

Studies on transmission of Indian peanut clump virus disease by *Polymyxa graminis**

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Summary

The plasmodiophoromycete fungus, *Polymyxa graminis* was observed in the roots of *Sorghum bicolor*, *S. sudanense*, *Pennisetum glaucum*, *Triticum aestivum*, *Cyperus rotundus*, *Eleusine coracana*, *Zea mays*, *Tridax procumbens* and *Arachis hypogaea* collected from Indian peanut clump virus (IPCV)-infested fields. Examination of roots of IPCV-infected *S. bicolor*, *S. sudanense*, *P. glaucum* and *T. aestivum* grown in previously air dried field soil also showed the presence of cystosori of *P. graminis*. IPCV-infested soil stored at room temperature for 3 years transmitted the virus to *A. hypogaea*, *T. aestivum* and *S. bicolor*. Roots extracted from IPCV-infected *P. glaucum* and *S. bicolor* containing cystosori, and dried root fragments incorporated into sterile soil, transmitted the virus to *A. hypogaea* and *T. aestivum*. The root extracts contained primary zoospores of the fungus, presumably arising from cystosori. Utilising root fragments of *S. sudanense* containing cystosori as inoculum *P. graminis* was shown to infect both monocotyledonous and dicotyledonous plants. Profuse cystosorus production in rootlets only occurred in monocotyledonous plants. In dicotyledonous plants, in general, only few rootlets showed cystosori. Indian isolates of *P. graminis* appear to differ from isolates from temperate soils in that they can infect dicotyledonous plants and have a much wider host range.

Key words: Indian peanut clump virus, *Polymyxa graminis*, soil-borne, furoviruses, cystosori, fungal transmission, plasmodiophoromycete

Introduction

Peanut clump disease was first described in India by Sundararaman in 1927, and in West Africa by Trochain in 1931. The causal agent was shown by Thouvenel, Germani & Pfeiffer (1974) to be a virus and was named peanut clump virus (PCV). Reddy *et al.* (1979) reported the occurrence of a "clump" disease of groundnut (*Arachis hypogaea* L.) in many parts of Punjab state in India with symptoms similar to those described for West African PCV (WAPCV). The PCV occurring in India was shown to be serologically different from that in West Africa, and was named Indian PCV (IPCV) by Reddy *et al.* (1983) and Reddy, Robinson, Roberts & Harrison (1985). IPCV particles were similar in size to those of WAPCV (Reddy *et al.*, 1983). Both IPCV and WAPCV are soil- and seed-borne (Thouvenel,

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Dollet & Fauquet, 1976; Thouvenel, Fauquet & Lamy, 1978; Reddy *et al.*, 1983). PCV belongs to the furovirus group (Reddy *et al.*, 1988; Fauquet, Desbois, Fargette & Vidal, 1988).

Polymyxa graminis was the first plasmodiophoromycete to be identified as a vector of a plant virus (soil-borne wheat mosaic virus, WMV) (Brakke, Estes & Schuster, 1965). It is an obligate root endoparasite of many species of Gramineae (Barr, 1979) and is now known to transmit several other furoviruses (Shirako & Brakke, 1984) including barley yellow mosaic (Inouye & Saito, 1975), oat mosaic (Hebert & Panizo, 1975), rice necrosis mosaic (Inouye & Fujii, 1977) and wheat spindle streak mosaic (Slykhuis & Barr, 1978) viruses. Air-dried infested soil and roots of host plants containing cystosori of the fungus can transmit WMV (Rao & Brakke, 1969).

Thouvenel & Fauquet (1981) suspected that peanut clump virus could be transmitted by *Polymyxa graminis* in West Africa, but they could not find the fungus in groundnut roots. They did, however, observe cystosori of the fungus in roots of *Pennisetum glaucum* (pearl millet) and other graminaceous species commonly grown in the region.

P. graminis was observed in the roots of graminaceous plants collected from all IPCV-infested areas in India (Nolt & Reddy, 1985). This paper reports the results of subsequent studies on the possible transmission of IPCV by *P. graminis*. The host range of *P. graminis* is also reported.

