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RESEARCH ARTICLE

Survey of the incidence and distribution of five viruses infecting yams in the major yam-producing zones in Benin

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Cucumber mosaic virus; Dioscorea alata bacilliform virus; Dioscorea sansibarensis bacilliform virus; Yam mild mosaic virus; Yam mosaic virus; ELISA; IC-PCR; IC-RT-PCR.

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Abstract

Surveys were conducted in 2004 and 2005 to determine the incidence and distribution of viruses infecting yams in four major yam-producing agro-ecological zones in Benin. Yam leaves collected from 69 fields and one experimental screen house were indexed for Cucumber mosaic virus (CMV), Dioscorea mottle virus (DMoV), Yam mild mosaic virus (YMMV), Yam mosaic virus (YMV) and yam-infecting badnaviruses [Dioscorea alata bacilliform virus (DaBV) and Dioscorea sansibarensis bacilliform virus (DsBV)] by enzyme-linked immunosorbent assay and immunocapture polymerase chain reaction. Eighty-two per cent and 66% of leaf samples tested in 2004 and 2005, respectively, were infected with CMV, YMMV, YMV and/or badnaviruses. DMoV was not detected. Yam-infecting badnaviruses were the most prevalent virus infection, detected in 45% of the total leaves sampled followed by YMV (31%), YMMV (27%) and CMV (2%). Although the occurrence of CMV was low, this is the first record of CMV in yams in Benin. Mixed virus infections were detected in 48% (2004) and 39% (2005) of the infected leaves. A mixture of YMMV and badnaviruses (DaBV or DsBV) was the most common mixed infection detected. *Dioscorea alata*, with a higher incidence of badnavirus infection (81%), YMMV (51%) and CMV (8%) was more heavily infected than *Dioscorea rotundata*.

Introduction

Yams occupy a very important position as a food crop in Benin and were ranked first among 20 most important food and agricultural commodities of Benin in 2005 (FAO, 2005). Benin is the fourth largest producer of yams in the world, cultivating about 195 747 hectares of yams in 2006 (FAO, 2007). Because of the continued and increasing dependence on yams for food in Benin, its importance for food security and the need for improvement in yam production, farmers in Benin are continuously boosting the diversity of their plots by domestication of wild species (Dumont & Vernier, 2000) and a highly developed yam chips production system has emerged for storage and preservation of the valuable tubers (Vernier, 1998). Major yam production is in Zone Vivrière du Sud Borgou and Zone Ouest-Atacora; yams are also produced in Zone Cotonnière du Nord Bénin and Zone Cotonnière du Centre Bénin. Very little production (0–1%) is observed in the southern zones (Zone des Terres de Barre, Zone des Pêcheries and Zone de la Dépression), and yams are not produced in the Zone Extrême Nord Bénin (Akker, 2000).

Pests and disease are among the most important of the many factors that have deleterious effects on yam tuber yield and quality. Diseases caused by viruses, fungi, nematodes and bacteria either singly or in combination are responsible for yield losses (Nwankiti & Arene, 1978; Onwueme, 1978; Ng, 1992; Hughes *et al.*, 1997). Viruses are of particular concern because, apart from causing significant reduction in tuber yield and quality, they restrict international exchange of germplasm.

The use of infected vegetative propagules and uncontrolled introduction and exchange of infected planting materials by farmers through permeable land borders have resulted in the presence of yam viruses in most of the yam-growing areas in West Africa (Thouvenel & Fauquet, 1979; Thouvenel & Dumont, 1990; Goudou-Urbino *et al.*, 1996; Hughes *et al.*, 1997; Olatunde, 1999; Odu, 2002). Viruses infecting yams belong to the *Potyvirus*, *Badnavirus* and *Cucumovirus* genera, while a number of yam viruses remain unclassified. Viruses reported to infect yams in West Africa include Yam mosaic virus (YMV), genus *Potyvirus*, Yam mild mosaic virus (YMMV), genus *Potyvirus*, Dioscorea dumetorum virus, genus *Potyvirus*, Dioscorea alata bacilliform virus (DaBV), genus *Badnavirus*, Cucumber mosaic virus (CMV), genus *Cucumovirus*, Dioscorea mottle virus (DMoV), genus *Comovirus* and the recently described Dioscorea sansibarensis virus (DsBV), genus *Badnavirus* (Seal & Muller, 2007).

Although there have been reports of yam viruses in Benin (Goudou-Urbino *et al.*, 1996; Phillips *et al.*, 1999), knowledge of the incidence, distribution and virus identities implicated in these yam virus diseases is lacking. Previous surveys of yam fields in Benin had concentrated on assessing farmer's knowledge and practices on the domestication of yams and on identification and morphological diversity of cultivated species (Dansi *et al.*, 1998, 1999, 2000; Dumont & Vernier, 2000). We report yam virus surveys conducted in the major yam-producing zones in Benin. This is the first attempt to study the occurrence and distribution of viruses infecting yams in Benin.

