Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Food Microbiology 93 (2004) 31–40
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Analysis of population structure of *Aspergillus flavus* from peanut based on vegetative compatibility, geographic origin, mycotoxin and sclerotia production

M. Belén Pildain[a,*], Graciela Vaamonde[b], Daniel Cabral[a]

[a] *Laboratorio de Micología, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, piso, Lab. 69, (EHA1428), Buenos Aires, Argentina*
[b] *Departamento de Química Orgánica, Lab. de Microbiología de Alimentos Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 4^to piso, (EHA1428), Buenos Aires, Argentina*

Received 23 January 2003; received in revised form 15 July 2003; accepted 2 October 2003

Abstract

Isolates of *Aspergillus flavus* obtained from a new growing peanut region in Argentina (Formosa province) were examined for aflatoxin types B and G and cyclopiazonic acid (CPA) production. Sclerotia diameters and the number of sclerotia produced per square centimetre were also determined for each isolate. They were tested by vegetative compatibility group analysis to investigate their genetic relatedness and correlate the results with vegetative compatibility groups previously described from the major peanut-growing area (Córdoba province) in our country. Two isolates were considered atypical because they simultaneously produce aflatoxins B and G and CPA.

A. flavus population from Formosa province was very diverse genetically. Vegetative compatibility groups (VCGs) formed by typical isolations of *A. flavus* were different among agroecological sites. Formosa isolates could not be grouped to any of the Córdoba VCGs, while that one of the VCGs that contain atypical isolates included strains from the two geographical regions. Each VCG included isolates of the same mycotoxin and sclerotia production pattern. The two regions analysed have different climatic conditions, soil type, crop sequence history and also are in different latitude. These parameters may reflect different geographic adaptation between isolates from both sites.

Keywords: *Aspergillus flavus*; Vegetative compatibility; Mycotoxins; Peanuts

1. Introduction

Peanuts, corn and cottonseed are often invaded before harvest by *Aspergillus flavus* Link. This fungi produce aflatoxins, potent natural carcinogens, and cyclopiazonic acid (CPA), which is toxic to a variety of animals and has been implicated in human poisoning (Rao and Husain, 1985; Peraica et al., 1999). Date from different geographical areas demonstrate a great variability in the mycotoxin producing potential of *A. flavus*. It has been suggested that strains with different aflatoxin-producing ability may

* Corresponding author. Tel./fax: +54-11-4-4787-2706.
E-mail address: belen@bg.fcen.uba.ar (M.B. Pildain).

0168-1605/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2003.10.007

interact with host genotypes to influence rate of aflatoxin production. Knowledge of subpopulation of toxicogenic *A. flavus* species in each region would be very useful in local agricultural management practices.

A. flavus can be divided into two groups of strains. The S strain, produces high levels of aflatoxins and numerous small sclerotia that are, on average, < 400 μm in diameter (Cotty, 1989). This isolates has been referred to as atypical (Saito et al., 1986) and also named *A. flavus* var. *parvisclerotigenus* (Saito and Tsuruta, 1993). The L strain produces fewer, larger sclerotia that are >400 μm in diameter and, on average, less aflatoxin (Saito et al., 1986; Cotty, 1989; Bayman and Cotty, 1993). S strain isolates from Thailand, Australia, Benin and Argentina are producers of AFB, or AFB and AFG (Hesseltine et al., 1970; Saito et al., 1986; Blaney et al., 1989; Cotty and Cardwell, 1999; Vaamonde et al., 2003). However, in North America, only S strain producers of AFB have been reported (Cotty and Cardwell, 1999; Horn and Dorner, 1999). Phylogenetic studies of *A. flavus* have shown that it consists of two subgroups (groups I and II) (Geiser et al., 1998; Tran-Dinh et al.,1999): Most group I strains produced AFB, and most group II strains produced both AFB and AFG. All group II isolates presented S sclerotium phenotype, whereas group I strains consisted of both S and L isolates (Geiser et al., 2001).

Gene flow within species is limited by a vegetative (also termed somatic or heterokaryon) compatibility system (Papa, 1986; Bayman and Cotty, 1991a). Populations of *A. flavus* comprise numerous subpopulations called vegetative compatibility groups (VCGs) that have been associated with many morphological and physiological features (Bayman and Cotty, 1993; Horn and Greene, 1995; Horn et al., 1996; Novas and Cabral, 2002).

Knowledge of regional differences in the toxigenicity and genetic diversity of *A. flavus* populations may help in understanding the population dynamics and also give important information that could be used in determination of which control measures are most effective in reducing preharvest aflatoxin contamination. For example, the use of atoxigenic *A. flavus* strain, as a biological control agent, to prevent contamination through competitive exclusion of toxigenic strains during infection (Cotty, 1994; Garber and Cotty, 1997). Regional differences in aflatoxin contamination of crops may be attributable to climatic conditions and to agricultural practices that increase susceptibility of plants to invasion by *A. flavus*. Drought stress accompanied by elevated temperatures during seed development promotes *A. flavus* invasion and subsequent aflatoxin contamination of peanuts (Diener et al., 1987; Horn and Dorner, 1999).

