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OCCURRENCE OF OOSPORES OF *PHYTOPHTHORA INFESTANS* IN THE FIELD AND UNDER CONTROLLED CONDITIONS

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ABSTRACT

Both A1 and A2 mating types of *Phytophthora infestans* were detected in El-Behera governorate and the surrounding area where potato and tomato were intensively cultivated during the 2005-2006 growing season. The A2 mating type constituted 14.8% of the total isolates recovered (162) while A1 mating type constituted 83.9%. A 7.4% of the total potato and tomato fields (94) surveyed yielded A2 mating type while 89.3% of the fields yielded A1 mating type and three fields only yielded both A1 and A2 mating types. Investigations were focused on fields where both mating types were occurred as opportunity for oospore formation presumably high. No oospores were detected in any of the blighted potato tubers and tomato fruits as well as the blighted potato and tomato leaves collected at harvest from fields showed both mating types. This was despite that two of 63 tubers sampled yielded both the A1 and A2 mating types and abundance of oospores were produced *in vitro* on a variety of culture media testes particularly the Rye A and V8 Juice. The artificial inoculation of both mating types into whole freshly harvested (dormant) and old (sprouting) potato tubers as well as the tomato fruits resulted in a successful blight but no oospores were produced in the dormant tubers while the old sprouting ones showed little number of oospores. The tomato fruits, however, supported more oospores with the same isolate pairings and also the inoculated leaf discs of both potato and tomato yielded more oospores. Temperature ranging from 10 to 23 °C supported oospore formation with maximum oospore production was at 10°C. Wetness exhibited a crucial role for oospore

formation as free moisture (floating leaf discs) supported oospore production in inoculated leaf discs while no oospores were formed in leaf discs on moist filter paper. Meantime, aeration supported oospore formation in inoculated leaf discs in nonsealed Petri dishes while sealed Petri dishes suppressed oospore formation in most pairings (five out of the six pairings conducted). The ratio of coinoculated A1 and A2 mating types was not an important factor in production of oospores. Even a ratio of 5A1 : 95A2 or 95A1 : 5A2 was resulted in oospore production which indicated that a low proportion of A2 mating type in the field may suffice for sexual reproduction of the pathogen and oospore formation. The apparently absence of oospores of *P. infestans* under field conditions in Egypt could be attributed to the absence of prolonged rainy season and the lack for A2 mating type in most fields.

keywords: *Phytophthora infestans*, mating type, oospores, potato, tomato.

INTRODUCTION

The role of oospores of *Phytophthora infestans* in the epidemiology of late blight in potato and tomato is still unclear (Hansen *et al.*, 2006). The conditions under which oospores can be produced *in planta* are only partially known (Levin *et al.*, 2001). The occurrence of A1 and A2 mating types in the field in several parts of the world (Shattock *et al.*, 1990, Mosa *et al.*, 1991; Drenth *et al.*, 1993, Flier *et al.*, 2001) created an opportunity for the fungus to reproduce sexually. Early observations of oospore production *in planta* were recorded in the highlands of Central Mexico, where the two mating types occur in equal ratios (Gallegly and Galindo, 1958). Later studies in the Netherlands (Frinking *et al.*, 1987), Germany (Göts, 1990), and Japan (Mosa *et al.*, 1991) revealed the occurrence of oospores in stems and leaves of potato and tomato plants grown in greenhouse. Oospores of *P. infestans* also reported in field grown potato and tomato in several countries (Niederhauser, 1991; Drenth *et al.*, 1995; Smirnov and Elansky, 1999; Fernández-Pavía *et al.*, 2004).

Therefore, oospores constitute a threat to potato and tomato cultivation worldwide. Oospores could serve as a primary inoculum (Anderson *et al.*, 1998, Lehtinen and Hannukkala, 2004), survival propagules (Mayton *et al.*, 2000; Turkensteen *et al.*, 2000), and a source of recombination and generation of new genotypes of *Phytophthora infestans* (Gavino *et al.*, 2000; Grünwald *et al.*, 2001). Potato tubers are globally traded in large quantities, thereby, oospores may introduce new recombinant genotypes of the fungus to new areas. Our objective for the present study was to investigate (i) potentiality of *P. infestans* isolates to produce oospores in potato tubers and tomato fruits in the field, in Egypt, and under controlled laboratory conditions, (ii) the influence of some factors (host, isolate origin, temperature, wetness, aeration) on oospore production *in planta*, (iii) and the marginal ratios of sporangia for the A1 and the A2 mating types that could enhance oospore formation. Conclusions will be drawn regarding the formation of oospores in the field in Egypt.

