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Differences in temperature requirements between *Polymyxa* sp. of Indian origin and *Polymyxa graminis* and *Polymyxa betae* from temperate areas

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Abstract

The temperature requirements of three single cystosorus strains of *Polymyxa* sp. from India were studied at 15–18, 19–22, 23–26 and 27–30 °C (night-day temperature), and compared with the temperature requirements of three strains of *P. graminis* from Belgium, Canada and France and two strains of *P. betae* from Belgium and Turkey. Sorghum was used as the host-plant for the Indian strains; the strains of *P. graminis* and *P. betae* from temperate areas were cultivated on barley and sugar beet, respectively. The cystosori germination and the development of plasmodia, zoosporangia and cystosori of *Polymyxa* sp. from India were optimal at 27–30 °C. Infection progression was slower at 23–26 °C than at 27–30 °C. At 19–22 °C, infection was insignificant. No infection occurred below 19 °C. In contrast, the infection of barley with *P. graminis* strains from temperate areas was optimal at 15–18 °C, but at 19–22 °C the progression appeared inconsistent and infection stayed low. Above 22 °C, infection was insignificant. *P. betae* strains showed consistent infection in the range of 15–18 °C to 27–30 °C. Plasmodia formation and cystosori detection of the Belgian strain were slightly advanced at 23–26 °C compared to 19–22 °C but clearly restrained at 27–30 °C. Fungus development of the *P. betae* strain from Turkey was almost as high at 27–30 °C as at the lower temperatures. These results strengthen the case for distinguishing between *Polymyxa* sp. from India and *P. graminis* or *P. betae* from temperate areas.

Abbreviations: AIS – automatic immersion system; (I)PCV – (Indian) peanut clump virus.

Introduction

Peanut clump affects groundnut (*Arachis hypogaea* L.) in tropical semi-arid areas. It is caused by the peanut clump virus (PCV) in West Africa (Thouvenel et al., 1976) and by the Indian PCV (IPCV) in the Indian subcontinent (Reddy et al., 1983). Transmission studies have shown that these soil-borne viruses are vectored by a plasmodiophoraceous fungus of the genus *Polymyxa* (Thouvenel and Fauquet, 1981; Ratna et al., 1991). The vector was identified as *P. graminis* Ledingham by Thouvenel and Fauquet (1980) and Nolt and Reddy (1985) who detected it in graminaceous plants grown on PCV and IPCV infested soils, respectively.

The genus *Polymyxa* currently includes two species, *P. graminis*, first described by Ledingham (1939) as parasite on wheat (*Triticum aestivum* L.) roots in Canada, and *P. betae* Keskin (1964), described from sugar beet (*Beta vulgaris* L.) in Europe. These species are distinguished by their restricted and different host ranges, *P. graminis* infecting only monocotyledonous species and *P. betae* dicotyledonous ones (Barr, 1979). They attracted particular attention when they were shown to be involved in the transmission of economically important plant viruses in temperate areas (Maraite, 1991). The characterization of the *Polymyxa* sp. isolates involved in PCV and IPCV transmission is of particular interest, for three reasons. First, these iso-

lates detected on graminaceous plants are able to vector PCV and IPCV to groundnut plants, in which the fungus has been rarely and sparsely detected (Ratna et al., 1991). Second, their host range is wide and includes monocotyledonous and dicotyledonous plants (Thouvenel and Fauquet, 1980; Ratna et al., 1991; Delfosse et al., 1996). Third, they originated from tropical areas and therefore may have temperature requirements that differ from those of *P. graminis* and *P. betae* from temperate areas. For the latter, temperature optima around 15–20 °C (Slykhuis and Barr, 1978; Maraite et al., 1988) and 20–25 °C (Blunt et al., 1991; Goffart and Maraite, 1992) were reported, respectively. Because of the uncertainty about the taxonomic status of the Indian isolates (Legrève et al., 1996), we are using the term IPCV-*Polymyxa* when referring to *Polymyxa* isolated from IPCV infested soils.

In order to specify the ecology of IPCV-*Polymyxa*, we have initiated studies on its temperature requirements. Reddy et al. (1988) and Thouvenel and Fauquet (1980) reported that the fungus could be detected at temperatures ranging from 23 to 30 °C, but little is known about the fungus behaviour within a wider temperature range. For this reason and because of the importance of temperature in the epidemiology and management of peanut clump, the development and multiplication rates of IPCV-*Polymyxa* from India on sorghum (*Sorghum bicolor* (L.) Moench) were studied at temperatures between 15 and 30 °C and compared with the development of *P. graminis* from Belgium, Canada and France on barley (*Hordeum vulgare* L.), and of *P. betae* from Belgium and Turkey on sugar beet studied under the same conditions.

As obligate root parasites, *Polymyxa* spp. can be isolated only by growing bait-plants on infested soils. Other soil fungi, such as *Olpidium* sp., *Phialophora* sp., *Lagena* sp., *Pythium* sp. and vesicular-arbuscular endomycorrhizes, as well as nematodes, also colonized the roots of the bait-plants. Because of their possible interference in *Polymyxa* spp. development, it was decided to produce and analyse *Polymyxa* strains derived from a single cystosorus.

