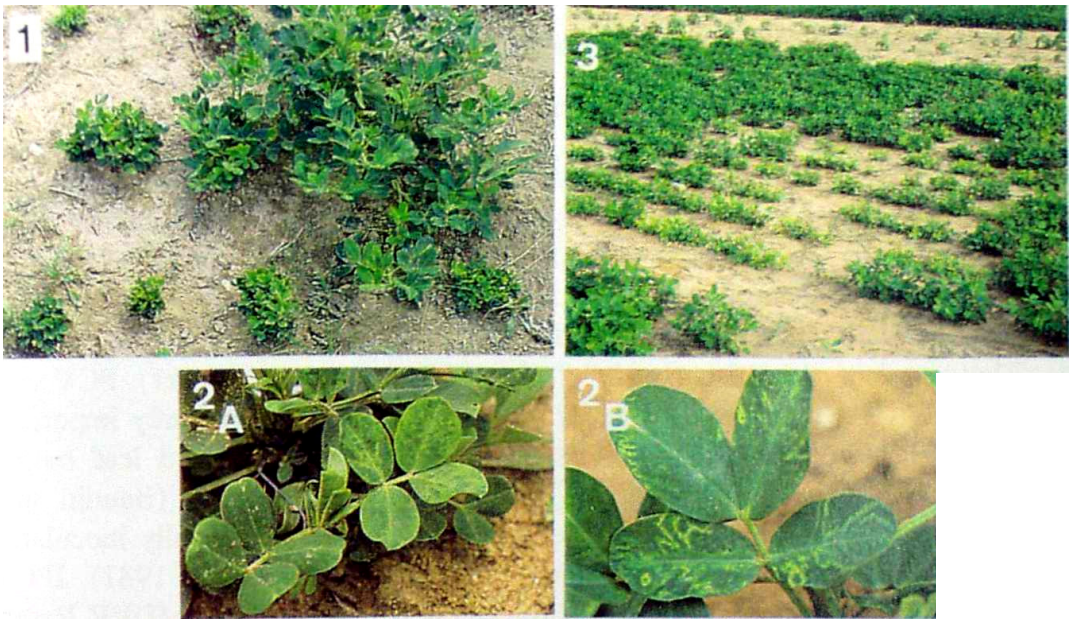

Figure 1-3: Major features of the peanut clump disease. **1**. Severe stunting and dark green appearance; **2**. Chlorotic lesions on young leaves (**2-a**) faint mottling, (**2-b**) marked chlorotic lines and rings; **3**. Patchy appearance in an infested field.

Figure 4: Peanut clump symptoms in an infested field of the Durgapura Agricultural Research Station, Jaipur, Rajasthan. In 1964, the problem was reported as "rosette disease of groundnut". (courtesy of Prof. LC Sharma).

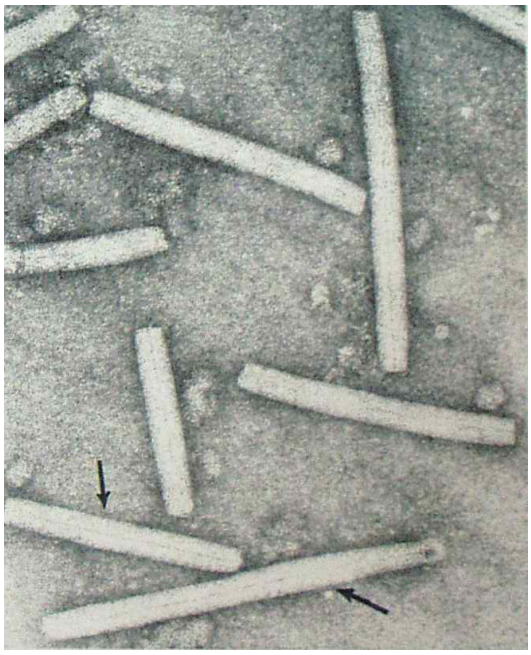

Figure 5: Virus particles of the causal agent, the Indian peanut clump virus. Particles are rod-shaped, with two predominant lengths of 249 and 184 nm.

