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Mating Populations and Effective Population Number in *Gibberella fujikuroi* species Complex of Rotted Maize Ears under Egyptian Conditions

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Maize growing fields surveyed for the infection with ear rots throughout 8 locations in Egypt indicated the prevalence of ear rots caused by *Fusarium* spp. The toxigenic isolates from the whole number recovered from this group of fungi were subjected to identification biologically. Two mating populations were only identified as MAT-A (*F. verticillioides)* and MAT-D (*F. proliferatum*). MAT-A prevailed in all locations, but only 5 isolates were identified as MAT-D. Isolates belong to MAT-A produced considerable amounts of fumonisin compared to those belong to MAT-D. Female fertile strains could produce higher amounts of the toxin than female sterile. Through measuring the parameters of the effective population number in biological species recovered from maize ears, it was found that the female sterility was lesser than 50%. This caused an increase in the inbreeding effective population number and decrease in the variance effective population number overall locations.

Key words: Ear rot, fumonisin, *Fusarium* spp., *F. proliferatum*, *F. verticillioides*, maize and mating populations.

Kernel and ear rot is one of the most prevalent diseases of maize that threaten productivity in Egypt. Surveying harvested maize grains done by many investigators (Munkvold and Desjardins, 1997; Desjardins *et al.*, 2000; and El-Shabrawy, 2001), who reported that *Fusarium spp.* is the major fungal population recovered from the rotted maize ears. *F. verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* Sheldon) is the most dominant species and earlier colonist of the pre-harvest maize ears before other moulds such as *Penicillium* spp. and *Aspergillus* spp. (El-Shabrawy, 2001). *F. verticillioides* and *F. proliferatum*, to some extent, are responsible for the production of fumonisin which have become the most contaminants of maize grain and maize commodities (Chelkowski and Lew, 1992; Musser and Plattner, 1997). This group of mycotoxins are of concern due to their toxicological implications to man and animal (Franceschi *et al.*, 1990 and Abbas *et al.*, 1999). *Gibberella fujikuroi* (Sawada) is the teleomorph for many of the species of conidial anamorphs in *Fusarium* section *Liseola* including *F. verticillioides* and *F. proliferatum*.

Equilibrium considerations of mixed modes of reproduction that affect the evolution of new stains from *G. fujikuroi* species complex in the consecutive generations were studied in the present investigation. The effective population size parameters for the *G. fujikuroi* species complex exist on rotted maize ears were calculated to assess the shifting species from sexual to asexual reproductive modes were also estimated.

Materials and Methods

Affected sample collection, isolation and identification of the causal organisms:

Moulded maize ears were obtained from three growing maize fields, representing each of the surveyed locations, at harvest time in 2001 and 2002. Ear grains were thoroughly mixed and 500g sub-samples from each were used in this study. Isolations of the prevalent fungi on grain were isolated on PDA, purified using single spore technique and microscopically identified. Obtained isolates were kept under -80°C for further studies.

Detection and determination of fumonisin (FB1) produced by Fusarium isolates:

For measuring the potentiality of the recovered Fusarium isolates to produce FB1, they were grown on coarsely cracked yellow maize grains as described by Nelson *et al.* (1993). Isolates were screened for their ability to produce FB1 by visual scanning under UV (365 nm) according to Ross *et al.* (1990). Quantitative determination of FB1 was carried out as the method adopted by Dupuy *et al.* (1993). Quantification was performed with a spectrophotodensitometer set at 600 nm.

Biological identification of mating populations, mating types and female fertility in Gibberella fujikuroi species complex:

Following methods described by Klittich and Leslie (1988), Burgess *et al.* (1994) and Leslie (1996), work was done in this part of investigation. The ability to cross and to produce the teleomorph sexual or perfect stage with standard testers of defined species groups is the ultimate assurance of correct species identification. Cross fertility for species identification was done with strains that are members of *G. fujikuroi* species complex and were done by crossing the unknown strains of *Fusarium* spp. as the male parent with the standard tester strains of known species and mating type which serve as a female parent. Tester strains included two mating populations, *i.e.* MAT-A and MAT-D, both of them consists of the two mating types MAT-1 and MAT-2, were kindly provided from Prof. John Leslie, Kansas State Univ., Kansas, USA. Reciprocal crosses were carried out using unknown strains, as both male and female, to study the female fertility.