In Argentina, the major peanut-growing region is localized on the center of Córdoba province, but due to the increasing economical importance of this legume (Harvez, 1996), new regions, like Formosa province, are seeking to introduce this crop.

In the present study, we present the results of VC experiments of *A. flavus* isolates from peanut seed sampled from a peanut field in Formosa province, VCG diversity and aflatoxin production also were analyzed. Furthermore, we evaluated any relationship between VCGs and mycotoxin production, sclerotia size and number and geographical origin, comparing with VC results from Córdoba province previously obtained by Novas and Cabral (2002).

2. Materials and methods

2.1. Isolation of Formosa strains

Undamaged pods were collected in 2001 harvest from a single peanut field at Las Lomitas in Formosa province. This was a experimental field, and it represents the first report of a peanut field grown in Formosa, Argentina.

The sample was processed immediately after harvest, undamaged pods were selected and mixed uniformly. They were first surface sterilized in 2% NaOCl with 100 μl/l Tween 20 (1 min) followed by a brief sterile water rinse and then hand shelled, 600 seeds from different pods were individually surface sterilized in test tubes in the same manner followed by three water rinses. Seeds were plated on Dichloran-18% glycerol agar (DG18) and incubated 7 days at 25 °C.

Species of *Aspergillus* that developed from the seeds were identified directly on the plates according to Klich and Pitt (1988) and Pitt and Hocking

(1997). *A. flavus* isolates were transferred to slants of Czapek-Dox agar (Cz) and incubated for 14 days at 25 °C; S and L strains were included in this study.

2.2. Sclerotia production

Plates 6 cm in diameter (three replicate per isolate) containing Cz were inoculated with mycelia and incubated at 30 °C in darkness for 14 days, sclerotia were obtained according Novas and Cabral (2002).

When an isolate did not produce any sclerotia under the culture conditions of this study, we incubated this isolate on peanut seed previously autoclaved with distilled water. If this procedure failed, we considered that this isolate did not produce sclerotia.

2.3. Sclerotia size and number

A Petri dish, 60 × 15 mm with 10 mm grid, was used like gridline. To assess diameters, sclerotia were spread out on the gridline plate and measurements of 30 sclerotia per replicate were recorded. It was assumed that sclerotium shape was approximately a prolate spheroid. Isolates were classified as S or L strain (Cotty, 1989; Novas and Cabral, 2002). Sclerotia were enumerated by counting four squares of 1 cm^2 each, chosen randomly, from each replicate.

2.4. Aflatoxin and CPA analysis

The production of AFB, AFG and CPA was tested using the screening method of Filtenborg and Frisvad (1980) and Filtenborg et al. (1983). A small agar-plug from 7 days pure culture growing over a solid substrate, Czapek-yeast extract agar (CYA), at 25 °C was removed and transferred directly to a thin-layer chromatography (TLC) plate.

Mycotoxin detection was performed using TLC on silica gel G60 plates (20 × 20 cm, 0.25 mm thick, Merck5721, Germany). Chloroform–acetone (90:10) was used as developing solvent for aflatoxins and ethyl acetate–2-propanol–ammonium hydroxide (40:30:20) for CPA (Fernandez Pinto and Patriarca, 2001). Aflatoxins were visualized under long-wave UV light (366 nm) and CPA in daylight after treatment of the plates with Erlich's reagent (1 g of 4-dimethylaminobenzaldehyde in 75 ml ethanol and 25 ml concentrated HCL), with subsequent development of blue spots.

2.5. Isolation and complementation of mutants

Complementary nitrate non-utilizing (*nit*) mutants were obtained on plates of Cz containing 25 g/l of potassium chlorate (Bayman and Cotty, 1991a) with pH unadjusted. Cultures were incubated at 30 °C for 1–3 weeks until chlorate-resistant sectors arose.

Hyphal tips from fast growing sectors were transferred to plates of Cz (nitrate medium) and tested for auxotropy. Phenotypes were determined by growth on Cz with nitrate replaced by other nitrogen sources (Cove, 1976). *nit* Mutants were identified as: *niaD* (nitrate non-utilizing), *nirA* (nitrite and nitrate non-utilizing) and *cnx* (hypoxantine and nitrate non-utilizing).

Complementary pairings of *niaD*, *nirA* and *cnx* mutants within each isolate were initially made to establish self-compatibility (Correl et al., 1989). Isolates that did not demonstrate self-compatibility were excluded from the analyses.

Pairs of mutants were inoculated 15 mm apart in the centre of Cz plates and incubated for 21 days at 30 °C. Complementation was indicated by a zone of dense, wild type growth, often accompanied by sclerotium formation. For each isolate, a *niaD*, a *nirA* and/or a *cnx* were selected as testers for pairing with new *nit* mutants.

2.6. VCGs

To establish Formosa VCGs, testers were paired in all possible combination and isolates that complemented each other were assigned to the same VCG; as each group of new compatible *nit* mutants was generated, they were paired against all testers for VCGs recognised at time. All positive reactions were repeated for verification.