The causal agents

PCV and IPCV have been shown to be soil- and seed-borne. They can persist in the soil for many years even in the absence of crop cultivation. PCV and IPCV are among the very few plant viruses (over 3000 plant viruses have been recorded so far) that readily infect a range of both monocotyledonous and dicotyledonous plants (Thouvenel *et al.*, 1981, Thouvenel *et al.*, 1988, Reddy *et al.*, 1988, Ratna *et al.*, 1991). PCV and IPCV have the potential to cause disease in other economically important crops. For example, PCV is responsible for sugar cane red leaf mottle (SCRLM) disease in Senegal, Burkina Faso and Sudan (Baudin and Chatenet, 1988, Chatenet and Saeed, 1995). PCV mechanically inoculated onto wheat causes stunting and mottling (Thouvenel *et al.*, 1981). IPCV causes stunting and chlorosis in chillies under field conditions (DVR Reddy personal communication). Both viruses were initially classified in the *Furovirus* group (Brunt, 1988) of plant viruses because of their transmission by a **fu**ngus vector, the soil-inhabiting root parasite *Polymyxa graminis* Ledingham (1939), and their **ro**d-shaped particles. Today, because of their specific genome organisation, taxonomists have agreed to class them in a new genus, *Pecluvirus (***Pe**anut **clu**mp virus)*,* which includes both the species, PCV and IPCV (Torrance and Mayo, 1997). The only clear feature that distinguishes PCV from IPCV is the geographical location in which the virus is found. Indeed the variability in genome sequence between any serotype of IPCV is as great as that between IPCV and the one isolate of PCV sequenced to date (Reddy *et al.*, 1999). It is also worth to note that to date, *Pecluviruses* have not been reported from South America, the centre of origin of the genus *Arachis*.

Research done in India at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and at the Scottish Crop Research Institute (SCRI), established the identity of IPCV. Its biological and physico-chemical characteristics as well as its serological properties were described (Reddy *et al.*, 1979, Reddy *et al.*, 1983, Reddy *et al.*, 1985, Nolt *et al.*, 1988, Reddy *et al.*, 1988, Wesley *et al.*, 1994, Miller *et al.*, 1996, Naidu *et al.*, 1996). All IPCV isolates have rod-shaped particles, 24 nm in diameter, with two predominant lengths of 249 and 184 nm (Fig. 5), which contain two single-stranded RNA species (RNA 1 and RNA 2) with approximate molecular weights of 1.83×10^6 and 1.36×10^6 respectively. The 249 nm particles are considered to contain the larger RNA species, RNA 1, and the 184 nm particles contains the smaller RNA species, RNA 2. Both RNA 1

and RNA 2 are required for successful infection. The virus coat protein of 24 kDa is encoded by RNA 2 (Fig. 6).

