Epidemiology

Chapter 2

Geographical distribution of the peanut clump disease in Asia

Abstract

Surveys were conducted in Asia to determine the geographical distribution of the peanut clump disease. The countries visited included India, Pakistan and Thailand. The Durgapura isolate (IPCV-D) is a member of the most widespread serotype of IPCV. Viruses serologically related to IPCV-D occur in the Indian States of Gujarat (Talod), Rajasthan (Boraj, Dausa, Durgapura, Kallavas and Ranoli), Andhra Pradesh (Bapatla, Chinnaganjam, Ganapavaram, Pallipalem, Ramapuram and Tsundupalle), Tamil Nadu (Pondicherry) and in the Punjab Province of Pakistan in Attock, Chakwal and Rawalpindi districts. A second serotype, which occurs in Punjab in India (Jalandhar, Ludhiana and Sangrur), is represented by the Ludhiana isolate (IPCV-L). It is also present in Pakistan in Attock district. In Hyderabad, Andhra Pradesh, a third serotype occurs (IPCV-H). The disease in India is very severe in Rajasthan where groundnut is rotated with cereals and particularly with wheat or barley. In Punjab the disease caused severe damage to groundnut until the early eighties when the wheat-groundnut rotation was replaced by a wheat-rice system. The disease was not encountered in Thailand where groundnut is mostly grown during the post rainy season and rotated with rainy season crops of rice.

Introduction and Methodology

Knowledge on the occurrence of clump disease in Asia was limited because virus detection in the developing countries where clump disease presumably occurs is often hampered by a lack of equipment and skills for proper identification of viruses by the national agricultural research systems (NARS) (Reddy, 1990, Reddy and Gowda, 1996, Reddy *et al.*, 1997). In the absence of sufficient data on the distribution and spread of peanut clump disease, the necessity of conducting research on this virus disease and its economic importance were sometimes questioned. Therefore, surveys were conducted in India, Pakistan and Thailand in Asia to collect the necessary information to evaluate the disease importance. The surveys in India and Pakistan were mostly requested by NARS, whereas the survey conducted in Thailand was opportunistic and conducted at the occasion of the 4th Meeting of the International Working Group on Groundnut Viruses (Khon Kaen University, Thailand, 12-14 March 1995).

Surveys were conducted in fields along or near roads. The fields were scored for clump symptoms by taking observations across the two diagonals of the field and at the borders. Groundnut and weed plants as well as soil samples were collected from disease sites. In most cases, whole plants were uprooted, labelled, sealed in polythene bags and kept on ice in an ice-box. Back to the laboratory, all plants were analysed for virus presence by ELISA using the three sera available for IPCV detection and a serum produced against a PCV isolate (Nolt *et al.*, 1988, Reddy *et al.*, 1998). Groundnut plants were further analysed by bioassays such as sap inoculation onto leaves of french bean plants in which IPCV causes veinal necrosis, or by grafting branches onto healthy groundnut rootstock followed by observation of symptoms in newly formed branches. Soils sampled at the disease sites were analysed to determine their texture (Table 2). The soil from Pakistan was sent to Belgium for *P. graminis* isolation (Legrève et al., 1999). A summary of the survey sites in Pakistan and Thailand is presented in Table 1 and illustrated in Fig. 1 and 2.

Survey for peanut clump disease in North Thailand in postrainy season crops of groundnut

Groundnut is grown in the postrainy season in central and north Thailand, followed by rice, the latter being the major rainy season crop in the

country. In March 1995, we surveyed the regions of Chiang Mai, Langpang, Kalasin and Wang Nam Yen. Groundnut is grown only if irrigation is available, near rivers, lakes, canals or at the foot hills. The soil in paddy fields is heavy and if allowed to dry may prevent entry of pegs. For this reason the crop is irrigated by flooding the field as in case of rice, at least 3 times during the growing season. In all the fields visited, the crop was found to be quite healthy. The peanut clump disease has never been observed. *Peanut bud necrosis virus* (PBNV) affected the crop up to 5 % in some fields. *Peanut Stripe Virus* (PStV) was rarely present. Aphids were sometimes heavily present. The main problem that we noticed was excessive weeds in most of the fields. Weeding is rarely done and in crops at harvesting stage we counted only 2 to 3 pods per plant. From our observations, the peanut clump disease is not present in postrainy season crops of groundnut. *P. graminis* could not be detected in graminaceous weeds collected during the survey in Thailand.

Table 1. Areas surveyed in Thailand (March 1995) and Pakistan (July 1995) for peanut clump disease.

Country	City or Village	Number of fields	Crop stage	Observation	IPCV serotype
Thailand	Lampang area:				
	Bandan	3	60 days	PBNV (5%), PStV (<5%)	-
	Ban Huey Rai	2	harvest	weeds	-
	Sanklang	3 small fields	60 days	weeds	-
	Hangchat	1	60 days	PBNV, aphids	-
	Chiang Mai area:	3	60 days	PBNV, mites	-
	Kalasin area:	5	>40 days	PBNV, PStV, mineral deficiency	-
	East of Bangkok:	2	60 days	PBNV	-
Pakistan	Chakwal area:				
	Dudhial	3	90 days	IPCV < 10%, PBNV	IPCV-D
	Bhaun	3	90 days	stunting <1%	-
	ARF of Bhaun	2	90 days	stunting <1%	-
	BARI	3	90 days	stunting <1%	IPCV-D IPCV-L
	Mari	1	90 days	-	-
	Chach	3	90 days	stunting <1%	-
	Chach to Islamabad	>20	90 days	stunting <1%	-
	Fateh Jang area:	19	90 days	PBNV 5-10%	-
	Attock area: 60 000 ha of groundnut	>20 large fields	60-90 days	Very healthy looking crops	
	NARC-Islamabad	1	2-3 weeks	IPCV >20%, chlorosis due to Fe deficiency	IPCV-D

PBNV: *Peanut bud necrosis virus*, PStV: *Peanut stripe virus*.
BARI: Barani Agricultural Research Institute, Chakwal, Pakistan.
NARC: National Agricultural Research Center, Islamabad, Pakistan.
ARF Bhaun: Adaptive Research Farm of Bhaun, Pakistan.
IPCV-Talod and IPCV-Durgapura are serologically related (serotype IPCV-D).

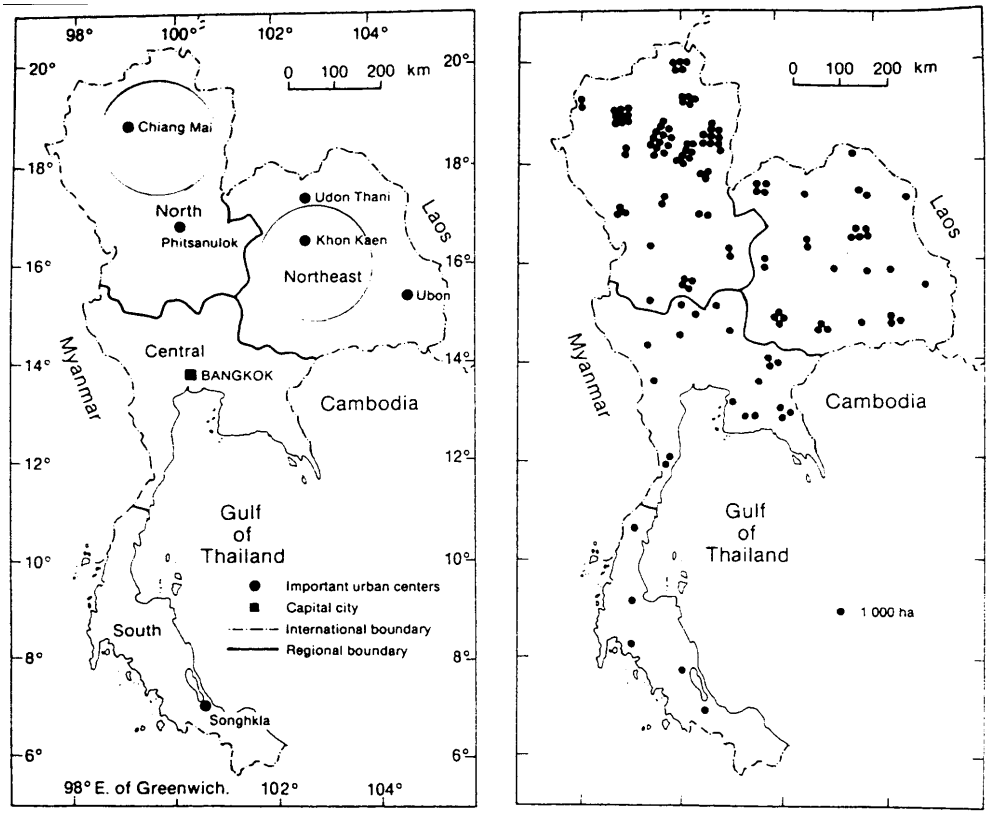

Figure 1. Administrative divisions of Thailand (left), groundnut distribution (●) and areas surveyed in March 1995 (right).

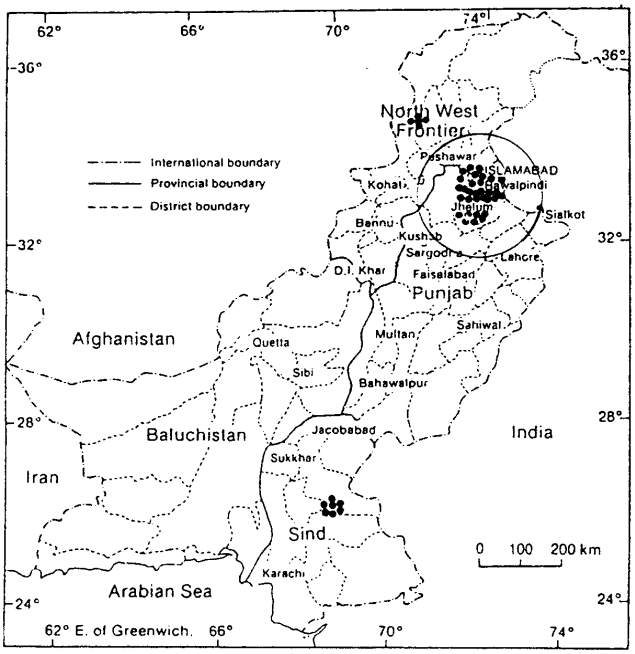

Figure 2. Administrative divisions of Pakistan, groundnut distribution (●) and areas surveyed in July 1995.

Survey of Groundnut Crop for Virus Diseases in Pakistan[3]

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In July, 1995 a survey for virus diseases was conducted in the major groundnut producing areas in Pakistan, including Attock, Chakwal and Rawalpindi districts (Figure 2). In Pakistan, the groundnut crop is mainly planted in the months of April and May. Groundnut-fallow-groundnut or groundnut-fallow-wheat are the main rotations used. However, a few farmers plant groundnut after harvesting wheat if there is enough moisture. Some farmers in the Pothar area plant groundnut in the month of July at the onset of monsoon. More than 98 % farmers plant the well adapted spreading variety, No 334. In the major groundnut producing areas the soils are sandy or sandy loam. Planting is done either by broadcasting the seed followed by mouldboard ploughing or by using a tractor driven drill in which the seed is dropped manually. Low plant population, drought stress in the month of June, weeds, lack of proper machinery for planting and harvesting and damage by boars and other wild animals are the major problems for groundnut production in Pakistan.

It was apparent from this survey that diseases are not a major constraint to groundnut production. However in one field near Dhudial on the way to Chakwal from Mandhra, the peanut clump virus disease (PCV) was observed with an incidence ranging from 4-10 %. The diseased plants occurred in patches and were severely stunted with typical symptoms of mottling on the younger leaflets and dark green colour for the lower leaves. The soil was sandy and planting was done in the month of April. The variety was the local spreading type. In one field, 10 Km away from Fateh Jang en route to Talaganh, we observed 5-15 % incidence of peanut bud necrosis virus (PBNV). The crop was planted in rows by agriculture extension staff as a demonstration plot. The variety was semi-spreading mixed with a local spreading type. It was also planted early. The virus infected plants showed typical symptoms of PBNV with chlorotic line and ring patterns. Some plants showed complete necrosis of the buds. PBNV was observed in every

[3] The survey in Pakistan was published as: Delfosse P, Bashir M, Malik SN, Reddy AS. 1995. Survey of groundnut virus diseases in Pakistan. *International* Arachis *Newsletter* **15**: 51-52.

field surveyed in Pothar area but always at a very low incidence (less than 1 %). In many fields, we also found a few scattered stunted plants. These plants were suspected to be affected by PCV but mottling symptoms were not clear on young leaflets. PBNV was also observed in the groundnut fields on the road to Tarbela Dam, Hazro Tehsil, near Kamra, Attock, Fateh Jang and Chakwal. At Barani Agricultural Research Institute (BARI), Chakwal and at NARC, Islamabad, a few plants suspected to be infected by PBNV and PCV were recorded. Due to the presence of severe iron deficiency which causes a yellowing of the leaflets it was difficult to detect symptoms of virus diseases. Samples were collected in all places surveyed and tested by ELISA with antisera raised against PBNV and PCV. The ELISA results confirmed the presence of PBNV and PCV in Pakistan. Two serotypes of PCV were identified. At BARI, two suspected plants reacted with an antiserum raised against the Ludhiana isolate of Indian PCV. Another plant also collected at BARI reacted with antiserum produced for the Talod isolate of Indian PCV. The plants from Dhudial and NARC also reacted with antiserum for the Talod isolate. None of the samples reacted with antisera raised against the Hyderabad or West-African isolates.

On the basis of this survey, it is concluded that in Pakistan two virus diseases : including at least two known serotypes of PCV, and PBNV are present in farmer's fields. The overall crop condition was very good and no fungal diseases were observed except for crown rot in some fields. Other viral diseases such as peanut stripe, peanut mottle and cowpea mild mottle viruses were not observed in the fields surveyed.

Short or medium duration varieties are likely to be introduced in the near future to help the farmers to obtain two crops per year, wheat in post rainy season and groundnut in the rainy season. We suggest that new introduced varieties should have resistance to PBNV. Fortunately the local variety appears to have tolerance to PBNV. This is based on comparative field observations with a newly introduced semi-spreading variety which showed very high PBNV incidence. This variety has not been identified. Regarding PCV, the new rotation proposed to the farmers of wheat-groundnut will create favourable conditions to increase the disease incidence which has already happened in the states of Punjab and Rajasthan in India. In July at NARC various groundnut lines were sown in a sandy soil block where wheat is grown once in every two years. In September the crop was severely affected by PCV (30% of incidence). We suggest that a non-preferred host for the fungal vector of PCV, *Polymyxa* sp., such as rapeseed should be grown prior to groundnut in PCV infested fields.

Geographical distribution of the Indian peanut clump virus (IPCV) in Rajasthan: soil characteristics and farming practices influencing the disease occurrence. [4]

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Abstract

Surveys were conducted along roads in Rajasthan in areas infested with IPCV, a soil-borne and seed-transmitted virus. Furthermore, farmers in Rajasthan were sent a questionnaire illustrated with pictures of typical symptoms. Over 500 questionnaires were sent and 72 answers were received covering the districts of Jaipur, Dausa, Tonk and Sikar. It is apparent from the survey that the disease occurs mainly in sandy and sandy-loam soils. The majority of the farms were irrigated and showed a disease incidence ranging from 10 to 20 %, which in most instances, extended over the entire farm. The problem is not recent but seems to have extended during the last 5 years. The longer the farmers had been growing wheat or barley the higher the incidence. If groundnut, millet and maize produced on a farm are reused for seed, it increases the disease incidence on the farm. About 250 000 ha are under groundnut cultivation in Rajasthan and peanut clump disease is considered to be the second-most limiting factor to groundnut production in the state, the major problem being damage caused by white grubs.

Introduction

Peanut clump viruses are among the most damaging soil-borne pathogens of groundnut, causing crop losses estimated at over US$ 38 millions per annum world-wide. The viruses occur in semi-arid areas of western Africa and the Indian subcontinent. In India, the virus is *Indian peanut clump virus* (IPCV). IPCV has been shown to be transmitted through seeds of groundnut and such cereals as, pearl millet, finger millet, foxtail millet, maize and wheat . It is also transmitted in a persistent way by the root obligate

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endoparasite, *Polymyxa graminis* Led. Resistance to clump could not be identified in groundnut germplasm. Biocides, though effective in reducing disease incidence, are hazardous and not economical. The two main management options left are to devise cultural practices which lead to reduction of IPCV incidence or to induce host-plant resistance by non-conventional approaches (Reddy *et al.*, 1999). In India, the disease was reported from the states of Andhra Pradesh, Gujarat, Punjab and Tamil Nadu, and it is considered to be one of the major constraints to groundnut production in sandy soils in the state of Rajasthan (Mathur & Sobti, 1993). The disease in Rajasthan has been known since the late 60's but at that time it was identified as "rosette" disease (Mathur *et al.*, 1971, L.C. Sharma, personal communication). During the last 5 years, farmers and agricultural extension officers regularly reported the problem to scientists at the Durgapura Agricultural Research Station, Jaipur. Little was known about the actual spread and severity of clump disease in Rajasthan where over 250 000 ha are under groundnut cultivation each rainy season. Therefore systematic surveys were conducted in Rajasthan to determine the economic importance of clump disease, to identify farming practices that could be responsible for high disease incidence, and to inform Rajasthan farmers about the causal agent and the methods available to date to contain the spread of this pernicious soil-borne disease.

Materials and Methods

Systematic surveys were conducted by road in Rajasthan in groundnut growing areas during the rainy seasons 1994 to 1997. The districts covered by the surveys and the respective areas under groundnut cultivation in 1997 were: Bikaner (23 200 ha), Bhilwara (19 100 ha), Chittorgarh (48 754 ha), Dausa (16 200 ha), Jaipur (20 700 ha), Swaimadhopur (18 390 ha), Tonk (20 460 ha), Sikar (2 660 ha) and Churu (2 000 ha). Districts are divided into "tehsils" which group 5-10 villages and each tehsil is under the supervision of one agricultural extension officer (AEO). On average about 4 tehsils regarded as important groundnut growing areas, were surveyed in each district. In every tehsil, at least 3 villages identified by the local farmers as clump problematic areas and accessible by road were visited. Finally in each village, 3 fields were scored for IPCV incidence. Groundnut samples were collected and tested for IPCV presence by ELISA. The disease sites were mapped to illustrate IPCV importance and distribution in the state of Rajasthan.

Furthermore, farmers in Rajasthan were sent a questionnaire, through AEO, written in Hindi and illustrated with color photographs of typical symptoms. Over 500 questionnaires were distributed. In most of the cases,

the questionnaire was filled in with the help of the AEO of the region. Some were also filled during the aforementioned surveys. Data from the inquiry were arranged in summary frequency tables and a correlation matrix (Pearson correlation coefficient) was established between IPCV incidence and the various factors studied.

Results

Surveys conducted by road. A map of Rajasthan showing all the disease sites observed during the surveys is presented in Fig. 3. It is apparent from the surveys that peanut clump disease is widely spread in groundnut growing areas of Rajasthan. Most of the crops surveyed were sown in June-July and most of the farms were provided with irrigation facilities (ground water). In the Indira Gandhi Nehar Project (IGNP, Bikaner district), crops were sown in April-May under irrigation provided by a canal. Groundnut leaf samples collected during the survey reacted positively with a polyclonal serum raised against the Durgapura isolate of IPCV (IPCV-D). One sample from Ranoli (Dausa district) did not react with any of the sera available for IPCV detection.

Survey conducted with the help of a questionnaire. Seventy two farmers replied to the questionnaire. This represents a participation of about 15%. Farmers originated from Dausa (36%), Jaipur (32%), Tonk (18%) and Sikar (14%) districts. According to the answers, most of the farms were located in sandy (33%) and sandy-loam (55%) soil areas, others (12%) were in loamy soil. Irrigation facilities were available in most of cases (71%) and the farms were generally of small size. Forty one (57%) had a surface not exceeding 2 ha, 23 farms (32%) had a surface of between 2 and 5 ha and 6 farms (8%) had a surface of 5 to 15 ha. The number of fields on the farms of 53 farmers (74%) was less than 5.

To the question “What are the major post-rainy season crops that you grow?”, mustard was quoted in 54 cases (75%), wheat in 52 (72%), barley in 36 (50%), and chickpea in 35 (49%). Other crops with minor importance were various vegetables including pea (30%). The major rainy season crops quoted by Rajasthan farmers were groundnut (93% of the answer), pearl millet (58%), cluster bean (*Cyamopsis tetragonoloba*) (36%), maize (28%), and sorghum (10%).

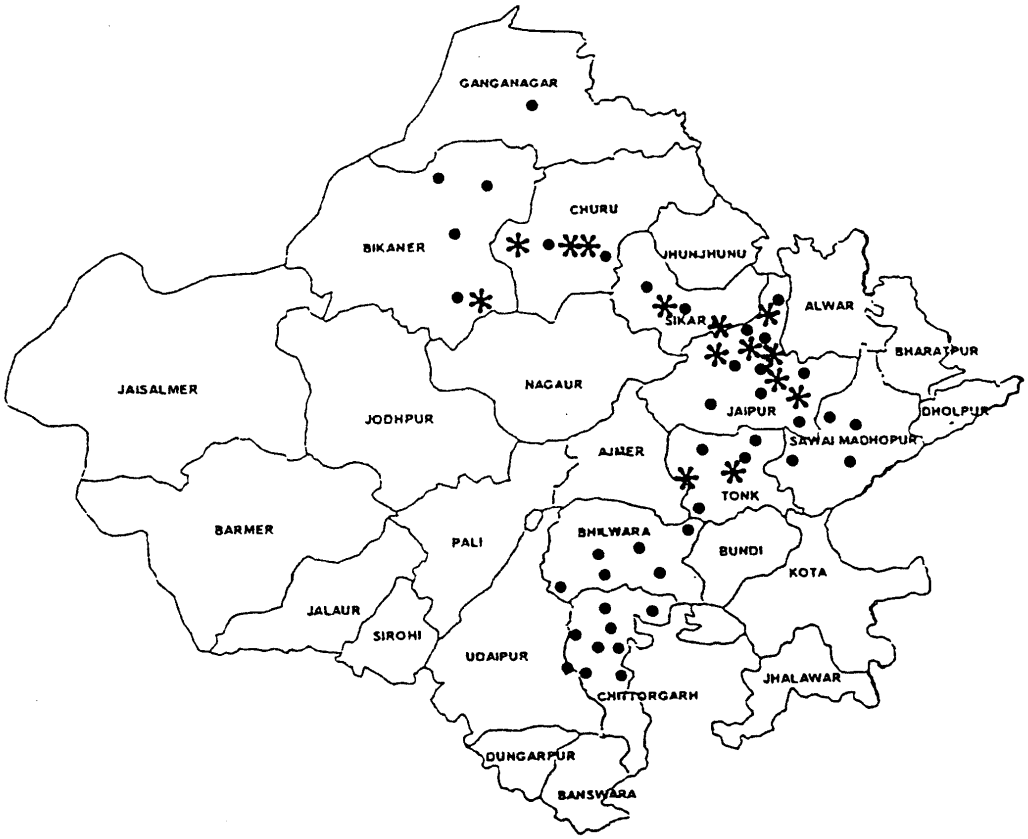

Figure 3. Peanut clump distribution in Rajasthan. Disease sites observed during the 1994 to 1997 rainy seasons in major groundnut growing areas. Incidence: ( ) below 20%; ( ) 20-70%

Regarding peanut clump disease, usually all the fields of a farm were infested (43 farms representing 60% of the answers). Farms located in loamy soil showed the lowest spread of IPCV on the farm (percentage of infested field). According to the farmers, IPCV incidence on their farm ranged from 10-20% in 56% of the case, and from 20-50% in 32%. A minority reported incidences of 0-10% (7%) and 50-100% (5%). The problem is not recent but seems to have extended during the last 5 years. Indeed, most of the farmers (67%) noted peanut clump as a problem that had affected their farm in the course of the last five years. Twelve farmers (17%) had encountered clump disease for the last 20 years and few (8%) noticed the disease about 5 to 10 years ago. The longer the farmers had been growing wheat or barley the higher the incidence. If groundnut, millet and maize produced on a farm had been reused for seed, the disease incidence increased (Pearson $r = 0.334$, $P = 0.0190$, *d.f.*=n-2=70) on the farm. On the contrary, if groundnut seed had been purchased from the store, IPCV incidence was low (Pearson $r = 0.524$, $P = 0.0001$, *d.f.*=n-2=70).

Discussion

The surveys conducted in the state of Rajasthan confirmed that peanut clump disease (IPCV-D serotype) is widely spread in the major groundnut growing areas. For an investigation of this type, there was a satisfactory participation of the farmers in filling in the questionnaires, which indicated their concern about this pernicious disease. The surveys confirmed previous reports that peanut clump disease occurs mainly in light soil, sandy and sandy-loam soil (Reddy *et al.*, 1999). Heavier soil texture, such as loam, was negatively correlated with the spread of the disease. IPCV is transmitted through seed of groundnut and cereals (Reddy *et al.*, 1998, Reddy *et al.*, 1999) but little is known about the role of seed inoculum in disease spread and disease establishment in soil harboring non-viruliferous *P. graminis*. This survey among the farmer community in Rajasthan suggested that the use of groundnut, millet and maize produced in infested farms for seed is linked to higher disease incidence on the farm, whereas the use of certified groundnut seed reduced the risk of having high incidence in groundnut crops. Groundnut is not a good host for *P. graminis*. Therefore, groundnut seed carrying the virus is not, a priori, considered as a serious threat that will support establishment of the disease in new areas. However, there is no doubt that the use of virus-infected seed can result in a higher disease incidence in groundnut crops. Maize, pearl millet and wheat are excellent hosts for *P. graminis* isolates from Rajasthan (Legrève *et al.*, 1999) and their seed is suspected to play an important role in disease spread and establishment in new areas.

About 250 000 ha are under groundnut cultivation in Rajasthan where groundnut is an important cash crop and a source of protein in the human diet. Peanut clump disease is considered the second-most limiting factor to groundnut production in the state, the major problem being damage caused by white grubs. The surveys indicated that the problem has extended during the last five years, which corresponds to the introduction of groundnut, a host expressing overt symptoms, to new irrigated areas where pearl millet, a symptomless host, was previously the major rainfed crop. It is likely that the cropping systems in force in Rajasthan, which implies rotation of groundnut with cereal hosts for *P. graminis* and IPCV, such as millet, maize and wheat, favor the increase in spread and incidence of peanut clump virus disease. The results of these surveys corroborate epidemiological studies conducted on IPCV and its vector *P. graminis*.