Materials and methods

Survey

Surveys of the four major yam-producing agro-ecological zones in Benin were conducted in October 2004 shortly after milking for early maturing yam cultivars and in August 2005 before the milking period (Fig. 1). In 2004, yam fields were surveyed in each of the location by walking through the fields and inspecting yam plants for symptoms of virus infection. Young leaves of symptomatic plants were collected moving diagonally across the field and stored at 4°C. Collected leaf samples were later transferred into Eppendorf tubes and stored in liquid nitrogen before indexing. Three plants with no obvious symptoms of virus infection were also sampled in two of the locations visited. Leaves were collected from these plants for indexing to exclude the possibility that plants were infected but tolerant (Cooper & Jones, 1983). The species of each plant was recorded and for symptomatic plants, symptom type(s) was recorded. Leaves were also collected from 23 yam plants (Benin landraces) in an experimental screen house of the International Institute of Tropical Agriculture in Cotonou, Benin. In 2005, yam fields were surveyed by walking across a diagonal and scoring plants for the presence or absence of virus symptoms. The species of each plant was recorded, and for symptomatic plants, symptom type(s) was recorded. Young leaves were collected both from symptomatic and non-symptomatic plants along the diagonal, 5–10 paces apart, depending on the field size. One or two plants outside the diagonal showing symptoms that were not encountered along the diagonal were sampled and collected. Leaf samples were stored in a mobile refrigerator at 4°C while in the field. Some leaves from each plant were later transferred into Eppendorf tubes and stored in liquid nitrogen for PCR, while others were dried over calcium chloride for later processing. All the yams sampled varied in the time of sowing but were mostly at the mid-developmental stage and were of four different varieties: *Dioscorea alata* L., *Dioscorea cayenensis* Lam., *Dioscorea dumetorum* (Kunth) Pax and *Dioscorea rotundata* Poir.

Enzyme-linked immunosorbent assay

Triple antibody-sandwich (TAS) enzyme-linked immunosorbent assay (ELISA) was used for the detection of YMV and DMoV, while protein-A sandwich (PAS) ELISA was used for the detection of badnavirus (DaBV and DsBV), YMMV and CMV. Rabbit polyclonal antisera and monoclonal antibodies against YMV (YMV-M24), known to detect YMV isolates from seven West African countries, were used for YMV detection (Njukeng *et al.*, 2002). For YMMV detection, rabbit polyclonal antibodies working at 1:250 dilutions for trapping and 1:6400 for detecting in PAS-ELISA and known to show no cross-reactivity with other potyviruses were used (Odu *et al.*, 1999). Rabbit polyclonal antisera against CMV, DaBV and DMoV and monoclonal antibodies to DMoV routinely used for the certification of virus-tested plantlets at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, were used for the detection of these viruses.

The rabbit polyclonal antibody against DaBV used in this study is now known to detect, but not differentiate, the two characterised yam-infecting badnavirus, DaBV and DsBV (unpublished data). Therefore, subsequent mention of badnavirus would refer to DaBV and/or DsBV.

Triple antibody-sandwich enzyme-linked immunosorbent assay was carried out as previously described by Thottappilly *et al.* (1998) with slight modifications. Wells of microtitre plates were coated with 100 μL of

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YMV or DMoV rabbit polyclonal antibodies diluted 1:1000 in coating buffer for trapping, and 100 μL of YMV or DMoV monoclonal antibodies diluted in phosphate buffered saline containing 0.05% (v/v) Tween-20 (PBS-T) were used as detecting antibodies in place of mouse polyclonal antibodies. PAS-ELISA followed the method described by Edwards & Cooper (1985). CMV, YMMV and badnavirus rabbit polyclonal antibodies were diluted 1:3000, 1:250 and 1:1000, respectively, for trapping and 1:3000, 1:6400 and 1:1000, respectively, for detecting. Healthy virus-tested yam leaves used as negative controls and positive controls used for each of the viruses were from IITA. The A_{405} for the substrate in each well was measured in a DYNEX MRX microplate reader after 1 h.

Immunocapture polymerase chain reaction/ immunocapture reverse transcription polymerase chain reaction

Immunocapture polymerase chain reaction was used for the detection of badnavirus, while immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) was used for CMV (only in 2005), YMV and YMMV. PCR was not used for detecting DMoV. Immunocapture was carried out using polyclonal antibodies diluted as in PAS-ELISA and TAS-ELISA. The following primer sets were used: CMV primer 1, 5′ GCC GTA AGC TGG ATG GAC AA 3′; primer 2, 5′ TAT GAT AAG AAG CTT GTT TCG CG 3′ (Wylie *et al.*, 1993); Badna FP, 5′-ATG CCI TTY GGI ITI AAR AAY GCI CC-3′; Badna RP, 5′-CCA YTT RCA IAC ISC ICC CCA ICC-3′ (Seal & Muller, 2007); YMMV forward, 5′-GGC ACA CAT GCA AAT GAA RGC-3′; reverse, 5′-CAC CAG TAG AGT GAA CAT AG-3′ (Mumford & Seal, 1997); YMV forward, 5′-ATC CGG GAT GTG GAC AAT GA-3′; reverse, 5′-TGG TCC TCC GCC ACA TCA AA-3′ (Mumford & Seal, 1997).