MATERIALS AND METHODS

1. Survey for A1 and A2 mating types of *Phytophthora infestans*.

- **Sampling, isolation, and maintenance methods.** During the 2005-2006 growing season, a survey was conducted for mating type where potato and tomato were grown in El-Behera governorate and the surrounding area. Blighted foliage was collected wherever late blight symptoms were detected and *P. infestans* was isolated on Rye-A medium (Caten and Jinks, 1968) amended with a mixture of antibiotics (nystatin at 25ug/ml, vancomycin at 100ug/ml, and rifampicin at 20ug/ml). The plates were incubated in the dark at 18 °C for 5-7 days and examined for *P. infestans* colonies. A single isolate was corresponding to a single separate lesion. Isolates were maintained on Rye-A and for short period storage were stored under sterilized mineral oil.

- **Mating type determination.** Mating type of all recovered isolates was determined according to Shaw *et al.* (1985) using tester isolates of known mating type kindly supplied by D. S. Shaw, University of Wales, UK. Unknown field isolates were paired against each of the A1 and A2 tester isolate using mycelial plugs (5mm in diameter) and

placed 3cm apart onto plates of Rye-A and incubated at 18°C in the dark. Oospore formation with one tester indicated that the isolate was of the opposite mating type.

2. Natural occurrence of oospores in blighted potato tubers and tomato fruits.

At the harvest of the 2005-2006 growing season, blighted potato tubers and tomato fruits were collected from the fields where both A1and A2 mating types were detected in the same field in the earlier survey. This where opportunity for sexual reproduction and oospore formation presumably high. Tubers were examined for presence of oospores by slicing the blighted area into thin slices (1×1×0.1 cm). The slices were, then, boiled for 30 min in 1% HCl, rinsed in water, and mounted on glass slides in 50% glycerol and examined under dissecting microscope. Tomato fruit was cut along the stem scar. The internal necrotic tissue was removed with the aid of a scalpel, blended in adequate amount of water using a Polytron homogenizer, and examined under light microscope for oospore formation. Oospores were microscopically counted according to Hanson and Shattock (1998) in 0.01 ml of the suspension and total number of oospores was calculated. Blighted potato and tomato leaves were also sampled for comparison, leaflets were clarified in boiling ethanol for 5 min. and mounted in 50% glycerin solution and examined for oospores under dissecting microscope. Meantime, blighted portions of potato tubers, tomato fruits, and their leaves were taken for isolation and mating type determination of the associated fungal isolates as previously described.

3. Oospores formation under controlled conditions.

3.1. The *in vitro* oospore formation.

Four A1 and four A2 mating type isolates of *Phytophthora infestans* of potato and tomato origin recovered in survey conducted earlier were used. Isolates were maintained on Rye A agar medium at 18°C in the dark. Six combinations of six pairings were conducted on three media known of inducing oospore formation of *Phytophthora infestans* (Ribeiro, 1978). These media were Rye A agar (Caten and

Jinks, 1968), V8 Juice agar and PDA amended with β-sitosterol (Ribeiro, 1978). Pairings were conducted on plates of the different media in for replicate plates for each pairing and incubated at 18 ^{0}C in the dark. Three weeks later, a 2-cm-diameter disc was excised from the area between two inocula of each pairing, blended in 10ml distilled water and oospores were counted using 10x light microscope. Four counts were conducted for each culture disc.

3.2. Oospore formation in whole potato tubers and tomato fruits.

Fungal inoculums, of the same isolates used above in the *in vitro* test, were prepared on potato leaves (cv. Rossetta) at 15°C in the dark according to Levin *et al.* (2001). Sporangia were harvested after 7 days and sporangial suspension was adjusted to 2×10^3 per ml. Meantime, newly harvested healthy tubers (cv. Rossetta) as well as old sprouting tubers (stored at 4 °C for 20 weeks and left for one week at room temperature, 22 ± 3 °C, for sprouting) were obtained from Dept., of Vegetables Crop Research, the Horticultural Research Institute (South El-Tahrir). Tubers were surface disinfested with 95% ethyl alcohol, and incision-inoculated (10mm deep), in one middle eye, with 0.1 ml sporangial suspension (2×10^3/ ml) of a mixture of A1 and A2 mating type (1:1 ratio) of the *P. infestans* isolate pairings. Inoculated tubers were then incubated at 15°C in humid chamber in the dark for three weeks. Also, tomato fruits at the mature green stage, obtained from Dept. of Vegetables Crop Research, the Horticultural Research Institute, were surface disinfested with 95% ethyl alcohol and incision-inoculated (10mm deep) into the stem scar with the same previously prepared sporangial suspension of the tested isolate pairings. Inoculated fruits were placed in humid chamber at 15°C in the dark with 12 h/day fluorescent light illumination. Three weeks later, inoculated potato tubers and tomato fruits were examined for oospore formation by the same methods described above.

