18. Peanut clump virus in West Africa

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SUMMARY

Peanut clump disease occurs in several places in West Africa: Burkina Faso, Gambia, Ivory Coast and Senegal. The disease is caused by a soil- and seed-transmitted agent, peanut clump virus (PCV) which has properties typical of the furovirus group. The particles are rod-shaped and of two predominant lengths, 190 and 245 nm. Experimentally, PCV can be mechanically transmitted and infects numerous species in eight plant families. The plasmodiophoromycete fungus *Polymyxa graminis* is thought to be the natural vector of PCV. Great millet (*Sorghum arundinaceum*) plays a large part in the epidemiology of the disease as host for both the virus and the vector.

INTRODUCTION

Peanut clump disease, which attacks peanut or groundnut *(Arachis hypogaea)* was first reported in Africa from Senegal (Bouhot, 1967) but it is now known to occur in several other countries in West Africa: Burkina Faso, Gambia, and Ivory Coast (Thouvenel, Dollet & Fauquet, 1976). The disease was originally described in India in 1927 by Sundararaman where it occurs as a different strain (recently characterized by Reddy, Rajeshwari, Iizuka, Lesemann, Nott & Goto, 1983). The disease is caused by a soil- and seed-transmitted agent: peanut clump virus (PCV; Thouvenel & Fauquet, 1981b).

HISTORICAL BACKGROUND

The disease has been recognized for more than 50 years and numerous studies have been made since to try to define its aetiology. The following brief survey of these studies shows that there have been several theories as to the disease's cause.

The first observations made of the disease in Africa were those of Trochain (1931) at Bambey, Senegal. Infected plants displayed characteristic symptoms which he called 'lettuce head'. Trochain also commented upon the resemblance of this disease to a rosette disease of groundnut known from Madras (India) and named 'clump' (Sundararaman, 1927).

It was originally proposed that clump disease was physiological in origin or else resulted from attack by insects or by pathogens (Trochain, 1931). Chevalier (1934) suggested that it might be due to a physiological disorder resulting from injury, or to poor soil quality, or from sowing seed too deep at the time of planting. Bouffil (*in* Risbec, 1950) showed that clump was due neither to injury, nor to sowing depth. Rather, he inferred from the fact that the disease was patchily distributed and covered relatively small areas, that it was caused by some local nutrient imbalance, perhaps related to the sites of old ant or termite mounds or of dead trees.

Bouhot (1967) confirmed the experiments made by the 'Institut de Recherche sur les Huiles et Oléagineux' which showed that the disease was definitely linked to some feature of the soil: the disease always reappeared at the same sites, even after crop rotation. Also, groundnuts planted in soil samples taken from a depth of 20 cm from sites where the disease occurred developed typical disease symptoms. Analysis of soils taken from diseased plots and compared with samples taken from disease-free areas showed there were no differences in soil type. Infected plants replanted, after their roots had been washed, in soil from disease-free areas remained infected (Bouhot, 1967). Soil quality clearly was not the causal factor.

Bouhot next considered biological causes. As no pathogenic fungus or bacterium capable of causing the disease could ever be isolated, Bouhot (1968) went on to suggest a viral origin. He found that grafting of portions of uninfected plants into diseased plants resulted in symptom expression in the grafted part. At the same time, when grafts of diseased plant material onto healthy plants were made, the graft stayed infected but apparently no transmission of the agent from the graft to the stock took place. Furthermore, Spire (*in* Bouhot, 1968) produced necrotic lesions in *Chenopodium* leaves that had been mechanically inoculated with extracts from infected groundnut leaves. These observations all supported the idea of a viral origin for the disease although characterization of the pathogen and description of peanut clump virus was not achieved until some years later (Thouvenel, Germani & Pfeiffer, 1974).

THE VIRUS

Symptomatology. Bouhot (1967) gave the following description for clump disease from Bambey, Senegal. 'The entire plant is affected: overall it appears healthy but stunted, dark green in colour and densely bushy. All parts of the plant are stunted: thus while the total number of leaves is unchanged, they are all small in size, with a reduced length/width ratio and

are a darker shade of green than usual. Petiole length is reduced, as is shoot length, diameter and inter-node distance. The root system is equally stunted, with fewer secondary roots and root nodules. Flowers form as normal but are reduced both in number and size. Fruit begin to form but rarely reach maturity and never contain more than one seed' (Plate 6).