Materials and methods

Isolation of Polymyxa *from IPCV-infested soil*

Soil naturally infested with IPCV was collected in April 1993 in field RCW17A at the ICRISAT Asia-Center farm at Patancheru in India, air-dried and sent to UCL, Belgium. After preparation of a 1:4 (v/v) soil-autoclaved sand mixture as described by Goffart and Maraite (1991), 5 polyethylene pots were filled with the mixture and planted with 8 pre-germinated seeds of sorghum (cv. IRAT 204), wheat (cv. Capitaine) or barley (cv. Corona). Seeds were surface disinfected by soaking for 5 min in 2.5% sodium hypochloride solution, and rinsed for 30 min in running demineralized water. The seedlings were further maintained in controlled environment cabinets at 20–25 °C or 25–30 °C (night-day temperature) with a photoperiod of 12 h. The plants were watered with modified half-strength Hoagland solution (Ca $[NO_3]_2 \cdot 4H_2O$ 2.5 mM, KNO_3 2.5 mM, KH_2PO_4 0.5 mM, $MgSO_4$ 1mM, FeEDTANa 16 μM, 1ml of microelements solution [H_3BO_3 610 mg l^{-1}, $MnCl_2 \cdot 4H_2O$ 389 mg l^{-1}, $ZnSO_4 \cdot 7H_20$ 56 mg l^{-1}, $CuSO_4.5H_2O$ 49 mg l^{-1}, $(NH_4)_6Mo_7O_{24} \cdot 4H_20$ 122 mg l^{-1}] in 1 l of solution, pH adjusted to 7.2 with NaOH 1M). The soil was saturated once a week by filling the saucer placed under each pot. Periodically, some plants were removed from the soil to assess the presence of *Polymyxa*. The fungus was detected after cotton blue lactophenol staining (Maraite et al., 1988) by analysing the roots, spread under water in a tray, with a stereomicroscope Wild M3B (Heerbrugg, Switzerland) at $\times 16$ to $\times 40$ magnification. For further confirmation, fragments were analysed at higher magnification with a light microscope.

*Production of IPCV-*Polymyxa *single cystosorus strains*

The method used to produce single cystosorus strains was derived from Jones et al. (1982) and Haji Tinggal and Webster (1981) for single spore infection by *Plasmodiophora brassicae*. After storage for at least 2 months at room temperature, dried IPCV-*Polymyxa*-infected sorghum roots obtained as described above were rehydrated in sterile distilled water (SDW), cut into small fragments (<2 mm) and homogenized in 20 ml SDW for 1 min with a Virtis Omnimixer (Gardiner, New York, USA) at 15000 rpm. The suspension was then centrifuged for 20 min at 7500 rpm and the pellet re-suspended in 1 ml SDW. A few drops of the final suspension were spread on 2% agar in a Petri dish using a fine brush. Isolated cystosori were located by scanning with a Wild Makroskop M420 (Heerbrugg, Switzerland) at $\times 40$ to $\times 64$ magnification, and picked up with a sterile microspear. Each cystosorus was then deposited individually into a sterile glass culture tube (2.5 cm diameter, 15 cm height) filled up to two-thirds

Table 1. Origins of the *P. betae*, *P. graminis* and IPCV-*Polymyxa* strains used

Species code	Bait-plant[1]	Soil origin	Virus associated in the field[2]	Sampling date	Soil sampled by	Strain[3] isolated by
P. betae						
A2641	sugar beet	Opprebais, Belgium	–	1987	Goffart[7]	Goffart[7]
T17	sugar beet	Turkey	BNYVV	1989		Legrève
P. graminis						
B1	barley	Loupoigne, Belgium	BaMMV	1987	Bastin[7]	Shahin[7]
C1	barley	Ottawa, Canada	WSSMV	1987	Barr[4]	Shahin[7]
F11	barley	Carcassonne, France	SBWMV	1988	Signoret[5]	Shahin[7]
IPCV-*Polymyxa*						
I1-1	sorghum	Patancheru, India	IPCV	1993	Reddy[6]	Legrève
I1-20	sorghum	Patancheru, India	IPCV	1993	Reddy[6]	Legrève
I1-229	sorghum	Patancheru, India	IPCV	1993	Reddy[6]	Legrève

[1]Bait-plant used for isolation of *Polymyxa* strain from the soil and for subsequent purification and multiplication.
[2]BaYMV: Barley yellow mosaic virus; BNYVV: Beet necrotic yellow vein virus; IPCV: Indian peanut clump virus; SBWMV: Soil-borne wheat mosaic virus; WSSMV: Wheat spindle streak mosaic virus.
[3]Single cystosorus strain production.
[4]Soil sample provided by D.J.S. Barr, Biosystematics Research Institute, Ottawa, Canada.
[5]Soil sample provided by P.A. Signoret, ENSA INRA, Montpellier, France.
[6]Soil sample provided by D.V.R. Reddy, ICRISAT, Patancheru, India.
[7]Soils and/or roots samples provided by V. Bastin, J.P. Goffart and N. Shahin UCL-Unité de Phytopathologie, Louvain-la-Neuve, Belgium.