3.3. Oospore formation in potato and tomato leaves.

- **Fungal isolates.** Three pairings of A1 and A2 isolates of *Phytophthora infestans* showed potentiality to produce oospores *in vitro* and *in planta* in the previously conducted investigations and represented both potato and tomato origin were selected for the comming investigations. Fungal inoculum was propagated on potato leaves (cv. Rossetta) according to Levin *et al.* (2001).

- **Leaf materials.** Potato cv. Rossetta and tomato cv. Super strain B were obtained from the Vegetables Crop Research Dept., Horticultural Research Institute. Potato tubers were sown in 25-cm-diameter pots filled with peat -sand mixture in 3:1.also, tomato seeds were sown in nursery trays and then transplanted four weeks later to 25-cm pots filled with the same soil mixture. Pots were watered as needed and treated according to the normal agricultural practices.
- **Leaf inoculation.** This was conducted according to the leaf discs technique described by Drenth *et al.*, (1995). Leaflets from 6-8-week-old, potted potato (cv. Rossetta) and tomato (cv. Super strain B) plants were used to prepare 2-cm-diameter discs. These were placed on moist filter paper in 9 cm Petri dishes and inoculated at abaxial surface uppermost with A1+A2 (1:1 ratio, or otherwise states) sporangial mixture (2×10^3/ ml), one 10-µl droplet per disc. Dishes were incubated at 15°C (or otherwise stated) for one week. When symptoms appeared, water was added (or otherwise stated) to the plates in excess so all leaf discs were uniformly floating on the water and incubated for further two weeks. Plates were incubated nonsealed to insure the air flow (or otherwise stated). Four replicate leaf discs were prepared for each pairing and four replicate plates were conducted for each treatment. Leaf discs were investigated, three weeks after inoculation.

3.3.1. Effect of A1 and A2 ratios.

Potato and tomato leaf discs were prepared and inoculated as previously described except that leaf discs were inoculated with sporangial mixture consists of a series of A1 and A2 sporangial ratios, *i.e.* 5/95, 25/75, 50/50, 75/25, and 95/5.

3.3.2. Effect of temperature.

Potato and tomato leaf discs were prepared and inoculated as previously described except that inoculated plates were incubated at different degrees of temperature, *i.e.* 10 °C, 15 °C, and 23 °C.

3.3.3. Effect of wetness.

Leaf discs were prepared and inoculated as above described except that inoculated leaf discs were either placed in Petri dishes lined with moist filter paper or excessive water was added as described to keep inoculated leaf discs floating on free water.

3.3.4. Effect of aeration.

Leaf discs were prepared and inoculated as above described except that, one week after inoculation, dishes with floating discs were parafilm-sealed or nonsealed to investigate the effect of aeration on oospore formation.

Microscopic investigations and oospores counting.

Leaf discs were blended in adequate amount of water using a Polytron homogenizer (PT7 Humberside, UK) for 30 seconds and oospores were miroscopically investigated and counted according to Hanson and Shattock (1998).

Statistical analysis. The obtained data were statistically analysed according to Gomez and Gomez (1984) on the American Costat software.

RESULTS

1. Survey for A1 and A2 mating types of *Phytophthora infestans*.

The survey conducted on potato and tomato fields during the 2005-2006 growing season in El-Behera governorate and the surrounding area revealed the prevalence of the A1 mating type on potato and tomato foliage. Eighty four fields out of the ninety four potato and tomato fields investigated yielded A1 mating type isolates while only seven fields yielded the A2 mating type isolates. Both mating types (A1+A2), however, were recovered from two potato fields and one tomato field out of the total 94 fields surveyed. Meantime, only 24 isolates were of A2 mating type versus 136 A1 isolates were recovered over the survey which constituted 14.8% of the total. None of the mating types was linked to any of the potato or the tomato host (Table 1).

Table 1: Occurrence of A1 and A2 mating types of *Phytophthora infestans* in potato and tomato fields surveyed during the 2005-2006 growing season.