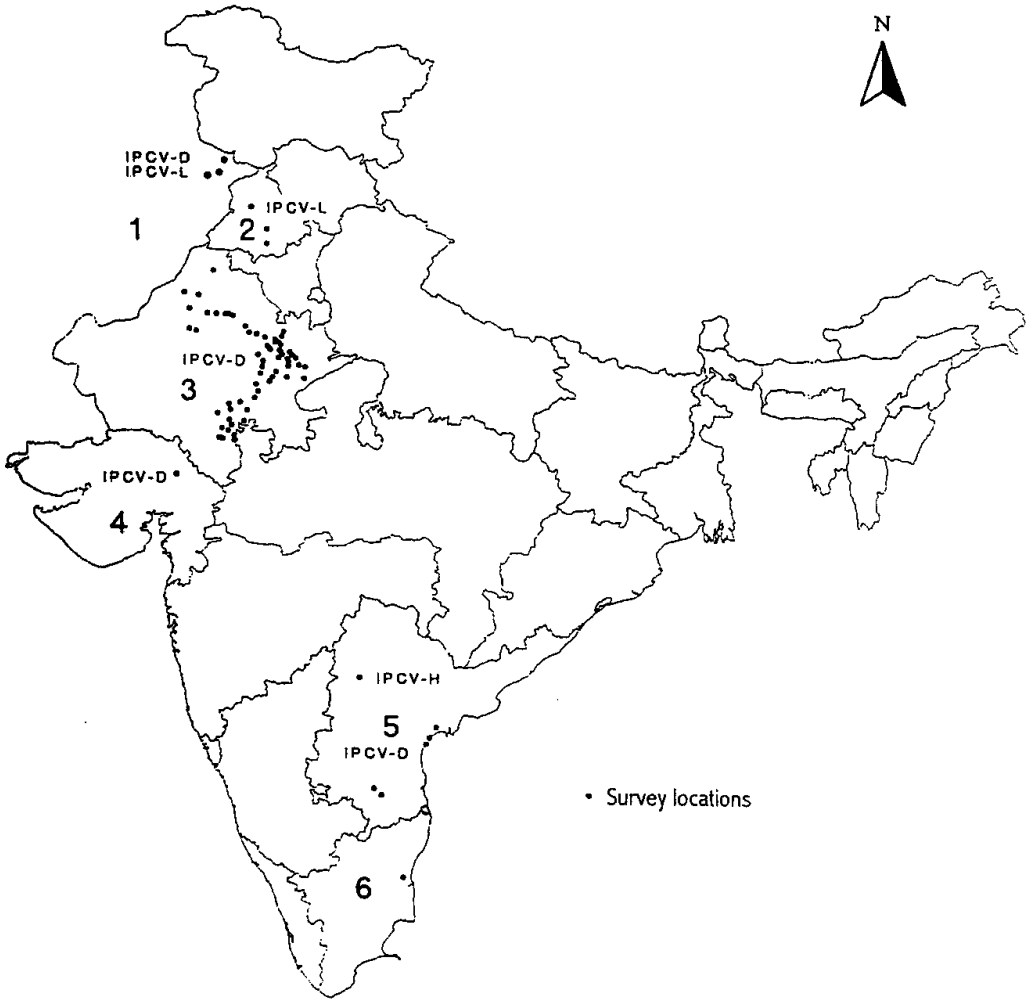

Figure 4. Distribution of the three known serotypes of the *Indian peanut clump virus* (IPCV) in the Indian sub-continent. The serotype IPCV-D (represented by the Durgapura isolate) was found in Pakistan (1), in India, in Rajasthan (3), Gujarat (4), Andhra Pradesh (5), and Tamil Nadu (6). The serotype IPCV-L (represented by the Ludhiana isolate) was found in Pakistan and in India in Punjab (2). The serotype IPCV-H (represented by the Hyderabad isolate) has so far been detected only in Andhra Pradesh.

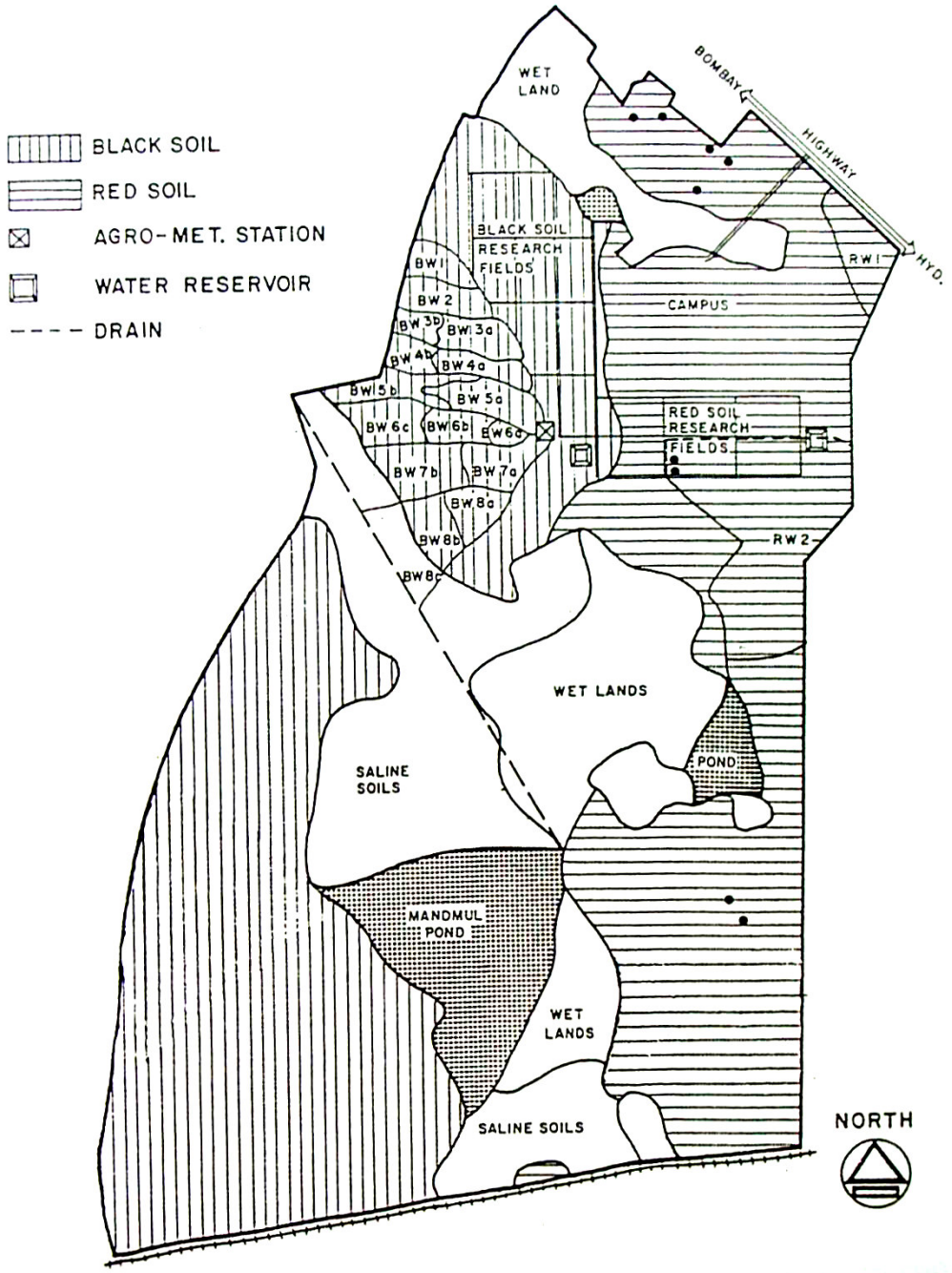

Figure 5. IPCV (IPCV-H) distribution on the ICRISAT farm at Patancheru, Andhra Pradesh. IPCV-H was detected in groundnut or pigeonpea crops grown in red soil fields RCW 13, 14, 17A, 18B, 18C, rain-out shelter plots, RM18C, and RP 14A and D.

Consolidated comments on IPCV distribution in Asia

The geographical distribution of IPCV in India and Pakistan is presented in Fig. 4. This is based on various surveys conducted from 1993 to 1998 in rainy season (Pakistan, Andhra Pradesh, Punjab, Rajasthan, Gujarat) and post-rainy season (Andhra Pradesh, sea shore area) crops of groundnut. The disease is wide-spread in the Indian subcontinent. It occurs (Rajasthan) or has occurred (Punjab) with high incidence in areas where groundnut is grown during the rainy season and rotated with post-rainy season crops of wheat or barley. In these areas pearl millet, a host for *P. graminis* and IPCV, is a major rainy season crops. High incidence in groundnut crops sown in July in Pakistan suggests that there is a possible outbreak of peanut clump disease if the traditional early sowing of groundnut in April is replaced by a sowing in July with the adoption of short duration varieties. Peanut clump disease was not encountered in post-rainy season crops of groundnut in Thailand. In this country groundnut is rotated with rice in heavy textured soils. For this reason, groundnut fields are flooded as in the case of rice to facilitate peg entry. It is probable that the current farming practices used in Thailand are not favorable to peanut clump disease. However, groundnut is also grown during the rainy season on small scale in the southern part of Thailand and surveys in these areas are required to clarify the status of peanut clump disease in Thailand.

At all disease sites, including PCV infested areas in West Africa, the soil was sandy, loamy sand or sandy-loam type (Table 2). During these surveys in Asia as well as during surveys conducted in West Africa, peanut clump disease was never observed in groundnut crops grown in soils with relatively heavy texture. Furthermore, at ICRISAT-Patancheru the presence of IPCV was noticed only in groundnut and pigeonpea, another crop severely affected by peanut clump disease (CHAPTER 3), fields located in light textured alfisol (red soil) areas (Fig. 5). There was no marked differences in texture between infested and healthy areas of a same field (Table 2). Because groundnut prefers light textured soil for peg establishment, it is normally not grown in soils of the vertisol type. However, pigeonpea fields within the vertisol area (black soil, often clay or clay-loam) were surveyed during the 1995 to 1998 rainy seasons but peanut clump disease was not observed. Based on these observations we conclude that peanut clump disease prefers light textured soil that probably facilitates the movement of *P. graminis* zoospores. Nevertheless, there is a need to confirm if under the tropics, *P. graminis* is present exclusively in light soils or if it can live in soils with heavy texture. Indeed in temperate

areas, *Polymyxa betae* infects sugar beet in sandy soils as well as in clay soils (Goffart, 1992). Alternate dry and wet conditions in the soil are more favorable for resting spore germination than when the soil holds high moisture content for long period. This has been shown for *Spongospora subterranea* (Cooper *et al.*, 1976), for *P. graminis* in lab experiments (Ledingham, 1939) and in the case of rice necrosis mosaic virus, also transmitted by *P. graminis,* in field experiments (Inouye, 1977). These requirements were simulated to improve condition for culturing *Polymyxa* spp. (Adams *et al.*, 1986 and Legrève *et al*., 1998). The rainfall is well distributed all through the year in temperate areas, thus helps to avoid water logging while in India for example, usually 600 to 800 mm of rain is recorded during the 4 months of the groundnut growing season in the semi-arid regions. Tropical soils with heavy texture remain saturated with water for long periods compare to light textured or temperate soils, and presumably are less favorable to *Polymyxa* sp. survival by not providing the alternate dry and wet conditions. In conclusion suitable soil texture is essential to avoid continuous water logging during heavy rainfall, characteristic of the tropics and to allow the movement of zoospores.

Table 2. Texture type of soils sampled at peanut clump disease sites during various surveys conducted in India, Pakistan, West Africa, and in IPCV-H infested fields at ICRISAT-Patancheru.

Sites	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture type[1]
India					
Karlapalem, AP	58.03	34.44	5.02	2.51	Sand
Ganapavaram, AP	24.76	66.41	5.05	3.78	Sand
Pallipalem-SV, AP	68.55	27.06	3.14	1.25	Sand
Pallipalem-SubaRao, AP	64.74	30.25	3.76	1.25	Sand
Pallipalem-Linni Yanadi, AP	68.9	27.97	3.13	0	Sand
Pallipalem-Thirupal, AP	67.13	28.48	3.13	1.25	Sand
Boraj Field 1, RAJ	15.88	74.05	5.67	4.4	Sand
Boraj Field 2, RAJ	9.95	78.72	6.3	5.04	Sand
Boraj Field 3, RAJ	11.42	80.42	5.65	2.51	Sand
Durgapura, RAJ	7.85	83.36	5.02	3.77	Sand
Rampura, RAJ	5.16	88.56	5.02	1.26	Sand
Rampal, RAJ	10.24	86	2.51	1.25	Sand
Rampura, RAJ	5.33	87.11	5.03	2.53	Sand
IGNP Bikaner- Nokha, RAJ	11.69	80.77	5.02	2.51	Sand
IGNP Bikaner-Hussein Sir, RAJ	10.53	74.36	8.81	6.29	Sand
Bhilwara, RAJ	47.51	29.73	12.7	10.12	Loamy sand

to be continued

Table 2: continuation

Sites	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture type[1]
Pakistan					
NARC, Islamabad	12.04	70.37	11.3	6.28	Loamy sand
Dudhial	26.36	50.84	13.9	8.87	Sandy loam
West Africa					
Toussiana, Burkina Faso	33.02	56.93	6.28	3.77	Sand
Zinzana, Mali	41.02	50.84	3.76	4.38	Sand
Sadore, Niger	38.17	56.82	3.76	1.25	Sand
ICRISAT farm					
-Red soils					
RCW13 Infested	47.43	35.59	8.17	8.8	Loamy sand
RCW14 Infested	42.71	26.79	10.2	20.34	Sandy clay loam
RCW17A Infested	50.75	30.38	9.43	9.43	Loamy sand
RCW18B Infested	45.42	29.17	8.89	16.52	Sandy loam
RCW18C Infested	45.61	30.33	8.86	15.2	Sandy loam
RM18C Infested	51.13	36.95	6.9	5.02	Sand
RP14A Infested	44.63	33.92	8.83	12.62	Sandy loam
RP14D Infested	42.93	32.92	11.4	12.71	Sandy loam
RCW13 Healthy	51.99	32.94	8.16	6.91	Loamy sand
RCW14 Healthy	42.07	33.25	6.96	17.72	Sandy loam
RCW17A Healthy	50.71	30.35	8.84	10.1	Loamy sand
RCW18C Healthy	43.74	33.56	7.57	15.14	Sandy loam
RM18C Healthy	46.36	38.53	6.3	8.81	Loamy sand
RP14A Healthy	40.92	25.76	8.97	24.35	Sandy clay loam
-Black soils (all healthy)					
BM13D	9.85	13.42	21.9	54.81	Clay
BM15A	35.43	22.12	11.1	31.35	Sandy clay loam
BM16B-east	16.67	17.74	20.7	44.93	Clay
BP13A	11.75	16.09	21.7	50.51	Clay
BP13B	11.94	15.34	21.8	50.9	Clay
BP14B	15.76	14.31	23.3	46.62	Clay
BP14C	15.38	14.68	23.3	46.63	Clay
BM21A-W-ISO01	14.96	23.22	23	38.86	Clay loam
BM25C-ISO02	18.38	18.2	22.3	41.09	Clay
BP01-ISO05	16.21	14.89	24.2	44.74	Clay
BR01A-ISO01	9.88	11.68	21.9	56.55	Clay
BR02J-ISO02	9.48	14.18	23.6	52.72	Clay
BR04D-ISO01	11.2	13.01	21.7	54.13	Clay

AP: Andhra Pradesh, RAJ: Rajasthan.

[1]. Soil texture analysis with classes of particle size defined as: coarse sand (2mm-200µ), fine sand (200-50µ), silt (2-50µ), and clay (<2µ).

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Survey in Thailand

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Survey in Pakistan

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Survey in Rajasthan

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Chapter 3

Natural host range of the Indian peanut clump virus (IPCV) and its vector *P. graminis*

Abstract

The host range of IPCV and *P. graminis* was evaluated under natural conditions. We confirmed that monocotyledonous hosts were the "preferred" hosts for *P. graminis*. The parasite was found to colonize sorghum, pearl millet and maize roots with high intensity. It was also found to infect various naturally occurring monocots. However *P. graminis* was generally not detected in roots of dicotyledonous plants. If infection occurred in dicots, it did not result in high intensity multiplication. Dicotyledonous plants were considered as "fortuitous" hosts for *P. graminis*. In addition to groundnut, IPCV was shown to cause a severe disease in post-rainy season crops of wheat and barley and in rainy season crops of pigeonpea. Virus infection causes yield loss in maize. Maize showed growth reduction in IPCV infested fields but symptoms could not be correlated with the presence of the virus. Rhizomes of *Cynodon dactylon* and seeds of groundnut containing IPCV-H were evaluated as possible primary source of inoculum which can lead to the establishment of the disease in new areas.

3-1. Weeds, dicotyledonous crops, maize, *Sorghum* spp., and *Pennisetum* spp. hosts for IPCV and *P. graminis*[5]

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Abstract

The host range of IPCV has been studied in rainy and postrainy seasons in India. The virus produces severe overt symptoms not only on groundnut but also on pigeonpea. Cowpea, chickpea, soybean, mungbean and mustard were infected by the virus without showing any overt symptoms. Sunflower was not infected by the virus under natural conditions but mechanical inoculation was successful in transmitting the virus. Various monocotyledonous weeds naturally infected by IPCV were *Cynodon dactylon*, *Dactyloctenium aegyptium*, *Digitaria ciliaris*, *Eragrostis ciliaris* and *E. uniloides* while the dicotyledonous weeds infected were *Celosia argentea*, *Macroptilium atropurpureum* and *Oldenlandia corymbosa*. Maize was a good host for IPCV and showed a virus transmission through seed at a frequency of 0.5%. A selected number of *Sorghum* spp. and *Pennisetum* spp. accessions from the ICRISAT genebank, including wild taxa, varied in their susceptibility to IPCV infection. *P. graminis* was often detected in roots of most of the monocotyledonous hosts but it was not detected in roots of groundnut and other dicotyledonous plants collected from IPCV infested fields.

[5] To be submitted for publication

Introduction

Indian peanut clump virus (IPCV) is a seedborne (Reddy *et al.*, 1998) and soilborne virus causing severe damage to groundnut crops in semi-arid areas of the Indian subcontinent (Reddy *et al.*, 1988). Resistance to IPCV has so far not been identified in cultivated or wild *Arachis* sp. and chemical controls have met with little success (Reddy *et al.*, 1999). Control may have to rely on cultural practices and information generated from epidemiological studies including the host range is likely to guide disease management strategies. IPCV is known to infect many monocotyledonous and dicotyledonous plants among wild and cultivated species (Reddy *et al.*, 1988; Ratna *et al.*, 1991). The plasmodiophorid, *Polymyxa graminis* Led., which was shown to be the vector of IPCV, is known to prefer monocotyledonous plants to complete its life cycle (Ratna *et al.*, 1991, Legrève, 1999). In this respect, peanut clump disease differs from other viral disease transmitted by plasmodiophorid vectors (Adams, 1988). Indeed IPCV causes an economically important disease in a crop - groundnut - which is not a natural reservoir for the vector. The disease cycle therefore requires involvement of monocotyledonous species, hosts for IPCV and its vector. However, those monocots which play a crucial role in the perpetuation of both *P. graminis* and virus inoculum are yet to be identified. IPCV has been shown to be seed-transmitted in groundnut and millets (Reddy *et al.*, 1998). Seed-borne inoculum in monocotyledonous hosts is likely to initiate the disease in areas where non-viruliferous populations of *P. graminis* exist.

Polymyxa sp. resting spores were seldom observed in dicotyledonous hosts grown in fields naturally infested with peanut clump disease. In such hosts only the resting stage of the fungus was observed and the number of sporosori was always very low (Ratna *et al.*, 1991). This was later confirmed by laboratory experiments using *P. graminis* isolates from clump infested soil (Legrève, 1999). For these reasons, dicotyledonous plants are considered as fortuitous hosts for *P. graminis* and their use in the rotation is suspected to reduce the disease incidence in groundnut crop (Delfosse *et al.*, 1996).

Sorghum (*Sorghum bicolor* [L.] Moench) and pearl millet (*Pennisetum glaucum* [L.] R. Br.) are considered to be the best hosts to support *P. graminis* multiplication on the basis of field observation, where heavy infection was recorded (Ratna *et al.*, 1991, Delfosse *et al.*, 1996), and in laboratory experiments conducted under controlled environmental

conditions in Belgium. However, accessions of *Sorghum* spp. and *Pennisetum* spp. differed in their susceptibility to infection by *P. graminis* (Legrève, 1999). They are also suspected to differ in their susceptibility to IPCV infection. Indeed, the pearl millet cultivar WCC 75 showed systemic infection by IPCV and transmitted the virus though seed. In contrast the pearl millet cultivar ICMH-451 and the sorghum cultivar ICSV 88036 were transient hosts for IPCV and the virus was not transmitted through their seeds (Delfosse *et al.*, 1996 and Delfosse *et al.*, unpublished data). Growing monocotyledonous crops which support *P. graminis* multiplication but show immunity to IPCV could also prove useful to render *P. graminis* non-viruliferous. It is essential to identify crop plants with such features. It is possible that growing such crops immune to IPCV, will lead to gradual reduction of virus inoculum and concomitant reduction in disease incidence.
IPCV infects a number of graminaceous weeds (Ratna *et al.*, 1991) and these often proliferate in clump infested fields because they do not need to compete for light and nutrients with groundnut plants whose growth is affected by the disease. They are suspected to play an important role in the disease perpetuation.

Even though the host range of IPCV and its vector has already been studied (Reddy *et al.*, 1988; Ratna *et al.*, 1991; Legrève, 1999), a detailed analysis of the susceptibility of the various hosts to *P. graminis* and IPCV infection, was considered to be an essential step towards the management of peanut clump disease through cultural practices.

Methodology and Results

Weeds

Various naturally occurring weeds were collected from clump infested areas in India. Samples were taken from Bapatla (post-rainy season) and Patancheru (rainy season) in Andhra Pradesh, and Boraj (rainy season) in Rajasthan). They were tested for the presence of IPCV in leaves and roots by the double antibody sandwich form of enzyme linked immunosorbent assay (DAS-ELISA) (Reddy *et al.*, 1998), ISEM (Nolt *et al.*, 1988) and by DNA hybridisation with a cDNA probe which can detect all the currently known isolates of IPCV (Wesley *et al.*, 1996). Roots were tested for *P. graminis* presence by light microscopy (Maraite *et al.*, 1988). Results for monocotyledonous weeds showed that they were all symptomless hosts for IPCV and hosts for *P. graminis* (Table1). Only the resting stage of *P. graminis* (sporosori) was identified in their roots.

Profuse *P. graminis* sporosori occurred in *Cyperus rotundus.* However its roots were rarely found to be infected by the virus and tubers and leaves were never found to be positive in ELISA and ISEM. Nevertheless the virus could be detected by DNA hybridisation indicating that the virus occurred in *C. rotundus* at a low concentration.
All vegetatively produced rhizomes of *Cynodon dactylon* arising from an infected plant contained the virus. *P. graminis* was detected in *C. dactylon* roots but the degree of colonisation by sporosori was not as high as that recorded for other graminaceous weeds.
Seeds were collected from virus infected plants of *Dactyloctenium aegyptium*, *Digitaria ciliaris* and *Eragrostis uniloides* to assess if IPCV was seed-transmitted in these hosts. Unfortunately germination of seeds from weeds is often hampered by dormancy and attempts to evaluate the seed transmission frequency of IPCV in weeds by grow-out tests were not successful.
Various dicotyledonous weeds collected in IPCV infested areas did not show the presence of the vector in their roots (Table 1). *Celosia argentea* and *Oldenlandia corymbosa* were symptomless hosts for the virus. In addition to the weeds listed in Table 1, *Macroptilium* sp. (Benth.), a member of the *Leguminosae*, was also found to be a symptomless host for IPCV.

Dicotyledonous crops

Groundnut (*Arachis hypogaea* L.), pigeonpea (*Cajanus cajan* [L.] Millsp.), chickpea (*Cicer arietinum* L.), cowpea (*Vigna unguiculata* [L.] Walp.), mung bean (*Vigna radiata* [L.] R. Wilczec.), soya bean (*Glycine max* [L.] Merr.), mustard (*Brassica juncea* [L.] Czerniak.) and sunflower (*Helianthus annuus* L.), were sown in an IPCV-H infested field on the ICRISAT farm during the 1995 rainy season. The crops were sown on July 1 in rows randomly distributed in two infested patches and were scored at regular intervals for symptoms and presence of the virus in their leaves by DAS-ELISA.

Groundnut and pigeonpea showed the highest virus incidence (over 25%). The other crops were infected at very low incidence and none of 165 sunflower plants tested were positive in DAS-ELISA. Infected plants of pigeonpea were severely stunted compared to healthy plants. Mosaic symptoms appeared on young leaves and the older leaves were dark green in colour. The symptoms were thus very similar to those observed on groundnut. The percentage of infection was similar for groundnut and pigeonpea but the symptoms appeared approximately 3 weeks sooner in the case of pigeonpea.

Table 1. Detection of *P. graminis* in roots of various weed plants collected from peanut clump infested fields in India, and detection of IPCV in their roots and leaves by ELISA and by DNA hybridization with a broad specific cDNA probe.

	Number of plants infected /Number of plants tested (ni/N)					
	Polymyxa spp.		IPCV-H			
Families			ELISA		c-DNA probing	
Species	ni/N	Degree[1]	Leaves	Roots	Leaves	Roots
DICOTYLEDONOUS WEEDS						
Amaranthaceae						
Celosia argentea L.	0/24	-	7/24	0/24	5/9	0/9
Asteraceae						
Eclipta alba (L.) Hassk.	0/32	-	0/32	0/32	2/10	0/10
Flaveria australasica Hook.	0/8	-	0/8	0/8	1/6	1/6
Tridax procumbens L.	0/31	-	0/31	0/31	0/10	0/10
Vernonia cinerea (L.) Less	0/26	-	0/26	0/26	0/11	0/11
Convolvulaceae						
Merriemia gangetica (L.) Cufod.	0/12	-	0/12	0/12	0/6	0/6
Euphorbiaceae						
Euphorbia hirta L.	0/23	-	0/23	1/23±	0/9	0/9
Phyllanthus niruri L.	0/26	-	0/26	0/26	2/10	1/10±
Leguminosae						
Desmodium dichotomum (Willd.) DC.	0/1	-	0/1	0/1	/	/
*Indigofera sp.*L.	0/5	-	0/5	0/5	0/1	0/1
Rubiaceae						
Oldenlandia corymbosa L.	0/5	-	2/5	1/5	2/3	2/3
MONOCOTYLEDONOUS WEEDS						
Cyperaceae						
Cyperus rotundus L.	23/32	+ to +++	0/32	2/32±	10/10	6/10
Cyperus difformis L.	½	+++	0/2	0/2	/	/
Poaceae						
Cynodon dactylon (L.) Pers	7/30	+ to ++	11/30	12/30	3/10±	9/10±
Dactyloctenium aegyptium (L.) P. Beauv.	17/26	+ to +++	5/26	6/26	5/10	4/10
Digitaria ciliaris (Retz.) Koeler.	3/26	+ to +++	1/26	2/26±	1/8	2/8
Eragrostis uniloides (Retz.) Nees ex Steudel	2/22	++ to +++	10/22	11/22	6/6	5/6
Eragrostis ciliaris	3/13	++ to ++	11/13	11/13	/	/

[1] : degree of infection. - : not detected, , + : low infection, ++ : medium infection, +++ : high infection.
± : weak reaction.
/ : not tested.