Effective population number (Ne) in G. fujikuroi species complex:

As stated by Crow (1954) and Crow and Denniston (1988), two commonly used effective population numbers are defined: the inbreeding effective population number $Ne(f)$, which is based on the probability of identity due to common ancestry (differences in mating types or female sterile / hermaphrodite), and the variance effective population number $Ne(v)$ which is based on the amount of allele frequency drift per generation as measured by its variance. Effective population number equations proposed by Leslie and Klein (1996) used in this study are shown below:

1- Inbreeding effective population number:

a- Based on mating type: $Ne = (4NmNf) / (Nm+Nf)$

Whereas, Nm = MAT-1 and Nf = MAT-2.

b- Based on female sterile/hermaphrodite polymorphism:

$Ne(f) = (4N^2Nh) / (N+Nh)^2$

Whereas, N = female sterile and Nh = hermaphrodite.

2- The variance effective population number: $Ne(v) = N^2/(N+Nh)$

Whereas, N = female sterile and Nh = hermaphrodite.

Results

Results in Table (1) indicate that a total number of 288 Fusarium isolates could be recovered from the samples of moulded maize grains obtained from the surveyed locations under study. These isolates were visually screened under UV (365nm) for their ability to produce the mycotoxin fumonisin (FB1). It was found that only 177 Fusarium isolates were able to produce FB1 toxin.

Table 1. Toxigenic Fusarium isolates collected from infected grain samples

Location	No. of collected isolates	No. of toxigenic isolates	Toxigenic isolates (%)
Beheira	50	43	86.0
Beni-Suef	37	15	44.0
Dakahliya	27	15	55.0
Gharbiya	32	15	46.8
Kafr El-Sheikh	45	29	64.4
Minufiya	25	16	64.0
Nubariya	43	27	62.7
Sharkiya	29	17	58.6
Total	288	177	61.5

According to the intensity of the fluorescence on TLC plates under UV (365nm), the toxigenic isolates of the recovered *Fusarium* spp. varied from very low (+) to very high (+++++) in producing FB1 toxin. Also, data (Table 1) reveal that toxigenic isolates percentages were differed according to different locations. For instance, isolates obtained from Beheira followed by Kafr El-Sheikh, Minufiya and Nubariya were highly efficient in producing FB1 comparable to isolates collected from the other locations. The least percentage of toxigenic Fusarium isolates was observed from Beni-Suef.

Out of the 177 toxigenic Fusarium isolates, 120 ones, varied (from very low to very high) in their efficiency to produce FB1 toxin and represented all the surveyed locations (15 isolates for each), were biologically identified using the sexual cross fertility with both of the two tester mating populations, *i.e.* MAT-A (*F. verticillioides*) and MAT-D (*F. proliferatum*), that included the two mating types MAT-1 and MAT-2. Results presented in Table (2) indicate that MAT-A was found to be included 115 isolates that consisted of 97 strains of mating type 1 (MAT-1) and 18 strains of mating type 2 (MAT-2). Meanwhile, MAT-D was found to be included 5 isolates that consisted of only 3 strains of MAT-1 and 2 strains of MAT-2.

Table 2. Biological identification (mating population, mating type, female fertility and levels of fumonisin) of toxigenic Fusarium strains isolated from maize ears obtained from different locations