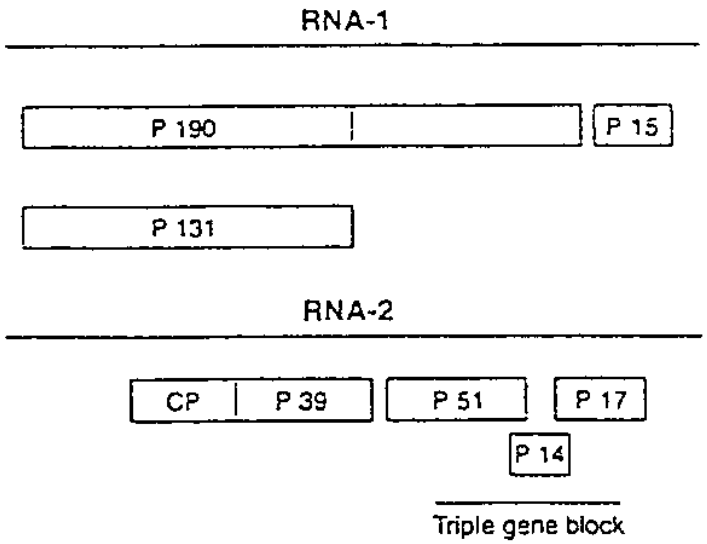

Figure 6: Genome organization of the Pecluviruses. Horizontal lines represent RNA molecules, open bars represent proteins and the figures are the protein weights in kDa. RNA 1: P 131 has methyl transferase and RNA helicase homologous domains; P 190 includes the sequence of P 131 and is thought to have RNA polymerase activity; the activity of P 15 remains unknown. RNA 2: CP is the virus coat protein; proteins encoded by the triple gene block are thought to be involved in virus movement within infected plants; P 39 is suspected to be involved in the transmission by *P. graminis*. Adapted from Reddy *et al.*, (1999).

Serological tests with polyclonal antisera using enzyme linked immunosorbent assay (ELISA) and immunosorbent electron microscopy (ISEM) demonstrated that the virus isolates, so far reported in India, can be grouped into three serotypes represented by typical isolates, namely IPCV-Hyderabad (IPCV-H), IPCV-Durgapura (IPCV-D), and IPCV-Ludhiana (IPCV-L) (Nolt *et al.*, 1988, Reddy *et al.*, 1988). Diversity among isolates was further enforced by their host range and the specific symptoms they cause in several hosts. IPCV-D is the most widespread serotype of IPCV. It occurs in the Indian states of Gujarat (IPCV-Talod, IPCV-T), Rajasthan (IPCV-D), and in the sea shore areas of Andhra Pradesh (IPCV-Bapatla, IPCV-B and IPCV-Chinnaganjam, IPCV-C) and Tamil Nadu near Madras. IPCV-L serotype occurs in Punjab. IPCV-H has so far only been reported from Andhra Pradesh near to Hyderabad. All IPCV isolates are serologically distinct from PCV isolates and vice versa. The Fig. 7 illustrates the geographical distribution of PCV in Africa and IPCV in India.