The viral antigen concentration was higher in pigeonpea than in groundnut, as measured in DAS-ELISA. The virus in pigeonpea was also detected by probing the viral RNA with a cDNA probe. The virus particles were readily trapped by ISEM from pigeonpea leaf extract showing stunting and mosaic symptoms. No particles were observed from the apparently healthy plants. Seeds of infected pigeonpea plants were collected during crop growth to assess if the virus is seed transmitted in pigeonpea. Out of 854 seeds kept for germination, 539 germinated and all gave rise to healthy plants as assessed by ELISA tests on leaves. Roots of 25 infected pigeonpea plants collected 2 months after sowing were analysed for the presence of *P. graminis*. None of the roots contained the vector. IPCV caused yield loss in pigeonpea, this point is addressed in Chapter 5-1. The other crops showed symptomless infection.

Table 2. IPCV-H incidence in dicotyledonous crops suspected to be fortuitous hosts of *P. graminis* and grown in a clump infested field (RCW 17a) on the ICRISAT farm during the 1995 rainy season. Number and percentage of plants infected by the virus and symptoms caused by the viral infection.

		Ni/N[1]				
Crops	Cultivars	Patch A	Patch B	Total	%[2]	Symptoms
Groundnut	NCAC-17090	18 / 84	24 / 65	42 / 149	28	stunting + mosaic
Pigeonpea	ICP-8863	10 / 99	43 / 107	53 / 206	26	stunting + mosaic
Chickpea	K-850	3 / 69	2 / 56	5 / 125	4	none
Cowpea	C-152	2 / 86	8 / 82	10 / 168	6	none
Mungbean	Unknown	0 / 124	3 / 110	3 / 234	1	none
Soya bean	Bragg	0 / 67	6 / 90	6 / 157	4	none
Rapeseed	Local in A.P.	6 / 510	2 / 322	8 / 832	1	none
Sunflower	MSFH-8	0 / 74	0 / 91	0 / 165	0	-

[1] : Number of plants infected by the virus / number of plants grown and tested by DAS-ELISA one month after emergence. Young leaves were used for ELISA test.

[2] : Percentage of plants whose leaves were infected by the virus, based on ELISA tests and calculated on the basis of 2 observations in patches A and B.

Monocotyledonous crops

Maize. Relatively high virus incidence was recorded in leaves and roots of maize plants grown during the 1996 rainy season. Plants that showed the presence of *P. graminis* presented intense colonisation of roots by sporosori (Delfosse *et al.*, 1996). Maize was grown during 1998 rainy seasons in a IPCV-H infested field on the ICRISAT farm. It was unclear if IPCV-H infection caused symptoms in maize. Indeed infected plants tested two months after sowing by ELISA showed either normal growth or severe stunting and/or presence or absence of chlorotic lesions on leaves. Stunted plants were distributed in infested patches in areas where the disease occurred in groundnut crops during the previous rainy season. However symptoms could not exactly be correlated with presence of the virus in leaves as assessed by ELISA. To determine if IPCV-H caused yield reduction in maize, yield was compared between plants tested positive and from plants tested negative by ELISA, irrespective of their size or the presence of chlorotic symptoms. The results indicated that IPCV-H infection caused yield loss in maize (Table 3). Plants tested positive by ELISA produced less biomass and seed weight per plants compared to plants that were tested negative by ELISA. There was a higher proportion of non-bearing seed plants among the ELISA positive plants.

IPCV-H was found to be transmitted through maize seed. Out of 1847 seeds collected from IPCV-H infected maize plants, 1573 germinated and 7 (0.5%) produced progeny containing the virus in both leaves and roots.

Table 3. Effect of IPCV-H presence in leaves as assessed by ELISA, on yield of maize plants grown during the 1998 rainy season in a naturally infested field on the ICRISAT farm.

Yield Dry matter	Healthy plants (n=491)	Virus infected plants (n=226)	Probability *P*
Aerial biomass per plant (g)	51	34	0.0026**
Confidence interval (95%)	47 to 55	30 to 39	
Seed weight per plant (g)	14	9	0.0382*
Confidence interval (95%)	12 to 16	6 to 11	
Number of seed per plant	58	37	0.0667^{NS}
Confidence interval (95%)	51 to 65	28 to 45	
Weight of 1000 seeds (g)	243	224	0.0679^{NS}
Confidence interval (95%)	228 to 259	213 to 235	
Plants bearing seeds (%)	62	43	

Maize was sown in 1m broad beds. Plants were harvested from each bed and infected plants separated. The effect of IPCV-H on yield was analysed by ANOVA 2-ways with one random factor (bed of origin), and one fixed factor (infected or not).

***Sorghum* spp. and *Pennisetum* spp.** At ICRISAT Patancheru, two sets of *Sorghum* and *Pennisetum* germplasm accessions, including wild taxa, were tested under field conditions to assess if they could become infected by *P. graminis* and IPCV while at UCL the accessions were tested under controlled environmental conditions for infection by *P. graminis* (isolate $I_{1\text{-}1}$) isolated from the same experimental field (Legrève, 1999). The accessions were selected from the ICRISAT genebank with the help of KE Prasada Rao (Visiting Scientist, Genetic Resource Division, ICRISAT). The criteria of selection were mainly driven by the hypothesis that accessions which encounter IPCV for the first time could possibly be more susceptible to the virus than varieties commonly grown in IPCV infested areas. Therefore care was taken to include accessions from Asia and Africa, including both wild and cultivated species. Four uniformly and highly infested patches (IPCV-H incidence over 70%) were demarcated in the wheat crops (Delfosse *et al.*, 1999) raised during the 1995-1996 and the 1997-1998 postrainy seasons. In 1996, because the infested area was limited in size, only 10 accessions each for *Sorghum* and *Pennisetum* which showed the best seed viability were tested. In 1998, all the selected accessions were tested in the field. Sowing was done on 28 June 1996 and on 26 June 1998 in a randomised complete block design with four replications. Plots were 50x25 cm in size. Leaf samples were collected and tested by DAS-ELISA (Reddy *et al.*, 1998) for the presence of IPCV-H 2 to 3 months after sowing. At harvest, the roots were dried at room temperature for storage prior to analysis for *P. graminis* presence by the direct antigen coating form of ELISA (DAC-ELISA) as described in Chapter 1. Seeds were collected from all virus infected plants to assess seed transmission frequency of IPCV-H.

In 1996, in *Sorghum spp.*, the virus incidence reached 22.0% (13 infected plants of 59 tested), 2.7% (1 plant infected of 37 tested), and 24% (6 infected plants of 25 tested) for IS 18519, *S. bicolor* ssp. *drummondii* (S. 64) and *S. bicolor* ssp. *verticilliflorum* race *arundinaceum* (S. 162), respectively. Among *Pennisetum* spp. accessions, *P. pedicellatum* and *P. schweinfurthii* were found to be infected with an incidence of 1.4% (1 plant infected of 71 tested) and 18.2% (10 plants infected of 55 tested), respectively. In the case of *Sorghum bicolor* (IS 18519), *S. bicolor* ssp. *verticilliflorum* race *arundinaceum* and *P. schweinfurthii*, infected plants were present in all replications. However other accessions were found to be infected only in one or two replications.

At harvest, roots of many *Sorghum* and *Pennisetum* plants had lost their cortical layers. Consequently *P. graminis* was detected in a restricted number of plants of most accessions. Nevertheless, plants tested positive in ELISA showed high OD values, between 1 and 2, suggesting that numerous sporosori were present in these infected plants. Among *Sorghum* spp. accessions, *P. graminis* was not detected in *S. bicolor* ssp. *drummondii* (IS 720 and S. 64), in *S. bicolor* ssp. *verticilliflorum* (S. 01), and among *Pennisetum* spp. accessions, in *P. glaucum* ssp. *americanum* (IP 16793 and IP11902) and in *P. schweinfurthii*. *S. dimidiatum* was not tested in 1996 but in 1998 none of 52 plants tested in the field were infected by IPCV. This accession was also tested under controlled environmental conditions (Legrève, 1999) and the author reported only a trace of infection after inoculation with the *P. graminis* isolate $I_{1\text{-}1}$. In our field experiments only one plant of *P. pedicellatum* was found to be positive for the presence of *P. graminis* in 1996 and Legrève (1999) found only a trace of *P. graminis* sporosori in roots of *P. pedicellatum.*

In 1998, a higher number of accessions were positive in ELISA tests for the presence of IPCV-H as compared to the 1996 rainy season. However, various cultivated forms of sorghum, that were tested negative in 1996, were confirmed as non-hosts for IPCV-H in 1998. For *Pennisetum* spp., the 1998 results corroborated those obtained in 1996. Most cultivated pearl millet accessions did not show the presence of IPCV-H in their leaves. Only one plant of an Indian variety (Shebra) was found to be infected. Among the wild taxa, *P. sweinfurthii* was confirmed as a good host for IPCV-H in the four replication plots.

Out of 950 seeds collected in 1996 from ELISA positive sorghum and pearl millet plants, 614 germinated and none gave rise to infected seedlings when tested by ELISA.

Table 4. *Sorghum spp.* and *Pennisetum* spp. accessions from the ICRISAT genebank tested for IPCV-H infection in leaves and *P. graminis* in roots under field conditions, and comparison with the degree of *P. graminis* infection under controlled environmental conditions (Legrève, 1999).

Sp. ssp.	**Code**	**Origin (cv. or type)**	**Form**	**IPCV-H 1996**		**IPCV-H 1998**		***P. graminis* 1996**	***P. graminis* (Legrève, 1999)**
***Sorghum* spp.**				(%)	ni/N	(%)	ni/N	ni/N	DI
S. bicolor ssp. *Bicolor*	IS 3890	Mali	Cultivated	0	0/58	0	0/62	4/58	3
" "	IS 7871	Nigeria	Cultivated	0	0/34	0	0/43	2/34	3
" "	IS 24357	India (Maldandi)	Cultivated	0	0/68	29	16/55	5/68	3
" "	IS 40284	India (296 B)	Cultivated	-	-	6	3/49	-	3
" "	IS 2861	South Africa	Cultivated	0	0/58	0	0/41	4/58	2.9
" "	IS 3162	South Africa	Cultivated	-	-	0	0/58	-	2.5
" "	IS 18519	Uganda	Cultivated	22	13/59	17	9/54	5/59	3
" "	IS 18520	Uganda	Cultivated	-	-	22	13/59	-	3
S. bicolor ssp. drummondii	IS 720	USA (sudangrass)	Forage	0	0/56	10	5/49	0/56	2.9
" "	IS 722	USA (sudangrass)	Forage	-	-	0	0/57	-	2.8
" "	S. 64	Ethiopia	Weedy	3	1/37	6	1/18	0/37	2.9
S. bicolor ssp. *verticilliflorum*	S .01	Angola (race *verticilliflorum*)	Wild	0	0/37	19	8/41	0/24	3
" "	S .162	Unknown (race *arundinaceum)*	Wild	24	6/25	17	7/42	2/25	3
S. halepense	S. 77	Angola (Johnson grass)	Wild	0	0/53	43	3/7	3/52	2.7
S. dimidiatum	S. 307	Sudan	Wild	-	-	0	0/52	-	0.1
***Pennisetum* spp.**									
P. glaucum ssp. americanum	IP 3122	India	Cultivated	0	0/47	0	0/76	3/47	0.9
" "	IP 4021	India	Cultivated	-	-	0	0/61	-	1.8
" "	IP 13115	Niger, Zongo	Cultivated	0	0/68	0	0/58	5/68	1.5
" "	IP 8638	Sudan	Cultivated	0	0/92	0	0/61	8/92	1
" "	IP 16793	Zimbabwe	Cultivated	0	0/65	0	0/60	0/65	1.8
" "	IP 11902	Sierra Leone (forage type)	Cultivated	0	0/53	0	0/16	0/53	0.1
" "	P 1437 I	India. (Shebra)	Cultivated	0	0/73	1.3	1/77	3/61	-
P. glaucum ssp. Monodii	IPW 7	Niger (=*violaceum)*	Wild	0	0/3	0	0/7	1/3	1.8
P. polystachyon	IPW 401	Tanzania	Wild	0	0/27	0	0/46	1/27	-
P. pedicellatum	IPW 306	Cameroon	Wild	1	1/71	0	0/54	1/71	0
P. schweinfurthii	IPW 414	Sudan	Wild	18	10/55	88	50/57	0/53	1.3

(%): IPCV-H incidence; (ni/N): number of plants positive in ELISA on the number of plants tested (valid for virus and *P. graminis*); (-): not tested;
(DI): degree of infection by sporosori of *P. graminis* isolate $I_{1\text{-}1}$; none (0), slight (1), moderate (2), or severe (3), (Legrève, 1999).

Discussion

The results showed that *P. graminis* could infect many pernicious weeds commonly found in farmers' fields in India. Therefore clean cultivation appears to be important for the management of the disease. *C. dactylon* is a weed which can vegetatively reproduce by rhizomes. All the rhizomes arising from an infected plant contain the virus. Such rhizomes can act as reservoirs for the virus. Since they tend to spread in the soil, the new roots arising from them are likely to provide viral inoculum to non–viruliferous *P. graminis* and thus create new foci of the disease. This aspect will be dealt in detail in another chapter (Chapter 4).

In weeds, in most cases, when the virus was detected by DAS-ELISA, it was also detected by DNA hybridisation with a broad specific probe. However *C. rotundus* samples that were tested negative for virus presence by DAS-ELISA, reacted positively with the cDNA probe. These results indicated that *C. rotundus* contained a very low virus concentration not detectable by DAS-ELISA. *C. rotundus* is also the species which showed under natural conditions, the most severe colonisation of roots by *P. graminis* resting spores. What is the role of *C. rotundus* in peanut clump disease life cycle? Does this weed contribute to the perpetuation of viruliferous *P. graminis* or on the contrary does it lead to reduction of virus inoculum by being a poor host for virus replication? *C. rotundus* mostly reproduces vegetatively by tubers. It is a very common weed in the tropics and is among the first weeds to emerge following onset of monsoon rains. High infestation with this weed, however, could not be correlated with high disease incidence in a naturally infested field on the ICRISAT farm.

Among dicotyledonous crops infected by IPCV-H, pigeonpea plants were severely stunted compared to healthy plants and showed mosaic symptoms on young leaves. This is the first report showing that IPCV can cause disease in pigeonpea. *P. graminis* was not detected in its roots. As it has been reported for groundnut, *P. graminis* presumably transmits IPCV to pigeonpea without completing its life cycle. Seed transmission of IPCV could not be demonstrated in pigeonpea and virus infection was shown to cause severe yield loss in this crop (Delfosse *et al.*, unpublished; Chapter 5).

Although IPCV-H was detected in several dicotyledonous plants including some weeds, these hosts were not found to be colonised by sporosori of *P.*

graminis. Sporosori were only detected once in groundnut (Delfosse *et al.*, 1996). They were few in number so they probably contributed little to the increase in the incidence of IPCV-H. Additionally roots of naturally infected groundnut plants failed to induce incidence when incorporated into sterile sandy soils. The work of Legrève (1999), conducted under controlled environmental conditions, confirmed that only a trace of infection by a *P. graminis* isolated from IPCV-H infested soil, occurred on such dicots as groundnut and sugar beet. Therefore dicotyledonous crops are expected to be fortuitous hosts for the fungal vector. Such crops could be beneficially rotated with groundnut to reduce the vector inoculum in the soil. However it is preferable not to rotate groundnut with a legume to avoid favourable conditions for other pests. Mustard is a very interesting alternative. A mustard crop grown in the postrainy season in Hyderabad (Delfosse et al., unpublished) was also infected by the virus at a low incidence (7 plants infected of 3930 tested). Ratna *et al.* (1991) reported infection of *P. graminis* on *Brassica nigra* [L.] Koch and *B. oleracea* L. grown in soil infested with the Bapatla isolate of IPCV (sea shore area of Andhra Pradesh) but the infection was limited to presence of very few sporosori. Recent experiments under laboratory conditions showed that mustard (*B. juncea*) is resistant to *P. graminis* infection (Legrève, 1999). In infested fields, mustard, an important cash crop, could beneficially replace wheat and barley as a post-rainy season crop in the cereal-groundnut rotation practised in Rajasthan to reduce peanut clump disease. Higher income provided from this crop compared to wheat and barley should also bear incentive for farmers to grow mustard.

Sunflower was not infected by IPCV-H under field conditions. However it can be infected by sap inoculation of the virus. The infection caused chlorotic blotches on the inoculated leaves. The viral infection was not systemic in sunflower, based on the absence of symptoms as well as analysis by ELISA and ISEM. Therefore sunflower appears to be resistant to virus translocation. It is also possible that *P. graminis* may not infect sunflower under field conditions. Indeed, under lab conditions, sunflower was found to be resistant to *P. graminis* infection (Legrève *et al.*, 1999). Sunflower is also an interesting alternative to replace cereals in the groundnut rotation. However, due to the bird damage following seed setting, farmers are not keen on growing sunflower in areas such as Rajasthan.

In Niger, a similar study (AS Reddy and F Waliyar, personal communication) was carried out on the susceptibility of various crops to *peanut clump virus* (PCV), the virus reported from Africa [Thouvenel *et al.*, (1988)]). In addition to the crops known to be infected by IPCV

(maize, sorghum, pearl millet, pigeonpea, and cowpea), PCV was found in 8/12 plants of Bambara groundnut (*Vigna subterranea* [L.] Verdc.), 3/12 *Stylosanthes fruticosa* (Retz.) Alston, and 4/12 sesame (*Sesamum orientale* L.). However none of 12 sunflower plants were infected by the virus. The virus was detected in stunted and healthy looking plants with a similar incidence. Growth variability is a common problem in Niger and stunting may be the result of various causes such as nematodes, nutrient imbalance or peanut clump disease (Subrahmanyam *et al.*, 1993). These results confirmed the wide host range of PCV. However, neither PCV nor IPCV could be detected in leaves of sunflower grown in naturally infested fields.

A much wider host range was reported by Ratna *et al.* (1991) for *P. graminis* involved in IPCV-H transmission. In this case infested soil or roots of *Sorghum sudanense* stored at 37°C for at least two months were used as a source of inoculum (AS Ratna, personal communication). High temperature during storage of inoculum are known to be conducive for breaking the dormancy of resting spores (Legrève *et al.*, 1999) and it is possible that the storage conditions facilitated infection of several dicotyledonous hosts.

IPCV-H caused yield loss in maize. The crop showed good systemic virus infection and transmission of the virus through seed. The absence of clear symptoms in maize and its ability to support *P. graminis* multiplication well (Legrève, 1999, Delfosse *et al.*, 1996), suggests that growing maize may favour the build up of viruliferous inoculum and increase disease incidence.

Sorghum and pearl millet have been identified as favorable hosts for *P. graminis* multiplication (Ratna *et al.*, 1991, Legrève, 1999). These cereals are often rotated or inter-cropped with groundnut in semi-arid areas of the Indian sub-continent and in West Africa. Along with maize, they are expected to contribute to the build up of *P. graminis* inoculum potential in the soil (Legrève, 1999) and subsequently to high disease incidence in the ensuing groundnut crops. Moreover IPCV seed transmission could be demonstrated in maize, in the millet cultivar WCC 75 and in two other minor millets (Reddy *et al.* 1998). Therefore these cereals may contribute to virus spread over large distances. For these reasons, it would be very useful to identify sorghum and pearl millet genotypes which can resist *P. graminis* infection or at least do not favor fungus multiplication. Additionally sorghum and pearl millet which are immune to virus infection may render *P. graminis* non-viruliferous which will avoid the risk of inoculum build up as well as transmission through seed. It was very interesting to note that only two *Pennisetum* accessions showed systemic

infection by the virus, the wild taxa - *P. schweinfurthii* - and only one cultivar (Shebra). Although they showed variability in the intensity of root colonisation by the vector, all *Sorghum* spp. and *Pennisetum* spp. accessions were found to host *P. graminis* isolated from the field where we conducted this experiment (Legrève, 1999). Therefore the low virus incidence in aerial parts of these hosts could be attributed to restricted virus replication or movement. Nevertheless some sorghum accessions showed high virus incidence in leaves and the accessions IS 18519 and S 162 exhibited yellowing of the lamina under field conditions. Susceptible *Sorghum* accessions are suspected to contribute to an increase in viruliferous *P. graminis* populations.

Among the accessions tested in Belgium under controlled environmental conditions, *S. dimidiatum* and *P. pedicellatum* showed poor colonization by *P. graminis* resting spores (Legrève, 1999). The absence of virus in *S. dimidiatum* leaves in the 1998 experiment and low virus incidence in *P. pedicellatum* confirmed that these accession are probably poor hosts for the vector.

The study of the host range of IPCV and its vector *P. graminis* have shown that monocotyledonous hosts have the potential to act as carry-over hosts for peanut clump disease whereas dicotyledonous hosts are likely to contribute little to perpetuation of viruliferous *P. graminis*. These hosts, monocots versus dicots, need to be tested under field conditions to determine their effect on peanut clump disease perpetuation and incidence. This aspect will be dealt in another chapter on the management of the disease (Chapter 5).

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3-2. *Indian Peanut Clump Virus* (IPCV) Infection on Wheat and Barley: Symptoms, Yield Loss, and Transmission through Seed[6]

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Opening comments

Wheat and barley are two major post-rainy season crops rotated with groundnut in areas where peanut clump disease occurs with severe incidence. This rotation is practised in Punjab and Rajasthan in India, and Pakistan (Chapter 2). In temperate countries wheat and barley are known to host *P. graminis* which transmit several Furoviruses and Bymoviruses to the two crops (Jones, 1993). PCV and IPCV could be transmitted to wheat and barley under controlled environmental conditions (Thouvenel and Fauquet, 1981, Ratna *et al.*, 1991). However, the economic importance of peanut clump disease to these crops has so far not been investigated. Considering the high incidence observed in groundnut crops in areas were it is rotated with wheat or barley, these cereals were suspected to participate to the disease epidemiology. This study was therefore undertaken to investigate symptoms, crop losses and seed transmission caused by IPCV infection of the two cereals under field conditions.

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Indian peanut clump virus (IPCV) infection on wheat and barley: symptoms, yield loss and transmission through seed

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Wheat and barley crops were shown to be susceptible to Indian peanut clump virus (IPCV) under field conditions. In wheat, the Hyderabad isolate of IPCV (IPCV-H) induced symptoms resembling the rosette caused by soil-borne wheat mosaic virus, and these were apparent only three weeks after emergence. Early-infected plants were severely stunted and dark green, with chlorotic streaks on the youngest leaves, which turned necrotic as the plants aged; most of these plants died. Late-infected plants were also stunted and were conspicuous in the field because of their dark green appearance as a result of delayed maturity. The virus was detected by ELISA and nucleic acid hybridization in all plants with symptoms. These plants usually produced fewer tillers than healthy ones. Spikes were malformed, often did not emerge from the flag leaf, and they contained few, shrivelled seeds. Grain yield was decreased, on average, by 58%. In barley, IPCV-H caused severe stunting and general leaf chlorosis. As the plants aged, the leaves became necrotic and the few infected plants that reached maturity produced small spikes. IPCV-H antigens were detected by ELISA in every wheat seed from infected plants and the virus was transmitted through wheat seed at a frequency of 0·5–1·3%. Storage at 4°C for more than a year did not affect seed transmission frequency. The virus was detected in leaves and roots of seed-transmitted plants. Seed transmission was not detected in barley. The Durgapura isolate (IPCV-D) was detected in wheat crops (cv. RR-21) at 3 different locations in Rajasthan State, India. Infected plants showed reduced growth without any overt symptoms.

Keywords: Arachis hypogaea, barley, Indian peanut clump virus, peanut, seed transmission, wheat

Introduction

Peanut clump disease is caused by viruses of the genus *Pecluvirus* (Torrance & Mayo, 1997). The disease occurs naturally in peanut or groundnut (*Arachis hypogaea*) in West Africa (Thouvenel *et al.*, 1988) and the Indian subcontinent (Reddy *et al.*, 1988; Mathur & Sobti, 1993; Delfosse *et al.*, 1995a). Annual losses caused by clump disease in peanut globally have been estimated to exceed US$ 38 million (Reddy *et al.*, 1999). The virus isolates that cause clump disease in West Africa and the Indian subcontinent are referred to as peanut clump virus (PCV) and Indian peanut clump virus (IPCV), respectively. IPCV isolates are named after the place where they were first reported in India, and fall into three distinct serotypes, IPCV-D (Durgapura isolate, Rajasthan), IPCV-H (Hyderabad isolate, Andhra Pradesh) and IPCV-L (Ludhiana isolate, Punjab) (Reddy *et al.*, 1983; Nolt *et al.*, 1988). All the currently known members of pecluviruses are seed- and soil-transmitted (Reddy *et al.*, 1988; Konaté & Barro, 1993) and have bipartite, positive-sense RNA genomes (Reddy *et al.*, in press). IPCV was shown to be transmitted by the fungus *Polymyxa* sp. (Ratna *et al.*, 1991) and PCV is suspected to have the same vector. IPCV and PCV have extremely wide host ranges which include many monocotyledonous plants (Ratna *et al.*, 1991; Delfosse *et al.*, 1996). In most production systems, peanut is either grown in rotation or as a mixed crop with cereals such as maize, millet or sorghum. Clump disease occurs at a fairly high incidence in Rajasthan, where ≈ 250 000 ha of peanut are rotated with irrigated wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) crops, grown during the post-rainy season. However, the economic importance of IPCV to these crops has so far not been investigated.

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Recently, IPCV was shown to be seed transmitted in three millets (Reddy *et al.*, 1998). This study was therefore undertaken to investigate symptoms, crop losses and seed transmission in wheat and barley caused by IPCV infection.

