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# Host range of tropical and sub-tropical isolates of *Polymyxa graminis*

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**Abstract**

The host range of *Polymyxa graminis* isolates originating from peanut clump-infested areas in India (Andhra Pradesh and Rajasthan), Pakistan and Senegal was studied on monocotyledonous and dicotyledonous cultivated species, using known quantities of sporosori as inoculum. Profuse multiplication occurred only on some graminaceous species, but the various isolates showed different host specificity. All the isolates produced high infection on sorghum and pearl millet, and all but one isolate from Rajasthan infected maize. Wheat, rye and barley were susceptible to some of the tested isolates. The isolates from Rajasthan and Pakistan produced moderate to severe infection on at least one of these species. On rice, groundnut and sugar beet, only traces of infection by some isolates were detected, whereas no infection was observed on mustard and sunflower. Differences of susceptibility in *Pennisetum* spp. and *Sorghum* spp. were demonstrated. The variations in host specificity among isolates from peanut clump-infested areas may result from an adaptation of *P. graminis* populations to various biotopes. The implications of these results for the management of peanut clump disease are discussed. A comparison of the host ranges of isolates of *P. graminis* and *P. betae* from temperate areas demonstrated that distinct types of *Polymyxa* might be identified based on their relative ability to multiply on susceptible species. Nevertheless, overlapping in the host ranges among the different *Polymyxa* types, characterised by distinct ecological and genomic features, raises doubts about the host range as a classification criterion for the *Polymyxa* genus.

*Abbreviation:* (I)PCV – (Indian) peanut clump virus.

## Introduction

*Polymyxa graminis* Ledingham, a member of the Plasmodiophorales (Karling, 1968), is an endoparasite of roots of graminaceous plant species. It occurs worldwide and is economically important as a vector of plant viruses causing severe diseases in several major food crops. It was first recognised as a vector of viruses on cereals, such as barley yellow mosaic virus (Inouye and Saito, 1975), oat mosaic virus (Hebert and Panizo, 1975), oat golden stripe virus (Plumb et al., 1977), rice necrosis mosaic virus (Inouye and Fuji, 1977), soil-borne wheat mosaic virus (Estes and Brakke, 1966) and wheat spindle streak mosaic virus (Slykhuis, 1976). More recently, it has also been recognised in sub-tropical countries and associated with the transmission of peanut clump virus (PCV) in West Africa (Thouvenel and Fauquet, 1981) and Indian peanut clump virus (IPCV) (Nolt and Reddy, 1985) in India causing peanut clump disease. Fauquet et al. (1988) reported that *P. graminis* might also be involved in the transmission of the rice stripe necrosis virus in Côte d'Ivoire.

At the epidemiological level, peanut clump disease differs from the other viral diseases transmitted by *P. graminis* in that the plant species affected by the

virus – groundnut – is not a natural reservoir of the vector. *P. graminis* was not (or only rarely) detected in groundnut roots grown in infected soils (Thouvenel et al., 1988; Ratna et al., 1991; A. Legrève and P. Delfosse, unpubl. data). The natural disease cycle, therefore, requires the involvement of alternate hosts of the virus and its vector. The transmission of PCV and IPCV by *P. graminis* was demonstrated by indirect evidence, based on the infection of groundnut grown in sterile soils containing dried root fragments of graminaceous plants, such as pearl millet (*Pennisetum glaucum* (L.) R. Br.), great millet (*Sorghum arundinaceum* (Desv.) Stapf.), sorghum (*Sorghum bicolor* (L.) Moench), foxtail millet (*Setaria italica* (L.) P.B.) and/or wheat (*Triticum aestivum* L.), which were obtained from clump-infested soils and contained *P. graminis* sporosori (Thouvenel and Fauquet, 1981; Thouvenel et al., 1988; Reddy et al., 1988; Ratna et al., 1991). It was assumed that *P. graminis* zoospores infect groundnut and transmit the virus without (or only rarely) subsequent development in groundnut (Thouvenel and Fauquet, 1981).

In addition to groundnut, PCV and IPCV were detected on species of different plant families of dicotyledonous and graminaceous plants (Thouvenel and Fauquet, 1981; Ratna et al., 1991; Delfosse et al., 1996). Most of these natural hosts are symptomless, but recent studies have shown that symptoms on wheat, barley (*Hordeum vulgare* L.), pigeonpea (*Cajanus cajan* L.) and chilli (*Capsicum annuum* L.) were associated with the presence of IPCV in India (Delfosse et al., 1995; 1999; P. Delfosse and H. Maraite, pers. comm.). In Africa, the red leaf mottle disease occurring on sugarcane (*Saccharum* L. interspecific hybrids) was shown to be caused by PCV (Baudin and Chatenet, 1988; Chatenet, 1995). *P. graminis* was also detected on a wide range of species in an IPCV-infested field, including monocotyledonous and dicotyledonous plants, but profuse sporosori production occurred only on monocotyledonous species (Ratna et al., 1991). In particular, sorghum, pearl millet and several grassy weeds, such as *Cynodon dactylon* (L.) Pers., *Cyperus rotundus* L. and *Eragrostis uniloides* (Retz.) Steud., have been identified as favourable hosts for the multiplication of *P. graminis* from IPCV-infested areas in Andhra Pradesh (Delfosse et al., 1996; Legrève et al., 1996). In the PCV-infested areas in West Africa, *P. graminis* was readily detected in roots of great millet bait plants that became infected with the virus. Thouvenel and Fauquet (1980) also reported that one or more stages of *P. graminis* were observed on six other *Sorghum* spp., on oat (*Avena sativa* L.), on wheat (*T. aestivum* L. and *T. durum* Desf.) and on barley. As cereals are often grown in rotation with groundnut in the semi-arid areas, it is likely that they contribute to a build-up of viruliferous inoculum potential of *P. graminis* in the soil and subsequently to high peanut clump disease incidence in the ensuing groundnut crops. Some highly pernicious weeds, hosts of both the virus and the vector, are also presumed to act as a carry-over for the virus and its vector (Dollet et al., 1993; Delfosse et al., 1996).