Materials and Methods

Detection of *P. graminis* in soils. Pot experiments were conducted in a greenhouse in which air temperatures ranged from 25 °C to 30 °C. The presence of *P. graminis* in soils was tested by layering 3 to 4 cm of IPCV-infested field soils onto a mixture of sterilised soil, sand and vermiculite in 15 cm diameter plastic pots. Seeds of *Sorghum bicolor* (sorghum), *S. sudanense*, *P. glaucum* (pearl millet) and *Triticum aestivum* (wheat) were sown in separate pots. After 4–6 wk, plants were uprooted and young rootlets removed and thoroughly washed. The roots were then stained with 1 g/litre acid fuchsin in lactophenol (Linford & McKinney, 1954) and observed for the presence of cystosori of *P. graminis* under a compound microscope at 400 × magnification.

Host range of *P. graminis*. The host range of *P. graminis* was investigated in two ways: (a) by sowing seeds of dicotyledonous and monocotyledonous species in pots with IPCV-infested soil, (b) by finely chopping dried roots of *S. sudanense* containing cystosori which had been dried for at least 1 year, mixing them with sterilised soil in pots, and sowing seeds of test plants in the pots. Sample plants were uprooted daily from day 4 to day 12 after germination and subsequently at weekly intervals. Rootlets were removed, washed in sterile water, stained as described above, and examined for the zoosporangial and plasmodial stages of *P. graminis*, which are known to precede cystosorus formation, and, beginning 2 wk after germination, for the presence of cystosori.

Transmission of IPCV from air dried soil. Soil samples collected from fields infested with Bapatla (B-IPCV) and Hyderabad (H-IPCV) isolates (Nolt *et al.*, 1988) were air-dried and stored for 3 years at room temperature. Stored soil was then placed in pots and seeds of *S. bicolor*, *T. aestivum* and *A. hypogaea* sown in them. After 4 wk leaves were tested, using appropriate IPCV antisera, by direct antigen coating ELISA (DAC-ELISA) as described by Hobbs, Reddy, Rajeshwari & Reddy (1987). Roots of plants containing viral antigens were stained and examined for the presence of cystosori of *P. graminis*.

Transmission of H-IPCV from dry root fragments. Roots of *P. glaucum* were collected from plants raised in pots containing H-IPCV-infested soils, air-dried and random samples of root segments checked for the presence of cystosori of *P. graminis*. Other root segments were dried at room temperature for 7 days and used as a source of inoculum. Seeds of *T. aestivum* and *A. hypogaea* were germinated in Petri dishes and the seedlings were transplanted into pots containing sterilised soil and root fragments of *P. glaucum* containing cystosori. Pots were placed in a greenhouse at temperatures ranging from 25 °C to 30 °C. After 4 wk the leaves of the plants were tested for the presence of H-IPCV by DAC-ELISA. *T. aestivum* and *A. hypogaea* seedlings raised in sterilised soil to which was added root fragments from *P. glaucum* plants mechanically inoculated with H-IPCV, and which did not contain cystosori of *P. graminis*, were used as controls.

Transmission of virus through root extracts. Root extracts were prepared from dried, H-IPCV-infected roots (at least one year old) of *S. bicolor* and *P. glaucum* containing cystosori of *P. graminis*. After incubating the rootlets in distilled water containing 200 mg/litre kinetin and 2 g/litre streptomycin sulphate, the extract was filtered through cheese-cloth. Seedlings of *T. aestivum* and *A. hypogaea* with roots 2–3 cm long were immersed in root extracts for 2 days. The extracts were checked for the presence of primary zoospores of *P. graminis* using a phase contrast microscope. The inoculated seedlings were then transferred to pots containing thoroughly washed, sterilised white sand and maintained in incubators at 30 °C. Plants were provided with a Hoaglands' nutrient solution (Hoagland & Arnon, 1950). After 4 wk the leaves of the plants were tested for H-IPCV infection by DAC-ELISA.

Results

Infection by *P. graminis*

P. graminis was observed in the roots of 14/70 *S. bicolor*, 142/168 *S. sudanense*, 20/100 *P. glaucum* and 14/50 *T. aestivum* plants raised in H-IPCV-infested soil. Viral antigens were detected in the leaves of all plants which contained *P. graminis*. Plasmodia were first observed in the roots of *P. glaucum* and *S. sudanense* on the sixth or seventh day after germination. A few plasmodia began to differentiate into sporangia within 7 days, and by 8 or 9 days after germination a few sporangia had developed fully and formed characteristic exit tubes (Fig. 1a). Cystosori began to develop from the eleventh day after germination. The

Table 1. *Presence of cystosori of Polymyxa graminis in roots of weeds and crop plants from H-IPCV-infested fields*