Materials and methods

Cultural practices in wheat and barley cultivation

The study was conducted during three consecutive post-rainy seasons (1994–95, 1995–96 and 1996–97) on the ICRISAT-Patancheru farm (near Hyderabad) in an IPCV-H-infested field. The soil was a sandy alfisol with a pH (H_2O) close to neutral. Certified seed of wheat (cv. RR-21) and barley (cv. RD 103) was treated with thiram at 3 g kg^{-1} seed and sown (≈ 100 kg ha^{-1}) in 1 m broad beds with four rows per bed during the last week of November or first week of December. Di-ammonium phosphate (80 kg ha^{-1}) was applied at the time of sowing. Urea (70 kg ha^{-1}) was applied as a top dressing at 2 weeks and 2 months after emergence. The crop received two 30-mm irrigations each week. Barley was only grown in the 1994–95 season.

Sample collection and analysis of yield components

During the 1994–95 season, one week after emergence, leaf samples were collected from wheat plants with and without symptoms, grown in the areas of the field where clump disease had occurred in peanut crops during the rainy season. One month after sowing, and subsequently at various stages of the crop growth, plants exhibiting severe stunting and dark green leaves with chlorotic stripes, as well as healthy-looking plants, were collected from 4 IPCV-H-infested patches, and tested for the presence of the virus by enzyme-linked immunosorbent assay (ELISA) and by nucleic acid hybridization assay with a nonradioactive probe. A limited number of plants were also assayed by immunosorbent electron microscopy (ISEM) and bioassay. Roots of 35 plants that tested positive in ELISA were examined for the presence of *Polymyxa* sp. During the 1994–95 season, only a few infected plants were recorded in each

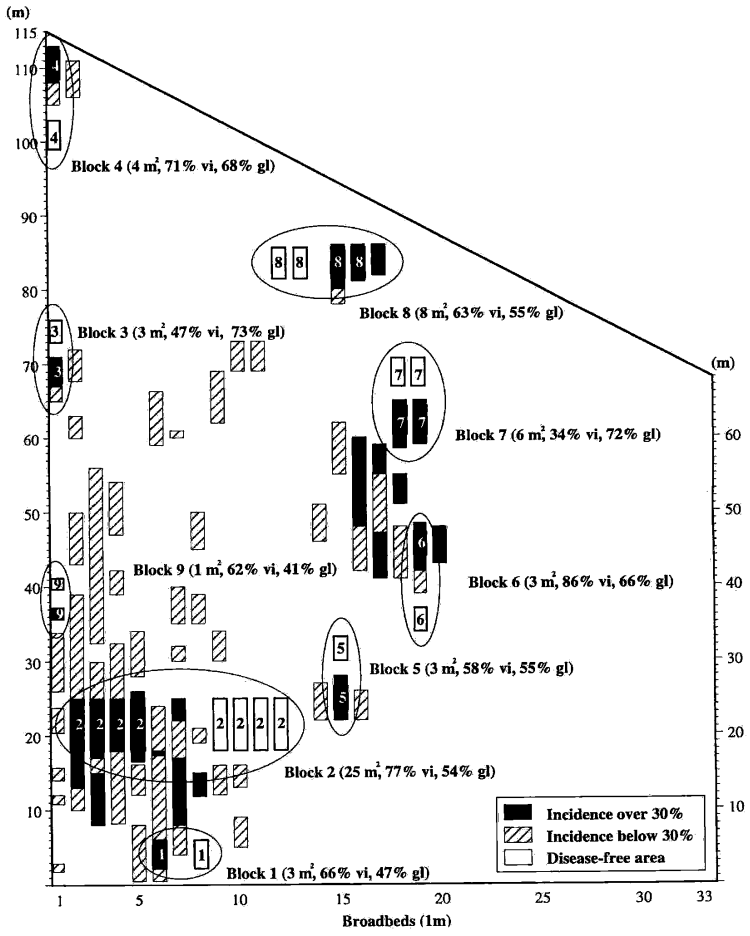

Figure 1 Clump disease distribution in wheat crop grown during the 1995–96 post-rainy season in a field naturally infested with the Hyderabad isolate of Indian peanut clump virus (IPCV-H). The entire field was under wheat cultivation and the virus incidence based on visual observation and ELISA is shown. For the assessment of yield loss caused by virus infection under field conditions, 9 replication blocks were selected, each containing an infested and a healthy plot. In each block, an equal area of wheat was harvested from both infested and healthy plots. For each block, the area that was harvested (m^2), the virus incidence (% vi) in the infested plot, and the grain yield loss (% gl) (infected vs. healthy) are indicated in brackets.

infested patch, and it was therefore difficult to study the effect of IPCV-H infection on yield on an area basis. Therefore, spikes were collected from barley and wheat plants that tested positive in ELISA on the flag leaf, and from healthy plants, to assess the number and weight of kernels per spike and the weight of 1000 kernels.

High disease incidence during the 1995–96 post-rainy season allowed the yield loss caused by IPCV-H infection to be studied on an area basis. The experiment was conducted in a randomized block design with 9 replicates for each treatment (infested and healthy) (Fig. 1). The plot size was identical within a block but varied from 1 to 25 m^2 for each block (according to the size of the infested patch), with an average size of 6·2 m^2. The infested patches chosen for the analysis showed a uniform distribution of infected plants with over 30% incidence. IPCV-H incidence was measured by visual symptoms and ELISA tests. For measurements and sample collection in infested plots, care was taken to leave a border of infected wheat plants between infested and healthy areas. The healthy plot chosen for yield comparison was selected in a disease-free area located as close as possible to the corresponding infested plot. After measuring plant height (10 plants per plot) and plant population per m^2, wheat was harvested manually from the whole plot. Various yield components were measured for each plot (Table 1). Small, shrivelled, dark-coloured seeds were considered as immature and were separated from mature seeds by a sieve with 3-mm holes to facilitate the determination of immature seed weight. During the 1996–97 post-rainy season, only the number and weight of kernels per spike and the weight of 1000 kernels were studied for spikes collected from infected and healthy plants.

Surveys of wheat crops in Rajasthan

Surveys for IPCV incidence in wheat crops were undertaken in the Boraj, Durgapura and Rampura regions in the Jaipur district of Rajasthan. During the 1994–95 and 1995–96 post-rainy seasons, the wheat cultivars in the fields surveyed were RAJ 3077 and RAJ 1482, and during the 1996–97 post-rainy season the cultivar was RR-21. Samples collected during the surveys were tested by ELISA for the presence of IPCV-D. To ascertain if IPCV-D infection occurred in plants that tested negative by ELISA, the plants were transferred to sterile soil in pots and maintained at a temperature of 25–30°C, which is known to favour IPCV multiplication.

ELISA and ISEM

The samples were assayed by the penicillinase-based (Sudarshana & Reddy, 1989) double-antibody sandwich ELISA procedure, using IPCV-H or IPCV-D antisera, similar to that described by Reddy *et al.* (1998). Results were recorded after 30 min to 1 h of substrate reaction time. Readings were considered positive if the difference in the absorbance value at 620 nm between infected and control sample exceeded 1 OD unit. For immunosorbent electron microscopy (ISEM) IPCV particles were trapped and decorated following the procedure described by Nolt *et al.* (1988).

Nucleic acid hybridization assay

Cloned cDNA of IPCV-H RNA-1, corresponding to the sequence from position 5,099–5,841, and labelled with digoxigenin, was used as a probe to detect IPCV-H in

Table 1 Effect of Indian peanut clump virus (Hyderabad isolate, IPCV-H) on yield components of the wheat cultivar RR-21 during the 1995–96 post-rainy season at ICRISAT-Patancheru

	Healthy[a]			Infected				
Parameter	Mean[b]	s.d.	Range	Mean[b]	s.d.	Range	% Loss	*F* *P*-value
Plant height[c] (cm)	99	5	91–105	36	10	27–49	64	<0·001
Population × 1000 ha^{-1}	821	335	435–1540	657	305	263–1080	20	0·021
Spikes per m^2	368	76	236–526	269	93	165–411	27	0·010
Total biomass (kg/ha)	8045	1813	4919–10610	4685	1886	2660–8506	42	<0·001
Straw yield[d] (kg/ha)	3662	924	2042–4802	2509	1111	1327–5012	31	0·014
Grain yield (kg/ha)	3121	722	2107–4067	1305	576	644–2360	58	<0·001
Harvest index	0·39	0·04	0·33–0·44	0·28	0·04	0·19–0·3	–	<0·001
Test weight (g L^{-1})	835	20	795–864	780	27	736–821	7	<0·001
1000-kernel weight (g)	33	3	28–38	28	3	23–33	14	<0·001
Immature grains (%)	7·0	4·9	2·5–18·6	21·9	7·8	12–35	–	<0·001
IPCV-H incidence				63	15	34–86		

[a]These plots showed apparently healthy plants and were located in areas known to be disease free.
[b]Means and standard deviations from 9 replicated plots varying from 1 to 25 m^2 with an average of 6·2 m^2.
[c]Mean height of 10 plants randomly measured in each plot.
[d]The straw weight did not include the weight of husks and rachis.

Figure 2 Symptoms caused by IPCV-H infection on wheat, cv. RR-21 (a, b, c, d) and barley, cv. RD-103 (e, f). (a) An early-infected wheat plant showing severe stunting compared to healthy plants. (b) An early-infected wheat plant showing rosette, chlorotic streaks and dark green leaves. (c) Chlorotic streaks on the flag leaf of a late-infected wheat plant. (d) The startling yield difference between (left) healthy and (right) infected plants. (e) Chlorotic leaves and early senescence in an early-infected barley plant. (f) Chlorotic streaks on the flag leaf of a late-infected barley plant.

leaves of wheat plants as previously described (Wesley *et al.*, 1996).

Biological assays

Leaf extracts from wheat plants infected with IPCV-H were mechanically inoculated onto carborundum-dusted leaves of *Phaseolus vulgaris* (cv. Topcrop), a good diagnostic host for IPCV (Reddy *et al.*, 1998). In experiments to assess whether inoculation of IPCV-H under laboratory conditions could reproduce the symptoms observed under field conditions on wheat, inoculum prepared from *P. vulgaris*, containing $1\,g\,L^{-1}$ diatomaceous earth (grade II, Sigma D-5509, Sigma Chemicals, St. Louis, MO, USA), was sprayed with an air-brush onto roots of one-week-old wheat seedlings. These were then transplanted into pots containing sterile sand and maintained in a glass-house at 25–30°C. Fifteen days after inoculation the plants were scored for symptoms and assayed by ELISA.

Determination of seed transmission frequency and viability of wheat and barley seed from infected plants

Kernels from plants that tested positive and from those that tested negative in ELISA were stored at 4°C until

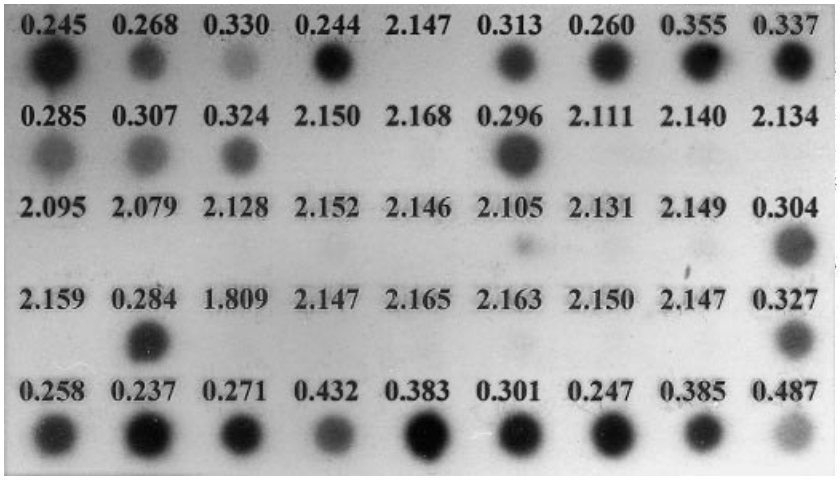

Figure 3 Detection of Indian peanut clump virus, Hyderabad isolate (IPCV-H), in extracts of wheat leaves. Samples of 50 μL of total RNA from wheat leaves spotted on the membrane and hybridized to digoxigenin-labeled probe. Samples of the same plants were tested for the presence of IPCV-H coat protein by the penicillinase-based system of enzyme-linked immunosorbent assay (ELISA), and the absorbance values (A620) are shown: high absorbance values indicate that no conjugate was bound whereas low values mean that the conjugate was bound, i.e. IPCV-H was present.

used. Kernels from each individual spike were pooled and soaked overnight in sterile distilled water to facilitate grinding. Presence of viral antigen in seeds was assessed using the extract from a single seed for each well of the ELISA plates. To eliminate any externally contaminating virus, seeds from infected plants were repeatedly soaked in 10 g L^{-1} Na_3PO_4 solution and rinsed several times with distilled water. Kernels from a certified seed lot were also included in ELISA tests.

To determine the frequency of seed transmission of IPCV-H in wheat and barley and the effect of virus infection on germination, seeds from individual infected plants and from healthy plants were germinated on moist paper towel in Petri dishes (a separate dish for the seeds from each individual spike). Germination percentage was recorded after 10 days. Seedlings were transplanted into pots containing sterile sand and maintained in a growth chamber at 25–30°C, using modified Hoagland nutrient solution. Two-week-old seedlings were processed by ELISA, initially in groups of five for each well of the ELISA plate. Individual plants were tested from groups that gave a positive reaction. Randomly chosen ELISA-positive wheat seedlings were also tested by infectivity assays.

Detection of *Polymyxa* sp.

Polymyxa infection in wheat and barley roots was assessed under natural conditions by examining root samples for the presence of cystosori by light microscopy (Maraite *et al.*, 1988).

Data analysis

The effect of IPCV-H infection on yield components was evaluated by analysis of variance for the data collected during the 1995–96 season and by the Mann–Whitney rank sum test for the yield data recorded during 1994–95 and 1996–97. The effect of IPCV-H infection on seed viability was analysed by a comparison of the proportion in independent samples for the binomial distribution (Z statistic) (Snedecor & Cochran, 1980).

Results

Symptomatology

In wheat, symptoms were first noticed 2–3 weeks after emergence. All early-infected plants were stunted and rosetted, with dark green leaves (Fig. 2a,b). These symptoms resembled those caused by the soil-borne wheat mosaic virus (SBWMV) (Wiese, 1977; Brakke & Langenberg, 1988). Chlorotic streaks (Fig. 2c) were noticed on newly emerged leaves, which subsequently became necrotic. The root system was poorly developed in early-infected plants, and most of these plants died. Plants infected later remained stunted, with dark green old leaves and young leaves showing chlorotic streaks. They produced malformed spikes, sometimes enclosed in a curled flag leaf, and fewer tillers than healthy ones. The spikes were not properly filled, and kernels from infected plants were shrivelled and dark brown. IPCV-H-infected barley plants were stunted and bushy, with chlorotic or necrotic leaves (Fig. 2e,f). Most of the

Table 2 Effect of Indian peanut clump virus (Hyderabad isolate, IPCV-H) on 3 yield components of wheat (cv. RR-21) and barley (cv. RD 103) during the 1994–95 and 1996–97 post-rainy seasons at ICRISAT-Patancheru

Crop and season[a]	Treatment[b]	Number of kernels per spike		Kernel weight per spike (g)		1000-kernel weight (g)	
		Mean[c]	Range	Mean[c]	Range	Mean[c]	Range
wheat							
1994–95	Healthy	36	33–40	1·29	1·25–1·59	36	35–42
	Infected	12	1–40	0·28	0·01–1·66	21	2–51
1996–97	Healthy	35	13–56	1·22	0·30–2·20	34	11–52
	Infected	18	1–46	0·33	0·01–1·60	17	1–47
barley							
1994–95	Healthy	36	21–46	1·64	0·70–2·20	45	21–60
	Infected	18	5–46	0·69	0·10–2·10	38	5–52

[a]For both apparently healthy ($n=289$) and infected ($n=351$) wheat spikes for the 1994–95 and 1996–97 seasons, respectively, and 50 barley spikes were individually analysed.

[b]When tested by ELISA, all infected plants contained the viral antigen in the flag leaf.

[c]Means within a column for one season differ significantly (rank sum test, T significant at $P<0·001$).

infected plants died. Those that reached maturity produced poorly developed spikes.

The virus was readily detected by ELISA in roots and leaves of wheat seedlings (4 out of 90 tested) collected 2 weeks after emergence, although these plants did not show any overt symptoms. Subsequently, and until harvest, all 191 plants with symptoms tested positive, whereas 319 apparently healthy plants tested negative by ELISA. In ISEM, seven naturally infected plants were tested: typical IPCV particles from the five wheat plants and two barley plants could be trapped and were fully decorated with IPCV-H antiserum. No virus particles could be trapped from two apparently healthy plants. All the ELISA-positive plants also contained IPCV-H RNA, as tested by nucleic acid hybridization tests (Fig. 3).

P. vulgaris inoculated with leaf extracts from naturally infected wheat plants developed typical symptoms. Wheat plants, root-inoculated (with the help of an airbrush) with virus isolated from peanut and multiplied on *P. vulgaris*, showed dark green leaves and stunting, but symptoms were less severe than those observed under field conditions. All plants tested positive by ELISA.

Yield loss and seed quality

The yield components studied during the 1995–96 season are presented in Table 1. IPCV-H infection caused severe losses of wheat yield. Plant height was reduced by more than half compared with healthy plants. Infected plants produced 42% less total biomass, including 31% loss of straw and 58% loss of grain (Fig. 2d). The plant population was affected because of the death of early-infected plants. Grain was of poor quality compared with that of healthy plants and contained a larger proportion of immature kernels. The harvest index was lower for infested patches than for healthy ones. During the 1994–95 and 1996–97 seasons, IPCV-H infection severely reduced the number and weight of kernels per spike as well as the weight of 1000 kernels (Table 2). IPCV-H significantly reduced the germination of wheat and barley seed (Table 3) although wide variability from plant to plant was observed in the percentage of germination.

Table 3 Effect of IPCV-H infection on wheat (cv. RR-21) and barley (cv. RD 103) seed viability

Crop	Season	Number of seeds germinated/ number of seeds tested			Mean germination (% (range))		
		Date of test	Healthy plants[a]	Infected plants	Healthy plants[a]	Infected plants	P
Wheat[b]	1994–95	25/11/1996	598/600	538/600	99·7 (97–100)	89·7 (50–100)	<0·001
Wheat	1995–96	16/05/1997	582/600	398/600	97·0 (80–100)	66·3 (0–100)	<0·001
Barley[c]	1994–95	09/05/1997	58/100	37/86	58·0 (20–90)	43·0 (32–71)	0·041

[a]All apparently healthy plants were sampled in disease free areas and the flag leaf of all infected plants tested positive by ELISA.

[b]Seeds collected from 29 infected and 20 healthy spikes for the season 1994–95 and from 20 healthy and 35 infected spikes for the season 1995–96.

[c]Seeds collected from 5 infected and 3 healthy spikes.

Table 4 Frequency of seed transmission of IPCV-H in wheat (cv. RR-21)

Season	Date of ELISA test	Number of seeds germinated/number of seeds tested	Germination (%)	Number of seedlings tested positive	Seed transmission (%)
1994–95	11/10/1995	854/1017	84	4	0·47
	08/11/1996	787/934	84	10	1·27
	25/11/1996	1172/1247	94	4	0·34
1995–96	16/05/1997	1240/2181[a]	57	13	1·05
1996–97	06/05/1997	737/2518[a]	29	8	1·08
Total		4790/7897	61	39	0·81

[a]Kernels derived from 250 spikes.

Frequency of IPCV-H seed transmission in wheat and barley

All the seeds collected from infected wheat and barley plants, either treated with Na_3PO_4 or not, contained the viral antigen, as tested by ELISA. The frequency of seed transmission in wheat is presented in Table 4 for seed lots collected during the three consecutive post-rainy seasons. Although infection by IPCV-H resulted in poor germination, seed transmission was observed in about 1% of the seedlings. Wheat seeds stored for more than a year at 4°C still transmitted the virus. Seedlings infected through seed contained the virus in both leaves and roots. Symptoms were somewhat similar to those on inoculated plants maintained under glasshouse conditions. The seeds that transmitted IPCV-H originated from different plants, thus excluding the possibility of cross-infection during the growth of the seedlings in Petri dishes or pots. Virus presence in ELISA positive seedlings was confirmed by infectivity assays. Out of 86 barley seeds collected from infected plants, 37 germinated. None of the seedlings was found to be infected by the virus when tested by ELISA.

Surveys of wheat crops in Rajasthan

During the surveys conducted in 1994–95 and 1995–96, the virus could not be detected either in leaves, roots or seeds of cultivars Raj 3077 and Raj 1482 from a number of samples collected from three locations (558 plants tested). However, during 1996–97, when RR-21 was grown in IPCV-D-infested fields, the plants showed uniform stunting in known infested patches, without any overt symptoms. In each of the three locations, IPCV-D was detected in roots and leaves of a restricted number of RR-21 plants (3/228). Wheat plants testing negative at the time of sampling, and then maintained for a month in a glasshouse, also gave negative results by ELISA. IPCV-D could not be detected in more than 900 wheat seeds of cv. RR-21 collected from infested plots.

Polymyxa sp. detection

A few resting spores of *Polymyxa* sp. were observed in roots of a limited number of IPCV-H-infected wheat plants (3/35), but none could be detected in barley roots (0/10).

Discussion

When mechanically inoculated onto wheat, PCV caused systemic mosaic and stunting symptoms (Thouvenel & Fauquet, 1981). Using *Polymyxa*-infested soil or *Polymyxa*-infected roots as inoculum, IPCV could be transmitted to wheat under glasshouse conditions (Ratna *et al.*, 1991) but the authors did not mention any symptoms. Earlier studies (Delfosse *et al.*, 1995b,c) and the present study showed for the first time that infection by IPCV can cause diseases in wheat and barley crops under natural conditions. Symptoms on wheat are similar to those caused by SBWMV. However, there is no serological relationship between the two viruses (Reddy *et al.*, 1985) and their genome organization differs substantially (Wesley *et al.*, 1994; Miller *et al.*, 1996; Naidu *et al.*, 1996).

The yield reduction in wheat infected with IPCV-H was very severe and consistent over the 3-year period. Grain yield loss caused by IPCV-H infection was as high as 58% (equal to a yield reduction of 1800 kg ha^{-1}). This is similar to the wheat loss caused by severe infection by two other *Polymyxa*-transmitted viruses in North America: SBWMV in Florida, Kansas and Nebraska (Kucharek & Walker, 1974; Campbell *et al.*, 1975; Palmer & Brakke, 1975; Nykaza *et al.*, 1979) and wheat spindle streak mosaic virus (WSSMV) in New York and Georgia (Cunfer *et al.*, 1988; Miller *et al.*, 1992). It was difficult to assess the effects of SBWMV and WSSMV on yield in controlled experiments because these viruses are difficult to transmit mechanically and their vector, *P. graminis*, is mostly prevalent in low-lying, poorly drained areas of the fields where waterlogging can also affect the yield (Bays *et al.*, 1985; Miller *et al.*, 1991). As IPCV disease occurs mainly in well-drained, sandy soils or sandy loam soils (Delfosse *et al.*, 1997), this conflict was not encountered. However, care was taken to compare plants with and without symptoms within similar soil environments.

In Andhra Pradesh, IPCV-H infection in wheat and barley caused severe symptoms and yield loss under the prevailing climatic conditions. In Rajasthan, however,

IPCV-D could not be detected in currently grown wheat cultivars. It was found only at low incidence, in wheat cv. RR-21, which showed stunting in areas of the fields known to be infested with IPCV-D. This occurred in the same patterns as that of peanut clump disease in peanut during the previous rainy season. There are thus indications that some wheat cultivars may show some resistance to IPCV-D. Yield loss caused by IPCV-D in wheat and barley crops has yet to be investigated in Rajasthan. Temperature seems to be an important factor regulating disease incidence. Winters are cooler in Rajasthan than in Hyderabad. The normal minimum, mean and maximum air temperatures are 9, 16 and 24°C in Jaipur, Rajasthan, and 15, 21 and 28°C in Hyderabad, Andhra Pradesh, in December, and 8, 15 and 22°C, and 15, 22 and 29°C, respectively, in January.

At the same time, IPCV-H incidence in the wheat crop grown in 1994–95 in Hyderabad was lower than in the crop grown during the 1995–96 and 1996–97 seasons. In 1994–95, infected plants were scattered among healthy ones, and occurred only in the areas where high disease incidence was recorded in peanut crops raised in previous rainy seasons. However, for the successive seasons, the infection was uniformly distributed and was present in areas of the field where the disease has never been observed on peanut crops. It is likely that temperature affected the infection by *Polymyxa* sp., and, consequently, the virus transmission. The 1994–95 season in Hyderabad was characterized by lower temperatures than the other two seasons. The minimum, mean and maximum air temperatures for December 1994 were 7, 19 and 29°C while for December 1995 and 1996 they were 10, 21 and 30°C and 10, 20 and 29°C, respectively. Legrève *et al.* (1998) observed that the *Polymyxa* isolated from the same experimental field of the ICRISAT-Patancheru farm had a very narrow temperature range, with an optimum between 27°C and 30°C, delayed development at 23–26°C, and almost no development at 19–22°C. The low temperatures prevalent during the 1994–95 post-rainy season at Hyderabad may not have been conducive for *Polymyxa* activation, and therefore virus transmission to wheat was low. If the *Polymyxa* occurring in Rajasthan has a similar temperature requirement to that of the Hyderabad isolate, temperatures prevalent in Rajasthan would not be conducive for fungus infection and virus transmission to winter wheat. Wheat is also grown below the tropic of Cancer in Madhya Pradesh, Maharashtra, Gujarat and Karnataka. Winters in these areas are not as cold as in Rajasthan, having temperatures similar to Andhra Pradesh. Therefore if IPCV is present in these areas it is likely that it can infect wheat crops.

Even though wheat and barley showed high incidence of IPCV-H under natural conditions they do not appear to be as good hosts of *Polymyxa* sp. as sorghum and pearl millet (Legrève *et al.*, 1996). The fungus was detected only as a trace of infection on wheat plants infected under natural conditions. As in the case of peanut, wheat and barley may act as fortuitous hosts, leading *Polymyxa* to enter the resting spore stage preferentially rather than favouring fungus multiplication through secondary zoospores. These results contradict those of Ratna *et al.* (1991) and Nolt *et al.* (1988), who readily detected *Polymyxa* sp. in wheat roots from plants grown on IPCV-H-infested soil. It is presumed that the continuous use of peanut, a fortuitous host for *Polymyxa*, as a sole crop since 1987, induced a reduction in *Polymyxa* inoculum potential in the experimental field, thereby probably reducing the chance of *Polymyxa* infection to wheat and barley from resting spores.