with sterile sand, by sprinkling the microspear with approximately 3 ml SDW over the sand. After adding sand up to 2.5 cm from the top of the tube, a single 2-day-old seedling was transplanted into each inoculated tube at a depth of 1 cm. In some cases, the single cystosorus was immediately deposited onto the root of the seedling, before transplantation into the culture tube. The culture tubes were placed in opaque polyethylene boxes (56 cm long × 37 cm wide × 23 cm high; 84 tubes/box) covered by a 5 cm thick polystyrene sheet pierced with holes 2.5 cm in diameter. The plantlets were grown for 10 weeks in controlled environmental cabinets at 25–30 °C. After 1 week, the roots were flooded weekly by adding the nutrient solution up to 0.5–1 cm above sand level to maintain soil saturation for at least 24 h. Between soil saturation periods, nutrient solution was added moderately in order to prevent any water stress. After incubation for 8 weeks, watering was reduced for 1 week, then stopped for the last 5–6 days. The roots were carefully washed free of sand under running tap water and divided longitudinally into two parts. One part was stained in cotton blue lactophenol and examined under a microscope to detect the presence of *Polymyxa* cystosori and to verify the absence of other contaminants. The other part was air-dried at room temperature and stored in darkness.

P. graminis *and* P. betae *strains*

Three *P. graminis* strains and two *P. betae* strains from temperate areas, produced at UCL's Unité de Phytopathologie in Belgium using the procedure described above, were selected on the basis of the origins of the soils from which they were isolated (Table 1). The *P. graminis* strains were obtained with barley as the bait-plant at 15–20 °C, and the *P. betae* ones with sugar beet at 20–25 °C.

Multiplication of Polymyxa *spp. strains*

High quantities of cystosori of each strain were produced by growing bait-plants on a sand-cystosori mixture using the automatic immersion system (AIS) (Figure 1). The system used is an adaptation, as an independent and easily transportable unit, of the one described by Adams et al. (1986). Two-day-old seedlings were each transplanted into handmade poly-vinyl-chloride culture tubes (10 cm long × 3 cm internal diameter) previously filled with autoclaved sand and up to 5 ml of a cystosori suspension. The tubes were closed on one side with a fine-mesh (60–120 μm, pore size) polyamid netting, supporting the substrate and permeable to nutrient solution and *Polymyxa* zoospores. The

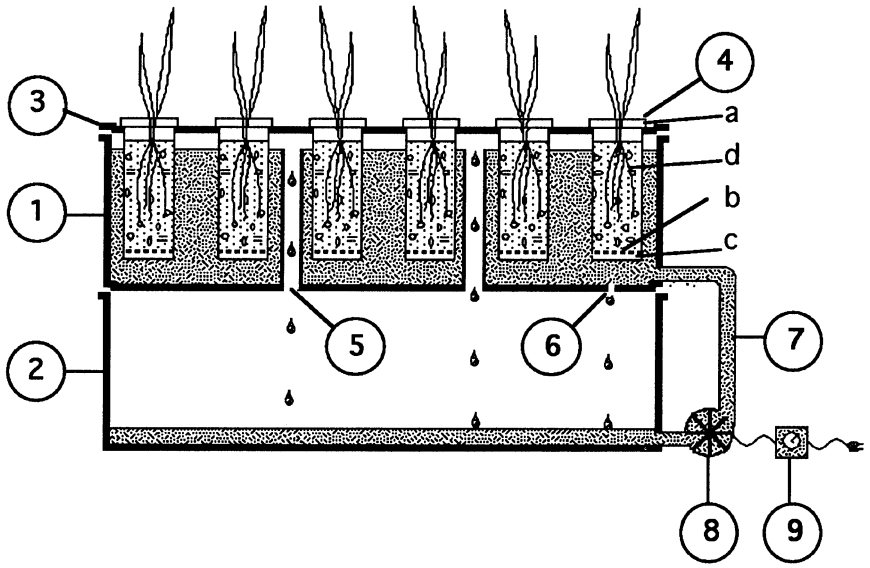

Figure 1. Diagram of the automatic immersion system (immersion phase) used for the multiplication of single cystosorus strains of *Polymyxa* sp. 1) Upper culture tank; 2) lower reservoir tank; 3) removable opaque lid on the upper tank with openings for insertion of culture tubes; 4) culture tube with (a) 32 mm ring (internal diameter) sealed to the tube, (b) a polyamid netting sealed to the tube with a 32 mm ring (external diameter) (c) and supporting the sand inoculum mixture (d); 5) overflow tubes sealed to the bottom of the culture tank; 6) emptying hole; 7) PVC tubing; 8) centrifugal pump; 9) timer for regulation of the alternate watering-drainage.