Immunocapture was performed using a modification of the coating and trapping method by Clark & Adams (1977). PCR tubes (200 μL; ABgene, Ebsom, UK) were coated with antibodies, incubated and washed three times at 3-min intervals with PBS-T. The coated tubes were loaded with 200 μL of sap prepared by grinding the leaves in grinding buffer [phosphate buffered saline containing 0.05% (v/v) Tween-20 (PBS-T), 0.5 mM polyvinyl pyrrolidone (PVP)-40 and 79.4 mM Na_2SO_3] and incubated at 4°C overnight. After washing three times with PBS-T and once with distilled water, 25 μL of reaction cocktail consisting of 1× Taq reaction buffer [10 mM Tris–HCl (pH 8.8), 50 mM KCl, 1.5 mM MgCl and 0.01% Triton-X 100], 0.25 mM of each dNTP, 50 pmol each of badnavirus forward and reverse primers and 1.25 U of Taq DNA polymerase (Promega, Madison, WI, USA) was added to each tube and PCR carried out for the detection of DBV in a PTC-200 Peltier Cycler (MJ Research Inc., Waltham, MA, USA) using the following thermocyclic regime, 94°C for 4 min, 94°C for 30 s, 50°C for 30 s and 72°C for 30 s. The cycle was repeated 40 times excluding the first step, and then a final extension was performed at 72°C for 5 min.

For the detection of YMV and YMMV in 2004, after the immunocapture step as described above, RT-PCR was performed as described by Mumford & Seal (1997). In 2005, after the immunocapture step, RT-PCR for the detection of CMV, YMV and YMMV was performed using QIAGEN Onestep RT-PCR kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol.

Ten microlitres of IC-PCR and IC-RT-PCR products were analysed on a 1.5% agarose gel using TAE (40 mM Tris–acetate pH 8.3, 1 mM ethylenediaminetetraacetic acid) at 100 V for 1 h. The 1-Kb plus DNA ladder (Invitrogen, CA, USA) was ran along with the PCR products and used for size estimation. The gels were stained in ethidium bromide and observed on a UV transilluminator.

Results

Virus survey

A total of 204 yam leaf samples were collected from 24 of the 29 locations visited in 2004. Leaf samples were not collected from the other five locations because obvious symptoms of virus infection were not observed in the fields. Twenty-three leaf samples were collected from the screen house. In 2005, 349 leaf samples were collected from symptomatic yam plants and 210 were collected from non-symptomatic plants. Thirteen different symptom phenotypes occurred at different proportions in 83% of the fields surveyed in 2004 and 100% of fields in 2005. Leaf mosaic (Fig. 2C) and chlorosis/chlorotic spots (Fig. 2A) were the most common symptoms observed in both years accounting for 75% and 58% of the field symptoms in 2004 and 2005, respectively. Bleaching, crinkling, distortion, leaf curl, mottle, puckering, shoe stringing, vein banding, vein clearing, whitish bleaching and leaves showing retarded growth accounted for the remaining 25% and 42% of the field symptoms.

Distribution of symptom types varied across species both in 2004 and in 2005. In 2004, chlorosis and mosaic were the major symptoms observed in all three yam species although very few *D. cayenensis* (eight plants) were assessed. In 2005, puckering was the most common symptom type observed in *D. alata* followed by chlorosis, mottling and mosaic. Chlorosis and mosaic remained the more common symptom types in *D. rotundata*. The proportion of the other symptom types varied across the