	Host	Total	A1	A2	A1+A2
Fields	Potato	71	64	5	2
	tomato	23	20	2	1
	Total	94	84	7	3
	%*		89.3	7.4	3.1
Isolates	Potato	108	86	20	2**
	tomato	54	50	4	-
	Total	162	136	24	2**
	%*		83.9	14.8	

Values are number of fields surveyed or isolates recovered. * percentage of mating types in the total fields or isolates. ** homothallic (A1A2) as the isolate formed oospores in pairing with both A1 and A2 testers.

2. Natural occurrence of oospores in blighted potato tubers and tomato fruits.

Further investigations for oospore formation were conducted at harvest in blighted potato tubers and tomato fruits (Fig. 1a) as well as their leaves for comparison. This was conducted where both mating types (A1+A2) were detected in the same field where opportunity for sexual reproduction presumably high. None of the potato tubers, tomato fruits or leaves of potato and tomato showed oospore in their tissues despite that two blighted tubers yielded both the A1 and A2 mating types. Meantime, the A1 mating type was prevalent as occurred in 79.4% in the total samples (190) collected while the A2 mating type occurred in 19.4% of the total samples (Table 2).

Table 2: Occurrence of oospores and mating types in blighted potato and tomato samples collected at harvest from fields where A1+A2 mating types were detected during the 2005-2006 growing season.

Host	Samples	Total samples investigated	Occurrence of oospores	Mating Type		
				A1	A2	A1+A2
Potato	Tubers	63	None	44	17	2
	Leaves	50	None	41	9	0
Tomato	Fruits	27	None	27	0	0
	Leaves	50	None	39	11	0
	Total	190		151	37	2
	%*			79.4	19.4	1.05

* percentage of mating types in the total samples collected.

3. Oospores formation under controlled conditions.

3.1. The *in vitro* oospore formation.

All pairings of A1 and A2 mating types with isolates of potato and tomato origin produced oospores *in vitro* in the culture media tested. The V8 Juice agar medium exhibited the highest oospore formation as 35.3 – 249.6 $x10^3$ oospore per 2-cm-diameter culture disc was recorded followed by 29.9 - 156.4 $x10^3$/ disc for Rye A while PDA amended with β-sitosterol exhibited oospore formation of 5.3 - 11.2 $x10^3$ /disc. No distinct variations were revealed for number of oospores produced with isolates of potato or tomato origin or between the different pairings (Table 3).

Table 3: The *in vitro* oospore formation in different media with different A1 and A2 pairings of isolates of potato and tomato origin.

Pairings A1x A2 Isolate Code No.	Source	Rye A ($x10^3$/disc)	V8 Juice agar ($x10^3$/disc)	PDA + β-sitosterol ($x10^3$/disc)
5P/05 x 24P/05	P x P	86.4±33.7	95.4±23.3	7.6±3.1
29P/06 x 41P/06	P x P	117.5±26.2	249.6±122.4	11.2±4.7
8T/05 x 12T/05	T x T	123.6±18.9	82.8±27.5	5.8±1.3
21T/06 x 23T/06	T x T	86.8±26.3	176.4±59.9	6.1±1.6
5P/05 x 12T/05	P x T	29.9±13.4	35.3±12.5	5.3±2.8
21T/06 x 29P/06	T x P	156.4±27.8	169.6±36.8	9.3±3.2

Values are number of oospores formed, ± standard deviation . Oospores were counted in 2-cm-diameter culture discs taken from the area between the two inocula of each pairing, three weeks after inoculation. Four counts were conducted for each disc. Four replicate plates were prepared for each pairing. P = potato, T = tomato.

3.2 Oospore formation in whole potato tubers and tomato fruits.

Data in table (4) showed that no oospores were formed in dormant (freshly harvested) whole potato tubers inoculated with the different pairings tested. However, inoculated sprouting tubers showed oospores (Fig. 1b) ranged between 28.7 and 73.8 in the blighted area while two of the pairings (5P/05 x 24P/05 ; 21T/06 x 23T/06) out of the six pairings conducted failed to produce oospores in these sprouting old tubers. More oospores were formed in the inoculated tomato fruits (Fig. 1c). Source of the isolates, *i.e.* their potato or tomato origin, engaged in the conducted pairings did not exhibit a distinct effect in this respect (Table 4). The most consistent three pairings were selected for further investigations for oospore formation on potato and tomato leaves.