tubes were suspended from an opaque lid set on an opaque high-density polyethylene tank, 30 tubes in a large version of the tank (40 cm long × 30 cm wide × 11.8 cm high) and 8 in a small version (20 cm long × 15 cm wide × 11.8 cm high). This culture tank was superimposed on an identical tank which served as a nutrient solution reservoir. The two tanks were connected externally by PVC tubes (8 mm internal diameter) to a centrifugal pump (Rena, type C20, Annecy, France) which, in the immersion phase, transferred the nutrient solution from the reservoir to the culture tank at a rate of 200 l per hour. The culture tank was equipped with two overflow tubes to control liquid level during the immersion phase. Alternate irrigation-drainage phases were regulated by an electric timer connected to the pump. When the pump stopped, the nutrient solution receded into the reservoir by counterflowing through the pump. Complete emptying of the culture tank between two flooding periods was achieved through a hole, 2 mm in diameter, in the bottom of the tank. On the basis of preliminary tests, independent AISs were placed in environmental cabinets at 15–20 °C for *P. graminis*, 20–25 °C for *P. betae* and 25–30 °C for IPCV-*Polymyxa*. In order to allow a vigorous plant growth, the tubes were flooded for 1 hour every 6 h during the first week after seedling transplantation. From the second week onwards, the tubes were flooded for 6 h every 12 h in order to stimulate *Polymyxa* development. The nutrient solution was replaced weekly.

Temperature requirements

The influence of temperature on the development stages of IPCV-*Polymyxa*, *P. betae* and *P. graminis* strains from various origins (Table 1) was analysed by growing host-plants in glass culture tubes on a sand-cystosori mixture in growth cabinets. After preliminary trials conducted in the range of 10 to 35 °C, four temperatures were chosen: 15–18 °C, 19–22 °C, 23–26 °C and 27–30 °C (night-day temperature), with a 12 h photoperiod. Cystosori suspension preparation, plant culture in tubes and nutrient solution were the same as described for single cystosorus strains production. Cystosori concentration was assessed using a Fuchs-Rosenthal haemacytometer. About 2500 cystosori suspended in 1 ml water were inoculated per tube (i.e., about 40 cystosori per gram of sand). The watering was regulated to provide a moderate supply of nutrient solution each day and a saturation of the substrate over 48 h once a week. After 15, 25, 35, 46 and 56 days of incubation, 10 tubes were sampled for each temperature. Ten seedlings were sown per date of sampling/temperature range/strain, but because of

irregular germination the number of plants effectively analysed was sometimes lower than 10. The percentages of infected plants and the infection indices of roots by *Polymyxa* plasmodia, zoosporangia and cystosori were assessed after cotton blue lactophenol staining as described above. The infection level in each root system was assessed on a 0–3 scale, where 0 = no infection, 1 = low infection (some rare [<10] root cells infected by the particular development stage), 2 = moderate infection (a few groups of some [>10 to 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 root). An infection index for each *Polymyxa* stage at each incubation temperature and for each assessment time was calculated using the method described by Somé et al. (1996) for assessment of *Plasmodiophora brassicae* infection, but modified as follows: infection index = (0 × n0 + 1 × n1 + 10 × n2 + 100 × n3) / (n0 + n1 + n2 + n3), where n0 = number of plants not infected, n1 = number of plants infected at infection level 1, n2 = number of plants infected at infection level 2, and n3 = number of plants infected at infection level 3. The infection index could vary from 0 to 100.

The various tested *Polymyxa* strains were grown under the same conditions except for the host-plants: sorghum for the IPCV-*Polymyxa* strains, sugar beet for the *P. betae* strains and barley for the *P. graminis* strains. Because of the small size of the growth cabinets, the eight strains were tested over four consecutive time periods, the strains growing on the same host being separated in time.

Results

Isolation of Polymyxa *from IPCV-infested soil*

IPCV-*Polymyxa* cystosori were observed only in roots of sorghum grown for up to 3 months at 25–30 °C in the IPCV-infested soil-sand mixture from Patancheru, India. Attempts to isolate *Polymyxa* from IPCV-infested soil at 20–25 °C or by using wheat or barley as bait-plants were unsuccessful. The infected roots sampled in August 1993 were used in October 1993 to produce single cystosorus strains.

*Production and multiplication of IPCV-*Polymyxa *single cystosorus strains*

Cystosori of IPCV-*Polymyxa* were detected on three of the 316 sorghum plants each inoculated with a single cystosorus. The three IPCV-*Polymyxa* strains obtained in January 1994 were labelled I1-1, I1-20 and I1-229 (Table 1). The first two strains were produced using the method whereby the single cystosorus was deposited in the sand, the third strain by direct inoculation on the root. Ten weeks after the single cystosorus inoculation, the infection level of the three roots infected with cystosori was moderate to fairly high and no fungal contaminant was detected. After air-drying for 2 months at room temperature, the three single cystosorus strains were multiplied in separate AISs. Eleven weeks after inoculation, abundant cystosori production was observed for the three strains. Infected sorghum roots were air-dried and used for further studies.