Isolates of *A. flavus* from Formosa province were paired against isolates from Córdoba province to test the consistency of regional differences in aflatoxigenicity within VCGs. These included Formosa VCGs established in this study, isolates in

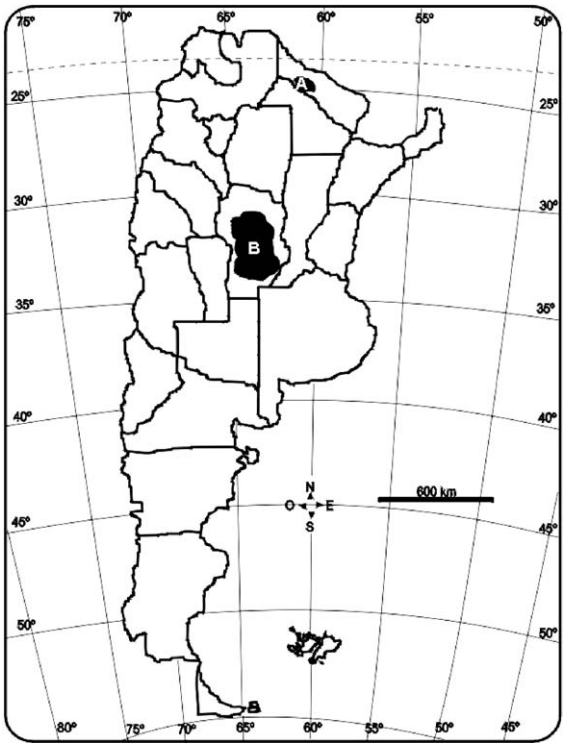

Fig. 1. Map of Argentina Republic showing agroecological zones from which *A. flavus* peanut seed isolates were obtained. (A) Las Lomitas, Formosa province; (B) Córdoba province.

each of 13 previously determinate VCGs (Novas and Cabral, 2002) and one S strain (M5N9) that produce AFB and AFG, not included before. All of them came from seeds of 19 peanut fields in Córdoba and 1 experimental field in Formosa province.

All members of each Formosa VCG were tested for complementation against all isolates or only one (in cases where VCG was represented by one single isolate) of each VCG formed by Novas and Cabral (2002).

2.7. Sampling zones climatic conditions

The two different agroecological zones in the provinces of Córdoba and Formosa have the same seasonal pattern (November–May) but distinct rainfall and maximum temperature. Las Lomitas, Formosa province (24–25°, South latitude), has subtropical climate, average rainfall between 700 and 800 mm and high maximum temperature (December, January, February) of 28–45 °C. Córdoba peanut region (31–33.5°, South latitude) has template weather, average rainfall between 800 and 900 mm and maximum temperature (December, January, February) of 25–32 °C (De Fina, 1992) (Fig. 1).

2.8. Data analysis

The correlation for aflatoxin and sclerotia production was determined using the Fisher's exact test for dichotomous nominal-scale data ($P<0.005$) and the Cramer coefficient (ϕ). To compare percentages of *nit* mutants, proportion test was utilized ($P<0.005$) (Zar, 1996). Sclerotia data (number and diameter) of VCGs with two or more isolates were statistically analysed using an unbalanced hierarchically two level nested ANOVA design, followed by least significant difference (LSD) mean separation tests ($P=0.05$). The statistical analyses were performed by using Statistix for Windows, version 2.1 Analytical Software.

3. Results

3.1. Isolates characteristics

Table 1 shows characteristics of *A. flavus* strains ($n=40$) isolated from peanuts seeds from Formosa, Argentina. Seventy eight percent of the isolates were sclerotia producers under our culture conditions and on peanuts seeds. Three of these sclerotia producers were classified as S strains (sclerotia

Table 1
Production of sclerotia, type B and G aflatoxins (AFB, AFG) and CPA by isolates of *A. flavus* from Formosa province, Argentina

AFB production	AFG production	CPA production	Percent of isolates		
			L strain ($n=28$)	S strain ($n=3$)	None[a] ($n=9$)
+	–	+	89.3	33.3	44
–	–	+	10.7	0	56
+	+	+	0	66.7	0

[a] None = sclerotia were not produced under our culture conditions.

< 400 μm in diameter). Eighty-nine percent of the L strain isolates produced AFB and CPA, and no isolates that produced only aflatoxin were observed.