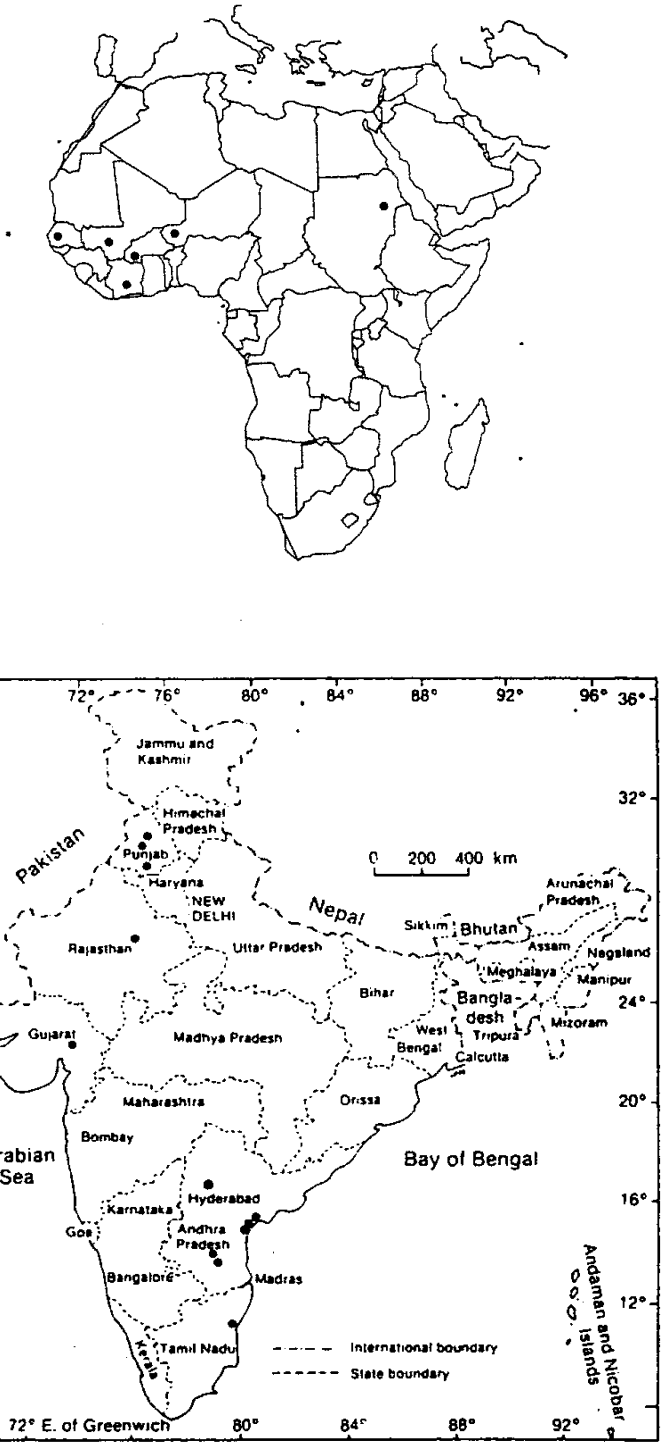

Figure 7: Geographical distribution of the Pecluviruses (●). PCV was reported from Senegal, Mali, Burkina Faso, Niger and Sudan in Africa (Thouvenel *et al.*, 1988, Chatenet and Saeed, 1995). IPCV occurs in the states of Punjab, Rajasthan, Gujarat, Andhra Pradesh, and Tamil Nadu in India (Reddy *et al.,* 1988).

Transmission

PCV and IPCV are transmitted through peanut seed at a rate exceeding 10% (Thouvenel *et al.*, 1978, Konaté and Barro, 1993, Reddy *et al.*, 1988). Additionally Reddy *et al.* (1998) have shown that the transmission rate in seeds obtained from plants infected through seed exceeded 50%. The same authors also demonstrated the transmission through seed of monocotyledonous crop species such as finger millet (*Eleusine coracana* [L.]. Gaertn.), pearl millet (*Pennisetum glaucum* [L.] R. Br., and foxtail millet (*Setaria italica* [L.] Beauv.).

The virus was shown to be transmitted by the root obligate parasite, *P. graminis* (Ratna *et al.*, 1991). Direct evidence for transmission of PCV by *P. graminis* has so far not been reported but circumstantial evidence indicated that *P. graminis* is also the vector of PCV (Thouvenel *et al.*, 1981).

IPCV is transmitted by mechanical inoculation with sap from infected plants (Reddy *et al.*, 1988, Nolt *et al.*, 1988, Reddy *et al.*, 1998). However, difficulties were encountered while using extracts from some infected plants, such as groundnut. Grafting of infected branches onto healthy root stock is an efficient way to maintain and propagate a virus isolate in groundnut (Germani *et al.*, 1975, DVR Reddy, and M Dollet, personal communication).

The vector *P. graminis*

Polymyxa is an obligate root parasite of vascular plants. The genus belongs to the class of the Plasmodiophoromycetes (Karling, 1968). Previously considered as lower fungi, *P. betae* (Keskin, 1964) and *P. graminis* (Ledingham, 1939), the two species so far recognised in the genus, were recently placed in the protozoa kingdom (Braselton, 1995). *P. betae* and *P. graminis* have similar morphological features and are separated only on the basis of their host range. *P. graminis* infects preferentially various monocotyledonous plants such as barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), maize (*Zea mays* L.), millets, rice (*Oryza sativa* L.), rye (*Secale cereale* L.), sorghum (*Sorghum bicolor* [L.] Moench) and wheat (*Triticum aestivum* L.) whereas *P. betae* infects such dicotyledonous plants as sugar beet (*Beta vulgaris* L.), *Chenopodium* sp. and spinach (*Spinacia oleracea* L.). Although parasitic, *Polymyxa* spp. do not induce external symptoms in infected plants under natural conditions. However the genus attracts particular attention because of its ability to transmit various plant viruses

affecting a number of cereal crops, sugar beet and groundnut (Maraite, 1991, Adams , 1990, Jones, 1993).