A better knowledge of the host specificity of *P. graminis* populations occurring in peanut clump-infested areas and of the rates of colonisation on different host species is thus essential for making a better assessment of the influence of different plant species rotated with groundnut on clump incidence, and for designing cultural practices to limit the vector multiplication in infested soils and restrict the spread of peanut clump disease (Reddy et al., 1988; Dollet et al., 1993). None of more than 9000 tested *Arachis hypogaea* genotypes showed resistance to IPCV; and biocides, though sometimes effective, are not economic. Some cultural practices were shown to limit the progression of the viruliferous inoculum potential in soils or its expression when the groundnut crop was planted. Thouvenel et al. (1988) reported that the incidence of the disease can be reduced by using a cropping system in which great millet is replaced by pearl millet. Another method involving growing pearl millet for 15 days as a trap crop prior to sowing groundnut was shown to result in a reduced incidence of the disease in the ensuing groundnut crop (Delfosse et al., 1997).

The host range is the basis for the classification of *Polymyxa* species. Two species have been described in the genus *Polymyxa* – *P. graminis* Ledingham (1939) and *P. betae* Keskin (1964). There is no apparent morphological distinction between them, and based on their morphology they could belong to the same species (Barr, 1988). Nonetheless, Barr (1979, 1988) retained them as separate species because their reported host ranges were both restricted and distinctive, *P. graminis* infecting graminaceous plants whereas *P. betae* colonises various species of Chenopodiaceae or associated plant families. The infection of groundnut roots by *P. graminis* from clump-infested areas and the differences between the host ranges, temperature requirements and genomic characteristics of some Indian isolates compared to isolates of *P. graminis*

and *P. betae* from temperate origins (Legrève et al., 1996; 1998) indicated the need for more in-depth studies on the host ranges of *Polymyxa* spp. and for a re-assessment of the validity of the distinction between the two species.

In this paper we report experiments conducted in controlled conditions with known quantities of purified inoculum on the host specificity of *P. graminis* isolates from IPCV-infested areas in the Asian sub-continent and from a PCV-infested area in Senegal. The rates of colonisation of host plant species by *P. graminis* isolates were estimated to assess their role in peanut clump epidemiology as well as the diversity among isolates from different areas infested by clump disease. The susceptibility of various *Pennisetum* and *Sorghum* germplasm accessions, including wild taxa, was also assessed to identify a potential source of resistance. Some isolates of *P. graminis* and *P. betae* from temperate areas were included in this study to explore the range of variation of *Polymyxa* spp. populations and the pertinence of the usual classification criterion for distinguishing the species.

## Materials and methods

### *Origin of the* Polymyxa *isolates*

The six isolates used for this work were obtained from bait plants grown in *Polymyxa*-infested soils originating from clump-infested areas in South Asia and Africa (Table 1). They were subsequently separated from fungal root contaminants by the inoculation of a single sporosorus or group of sporosori extracted from root fragments infected only by *Polymyxa* to seedlings grown in sand culture, and multiplied by repeated transfer to seedlings using the automatic immersion system (Legrève et al., 1998). These isolates were compared with isolates of *P. graminis* on barley from Canada and France and of *P. betae* on sugar beet from Belgium and Turkey (Legrève et al., 1998). The origins of isolates and the conditions under which they were propagated are summarised in Table 1.

### *Host specificity of* Polymyxa *isolates*

*Polymyxa* isolates were tested for host specificity by growing plants in single culture tubes (volume $\pm$ 65 ml) on autoclaved sand inoculated with defined quantities of sporosori. A sporosori suspension was prepared for each isolate as described by Legrève et al. (1998). Ten thousand sporosori were inoculated in culture tubes by the addition of 0.5–2 ml of sporosori suspension. Pre-germinated seeds of the different plant species and cultivars tested (Table 2) were transplanted into 10 (sometimes 6) tubes inoculated with each *Polymyxa* isolate. For some combinations of plant species/isolates, two other sporosori concentrations (2000 and 400 sporosori/tube) were used as inoculum. Control plants were transplanted into non-inoculated tubes. The temperature requirements of *Polymyxa* isolates originating from different areas being distinct (Legrève et al., 1998), the plants were grown in various conditions depending on the