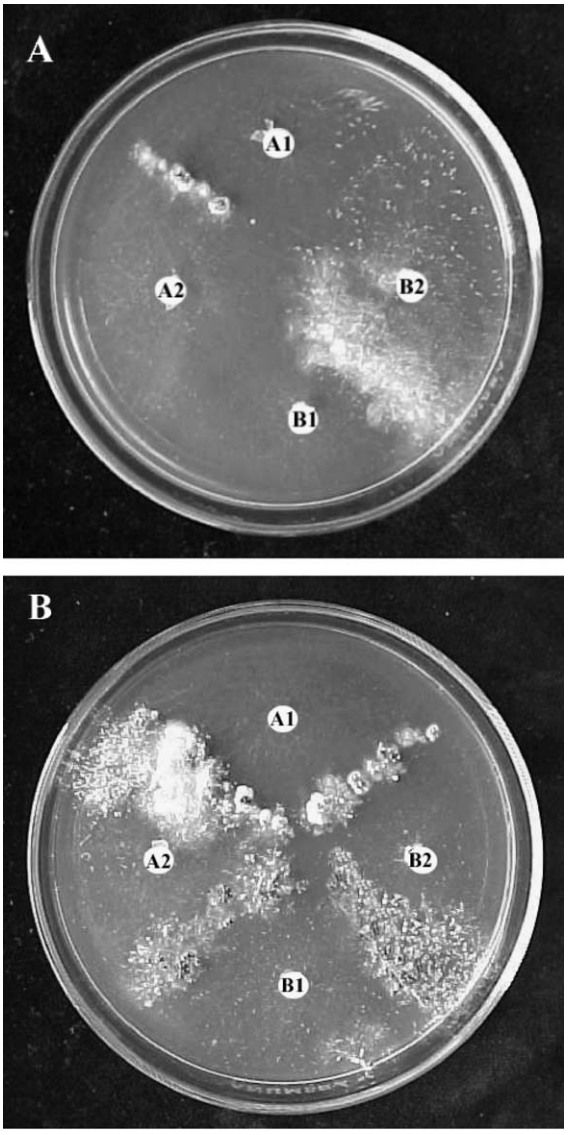

Fig. 2. Pairings on Cz of nitrate nonutilizing (*nit*) mutants of *A. flavus* peanut seeds isolates for VCG determination. Plates were photographed after 15 days of incubation. Lines of robust mycelial grouth between *nit* mutants indicate compatible reactions. In (A), self-compatible isolates LLF9 (A1 = *cnx*; A2 = *niaD*) and LLF40 (B1 = *cnx*; B2 = *niaD*) are in differents VCGs. (B) Heterokaryon formation between self-compatible isolates, LLF9 (A1 = *cnx*; A2 = *niaD*) and LLF8(B1 = *cnx*; B2 = *niaD*), since they belong to the same VCG.

Table 2
A. flavus isolates VCG, toxicogenic and morphological characteristics

Isolates[a]	Sclerotia mean[b]	Sclerotium diameter (μm) ± S.D.[c]	Strain isolate[d]	Category[e]	VCG[f]
LLF8	29.83[b]	564 ± 188.8[b]	L	I	N
LLF9/ 1005[g]	22.25[b,c]	463.9 ± 136.8[b]	L	I	N
LLF25	2.25[d]	566.4 ± 148.7[a]	L	I	N
LLF36	2.42[d]	864.3 ± 186.4[a]	L	I	N
LLF3/ 001[g]	None[h]	None[h]	–	I	O
LLF17	None[h]	None[h]	–	I	O
LLF22	None[h]	None[h]	–	I	O
LLF35	None[h]	None[h]	–	I	O
LLF14	21[b,c]	472 ± 218.4[c]	L	I	P
LLF40/ 400[g]	6.83[c,d]	730.6 ± 94.9[a]	L	I	P
LLF5/ 002[g]	None[h]	None[h]	–	IV	Q
LLF30	None[h]	None[h]	–	IV	Q
LLF37/ 792[g]	230.75[a]	259.8 ± 81.8[d]	S	II	R
M5N9/ 769[g]	224.33[a]	238 ± 59.18[d]	S	II	R
LLF1	None[h]	None[h]	–	IV	S
LLF7	6.44	595.2 ± 156.6	L	IV	T
LLF10	78.33	542 ± 203.5	L	I	U
LLF13	0.42	538.93 ± 152.5	L	IV	V
LLF15	None[h]	None[h]	–	I	W
LLF21	62.5	522 ± 215	L	I	X
LLF16	34.92	437.6 ± 136	L	I	Y
LLF18	3.83	530 ± 151	L	IV	Z
LLF33	None[h]	None[h]	–	IV	A1
LLF34	32.25	554.5 ± 151.5	L	I	b1
LLF39/ 1076[g]	224.27	232.4 ± 69.7	S	II	C1

[a] *A. flavus* culture designation.

[b] Mean per square centimeter, based on four square centimeters, chosen randomly, from each of the three replicate plates per isolate on Cz. Means that not sharing a commom letter are significantly different ($P < 0.05$) according to a least significant difference test.

[c] Means based on 30 sclerotia, chosen randomly, from each of the three replicates plates per isolate. Means that not sharing a commom letter are significantly different ($P < 0.05$) according to a least significant difference test.

[d] L strain produce sclerotia >400 μm in diameter, S strain produce sclerotia <400 μm in diameter (Cotty, 1989).

[e] I: isolates produce type B aflatoxinsand CPA; II: isolates produce types B and G aflatoxins and CPA; IV: isolates produce only CPA (Novas and Cabral, 2002).

[f] VCG designation correlative with Novas and Cabral (2002).

[g] BAFC cult: Culture Collection number, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

[h] None = sclerotia were not produced under our culture conditions.

Two of the three S strain isolates produced AFB, AFG and CPA (Table 1).

Production of AFB and sclerotia for the Formosa isolates was significantly correlated according to the Fisher's exact test ($P < 0.005$), and there was also a positive association for the Cramer coefficient ($\phi = 0.57$; $\phi_{max} = 1$); $n = 40$.

3.2. nit Mutants

They were obtained from 38 isolates of the Formosa field. Two additional isolates never produced *nit* sectors and were discarded. Out of 38 isolates, from which at least two types of *nit* mutants were recovered, 24 were self-compatible.