The taxonomy of *Polymyxa* has often been the source of controversy (Barr, 1988, Braselton, 1995). Recent research conducted by Legrève (1999) has shown that *Polymyxa* spp. from different parts of the world, exist as distinct isolates which differ in their temperature requirements for optimum development, host range, the virus they transmit, and in the nucleotide sequence of internal transcribe spacer regions of ribosomal DNA. Because the genomic distance between *P. graminis* isolates was sometimes higher than between *P. betae* and *P. graminis,* Legrève proposed a new organisation of the genus *Polymyxa*. Instead of separating *P. graminis* and *P. betae* on the basis of their specific host range, the author suggested the consideration of only one species, *P. graminis,* the first to be described. Within the one species, Legrève proposed 5 *formae speciales* based on their geographical origin, and ecological and genomic characteristics. According to this nomenclature, *P. graminis* isolates involved in the transmission of IPCV belong to two distinct *formae speciale*: *P. graminis* Ledingham f. sp. *tropicalis* from regions situated in the tropical areas (e.g. isolates from Andhra Pradesh in India and Senegal), and *P. graminis* Ledingham f. sp. *subtropicalis* from the subtropical regions of the Indian sub-continent (e.g. isolates from Rajasthan in India and Punjab in Pakistan). This new taxonomic organisation is not yet accepted and may require analysis of a larger number of *Polymyxa* isolates prior to validation. We refer to this parasite as *Polymyxa* sp. in papers published prior to this proposal. We now prefer to refer to it as *P. graminis*.

The life cycle of *Polymyxa* was first described by Ledingham (1939) in the case of *P. graminis* and additional information was given by Barr (1988), Chen *et al.* (1998), and Littlefield *et al.* (1998). *Polymyxa* survives in the soil in the form of thick-walled resting spores aggregated in clusters commonly called cystosori or, more recently, sporosori (Braselton, 1995). The sporosori are released in soil during root decay. They can survive in the soil for several years and germinate under favourable conditions, which are not yet fully understood. It is commonly accepted that root systems of favourable hosts stimulate the germination of resting spores in their surrounding. Each resting spore gives rise to a single primary zoospore. If there is enough free water in the soil, the zoospores swim towards root hairs or epidermal cells in the root hair zone. The distance that a zoospore (*P. betae*) can swim is only a few millimetres (Tuitert, 1993). The zoospores form a cyst on the host cell and develop an inner dagger-like body, the stachel, and an adhesive appendage to facilitate cyst attachment

to the host cell. The stachel perforates the host wall and membrane, and the zoospore content is drawn through the opening into the host cytoplasm. The plasmodium, cytoplasmic mass and a nucleus surrounded by a thin membrane, moves within the host cell by cytoplasmic streaming. There is a rapid enlargement of the fungal nucleus and cytoplasm followed by active nucleus division. A cell wall is laid down on the fungal membrane giving rise to a structure referred to as zoosporangium. Zoosporangia discharge zoospores outside the root cell through small exit tubes (small and specialised cells of *Polymyxa*). These secondary zoospores can reinfect the root either internally or externally and in turn produce new zoosporangia leading to rapid multiplication of the fungus. Instead of multiplication through secondary cycles, *Polymyxa* can also enter into a resting stage. Conditions which favour the production of sporosori are currently not known. It is presumed that factors such as water stress, plant age or infection of a non-favourable host, leads to production of sporogenic plasmodia and formation of thick walled resting spores as a result of meiotic divisions. Virus transmission occurs if viral particles are present in the fungal cytoplasm of primary or secondary zoospores that are introduced into the host cell cytoplasm during the process of infection (Chen *et al.*, 1991). The acquisition of the virus by a non-viruliferous *Polymyxa* presumably occurs when the fungus infects and multiplies in a plant root containing the virus transmitted by previous infection or transmitted through seed-borne inoculum (Rao and Brakke, 1969, Adams *et al.*, 1988).