*Table 1.* *Polymyxa graminis* and *P. betae* isolates

| Code | Origin of the soil | Isolation | | Propagation | |
|---|---|---|---|---|---|
| | | Plant used | Year | Plant used | Temperature °C |
| *P. graminis* | from IPCV- or PCV-infested soils | | | | |
| $I_{1-1}$* | India, Andhra Pradesh, Patancheru | Sorghum | 1993 | Sorghum | 25–30 |
| $I_{1-229}$* | India, Andhra Pradesh, Patancheru | Sorghum | 1993 | Sorghum | 25–30 |
| $I_9$ | India, Rajasthan, Boraj | Sorghum | 1997 | Sorghum | 25–30 |
| $I_{10}$ | India, Rajasthan, Rampura | Sorghum | 1997 | Sorghum | 25–30 |
| $P_1$ | Pakistan , Punjab | Sorghum | 1996 | Sorghum | 25–30 |
| $S_6$ | Senegal, Bambey | Pearl millet | 1995 | Sorghum | 25–30 |
| *P. graminis* | from temperate origins | | | | |
| $C_1$* | Canada, Ottawa | Barley | 1987 | Barley | 15–20 |
| $F_{11}$* | France, Carcassonne | Barley | 1988 | Barley | 15–20 |
| *P. betae* | | | | | |
| $A_{26-41}$* | Belgium, Opprebais | Sugar beet | 1987 | Sugar beet | 20–25 |
| $T_{17}$* | Turkey | Sugar beet | 1989 | Sugar beet | 20–25 |

*Single-sporosorus isolates.

*Table 2*. Host range of isolates of *Polymyxa graminis* and *P. betae* from different origins. Mean infection degree on 6–10 plants, each grown on sterile sand inoculated with $10^4$ sporosori per tube

| | Degree of infection[1] by | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *P. graminis* from PCV- and IPCV-infested areas | | | | | | *P. graminis* | | *P. betae* | |
| | $I_{1-1}$ | $I_{1-229}$ | $I_9$ | $I_{10}$ | $P_1$ | $S_6$ | $C_1$ | $F_{11}$ | $A_{26-41}$ | $T_{17}$ |
| Monocot. species[2] | | | | | | | | | | |
| *A. sativa* | – | – | – | – | – | – | – | 0.0a | 0.0a | 0.0a |
| *H. vulgare* | 0.3a[3] | 0.0a | 2.8c | 1.1b | 0.3a | 0.3a | 2.9b | 3.0d | 0.0a | 0.0a |
| *O. sativa* | 0.0a | 0.0a | 0.3a | 0.1a | 0.0a | 0.0[5]a | 0.0a | 0.0a | 0.0a | 0.0a |
| *P. glaucum* IC | 3.0d | 2.6c | 3.0c | 2.6c | 2.3cd | 3.0b | (0.0a) | 0.0a | 0.0a | 0.0a |
| *P. glaucum* IP | 2.2c | 1.2b | – | – | (1.3abc) | 2.5b | – | – | – | – |
| *S. bicolor* | 2.9d | 2.9c | 3.0c | 2.1c | 2.4cd | 2.8b | 0.0a | 0.4abc | 0.0a | 0.0a |
| *S. cereale* | 1.2b | 0.5a | 3.0c | 1.1b | 1.8bcd | 0.8a | 0.7a | 0.8bc | 0.0a | 0.0a |
| *S. dimidiatum* | 0.0a | 0.4a | – | – | (1.0ab) | 0.0a | – | – | – | – |
| *T. aestivum*-RR | 0.3a | 0.1a | 2.3b | 0.0a | (0.8ab) | 0.5a | 0.8a | 1.0c | 0.0a | 0.0a |
| *T. aestivum*-Cap. | – | – | – | – | – | – | 0.2a | 0.5abc | 0.0a | 0.0a |
| *Z. mays* | 2.4cd | 2.9c | 3.0c | 0.0a | 2.9d | 2.6b | 0.0a | 0.2ab | 0.0a | 0.0a |
| Dicot. species | | | | | | | | | | |
| *A. hypogaea* | 0.0[4]a | 0.0a | 0.2a | 0.0a | 0.0a | 0.0a | (0.0a) | 0.0a | 0.0a | 0.0a |
| *B. juncea* | 0.0a | 0.0a | – | – | 0.0a | 0.0a | – | – | – | – |
| *B. vulgaris* | 0.0a | 0.2a | – | – | 0.2a | 0.0a | 0.0a | 0.2ab | 2.8b | 3.0b |
| *H. annuus* | 0.0a | 0.0a | – | – | 0.0a | 0.0a | – | – | – | – |

[1]Degree of infection of a root system: 0 = no infection; 1 = low infection (a few (< 10) root cells infected by the particular development stage); 2 = moderate infection (a few groups of some (> 10–100) root cells infected or a single part of the root heavily infected (> 100 root cells infected)); and 3 = high infection (infection in all parts of the roots). – = not tested. Results in parentheses indicate mean degree of infection calculated on fewer than 6 plants.

[2]Monocotyledonous species: *Avena sativa* cv. Tornade; *Hordeum vulgare* cv. Narcis; *Oryza sativa* cv. Rasi; *P. glaucum* IC = *Pennisetum glaucum* cv. ICMV 8790; *P. glaucum* IP = *P. glaucum* IP 11902; *Sorghum bicolor* cv. ICSV 88036; *Secale cereale* cv. Halo; *Sorghum dimidiatum* S 307; *T. aestivum* RR = *Triticum aestivum* cv. RR21; *T. aestivum* Cap. = *T. aestivum* cv. Capitaine; *Zea mays* cv. DHM 103. Dicotyledonous species: *Arachis hypogaea* cv. NCAc 17090; *Brassica juncea*; *Beta vulgaris* cv. Cadyx; *Helianthus annuus* cv. MSFH-8.

[3]Means in a column not followed by the same letter differ significantly according to the Student–Newman–Keuls test ($P = 0.05$).