Mutants ($n = 235$) were tested for growth on different nitrogen sources and classified according to Cove (1976): 71.7% were *niaD* mutants, 16.6% were *cnx* mutants and 11.7% were *nirA* mutants.

3.3. VCGs

Sixteen VCGs were revealed from complementation test, with a total of 25 isolates (including one isolate from Córdoba province). As previously mentioned, compatibility was identified by a line of wild-type growth at the zone of interaction (Fig. 2). The remaining isolates ($n = 14$) could not be assigned to any VCG, because complementary pairs of mutants were not obtained or the isolates pairings failed to generate the heterokaryon (vegetative incompatibility).

Isolates within each VCG are all sclerotia producers, or they are all nonproducers. Furthermore, sclerotia producers within each VCG group are either L or S strain, not both. Analyses of variance showed significant differences among VCGs ($P < 0.05$) in the number of sclerotia per square centimeter and in sclerotia size. However, isolates within a VCG were also significantly different in sclerotia size and number, except in VCG R (Table 2). All Formosa *A. flavus* isolates from VCG N, O and P produced AFB and CPA, while the isolates of VCG R and C1 produced AFB, AFG and CPA. The largest Formosa VCGs (N, O) included 4 isolates, 3 included two (P, Q, R) and the last 11 were represented by a single isolate (Table 2). Formosa VCG diversity index, expressed as the number of groups divided by the total numbers of isolates (Horn and Greene, 1995) was 0.64.

Fig. 3 shows the distribution of the 24 Formosa isolates among 16 VCGs and 26 Córdoba isolates (25 which composed 13 VCGs, and M5N9). None of the isolates from the 16 VCGs of Formosa complemented with isolates from the 13 VCGs formed by Novas and Cabral (2002) in *A. flavus* isolates from Córdoba, except atypical isolate LLF37 that was compatible with M5N9 (VCG R) from Córdoba province, not included by Novas and Cabral (2002). However, VCG R members were incompatible with the VCG (C) formed with the rest of the Córdoba atypical strains, and they were also incompatible with the other atypical isolate from Formosa (LLF39 in VCG C1) (Fig. 3).

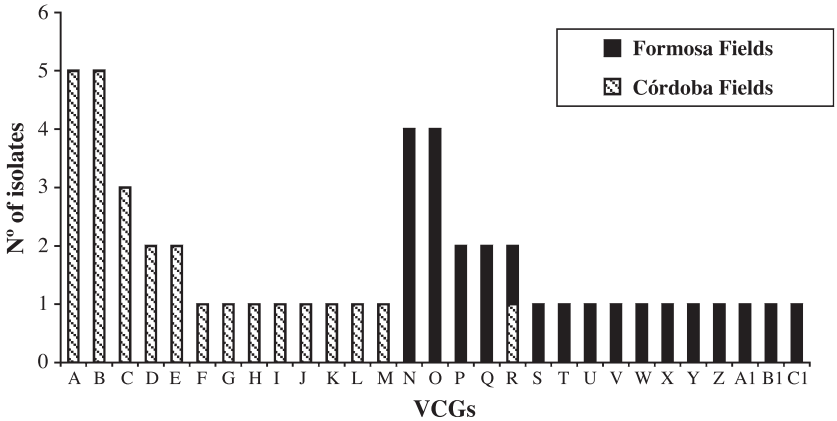

Fig. 3. Distribution of *A. flavus* peanut isolates from Las Lomitas and from Córdoba fields among vegetative compatibility groups (VCGs).

4. Discussion

Scarce studies have been carried out on *A. flavus* in peanut seeds in Argentina and South America. In 1979, Vaamonde and Varvsavsky reported the isolation and characterization of *A. flavus* and *A. parasiticus* strains from different peanut varieties grown in Argentina and Paraguay. Vaamonde et al. (1995) presented a morphological and toxicogenic description of 35 isolates from Córdoba fields, which were later utilized by Novas and Cabral (2002) who determined their VCG diversity.

The present work is the first report where mycotoxin, sclerotia production, genetic diversity and agroecological origin were determined and compared. Our results show that in the two agroecological zones the incidence of S strains was similar and also lower than the L strains. Orum et al. (1997) reported ranges of S strain incidence from less than 5% to more than 90%, and the association with cotton cultivation in the Southern United States. In Arizona S strains, incidence is inversely correlated with elevation (Bigelow et al., 2000).

Greater than 75% of the *A. flavus* isolates from Formosa peanut produced aflatoxins, being this frequency higher than that obtained from Córdoba province (Vaamonde et al., 1995). CPA production was more uniform, and it was registered in all the Formosa isolates, this results are similar to the high percentage found in other studies (Horn and Dorner, 1999; Horn and Greene, 1995; Geiser et al., 2001). It is known that atypical strains AFB, AFG and CPA producers are present in several countries, all of them in the South Hemisphere or below of the tropic of Cancer (Hesseltine et al., 1970; Saito et al., 1986; Cotty and Cardwell, 1999). From our study, we can observe that in the American continent, they are present at least between the 24° and 33° South latitude.