IPCV infection affected the germination and induced variability in the germination percentage in wheat and barley. Nevertheless, wheat seedlings infected through seed grew well under glasshouse conditions and the frequency of seed transmission in wheat was close to 1%. As wheat is sown at a rate of $\approx 100\,kg\,ha^{-1}$, corresponding to $2{\cdot}5 \times 10^6$ seeds/ha, there is a high risk of spreading the virus if seed is collected from infested fields. The virus was detected in roots of seedlings infected through seed. Therefore it is most likely that isolates of *Polymyxa* that infect and multiply on wheat can acquire the virus from plants infected through seedborne inoculum. Preliminary experiments have shown that nonviruliferous *Polymyxa* could acquire the virus from wheat and maize plants infected by IPCV-H through seed-borne inoculum, and transmit the virus to plants grown in an automatic immersion tank system (Delfosse & Legrève, unpublished). IPCV has a wide temperature range and mechanical inoculation onto wheat results in infection at temperatures between 15 and 30°C (Reddy *et al.*, 1988). Thus, there is a potential risk to wheat if IPCV is established in temperate areas where *Polymyxa* sp. is adapted to wheat and to low temperatures.

Few rod-shaped, *Polymyxa*-transmitted viruses were reported to be transmitted through seed. These include: *Nicotiana veluntina* mosaic, a proposed furovirus (Randles, 1978); potato mop top virus, which is vegetatively transmitted through seed tubers; and PCV and IPCV (Mink, 1993). To our knowledge, seed transmission of SBWMV has been investigated for low temperature strains that are usually no longer detected in aerial parts of plants when the temperature rises in late spring and summer. The virus was reported not to be seed transmitted (Brakke, 1971). However, Wiese (1977) mentioned that SBWMV sometimes spreads more rapidly and over larger distances than can be explained by soil movement. Rubies-Autonell & Vallega (1991), reported the presence, in the region of Rome, Italy, of SBWMV particles in wheat leaves analysed by ISEM, well beyond the heading stage. They could even detect them in immature seed of cv. Valnova. More recently a Polish isolate of SBWMV was found to be seed-transmitted in rye, and seedborne, but not seed-transmitted, in wheat (Jezewska, 1995). Considering that IPCV is seed-transmitted in wheat, three millets (Reddy *et al.*, 1998), and maize (Delfosse *et al.*,

unpublished), and that high-temperature strains of SBWMV exist, it is suggested that the seed transmission of *Furovirus* be reinvestigated.

Polymyxa sp. is a ubiquitous fungus. Care should be taken in germplasm movement to avoid the spread of furoviruses and pecluviruses. The risk of IPCV acquisition by several temperate strains of *Polymyxa* sp. is currently being investigated.

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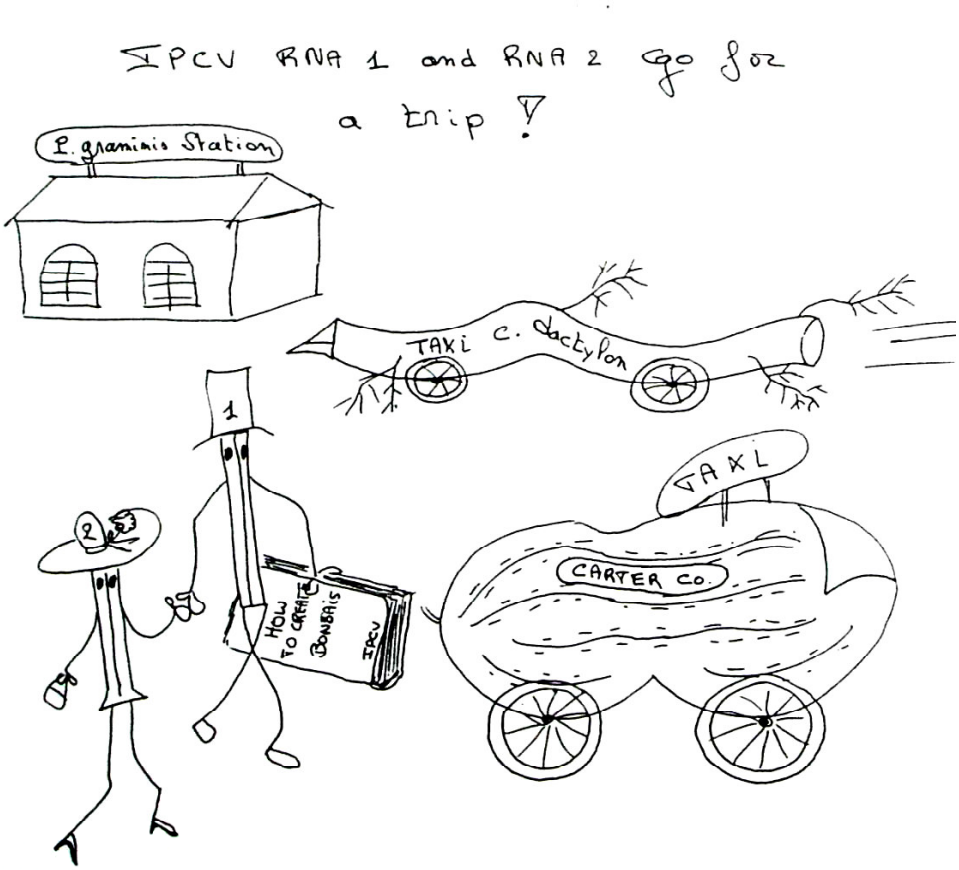

Pecluviruses have rod-shaped particles, 24 nm in diameter, with two predominant lengths of 249 nm and 184 nm. The long particle (the man with the book "How to create bonzaïs" in the hand) carries the RNA 1. The short particle (the female with a cute flower hat) carries the RNA 2. While groundnut seed (not a fast car indeed) carries the virus particles, it is doubtful that it contribute to the spread and establishment of the virus to new areas. Indeed groundnut is a poor host for the virus vector, *Polymyxa graminis*. On the contrary, rhizomes of *Cynodon dactylon* (a well profiled rocket car), hosting both the virus and its vector, contributes to the spread and establishment of peanut clump disease to new areas. Seed of cereals such as millets, maize and wheat are highly suspected to contribute to the disease spread.

3-3. The role of groundnut seed and *Cynodon dactylon* rhizomes in the establishment of peanut clump disease.[7]

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Abstract

Experiments were conducted on the ICRISAT farm at Patancheru, India, to assess if virus-containing groundnut seed and *Cynodon dactylon* rhizomes could serve as a source of primary inoculum to the vector, *P. graminis*, and contribute to the establishment of peanut clump disease. Areas apparently free of disease were either sown with groundnut seed or planted with *C. dactylon* during the 1995 rainy season. Material containing IPCV-Hyderabad isolate (IPCV-H) was used as a source of inoculum and virus-free material as a control.. Disease establishment was assessed by growing indicator crops of groundnut and pigeonpea during the following 1996 and 1997 rainy seasons. Plots treated with virus-free groundnut and *C. dactylon* rhizomes showed respectively incidence of 0.1 and 1.4% in 1996, and 0.0 and 1.1% in 1997. Where virus containing seed and rhizomes were introduced, virus incidence was respectively 3.2 and 9.1% in 1996, and 3.1 and 7.7% in 1997. IPCV-H-containing rhizomes of *C. dactylon* contributed to the establishment of the disease on the basis of significantly higher incidence recorded in the indicator crops. Low virus incidence in plots treated with virus-infected groundnut seeds, did not permit to conclude if groundnut seeds contributed to disease establishment.

[7] To be submitted for publication

Introduction

In the Indian sub-continent, peanut clump disease occurs naturally in groundnut or peanut (*Arachis hypogaea* L.) in light textured soils (Reddy *et al.*, 1988; Mathur & Sobti; 1993; Delfosse *et al.*, 1995). It is caused by the Indian peanut clump virus, a member of the genus *Pecluvirus* (Torrance & Mayo, 1997). IPCV has been shown to be transmitted by the soil-borne parasite, *Polymyxa graminis* Led. (Ratna *et al.*, 1991). Additionally, IPCV is seed-transmitted at a high frequency in groundnut and therefore is regarded as high risk pathogen for germplasm exchange (Reddy *et al.*, 1988). IPCV has an extremely wide host range which include many monocotyledonous plants. These include various grassy weeds which were found to support good multiplication of *P. graminis* (Reddy *et al.*, 1988, Ratna *et al.*, 1991; Delfosse *et al.*, 1996, Reddy *et al.*, 1999). In the majority of the production systems, peanut is either grown in rotation or as a mixed crop with such cereals crops as maize, millets and sorghum. Recently the virus was shown to be transmitted through seeds of three millet crops (Reddy *et al.*, 1998) and in wheat (Delfosse *et al.*, 1999) at frequencies ranging from 1 to 10%. It is essential to determine if seed-borne inoculum in either groundnut or cereals can contribute to the establishment of the disease. On the other hand, *Cynodon dactylon* [L.] Pers. is a very common weed in tropical areas. It spreads through rhizomes and it was identified as a host for both the virus and the fungus (Ratna *et al.*, 1991, Delfosse *et al.*, 1996). All the rhizomes arising from an infected plant contained the virus. Since they tend to spread in the soil, the new roots arising from them were suspected to provide viral inoculum to non-viruliferous *P. graminis*, thus creating new foci for the disease spread. As a first step towards the identification of the primary sources of virus inoculum, this study was undertaken to investigate if virus carrying groundnut seed and *C. dactylon* rhizomes could contribute to the establishment of the peanut clump disease.

Materials and Methods

Selection of C. dactylon *and groundnut seeds*. IPCV infected and uninfected rhizomes of *C. dactylon* were collected in the 1994 rainy season on the ICRISAT farm in the field RCW17A, infested with IPCV Hyderabad isolate (IPCV-H). The plants were vegetatively reproduced by planting rhizomes segments in receptacles of automatic immersion systems (AIS) (Legrève *et al.*, 1998) and maintained during one year in a glasshouse at 25-30°C in order to produce 36 shoots of both infected and

virus-free plants without *P. graminis*. To achieve this, the new rhizomes arising from the mother plants, which were grown in pots kept aside the AIS, were partially buried into sterile sand in an individual receptacle of the AID in a manner that they never enter in contact with soil to avoid contamination by *P. graminis*. Rhizomes were separated from the mother plant after root establishment. After one year all the rhizomes arose from the IPCV-H infected source were still containing the virus in both leaves and roots when tested by double antibody sandwich form of enzyme-linked immunosorbent assay (ELISA) (Reddy *et al.*, 1998) or by DNA hybridisation with a broad specific cDNA probe raised against all known serotype of IPCV (Wesley *et al.*, 1996). *C. dactylon* roots randomly collected in the AID were analysed by light microscopy to confirm the absence of *P. graminis* (Maraite *et al.*, 1988).
Groundnut (NCAc 17090) seeds were collected from mother plants that were infected by IPCV-H either from soil or seed inoculum. Seeds were selected for the presence of the virus in their cotyledons and embryos as assessed by ELISA (Reddy *et al.*, 1998).

Field design and establishment of C. dactylon *and groundnut*. The experimental design was a split-split-plot (Gomez and Gomez, 1984) with "source of virus" (groundnut versus *C. dactylon*) as main plot; the "virus status" (infected versus healthy) as subplot; and the "indicator host" as sub-subplot (groundnut cv.: NCAc 17090 and TMV-2, pigeonpea cv.: ICP-8863 and ICPL-87). Sixteen subplots of 3 x 4 m were demarcated in a virus-free area of RCW 17A where the disease has never been observed in rainy season crops of groundnut during the past 7 years. The plots were arranged in a square design and were separated by 50 cm. In eight subplots, 9 plants of *C. dactylon* were transplanted (3 lines of 3 plants) on 1 July 1995, 4 plots received H-IPCV infected *C. dactylon* plants and the 4 others received virus-free plants. *C. dactylon* was maintained for one year in the field and in June 96, the rhizomes completely covered the surface of the experimental subplots. Groundnut was sown in the remaining eight subplots in 5 rows (4 m long) each of 25 seeds. Distance between row was 50 cm, and between plants 16 cm. Four subplots were sown with 4 rows of IPCV-H infected seeds and a fifth central row, was sown with healthy seeds to confirm the absence of clump disease in the plots. The four remaining subplots were sown in a similar way using 5 rows of healthy seeds. Groundnut was harvested at the end of the growing season in November 1995 and the plots were kept under fallow during the 1996-1997 post-rainy season. In 1996, subplots planted with *C. dactylon* were surrounded with aluminium sheets arising 30 cm above ground and buried partially into the soil (20 cm), to avoid the spread of rhizomes. In 1997 the sheets were replaced by a permanent concrete wall.

Assessment of the disease establishment: In June 1996, *C dactylon* rhizomes were cut above soil surface leaving roots in the soil. The soil was manually prepared for sowing with 2 groundnut lines (NCAC-17090 and TMV-2) and 2 pigeonpea lines (ICP-8863, medium duration and ICPL-87, short duration) on 28 June 1996. Groundnut and pigeonpea were chosen because they are excellent indicator hosts under conditions prevailing during the rainy season. The four accessions were sown in 4 sub-subplots arranged in a random design in each subplot. Accessions were sown in 3 rows to reach a population of approximately 50 groundnut or 100 pigeonpea plants per sub-subplot. The groundnut and pigeonpea crops were scored 2 months after sowing for IPCV incidence based on visual symptoms (groundnut) and ELISA tests on leaves (pigeonpea). In groundnut visual symptoms were corroborated by ELISA tests conducted on leaves of plants chosen at random.
After harvesting groundnut and pigeonpea indicator hosts, in *C. dactylon* plots, the rhizomatous weed was allowed to re-established. The long term effect of growing groundnut and *C. dactylon* carrying the virus was analysed by repeating the sowing of the indicator crops in the 1997 rainy season.

Data analysis. The data were analysed by ANOVA on angular transformed percentage of incidence for a split-split-plot design (Gomez and Gomez, 1984).

Results

IPCV-H incidence in groundnut plants during establishment in 1995. IPCV-H was not recorded in groundnut plants arose from virus-free seed including in central lines sown with healthy seeds in plots where infected groundnut seed were sown. On the opposite, all groundnut plants produced by virus-carrying seeds showed severe chlorotic lesions on young leaves and were stunted compared to healthy plants.

IPCV-H incidence in the indicator groundnut and pigeonpea crops during the 1996 and 1997 rainy seasons. Virus incidence on the basis of visual symptoms in groundnut and ELISA tests on pigeonpea leaf extract was low (below 10%) and similar for 1996 and 1997 (Table 1). The source of virus inoculum (groundnut versus *C. dactylon*) had a significant effect on virus incidence, the incidence being highest for plots treated with *C. dactylon* ($P \leq 0.05$). IPCV-H incidence was significantly higher in plots where virus-infected material was used compared to virus-free one ($P \leq 0.01$). The

highest IPCV-H incidence was observed for plots treated with virus-carrying rhizomes of *C. dactlyon* (interaction source x virus significant at $P \leq 0.01$). The indicator host used (groundnut versus pigeonpea) had no significant effect on IPCV-H incidence (1996: $P=0.2693$), 1997: $P=0.2165$). A surprising result is the presence of infected plants among the indicator host in plots where virus-free material was introduced. In 1996 one pigeonpea plant was found in plots treated with healthy groundnut seeds and 5 groundnut and 12 pigeonpea plants were found in plot treated with virus-free *C. dactylon*.

Table 1. Groundnut seed and *C. dactylon* rhizomes as primary source of IPCV-H inoculum for the establishment of peanut clump disease. Mean IPCV-H incidence (Θ = angular transformation) and total proportion of infected plants (ni/N) in groundnut and pigeonpea indicator crops grown during the ensuing 1996 and 1997 rainy seasons.

	Groundnut seeds		*C. dactylon* rhizomes		Mean-Virus	
	ni/N	Θ	ni/N	Θ	ni/N	Θ
1996						
Virus-free	1/1272	0.68	17/1222	4.45	18/2494	2.57
Virus-infected	45/1173	6.59	103/1149	15.11	148/2322	10.85
Mean-Source	46/2445	3.64	120/2371	9.78		
1997						
Virus-free	0/1443	0.31	32/2774	4.11	32/4217	2.21
Virus-infected	45/1454	7.08	196/2728	14.15	241/4182	10.61
Mean-Source	45/2897	3.69	228/5502	9.13		

SEDs (1996): source ± 2.86, virus ± 3.34, source x virus ± 4.72
SEDs (1997): source ± 1.89, virus ± 2.06, source x virus ± 2.92

Discussion

Cynodon dactylon is a very common weed in both tropical and sub-tropical regions of India. It spreads through rhizomes and is therefore often appreciated by the farmers to strengthen field borders and irrigation dams in sandy soil areas. It was identified as a host for both the virus and the fungus (Ratna *et al.*, 1991, Delfosse *et al.*, 1996). All the rhizomes arising from an infected plant contained the virus and they can act as a virus reservoir. We have shown that virus infected rhizomes of *C dactylon* led to disease establishment by providing viral inoculum to non-infested soil and therefore creating foci for further disease spread. The weed is often moved from field to field by farm implements. For these reasons, *C dactylon* is likely to present a high risk for virus spread to new areas.

A serious side effect of the presence of diseased plants in a clump infested field is an increased crop weediness. Indeed, IPCV induced severe growth reduction in groundnut plants. As a consequence weeds do not need to compete anymore with the crops for availability of light and nutrient. This phenomenon was often observed in severely infested groundnut fields in Rajasthan where grassy weeds, mostly *Dactyloctenium aegyptium* [L.] Willd. and *Eragrostis spp.* Wolf., proliferate in infested areas. It is likely that profusion of such monocotyledonous weeds that host both IPCV and its vector (Delfosse *et al.*, 1996), can contribute to increase in disease incidence. This was evidenced by a higher incidence in plots treated with healthy *C. dactylon*, a good host for *P. graminis*, than in plots treated with healthy groundnut seeds, a fortuitous host for the vector. Clean cultivation is therefore strongly recommended in infested field eventhough the farmer usually lacks motivation to invest in a crop of little prospect.

Virus containing groundnut seed did not appear to be a good source of inoculum to the vector. *P. graminis* resting spores are seldom observed in groundnut roots, and it does not reproduce in this host, reducing the risk for seed-borne virus to establish as soil-borne inoculum in new sites. Although very low, the risk of establishing peanut clump disease to a new site may exist and it is therefore not recommended to use groundnut crops raised in infested fields for seed purposes. Seed transmission frequency can be very high in groundnut. Field-infected plants produce 10 to 14% of seeds carrying the virus whereas plants arising from infected seed produce up to 50% of seeds with the virus in their embryos (Reddy *et al.*, 1998). Plots treated with virus-infected groundnut seeds were not expected to show peanut clump disease presence because groundnut, a fortuitous host, does not support intense multiplication of *P. graminis* in its roots (Ratna *et al.*, 1991, Legrève, 1999). Nevertheless few indicator plants were found to be infected by the virus in these plots. Because of the low incidence, it is still unclear if virus established in the area or if weediness contributed to an increase in viruliferous inoculum, that was previously undetected, to a threshold which permitted disease occurrence in the indicator plants. *Eragrostis uniloides* (Retz.) Nees ex Steud, *D. aegyptium*, *Digitaria ciliaris* (Retz.) Koeler, *C. dactylon* and *Eleusine indica* (L.) Gaertner, hosts for both IPCV and *P. graminis* (Delfosse *et al.*, 1996), were found to occur naturally in the infested field. Climatic conditions prevailing during the 1996 rainy season were very conducive for *P. graminis* multiplication (Delfosse *et al.*, unpublished) and it is therefore possible that weeds contributed to build up viruliferous inoculum.

Transmission of IPCV through seed of cereals, preferred hosts for *P. graminis*, are likely the present a higher risk than groundnut seed for virus

spread and establishment of peanut clump disease in new areas. These include finger millet (*Eleusine coracana* [L.] Gaertner), Foxtail millet (*Setaria italica* [L.] Pal.), maize (*Zea mays* L.), pearl millet (*Pennisetum glaucum* [L.] R. Br.), and wheat, which were shown to transmit IPCV through seed at frequencies of 5, 10, 1, 1, and 1%, respectively (Reddy *et al.*, 1998, Delfosse *et al.*, 1999). Considering the number of species that show seed transmission of IPCV, seeds of monocotyledonous weeds can also be suspected to carry the virus since they usually support systemic infection. Germination of seeds from weeds is often hampered by dormancy and attempts to evaluate the seed transmission frequency of IPCV in *D. aegyptium*, *D. ciliaris* and *E. uniloides* were not successful (Delfosse *et al.*, unpublished). Nevertheless, the primary source of virus inoculum is considered as a very important target in the development of an integrated strategy towards the management of peanut clump. Efforts will be pursued to evaluate monocotyledonous hosts for seed transmission frequency and for their potential to serve as a source of inoculum for the dissemination and establishment of the disease.

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Chapter 4

Dynamics of *Polymyxa graminis* and *Indian peanut clump virus* (IPCV) infection on various monocotyledonous crops and groundnut during the rainy season.[8]

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Abstract

The progress of IPCV-H (Hyderabad isolate) and *P. graminis* incidence in various monocots and groundnut was studied during the 1994, 1995, and 1996 rainy seasons in a naturally infested field. Concomitantly, the role of rainfall and temperature on the dynamics of infection by both the virus and its vector was analysed by exposing young seedlings for short periods in the field. Among all the hosts studied, wheat followed by barley showed the highest virus incidence. They supported systemic virus infection although the *P. graminis* isolate involved in IPCV-H transmission was rarely observed in roots of wheat and was not detected in those of barley. The roots of maize, pearl millet and sorghum plants infected by *P. graminis* showed intense colonisation by sporosori. IPCV accumulated in systemically infected maize plants; the sorghum and pearl millet cultivars studied were transient hosts for IPCV-H. Rice was seldom infected by the virus and *P. graminis* was not detected in its roots. Groundnut was a good host for the virus although during these experiments no *P. graminis* was found in its roots. Groundnut appeared to be susceptible to infection mostly at early stages of crop development and the rate of IPCV-H transmission in seeds was highest (13%) for groundnut plants infected when young. The seed transmission rate quickly decreased in plants which showed symptoms one month after sowing. The quantity and distribution

[8] To be submitted for publication

of rainfall were shown to be the key factors that influenced the incidence of IPCV-H and *P. graminis*. High rainfall resulted in high virus and *P. graminis* incidence. A weekly rainfall of 14 mm was sufficient *P. graminis* to initiate infection. Temperatures prevailing during the rainy season were found to be conducive for natural virus transmission. These results suggested measures to be experimented for controlling the peanut clump disease.

Key words: Epidemiology, rainfall, temperature, *Polymyxa graminis*, IPCV, groundnut, sorghum, millet, maize, wheat, barley, rice.

Introduction

Peanut clump disease causes significant losses to groundnut (peanut, *Arachis hypogaea* L.) crops in West Africa and in the Indian sub-continent. The disease causes annual losses estimated to exceed 38 million US dollars (Reddy *et al.*, 1999). *Indian peanut clump virus* (IPCV), a member of the genus *Pecluvirus* (Torrance and Mayo, 1997 and Reddy *et al.*, 1999), is the causal agent of the disease in India and Pakistan (Reddy *et al.*, 1988, Delfosse *et al.*, 1995). All currently known pecluviruses are seed- and soil-transmitted (Reddy *et al.*, 1988, Konaté and Barro, 1993). IPCV is transmitted in seeds at least of groundnut, millets, wheat and maize and therefore is of particular importance for germplasm exchange (Reddy *et al.*, 1998, Reddy *et al.*, 1999, Delfosse *et al.*, 1999). IPCV has been shown to be transmitted by the obligate parasite *Polymyxa graminis* Led. which thrives in the soil (Ratna *et al.*, 1991). The soil-borne habit of the vector and its survival as highly resistant resting spores are responsible for the patchy appearance of the disease and its recurrence year after year in nearly the same areas of a field. IPCV induces severe stunting in groundnut indicating that infection usually occurs at an early stage of crop growth (Thouvenel *et al.*, 1988, Reddy *et al.*, 1988). However, various degrees of stunting severity were noticed during surveys conducted in Asia and West Africa and it was though that stunting severity depended on the age of the plants when they were infected (Dollet *et al.* 1993, Manohar *et al.*, 1995, Delfosse *et al.*, unpublished). In India, Reddy *et al.*, (1988) reported influence of the date of sowing on disease incidence. Groundnut crops grown during the post-rainy season, and crops sown in the rainy season well beyond the onset of monsoon rains, mostly escaped the disease (Reddy *et al.*, 1988). The authors concluded that temperature was an important factor influencing natural virus transmission.

IPCV and its vector, *P. graminis* have wide host ranges which includes many monocotyledonous as well as dicotyledonous weeds and crops. Although *P. graminis* transmits the virus to dicotyledonous crops, it does not extensively colonise their roots. Sporosori (resting spore clusters or cystosori) were rarely detected in such plants and were few in number (Ratna *et al.*, 1991, Delfosse *et al.*, 1996, Legrève, 1999). Also, roots of naturally virus-infected groundnut plants failed to induce the disease when incorporated into sterile sand whereas infected sorghum and pearl millet roots were sources of inoculum (Thouvenel *et al.*, 1988, Ratna *et al.*, 1991, Delfosse *et al.*, 1996). For these reasons, dicotyledonous plants are considered as "fortuitous" hosts that are unlikely to contribute to build up of clump disease inoculum. In contrast, monocotyledonous hosts such as maize, pearl millet, and sorghum are regarded as "preferred" hosts for *P. graminis* due to its relatively high incidence and multiplication as measured by the number of sporosori present in their roots (Ratna *et al.*, 1991, Delfosse *et al.*, 1996, Legrève, 1999). They also are hosts of IPCV and some of them transmitted the virus through seed. Preferred hosts are therefore suspected to play an important role in the perpetuation and spread of virus inocula (Reddy *et al.*, 1999).

Resistance to IPCV could not be identified in any of nearly 9000 *Arachis* germplasm lines tested. Attempts to control the disease by the application of soil biocides and soil solarization, although effective, were found to be either hazardous or uneconomical (Reddy *et al.*, 1988, Dhery *et al.*, 1975, Reddy *et al.*, 1999). Therefore the work reported here was undertaken mainly to study the epidemiology of peanut clump disease with the hope of formulating cultural methods of disease control. Various parameters studied were the role of monocotyledonous crops, which are often rotated or inter-cropped with groundnut, in the establishment, survival and spread of the peanut clump disease, and the factors crucial for transmission by *P. graminis*. The results show the important role played by the quantity and distribution of rainfall and, to a lesser extent, by temperature on *P. graminis* and IPCV incidence in various monocotyledonous crops and groundnut. The importance of our findings for disease management are discussed.