[4]Trace of infection (few sporosori) detected in seminal roots in a previous assay in roots inoculated with $1.5 \times 10^4$ sporosori per plant.

[5]Trace of infection (isolated sporosori or zoosporangia) detected in a previous assay in 2 roots on 20 tested, each inoculated with $5 \times 10^3$ sporosori.

tested isolates. They were placed in growth chambers at 25–30 °C for 6 weeks when grown with *P. graminis* isolates from India, Pakistan and Senegal, at 20–25 °C for 6 weeks with *P. betae* isolates, and at 15–20 °C for 7 or 8 weeks with *P. graminis* from Canada and France. The plants were watered with modified half-strength Hoagland solution (Legrève et al., 1998). The watering was moderated during the first 10 days of culture to favour root development. Afterwards, the cultures were abundantly watered by a daily supply of nutrient solution, and a saturation of the tube at least twice a week to promote *Polymyxa* development. After the saturation periods, the watering was reduced slightly to avoid asphyxia of the roots. After the growing period, each plant was uprooted. One-fifth of the volume of each root system was detached and air-dried as a potential source of inoculum. The development of *Polymyxa* in the rest of each root system, stained in aniline blue lactophenol, was analysed microscopically (16–500×) by systematically scrutinising the roots over their entire length. The *Polymyxa* development stages observed in the roots were recorded and the degree of root infection was scored on a 0–3 scale: 0 = no infection; 1= low infection (a few (< 10) root cells infected); 2 = moderate infection (a few groups of some (> 10–100) root cells infected or a single part of the root heavily infected

(> 100 root cells infected)); and 3 = high infection (infection in all parts of the roots).

### *Resistance to* P. graminis *isolate* $I_{1-1}$ *in* Sorghum *spp. and* Pennisetum *spp.*

The susceptibility of 16 accessions of *Sorghum* spp. and 10 of *Pennisetum* (Table 4) to infection by the *P. graminis* isolate $I_{1-1}$ was tested in controlled conditions, following a procedure similar to the one described above. Twenty pre-germinated seeds of each germplasm accession were transplanted separately into individual culture tubes, each inoculated with 2000 sporosori. Five other seeds were transplanted into non-inoculated culture tubes as the control. After 6 weeks growth at 25–30 °C, the plants were removed from the sand and the roots cut into two halves. One half was stained in aniline blue lactophenol and *P. graminis* infection was assessed microscopically, as described above. The other half was air-dried and weighed. Four composite samples from 5 roots were prepared from the air-dried roots of each accession. The root samples were weighed and placed in a defined volume of water. Sporosori were extracted from the roots as for inoculum preparation, and the sporosori concentration was assessed using a Fuchs Rosenthal counting chamber. Eight counts were done for each of the four sporosori suspensions prepared per accession.

## Results

### *Host specificity of* Polymyxa *isolates from different origins*

All the *P. graminis* isolates originating from clump-infested areas grew well on *S. bicolor*, on *P. glaucum* cv. ICMV 8790 and, apart from the isolate $I_{10}$, on *Z. mays* (Table 2). Six weeks after inoculation of $10^4$ sporosori per plant, with the exception of $I_{10}$ on *Z. mays*, large numbers of zoosporangia and sporosori of these isolates were produced on these species, indicating that both phases of the life cycle occurred. Nevertheless, for $I_{1-1}$, $I_{1-229}$, $P_1$ and $S_6$, assays with two lower inoculum concentrations demonstrated some differences in the plant species–isolate interaction (Table 3). On *S. bicolor*, the degree of infection was high regardless of the amount of inoculum used, but on *P. glaucum* cv. ICMV 8790 and *Z. mays* the infection was sometimes moderate to low, especially when 400 sporosori were used as inoculum (Table 3). On *P. glaucum* cv. IP 11902, the growth of these isolates was sometimes significantly lower than on *S. bicolor* and *P. glaucum* cv. ICMV 8790. There was usually little or no growth on *S. dimidiatum*.

The infection produced by the six tested isolates originating from clump-infested areas on *T. aestivum*, *H. vulgare* and *Secale cereale* varied strongly depending on the isolate (Tables 2 and 3). Isolate $I_9$ multiplied well – a large amount of sporosori and some

*Table 3.* Effect of sporosori concentration on infection of some plant species by four *Polymyxa graminis* isolates from PCV- or IPCV-infested areas. Mean degree of infection on 6–10 plants

| Plant species[1] | Sporosori concentration | Degree of infection by *P. graminis* isolates[2] | | | |
|---|---|---|---|---|---|
| | | $I_{1-1}$ | $I_{1-229}$ | $P_1$ | $S_6$ |
| *P. glaucum* IC | 10000 | 3.0b[3] | 2.6 | 2.3 | 3.0 |
| | 2000 | 2.8b | 2.6 | 2.4 | 3.0 |
| | 400 | 1.6a | 2.3 | 1.6 | 2.8 |
| *P. glaucum* IP | 10000 | 2.2b | 1.2ab | (1.3)[4] | 2.5 |
| | 2000 | 1.7b | 1.8b | 2.6 | 2.8 |
| | 400 | 0.2a | 0.7a | 1.0 | 3.0 |
| *S. bicolor* | 10000 | 2.9 | 2.9 | 2.4 | 2.8 |
| | 2000 | 3.0 | 2.4 | 2.6 | 2.9 |
| | 400 | 3.0 | 2.5 | 2.5 | 2.8 |
| *S. dimidiatum* | 10000 | 0.0 | 0.4 | (1.0) | 0.0 |
| | 2000 | 0.0 | 0.0 | 0.2 | 0.0 |
| | 400 | 0.0 | 0.0 | 0.0 | 0.0 |
| *T. aestivum* | 10000 | 0.3 | 0.1 | (0.8)b | 0.5 |
| | 2000 | 0.0 | 0.0 | 0.1a | 0.0 |
| | 400 | 0.0 | 0.0 | 0.0a | 0.0 |
| *Z. mays* | 10000 | 2.4 | 2.9 | 2.9b | 2.6b |
| | 2000 | 2.4 | 3.0 | 2.3b | 2.4b |
| | 400 | 2.3 | 2.8 | 1.0a | 1.6a |