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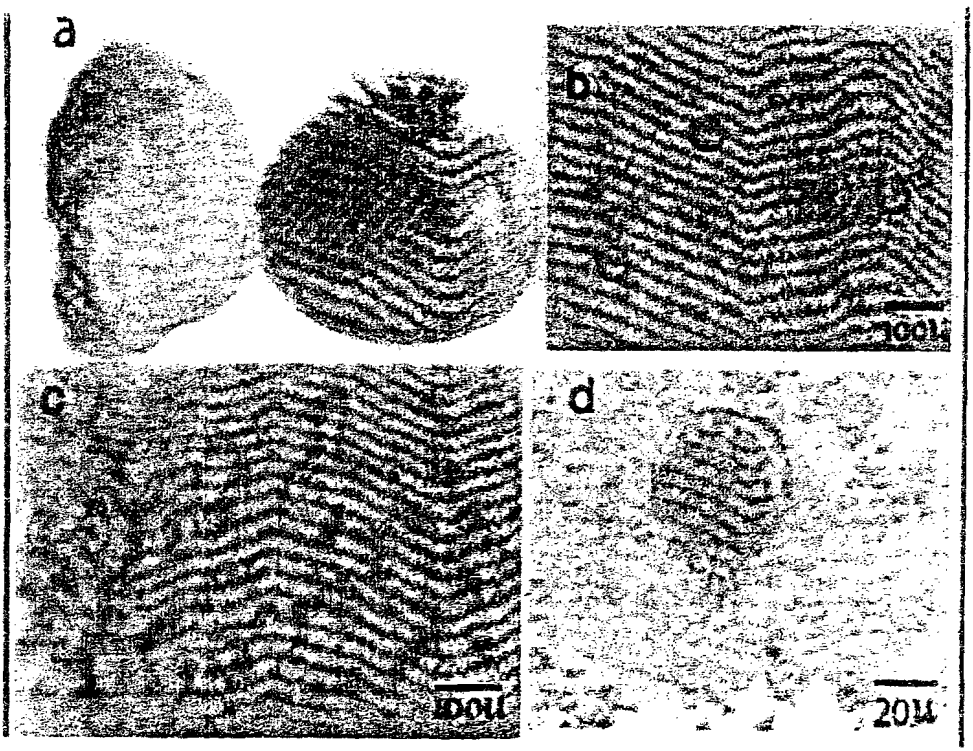

Fig.1. (a). Blighted potato tubers and tomato fruits sampled from fields in which both mating types (A1+A2) were detected during 2005-2006 growing season, (b). oospore produced in leaf discs of tomato cv. Super strain B, inoculated with 21T/06 x29P/06 pairing.

Table 4: Number of oospores produced in whole potato tubers and whole tomato fruits inoculated with different A1 and A2 isolate pairings.

Pairings A1x A2 Isolate Code No.	Source	Potato tubers		Tomato fruits
		Freshly harvested (Dormant)	Old sprouting	
5P/05 x 24P/05	P x P	0.0	0.0	338.4±92.5
29P/06 x 41P/06	P x P	0.0	73.8±3.5	142.6±53.6
8T/05 x 12T/05	T x T	0.0	28.7±12.4	438.3±77.4
21T/06 x23T/06	T x T	0.0	0.0	169.4±85.6
5P/05 x 12T/05	P x T	0.0	34.6±18.5	287.5±131.3
21T/06 x 29P/06	T x P	0.0	55.3±21.2	536.2±56.8

Values are total number of oospores produced in the blighted area of potato tubers or tomato fruits tissues, ± standard deviation. Potato tubers were of cv. Rossetta, while tomato fruits were of cv. Super strain B. Dormant tubers were freshly harvested potato tubers while sprouting tubers were tubers stored at 4°C for 20 weeks and left for sprouting for one week at room temperature (22 ± 3°C). P = potato, T = tomato.

3.3. Oospore formation in potato and tomato leaves.

3.3.1. Effect of the A1/A2 mating type ratio.

Inoculum of A1 and A2 sporangial mixture in the different ratios tested produced oospores in potato and tomato leaf discs (Fig 1 and Table 5). Number of oospores produced was the highest (256 – 714 /leaf disc) with the 50/50 ratio of A1/A2 mating types. The different deviations from this ratio were still producing oospores but at lower values which increased towards the 50/50 ratio. This was evident on both potato and tomato leaves and regardless of source of the involved isolates, *i.e.* potato and tomato origin (Table 5).