Isolate No.	Location	MAT *	F.F **	FB1***	FB1 ppm
1	Beheira	A+	No	++	ND
2	„	A+	Yes	++	„
3	„	A-	Yes	++	„
4	„	A+	Yes	+++	263
5	„	A+	No	++	110
6	„	A+	No	++	109.2
7	„	A+	No	++	94.6
8	„	A+	Yes	++++	ND
9	„	A+	Yes	++++	„
10	„	A+	Yes	++++	„
11	„	A+	Yes	+++	„
12	„	A+	Yes	++++	„
13	„	A+	Yes	+++++	537
14	„	A+	No	++	63
15	„	D+	Yes	++	104
16	Beni-Suef	A+	Yes	++++	479
17	„	A+	No	+	ND
18	„	A+	Yes	++	122
19	„	A+	No	++	ND
20	„	A+	No	++	73.3
21	„	A+	Yes	+++	ND
22	„	A+	No	+	57
23	„	A+	Yes	++	ND
24	„	A+	No	++	„
25	„	A+	Yes	+++	161
26	„	A+	No	+	ND
27	„	A+	Yes	++	„
28	„	A+	Yes	++	„
29	„	A+	No	+	„
30	„	A-	No	+	37
31	Dakahliya	D+	Yes	+++	244
32	„	A+	Yes	+++	281
33	„	D-	No	++	73
34	„	A+	Yes	++	ND
35	„	A+	Yes	+	6.4
36	„	A+	No	+	13
37	„	A+	No	++	ND
38	„	A-	Yes	+++	„
39	„	A+	No	+	63.6

Table 2. Continued

40	Dakahliya	A-	Yes	++	ND
41	,,	A+	Yes	++	,,
42	,,	A-	No	+	,,
43	,,	A+	Yes	++	,,
44	,,	A-	No	++	,,
45	,,	A-	Yes	++	,,
46	Gharbiya	A+	Yes	++	,,
47	,,	A+	Yes	+++	,,
48	,,	A+	Yes	+++	332
49	,,	A+	Yes	++	83.5
50	,,	A+	Yes	+++	ND
51	,,	A+	Yes	++	,,
52	,,	A-	No	+	51
53	,,	A+	Yes	++	ND
54	,,	A+	Yes	++	,,
55	,,	A+	No	+	53
56	,,	A-	Yes	+	ND
57	,,	A+	Yes	+++	227
58	,,	D-	No	++	64
59	,,	A+	Yes	++	ND
60	,,	A+	Yes	++	,,
61	Kafr El-Sheikh	A+	Yes	+++++	607
62	,,	A+	Yes	+++	321
63	,,	A+	Yes	+++	ND
64	,,	A+	Yes	++++	,,
65	,,	A+	No	++	,,
66	,,	A+	No	++	,,
67	,,	A-	No	++	102
68	,,	A+	Yes	++	ND
69	,,	A+	Yes	+++++	603
70	,,	A+	Yes	+++	ND
71	,,	A+	No	++	78.9
72	,,	A+	No	++	172
73	,,	A+	Yes	+++	ND
74	,,	A+	Yes	++	,,
75	,,	A+	Yes	++	,,
76	Minufiya	A+	Yes	++	,,
77	,,	A+	No	++	73.4
78	,,	A+	Yes	++++	681.4
79	,,	A+	Yes	++	ND
80	,,	A-	Yes	+	,,
81	,,	A+	Yes	++	,,
82	,,	A-	No	++	,,

Table 2. Continued

83	Minufiya	A-	Yes	+++	ND
84	„	A+	No	++	„
85	„	A+	Yes	+++	344
86	„	A+	Yes	++	ND
87	„	A+	Yes	+++	„
88	„	A+	Yes	+++++	689.7
89	„	A+	No	++	117.4
90	„	A-	No	++	64.3
91	Nubariya	A+	No	++	114
92	„	A+	Yes	+++++	887.4
93	„	A+	Yes	+++	ND
94	„	A+	Yes	++	„
95	„	A+	No	++	145
96	„	A+	Yes	++	ND
97	„	A+	No	++	„
98	„	A+	No	++	14.3
99	„	A+	Yes	++	ND
100	„	A+	Yes	+++++	1020
101	„	A+	Yes	+++++	765
102	„	A-	Yes	++	ND
103	„	A-	Yes	+++	„
104	„	A+	Yes	++	„
105	„	A+	Yes	+++	„
106	Sharkiya	A-	Yes	++	„
107	„	D+	Yes	+++	242
108	„	A+	Yes	+++	ND
109	„	A+	Yes	+++	„
110	„	A+	No	+++	„
111	„	A+	Yes	++	„
112	„	A+	No	+++	„
113	„	A+	Yes	++++	418
114	„	A-	No	++	ND
115	„	A+	Yes	++	„
116	„	A+	Yes	++++	„
117	„	A+	No	++	94
118	„	A+	No	+	57
119	„	A+	No	+	54.4
120	„	A+	Yes	+++	175.4