Effect of temperature

The tested IPCV-*Polymyxa* strains developed almost exclusively at temperatures above 23 °C (Figure 2). At 27–30 °C, 80% (mean of the three strains) of the plants were infected 15 days after planting on the inoculated sand. At this time, the three fungal stages were already observed, but zoosporangia were predominant (Figure 3). After 25 days of incubation, an intensification of root colonization and, in particular, a higher frequency of differentiation from the plasmodium stage into the zoosporangium and cystosorus stages were obvious. The infection progressed continuously with an increase in cystosori production in the roots up to 35 days after inoculation (Figure 3). Afterwards, plasmodia and zoosporangia became rare, and infection was detected mainly as cystosori (Figure 3). At this temperature a degradation of the root cortex was observed after 46 days growth and probably related to the small volume of the culture tube. At 23–26 °C, progression of the fungal infection was slower than at 27–30 °C. Only 10% of the plants were infected 15 days after inoculation, and 38% after 25 days (Figure 2). After 56 days, infection could be detected on 67–83% of the plants for I1-229 and I1-1 and on 100% of the plants for I1-20. At this temperature, differentiation into cystosori was detected 25 days after inoculation for I1-229, and only after 35 days for I1-1 and I1-20, but increased thereafter (Figure 3). At 56 days after inoculation, infection indices with cystosori at

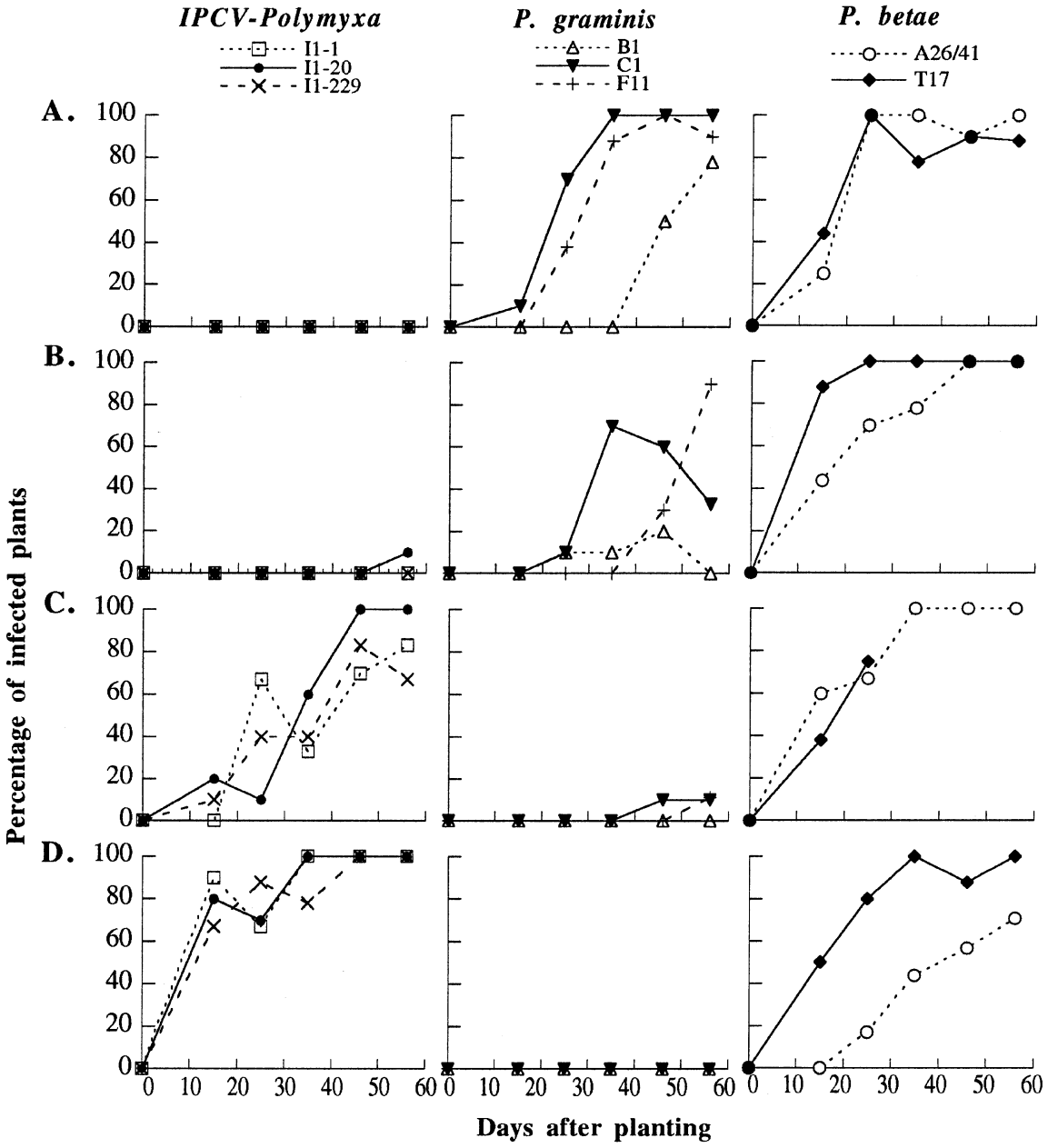

Figure 2. Temperature requirements of IPCV-*Polymyxa* strains I1-1, I1-20 and I1-229 from India grown on sorghum, compared with *P. graminis* strains B1 from Belgium, C1 from Canada and F11 from France grown on barley, and *P. betae* strains A26-41 from Belgium and T17 from Turkey grown on sugar beet. Evolution of percentage of infection at (A) 15–18 °C, (B) 19–22 °C, (C) 23–26 °C and (D) 27–30 °C. Plants were grown in single tubes on sand contaminated with ± 40 cystosori/g of sand. Each point is based on the observation of 7–10 plants or, exceptionally, 4–6 plants.