Table 5: Effect of the A1/A2 mating type ratio in sporangial inoculum on number of oospores of *Phytophthora infestans* produced in potato and tomato leaf discs with different isolate pairings.

Pairings A1x A2 Isolate Code No.	Source	Host*	A1/A2 Ratio (%)				
			5/95	25/75	50/50	75/25	95/5
29P/06 x 41P/06	P x P	Potato	87±23	292±86	326±131	217±113	125±53
		Tomato	119±112	147±69	412±128	338±126	187±62
8T/05 x 12T/05	T x T	Potato	34±16	182±24	256±117	417±182	136±115
		Tomato	114±65	197±46	316±194	186±63	162±23
21T/06 x 29P/06	T x P	Potato	163±109	203±23	273±163	264±129	172±47
		Tomato	115±42	317±121	714±226	457±213	143±58

Values are number of oospores per 2-cm-diameter potato or tomato leaf discs, ± standard deviation, *Potato cv. Rossetta; Tomato cv. Super strain B. P = potato, T = tomato.

3.3.2. Effect of temperature.

Temperature ranging from 10 to 23°C allowed oospore formation in potato and tomato leaf discs. Number of oospores produced at 10°C was the highest (253.9 – 612.7/leaf disc) in the different pairings tested. This was followed by the 15°C where numbers of oospores produced was in the range of 183.3-519.8/ leaf disc. At the 23°C, however, lower number (127.6-325.8 / leaf disc) of oospores were produced in potato or tomato leaf discs while two out of the six pairings conducted failed to produce oospores at this temperature degree (Table 6).

Table 6: Effect of temperature on number of oospores of *Phytophthora infestans* produced in potato and tomato leaf discs with different isolate pairings.

Pairings A1x A2 Isolate Code No.	Source	Host*	Temperature (°C)		
			10	15	23
29P/06 x 41P/06	P x P	Potato	418.3±194.3	264.4±65.7	0.0
		Tomato	612.7±252.8	502.5±213.8	215.4±81.6
8T/05 x 12T/05	T x T	Potato	253.9±97.5	197.9±56.3	196.2±87.4
		Tomato	425.2±124.6	316.2±94.6	127.6±63.6
21T/06 x 29P/06	T x P	Potato	393.6±161.4	183.3±61.7	0.0
		Tomato	526.8±226.3	519.8±315.4	325.8±97.4

Values are number of oospores per 2-cm-diameter potato or tomato leaf discs, ± standard deviation, *Potato cv. Rossetta; Tomato cv. Super strain B. P = potato, T = tomato.

3.3.3. Effect of wetness.

No oospores were produced in potato and tomato leaf discs placed in Petri dishes lined with moist filter paper and inoculated with sporangial suspension of the different isolate pairings. However, the inoculated floating leaf discs of potato and tomato in Petri dishes resulted in abundant number of oospores ranged between 216.9 /leaf disc and 546.2/ leaf disc for the different pairings conducted (Table 7).

3.3.4. Effect of aeration.

Sealing Petri dishes one week after inoculation and development of blight symptoms on leaf discs was resulted in suppressing of oospore formation in all pairings conducted on potato leaf discs while only one pairing on tomato out of the three pairings conducted produced little number of oospores (4.3 oospore/leaf disc). The nonsealed Petri dishes, however, produced abundant oosopres on potato and tomato leaf discs (Table 7).

Table 7: Effect of wetness and aeration on number of oospores of *Phytophthora infestans* produced in potato and tomato leaves in different isolate pairings.

Pairings A1x A2 Isolate Code No.	Source	Host*	Wetness		Aeration	
			Moist filter paper	Floating discs	Sealed dishes	Nonsealed dishes
29P/06 x 41P/06	P x P	Potato	0.0	397.4±112.5	0.0	285.4±87.2
		Tomato	0.0	546.2±142.4	0.0	573.5±342.8
8T/05 x 12T/05	T x T	Potato	0.0	216.9±105.6	0.0	182.9±42.6
		Tomato	0.0	391.2±123.7	4.3±3.8	412.2±213.5
21T/06 x 29P/06	T x P	Potato	0.0	264.3±52.8	0.0	219.5±111.3
		Tomato	0.0	522.8±133.6	0.0	446.8±241.7

Values are number of oospores per 2-cm-diameter potato or tomato leaf discs. ± standard deviation, *Potato cv. Rossetta; Tomato cv. Super strain B., P = potato, T = tomato.