'It is easy to identify infected plants as their small size and darker colour render them conspicuous from afar. If the leaves of an infected plant are examined carefully, a faint mosaic is often seen. It is diffuse and ill-defined and takes the form of small patches of light green, paler than the rest of the leaf. This pattern cannot be seen in direct sunlight but is evident when the leaf is in shade or in silhouette.'

The description of the disease from Burkina Faso (Germani & Dhéry, 1973) agrees well with that given above but a few minor differences are noteworthy. The principal one is that fruits develop more or less normally, but are reduced in size and contain up to three, usually two, seeds.

In all cases, clump disease occurs in localized patches of variable size which enlarge slowly with time. While only groundnut is affected, it has been noted that disease symptoms are more marked when a groundnut crop succeeds a great millet (*Sorghum arundinaceum*) crop.

In Burkina Faso, disease symptoms start to appear 17 days after sowing: by 25 days, infected plants have assumed the characteristic clump shape which persists until harvest. The proportion of plants affected in a given disease area is variable and some plants have an appearance that is intermediate between healthy and diseased.

Natural host plants. The principal economic host of PCV is peanut, in which the effect on crop yield is considerable. Yield diminution in Burkina Faso has been estimated to be between 40 and 70% (Germani & Dhéry, 1973). In India, where plants infected early in their development do not produce pods of commercial value and where even plants that are infected late may have yield losses up to 60% (Reedy *et al.*, 1983), the economic impact seems greater.

Surveys for alternative hosts of PCV in areas with the disease revealed that the virus also occurred in great millet, a crop which is often grown in rotation with groundnut. Normally great millet is a symptomless host under natural conditions but it takes a prominent role in the epidemiology of the disease (Dollet, Fauquet & Thouvenel, 1976).

Mechanical transmission. The virus can be mechanically transmitted to a wide host range in the following plant families: *Aizoaceae, Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Gramineae, Leguminosae, Scrophulariaceae*, and *Solanaceae*. Symptoms in *Chenopodium amaranticolor* (concentric ringspots and line-patterns extending along the veins) are characteristic and useful for identification though somewhat similar to those caused by potato mop-top virus.