Host	No. of plants containing Cystosori/No. of plants tested
<i>Amaranthus</i> sp.	0/20
<i>Arachis hypogaea</i>	5/100
<i>Cynodon dactylon</i>	3/20
<i>Cyperus rotundus</i>	15/25
<i>Eleusine coracana</i>	10/20
<i>Pennisetum glaucum</i>	30/40
<i>Sorghum bicolor</i>	60/75
<i>Sorghum sudanense</i>	60/60
<i>Tridax procumbens</i>	5/15
<i>Triticum aestivum</i>	35/50
<i>Zea mays</i>	35/370

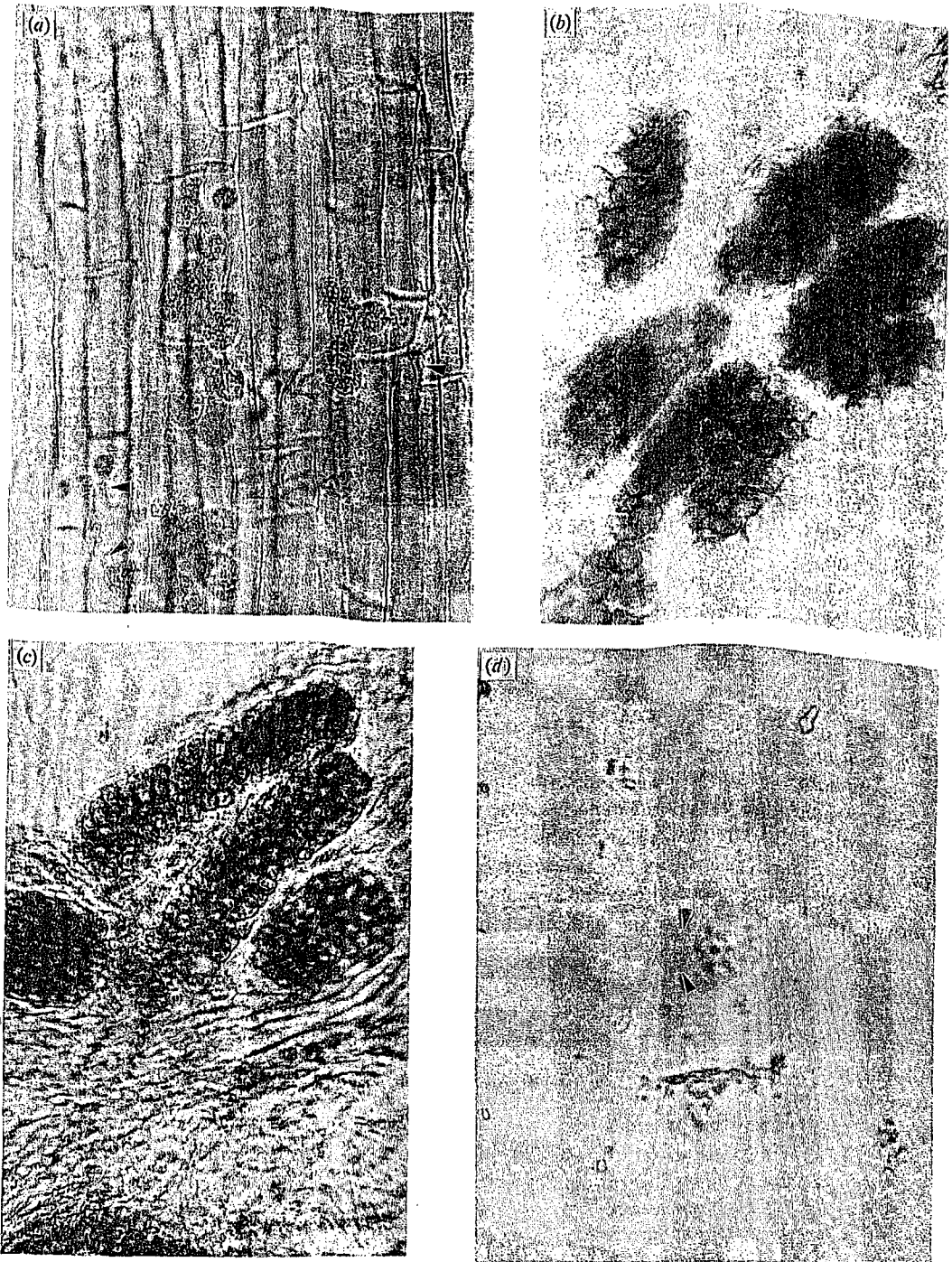


Fig. 1 (a) Sporangia of *Polymyxa graminis* with characteristic exit tubes (marked with arrows) in the roots of *Pennisetum glaucum*, $\times 528$.