Materials and Methods

The experiments were conducted during the consecutive 1994, 1995 and 1996 rainy seasons in a field (0.35 ha) naturally infested with the Hyderabad isolate of IPCV (IPCV-H) (Nolt *et al.*, 1988) on the ICRISAT farm (18° N, 78° E), Andhra Pradesh, India. The soil was sandy with 84%

of sand, 10% of loam and 6% of clay and showed a pH close to neutral. In the first experiment the progress of *P. graminis* and IPCV-H incidence was studied for plants grown under field conditions and uprooted at regular intervals during the rainy season to assess the influence of climatic factors on infection. A second experiment was conceived to refine the study of the climatic factors influencing peanut clump disease incidence. The trial consisted in exposing young plants for short periods in the field during which period, rainfall and temperature were recorded. After the field exposure, plants were transplanted to a glasshouse in conditions conducive for IPCV-H and *P. graminis* multiplication to facilitate virus and vector detection irrespective of when they had infected the plants. Therefore the climatic factors which prevailed during the period of exposure in the field are expected to contribute to infection by *P. graminis* and H-IPCV and influence their incidence. The experimental details are summarised in Table 1. For each season four new replication plots were demarcated in areas where IPCV-H incidence ranged from 60 to 80% in groundnut crops grown during the rainy season preceding each of the experimental season. Because the areas with homogenous infestation were small, the number of plants analysed was limited (Table 1). In 1994 four virus-free plots, near to infested one, were also demarcated in the same field in an area where IPCV-H had never been recorded in groundnut crops during the past 7 years. These plots were included to assess if *P. graminis* occurs with similar incidence in infested and virus-free soils.

Progress of IPCV-H incidence in the groundnut crops

For each season the remaining part of the field was sown with groundnut to reach a population of approximately 35 x 10^3 plants. Seeds were treated with thiram at 3g kg^{-1} seed to prevent damping-off. Groundnut was grown to monitor the progress of clump disease incidence over time by scoring the crop for typical symptoms (Reddy *et al.*, 1988). Infected plants were marked according to the date they showed symptoms. In 1996 the entire groundnut production was harvested 4 ½ months after sowing from four major virus infested areas and the pods were sorted according to the date symptoms appeared. The yield and the seed transmission rate (Reddy *et al.*, 1998) were calculated for each group and compared to those of healthy plants.

Progress of P. graminis and IPCV-H incidence in field-grown plants

For each season, various crops listed in Table 1 were sown in the field. Crop plants were carefully uprooted at weekly intervals (or every other week from 31 July onwards in 1995) in each plot. The roots were washed free of soil under tap water, stained and analysed by light microscopy for the presence of *P. graminis* sporosori as described by Maraite *et al.* (1988). For the assessment of *P. graminis* incidence, only the plants containing sporosori in their roots were taken into account. Indeed, in naturally infected plants, sporosori were the predominant stage observed and identification of *P. graminis* on the basis of the plasmodial and zoosporangial stages was found to be unreliable since other soil organisms produce similar structures in roots. As the season progressed the root system grew substantially and therefore root fragments picked up at random over the whole root system to represent approximately 10% of the root, were stained and analysed. A portion of the youngest leaf and root samples were collected and tested by enzyme linked immunosorbent assay (ELISA) to assess the presence of the virus. In 1994 only leaves were assayed.

Plants exposed in the field for short periods to determine the conditions conducive for virus and P. graminis infection

Seeds of the various crop plants listed in Table 1 were disinfected by soaking for 10 min in 5% sodium hypochlorite (commercial solution), rinsed thoroughly with distilled water and germinated. Two day old seedlings were transplanted to polyvinyl chloride pipes (32 mm diameter, 100 mm long) closed on one end with a piece of sterile cotton cloth held with a rubber band. The pipes were filled with sterile sand (1 to 2 mm particles size). The seedlings were watered daily with half strength Hoagland solution, pH 6.5 (Adams *et al.*, 1986). The pipes were held in a tray to facilitate watering and transport. One week old seedlings were watered, brought to the field and transplanted. Transplanting was done by drilling a 40 mm hole in the soil with a small soil sampler. Cloth holding the seedling was removed and the seedling was slipped into the hole without causing much disturbance to the root system or to the aerial part of the plant. The seedlings were exposed in the field for one week and then carefully uprooted. In 1995, plants collected from 31 July onwards were exposed for two weeks. When a set of exposed plants was uprooted, a new set of seedlings was transplanted to the field the same day.

Table 1. Experimental details of the field trials in 1994, 1995 and 1996 rainy seasons

	1994	1995	1996
Cultivations			
Sowing	1 July	24 June	25 June
First sampling/transplanting from/to the field	11 July	6 July	12 July
Last sampling for the plants grown in the field	5 Sept.	11 Sept.	20 Sept.
Last sampling for the plants exposed in the field	5 Sept.	9 Oct.	8 Oct.
Irrigation as specified in the figures			
Experiment repeated in virus-free area	yes	no	no
Scoring for clump symptoms in groundnut crops			
July	14, 29	31	12, 19, 26
August	12, 30	23	9, 23
September	-	28	27
Harvest of groundnut	First or second week of November		
Crops and cultivars studied			
Sorghum (*Sorghum bicolor* [L.] Moench) ICSV-88036	yes	yes	yes
Pearl millet (*Pennisetum glaucum* [L.] R. Br.) ICMH-451	yes	yes	yes
Finger millet (*Eleusine coracana* [L.] Gaertner) HR-374	yes	yes	no
Maize (*Zea mays* L.) CHM-103	no	yes	yes
Wheat (*Triticum aestivum* L.) RR-21	no	yes	yes
Barley (*Hordeum vulgare* L.) RD-103	no	yes	no
Rice (*Oryza sativa* L.) Rasi	no	yes	yes
Groundnut (*Arachis hypogaea* L.) NCAc 17090	no	yes	yes
Design[a]			
Number of plants analysed per observation[b]	4	4 or 8	6
Number of observations over time	9	7	10
Subplot size (1 replicate x 1 species)[c]	2 m row	4 m row	1 x 1 m
Whole plot size (m)[c]	2 x 2	4 x 2	6 x 1
Distance within row (cm)	5	5	5
Distance between rows (cm)	25	25	25
Virus detection -in leaves	yes	yes	yes
-in roots	no	yes	yes

[a] Randomised block design with 4 replicates.
[b] This number applies to the field-grown plants. For the plant exposed for short period, this number is the optimum targeted but often it varied according to the survival rate of the plants.
[c] Plot size and shape were adjusted to fit the area available in IPCV-H infested patches. Exposure in the field was for one week except in 1995, plants collected from 31 July onwards were exposed for two weeks.

Leaf samples were collected from the exposed plants and tested by ELISA, the roots were not tested at the time of retrieval from the field because sampling could have resulted in the loss of young *P. graminis* infection. Instead, the plants were transplanted to sterile sand in 50 ml glass culture tubes (1994 and 1995 seasons) (Maraite *et al.*, 1988) or in the 1996 season, to automatic immersion system with individual watering for each plant (Delfosse *et al.*, unpublished). Transplanted plants were maintained in a

glasshouse for a few weeks (8 to 10 wk in 1994, 6 wk in 1995, and 4 to 5 wk in 1996) at 25-30°C, a temperature range known to favour IPCV and *P. graminis* multiplication (Reddy *et al.*, 1988, Legrève *et al.*, 1998). After the incubation period the roots were analysed for the presence of *P. graminis* and tested by ELISA to assess the presence of the virus in both leaves and roots. In this experiment IPCV-H and *P. graminis* incidences were measured on the basis of plants that survived after the incubation period in a glass house.

ELISA.

IPCV-H was detected by the penicillinase based form of double antibody sandwich ELISA (Sudarhana *et al.*, 1989, Reddy *et al.*, 1998). For the field-grown and field-exposed plants whenever reference is made to virus incidence it actually refers to the number of plants that tested positive in ELISA. For the groundnut crop sown in the remaining part of the field, virus incidence was based on visual symptoms corroborated by random tests on leaf extracts by ELISA.

Weather data.

Daily mean soil temperature was recorded at 10 cm below the soil surface. Daily rainfall was recorded in the field with a tipping bucket rain gauge (Texas Electronics Inc., Dallas, Texas, USA - Model: TR5251) or was obtained from the Meteorological Observatory of ICRISAT-Patancheru. Dataloggers (Campbell Scientific, Inc., Logan, Utah, USA - Model: CR 10) were used to record the weather data in the field.

Data analysis.

For the plants grown in the field and uprooted at regular intervals during the rainy season, the number of plants analysed was consistent for all the observations allowing a data analysis using ANOVA on angular transformed percentage (arc sine transformation: θ = arc sine [$\sqrt{x}$]) developed for binomial proportions (Snedecor and Cochran, 1967, Gomez and Gomez, 1984). For the plants exposed for short periods in the field, the number of plants that survived varied greatly in 1995. Therefore the data were not analysed statistically. In 1994 and 1996, the mortality was low and the data could be analysed by ANOVA. The influence of the moment of infection by IPCV-H on yield and seed transmission frequencies (angular transformation) in groundnut was also analysed by ANOVA.

Results and interpretation

Weather data

Temperature. During the predominantly dry summer season (April-June) soil temperatures above 45°C were commonly recorded and they rapidly decrease with the onset of monsoon rains in June. High temperature and drought were shown to be conducive for breaking down *P. graminis* resting spore dormancy (Legrève *et al.*, 1999). Therefore, the summer season was favourable to disease occurrence in rainy season crops. For the three rainy seasons the mean soil temperature remained in the range of 23-30°C known to be conducive for *P. graminis* development (Fig. 1 to 6) (Legrève *et al.*, 1998). However, the same authors have shown that initiation of infection by the plasmodiophorid is favoured by relatively high temperatures in the range 27-30°C. The only period when mean soil temperature was clearly below 25°C, thus not favouring infection by *P. graminis*, occurred in early July 1994. The soil temperature was found to be closely related to sunshine and rainfall, the temperature decreasing during cloudy or rainy days.

Table 2. Monthly rainfall (mm) including irrigation for the 1994,1995 and 1996 rainy seasons and long term average rainfall at Patancheru (1974-1998).

	June	July	August	September	October	Total
Average 1974-1998[1]	118	184	217	157	105	781
1994	144	143	220	66	247	820
1995	136	252	245	128	376	1137
1996	87	301	466	191	83	1128

[1] More than 85% of the average annual rainfall occurs during June-October at Patancheru

Rain fall. The monthly rainfall for each season is summarised in Table 2. More than 85% of the average annual rainfall (long term average 1974-1998) occurred during June to October at Patancheru. The 1994 season was the driest with the highest daily rainfall reaching only 40.4 mm during the last week of August. The 1995 season was wetter with a regular distribution of rainfall. There was at least one important daily rainfall recorded each month. In 1996 the rainfall was not as evenly distributed as it was for 1994 and 1995. It was necessary to irrigate the crops in July and at the end of the season in September and October while August was characterised by a heavy rainfall. June was drier in 1996 than in 1994 and 1995 and a rainfall of 35 mm occurred just after completing sowing in the

field on 25 June 1996. Rains occurred from 11 to 15 July coinciding with the early development of the 1996 groundnut crop. A very high rainfall (107 mm) was recorded during the last week of August 1996.

Progress of IPCV-H incidence in the groundnut crops

During the 1994 season, the field where the experiment was being conducted showed a few small patches with incidence ranging from 60 to 80 %. This low incidence is attributed to the continuous monoculture of groundnut, a fortuitous host for *P. graminis,* for 7 years. Clump disease symptoms on groundnut appeared 15 days after sowing (Figure 1-B). The incidence was low (15 plants detected in a population of 35 x 10^3 plants) and only in patches where the disease was known to recur year after year with high incidence. The number of infected plants increased progressively and reached a plateau at the end of August (Figure 1-B). Because of a two to three week delay between virus infection and symptom detection (Reddy *et al.*, 1988), all the plants detected with symptoms until mid-August were most probably infected in July. These plants represented 80% (445/563) of the total plants infected during the growing season.

In 1995, during the first scoring (approximately 5 WkAS) 474 groundnut plants exhibited clump disease symptoms. In August (8 WkAS) 286 additional plants and in September (12 WKAS) only 15 new plants were found to be symptomatic. For the whole field 775 clump infected plants were present at harvesting time. From the total of plants infected by IPCV-H during the 1995 season, 61% were observed during the month of July, 37% in August and only 2% in September.

During the 1996 season, infected groundnut plants were recorded in new areas of the field. The cumulative numbers of symptomatic plants recorded during the rainy season 1996 are presented in Table 3. The summer 1996 until end of June was predominantly dry (Table 2). As a result there was no opportunity for P. graminis sporosori to germinate and infect alternate hosts such as wild grasses prior to sowing the groundnut crop. A high rainfall occurred on 25 June coinciding with sowing of groundnut and favourable soil moisture conditions were assured by irrigation throughout the 1996 rainy season. Exceptional high rainfalls were recorded in August (Table 2). These conditions may have contributed to high disease incidence in 1996. It was the highest disease incidence (4242 infected plants) ever observed in this field since ICRISAT initiated research activities on clump in 1983. From the total number of infected plants, 17% were detected in July, 66% in August and the remaining 17% in September. Infected plants were analysed for their yield and the rate of virus transmission through seed (Table 3). It is apparent that there was no

statistically significant difference in pod and seed yield between plants found to be symptomatic early or late during the season. There was only a slight tendency for the plants infected later in the season to produce more yield. Irrespective of the age when plants showed symptoms, the yield loss in infected plants compared to healthy plants was always above 60%. However the frequency of virus transmission through seed was significantly lower in plants that were infected later in the season compared to early-infected ones (Table 3).

Table 3. Yield components and seed transmission rate in groundnut plants that were found symptomatic during successive scorings of the crop grown during the 1996 rainy season..

Scoring date	Cumulative No. of symptomatic plants	Pod yield [1] (kg/ha)	No of seed per plant [1]	Seed yield per plant [1](g)	Seed transmission[2] ni/N	(%)
12 July	110	235±27[a]	6.9±1.3 [a]	1.0±0.2 [a]	56/413	(13.5±1.9)[a]
19 July	298	213±13 [a]	4.7±1.7 [a]	1.0±0.1 [a]	107/808	(13.2±2.4)[a]
26 July	716	221±134 [a]	6.8±3.0 [a]	1.4±0.2 [a]	222/3228	(6.9±1.9)[b]
9 Aug.	1776	326±44 [a]	8.2±1.4 [a]	1.6±0.3 [a]	138/5680	(2.4±1.2)[c]
23 Aug.	3540	321±18 [a]	9.2±0.4 [a]	1.7±0.1 [a]	9/475	(1.9±1.0)[c]
27 Sept.	4242	394±83 [a]	10.1±1.6 [a]	1.9±0.3 [a]	7/405	(1.7±0.9)[c]
Healthy[3]	-	1258±547 [b]	23.6±10.8 [b]	6.4±3.0 [a]	-	

[1] Means and standard deviations from four replications. If followed by the same letter, means in a column do not differ in Tukey honestly significant difference test ($P \leq 0.05$).
[2] Number of seed infected / number of seed tested (ni/N) and percentage (%).
[3] Four sets of 50 plants each analysed.
The crop was sown on 28 June and harvested on 18 Nov
The plant population was approximately 110 x 10^3 plants/ha.

Progress of P. graminis and IPCV-H incidence in plants grown in the field and uprooted at regular intervals

1994. Incidence of *P. graminis* and IPCV-H in finger millet, pearl millet and sorghum is given in Table 4. Over the entire season finger millet and pearl millet sampled in the virus-free plots showed a lower *P. graminis* incidence than sorghum. In the virus-infested area, finger millet showed a lower *P. graminis* incidence than the two other cereals. In the virus-infected area, the three cereals showed similar and low IPCV-H incidence (below 7%). For both *P. graminis* and IPCV-H incidence, the three crops responded in a consistent manner over time (the crop x date of sampling interaction was not significant). When conditions were favourable for infection of sorghum, they were also favourable for infection of the other two crops.

It is apparent that the progress of incidence during the rainy season is quite irregular for both the virus and the fungus (Fig.1-A and 1-B). Virus and

vector incidence were not accumulative. This may be due to the sampling of young leaf and root tissues in which *P. graminis* and IPCV-H were not yet detectable. The variation in *P. graminis* incidence appeared to be influenced by the distribution and quantity of rainfall. Indeed *P. graminis* incidence in the roots of the three cereals combined could be correlated with the weekly rainfall (WR) that occurred 2 weeks before sampling (Fig. 1-A and 1-D). This time interval corresponds to the time required for *P. graminis* to produce resting spores after infection has occurred (Ratna *et al.*, 1991, Legrève, 1999). The logarithmic regression between *P. graminis* incidence in the roots of the cereals and the weekly rainfall (Fig 1-D), suggests that a WR higher than 10.3 mm is required to induce *P. graminis* infection. During the experiment the lowest WR observed reached already 14 mm and was sufficient to induce infection.

Table 4. *P. graminis* and IPCV-H incidence (angular transformation) in cereals grown in the field during the 1994 rainy season, uprooted at weekly interval and directly analysed.

	IPCV-H[1]	*P. graminis*[2]	
		IPCV-H infested area	Virus-free area
Crops	n=144	n=144	n=144
Finger millet	8 (8.2)	7 (5.5)	5 (3.8)
Pearl millet	9 (7.8)	31 (21.0)	3 (2.5)
Sorghum	5 (5.0)	27 (17.7)	18 (12.5)
$SED_{\text{crop (2 d.f.)}}$	2.6 ^{NS}	6.1 *	2.9 **
$SED_{\text{date of sampling (8 d.f.)}}$	4.5 **	10.5 ^{NS}	5.1 *
$SED_{\text{crop x date (16 d.f.)}}$	7.8 ^{NS}	18.3 ^{NS}	8.8 ^{NS}

[1] IPCV-H incidence was assessed by ELISA only in the virus infested area.
[2] *P. graminis* incidence was assessed in both the areas.
Combined data from 9 samplings collected from 11 July to 5 September. The figures represent the number of infected plants. The number of plants analysed (n) is indicated for each category and the angular transformed percentage is presented in brackets.
Treatments were: ^{NS} not significant, ** significant at 1% level, * significant at 5% level.

In the three cereals, the vector was detected in the roots before the virus could be detected in the leaves. The progress of the virus incidence in leaves followed nearly that of *P. graminis* in roots with approximately 1 to 2 wk delay (compare Fig. 1-A to Fig. 1-B). Finger millet showed a virus incidence of 20% on 1 August while none of these plants showed the presence of *P. graminis* (Fig. 1-A and 1-B). For the three crops the virus incidence was low during the first three weeks of August when the mean soil temperature was high and the maximum mean air temperature was close to 30°C a temperature very favourable to the growth of these cereals from tropical origin. In mid-September the plants remaining in the infested patches were all tested by ELISA for the presence of the virus in leaf

extracts. The results showed that the virus was detected in 13/100 finger millet, 0/380 pearl millet and 1/330 sorghum plants.

1995. The progress of virus incidence in the various hosts is presented in Fig. 2. The virus was detected in the first root samples collected 12 days after sowing. All species but pearl millet were hosts for IPCV-H based on detection of virus antigen in root extracts. The virus was also detected in the leaves of wheat, barley, and maize. Wheat showed the highest incidence in the roots (94%) followed by maize (50%). The virus incidence in wheat reached almost maximum incidence for the second sampling done 2 WKAS. At the time of the second sampling the incidence in wheat leaves (88%) was similar to that observed in roots (94%). There was thus approximately a week delay for virus migration from roots to leaves during the early stage of wheat growth. During the experiment wheat remained infected in both leaves and roots. Surprisingly in September, the virus was detected in the leaves of groundnut plants that did not show the presence of the virus in their roots. Otherwise in most cases, irrespective of the species the virus incidence was higher in the roots than in the leaves. This was significant for maize and sorghum (Table 5). The virus was never detected in leaves of the pearl millet cultivar studied and it occurred with a very low incidence in the leaves of sorghum and rice. In the case of groundnut, the virus in the leaves was detected with a delay of approximately 3 weeks compared to the roots (Fig. 2). The virus was already detected in groundnut roots on 6 July (2 WKAS) while in leaves, the first virus detection was recorded on 31 July (5 WKAS). Additionally, virus detection in groundnut leaves coincided with the appearance of symptoms as assessed by scoring the groundnut crop.

The various crop plants were also analysed for the presence of *P. graminis*. From a total of 160 plants analysed, 1 maize, 10 pearl millet, 1 finger millet and 10 sorghum plants were found to be infected by the fungus. Those plants with infection showed a high number of sporosori in their roots. Although the other crops were infected by the virus, *P. graminis* could not be detected in their roots.

During the last week of October 1995 the plants remaining in the experimental infested plots were all tested by ELISA for the presence of the virus in leaf extracts. The results showed that 27/150 finger millet, 0/190 pearl millet, 0/100 rice and 3/155 sorghum plants tested positive.

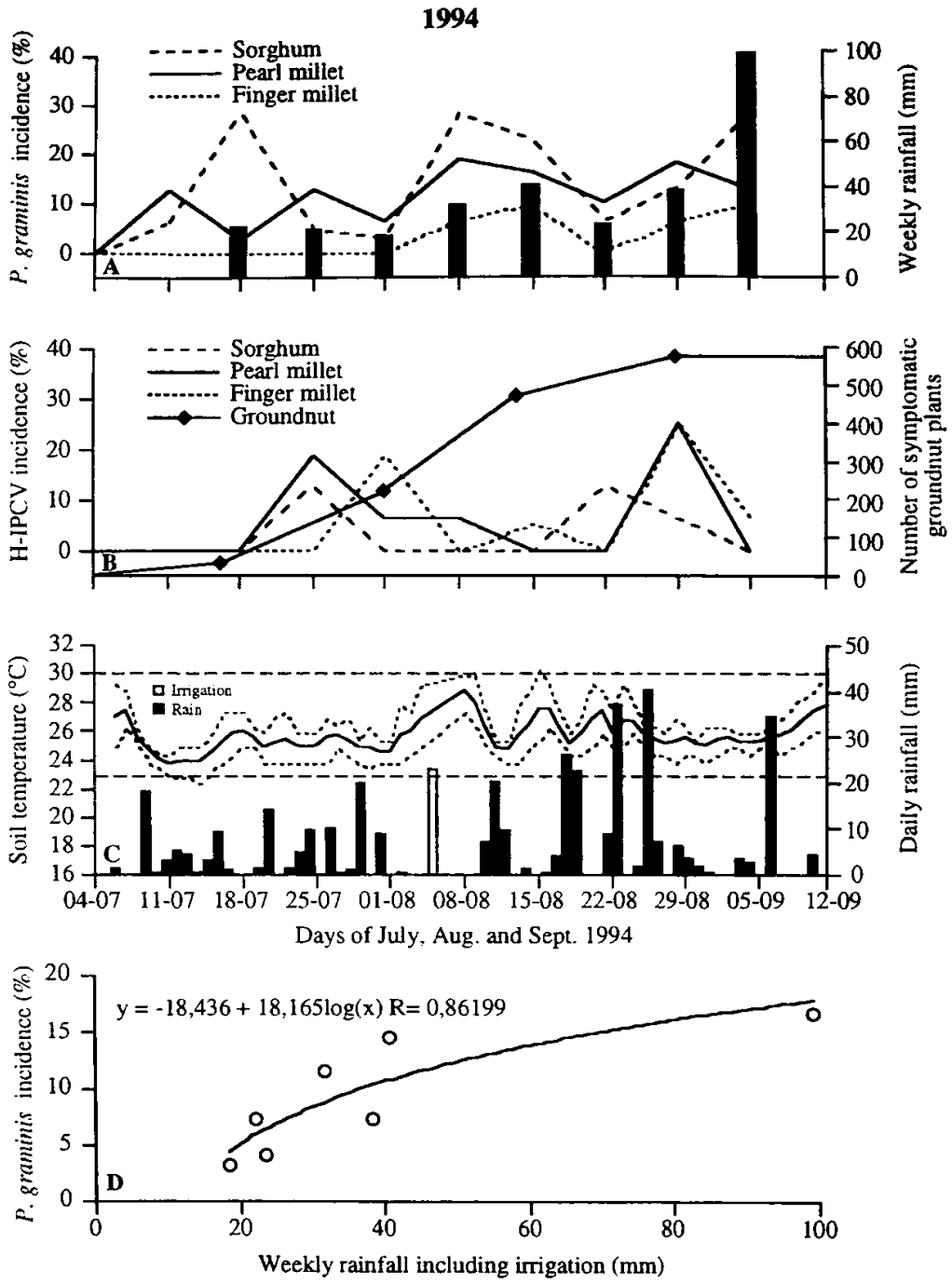

Figure 1. Progress of *P. graminis* incidence in three cereals sown on 4 July, grown in the field during the 1994 rainy season and uprooted at weekly intervals. **A**. Mean percentage calculated on the total of plants grown in IPCV-H infested and virus-free plots and weekly rainfalls (including irrigation) recorded 15 days before uprooting the plants, and **D**, the relationship between the two. **B**. Progress of IPCV-H incidence in leaves of plants grown in virus infested plots and number of symptomatic groundnut plants observed at the various scoring dates. **C**. Daily mean, minimum and maximum soil temperatures at 10 cm below soil surface (the horizontal lines border the temperature range known to be conducive for *P. graminis* development), daily rainfall (black columns) and irrigation (white columns). The SEDs are given in Table 4.

Table 5. IPCV-H. incidence (angular transformation) in various crop plants grown during the 1995 rainy season, uprooted weekly or every other week and directly assayed by ELISA.

	IPCV-H infected plants		Significance in ANOVA [1]	
Crops	Roots n=160	Leaves n=160	SEDs	(d.f.)
Barley	99 (51.0)	90 (45.4)		
Finger millet	40 (24.2)	33 (20.0)	crop ± 3.05 **	(7)
Groundnut	34 (21.3)	13 (9.3)	date of sampling ± 2.8 **	(6)
Maize [2]	61 (37.2)	6 (5.8)	organ ± 1.5 **	(1)
Pearl millet	5 (4.9)	0 (1.2)	crop x organ ± 4.3 **	(7)
Rice	4 (5.3)	1 (2.3)	crop x date ± 8.1 **	(42)
Sorghum [2]	41 (28.6)	10 (6.8)	organ x date ± 4.0 **	(6)
Wheat	136 (75.3)	122 (63.7)	crop x date x organ ± 11.4 NS	(42)
Total [2]	420 (31.0) (n=1280)	275 (19.3) (n=1280)		

Combined data from 7 samplings collected from 6 July to 14 September. The figures represent the number of infected plants. The number of plants analysed (n) is indicated for each category and the angular transformed percentage is presented in brackets.
[1] Treatments were: NS not significant, ** significant at 1% level.