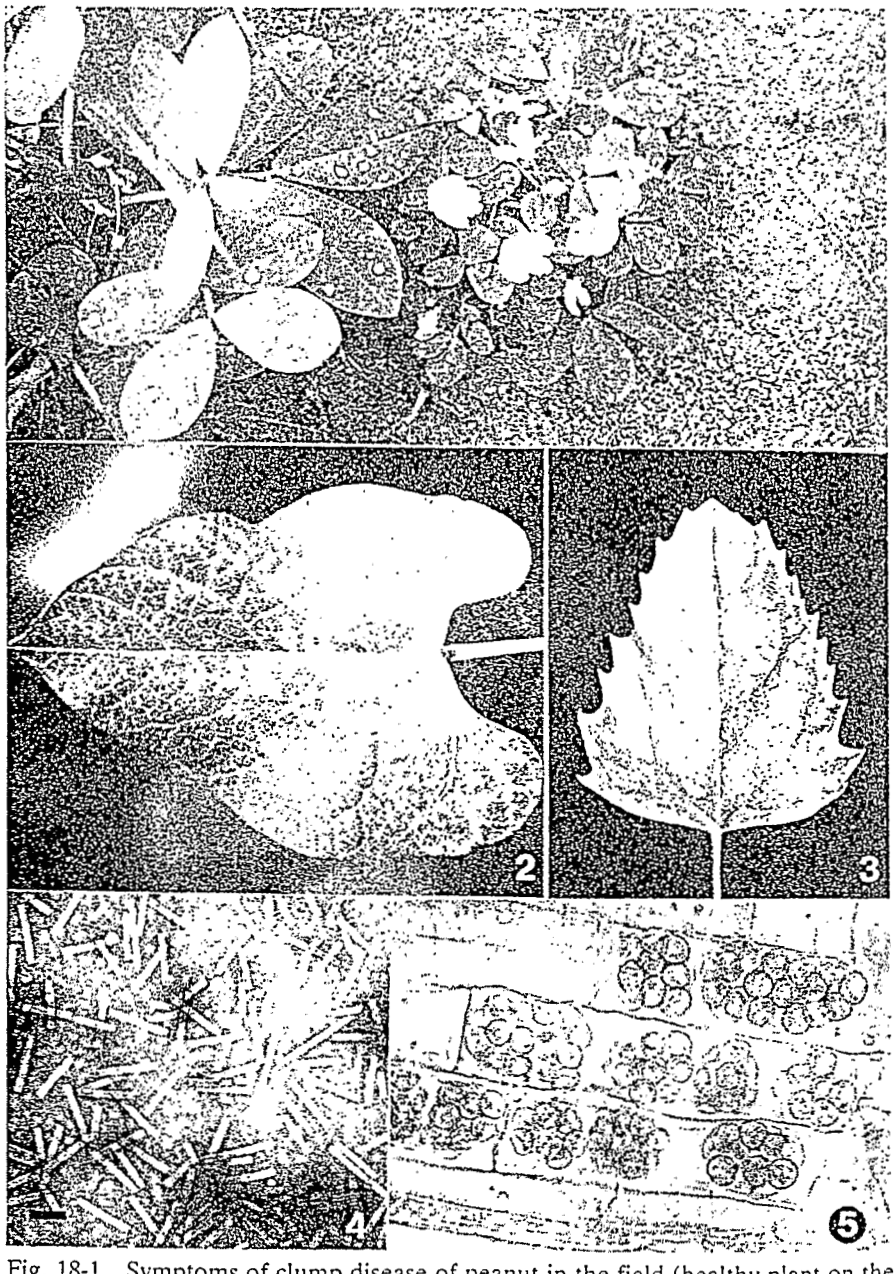

Fig. 18-1 Symptoms of clump disease of peanut in the field (healthy plant on the left).

Fig. 18-2 Symptoms of PCV in an inoculated leaf of *Phaseolus vulgaris*.

Fig. 18-3 Symptoms of PVC in an inoculated leaf of *Chenopodium amaranticolor*.

Fig. 18-4 Electron micrograph of purified PCV, stained with uranyl acetate (bar represents 100 nm).

Fig. 18-5 Cystosori of *Polymyxa graminis* in great millet roots.

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Causal agent. PCV is a virus with straight tubular particles of two predominant lengths, 190 and 245 nm. The virus is found in the cells of roots, stems and leaves of systemically infected plants. The virus particles occur in the cytoplasm, near the nucleus or along the plasmalemma, usually arranged in angled-layer aggregates. Purification is easier with high pH buffers (Thouvenel, Fauquet & Dollet, 1978). The nucleic acid (about 4% of the weight of the particle as estimated from the absorption spectrum) is single stranded RNA with molecular weights of 1.7×10^6 and 2.1×10^6, and the coat protein is a single polypeptide with a molecular weight of 23,000. The purified virus is a good antigen, and antiserum with a titre of 1/2048 has been produced (Thouvenel & Fauquet, 1981b). The virus has been assigned to the furovirus group (Shirako & Brakke, 1984; Reddy, Robinson, Roberts & Harrison, 1985).

Strains. In West Africa at least two different strains have been found. These are closely related serologically but produce slightly different coloration in groundnut leaves ('common' strain and 'yellow' strain). No serological relationship was found with any other previously described rod-shaped virus. The Indian isolate has the same properties as the common strain but was not serologically related, as judged by micro-precipitation tests. A distant relationship with the Indian isolate was found using cDNA (Reddy *et al.*, 1985).

ECOLOGY AND EPIDEMIOLOGY

Vector transmission. By sowing seeds of groundnut, great millet and wheat in soil collected from a disease outbreak area Thouvenel, Dollet & Fauquet (1976) confirmed the suggestion made by Germani & Dhéry (1973) that PCV was soil-borne; after three weeks the different seedlings developed symptoms typical of the disease. Furthermore, no transmission occurred when healthy groundnut seeds were grown in sterilized soil contaminated with lyophilized and crushed pieces of PCV-infected groundnut leaves. In a further experiment, groundnuts were sown in infective field soil previously air-dried at 25°C for 3 months, and they also developed clump disease symptoms.