[1] *P. glaucum* IC = *Pennisetum glaucum* cv. ICMV 8790; *P. glaucum* IP = *P. glaucum* IP 11902; *Sorghum bicolor* cv. ICSV 88036; *Sorghum dimidiatum* S307; *Triticum aestivum* cv. RR21, *Zea mays* cv. DHM 103.

[2] The analyses of variance of the *P. graminis* degree of infection on six plant species after 6 weeks growth with three different sporosori concentrations were tested separately for each isolate and showed that the plant species had a highly significant effect ($P \leq 0.01$) on the infection degree of each isolate, but for $I_{1-1}$ and $P_1$ the sporosori concentration also influenced significantly ($P \leq 0.01$) the infection degree. Furthermore, for $I_{1-1}$ and $S_6$ the interaction between both factors was highly significant ($P \leq 0.01$).

[3] For each isolate, the infection produced by the three sporosori concentrations on the same species were compared with the Student–Newman–Keuls test. Means not followed by the same letter differ significantly ($P = 0.05$).

[4] Numbers in parentheses indicate mean degrees of infection calculated from fewer than 6 plants.

zoosporangia were observed – on these three species, with a slightly lower infection on *T. aestivum* than on the other cereals. A great number of sporosori were produced by the isolate $I_{10}$ on some plants of *H. vulgare* and *S. cereale* but no infection of $I_{10}$ was observed on *T. aestivum*. The isolate $P_1$ produced only a trace of infection – presence of a few sporosori – on *H. vulgare*, but was able to produce moderate to high infection on 66% of the *S. cereale* plants and moderate infection on 40% of the *T. aestivum* plants. A recent assay, not described here, confirmed the ability of this isolate to grow on wheat, but its multiplication rate was lower on this species than on *Sorghum bicolor*. The infection produced by the three other isolates ($I_{1-1}$, $I_{1-229}$ and $S_6$) on *H. vulgare*, *Secale cereale* and *T. aestivum* was very limited (as sporosori and/or zoosporangia depending on the isolate/species interaction). On *S. cereale*, a slight to moderate infection (zoosporangia and sporosori) by isolates $I_{1-1}$ and $S_6$ was detected. On *H. vulgare* and on *T. aestivum*, the mean infection degree was very low, but the isolate $S_6$ produced a high infection (as zoosporangia and sporosori) on one wheat plant when using $10^4$ sporosori as inoculum. The restricted infection of wheat by the isolate $S_6$ was, nevertheless, confirmed in several other assays using $5 \times 10^3$ sporosori per plant as inoculum (A. Legrève, unpubl. data).

*O. sativa* was found to be infected by the isolates $S_6$, $I_9$ and $I_{10}$, but the plants always showed a very low degree of infection (Table 2).

On dicotyledonous plants, a very restricted infection was observed for some *P. graminis* isolates. *Beta vulgaris* was infected by isolates $I_{1-229}$ and $P_1$ and *A. hypogaea* by isolates $I_9$ and $I_{1-1}$ (Table 2). A single zoosporangium and a single sporosorus of $I_9$ were detected in *A. hypogaea* roots. In a previous assay, the presence of a few sporosori of isolate $I_{1-1}$ had been detected in seminal *A. hypogaea* roots after growth on sand inoculated with $1.5 \times 10^4$ sporosori per plant.

*P. graminis* isolates $C_1$ from Canada and $F_{11}$ from France showed distinct host specificity compared with the isolates of *P. graminis* originating from peanut clump-infested areas (Table 2). In addition to *H. vulgare*, these isolates infected the two tested cultivars of *T. aestivum* and *S. cereale*. The isolate $F_{11}$ also infected *Z. mays*, *Sorghum bicolor* and even *B. vulgaris* (a few zoosporangia and sporosori). The severity of infection for both isolates was significantly higher on *H. vulgare* than on the other monocotyledonous species. For these latter species, infected plants showed only a trace of infection (zoosporangia or sporosori), except for a single case of moderate infection on a *T. aestivum* cv. RR21 root for $F_{11}$ and a single case of high infection on *Secale cereale* for $C_1$ and $F_{11}$.

The two *P. betae* isolates tested showed a host range restricted to *B. vulgaris* (Table 2).