23–26 °C were higher for two of the three strains than at 27–30 °C. At 19–22 °C, IPCV-*Polymyxa* was detected in only one plant, as cystosori and 56 days after inoculation. No infection was observed on sorghum plants grown at 15–18 °C. In preliminary trials, only very sparse infection was detected at 30–35 °C but never at 10–15 °C.

In contrast with IPCV-*Polymyxa*, *P. graminis* from Belgium, Canada and France developed mainly at temperatures below 23 °C (Figure 2). At 15–18 °C, infec-

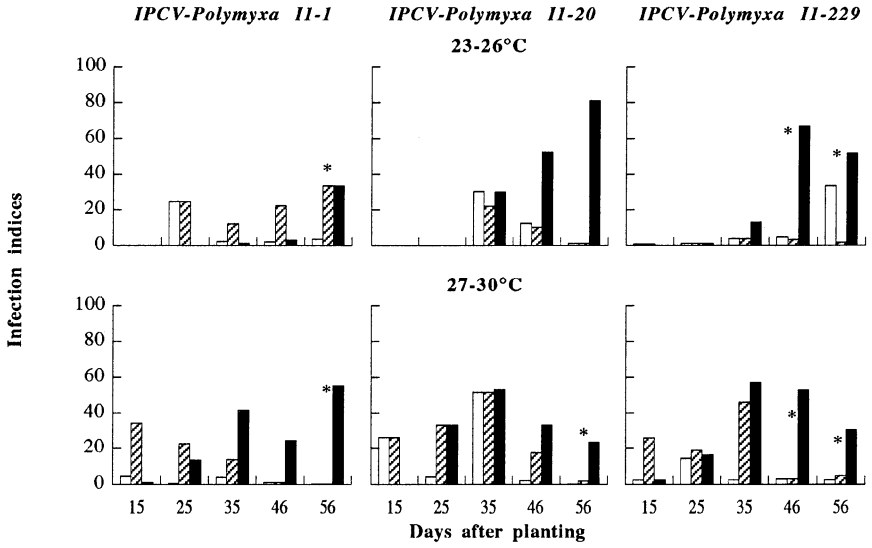

Figure 3. Evolution of infection indices of sorghum roots by plasmodia (white columns), zoosporangia (hatched columns) and cystosori (black columns) of IPCV-*Polymyxa* strains I1-1, I1-20 and I1-229 from India at 23–26 °C and 27–30 °C. Each column is based on the observation of 7–10 plants or, sometimes (*), 4–6 plants.

tion was detected earlier, and the proportion of infected plants as well as the infection indices of the fungus were higher than at 19–22 °C (Figures 2 and 4). The delay in infection of barley with strain B1 from Belgium was not observed in other trials with this strain (results not shown). At 19–22 °C, progression of infection was less consistent, and the infection indices remained very low (Figure 4). Infection was detected almost exclusively as plasmodia with strain C1. Some zoosporangia and cystosori were observed 46 and 56 days after inoculation, respectively, with strain F11. At 23–26 °C, only 10% of the plants showed some very sparse infection after 46 days incubation with strain C1, and after 56 days with strain F11. No infection by the *P. graminis* strains from temperate areas was detected at 27–30 °C.

Infection of sugar beet by *P. betae* strains occurred between 15 and 30 °C (Figure 2). With the Belgian strain, some infection was detected 15 days after inoculation at the three lower temperatures, and cystosori were already visible by this time at 19–22 °C and 23–26 °C (Figures 2 and 5). After 35 days of incubation, almost all the plants – 100% at 23–26 °C, 78% at 19–22 °C and 70% at 15–18 °C – were heavily infected by cystosori. At 27–30 °C, a few plasmodia were detected in 16% of plants only after 25 days (infection index = 0.16). The proportion of infected plants increased thereafter, reaching 70% 56 days after inoculation. Some zoosporangia were observed, but infection indices stayed low and no cystosori were detected at this temperature over the 56 days of the experiment. However, with *P. betae* strain T17 from Turkey, infection and cystosori differentiation occurred and reached a high level for each tested temperature range, even at 27–30 °C (Figure 5). The high mortality of plantlets grown at 23–26 °C did not allow a satisfactory analysis to be made of T17 multiplication at this temperature.