Failure to find any nematodes known to be virus vectors, such as *Trichodorus*, in infective field soil, plus the fact that soil remained infective after air-drying for 3 months, suggested that the vector was a fungus. When the roots of great millet plants from an infected field were examined, clusters of resting spores similar to those of a plasmodiophoromycete fungus were observed. Observations on the different stages of the life cycle of this fungus in culture, particularly the multinucleate plasmodia that develop into sporangia with exit tubes, indicated that it was *Polymyxa*

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graminis Ledingham (Thouvenel & Fauquet, 1980). This was the first record of this well known virus vector, as far as we have been able to ascertain, from Africa.

Resting spores of *P. graminis* were found in the roots of many other graminaceous plants grown in infective soil and these also showed symptoms of PCV infection, while in contrast, no spores were ever found in groundnut roots. However not all the grasses used in these tests occur naturally in West Africa; wheat for example. To date, all attempts to isolate the virus from the fungus have failed. Despite this, experiments demonstrated a correlation between the presence of *P. graminis* and the spread of PCV (Thouvenel & Fauquet, 1981a), so that while the fungus was not found in groundnut it is possible that it infected the groundnut roots without itself developing there. This hypothesis of incomplete development is supported by the fact that groundnut roots which became infected naturally with PCV were not sources of infection for other plants, whereas comparable roots of great millet were.

Factors that influence transmission. The factors that influence disease transmission are the same as those which affect development of the vector. The most important of these are soil moisture and relative humidity, soil pH and the length of the interval between successive cropping years.

Experiments made over a 3 yr period in southern Ivory Coast showed that the most favourable months for propagation of the fungus were December and January, whereas during the rest of the year growth of the fungus was very slow. In Ivory Coast during December and January, there is a dry north-easterly wind from the Sahara, the Harmatan. Temperatures remain about the same over this period as they are for the rest of the year, but relative humidity is reduced to about 40%, from the 80–90% level that usually prevails. It is during this period that climatic conditions in southern Ivory Coast most closely resemble those that prevail during the season of groundnut cultivation in Senegal and Burkina Faso to the north, where the disease occurs naturally.

The best results for propagating the fungus under artificial conditions were obtained with a soil pH of about 7.0. As soil in West Africa is naturally acid, addition of soil with a basic pH, from termite nests for example, to the loam used for transmission tests, stimulated development of the fungus. Soil pH is not only important in affecting the growth of *P. graminis*; it has also been reported to be of importance in the development of *P. betae* (Abe, 1974). Thus, *P. betae*, vector of rhizomania of sugar beet, induced little disease at pH 6.0 and below, but grew readily at pH 7.0 and above.

To investigate the persistence of infectivity, contaminated great millet roots were kept dry in the laboratory for 2 yr, then crushed and added to soil used in transmission tests. Whilst all the graminaceous hosts tested subsequently developed symptoms, groundnut remained disease free.

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However, field observations in Burkina Faso have indicated that soil can remain infective to groundnut for over two years in the absence of a crop.

Seed transmission. It has been demonstrated that in naturally infected groundnut cultivars, PCV is transmitted in seed at a rate varying between 6 and 14%, increasing to 24% with artificially infected plants (Thouvenel, Fauquet & Lamy, 1978). Seed transmission of PCV was not detected in *Sorghum arundinaceum, Phaseolous mungo* or *Nicotiana benthamiana*.

These observations probably help to explain the characteristic distribution pattern of the disease. It is found in large, well defined patches that occur in areas geographically remote from one another in Senegal, Burkina Faso and northern Ivory Coast.

CONTROL

Clump disease is easily prevented, using selected seeds and by soil treatment with fungicides prior to the planting of the crop. It is also controlled by treating the soil with fumigants such as dibromochlorophenol or dichloropropane-dichloropropene (Germani, Thouvenel & Dhéry, 1975). It is not controlled by the use of systemic nematicides.

Disease incidence can be reduced by using a crop rotation system that replaces great millet with pearl millet (*Pennisetum typhoides*), as the latter is a host for neither the virus nor the vector. Trials are now in progress in Burkina Faso to eradicate the disease with an appropriate crop rotation system as this technique seems the most practical and the cheapest method of control.

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