* MAT = mating population A and D. Mating type one= (+) and mating type two= (-).
** F.F: Female fertile strains.
*** FB1= Intensity of fluorescence under UV visual scanning.
FB1 ppm= Levels of fumonisin B1 quantitatively. ND= not detected.

Also, from reciprocal crosses between tester strains and the unknown Fusarium isolates which tested either as a male or as a female, it could be concluded that in mating population 'A', 75 isolates were found to be female-fertile or hermaphrodites, but 40 isolates functioned as male only. While, in mating population 'D', 3 isolates functioned as hermaphrodites and 2 isolates were female-sterile.

A number of 49 isolates were chosen, out of the total of the 120 toxigenic *Fusarium* isolates, according to their mating types, visual fluorescence intensity scanning for FB1 as well as fertility and locations, to determine quantitatively their efficiency to produce the FB1. Results in Table (2) indicate that a clear correlation was found between the intensity of the fluorescence under UV (visual scanning) and the quantities (ppm) of the toxin. In general, the high visual intense (+++++) was found to correlate with the high analyzed level of FB1 (1020-537ppm), while, the low degree of fluorescence intensity was correlated with the low level of the detected FB1 (about 6 to 100 ppm). Moreover, it was observed that Fusarium isolates, that chosen throughout this part of work from Nubariya, Kafr-El-Sheikh and Minufiya, had the potential to produce the highest average levels of fumonisin comparable to the isolates from the other locations. Also, it was found a correlation between the mating type and female fertility in relation to the amount of toxin production. The female fertile MAT-1 strains were able to produce high levels of FB1when compared with the female sterile MAT-1 or MAT-2 (Table 2).

Also, obtained results (Table 2) show that, in general, the recovered 5 strains of the mating population 'D' (*F. proliferatum*) were lesser in their efficiency to produce FB1 comparable to the strains belong to the mating population 'A' (*F. verticillioides*).

Effective population size in G. fujikuroi species complex:

The effective population number is a critical parameter used to estimate the effects of genetic drift during sexual reproduction and inbreeding, and to compare field populations to an idealized population (equations). From data of mating populations, mating types and female fertility shown in Table (2), and by the aid of using the equations of the effective population number (*Ne)* for differences based on mating type, female fertility *Ne(f)* and the variance effective population number *Ne(v)*, the relative rarity of sexual reproduction that may permit female sterile strains to accumulate to a level such that local population could completely lose sexuality and appear as asexual (imperfect) species could be illustrated in Tables (3 and 4).

Results in Tables (3 and 4) indicate that the two species (mating populations 'A' and 'D') all over the locations under study have female sterile (FS) strains in frequencies < 50%, where 34.7% FS were found in MAT-'A' and 40% in MAT-'D' (Table 3). But, among locations, data in Table (4) reveal that the FS percentage was high in populations of Beni-Suef and Sharkiya (46.7 and 100%, respectively) and low (from 21.4 to 35.7%) in other locations. The effective population number (*Ne*) based on the mating type was 50.4 in MAT-'A', where MAT-1/ MAT-2 (98/17). But, the *Ne* based on the FS/hermaphroditism was 95.6% and *Ne (v)* was 60.5%. Similar population size of the effective population number in MAT-'D' as in MAT-'A' was observed, except that the *Ne* based on the mating type was very high (96%).