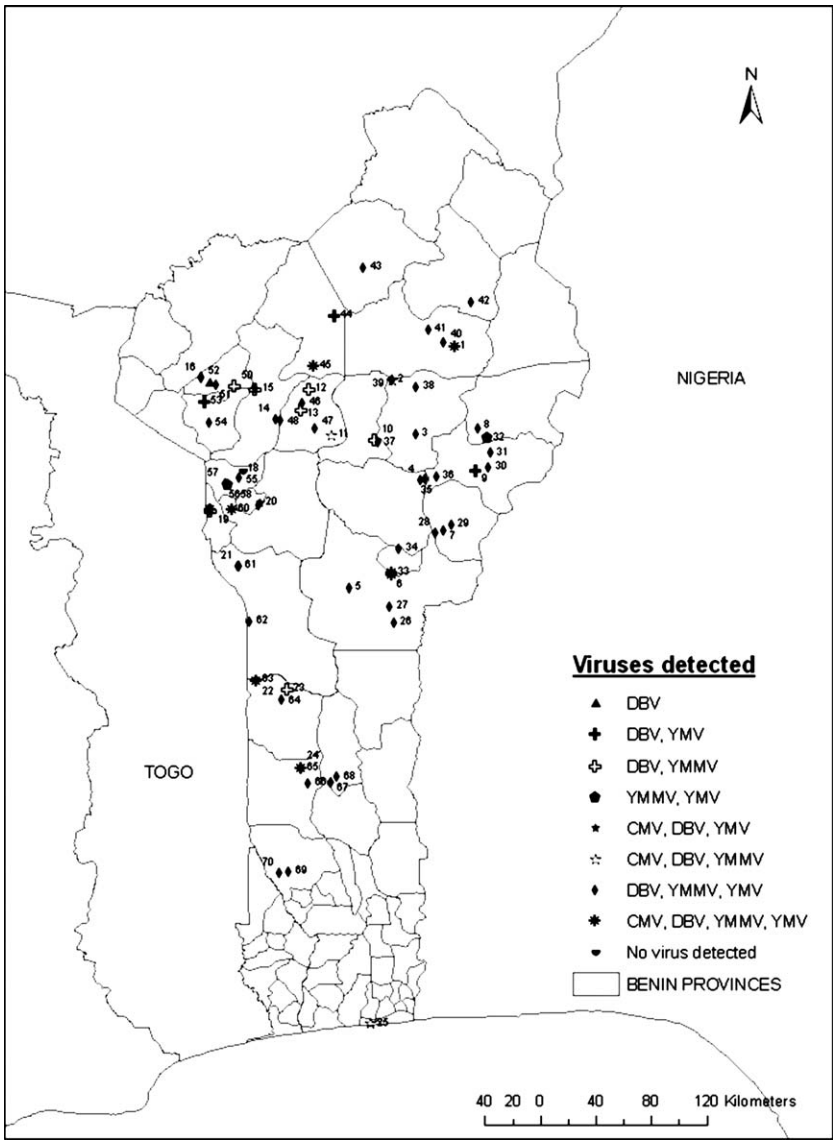

Figure 1 Map of Benin showing the locations sampled and viruses detected in each location. 1–29, 2004 locations and 30–74, 2005 locations. 1, Koutakourkou; 2, Zougou Pandrossi; 3, Sombouan; 4, Ouararou; 5, Sakarou; 6, Banigiri; 7, Bakperou; 8, Guinagourou; 9, Bouca; 10, Kassakpere; 11, Koukabou; 12, Sinaou; 13, Bonigourou; 14, Sayakrou; 15, Makrougourou; 16, Kabare; 17, Tchakalakou; 18, Koutounkouna; 19, Perporeyakou; 20, Tectibayarou; 21, Dikokore; 22, Kpassabeha; 23, Karoum; 24, Awanla; 25, Dendougou; 26, Nagayile; 27, Okoutaosse; 28, Banon; 29, Doyissa; 30, Guinirou; 31, Gokanna; 32, Guinagourou; 33, Perere; 34, Kassakpere; 35, Nikki; 36, Bouca; 38, Bakperou; 38, Kwasekam; 39, Sakarou; 40, Sonsonre; 41, Koukabou; 42, Bembereke; 43, Sombouan; 44, Zougou Pandurossi; 45, Bonigourou; 46, Koutakourkou; 47, Banikoara; 48, Boukoubou; 49, Kerou; 50, Sayakrou; 51, Gountian; 52, Makrougourou; 53, Kabare; 54, Kouarfa; 55, Tectibayarou; 56, Tchakalakou; 57, Koudengou; 58, Berecingou; 59, Kpasabeha; 60, Tanica Beri; 61, Karoum; 62, Dendougou; 63, Awanla; 64, Dangoussar; 65, Nagayille; 66, Bassila; 67, Okoutaosse; 68, Banon; 69, Doyissa; 70, Zounzonkanme; 71, Kaya-Glazoue; 72, Yawa-Glazoue; 73, Agonhohoun-Djidja; 74, Soulegri-Djidja.

different species but leaf distortion, leaf curl, leaf retardation and vein clearing were observed only in *D. rotundata* but not in the other two yam species. Most of the symptom types were associated with the four viruses detected; however, except for one leaf sample that tested positive to all four viruses, puckering in *D. alata* was always associated with DBV and/or YMMV and 20/30 (66.6%) *D. rotundata* leaf samples that tested positive to single infection of YMMV were non-symptomatic.