DISCUSSION

The occurrence of both A1 and A2 mating types of *Phytophthora infestans* in Egypt (Shaw *et al.*, 1985; El-Korany, 1994; El-Komy, 2007) was confirmed in the present study. This raised a question about the opportunity for the fungus to reproduce sexually and to produce oospores under field conditions in Egypt. However, no oospores were detected in the present study in any of the blighted potato tubers, tomato fruits, and potato and tomato leaves collected from fields showed both the A1 and A2 mating types, where opportunity for oospore formation presumably high. This was despite that two of the 63 tubers sampled yielded both the A1 and A2 mating types and also abundance of oospores were produced *in vitro* in a variety of culture media testes particularly with Rye A and V8 Juice. The artificial inoculation of both mating types into whole Freshly harvested (dormant) and old (sprouting) potato tubers as well as the tomato fruits resulted in successful blight. In the same time, no oospores were produced in dormant tubers while the old sprouting ones showed little number of oospores. The tomato fruits, however, as well as leaves of potato and tomato showed more oospores with the same isolate pairings. The poor oospore formation in potato tubers in

the present study, could be explained for their relatively low free water content as the free moisture exhibited crucial role in the oospore formation in the present study. This could explain also that more oospores were produced in tomato fruits upon coinoculation with A1+A2 sporangia as tomato fruits are much juicier and of less compact tissues compared to the tuber tissues. This also could explain, in a part, formation of oospores in the old sprouting potato tubers and not in the freshly harvested dormant ones as the young dormant tuber tissue is more compact than old sprouting potato tubers. Supporting this wiew finding of the present study in which sealing Petri dishes (reduced aeration) of inoculated leaf discs suppressed oospore formation of most pairings conducted. The finding that tuber tissue becomes more conducive with aging may also result from either the formation of stimulators, the degradation of inhibitors, or both (Elliot, 1983). The finding that aged tubers were more supportive to oospore formation seemed to have an epidemiological significance as mating between both mating types could occur in infected tubers stored for Nili plantation. The present study also showed that a constant supply of free moisture to blighted lesions was important for sexual reproduction and oospore formation of *P. infestans* in host tissue. The practical implication was that prolonged rainy periods in nature may favor oospore production. Temperatures ranging from 10 to 23°C supported oospore formation in the present study with maximum production at 10°C. This means that favorable temperature for late blight epidemic development (15 to 20°C) do not necessarily coincide with the most favorable for sexual reproduction. The ratio of coinoculated A1 and A2 mating types was not an important factor in production of oospores. Even a ratio of 5A1 : 95A2 or 95 A1: 5A2 was resulted in oospore production. The practical implication of this finding was that a low proportion of A2 sporangia in the field may suffice for sexual reproduction of the pathogen. The dogma that a ratio of 1:1 mating types is required for oospore formation probably comes from the fact that in central Mexico, where oospores are common, A1 and A2 exist at a 1:1 ratio (Niederhauser, 1991). This composition probably reflects the mode of inheritance of the mating-type trait rather than the ratio required for oospore production (Shaw and Shattock, 1991). Tomato generally supported more oospore production in leaves than potato. The effect of the host, however, and

origin of the isolate varied in an inconsistent manner. These findings generally were in harmony with Drenth *et al.* (1995), Zarzycka and Sobkowiak (1997), Hanson and Shattock (1998), Gavino *et al.* (2000), Mayton *et al.* (2000), Turkensteen *et al.* (2000), Levin *et al.* (2001), Fernandez-Pavia *et al.* (2004), Détourné *et al.* (2006), Hansen *et al.* (2006), Hannukkala *et al.* (2007), and Fahim (2007). It has been concluded that *P. infestans* oospores can readily be produce in detached tomato and potato leaves provided with constant free moisture. Oospore formation was favored by relatively low temperatures but can occur at 23°C. A small proportion of an opposite mating type is necessary for oospore formation. The apparently absence of oospores of *P. infestans* under field condition in Egypt could be explained mainly by absence of prolonged rainy seasons and the lack for A2 mating type in most fields.