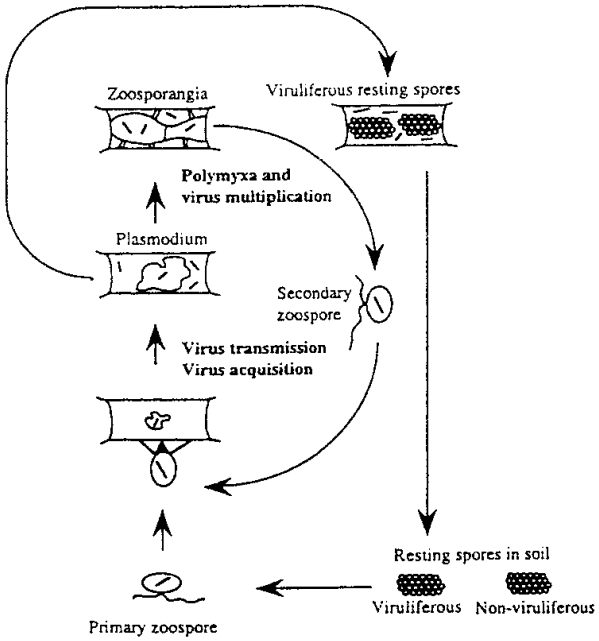

Figure 8: Life cycle diagram for *P. graminis* adapted from Reddy DVR, Mayo MA, and Delfosse P (1999). See text for explanation.

The life cycle of *Polymyxa* is one of the major factors that influences the epidemiology of the viral diseases it transmits (Schlösser, 1988, Usugi, 1988, Brakke and Langenberg, 1988, Goffart, 1992, Tuitert, 1993, Ohto and Naito, 1997, Carrol *et al.*, 1997). *P. graminis* survival in the soil, as highly resistant resting spores, is responsible for the recurrence of the disease in a patchy distribution in successive susceptible crops. Because *P. graminis* zoospores, the only mobile stage in the life cycle, are incapable of movement over large distances and require sufficient soil moisture for swimming, clump infested patches occurs in nearly the same areas of an infested field in succeeding years. Climatic factors, soil moisture, and cropping systems that may influence the *Polymyxa* life cycle, will also affect the disease epidemiology.

Control and Scope of Investigation

The main aims of this research work were first, to study *P. graminis* involved in transmission of IPCV in India for a better comprehension of the epidemiology of the disease, and secondly to collect basic information for the development of environmentally friendly methods, mostly based on cultural practices, for controlling peanut clump disease. An additional condition was that these strategies had to be suitable for small-scale farmers in the developing countries where this pernicious disease thrives.

Why to go for such a complex approach ? Viral diseases are usually controlled by the use of resistant varieties, or by controlling their vectors through chemical or physical treatments. These methods appeared to be the first choice to control clump disease in groundnut. However, in studies aimed at identification of the sources of resistance for conventional breeding, none of the approximately 9000 cultivated types of groundnut or 100 wild types screened to date has shown resistance to IPCV (Reddy *et al.*, 1988, Reddy *et al.*, 1999). Additionally an exclusive reliance on resistant cultivars, if they are identified, is hazardous because resistance may break down through virus synergism or if previously undetected strains of virus become common.

Attempts to control clump disease in groundnut by the application of biocides [e.g., dibromochloropropane (DBCP), carbofuran, aldicarb] or solarization of soil, although effective in reducing disease incidence, have met with little success (Dhery, *et al.*, 1975, Reddy *et al.*, 1988, Mathur and Sobti, 1993). They were suggested some decades ago, but they were not adopted by farmers, probably because these methods of defence against peanut clump were not economical for small-scale farming and not

environmentally friendly (Reddy *et al.*, 1999). Thus the inability to use conventional approaches for controlling the disease strongly justifies the importance of searching for new strategies to contain peanut clump disease.