(b) Cystosori of *P. graminis* in roots of *Arachis hypogaea*, $\times 1320$.

(c) Cystosori of *P. graminis* in roots of *Gomphrena globosa*, $\times 800$.

(d) Primary zoospore of *P. graminis* showing two whiplash flagella, $\times 1320$. Flagella are marked with arrows.

Table 2. *Host Range of Polymyxa graminis in laboratory tests*

Host name	Family	Nature of inoculum ^a	
		B-IPCV-infested soil	Roots of <i>S. sudanense</i>
Monocotyledonous plants			
<i>Allium porrum</i>	Liliaceae	3/110 ^b	Not done
<i>Eleusine coracana</i>	Poaceae	20/25	30/50
<i>Pennisetum glaucum</i>	Poaceae	20/25	75/100
<i>Setaria italica</i>	Poaceae	15/25	15/25
<i>Sorghum sudanense</i>	Poaceae	15/25	75/100
<i>Triticum aestivum</i>	Poaceae	25/25	80/100
<i>Zea mays</i>	Poaceae	49/200	19/20
Dicotyledonous plants			
<i>Beta vulgaris</i> cv. Globe	Chenopodiaceae	8/90	Not done
<i>B. vulgaris</i> cv. Detroit	Chenopodiaceae	28/100	0/20
<i>Brassica nigra</i>	Brassicaceae	22/100	Not done
<i>B. oleracea</i>	Brassicaceae	15/90	Not done
<i>Cleome spinosa</i>	Capparidaceae	39/150	Not done
<i>Celosia cristata</i>	Amaranthaceae	30/125	10/50
<i>C. plumosa</i>	Amaranthaceae	28/135	5/20
<i>Daucus carota</i>	Apiaceae	20/125	1/20
<i>Dolichos lablab</i>	Fabaceae	29/125	6/30
<i>Gomphrena globosa</i>	Amaranthaceae	5/85	Not done
<i>Lactuca sativa</i>	Asteraceae	34/125	1/20
<i>Lepidium sativum</i>	Brassicaceae	35/150	Not done
<i>Lycopersicum esculentum</i>	Solanaceae	23/140	1/20
<i>Medicago sativa</i>	Fabaceae	0/100	Not done
<i>Phaseolus lunatus</i>	Fabaceae	23/125	0/15
<i>P. vulgaris</i>	Fabaceae	31/175	3/60
<i>Pisum sativum</i>	Fabaceae	0/250	Not done
<i>Raphanus sativus</i>	Brassicaceae	35/175	0/10
<i>Spinacea oleracea</i>	Chenopodiaceae	27/125	1/20
<i>Tagetes patula</i>	Asteraceae	37/150	0/20

^a Experimental details given in the text. All plants incubated at 25–30°C.

^b No. of root segments containing *P. graminis* cystosori/No. examined.

formation of small, distinct thalli adjacent to cystosori at this stage indicates that some of the released zoospores may have reinfected the host cells.

Roots of *Cyperus rotundus*, *Eleusine coracana*, *S. bicolor*, *S. sudanense*, *P. glaucum* and *T. aestivum* collected from H-IPCV-infested fields also contained cystosori of *P. graminis*. The number of plants which contained the cystosori are given in Table 1. Cystosori of *P. graminis* were, for the first time, observed in the roots of IPCV-infected groundnut plants collected from H- and B-IPCV-infested fields. They were only observed in plants less than 6 wk old and even then only a small proportion of rootlets contained cystosori.

Of 19 dicotyledonous species raised in B-IPCV-infested soil in pots, 17 species showed moderate to low numbers of cystosori in rootlets (Table 2). All 7 monocotyledonous species tested had large numbers of *P. graminis* cystosori in their rootlets.

All six graminaceous species and eight of the 12 dicotyledonous species raised in sterilised soil with added H-IPCV infected roots of *S. sudanense* had *P. graminis* cystosori in their rootlets. Infection was common in monocotyledonous plants but uncommon in dicotyledonous plants (Table 2). Cystosori of the fungus in the roots of *A. hypogaea* are shown in Fig. 1b.