### *Resistance to* P. graminis *isolate* $I_{1-1}$ *in* Sorghum *spp. and* Pennisetum *spp.*

Fifteen of the 16 tested *Sorghum* accessions, including *S. bicolor* ssp. *bicolor*, *drummondii* and *verticilliflorum* and *S. halepense*, were abundantly infected after 6 weeks growth in sand inoculated with sporosori of the *P. graminis* isolate $I_{1-1}$ (Table 4). Some plasmodia and zoosporangia were observed in all the accessions, sometimes in high quantities, but the sporosorus stage was prevalent. The degree of colonisation by *P. graminis* was not significantly different for these accessions when assessed by observation of the stained portion of the root systems under the microscope. However, when the degree of infection was estimated by counting the sporosori concentrations per mg of dried roots using the Fuchs Rosenthal cell, significant differences were apparent. The multiplication of *P. graminis* in the roots was the highest for the cultivated form of *Sorghum*, and slightly reduced in *S. bicolor* spp. *drummondii* and *verticilliflorum* and in *S. halepense*. The sporosori concentration in roots after 6 weeks growth reached between 1100 and 4500 sporosori per mg of dried roots for all these accessions. The infection on *S. dimidiatum* was significantly lower than on the other accessions. Two plants of *S. dimidiatum* (out of 20) showed the presence of a few sporosori and one plant was infected with isolated zoosporangia.

In the *Pennisetum* accessions, the degree of infection and the sporosori concentrations were significantly lower than in the *Sorghum* species (Table 4). Diversity in the susceptibility of plants to *P. graminis* and in their ability to develop the infection occurred among *P. glaucum* cultivars as well as among the other *Pennisetum* species tested. The degree of infection in the roots, assessed by observation or by counting the sporosori concentration, was highest for *P. glaucum* cv. ICMV 8790 but the concentration of sporosori in roots did not reach that in the grain and forage *Sorghum* accessions. The mean degree of infection was moderate for the five other tested cultivars of *P. glaucum* (IP 3122,

*Table 4*. Susceptibility of 16 *Sorghum* accessions and of 10 *Pennisetum* ones to *Polymyxa graminis* isolate $I_{1-1}$

| Accessions | | | | Number of tested plants | Mean degree of infection[1] by | | | | Sporosori/ mg root |
|---|---|---|---|---|---|---|---|---|---|
| Sp.- ssp. | Form | Code | Origin | | Plasm. | Zoosp. | Spor. | Total | Mean |
| *S. bicolor* ssp. *bicolor* | Cultivated | IRAT 204 | | 20 | 0.65 | 1.30 | 2.90 | 2.95b | 2333e |
| *S. bicolor* ssp. *bicolor* | Cultivated | IS 3890 | Mali | 20 | 0.85 | 1.50 | 2.95 | 2.95b | 2397e |
| *S. bicolor* ssp. *bicolor* | Cultivated | IS 7871 | Nigeria | 20 | 1.45 | 2.00 | 3.00 | 3.00b | 2945f |
| *S. bicolor* ssp. *bicolor* | Cultivated | IS 24357 | India | 20 | 1.55 | 2.05 | 3.00 | 3.00b | 2997f |
| *S. bicolor* ssp. *bicolor* | Cultivated | IS 40284 | India | 20 | 1.25 | 2.35 | 2.95 | 3.00b | 4518i |
| *S. bicolor* ssp. *bicolor* | Cultivated | IS 2861 | South Africa | 20 | 1.90 | 2.10 | 2.90 | 2.95b | 4012h |
| *S. bicolor* ssp. *bicolor* | Cultivated | IS 3162 | South Africa | 20 | 1.60 | 2.15 | 2.55 | 2.90b | 4183hi |
| *S. bicolor* ssp. *bicolor* | Cultivated | IS 18519 | Uganda | 20 | 0.60 | 2.55 | 3.00 | 3.00b | 3617g |
| *S. bicolor* ssp. *bicolor* | Cultivated | IS 18520 | Uganda | 20 | 0.80 | 2.45 | 3.00 | 3.00b | 2758f |
| *S. bicolor* ssp. *drummondii* | Forage | IS 720 | | 20 | 0.35 | 2.25 | 2.90 | 2.95b | 1824cd |
| *S. bicolor* ssp. *drummondii* | Forage | IS 722 | | 20 | 1.85 | 1.70 | 2.85 | 2.90b | 1501c |
| *S. bicolor* ssp . *drummondii* | Weedy | S. 64 | | 20 | 1.50 | 2.80 | 2.90 | 3.00b | 1780cd |
| *S. bicolor* ssp. *verticilliflorum* | Race *verticilliflorum*, wild | S .01 | | 20 | 0.75 | 2.65 | 2.95 | 3.00b | 1982de |
| *S. bicolor* ssp. *verticilliflorum* | Race *arundinaceum*, wild | S .162 | | 20 | 1.15 | 2.50 | 3.00 | 3.00b | 2226e |
| *S. halepense* | Johnson grass | S. 77 | | 15 | 1.67 | 2.53 | 2.73 | 2.93b | 1134b |
| *S. dimidiatum* | | S. 307 | | 20 | 0.05 | 0.15 | 0.10 | 0.20a | 3a |
| *P. glaucum* | Cultivated | ICMV 8790 | | 20 | 0.10 | 1.50 | 2.35 | 2.75c | 220f |
| *P. glaucum* | Cultivated | IP 3122 | India | 20 | 0.10 | 1.40 | 0.90 | 1.70c | 47abc |
| *P. glaucum* | Cultivated | IP 4021 | India | 20 | 0.25 | 1.35 | 1.85 | 2.2bc | 53bc |
| *P. glaucum* | Cultivated | IP 13115 | Niger | 20 | 0.00 | 1.65 | 1.45 | 1.95bc | 78de |
| *P. glaucum* | Cultivated | IP 8638 | Sudan | 20 | 0.00 | 1.50 | 0.95 | 1.60b | 13ab |
| *P. glaucum* | Cultivated | IP 16793 | Zimbabwe | 17 | 0.24 | 1.18 | 1.82 | 2.24bc | 213f |
| *P. glaucum* | Cultivated | IP 11902 | Sierra Leone | 20 | 0.10 | 0.40 | 0.10 | 0.55a | 2a |
| *P. glaucum* ssp. *violaceum* | Wild | IPW 7 | | 6 | 0.00 | 1.00 | 1.83 | 2.00bc | 56bc |
| *P. pedicellatum* | Wild | IPW 306 | | 7 | 0.00 | 0.00 | 0.00 | 0.00a | 2a |
| *P. schweinfurthii* | Wild | IPW 414 | | 20 | 0.00 | 0.75 | 1.35 | 1.60b | 112e |