Enzyme-linked immunosorbent assay/ polymerase chain reaction

For ELISA, leaf samples with mean absorbance values at 405 nm (A_{405}) that were twice or more than those of the

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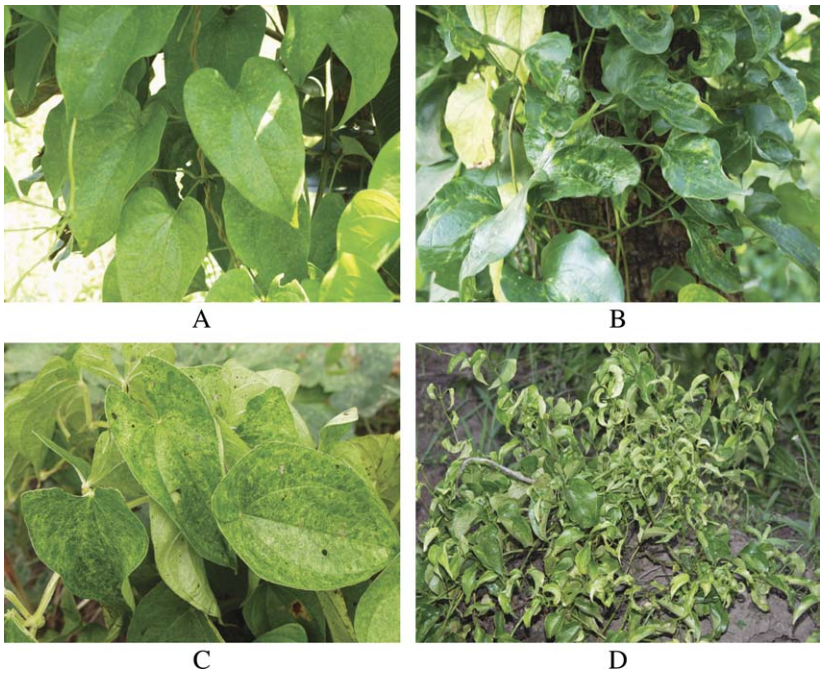

Figure 2 Symptoms of virus infection of yams in the Republic Benin. (A) Chlorotic spots on leaf sample with mixed infection of badnavirus and Yam mild mosaic virus (B) Mosaic and puckering on leaf sample with mixed infection of badnavirus and Yam mosaic virus (YMV). (C) Mosaic on leaf sample with mixed infection of badnavirus and YMV. (D) Shoe stringing on leaf sample with YMV.

healthy leaves were considered to be virus infected (Thottappilly *et al.*, 1998). Leaf samples that showed the expected band sizes of 500, 249, 586 and 579 bp, on gel after PCR, were considered positive for CMV, YMMV, YMV and badnavirus (DaBV or DsBV), respectively (Wylie *et al.*, 1993; Mumford & Seal, 1997; Seal & Muller, 2007).

Eighty-two per cent of the 227 leaf samples tested in 2004 and 60% leaf samples tested in 2005 were positive by ELISA and/or PCR for CMV, YMMV, YMV and/or badnavirus (DaBV and/or DsBV). DMoV was not detected in any of the leaf samples tested in both years (Table 1).

Badnavirus was the most prevalent virus infection in 2004 detected in 70.9% of the 227 leaf samples tested, in 23 of the 24 locations and in the screen house. The occurrence of YMV in the 2004 growing season was low, being detected in only 10.1% of 227 leaf samples from 14 locations, and no detection in the screen house. In comparison, YMV was the most prevalent virus infection in 2005, was detected in 39.7% of 559 leaf samples and was present in all fields in all locations. CMV was the least prevalent virus in both years detected only in 4.8% and 0.7% leaf samples tested in 2004 and 2005, respectively (Table 1).

Similar to the observation in 2004, when two of the three non-symptomatic leaf samples tested positive to badnavirus and/or YMMV, 46.6% of 210 non-symptomatic leaf samples tested in 2005 reacted positively to single or mixed infection of badnavirus, YMMV or YMV. Forty of 224 symptomatic leaf samples (17.8%) tested in 2004 and 79/349 symptomatic leaf samples (22.6%) from 2005 tested negative to all five viruses indexed for.

Distribution across species and across zones

Of the two major yam species tested for viruses during this study, incidence of YMV was higher in *D. rotundata* 232/659 (35.2%) compared with *D. alata* 12/116 (10.3%) in all four zones in both years. Incidence of badnavirus (DaBV and/or DsBV) was higher in *D. alata* 95/116 (81.9%) compared with *D. rotundata* 250/659 (37.9%) in most of the zones where both species were tested besides the observation in Zone Ouest-Atacora in 2004 where the incidence of DBV was higher in *D. rotundata*. As observed for badnavirus, the incidence of YMMV was also higher in *D. alata* 60/116 (51.7%) than in *D. rotundata* 154/659 (23.4%) in all the zones in both years except for Zone Vivrière du Sud Borgou in 2004. Both in 2004 and in 2005, the occurrence of CMV across the four zones varied greatly, however, incidence was higher in *D. alata* 10/116 (8.6%) compared with *D. rotundata* 5/659 (0.8%) (Fig. 3).