Table 6. IPCV-H. incidence (angular transformation) in various crop plants grown during the 1996 rainy season, uprooted at weekly interval and directly assayed by ELISA.

	IPCV-H[1]		Significance in ANOVA [1]	
Crops	Roots	Leaves	SEDs	(d.f.)
Groundnut	129/243 (48.2)	90/243 (35.9)		
Maize	137/241 (51.0)	100/241 (38.4)	crop ± 2.2 ***	(5)
Pearl millet	15/261 (6.7)	16/261 (8.0)	date of sampling ± 2.9 ***	(9)
Rice	10/252 (5.1)	7/252 (4.9)	organ ± 1.3 *	(1)
Sorghum	55/249 (21.8)	53/249 (22.4)	crop x organ ± 3.2 ***	(5)
Wheat	162/197 (69.1)	176/197 (75.5)	crop x date ± 7.1 ***	(45)
			organ x date ± 4.1 ***	(9)
Total	508/1443 (33.6)	442/1443 (30.8)	crop x date x organ ± 10.1 *	(45)

Combined data from 10 samplings collected from 12 July to 13 September. The figures represent the number of plants infected on the number of plants analysed for each category. The angular transformed percentage is presented in brackets.
[1] Treatments were: * significant at 5% level, *** significant at 0.1% level.

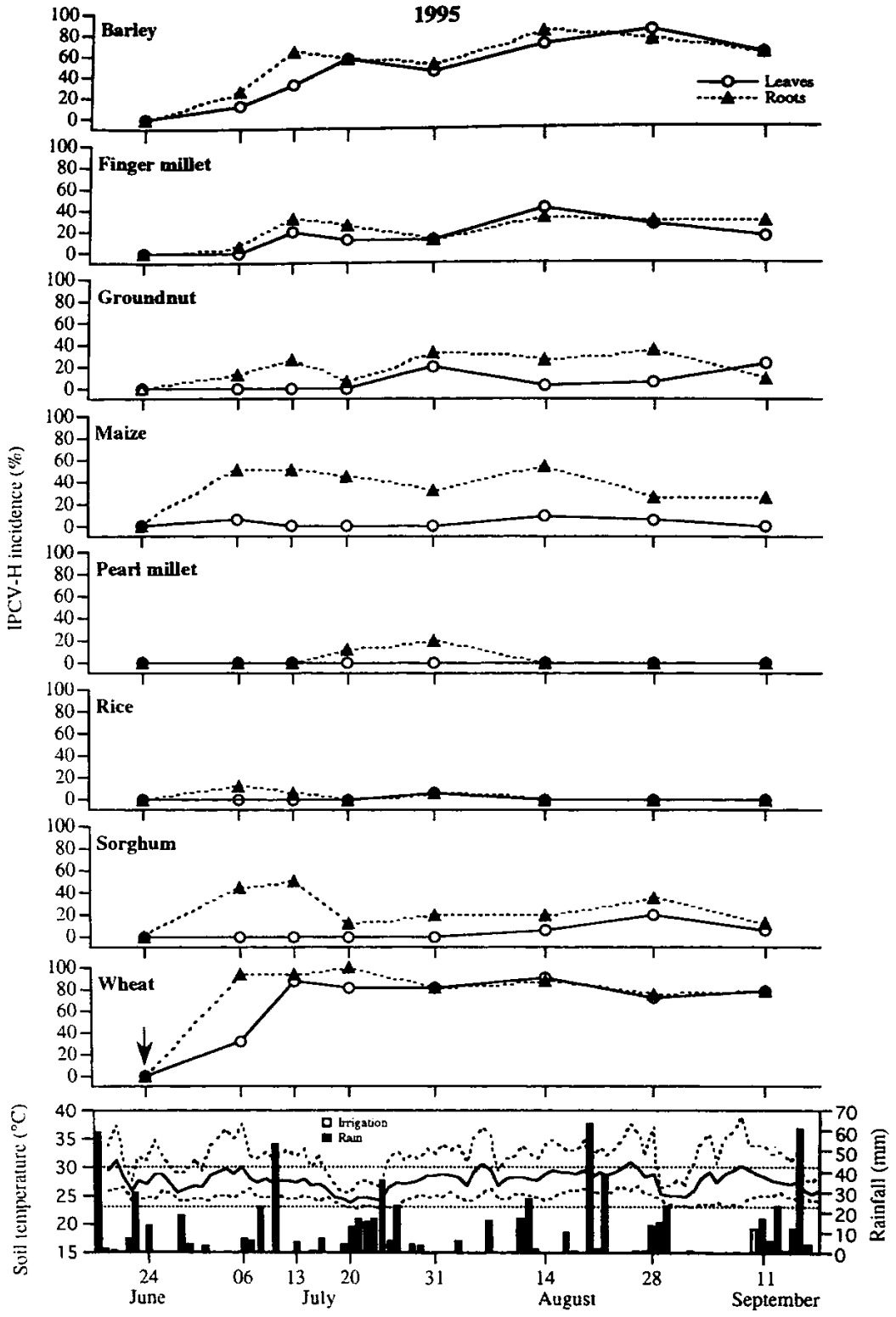

Figure 2. Progress of IPCV-H incidence in roots and leaves of various crop plants grown in the field during the 1995 rainy season and uprooted at weekly intervals (samples of 6, 13, 20 July) or two weeks intervals (samples of 31 July to 11 September). The arrow indicates the date of sowing. Daily mean, minimum and maximum soil temperatures at 10 cm below soil surface (the horizontal lines border the temperature range known to be conducive for *P. graminis* development), daily rainfall (black columns) and irrigation (white columns). The SEDs are given in Table 5.

1996. Among the six crops tested in 1996, wheat was again the one showing the highest virus incidence over the entire season (Table 6 and Fig. 3). It was true for both leaves and roots. A great number of wheat plants were moribund or dead at the end of the growing season. The virus was readily detected in all the crops right from the first sampling on 12 July, 17 days after sowing. It is probable that an incubation period of one week is necessary after the infection has occurred for the virus to be detectable by ELISA in root extract. Therefore virus incidence in roots must be linked to the weekly rainfall that occurred at least one week before uprooting the plants from the field. For instance, all the crops showed a reduced virus incidence in their roots for the sampling of 16 August which was preceded by a period of two dry weeks, the weekly rainfall recorded between 2 and 9 August reached only 20.6mm (Fig. 3). A daily rainfall superior to 40 mm on 10 August appeared to be conducive to an increased incidence in the plants sampled on 23 August. These plants were also exposed to a series of small rains a few days before the sampling. From 23 August to 13 September, wheat, maize and groundnut showed a relatively high virus incidence in both leaves and roots. This incidence could be linked to the relatively high weekly rainfalls recorded in August during the weeks 16-23 (173.7 mm), 23-30 (198.7 mm) and the rainfall that occurred on 3 September (46 mm).

Young groundnut plants showed similar virus incidence in both roots and leaves. At later stages during the season, an increasing delay was observed between the timing of detection of the virus in leaves compared to roots (Fig. 3). The peak incidence observed in leaves on 2 August already occurred in roots on 26 July (1 wk delay) while the peak of 23 August in leaves is most likely the result of systemic virus movement of root infection detected on 9 July (2 wk delay). At the end of the season the delay for virus detection in leaves compared to roots increased up to 3 wk. Virus incidence in leaves increased on 13 September while in roots the increase in incidence was noticed on 23 August. From the total of plants analysed during the 1996 season, groundnut and maize showed a higher virus incidence ($P \leq 0.05$) in roots than in leaves (Table 6).

During the first week of October the plants remaining in the plots were all tested by ELISA for the presence of the virus in leaves. The results showed that 34/50 groundnut, 45/141 maize, 3/202 pearl millet, 1/128 rice, and 43/197 sorghum plants were infected. All wheat plants were dead at that time.

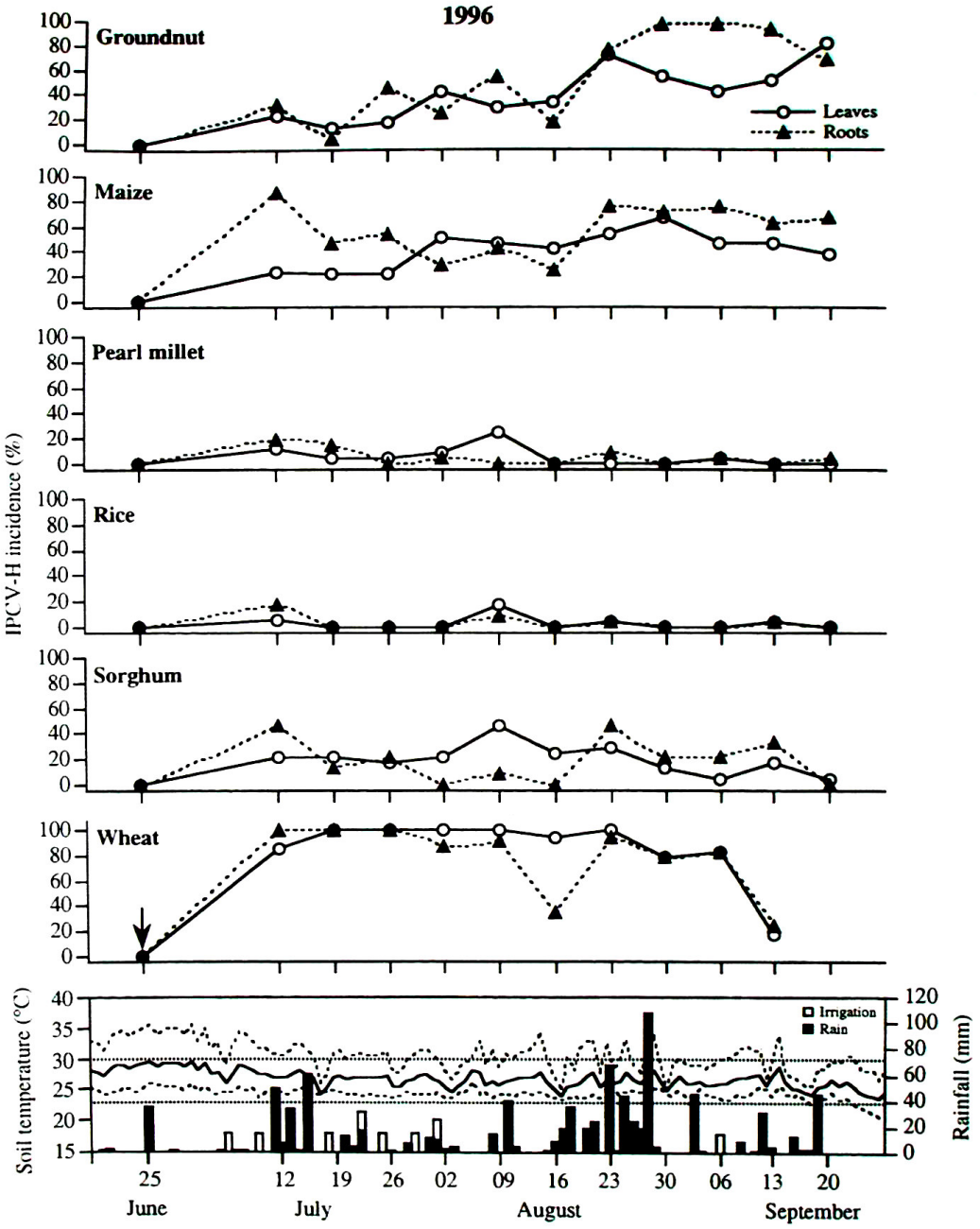

Figure 3. Progress of IPCV-H incidence in roots and leaves of various crop plants grown in the field during the 1996 rainy season and uprooted at weekly intervals. The arrow indicates the date of sowing. Daily mean, minimum and maximum soil temperatures at 10 cm below soil surface (the horizontal lines border the temperature range known to be conducive for *P. graminis* development), daily rainfall (black columns) and irrigation (white columns). The SEDs are given in Table 6.

Over the entire season, *P. graminis* was readily detected in roots of 16/227 maize, 38/225 pearl millet, and 140/249 sorghum plants. In sorghum *P. graminis* incidence was nil 1 WkAS, then it increased and already reached a plateau (over 50%) 3 WkAS. *P. graminis* incidence in pearl millet oscillated between 0 and 38%. Wheat (2/191) and rice (13/246) showed plants with few sporosori in their roots.

IPCV-H incidence in 1995 versus 1996. For all the crops combined virus incidence recorded in the roots in 1996 (508/1443 = 35%) was similar to that observed in 1995 (420/1280 = 33%) (P=0.1887, Chi square test, 1 d.f.). However, virus incidence in leaves was significantly higher in 1996 (442/1443 = 31%) compared to 1995 (275/1280 = 21%) (P<0.0001, Chi square test, 1 d.f.). This was mostly due to low virus incidence in leaves of sorghum, maize and groundnut in 1995 (Table 5). If systemic movement of IPCV occurs in part through xylem as it was reported for potato mop-top virus (PMTV), another furovirus (Jones, 1975), higher evaporation during the early stage of crop development in July 1996 (177.4 mm) compared to July 1995 (143.1 mm) may have favoured systemic virus infection in 1996.

P. graminis and IPCV-H incidence in plants exposed for a short period in the field

1994. IPCV-H was never detected in the leaves of field-exposed plants assayed at the time of retrieval from the field. The two species tested, sorghum and pearl millet, hosted *P. graminis* sporosori (Table 7). The plasmodiophorid was detected for the first time in the samples collected on 18 July (Fig. 4). Only sorghum was infected with 16.6% incidence. A peak of infection was observed on 1 August. Sorghum and pearl millet showed equal percentages of infection (37%) (Fig.4-A) and mainly the plants collected in the H-IPCV infested plots were infected (Fig.4-B). Based on the observations made on the entire season it was apparent that *P. graminis* incidence on the plants exposed during one week in the field was positively correlated with the weekly rainfall (WR) recorded during the period of exposure (Fig.4-A and 4-D). A logarithmic regression could be established between the incidence of *P. graminis* in the roots of sorghum and pearl millet plants combined and the weekly rainfall recorded during the week of exposure (*P. graminis* incidence = - 58.287 + 56.865 log (WR), R = 0.809 with 6 d.f.). According to this regression a WR superior to 10 mm is required to induce infection by the vector and the lowest weekly rainfall recorded during the season (14 mm) was sufficient to induce *P. graminis* infection.

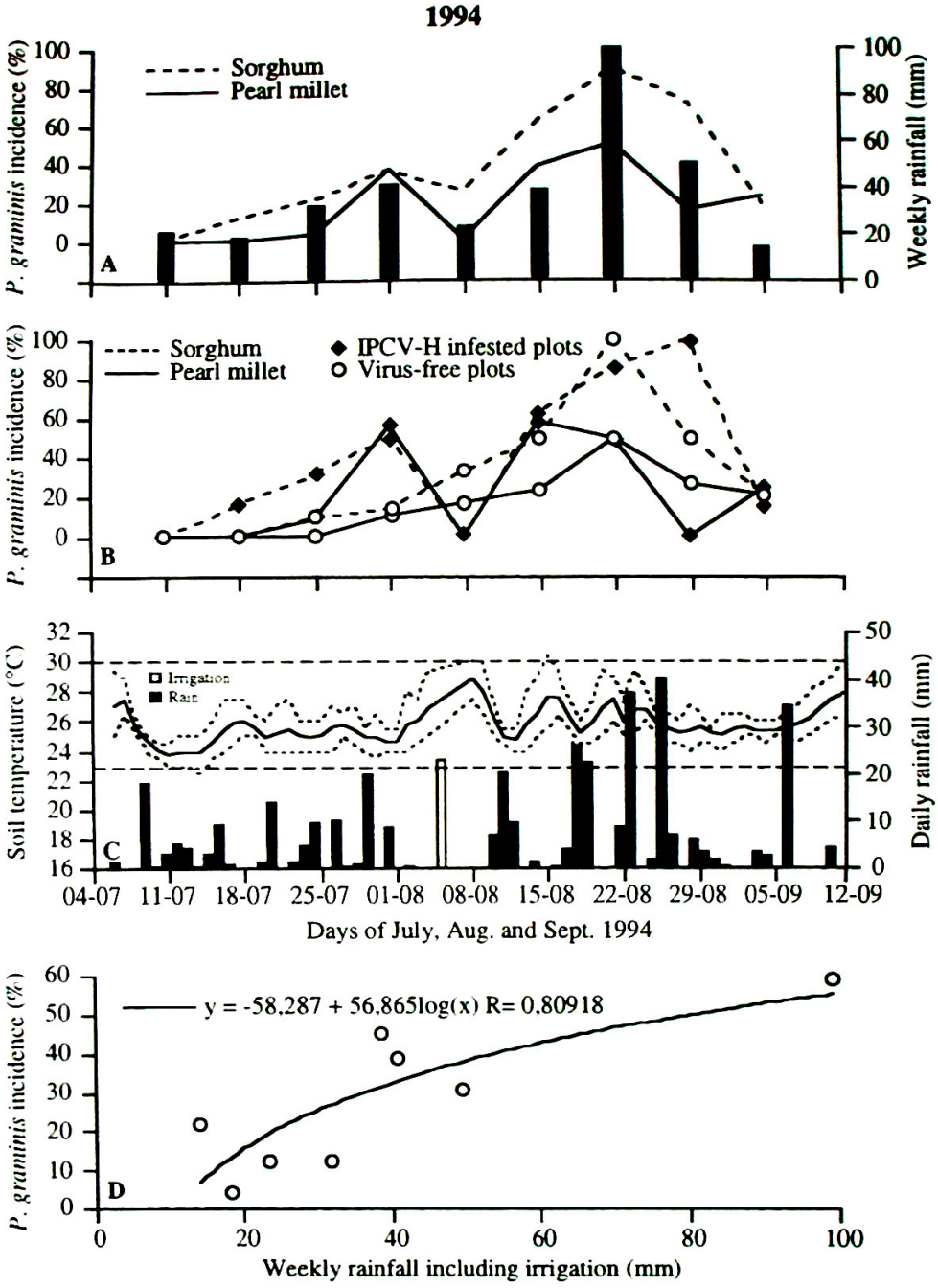

Figure 4. Progress of *P. graminis* incidence in roots of sorghum and pearl millet seedlings exposed for one week in the field during the 1994 rainy season, then maintained in a glasshouse for 8 to 10 weeks before analysis. **A.** Mean percentage calculated for the total of plants grown in IPCV-H infested and virus-free areas and corresponding weekly rainfall recorded during the period of exposure in the field, and **D**, relationship between the two. **B.** Percentage for the plants grown in IPCV-H infested and virus-free areas respectively. **C.** Daily mean, minimum and maximum soil temperatures at 10 cm below soil surface and daily rainfall (the horizontal lines border the temperature range known to be conducive for *P. graminis* development). The SEDs are given in Table 7.

Table 7. *P. graminis* incidence (angular transformation) in roots of plants exposed for one week in the field during the 1994 rainy season, then transplanted to sterile sand and maintained in a glasshouse to favour *P. graminis* development.

Crops	IPCV-H infested area[1]	Virus-free area
Pearl millet	29/111 (22.4)	18/104 (18.0)
Sorghum	27/80 (35.1)	13/51 (28.4)
$SED_{\text{crop (1 d.f.)}}$	5.5 *	6.1 NS
$SED_{\text{date of sampling (8 d.f.)}}$	11.6 **	13.0 **
$SED_{\text{crop x date (8 d.f.)}}$	16.9 **	18.4 NS

Combined data from 9 sets of plants exposed in the field. Retrieval from the field started 11 July and ended 5 September 1994. The figures represent the number of plants infected / number of plants analysed; the angular transformed percentage is indicate in brackets.

[1] None of these plants tested positive for IPCV-H presence in their leaves by ELISA at the time of uprooting from the field.

Treatments were: NS not significant, * significant at 5% level, ** significant at 1% level.

1995. All the crops except rice were found to be hosts for IPCV (Fig. 5). Wheat, barley and maize showed a high virus incidence in the leaves. Among all the crops wheat showed the highest virus incidence. It was also the crop along with barley for which a significant number of leaf samples tested positive directly after the period of plant exposure in the field. IPCV migration from roots to shoots was thus very fast in wheat and barley. Generally speaking after incubation in a glass-house, virus incidence in leaves was higher than in roots. This was probably due to root rotting in glass culture tubes during the incubation period and consequently virus infection was lost. Finger millet, groundnut, sorghum, pearl millet and rice showed a very weak virus incidence. *P. graminis* was detected in 7/141 sorghum, 7/137 pearl millet and 1/141 maize plants. The mortality of plants exposed in the field and then maintained in a glasshouse was relatively high during the 1995 season. The survival rate varied from 46% for wheat to 77% for maize and therefore no evident correlation appeared between virus incidence in the crops and rainfall (including irrigation) recorded during the period of exposure in the field.

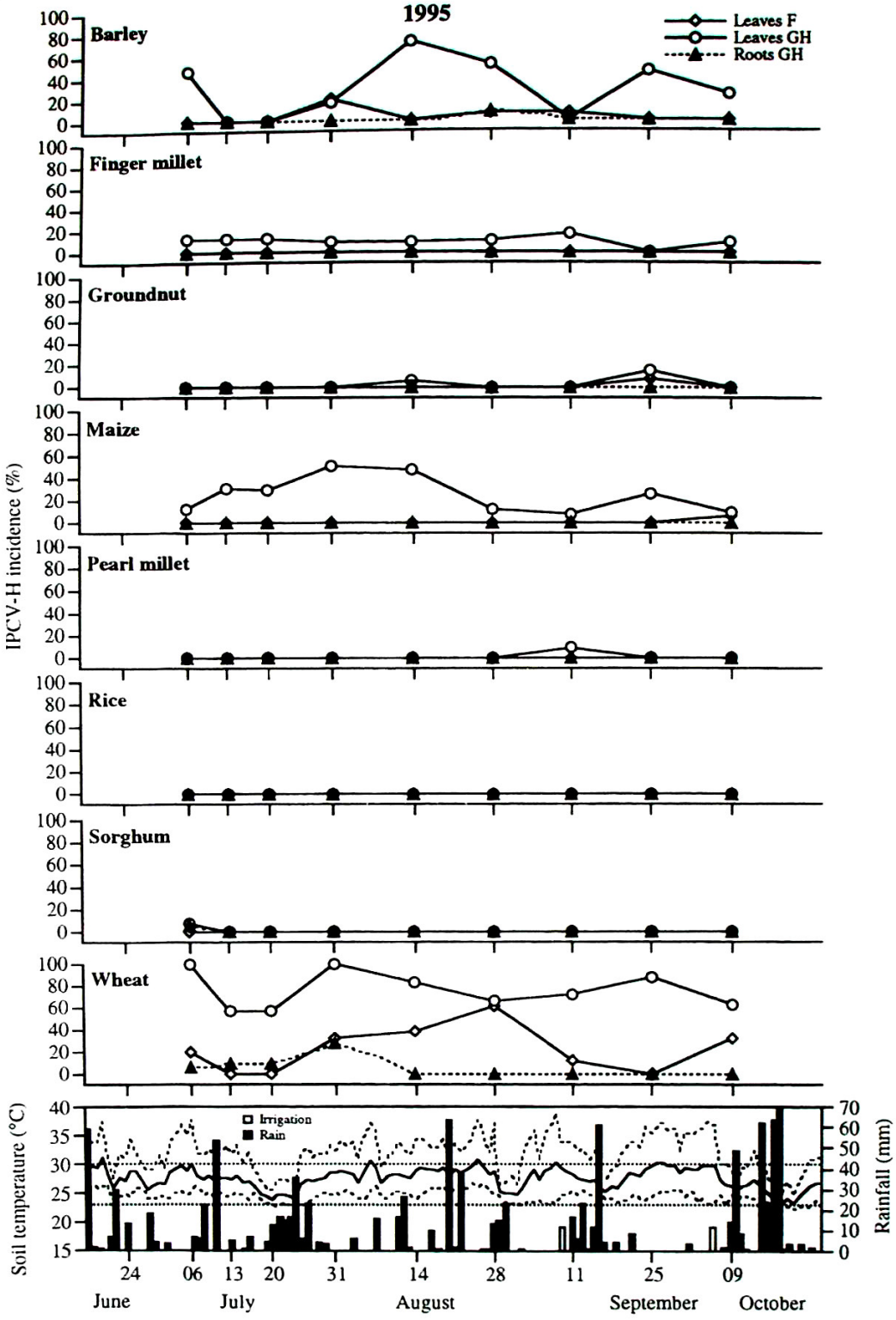

Figure 5. Progress of IPCV-H incidence in seedlings of various crops exposed for one week (samples of 6, 13 and 20 July) or two weeks (samples of 31 July to 9 October) in a infested field during the 1995 rainy season, then maintained in a glasshouse for 6 weeks before analysis. ELISA tests were performed on leaves immediately after the period of exposure in the field (♀) and, on leaves (♂) and roots (*) after the incubation period under glasshouse conditions. Daily mean, minimum and maximum soil temperatures at 10 cm below soil surface (the horizontal lines border the temperature range known to be conducive for *P. graminis* development), daily rainfall (black columns) and irrigation (white columns).

1996. All the crops were found to be hosts for IPCV (Table 8 and Fig. 6). *P. graminis* was detected exclusively in maize roots (11/372). The survival rate of these plants after field exposure was in the range of almost 90% for rice to 100% for groundnut (Table 8). After the incubation period in a glasshouse in an automatic immersion system, the survival rate was higher than in 1994 and 1995. It ranged from 91% for rice to 86% for wheat from the total of plants effectively transplanted to a glasshouse. Wheat showed again the highest rate of IPCV incidence (Table 8). Wheat showed a significantly higher incidence in leaves than in roots while for groundnut the contrary was true. For all the crops combined the incidence in roots was equivalent to that observed in leaves which is evidence of systemic virus infection. The total proportion of plants that became infected by the virus after exposure in the field was lower in 1996 (202/2013) than in 1995 (114/768) for the leaves (P=0.0004, Chi square test 1 d.f.). For the roots the contrary was observed, a higher virus incidence was recorded in 1996 (194/2088) than in 1995 (9/1088) (P<0.0001, Chi square test 1 d.f.)