[1]Degree of infection: none (0), slight (1), moderate (2) or severe (3) infection, by plasmodia (Plasm.), zoosporangia (Zoosp.) or sporosori (Spor.). Means in a column not followed by the same letter differ significantly according to the Student–Newman–Keuls test ($P = 0.05$).

IP 4021, IP 13115, IP 8638 and IP 16793) from India, Niger, Sudan and Zimbabwe and very reduced for the cv. IP 11902 originating from Sierra Leone. The concentration of sporosori varied from 2 to 213 sporosori per mg of dried root for the different cultivars. Zoosporangia were detected in all these accessions at frequencies sometimes higher than sporosori. The infection and the assessed sporosori concentration were in the same range for *P. schweinfurthii* Pilger and *P. glaucum* ssp. *violaceum* (Lam.) A. Rich. No infection was detected by observation of the stained portion of the root systems for *P. pedicellatum*, although a few sporosori were counted in the dried part of roots of this species. The number of plants that were analysed for the two latter species was lower than for all the other accessions because of reduced germination.

## Discussion

Our results confirmed the previous observations of Thouvenel and Fauquet (1980) and Ratna et al. (1991) that *P. graminis* isolates from clump-infested areas can be found in a wide number of species, including monocots and dicots. Furthermore, data presented here and in a previous report (Legrève et al., 1996), and obtained with defined quantities of purified inoculum, demonstrated that high levels of infection by these isolates occurred only on cereals but there is a real diversity in host specificity and aggressiveness among the isolates. Each isolate had a particular host range. All the isolates produced high infection on *S. bicolor* and on the cv. ICMV 8790 of *P. glaucum*, but the infection on the other tested plants varied depending on the isolate. Besides this diversity, our results revealed a

new notion that the inoculum threshold required for intense root colonisation varied according to the host species.

The origin of the diversity, which could be related to the ability of the isolates to germinate, to infect, to multiply or even to adapt to a host species, has yet to be determined. Nevertheless, this variability between the host range of the various isolates is not an experimental artefact. Indeed, the host range of the isolates corroborates with the plant species found to be infected when grown on naturally infested soils. For example, in experiments conducted in the field in Andhra Pradesh, *P. graminis* was found to invade with high intensity roots of sorghum, pearl millet and maize, but not wheat, barley and dicotyledonous plants (Delfosse et al., 1996). In controlled conditions, our attempts to isolate *P. graminis* from the soil of Patancheru succeeded when using sorghum and pearl millet as bait plants, but failed with wheat and barley (Legrève et al., 1998). From the soil of Pakistan, *P. graminis* could be isolated on sorghum and wheat. The quantity of sporosori produced on wheat was lower than on sorghum. Moreover, there is apparently a link between the host specificity of the isolates and the species usually cultivated in the areas whence the isolates originated. In Andhra Pradesh in India and in Senegal, located in the semi-arid tropics, sorghum, pearl millet and maize are the major cereal crops grown in the rainy season, whereas the cultivation of wheat and barley is negligible. The isolates originating from these areas showed a high colonisation rate on sorghum, pearl millet and maize, while the infection detected on wheat and barley was relatively insignificant. In Rajasthan and Pakistan, located in the northern part of the Indian sub-continent, millet, sorghum and maize are the main cereals planted during the rainy season, but wheat and barley are common crops grown during the post-rainy season. The isolates originating from these areas ($I_9$, $I_{10}$ and $P_1$) multiplied on most of these cereals. All these elements indicate that the diversity pointed out in our study is relevant.

These results on the host range of *P. graminis* isolates have some implications on the management of the peanut clump disease. Most of the monocotyledonous hosts studied were found to support well *P. graminis* multiplication and, therefore, may contribute to an increase of *P. graminis* inoculum in soils. Because all these cereals were also reported to be hosts of PCV or IPCV (Thouvenel and Fauquet, 1981; Thouvenel et al., 1988; Reddy et al., 1988; Delfosse et al., 1996), they are likely to play an important role in the spread and carry-over of the vector of peanut clump viruses.