We observed a positive association between aflatoxin and sclerotia in *A. flavus* isolates from Formosa, and similar results were found in Córdoba, Argentina (Novas and Cabral, 2002). Also, previous studies reported that aflatoxins may have a function correlated to sclerotia and that aflatoxin biosynthesis and sclerotia morphogenesis are interrelated (Wicklow and Shotwell, 1983).

In agreement with other *A. flavus* VCG reports (Bayman and Cotty, 1991a; Horn, et al., 1995; Novas and Cabral, 2002), *niaD* type of mutants was isolated in higher proportion than *cnx* and *nirA* mutants, but with the difference that in our study *cnx* was obtained easier than *nirA* mutants. *cnx* is known as the most reliable for compatibility tests between *nit* mutants (Bayman and Cotty, 1991a). The mechanism by which differences in number and type of *nit* mutants among isolates is observed is not understood. It has been suggested that could be related to the physical size of the genes or that some loci may be more susceptible to mutation than others (Klittich and Leslie, 1988). Also, Korolev and Katan (1997) in *Verticillium dahliae* related this difference with culture age and storage conditions of the isolates.

It was frequently observed that mutants of the same isolate were unable to complement with any of the other mutants derived from the same strain. Heterokaryon self-incompatible strains complicate VCG analyses of populations since it is usually difficult to assign such strains to a VCG. These isolates are potentially useful in biological control where it is undesirable for the released strain to be able to form a heterokaryon with any other strain in the population (Brooker et al., 1991).

In the present study, isolates within a VCG were similar in their production of aflatoxins, CPA and sclerotia, which is consistent with previous reports (Bayman and Cotty, 1993; Horn et al., 1996). This may be of major importance in developing a biocontrol strategy. Geiser et al. (1998) suggested that recombination has important implications for biological control strategies, particularly adding nontoxicogenic strains to fields where crops are grown, because of the potential for outcrossing between the introduced and native strains. Based on vegetative incompatibility barriers, the potential for genetic exchange would likely be must greater among isolates within a VCG than for isolates in different VCGs.

The Formosa *A. flavus* population is very diverse genetically and includes numerous VCGs ($n = 16$), which was reflected by a diversity value (0.64). This diversity was also indicated by the presence of many single-isolate VCGs ($n = 11$). The Formosa VCG diversity index is higher than Córdoba index, obtained by Novas and Cabral (2002). The size of the two peanut-growing areas are very different, the Córdoba region being bigger than the Formosa region. This result shows that a small area may be inhabited by

several to many genetically distinct individuals, and it is in agreement with the result shown by Bayman and Cotty (1991b).

As in other VCG studies, L isolates were not compatible with S isolates (Bayman and Cotty, 1993; Novas and Cabral, 2002). This is consistent with phylogenetic analysis suggesting that the S forms related to *A. flavus* var. *parvisclerotigenus* (Saito and Tsuruta, 1993) and the group II of *A. flavus* deserve recognition as a species (Geiser et al., 2001). None of the L Formosa isolates were compatible with Córdoba isolates; while the S isolate LLF37 from Formosa province was compatible with S form M5N9 from Córdoba province. These results may indicate that *A. flavus* genotype are widely dispersed, but isolates LLF37 and M5N9 belonging to the same VCG are clonal and genetically identical. In agreement with Horn and Dorner (1999), the geographically separated VCG R members may have diverged from one another while still maintaining the same alleles at loci that govern VC. Therefore, that none of the Formosa VCGs represented by L isolates were the same as that for Córdoba region suggests that selective pressure in *A. flavus* may instead occur in response to the multitude of environmental parameters (Horn et al., 1996).

Orum et al. (1997) postulated that temperature, soil condition, day length, crop sequence history, insect levels, rainfall frequency and management practice may influence *A. flavus* communities. All these factors and many other micro-climatic factors are different between these two regions in Argentina. The Formosa peanut-growing region is warmer and with relatively low rainfall than the Córdoba region (De Fina, 1992). In Córdoba province, peanuts have been planted for many years, but the Formosa peanut sample was obtained from an experimental field in which the agricultural soil was occupied by natural pasture for 20 years. Besides, this region is a cotton- and pumpkin-producing area with a natural vegetation formed by abundant leguminous trees, being mesquite (*Prosopis* spp.), the most frequent near to agricultural fields (Zuloaga and Morrone, 1999). *A. flavus* populations are natural contaminants of cottonseed and mesquite; furthermore, they may consider important reservoirs and significant sources of aflatoxin-producing fungi (Boyd and Cotty, 2001).

The latitude was also correlated with *A. flavus* divergence among communities in different areas (Cotty, 1997). Formosa province is in the north of Argentina (25° South latitude) and Córdoba province is in the center of the country (32° South latitude). Also, Carwell and Cotty (2002) reported that S strain isolates were frequently found in high-temperature regions with relatively low rainfall in North America, and postulated that the production of small sclerotia by the S strains may be a survival trait for an organism adapted to climatic fluctuations. While in our study, the VCG R, which included S strains, is the only one that group isolates of different geographical zones, and this may indicate differential adaptation and/or migration capacity between L and S forms.