Infection with IPCV

When tested by ELISA 32% (8/25 plants) of groundnut, 40% (10/25 plants) of *S. bicolor* and 80% (20/25 plants) of *T. aestivum* raised in B-IPCV-infested, air-dried soil contained virus in their leaves. All infected plants contained *P. graminis* cystosori in their rootlets.

When used as inoculum, the root fragments of H-IPCV-infected *P. glaucum* resulted in infection of *T. aestivum* (20/40 plants), and *A. hypogaea* (10/18 plants). In mechanical transmission tests over 80% of *P. glaucum* and *A. hypogaea* plants were infected.

Table 3. *Transmission of H-IPCV from extracts prepared from roots of Sorghum bicolor and Pennisetum glaucum*

Source of root extract	Host plant	No. of positives in ELISA/ No. of plants tested
<i>S. bicolor</i>	<i>Arachis hypogaea</i>	9/25
	<i>Triticum aestivum</i>	8/20
<i>P. glaucum</i>	<i>Arachis hypogaea</i>	4/10
	<i>Triticum aestivum</i>	35/84

Results of examination of root extracts prepared from H-IPCV-infected *S. bicolor* and *P. glaucum* plants are given in Table 3. The extracts from infected *S. bicolor* roots transmitted the virus to 36% of *A. hypogaea* plants and 40% of *T. aestivum* plants; the extracts from *P. glaucum* transmitted the virus to 40% of *A. hypogaea* plants and 38% of *T. aestivum* plants. Zoospores of the fungus had two whiplash type flagella of unequal length and showed a rotatory swimming movement, typical of *P. graminis*, when observed in the extracts (Fig. 1*d*).

Discussion

The above results indicate that the soil-borne fungus *P. graminis* infects *A. hypogaea* and is a vector of IPCV. Unsuccessful attempts in West Africa to locate cystosori of *P. graminis* in *A. hypogaea* roots (Thouvenel & Fauquet, 1981) may be explained on the basis that only young rootlets obtained from less than 6 wk old *A. hypogaea* plants contained cystosori. It is easy to lose these rootlets when uprooting the plants. Additionally only 5% of *A. hypogaea* plants contained cystosori. It is important to determine if *P. graminis* isolate from West Africa can also infect dicotyledons. The *P. graminis* strains adapted to tropical conditions, however, seem to differ from those occurring in temperate soils in that the optimum temperature requirement for their development is higher, at around 30 °C (Nolt & Reddy, 1985; Reddy *et al.*, 1988), and they have a much wider host range which includes both dicotyledonous and monocotyledonous plants. In areas of the semi-arid tropics where IPCV is important some of the most commonly grown crops, *S. bicolor*, *P. glaucum* and *E. coracana* are hosts of IPCV. Additionally common weeds such as *C. rotundus*, *Cynodon dactylon* and *T. procumbens* are also hosts of IPCV. Thus crop rotation is not expected to be effective for the control of IPCV.

This study points to the need to re-examine the taxonomic status of *P. graminis* and *P. betae*, which are morphologically identical (Barr, 1979), and possess the same number of synoptinomal complexes during nuclear division (Braselton, 1988). Hitherto they have been retained as separate species on the basis of host range, *P. graminis* infecting only monocotyledonous species and *P. betae* infecting only dicotyledonous hosts. Such a distinction no longer seems valid.