Pearl millet and sorghum are two major crops in sub-tropical and tropical areas, and, therefore, it is inappropriate to advise farmers to abandon the cultivation of these crops in clump-infested areas. The low susceptibility of *S. dimidiatum* and various accessions of *Pennisetum* to *P. graminis* found in this study could be used for the development of cultivars resistant to the multiplication of *P. graminis*. Nonetheless, recent tests have revealed that the reduced susceptibility of *S. dimidiatum* to the *P. graminis* isolate $I_{1\text{–}1}$ was not inherited in 20 lines issued from the crossing of *S. dimidiatum* and *S. bicolor* (A. Legrève, unpubl. data). Furthermore, the susceptibility of a cultivar of *P. glaucum* (cv. IP 11902) varied among isolates. It is, thus, not advisable to base disease management strategies only on the resistance to the vector. Indeed, the low level of infection in some of the *Sorghum* and *Pennisetum* genotypes may indicate the possibility for *P. graminis* to infect newly encountered hosts and adapt to them. In West Africa, pearl millet was reported to be host for neither PCV nor *P. graminis* (Thouvenel et al., 1988). These authors suggested substituting *S. arundinaceum*, a good host of both the virus and its vector, with pearl millet in the cropping system as a measure to reduce viruliferous inoculum in the soils. Nevertheless, the good infection of the Senegalese isolate of *P. graminis* on pearl millet and the successful isolation of the parasite from soil originating from several areas in Senegal (A. Legrève, unpubl. data) suggest that pearl millet is, on the contrary, an excellent host for *P. graminis*. It is, therefore, essential to pursue the search for genotypes harbouring resistance to both agents (virus and vector) involved in peanut clump disease.

To limit the incidence of clump disease in infested areas, it might be more appropriate to rotate groundnut with dicotyledonous crops that, as groundnut, do not contribute to the multiplication of *P. graminis* inoculum potential in soils. Our results showed that mustard and sunflower are resistant to *P. graminis*. Under natural conditions sunflower is not susceptible to IPCV, and mustard, although infected, did not exhibit symptoms. These two species could beneficially be rotated with groundnut in PCV- and IPCV-infested fields to reduce disease incidence (P. Delfosse, unpubl. data). This strategy should nevertheless be combined with the control of weeds that can act as carry-over hosts for the virus and its vector (Dollet et al., 1993; Delfosse

et al., 1996) and with cultural practices that limit the expression of the disease when groundnut is sown in infested soils. Growing a cereal catch-crop (e.g. pearl millet) for a short period just before sowing groundnut in infested fields was tested by Delfosse et al. (1997) in India as a way of limiting the incidence of the disease in groundnut. This management method resulted in the reduction of clump incidence in two different regions (Delfosse et al., 1997). Nevertheless, it is unlikely that such a method will lead to an exhaustion of the soil inoculum because of the high survival ability of *P. graminis* resting spores and the spread over time of the germination of *P. graminis* inoculum (Legrève et al., 1999).

On the biological level, the results indicate that *P. graminis* isolates from peanut clump-infested areas are clearly adapted for multiplying on graminaceous species. But their host specificity differed from that of the isolates from temperate areas tested for comparison in this study or reported by other authors (Ledingham, 1939; Barr, 1979; Langenberg, 1984; Bastin et al., 1989; Adams, 1990; Adams and Jacquier, 1994). Barr (1979) was the first to consider that there might be several *formae speciales* in *P. graminis* on the basis of the host range of various isolates. Adams and Jacquier (1994) also observed some differences in the host ranges of *P. graminis* isolates, but their attempts to demonstrate the existence of *formae speciales* adapted to different host species have been unsuccessful because only one of the isolates had a markedly different host range. Our data add to the diversity previously found within *P. graminis*, but the overlapping between host ranges of isolates from various areas (among isolates from warm and/or temperate areas) suggested that there is a continuum rather than a separation between the host ranges of the isolates. This is also the case for *P. betae* for which a great diversity has been reported for the host range (Barr, 1979; Abe and Ui, 1986; Goffart et al., 1989; Barr and Asher, 1992). Therefore, the distinction of *formae speciales* only on the basis of the host range of isolates does not appear valid. *P. graminis* isolates from clump-infested areas differed from the temperate isolates for their temperature requirements and their genome (Legrève et al., 1996; 1998; Ward and Adams, 1998; Legrève, 1999). Although nothing is known about the possibility for gene flow among the isolates from diverse origins, our results proved the existence of various ecotypes within *P. graminis*, adapted to various biotopes. If it is difficult to distinguish several *formae speciales* only on the basis of host range, the specificity of other characters (ecological and/or genomic) might also be appropriate to distinguish them.

In our experiments, some *P. graminis* isolates caused a trace of infection on dicots even though they did not produce infection on some of the graminaceous species. Such low levels of infection are insignificant compared with the high infection on graminaceous species by *P. graminis* isolates and on sugar beet by *P. betae*, and may, thus, be considered insignificant in the vector spread. However, they have a significant impact when the species infected by the vector (even without further development) is susceptible to the transmitted virus. This is the case for PCV and IPCV on groundnut. These observations also question the relevance of the host range as an adequate criterion for distinguishing between *P. graminis* and *P. betae*. Ratna et al. (1991), Adams and Jacquier (1994) and Legrève et al. (1996) already raised this issue. While there are still no isolates that grow well on both monocotyledonous and dicotyledonous hosts, we consider the question of the taxonomy of *Polymyxa* unresolved. Studies comparing the ability of distinct *Polymyxa* isolates to transmit different viruses should be very useful in assessing the specificity of the virus–vector interactions. This point is important for the epidemiology of the *Polymyxa*-transmitted viruses and for assessing the risk of the disease they cause. Unfortunately, such studies are still difficult to conduct because standardised methods for the transmission of several viruses by purified *Polymyxa* isolates, in controlled conditions, are not yet available.

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