Table 8. IPCV-H incidence in various crop plants exposed for one week in the field during the 1996 rainy season, then transplanted in steamed sand and maintained in a glasshouse to favour virus replication.

	After the period of exposure in the field	After the incubation period in a glasshouse at 25-30°C		Significance in ANOVA[1]	
	IPCV-H	IPCV-H[1]			
Crops	Leaves (n=360)	Leaves (n=360)	Roots[2] (n=360)	SEDs	(d.f.)
Groundnut	1/369 (2.7)	3/369 (3.4)	15/369 (9.1)	crop ± 0.91	(5)
Maize	0/373 (2.3)	16/366 (7.2)	21/372 (8.9)	date ± 1.44	(14)
Pearl millet	2/348 (3.1)	7/342 (5.0)	8/348 (4.6)	organ ± 0.64	(2)
Rice	0/320 (2.5)	10/292 (6.2)	4/303 (4.0)	crop x date ± 3.54	(70)
Sorghum	0/358 (2.4)	3/332 (3.6)	3/350 (3.4)	crop x organ ± 1.58	(10)
Wheat	10/362 (4.6)	163/312 (49.5)	143/346 (39.4)	date x organ ± 2.50	(28)
				crop x date x organ ± 6.13	(140)
Total	13/2130 (2.9)	202/2013 (12.5)	194/2088 (11.6)		

Combined data from 15 sets of plants exposed in the field for one week. Retrieval from the field started 2 July and ended 8 October. The figures represent the number of plants infected / number of plants analysed for each category and the angular transformed percentage is presented in brackets. (n) is the targeted number of plants exposed in the field for each crop. The number of plant effectively analysed gives information on the survival rate of the plants after exposure in the field or incubation in a glasshouse.

[1] The treatments, their two-by-two interactions and the interaction between the three were all significant (P≤0.001).

[2] These also included plants that were found moribund during incubation and tested for the presence of the virus in their roots.

1996

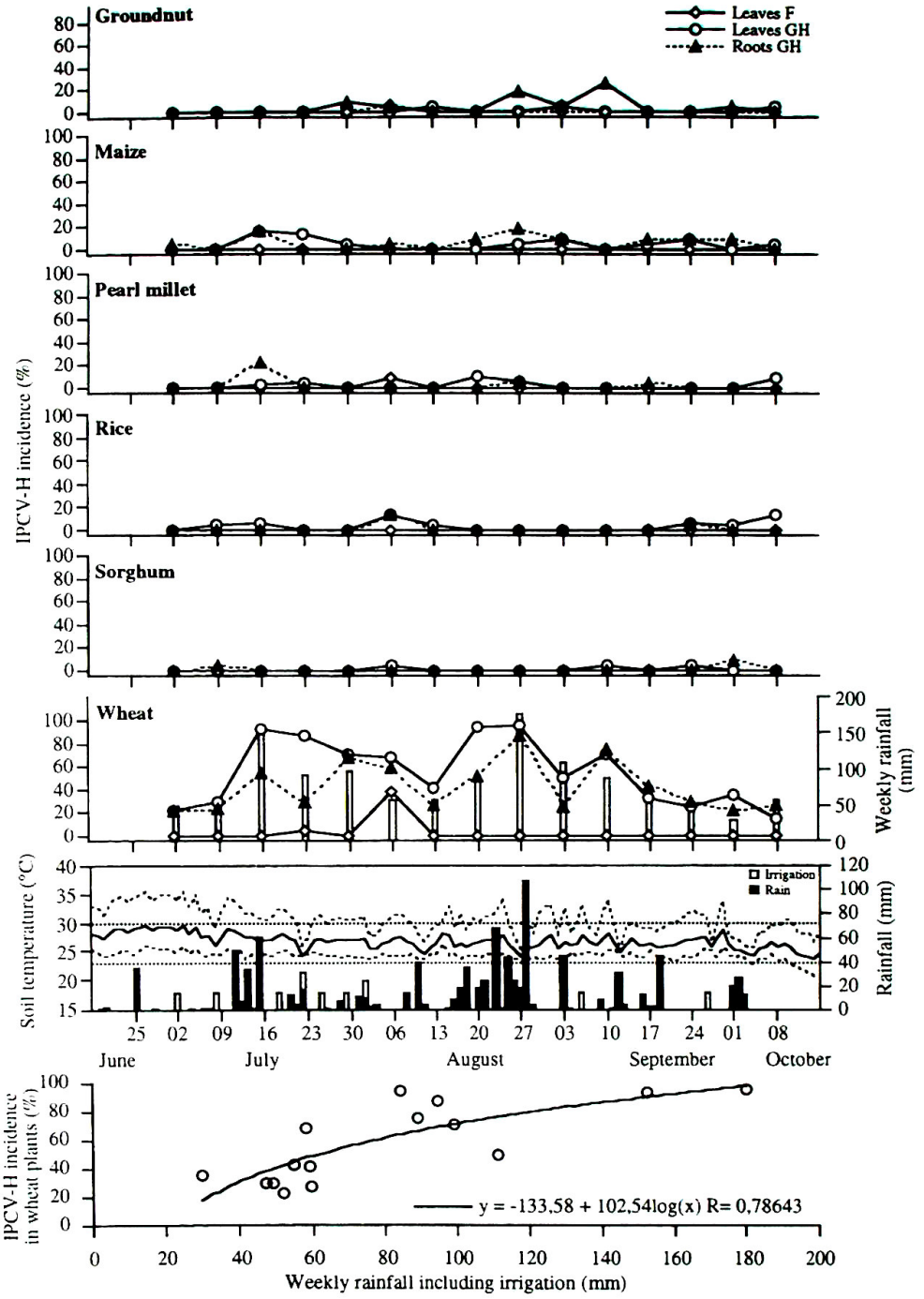

Figure 6. Progress of IPCV-H incidence in seedlings of various crops exposed for one week in a infested field during the 1996 rainy season, then maintained in a glasshouse for 4 to 5 weeks before analysis. ELISA tests were performed on leaves immediately after the period of exposure in the field (♀) and, on leaves (♂) and roots (⚥) after the incubation period under glasshouse conditions. Daily mean, minimum and maximum soil temperatures at 10 cm below soil surface (the horizontal lines border the temperature range known to be conducive for *P. graminis* development), daily rainfall (black columns) and irrigation (white columns). The weekly rainfalls (including irrigation) recorded during the period of exposure in the field are indicated in the figure given for wheat plants and the relationship between IPCV-H incidence in wheat plants and the weekly rainfall that occurred during exposure in the field is given in the last graphic. The SEDs are given in Table 8.

After the incubation period under glasshouse conditions, the progress of virus incidence in leaves was very similar to that of the roots and both were much higher than the incidence observed in the leaves tested immediately after retrieval from the field, the latter being not detectable for most of the crops (Fig. 6). Only wheat showed a peak of virus incidence in the leaves tested at the time of plant retrieval from the field on 6 August. This increase in incidence remained unexplained. The virus incidence in wheat plants tested after the incubation period in a glasshouse was closely related to the weekly rainfall (WR) that occurred during the period of exposure in the field (Fig. 6). There were two major periods with high virus incidence for the second week of July and for the last part of August. Both coincided with periods of high rainfall. A logarithmic regression could be established between virus incidence in wheat plants and weekly rainfall (WR) recorded during the period of exposure in the field [IPCV-H incidence = - 133.58 + 102.54 log (WR); $R = 0.7864$ with 13 d.f.].

Discussion

The number of plants analysed during these experiments was generally small and as a consequence it induced a high variability in the results. Nevertheless, the work was repeated over three rainy seasons allowing to know the major trends of the epidemiology of clump disease. Temperature, rainfall, and the hosts were major factors that influenced the epidemiology.

Temperature

The results indicated that conducive conditions occurred for infection during early stages of groundnut establishment in rainy season. High temperature during storage have been shown to increase expression of *P. graminis* inoculum from root (Legrève *et al.*, 1999). Drought and heat also favours infection by the virus. The results of a former bioassay using the most probable number method and sorghum (ICSV 88036) as bait plants grown on serial dilution of soil in sand, have shown that IPCV-H inoculum potential of a soil sample incubated at 38°C for 6 weeks was higher (89±31 infective units per litre of soil) than that of the same sample stored at room temperature (about 25°C) for the same period of time (32±17 infective units per litre of soil) (Delfosse *et al.*, unpublished). Dry and hot summers (April-June) in India, preceding the monsoon, are therefore conducive to the breakdown of the dormancy of *P. graminis* resting spores and consequently favour early infection and high virus incidence in groundnut crops sown with the onset of monsoon rains (Delfosse *et al.*, 1996).

Temperature during the rainy season did not appear to be a limiting factor for disease occurrence in Hyderabad area. The optimum temperature for infection by *P. graminis* is between 27 and 30°C. Below 23°C, infection is suppressed and the fungal development is delayed (Legrève *et al.*, 1998). During most of the three rainy seasons, the mean soil temperature varied from 23 to 30°C and was thus conducive for infection. The virus replicates well over the same range of temperature as the fungus but if mechanically inoculated, IPCV can also infect wheat at 15°C (Reddy *et al.*, 1988). Temperatures higher than 30°C are not favourable to the virus. Symptoms on IPCV infected groundnut plants collected in the field tended to disappear if the plants were maintained in a glasshouse at temperature above 30°C. After few weeks, the virus itself was difficult to detect by ELISA (Reddy, personal communication).

Seasonal variations in peanut clump disease incidence have been reported (Reddy *et al.*, 1988). Groundnut crops grown in post-rainy season are negligibly infected whereas wheat crops sown during the same season showed relatively high disease incidence (Delfosse *et al.*, 1999). Initially, low temperatures prevailing during the post-rainy season were suspected to be responsible for such low incidence in groundnut crops (Reddy *et al.* 1988). However high incidence recorded in wheat indicated that temperature was not the only factor involved and that the host was likely to play an important role.

Rainfall

The results showed clearly that rainfall played a preponderant role in natural infection by *P. graminis* and IPCV. This is probably due to the ecological requirement for completion of *P. graminis* life cycle which is known to be favoured by alternate watering and drainage (Adams *et al.*, 1986). On the contrary continuous water logging is suspected not to be favourable to plasmodiophorids (Ledingham, 1939, Cooper, *et al.*, 1976, Inouye, 1977). Soils where peanut clump disease occurs are generally light textured which assures excellent drainage even during the monsoon characterised by heavy rains. In such soils with high drainage, adequate levels of free water for zoospores movement become available with the occurrence of relatively high rainfall. In our field experiments it was apparent that if heavy rainfall occurred during the period the young seedlings were exposed in the field, it resulted in high *P. graminis* and IPCV incidence. The correlation was particularly evident for the 1994 and 1996 seasons. The correlation between *P. graminis* incidence and amount of rainfall was also observed for the plants grown exclusively in the field

and sampled at weekly interval. In this case *P. graminis* incidence was influenced by the weekly rainfall that occurred 15 days before sampling from the field, the time required for *P. graminis* to produce sporosori in the root cortex (Ratna *et al.*, 1991, Legrève, 1999). Maraite and Legrève (1994) reported that *P. graminis* from temperate areas infected barley plants grown in the field not at the first rain but usually at the second rain, then the number of *P. graminis* infected plants increased rapidly to reach a plateau 2 months after sowing. The authors suggested that *P. graminis* required a period of rain to provide enough soil moisture and prepare resting spores for germination. In our experiments, a succession of rainy days or a punctual high rainfall were favourable to *P. graminis* infection and to virus transmission.

Rainfall that occurred in June 1995 prior to sowing the groundnut crop were not favourable to high disease incidence. Rains allowed establishment of weeds, mostly *Cyperus rotundus* L., an excellent host for *P. graminis* (Delfosse *et al.*, 1996). *C. rotundus* may have trapped some of the inoculum before soil preparation for sowing. Similarly, in recent experiments, pearl millet was successfully used as a trap crop to reduce peanut clump disease incidence (Delfosse *et al.*, 1997).

The hosts

The various crops analysed during successive rainy seasons showed clearly different levels of susceptibility to infection by IPCV and *P. graminis*. The hosts showing the highest virus incidence were wheat, barley, maize and groundnut. Groundnut plants were susceptible to infection during the early stage of crop growth, mostly during the first month following the sowing of the crop. Because infection occurred early, the growth of young plants was suppressed by the virus and this explains why the majority of the plants show a severe stunting as compared to healthy plants. Groundnut roots are usually devoid of hairs and a distinct epidermis. Root hairs disappear few days after sowing (Porter *et al.*, 1984, Ramanatha Rao and Murty, 1994, Sprent, 1994). Groundnut roots present a drying and sloughing surface and absorption occurs mainly in young primary roots with active meristematic cells underlying the drying outer layer. A priori, a young epidermis should be more susceptible to infection by *P. graminis* than the sloughing and suberised layers that constitute the root surface of old groundnut plants. Recent experiments on the susceptibility of groundnut related to plant age, reinforced this reasoning. Groundnut susceptibility to IPCV infection decreased with age (Delfosse *et al.*, unpublished, Chapter 5-3).

As the groundnut crop is susceptible to infection when young, if climatic conditions are not conducive for infection when the crop is young, disease incidence and disease severity will be low even if conducive conditions occurs at later stages. This was confirmed in recent experiments with different dates of sowing. When groundnut was sown early (June) before the onset of monsoon rains and maintained with minimal irrigation not to favour *P. graminis* infection, IPCV incidence was very low and infected plants showed stunting less severe compared to crops sown with the onset of monsoon rains. On the other hand crops which were sown later during the rainy season in India (August) and exposed to relatively low rainfall during the first weeks of crop development also showed low disease incidence (Reddy *et al.*, 1988, Delfosse *et al.*, unpublished). It is however not advisable to delay the sowing of groundnut because low temperature or drought at the end of the cropping season can cause a delay in maturity or a plant weakness allowing seed infection by *Aspergillus flavus,* a fungus responsible for aflatoxin contamination (Mehan, 1988).

The majority of groundnut plants exhibit symptoms 2 to 3 wk after virus infection was detected in roots. These observations confirm the reports of Reddy *et al.* (1988) and Thouvenel *et al.* (1988) for IPCV and PCV respectively. Groundnut differed from wheat in this aspect. While wheat supported fast and systemic virus infection, IPCV movement in groundnut from roots to leaves was relatively slow. Groundnut extracts usually give lower absorbance in ELISA tests than wheat extracts probably as a result of a lower virus concentration in groundnut than in wheat tissue. The lower susceptibility to infection and a slower virus movement in old groundnut plants resulted in lower seed transmission frequencies for late infected plants compared to early infected ones. Therefore plants that were infected at early stage represent the major source of virus infected seed and seeds from such plant should not be harvested.

Sorghum (ICSV-88036) and pearl millet (ICMH-451) supported *P. graminis* multiplication well but systemic infection by the virus into leaves seldom occurred in the cultivars studied. In laboratory and field tests ICSV-88036 could be infected by the virus at seedling stage. However a few weeks later the virus could no longer be detected in newly produced leaves. Sorghum and pearl millet are transient and symptomless hosts for IPCV. However it is unclear if the virus causes any yield loss in these crops. Symptomless hosts for IPCV such as sorghum and millets and the absence of clear symptoms in maize increase the risk of spread and carry-over of clump disease if these crops are grown in IPCV infested fields. Indeed, they play an important role in the epidemiology of clump disease because they support *P. graminis* multiplication well and, in addition to

groundnut and wheat, the virus is also seed transmitted in millets and maize (Reddy *et al.*, 1998, Delfosse *et al.*, 1999). Recent experiments showed that sorghum and pearl millet accessions chosen from the ICRISAT gene bank for their distinct geographical origins, differed in their susceptibility to *P. graminis* and IPCV infection (Delfosse and Legrève, unpublished). Some sorghum and millet accessions strongly supported systemic virus infection and therefore may contribute to produce viruliferous sporosori of *P. graminis* in roots and consequently increase viruliferous inoculum potential in soils.

Wheat and barley are definitely the hosts showing the highest IPCV incidence during the rainy season under the conditions prevailing in Andhra Pradesh. When grown during the post-rainy season, wheat and barley crops also showed high virus incidence. In post-rainy season crops, the virus was seed transmitted in wheat and the viral antigen was detected in barley seed (Delfosse *et al.*, 1999). None of the barley roots analysed during the rainy season experiments contained *P. graminis* sporosori. Few *P. graminis* sporosori were detected in roots of 2 wheat plants. The plasmodiophorid was detected in rare roots of wheat plants grown in the post-rainy season (Delfosse *et al.*, 1999). In our experiments *P. graminis* was rarely detected in rice roots and infected plants did not show intense colonisation by sporosori. IPCV infection seldom occurred in this crop. Rice has been shown to host *P. graminis* in West African countries (Fauquet *et al.*, 1988) and in Japan (Usugi, 1988). Rice necrosis mosaic virus, thought to be transmitted by *P. graminis* (Inouye and Fujii, 1977) was reported to infect rice crops in India (Ghosh, 1981). We tested only a single rice cultivar and further tests are probably required to yield conclusive data on the ability of *P. graminis* from tropical India to develop on rice.

Detailed studies on the host range of *P. graminis* from tropical and subtropical regions of India confirmed our findings (Legrève, 1999). The author reported that only a trace of infection by *P. graminis* from tropical India occurred on wheat, barley, rice and groundnut. In both the present experiment conducted under field conditions and those conducted by Legrève under controlled environment, *P. graminis* from tropical India was mainly detected in sorghum, pearl millet and maize in which numerous sporosori were observed, and it occurred also in few plants of finger millet. The crops that support well multiplication of *P. graminis* from tropical India all have a C4 photosynthetic pathway and are of tropical origin. In contrast, *P. graminis* from temperate areas, including sub-tropical isolates from Rajasthan in India, has been reported to develop well on C3 plants

such as barley, wheat and oat (Barr, 1979, Bastin *et al.*, 1989, Adams and Jacquier, 1994, Legrève, 1999).

Other factors

Young plants exposed for one week in the field, uprooted and transplanted in a glasshouse to allow the fungus to continue its development showed higher colonisation by *P. graminis* in their roots than the plants grown in the field and directly analysed. The infection was generally severe with numerous sporosori of smaller size compared with the sporosori observed in the roots of the plants sown in the field. Transplanting experiments into and out of infested soil have suggested a higher susceptibility to infection in younger plants (Usugi, 1988 and Adams, 1990). The transplanting of young seedlings appears to be an excellent technique to study the epidemiology of the disease. The technique could also prove useful for isolation of *P. graminis* from tropical soils which has been shown to be difficult (Legrève, 1999).

The vector incidence was generally found to be lower than the virus incidence. A possible explanation for this is that chances to lose *P. graminis* infection is likely to be higher than the risk of losing the virus. Indeed, *P. graminis* multiplies and invades roots at much slower rate compared to virus replication and movement. Plants retrieved from the field were exposed to water stress before transplanting to a glasshouse. The stress resulted in the death of many of the roots. Most of the cereal plants can produce new roots to assure their survival but it is highly probable that *P. graminis* in its early stages of development will not survive in moribund or death roots and therefore the parasite infection was lost. For these reason it is recommended to reduce as much as possible water stress between sampling from the field and transplanting to a glasshouse by reducing the number of plants being handled.

The plasmodiophorid incidence on the plants grown in the field often progressed erratically during the season instead of increasing regularly to a plateau. Resting spores are localised in the root cortex and as the roots age, the suberisation progresses and the cortex peels off releasing sporosori in the soil (Adams, 1988). This may explain why *P. graminis* was detected only in the roots of young groundnut plants (3 wk old seedlings) Ratna *et al.* (1991) and not in older plants (Thouvenel *et al.*, 1988).

Virus incidence also varied greatly during the rainy season. We collected a portion of the youngest leaves for the ELISA tests. The crop growth can also influence the detection by ELISA. Crop plants such as maize, millet

and sorghum have a faster aerial and subterranean growth than barley, finger millet and wheat. If the plant growth is faster than the virus replication and movement, it is possible that ELISA could not detect the virus in the youngest leaves whereas it could have been present in older leaves. Cereal crops such as sorghum and pearl millet were transient for virus presence and usually one month after infection the virus may no longer be detected in plants which were originally tested positive. This was confirmed by ELISA tests conducted on leaves at the end of the growing season. Very rarely, sorghum and pearl millet plants were tested positive for virus presence. Groundnut, once infected, remained infected for life. This is in line with the regular increase in disease incidence in the groundnut crops based on visual symptoms during the rainy season before it reached a plateau by the end of August or beginning of September. In wheat and barley which showed very high virus incidence and systemic infection, the progress of virus incidence in field grown plants quickly reached a plateau close to maximum incidence and remained high throughout the rainy season with only minor variations.

Conclusion

We have shown that the various crops studied were all hosts for IPCV under natural environment. However virus incidence varied greatly according to the crop. Apparently crop species that were recently introduced to the Indian sub-continent (groundnut and maize) or crop species (wheat and barley) which were grown in Andhra Pradesh, a marginal area for their production, for the purpose of the experiment, showed the highest incidence. Groundnut and maize are considered to be relatively recent introductions to the Indian sub-continent. They are American in origin. The time of their introduction to India, probably the 15^{th} century, is still the source of controversy but it is a general consensus that their cultivation on significant scale in India was initiated about 150 years ago (Sinha and Bhagat, 1988, Norman *et al.*, 1984, Singh and Nigam, 1997). Pecluviruses were not reported to occur in America. In contrast, sorghum, pearl millet and finger millet are ancient and traditional crops of the Indian agriculture. They were introduced from western and eastern Africa (Norman *et al.*, 1984) where pecluviruses have been reported to occur (Thouvenel *et al.*, 1988 and Chatenet and Saeed, 1995). It is suggested that pecluviruses are not typical groundnut viruses but actually gramineae viruses that possibly co-evolved in tropical and sub-tropical areas with wild grasses and cereal crops such as millets and sorghum. This hypothesis is reinforced by the fact that the vector of IPCV, *P. graminis* multiplies well in millet and sorghum and is often detected in grassy weeds (Chapter 3). IPCV is a seed transmitted and soil-borne virus with a wide

host range and a complex epidemiology. The virus inoculum is built up in monocotyledonous hosts in which it causes little damage but when transmitted to susceptible crops such as groundnut, it causes an economically important disease.

The epidemiology of IPCV was found to be closely linked to the epidemiology of *P. graminis*. Various parallelisms were observed: (*i*) high rainfall resulted in high virus incidence but also induced high *P. graminis* incidence in monocotyledonous hosts, (*ii*) *P. graminis* from tropical India is favoured by relatively high temperatures in the range 23-30°C (Legrève *et al.,* 1998) and low temperatures in post-rainy season resulted in low disease incidence in groundnut crops, (*ii*) drought and heat during storage increases germinability of *P. graminis* resting spores (Legrève *et al.*, 1999) and groundnut crops sown after the dry and hot summer with the onset of monsoon rains were found to be severely affected by clump disease (Delfosse *et al.*, 1996). These observations corroborates the hypothesises that peanut clump disease epidemiology is closely influenced by the ecological requirements of its vector as it was reported for other virus diseases transmitted by the genus *Polymyxa* (Schlösser, 1988, Usugi, 1988, Brakke and Langenberg, 1988, Adams, 1990, Goffart, 1992, Tuitert and Homeester, 1992, Ohto and Naito, 1997, Carroll *et al.*, 1997).

Results of these epidemiological studies provided information to formulate new ways of controlling peanut clump disease. The hypothesises formulated were tested under field conditions during the last years. An early sowing of groundnut crop before the onset of monsoon rains, under judicious irrigation, was shown to be a simple and efficient cultural method to reduce disease incidence in irrigated areas (Delfosse *et al.*, unpublished). Inspired from the baiting technique used in these experiments to monitor IPCV and *P. graminis* infection, a trap cropping method using pearl millet was developed and tested successfully at different sites in India (Delfosse *et al.*, 1997). Since *P. graminis* multiplies intensively in monocots, cropping systems devoid of such hosts will not contribute to build up inoculum potential. We are currently evaluating various post-rainy season crops that could beneficially be rotated with groundnut to reduce peanut clump incidence.

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Conclusion on the epidemiology

We have shown that IPCV is an economically important seed-transmitted and soil borne virus with an extremely wide host range which includes both mono- and dicotyledonous species. Our observations on its epidemiology and the hypotheses they suggest for controlling the disease are summarized below.

- The **host range** of the virus was determined under natural conditions. The virus can infect many economically important crops which include wheat, barley, maize, sorghum, millets in addition to groundnut, pigeonpea various legumes and chillies. In fact it is the first time that conclusive evidences were presented to show that IPCV can cause severe disease in wheat, barley and pigeonpea. Additionally it has also been shown to cause crop losses in maize.

- The natural vector of IPCV, *P. graminis*, has been shown to have preference to monocotyledonous hosts. Our observation confirmed those of Legrève (1999) conducted under controlled environment. Monocotyledonous hosts for *P. graminis* include sorghum, pearl millet and maize which showed profuse number of sporosori in their roots. The parasite has also been shown to thrive in grassy pernicious weeds (*Cynodon dactylon*, *Cyperus* spp., *Dactyloctenium aegyptium*, *Digitaria ciliaris*, *Eleusine indica*, *Eragrostis* spp.).

- Since *P. graminis* do infect monocotyledonous hosts even when climatic conditions are not conducive for infection to dicotyledonous crops during the post-rainy season, it should be possible to use a preferred monocotyledonous host in a trap cropping strategy to reduce peanut clump disease incidence.

- Interestingly *P. graminis* infects but does not multiply intensively in dicotyledonous species including weeds and crops. This information has facilitated formulation of strategies to control the disease utilising crop rotation.

- We have shown that the amount and distribution of **rainfall** was a key factor that influenced the epidemiology of peanut clump disease. High rainfall resulted in high virus and *P. graminis* incidence. Groundnut plants appeared to be mostly susceptible to infection when they are young. If climatic conditions are not conducive for infection during the early stage of crop growth, disease incidence and severity will be low. This is again a valuable information for designing cultural practices for

controlling the disease. It suggested that an early sowing of groundnut crops, prior to the onset of monsoon rains, with the application of judicious irrigation, could possibly lead to significant reduction in disease incidence.

- We have identified some of the possible **primary sources** of virus inoculum which can lead to the establishment of the disease in new areas. To our knowledge, IPCV is the only plant virus which can be transmitted through seed of both dicotyledonous and monocotyledonous plants. Seeds of cereals which support well multiplication of tropical *P. graminis*, such as maize and millets, or wheat that support multiplication of sub-tropical *P. graminis*, present a high risk of establishing the disease to new areas in soil containing non-viruliferous *P. graminis*. A similar situation may occur with *C. dactylon* rhizomes which serve as a virus reservoir.