An option is to search for non-conventional resistance. Recent advances in the field of biotechnology have made it possible to transfer into crop plants a number of viral genes including those coding for virus coat proteins (Beachy *et al.*, 1990; Hull and Davies, 1992). These advances are contributing to strategies that may help to produce genetically transformed groundnut plants expressing resistance to IPCV. This option is currently being investigated by ICRISAT scientists in collaboration with SCRI. However, groundnut is a very difficult candidate for plant transformation and it may take several years before transformed groundnut plants, resistant to IPCV, will become available to farmers.

A more traditional approach, the one we chose, is based on strategies to control the vector, *P. graminis*, by cultural practices amenable to the small-scale farmers of the semi-arid tropics. In temperate areas, agronomic factors and cultural practices appeared to be of minor importance in regard to the control of virus diseases transmitted by *Polymyxa*. The very effective survival of *P. betae* and *P. graminis* and considerable inoculum potentials in soils has hampered disease control through such practices (Maraite, 1991, Goffart, 1992). Nevertheless, the situation could possibly be different in tropics where a rapid turn-over of the organic matter could maintain *P. graminis* inoculum potential at low level. Therefore it was worth investigating these options for the control of clump. We describe below, how investigation in various aspects of clump disease epidemiology, has lead to formulation of simple cultural practices that could lead to reduction of disease incidence. The work addressed in this thesis was conducted in India mostly under field conditions and it was backed up by experiments conducted under controlled environmental conditions by Anne Legrève and her colleagues in Belgium (Legrève, 1999).

Structure of the thesis

The thesis has been organised in three major parts to cover the main objectives of this research work, (i) the development of diagnostic tools, (ii) the study of clump disease epidemiology, and (iii) testing possible ways of managing the disease formulated on the basis of the results gained during the epidemiological study. The thesis structure does not reflect the time sequence of the achievements made during this research work but aid in rendering a logical presentation.

Tools

In any program of developing and exploiting methods of control of virus diseases, it is necessary to assay the causal viruses and its vector with precision and sensitivity. This is important for survey, for understanding the epidemiology of the disease, for safe exchange of germplasm, and for screening genotypes for resistance. Detection methods for IPCV based on serological techniques were available (Reddy *et al.*, 1983, Nolt *et al.*, 1988, Reddy *et al.*, 1998). *Polymyxa* detection has traditionally relied on microscopic observation of roots after staining (Maraite *et al.*, 1988). This method is laborious, time consuming and prohibitive if large number of samples are to be analysed such as in the case of epidemiological studies. Therefore, it was thought possible that diagnostic methods based on serology could also be developed for detecting *P. graminis* in plant roots. Serological methods offer the possibility of rapid assay as diagnostic tools and have a high degree of sensitivity. Therefore it can greatly facilitate the production of epidemiological results. The CHAPTER 1 describes serological methods developed for the detection and quantification of *P. graminis* in root samples. Serology was used for detecting *P. graminis* in various *Sorghum* and *Pennisetum* accessions (CHAPTER 3).
An automatic immersion system was designed for optimum infection and multiplication of *P. graminis* (CHAPTER 5-1). This apparatus allowed for the first time estimation of inoculum potential of the vector in soil samples, an estimation very valuable to conduct epidemiological studies and for evaluation of management methods.

Epidemiology

Knowledge on the occurrence of clump disease was limited because virus detection in the developing countries where clump disease presumably occurs is often hampered by a lack of equipment and skills for proper identification of viruses by the national agricultural research systems (NARS) (Reddy, 1990, Reddy and Gowda, 1996, Reddy *et al.*, 1997). Surveys were conducted in India, Pakistan and Thailand in Asia. Based on these surveys the geographical distribution of clump disease could be established with more accuracy (CHAPTER 2). The analysis of the soil characteristic at the disease sites indicated that clump disease occurs mostly in light texture soil. The farming practices were shown to be important factors that influence disease spread and incidence.