Table 3. The effective population number (*Ne*) in mating populations 'A' and 'D' isolated from maize ear rot overall locations

Biological species	Mating type *Mat-1/Mat-2*	*N(fs):N(h)**	*Fs* (%)**	*Ne****		
				M.T.	*Ne(f)*	*Ne (v)*
A	97/18	40/75	34.7	50.4	96.0	60.0
D	3/2	2/3	40.0	96.0	93.8	62.5

* *N(fs)*: Number of female sterile strains. *N(h)*: Number of female fertile strains.
** *Fs (%)*: Percentage of female sterile per total count.
*** (*Ne*): Effective population number.
(*M.T.*): Inbreeding effective population number based on mating types and expressed as percent of actual count.
Ne(f): Inbreeding effective population number based on number of males and hermaphrodites and expressed as percent of actual count.
Ne(v): Variance effective number based on number of males and hermaphrodites and expressed as percent of actual count.

Data presented in Table (4) show that the effective population number (*Ne*) differed from location to location, where low *Ne* was found in Kafr El-Sheikh, Beheira and Beni-Suef, according to the measured parameters based on the ratio between MAT-1 to MAT-2 (24.8 %). Dakahliya and Minufiya show the high *Ne* (94.7 and 78.2 %, respectively). Except in populations of Dakahliya, almost similar high results concerning the *Ne* based on FS/hermaphroditism and low results for *Ne(v)* were found. It could be concluded that, in general, the highest effective population number (*Ne*) was found in the population of Minufiya.

Table 4. The effective population number (*Ne*) in relation to the surveyed locations

Location	Mating type *Mat-1/Mat-2*	*N(fs): N(h)**	*Fs* (%)**	*Ne****		
				M.T.	*Ne(f)*	*Ne (v)*
Beheira	13/1	5/9	35.7	24.8	95.3	60.8
Beni-Suef	14/1	7/8	46.7	24.8	90.7	65.2
Dakahliya	8/5	1/0	100.0	94.7	00.0	100.0
Gharbiya	12/2	3/11	21.4	48.9	98.5	56.0
Kafr El-Sheikh	14/1	5/10	33.3	24.8	96.0	60.0
Minufiya	11/4	5/10	33.3	78.2	96.0	60.0
Nubariya	13/2	4/11	26.6	46.2	97.6	57.7
Sharkiya	12/2	6/8	21.4	48.9	92.6	63.6

* *N(fs)*, *Fs (%)*,(*Ne*), (*M.T.*), *Ne(f)* and *Ne(v)* as described in footnote of Table (3).

Discussion

Results obtained in the present investigation indicated that a number of 177 out of the total number of 288 Fusarium isolates recovered from rotted maize ears were shown by visual scanning under UV to have the potential to produce fumonisin. Among these toxigenic isolates, 120 individuals representing the surveyed locations were chosen for biological identification. Two biological species (MAT-'A' and MAT-'D') were identified throughout this work and MAT-'A' was the most common compared with MAT-'D'. This is in conformity with those obtained by Leslie (1995) and Kedera *et al.* (1999), who stated that *F. verticillioides* (MAT-'A') and *F. proliferatum* (MAT-'D') are the most prevalent strains within *Fusarium* section *Liseola* that affect maize. Isolates that belong to MAT-'A' were able to produce considerable amounts of FB1 if compared with isolates of MAT-'D'. The same findings were found by (Thiel *et al.*, 1991) who stated that *F. verticillioides* is considered the most dangerous strain in section *Liseola* as a toxigenic agent threatening man and animal health. Dealing with the toxigenic isolates of Fusarium in the present study showed clear correlation between the levels of fumonisin produced expressed as intensity of the fluorescence under UV in visual scanning, location from which these isolates were recovered from, mating population ('A' or 'D') and the female fertility in the population. Findings stated by Nelson *et al.* (1991) are consistent with these results. They found that the potential exists for production of fumonisins by such strains in agricultural commodities and other substrates is widespread in geographic areas. Alike findings obtained throughout this study, Leslie *et al.* (1992) reported that members of the biological species 'A' (MAT-'A') could produce an average of 786 ppm of the toxin, much higher than members of the 'D' population which averaged 636 ppm when grown on maize grains. It could also be emphasized by Leslie *et al.* (1992) that the female fertile strains produced considerable amounts of FB1comparable to that produced by the female sterile ones of the same species.