Table 1 Occurrence of four yam viruses in four agro-ecological zones sampled in Benin in 2004 and 2005

Agro-ecological Zone	N[a]	Yam Species	N[b]	Viruses Present[c]				
				CMV	Badnavirus	YMMV	YMV	Mixed
2004								
Zone Cotonnière du Centre Bénin	7	*Dioscorea alata*	21	2	19	15	2	15
		D. cayenensis	8	0	5	2	0	1
		Dioscorea rotundata	45	1	34	16	8	18
Zone Cotonnière du Nord Bénin	2	*D. rotundata*	22	1	13	9	2	7
Zone Ouest-Atacora	8	*D. alata*	5	0	3	1	0	1
		D. rotundata	12	1	9	1	2	4
Zone Vivrière du Sud Borgou	12	*D. alata*	13	1	12	5	0	5
		D. rotundata	78	1	57	42	9	35
Screen house	1	*D. alata*	23	4	9	2	0	4
2004 total	29		227	11	161	93	23	90
2005								
Zone Cotonnière du Centre Bénin	15	*D. alata*	29	1	27	18	5	17
		D. dumetorum	1	0	1	0	1	1
		D. rotundata	166	1	54	30	77	40
Zone Cotonnière du Nord Bénin	6	*D. alata*	11	1	11	8	3	9
		D. rotundata	56	0	8	9	22	8
Zone Ouest-Atacora	10	*D. alata*	3	1	3	3	0	3
		D. dumetorum	2	0	0	0	0	0
		D. rotundata	108	0	29	20	25	20
Zone Vivrière du Sud Borgou	14	*D. alata*	11	0	11	8	2	9
		D. rotundata	172	0	46	27	87	38
2005 total	45		559	4	190	123	222	145
2004 and 2005	75		786	15	351	216	245	235

CMV, Cucumber mosaic virus; YMMV, Yam mild mosaic virus; YMV, Yam mosaic virus.

[a]Number of fields sampled.

[b]Number of plants sampled.

[c]Number of plants infected.

Mixed infections

Mixed infections of two or more viruses were detected in 48.3% (90/186) and 39.2% (145/369) of infected leaf samples in 2004 and 2005, respectively. Percentage of mixed infections encountered in various yam species in each zone in relation to the number of infected leaf samples ranged from 17% to 75% in 2004 and from 0% to 100% in 2005 (Table 2). Distributions of mixed infection across the zones were similar in both years (Table 1). Dual infections were more common than triple infections in 2004 (78 leaf samples and 12 leaf samples, respectively) and 2005 (122 leaf samples and 21 leaf samples, respectively). The most frequent mixed infection detected in 2004 was badnavirus and YMMV followed by badnavirus and YMV. In 2005, badnavirus and YMV was the most common mixed infection followed by badnavirus and YMMV (Table 3).

A new severe leaf symptom, which the authors describe as whitish bleaching/patches, was observed on six yam plants in the field and two yam plants in the screen house in 2004 and five yam plants in 2005. Leaf samples taken from 84.6% (11/13) of yam plants showing this symptom tested positive for one or a mixture of the four viruses detected in this study. Two samples tested negative to all the viruses indexed for. This symptom was observed only in *D. alata* and *D. rotundata* but not in *D. cayenensis* or *D. dumetorum*.

Discussion

This paper reports the incidence and distribution of four viruses infecting yams in four major yam-producing agro-ecological zones in Benin. All four viruses that were detected in Benin have been reported in other countries in the West African yam zone (Thouvenel & Fauquet, 1979, Porth *et al.*, 1987; Hughes *et al.*, 1997; Odu *et al.*, 1999; Phillips *et al.*, 1999; Seal & Muller, 2007).

Although the specificity of the antisera used for the detection of these viruses have been previously evaluated and determined (Odu *et al.*, 1999; Njukeng *et al.*, 2002; unpublished data), all leaf samples were also tested by IC-PCR to ensure that plants with low virus load were not missed and that badnavirus detection is correctly reported. Firstly, IC-RT-PCR is an important method for the detection of low titre RNA viruses (Wetzel *et al.*,

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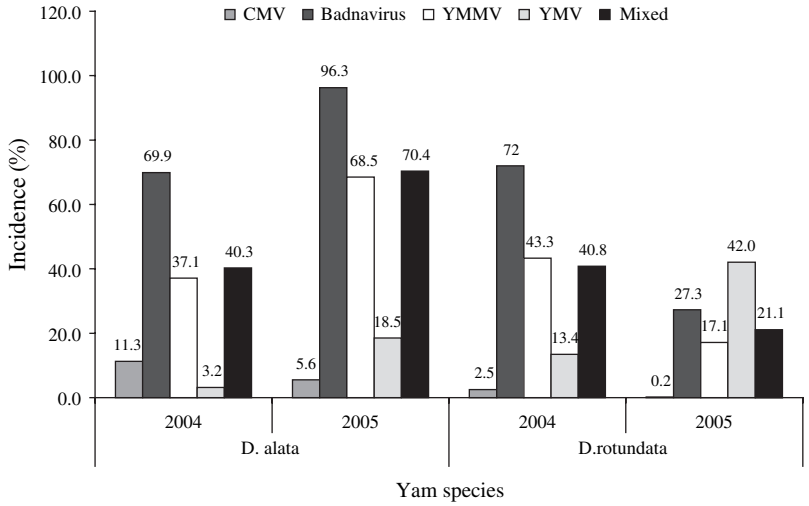

Figure 3 Prevalence (%) (2004) and incidence (%) (2005) of Cucumber mosaic virus (CMV), Yam mild mosaic virus (YMMV), Yam mosaic virus (YMV), yam-infecting badnaviruses [Dioscorea alata bacilliform virus (DaBV) or Dioscorea sansibarensis virus (DsBV)] and mixed infections in *Dioscorea alata* and *Dioscorea rotundata* in 70 locations sampled in Benin.