Presents results demonstrate that VCGs are an effective multilocus marker that has been particularly helpful for characterizing genetic structure in populations of *A. flavus* in Argentina, where cost and laboratory facilities may limit the number of isolates that can be analyzed by molecular methods. Detailed studies on VCGs may also help assess the degree to which horizontal gene transfer can occur between similar or different genotypes of *A. flavus*. A better understanding of genetic variability and population structure within and between field populations may reveal how we can most effectively manage this important pathogen.

Acknowledgements

We are grateful to Dr. P.J. Cotty for his critical suggestions to the manuscript. We are also thankful to C. Roggero and INTA, Formosa, for providing the peanut samples. This research was supported by the Agencia Nacional para la promoción de la Ciencia y Técnica, PRHIDEB-CONICET (Publicación No. 153) and the University of Buenos Aires.

References

Bayman, P., Cotty, P.J., 1991a. Improve media for selecting nitrate-nonuntilizing mutants in *Aspergillus flavus*. Mycologia 83, 311–316.

Bayman, P., Cotty, P.J., 1991b. Vegetative and genetic diversity in the *Aspergillus flavus* population of a single field. Can. J. Bot. 69, 1707–1711.

Bayman, P., Cotty, P.J., 1993. Genetic diversity in *Aspergillus flavus*: association with aflatoxin production and morphology. Can. J. Bot. 71, 23–31.

Bigelow, D.M., Orum, T.V., Cotty, P.J., Nelson, M.R., 2000. Monitoring *Aspergillus flavus* AF36 and S strain incidence in the desert Southwest. 2000 APS Pacific Division Meeting Abstracts (joint with Canadian Phytophatologycal Society), Victoria, BC, Canada.

Blaney, B.J., Kelly, M.A., Tyler, A.L., Connole, M.D., 1989. Aflatoxin and cyclopiazonic acid production by Queensland isolates of *Aspergillus flavus* and *Aspergillus parasiticus*. Aust. J. Agric. Resour. 40, 395–400.

Boyd, M.L., Cotty, P.J., 2001. *Aspergillus flavus* and aflatoxin contamination of leguminous trees of the Sonoran desert in Arizona. Phytopathology 91, 913–919.

Brooker, N.L., Leslie, Y.F., Dickman, M.B., 1991. Nitrate non-utilizing mutants of colletotrichum and their use in studies of VC and genetics relatedness. Phytopathology 81, 672–677.

Carwell, K.F., Cotty, P.J., 2002. Distribution of *Aspergillus* section Flavi among field soils from four agroecological zones of the Republic of Bénin, West Africa. Plant Dis. 86, 434–439.

Correll, J.C., Klittich, C.J.R., Leslie, J.F., 1989. Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniluforme*). Mycol. Res. 93, 21–27.

Cotty, P.J., 1989. Virulence and cultural characteristics of two *Aspergillus flavus* strains pathogenic on cotton. Phytopathology 79, 808–814.

Cotty, P.J., 1994. Influence of field application of an atoxigenic strain of *Aspergillus flavus* on the populations of *A. flavus* infecting cotton bolls and on the aflatoxin content of cottonseed. Phytopathology 84, 1270–1277.

Cotty, P.J., 1997. Aflatoxin-producing potential of communities of *Aspergillus* section Flavi from cotton producing areas in the United States. Mycol. Res. 101 (6), 698–704.

Cotty, P.J., Cardwell, K.F., 1999. Divergence of West African and North American communities of *Aspergillus* section Flavi. Appl. Environ. Microbiol. 65, 2264–2266.

Cove, D.J., 1976. Chlorate toxicity in *Aspergillus nidulans*: the selection and characterization of chlorate resistant mutants. Heredity 36, 191–203.

De Fina, A.L., 1992. Aptitud agroclimática de la República Argentina. Academia Nacional de Agronomía y Veterinaria, Buenos Aires, Argentina. 427 pp.

Diener, U.L., Cole, R.J., Sanders, T.H., Payne, G.A., Lee, L.S., Klich, M.A., 1987. Epidemiology of aflatoxin formation by *Aspergillus flavus*. Annu. Rev. Phytopathol. 25, 249–270.

Fernandez Pinto, V., Patriarca, A., 2001. Natural co-occurence of aflatoxin and cyclopiazonic acid in peanuts grown in Argentina. Food Addit. Contam. 18, 1017–1020.

Filtenborg, O., Frisvad, J.C., 1980. A simple screening method for toxigenic molds in pure cultures. Lebensm.-Wiss. Technol. 13, 128–130.

Filtenborg, O., Frisvad, J.C., Svendsen, J.A., 1983. Simple screening method for molds producing intracellular mycotoxins in pure cultures. Appl. Environ. Microbiol. 45, 581–585.

Garber, R.K., Cotty, P.J., 1997. Formation of sclerotia and aflatoxins in developing cotton bolls infected by the S strain of *Aspergillus flavus* and potential for biocontrol with an atoxigenic strain. Phytopathology 87, 940–945.

Geiser, D.M., Pitt, J.I., Taylor, J.W., 1998. Cryptic speciation and recombination in the aflatoxin producing fungus *Aspergillus flavus*. Proc. Natl. Acad. Sci. U. S. A. 95, 388–393.

Geiser, D.M., Dorner, J.W., Horn, B.W., Taylor, J.W., 2001. The phylogenetics of mycotoxin and sclerotium production in *Aspergillus flavus* and *Aspergillus oryzae*. Fungal Genet. Biol. 31, 169–179.