In CHAPTER 3, the host range of IPCV and *P. graminis* was evaluated under natural conditions. Monocotyledonous hosts were shown to be the "preferred" host for *P. graminis*. The parasite was found to colonise

sorghum, pearl millet and maize roots with high intensity. It was also found to infect various monocotyledonous weeds. However *P. graminis* was generally not detected in roots of dicotyledonous plants. If infection occurred in dicots, it did not result in high intensity multiplication Dicotyledonous plants were considered as "fortuitous" hosts for *P. graminis*. In the same chapter, virus infected groundnut seed and *Cynodon dactylon* rhizomes were evaluated as possible source of virus inoculum for the establishment of clump disease to new areas. The study of the host range was carried out because the information generated was expected to help in designing crop rotation that can lead to reduction of disease incidence. Additional information on the influence of the hosts on clump disease epidemiology is provided in CHAPTER 4 and 5-1.

CHAPTER 4 deals with dynamics of infection by *P. graminis* and IPCV on various monocotyledonous crops and groundnut during the rainy season. From the results obtained during this research, it is apparent that quantity and distribution of rainfall are factors that strongly influenced the disease incidence. This is a very important information because it suggests new ways of controlling the disease by adoption of an early sowing of groundnut crops prior to the onset of monsoon rains. This chapter provides also valuable information on the host range of IPCV and its vector.

Management

The information generated during the study of the disease epidemiology in the previous chapters was used for designing methods of defence against peanut clump disease. Various hypotheses were formulated for the management of peanut clump, strategies based on cultural practices were developed and their effectiveness to reduce of IPCV incidence was evaluated under field conditions (CHAPTER 5).

We have tested various crop rotations involving dicotyledonous crops, fortuitous hosts for *P. graminis*, which could be beneficially used to reduce IPCV incidence (CHAPTER 5-1).

Pearl millet, was shown to be a preferred host for *P. graminis.* Its use as a trap crop prior to sowing rainfed groundnut crops was therefore proposed as a possible way to reduce disease inoculum and consequently disease incidence. Trap cropping with pearl millet reduced IPCV incidence in the ensuing groundnut crop (CHAPTER 5-2).

Epidemiological studies have shown the important role played by the rainfall on IPCV incidence. High rainfall occurring when the groundnut crops are young resulted in high disease incidence. Therefore It appeared feasible to sow groundnut crop early, before the onset of monsoon rains and grow the crop under judicious irrigation until the monsoon starts. Early

sowing was shown to be a simple and efficient method for the management of clump disease for farms provided with irrigation facilities (CHAPTER 5-3). Finally the results presented in this manuscript will be collectively addressed in the general discussion.

Particularities of clump disease important for designing field experiments

As mentioned earlier, peanut clump is a soil-borne disease occurring in patches of various sizes and levels of incidence. This brings difficulties and limitations to conduct trials for epidemiological studies and evaluation of management practices. The best way to bring uniformity in the trials is to limit variability due to heterogeneous distribution of viruliferous inoculum in the fields. This can be achieved by replicating the experiments in numerous blocks of small size and showing high level of incidence. Unfortunately, this type of experimental design in small blocks implies a very dense sowing of groundnut used as indicator host, to reach populations suitable for statistical analysis on disease incidence. Consequently estimation of yield loss is relative and cannot be compared to current farming practices.

P. graminis is an obligate parasite and its detection in various hosts and quantification in soil have to be assessed by examination of roots for its presence after exposing host plants to soil inoculum. Although very valuable for epidemiological studies, this is very tedious and time consuming. This is the reason why most of the epidemiological experiments were conducted with a limited number of plants.

Now that the actors and their environment have been presented we invite the reader to enter the world of peanut clump disease.