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References

- Barr, D. J. S. (1979). Morphology and host range of *Polymyxa graminis*, *Polymyxa betae*, and *Ligniera pilorum* from Ontario and some other areas. *Canadian Journal of Plant Pathology* 1, 85–94.
- Brakke, M. K., Estes, A. P. & Schuster, M. L. (1965). Transmission of soil-borne wheat mosaic virus. *Phytopathology* 55, 79.
- Braselton, J. P. (1988). Karyology and systematics of Plasmodiophoromycetes. In *Developments in Applied Biology II. Viruses with Fungal Vectors*, pp. 139–145. Eds J. I. Cooper and M. J. C. Asher. Wellesbourne, UK: Association of Applied Biologists.
- Fauquet, C., Desbois, D., Fargette, D. & Vidal, G. (1988). Classification of furoviruses based upon the amino acid composition of their coat proteins. In *Developments in Applied Biology II. Viruses with Fungal Vectors*, pp. 19–35. Eds J. I. Cooper and M. J. C. Asher. Wellesbourne, UK: Association of Applied Biologists.
- Hebert, T. T. & Panlzo, C. H. (1975). Oat mosaic virus. *CMI/AAB Descriptions of Plant Viruses* 145, 3 pp.
- Hoagland, D. R. & Arnon, D. I. (1950). The water culture method for growing plants without soil. *California Agricultural Experimental Station Circular* 347.
- Hobbs, H. A., Reddy, D. V. R., Rajeshwari, R. & Reddy, A. S. (1987). Use of direct antigen coating and protein A coating ELISA procedures for detection of three peanut viruses. *Plant Disease* 71, 747–749.
- Inouye, T. & Fujii, S. (1977). Rice necrosis mosaic virus. *CMI/AAB Descriptions of Plant Viruses* 172, 4 pp.
- Inouye, T. & Saito, Y. (1975). Barley yellow mosaic virus. *CMI/AAB Descriptions of Plant Viruses* 143, 3 pp.
- Linford, M. B. & McKinney, H. H. (1954). Occurrence of *Polymyxa graminis* in roots of small grains in the United States. *Plant Disease Reporter* 38, 711–713.
- Nolt, B. L. & Reddy, D. V. R. (1985). First report of *Polymyxa graminis* in India. *Plant Disease* 69, 542.
- Nolt, B. L., Rajeshwari, R., Reddy, D. V. R., Bharathan, N. & Manohar, S. K. (1988). Indian peanut clump virus isolates; host range, symptomatology, serological relationships, and some physical properties. *Phytopathology* 78, 310–313.
- Rao, A. S. & Brakke, M. K. (1969). Relation of soil-borne wheat mosaic virus and its fungal vector, *Polymyxa graminis*. *Phytopathology* 59, 581–587.
- Reddy, D. V. R., Robinson, D. J., Roberts, I. M. & Harrison, B. D. (1985). Genome properties and relationships of Indian Peanut Clump Virus. *Journal of General Virology* 66, 2011–2016.
- Reddy, D. V. R., Iizuka, N., Subrahmanyam, P., Rajeshwari, R. & McDonald, D. (1979). A soil-borne disease of peanuts in India. *Proceedings of American Peanut Research and Education Society* 11, 49.
- Reddy, D. V. R., Rajeshwari, R., Iizuka, N., Lesemann, D. E., Nolt, B. L. & Goto, T. (1983). The occurrence of Indian peanut clump, a soil-borne virus disease of groundnuts (*Arachis hypogaea*) in India. *Annals of Applied Biology* 102, 305–310.
- Reddy, D. V. R., Nolt, B. L., Hobbs, H. A., Reddy, A. S., Rajeshwari, R., Rao, A. S., Reddy, D. D. R. & McDonald, D. (1988). Clump virus in India: isolates, host range, transmission and management. In *Developments in Applied Biology-II. Viruses with Fungal Vectors*, pp. 239–246. Eds J. I. Cooper and M. J. C. Asher. Wellesbourne, UK: Association of Applied Biologists.
- Shirako, Y. & Brakke, M. K. (1984). Two purified RNAs of soil borne wheat mosaic virus needed for infection. *Journal of General Virology* 65, 119–127.
- Slykhuis, J. T. & Barr, D. J. S. (1978). Confirmation of *Polymyxa graminis* as a vector of wheat spindle streak mosaic virus. *Phytopathology* 68, 639–643.
- Sundararaman, S. (1927). A clump disease of groundnuts. *Madras Agricultural Department Year Book*, 1926, pp. 13–14.
- Thouvenel, J. C., Dollet, M. & Fauquet, C. (1976). Some properties of peanut clump, a newly discovered virus. *Annals of Applied Biology* 84, 311–320.

- Thouvenel, J. C. & Fauquet, C.** (1981). Further properties of peanut clump virus and studies on its natural transmission. *Annals of Applied Biology* **97**, 99–107.
- Thouvenel, J. C., Fauquet, C. & Lamy, D.** (1978). Transmission of groundnut “clump” virus by the seed. *Oléagineux* **33**, 503–504..
- Thouvenel, J. C., Germani, G. & Pfeiffer, P.** (1974). Preuve de l’Origine virole du rabougrissement ou “clump” de l’Arachide en Haute Volta et au Sénégal. *Comptes-rendus hebdomadaire des seances de l’Academie des Sciences* **278**, 2847–2849.
- Trochain, J.** (1931). La “lepre” de l’arachides. *Revue de Botanique et Agriculture Tropicale* **11**, 330–334.

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