Discussion

Our experiments in controlled conditions showed that extensive development of the three IPCV-*Polymyxa* strains on sorghum requires temperatures above 23 °C and that the initiation of infection and the rates of fungal development were fastest at 27–30 °C. The infection progression was delayed at 23–26 °C but final root colonization indices were similar compared with 27–30 °C. Below 23 °C, almost no development occurred for IPCV-*Polymyxa*. Similar results were observed in three independent trials for the three single cystosorus strains isolated from the same soil sample. This indicates that the observed requirements are characteristic of *Polymyxa* strains occurring at this location. It could be argued that the used IPCV-*Polymyxa* strains are adapted to high temperatures because of their isolation at 25–30 °C. Several attempts to isolate *Polymyxa* from IPCV-infested soils, however, were unsuccessful

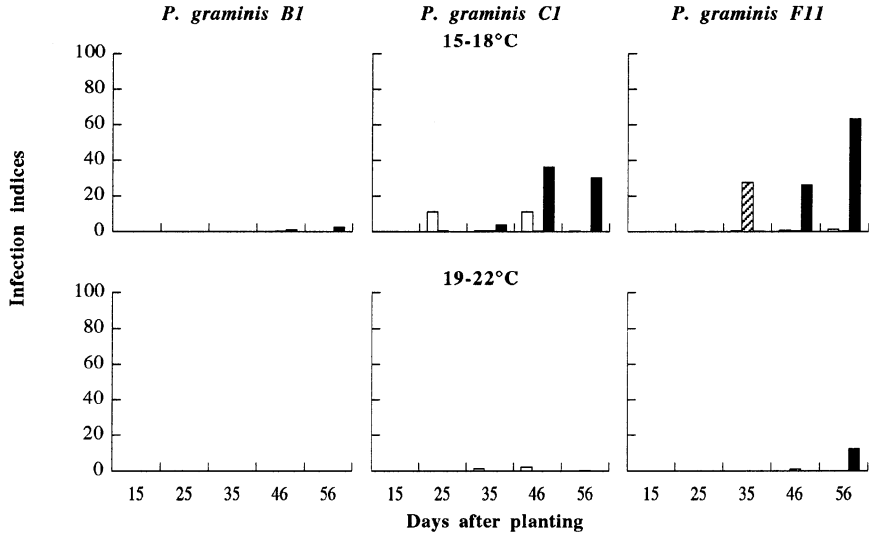

Figure 4. Evolution of infection indices of barley roots by plasmodia (white columns), zoosporangia (hatched columns) and cystosori (black columns) of *P. graminis* strains B1 from Belgium, C1 from Canada and F11 from France at 15–18 °C and 19–22 °C. Each column is based on the observation of 7–10 plants.

at 20–25 °C whereas it was readily isolated at 25–30 °C. Temperatures above 23 °C thus appear to be a specific requirement of the IPCV-*Polymyxa* strains. Our results indicate that small variations in temperature in the range 20–25 °C could greatly affect the activity and development of IPCV-*Polymyxa* and thus virus transmission. The seasonal variation of clump disease severity in groundnut crops is linked to the temperature, a high incidence occurring in the rainy season when soil temperatures range from 25 to 30 °C, while the disease is negligible in post-rainy season crops grown under irrigation at lower temperatures (Reddy et al., 1988). The same authors reported that at 15 °C IPCV was not naturally transmitted to wheat and groundnut, and *Polymyxa* was not detected in wheat roots. At 25–30 °C, however, both the virus and the fungus were detected on wheat, and groundnut became infected by IPCV. Nevertheless, plants mechanically inoculated with IPCV showed clump symptoms when incubated at both 15 and 30 °C, suggesting that low temperature does not restrict the virus replication but the transmission by the fungus.

In contrast with IPCV-*Polymyxa*, the development of *P. graminis* strains from Belgium, Canada and France is favoured at temperatures below 23 °C, with an optimum close to 15–18 °C. Our results agree with those reported by Slykhuis and Barr (1978) and Maraite et al. (1988) for *P. graminis* originating from Canada and Belgium, and obtained using unifungal culture or infested soil as inoculum, respectively.

The *P. betae* strains used in our experiments showed a broader temperature range than IPCV-*Polymyxa* strains or *P. graminis* strains from temperate regions. Previous studies on *P. betae* reported an optimum temperature between 20 and 25 °C (Blunt et al., 1991; Goffart and Maraite, 1992), but an infection capacity from 11.5 °C (de Heij, 1991) to 30 °C (Blunt et al., 1991; Goffart and Maraite, 1992). Ivanovic (1984) was the only one to report 30 °C as the optimum temperature for fungal infection and development. Our data confirm early production of *P. betae* cystosori in the 19–26 °C range. At 27–30 °C, development of the Belgian strain appeared strongly reduced compared with strain from Turkey. These differences in temperature requirements among *P. betae* strains highlight the need to explore further the differences in temperature requirements among *Polymyxa* strains associated with IPCV transmission in various parts of the Indian sub-continent. Nevertheless, to date *Polymyxa* isolates have been caught by sorghum bait-plants grown on soil samples from IPCV-infested areas only at temperatures above 25 °C (A. Legrève, unpubl.). This confirms the strong difference in temperature requirements of these isolates and *P. graminis* from temperate origins. These differences in ecological requirements, together with differences in host range (Legrève et al., 1996), minimize the likelihood of gene flow between the Indian *Polymyxa* sp.