1992) and three of the viruses tested for (CMV, YMMV and YMV) are RNA viruses. Secondly, IC-PCR favours amplification of DNA contained within capsids excluding DNA derived from other genomes thus ensures that only episomal virus sequences are amplified. This considerably decreases non-specific PCR amplifications particularly, for the detection of badnaviruses whose sequences have been reported to be integrated into host genomes (Harper *et al.,* 1999*a, b*; Geering *et al.,* 2001; Geering *et al.,* 2005). We found that virus detection by IC-RT-PCR/IC-PCR was

Table 2 Multiple infections detected in yams in four agro-ecological zones sampled in Benin in 2004 and 2005

Agro-ecological Zone	Yam Species	Occurrence[a]	Frequency[b]	Most Abundant Multiple Infections[c]
2004				
Zone Cotonnière du Centre Bénin	*D. alata*	15/21 (71.4)	15/20 (75.0)	A (12/15)
	D. cayenensis	1/8 (12.5)	1/6 (16.7)	A (1/1)
	D. rotundata	18/45 (40.0)	18/39 (46.2)	A (11/18)
Zone Cotonnière du Nord Bénin	*D. rotundata*	7/22 (31.8)	7/16 (43.8)	A (4/7)
Zone Ouest-Atacora	*D. alata*	1/5 (20.0)	1/3 (33.3)	A (1/1)
	D. rotundata	4/12 (33.3)	4/9 (44.4)	A (2/4), B (2/4)
Zone Vivrière du Sud Borgou	*D. alata*	5/13 (38.5)	5/12 (41.7)	A (4/5)
	D. rotundata	35/78 (44.9)	35/72 (48.6)	A (28/35)
Screen house	*D. alata*	4/23 (17.4)	4/9 (44.4)	C (2/2), D (2/2)
2005				
Zone Cotonnière du Centre Bénin	*D. alata*	17/29 (58.6)	17/29 (58.6)	A (12/17)
	D. dumetorum	1/1 (100.0)	1/1 (100.0)	B (1/1)
	D. rotundata	40/166 (24.1)	40/115 (34.7)	B (18/40)
Zone Cotonnière du Nord Bénin	*D. alata*	9/11 (81.8)	9/9 (100.0)	A (6/9)
	D. rotundata	8/56 (14.3)	8/31 (25.8)	A (4/8)
Zone Ouest-Atacora	*D. alata*	3/3 (100.0)	3/3 (100.0)	A (2/3)
	D. dumetorum	0/2 (0.0)	0/2 (0.0)	NA
	D. rotundata	20/108 (18.5)	20/51 (39.2)	B (7/20)
Zone Vivrière du Sud Borgou	*D. alata*	9/11 (81.8)	9/11 (81.8)	A (7/9)
	D. rotundata	38/172 (22.1)	38/117 (32.4)	B (22/38)

CMV, Cucumber mosaic virus; NA, not applicable; YMMV, Yam mild mosaic virus; YMV, Yam mosaic virus.

[a]Number of plants infected with more than one viruses/number of plants tested (%).

[b]Number of plants infected with more than one viruses/number of plants infected (%).

[c]A, Badnavirus + YMMV; B, Badnavirus + YMV; C, CMV + Badnavirus; D, CMV + Badnavirus + YMMV (number of plants with the specified multiple infection/total number of multiple infection).

Table 3 Combination of multiple infections encountered in four yam species in four agro-ecological zones in Benin and in the screen house in 2004 and 2005

		Zones[a]					Species[b]		
Combinations	Frequency	1	2	3	4	Screen House	1	2	3
2004									
CMV + Badnavirus	5	1	0	1	1	2	2	0	3
CMV + YMMV	1	0	1	0	0	0	0	0	1
Badnavirus + YMMV	62	4	32	2	24	0	17	1	44
Badnavirus + YMV	8	0	3	2	3	0	0	0	8
YMMV + YMV	2	0	1	0	1	0	0	0	2
CMV + Badnavirus + YMMV	5	0	1	0	2	2	5	0	0
Badnavirus + YMMV + YMV	7	2	2	0	3	0	1	0	6
2005									
CMV + Badnavirus	1	0	0	0	1	—	0	0	1
Badnavirus + YMMV	43	7	10	9	17	—	27	0	16
Badnavirus + YMV	54	4	23	7	20	—	3	1	50
YMMV + YMV	24	4	8	3	9	—	0	0	24
CMV + Badnavirus + YMMV	1	0	0	1	0	—	1	0	0
Badnavirus + YMMV + YMV	20	1	6	3	10	—	5	0	15
CMV + Badnavirus + YMMV + YMV	2	1	0	0	1	—	2	0	0

CMV, Cucumber mosaic virus; YMMV, Yam mild mosaic virus; YMV, Yam mosaic virus.