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الملخص العربي

تكون الجراثيم البيضية لفطر الفيتوفثورا إنفستانز فى الحقل وتحت الظروف المعملية

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فى دراسة حصرية فى الموسم الزراعى 2005 - 2006 بمحافظة البحيرة والمنطقة المحيطة حيث يزرع محصولى البطاطس والطماطم بشكل مكثف أكدت الدراسة تواجد الطراز الجنسى النادر A2 من فطر الفيتوفثورا إنفستانز حيث شكل 14.8 % من مجموع العزلات (162) التى تم عزلها من عينات مصابة من المجموع الخضرى لنباتات البطاطس والطماطم، بينما شكل الطراز الجنسى A1 83.9%، كما كشفت الدراسة عن إثنين من العزلات متماثلة الثالوس ،هذا كما كشفت الدراسة عن تواجد الطراز A2 فى 7.4 % من حقول البطاطس والطماطم المختبرة (94 حقل) ، بينما تواجد الطراز A1 فى 3. 89 % منها و تواجد الطرازين معا فى ثلاثة حقول فقط ، و بأخذ عينات من درنات البطاطس وثمار الطماطم و كذا أوراق البطاطس والطماطم المصابة عند الحصاد من الحقول التى تواجد بها الطرازين معا حيث يحتمل حدوث التكاثر الجنسى بين الطرازين وتكوين الجراثيم البيضية، لم تظهر الجراثيم البيضية فى أى من العينات (190 عينة) هذا بالرغم من عزل الطرازين معا من إثنين من الدرنات التى تم فحصها (63 درنة) ، و بأختبارالقدرة على التزواج بين مجموعة من عزلات الطرازين وتكوين الجراثيم البيضية معمليا على البيئات الصناعية ، تكونت الجراثيم البيضية بكثافة على بيئات الراى و V8 كما تكونت بنسبة قليلة على بيئة البطاطس المضاف إليها بيتا سيتو استيرول ، وبإ ختبار قدرة هذه التزاوجات نفسها على تكوين الجراثيم البيضية معمليا فى درنات بطاطس كاملة (حديثة الحصاد- قديمة عمر 21 اسبوع) صنف روزيتا وكذلك على ثمار طماطم كاملة صنف سوبر إسترين، وجد أنه بالرغم من تكشف أعراض اللفحة على الدرنات والثمار الملقحة إلا أنه لم تنجح أى من التزاوجات الستة المختبرة فى إنتاج جراثيم بيضية فى درنات البطاطس الحديثة بينما نجحت معظم التزاوجات (4 من 6) فى تكوين أعداد قليلة من الجراثيم بيضية (28.7 - 74.8 للدرنة) بالدرنات القديمة . هذا بينما نجحت التزاوجات الستة المختبرة جميعها فى تكوين الجراثيم البيضية فى ثمار الطماطم الملقحة وبأعداد أكبر(142.6- 536.2 للثمرة) بأماكن الإصابة ، وبإ ختبار قدرة عدد من هذه التزاوجات (التى أثبتت خصوبتها فى الإختبارات السابقة) على تكوين الجراثيم البيضية على أوراق نباتات البطاطس والطماطم بتلقيح أقراص أوراق البطاطس والطماطم تحت ظروف مختلفة من الحرارة والرطوبة ونسبة اللقاح A1 وA2 ، أظهرت الدراسة أن نسبة الطرازين A1وA2 فى اللقاح لم تكن ذات تأثير فى تكوين الجراثيم البيضية حيث تكونت الجراثيم تحت نسب مختلفة منها تراوحت بين 5A2:95A1 و 95A2:5A1 ، هذا كما تكونت الجراثيم البيضية فى المدى الحرارى المختبر من 10 - 30 درجة مئوية وأظهرت 10 درجة مئوية أكبر عدد من الجراثيم البيضية والذى إنخفض مع إرتفاع درجة الحرارة ،حيث لم تنجح إثنين من التزاوجات فى تكوين الجراثيم البيضية على 23 درجة

منوية ، هذا وأظهرت نسبة الرطوبة خلال التحضين تأثيرا مهما على تكوين الجراثيم البيضية فلم تتكون جراثيم بيضية فى أى من التزاوجات على أقراص أوراق البطاطس أوالطماطم الملقحة والموضوعة على أوراق ترشيح رطبة بينما تكونت الجراثيم البيضية بشكل جيد (236.6 – 546.2 لقرص الورقة) على الأقراص المعداة الطافية على مياه حرة فى الطبق ، هذا كما أدى غلق الأطباق الملقحة sealed الى تثبيط إنتاج الجراثيم البيضية فى معظم التزاوجات، هذا وفى ضوء النتائج المستخلصة يرجح أن غياب الجراثيم البيضية فى مصر إنما يرجع إلى قلة المطروقصر فتراته و غياب الطراز A2 فى معظم الحقول فى الأراضى المصرية .