Harvez, J., 1996. Análisis y perspectivas de la comercialización de maní. Informe para la Secretaría de Agricultura, Pesca y Alimentación

Hesseltine, C.W., ShotwellL, O.T., Smith, M., Ellis, J.J., Vandegraft, E., Shannon, G., 1970. Production of various aflatoxins by strains of the *Aspergillus flavus* series. Proceedings of the First US–Japan Conference on Toxic Microorganisms, UJNR Joint Panels on Toxic Microorganisms and the US Department of the Interior, Washington, DC, pp. 202–210.

Horn, B.W., Greene, R.L., 1995. Vegetative compatibility within populations of *Aspergillus flavus*, *A. parasiticus*, and *A .tamarii* from a peanut field. Mycologia 87 (3), 324–332.

Horn, B.W., Dorner, J.W., 1999. Regional differences in production of aflatoxin B_1 and cyclopiazonic acid by soil isolates of *Aspergillus flavus* along a transect within the United States. Appl. Environ. Microbiol. 65, 1444–1449.

Horn, B.W., Greene, R.L., Soboler, V.S., Dorner, J.W., Powell, J.H., Lyton, R.C., 1996. Association of morphology and mycotoxin production with vegetative compatibility groups in *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus tamarii*. Mycologia 88, 574–587.

Klich, M.A., Pitt, J.I., 1988. Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. Trans. Br. Mycol. Soc. 91, 99–108.

Klittich, C.J.R., Leslie, J.F., 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Giberella fujikuroi*). Genetics 118, 417–423.

Korolev, N., Katan, T., 1997. Improved medium for selecting nitrate-nonutilizing (*nit*) mutants of *Verticillium dahliae*. Phytopathology 87, 1067–1070.

Novas, M.V., Cabral, D., 2002. Association of mycotoxin and sclerotia production with compatibility groups in *Aspergillus flavus* from peanut in Argentina. Plant Dis. 86, 215–219.

Orum, T.V., Bigelow, D.M., Nelson, M.R., Howell, D.R., Cotty, P.J., 1997. Spatial and temporal patterns of *Aspergillus flavus* strain composition and propagule density in Yuma County, Arizona soils. Plant Dis. 81, 911–916.

Papa, K.E., 1986. Heterokarion incompatibility in *Aspergillus flavus*. Mycologia 78, 98–101.

Peraica, M., Radic', B., Lucic', A., Pavlovic', M., 1999. Toxic effects of mycotoxins in humans. Bull. World Health Organ. 77 (9), 754–763.

Pitt, J.I., Hocking, A.D., 1997. Fungi and Food Spoilage, 2nd ed. Aspen Publishers, Gaithersburg, MD, USA.

Rao, B.L., Husain, A., 1985. Precence of cyclopiazonic acid in kodo millet (*Paspalum scrobiculatum*) causing "koda poisoning" in man and its production by associated fungi. Mycopathologia 89, 177–180.

Saito, M., Tsuruta, O., 1993. Anew variety of *Aspergillus flavus* from tropical soil in Thailand and its aflatoxin productivity. Proc. Jpn. Assoc. Mycotoxicol. 37, 31–36.

Saito, M., Tsuruta, O., Siriacha, P., Kawasugi, S., Manabe, M., Buangsuwon, D., 1986. Distribution and aflatoxin productivity of the atypical strains of *Aspergillus flavus* isolated from soils in Thailand. Proc. Jpn. Assoc. Mycotoxicol. 24, 41–46.

Tran-Dinh, N., Pitt, J.I., Carter, D.A., 1999. Molecular genotype analysis of natural toxigenic and nontoxigenic isolates of *Aspergillus flavus* and *A. parasiticus*. Mycol. Res. 103 (11), 1485–1490.

Vaamonde, G., Varvsavsky, E., 1979. Producción de aflatoxinas sobre diferentes variedades de maní. An. Assoc. Quim. Argent. 67, 139–152.

Vaamonde, G., Degrossi, C., Comerio, R., Fernandez Pinto, V., 1995. *Aspergillus flavus* y *A. parasiticus* en maní cultivado en la provincia de Córdoba (Argentina): características diferenciales y capacidad aflatoxicogénica. Bol. Soc. Argent. Bot. 30 (3–4), 191–198.

Vaamonde, G., Patriarca, A., Fernáandez Pinto, V., Comerio, R., Degrossi, C., 2003. Variability of aflatoxin and cyclopiazonic acid production by *Aspergillus* section flavi from different substrates in Argentina. Int. J. Food Microbiol. 2738, 1–6.

Wicklow, D.T., Shotwell, O.L., 1983. Intrafungal distribution of aflatoxins among conidia and sclerotia of *Aspergillus flavus* and *Aspergillus parasiticus*. Can. J. Microbiol. 29, 1–5.

Zar, J.H., 1996. Biostatistical Analysis, 3rd ed. Pretince Hall, Upper Saddle River, NJ, USA.

Zuloaga, F.O., Morrone, O., 1999. Catálogo de las plantas vasculares de la República Argentina II. Missouri Botanical Garden Press, pp. 620–625.