[a]1, Zone Cotonnière du Nord Bénin; 2, Zone Vivrière du Sud Borgou; 3, Zone Ouest-Atacora; 4, Zone Cotonnière du Centre Bénin.

[b]1, *Dioscorea alata*; 2, *Dioscorea cayenensis* (2004) or *Dioscorea dumetorum* (2005); 3, *Dioscorea rotundata*.

more than the corresponding ELISA tests (data not shown), possibly because of the greater sensitivity of IC-RT-PCR/IC-PCR (Mumford & Seal, 1997; Olatunde, 1999; Njukeng *et al.*, 2005; Agindotan *et al.*, 2006). The lower sensitivity observed with the ELISA tests is similar to the findings of Mumford & Seal (1997) and Njukeng *et al.* (2005) and could be because of low virus concentration in yam leaf samples, particularly for the potyviruses (Brunt *et al.*, 1990) or because of interference of polyphenols and glutinous polysaccharides contained in yam leaves (Rossel & Thottappilly, 1985).

The detection of badnavirus in 67 of the 70 locations (95.7%) where viruses were detected and in all four yam species encountered in the study, and its presence in six of the eight mixed infections, makes the badnaviruses (DaBV and DsBV) very important and widespread yam virus in Benin. The observed higher incidence of badnavirus and YMMV in *D. alata* compared with *D. rotundata*, in Benin, is similar to the findings of Hughes *et al.* (1997) on the distribution of these viruses in Nigeria. The occurrence of a higher incidence of YMV in *D. rotundata* compared with the other yam species sampled is also similar to the distribution of YMV in Nigeria (Hughes *et al.*, 1997) and in Ghana (Olatunde, 1999). The extensive spread of CMV, YMMV, YMV and badnavirus and the high incidence of mixed infection observed in all the four zones surveyed in this study may be attributed to the exchange of infected planting materials both locally and internationally through permeable land borders. These exchanges may also account for the similarities in the incidences and distribution of these viruses in Nigeria (Hughes *et al.*, 1997) and in Ghana (Olatunde, 1999).

The high incidence of badnavirus and YMMV mixed infections in all four yam species tested and its detection in almost all the locations visited in both years is of concern. Some leaf samples tested positive for badnavirus but not for YMMV, in ELISA and in PCR, and vice versa. This shows that the tests conducted accurately discriminates between the two viruses in single and mixed infections. The occurrence of two types of mixed infection in the screen house in comparison with the field, where eight different types were detected, can be attributed to the controlled environment of the screen house, which excludes insect vectors, particularly aphids. Three (CMV, YMMV and YMV) of the four viruses detected in Benin during this study are reported to be aphid transmitted, except DBV, which is transmitted by the mealybug *Planococcus citri* Risso (Kenyon *et al.*, 2001).

Dioscorea alata was found to be the most heavily infected yam species in this study. Although it is the most widely distributed yam species globally, its high susceptibility to diseases is a major limitation to the profitable and sustainable production of *D. alata*, which is also a favoured alternative to *D. rotundata* for diabetic patients (Abang *et al.*, 2003; Riley *et al.*, 2006).

Leaf samples from some plants showing obvious symptoms of virus infection tested negative to all the viruses indexed for. Symptoms observed on these plants may be caused by other virus(es) for which tests were not

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performed or that are yet unidentified. Symptoms may also be because of abiotic agents causing virus-like symptoms. The detection of badnavirus, YMMV and YMV infections on non-symptomatic leaf samples shows that absence of visual symptoms on yam leaves may not be indicative of absence of virus infection, but laboratory diagnosis serves as a more sensitive and conclusive way of affirming the health status of potential breeding or planting materials. Most of the symptoms observed during this study have been reported (Thouvenel & Dumont, 1988; Odu *et al.*, 2001), but the previously unrecorded virus symptom of whitish bleaching/patches described in this paper has not been reported. The severity of this symptom and its effect on the green pigmentation of the yam leaves may lead to a severe reduction in the photosynthetic ability of affected plants, with a consequent reduction in yield.

The extensive spread of yam viruses within the West African sub-region needs to be addressed. Restricting movement and exchange of planting materials across land borders by enforcing registration and certification programmes, and the use of planting materials (seed tubers) obtained from healthy plants for yam cultivation would be a good initial approach to solving this problem. Some of the major challenges for farmers and researchers in choosing healthy planting materials and in screening of yam genotypes for multiplication as clean planting material both for local and for international distributions, include multiplicity of viruses and symptom types, confounding of virus symptoms with those of nutritional disorders, mixed virus infections and the absence of methods for rapid and reliable field diagnosis. A rapid field test for the certification of yam planting materials will be major progress for the control of yam viruses.

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