In studying the effective population number based on the mating types (MAT-1/MAT-2) ratios were different in both mating populations (A and D) from 1:1, resulting in large decreases in the inbreeding effective population number to 50.4% of the count for MAT-'A' and 96% in MAT-'D'. Concerning the effective population number based on FS / hermaphroditism, in both mating types were high, where it was 95.6% in MAT-'A' and 93.8% in MAT-'D' that because of the low percentage of FS proportion in both mating types, where it was 34.7 and 40% in MAT-'A' and MAT-'D', respectively. Also, the variance effective population number $Ne(v)$ was not differed in both mating populations, where it decreased due to increasing of female fertility. These results are in harmony with those of Leslie and klein (1996) who stated that the female fertility are at a selective advantage every time sexual reproduction occurs, and have an disadvantage during vegetative propagation.

It could be concluded, from results obtained throughout the present study, that strains within the mating population (MAT-'A') all-over the surveyed locations have the advantage to sexual reproduction occurs and the new recombination would be found, generally. As what emphasized by Leslie (1999), mating population D usually resembles mating population A in most these features, but is usually recovered in smaller quantities making such analyses more difficult. Hence, incomplete data derived during executing this work may be regarded to the insufficient frequency in MAT-'D'.

As regards to the effect of locations from which the field strains of *Fusarium* spp. were recovered from, the effective population number (*Ne*) based on the ratio of MAT-1/MAT-2 differed obviously from location to location, where it was higher greatly in Dakahliya population and lesser in that of Kafr El-Sheikh. Whereas, *Ne* based on FS/hermaphroditism, except in Dakahliya were always very high. FS/hermaphroditism was always <50%. It could be concluded from results derived from this study that sexual reproduction in mating population A (*Fusarium verticillioides*) recovered from affected maize ears throughout the studied locations, is common in higher frequencies in general. But, data of isolates obtained from Dakahliya suggest that sexual reproduction is not common, but widely dispersed clones would be expected.

In conclusion, there is a complex dynamic among populations of *Fusarium* spp. associated with grain moulds of maize as suggested by Mansuetus *et al.*(1997). Successful protection of maize from fungi associated with ear and grain mould will probably require an approach that combines host resistance mechanisms with other nonspecific control measures.

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العشائر التزاوجية والرقم المؤثر فى العشيرة من أنواع الجبريلا فوجيكيوروى على كيزان الذرة الشامية المصابة بالعفن تحت الظروف المصرية

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أثبتت الدراسة أن الأنواع من جنس الفيوزاريوم كانت هى السائدة على كيزان الذرة الشامية المصابة بالاعفان وذلك فى المناطق الذى اجرى فيها الحصر وهى ٨ مناطق مختلفة. وقد تم اختيار العزلات المفرزة للسموم (الفيومونيزين) الممثله لتلك المواقع موضع الدراسة بغرض تعريفها بيولوجيا. واظهرت طريقة التعريف البيولوجيه باستخدام العزلات القياسيه باجراء التزاوج وجود مجموعتين من العشائر فقط وهما مجموعة التزاوج (أ) الممثله لفطر فيوزاريوم فيرتيسيلويدس ومجموعة التزاوج (د) الممثله للفطر فيوزاريوم بروليفيراتم . وكانت افراد العشيره (أ) هى الاكثر تواجدا بكل المناطق بلا استثناء بينما لم يتواجد سوى ٥ افراد فقط من العشيره (د). كما امتلكت افراد العشيره (أ) مقدره عاليه على انتاج الفيومونيزين عن افراد العشيره (د). كذلك لوحظ ايضا ان العزلات الخصبه انثويا كانت ذات مقدرة عاليه على انتاج الفيومونيزين عن تلك العقيمة انثويا. كما اوضحت دراسة الرقم المؤثر فى التزاوج فى الانواع البيولوجيه المعزوله من كيزان الذرة الشامية ان هناك درجة منخفضه من العقم الانثوى تقل عن ٥٠% مما ادى زيادة الرقم المؤثر فى التزاوج نتيجة للتربيه الداخليه داخل العشيرة وقلة فى الرقم الراجع الى الاختلاف فى الاليلات الموجودة بالعشيرة بكل المواقع.