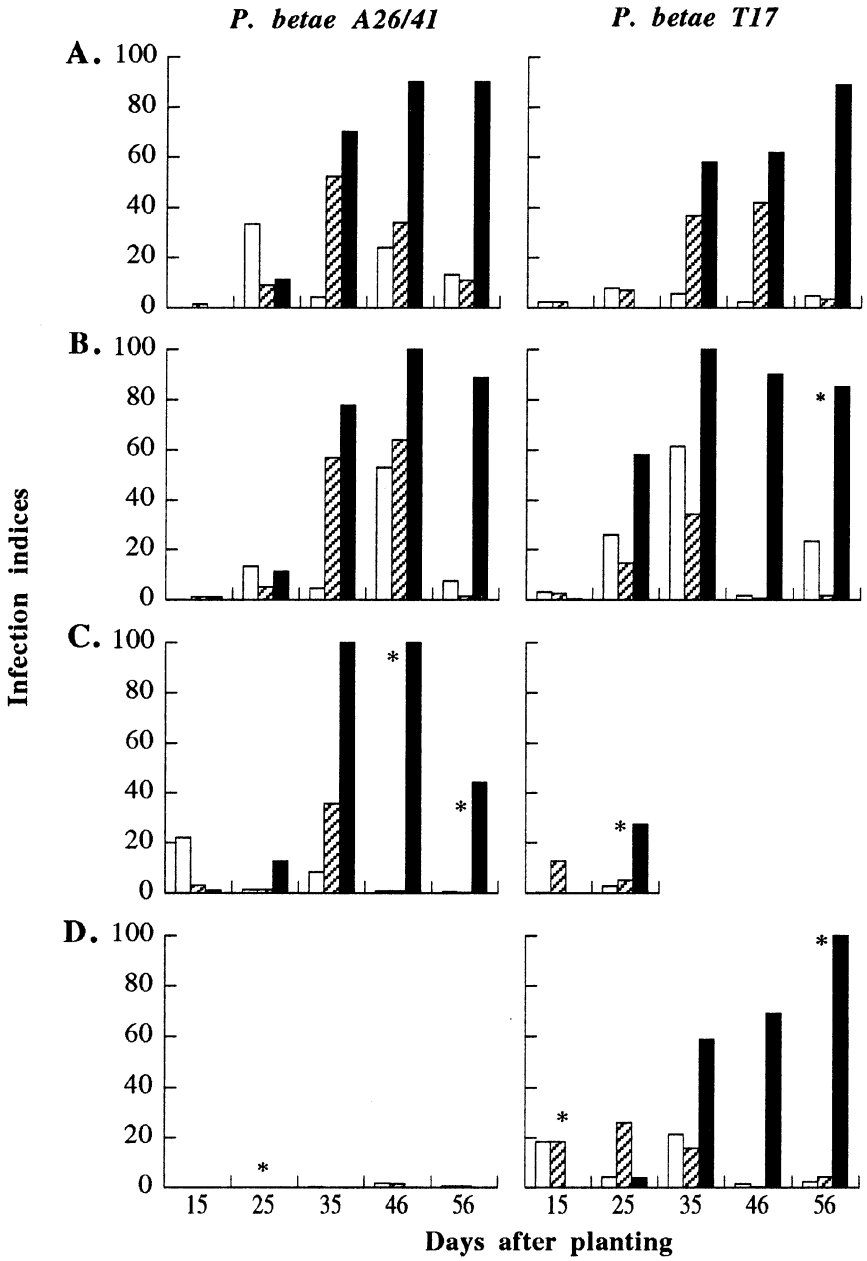

Figure 5. Evolution of infection indices of sugar beet roots by plasmodia (white columns), zoosporangia (hatched columns) and cystosori (black columns) of *P. betae* strains A26–41 from Belgium and T17 from Turkey at (A) 15–18 °C, (B) 19–22 °C, (C) 23–26 °C and (D) 27–30 °C. Each column is based on the observation of 7–10 plants or, sometimes (*), 4–6 plants.

populations and *P. graminis* populations from temperate areas. Genetic isolation was further confirmed by genomic differences (Legrève et al., 1996; Ward and Adams, 1996). Further characterization of *Polymyxa* isolates from intermediate geographical areas, such as *Polymyxa* sp. from (I)PCV-infested soils in northern India or in Senegal, should help to clarify the position of IPCV-*Polymyxa* within the genus.

The success rate for the production of single cystosorus strains from the IPCV-*Polymyxa* isolate was very low (<1%). This suggests that many cystosori did not germinate or did not succeed in infecting the

sorghum roots. The mechanisms involved in cystosori *Polymyxa* spp. maturation and germination are still unclear, despite the fact that the knowledge of the stimulus for effective germination might be useful for the design of control measures (Adams, 1990). Experiments are in progress to improve our understanding of the impact of temperature on the maturation and germination of cystosori.

The developed AIS, providing alternate immersion and drainage periods for the root system, appeared to be a very efficient technique for multiplying *Polymyxa* spp. from various areas. It offers the same possibilities for the production of zoospore inoculum as the set-up described by Adams (1986). It has the advantage of a modular design, allowing the easy handling of strains under various environmental conditions. The apparatus also permits the production of a high number of cystosori and maintains active *Polymyxa* spp. culture for long periods. It is easy to use and could be adapted for other zoosporic and aquatic fungi.

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