

Production of Single Oospore Progeny (SOP) of *Phytophthora infestans*: Inheritance of Mating Type and Mycelial Morphotypes

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Abstract: The aim of this study was to produce sexual progeny (oospores), of the causal agent of late blight, *Phytophthora infestans*, study the inheritance of mating type and morphological appearances of these progeny. This pseudofungus exists in two distinct mating types: A1 and A2 mainly in Mexico. However, reports indicate presence of both mating types in many countries all over the world which indicates possibilities of sexual reproduction. The experiment was conducted at the University of Wales, Bangor, in the School of Biological Sciences. Three A₁ parental isolates 96.89.43 and 96.69 (both UK) and P9463 (California, USA) were mated with a common A₂ isolate 96.70 (UK). About 545 Single Oospore Progeny (SOP) were obtained. The A1:A2 ratio was 3:1 for cross #1; 1:1 for cross #2; and 3:1 for cross #3. All A1 SOPs and 7% of the A2 SOPs had non-lumpy (-) morphology, the rest (93%) of the A2 SOPs exhibited lumpiness of varying degrees. Lumpiness varied from slightly lumpy (+) (17%), lumpy (++) (56%), to being very lumpy (+++) (20%). No visible sporangiophores observed among the very lumpy progeny. The A2 SOP were lumpy as opposed to non-lumpy A2 parent, which was a fluffy isolate just like the A1 parents. Scanning electron microscope revealed that the lumpiness was an aggregate of short-branching hyphae. Lumpiness of A2 isolates has not been reported in nature but observed in laboratories. This could be due to the failure by such isolates to survive as in this study such isolates were observed to produce very few sporangia.

Key words: Lumpiness, mating type, *phytophthora infestans*, sexual progeny, single oospore progeny, sporangiospore

INTRODUCTION

Following the discovery of the A2 mating type and subsequently novel genotypes of A1 mating types due to migrations out of Mexico, first to Europe in the 1970s (Niederhauser, 1991) and then to north America in the 1980s and 1990s (Spielman *et al.*, 1991; Fry *et al.*, 1993; Goodwin and Drenth, 1997), there have been many reports of oospore production both *in vitro* and *in vivo*. Oospores are produced for different studies, mainly for studies of inheritance of important monogenic traits, e.g. mating type, virulence, phenylamide sensitivity, and/or genetic diversity (Smoot *et al.*, 1958; Shattock *et al.*, 1986a; Shattock, 1988; Al-Kherb *et al.*, 1995; Hanson and Shattock, 1998; Turkensteen *et al.*, 2000; Knapova and Gisi, 2002; Knapova *et al.*, 2002; Oliver *et al.*, 2002). Oospores have been produced also to study their survival and/or viability (Shaw *et al.*, 1985; Pittis and Shattock, 1994; Drenth *et al.*, 1995; Flier *et al.*, 2001; Hammi *et al.*, 2001) and effects of host and fungicides on their production (Cohen *et al.*, 1996; Hanson and Shattock, 1998). In addition, oospores have been reported in nature in naturally blighted potatoes, *Solanum tuberosum*, and tomatoes (*Lycopersicon esculentum*) (Hammi *et al.*, 2001; Strömberg *et al.*, 2001) and also under experimental

treatments. Cohen *et al.* (2000) studied the effects of different sprinkler irrigation regimes on the formation of oospores on artificially inoculated potato plants. They observed that an increase in total rain (natural and irrigation), significantly enhanced oospore production in potato leaves, although the number of oospores were still less than those observed under laboratory conditions.

Characterisation of single oospore progeny had been studied as early as the 1960s (Romeo and Erwin, 1969), soon after the heterothallic nature of *P. infestans* was discovered in Mexico (Gallegly and Galindo, 1958). The majority of studies, like the first, have been restricted by the small numbers of established single oospore progeny (e.g., 2 and 34 in the study of Smoot *et al.* (1958) and Romeo and Erwin (1969)). Exceptions to this were studies by Shattock *et al.* (1986a), Shattock (1988), Smith (1993) and Al-Kherb *et al.* (1995).

This study describes the establishment of large numbers of single oospore progeny from three matings of one A2 of UK common RG57 fingerprint genotype, RF 040, (Purvis *et al.*, 2001; Shattock, 2002) and three A1s, of which two were UK isolates (RF 039 and RF 032, common and not common RG57 fingerprint genotype, respectively) and one American isolate (P9463), RF genotype not identified. The genotypes of RG57 have the

Table 1: Parental isolates used in this study and their characteristics

Isolates	Origin of isolates	Mating type	Mt DNA	Haplotype fingerprint RG 57	Mycelial morphotype
96.69	Beaumaris (Anglesey, UK)	A1	Ia	RF 039	Fluffy
96.70	Beaumaris (Anglesey, UK)	A2	Ia	RF 040	Fluffy
96.89.43	Pant y ddolen (Wales, UK)	A1	Ia	RF 032	Fluffy
P9463	California (USA)	A1	Ia	NT*	Fluffy

NT*: Not tested

prefix 'RF' for 'RG57 Fingerprint'. This study describes the establishment of three sets of these progeny, their mating type ratios and morphological characteristics. In the latter, some single oospore progeny of the A2-mating type have been observed to be 'lumpy' (Shaw *et al.*, 1985). Lumpy isolates were also observed among field isolates of *P. infestans* of A2-mating type (Shattock *et al.*, 1990) when grown *in vitro* on some nutrient agars but not on others. In this study, the A2 isolate used in the three crosses was non-lumpy.

MATERIALS AND METHODS

Choice and recovery of parent isolates: Parental isolates were recovered from the late blight collection stored in liquid nitrogen at the University of Wales, Bangor, Wales, Cryo-vials containing 3-4 frozen agar plugs, in 15% dimethylsulphoxide (DMSO), of *P. infestans* were thawed under sterile conditions (under laminar airflow). The melted agar plugs in DMSO were then transferred onto a Petri dish containing rye A agar (Caten and Jinks, 1968) amended with the antibiotics rifamycin (25 µg/mL), ampicillin (25 µg/mL) and nystatin (50 µg/mL) (RAN). The Petri dishes were then incubated at 18°C in the dark at a slanted position to drain excess DMSO.

A total of four parental isolates were selected to be used in the study. Three isolates 96.69, 96.89.43 (Purvis, 2000; Purvis *et al.*, 2001) and P9463 were A1-mating type. All these were mated with a single A2 isolate 96.70 (Purvis, 2000; Purvis *et al.*, 2001). These are shown in Table 1.

Production of oospores: Pairing of an A1 and an A2 isolate was on rye A agar plates involved placing 1.5-cm long agar strips from edges of actively growing cultures 1 cm apart on rye A agar in Petri dishes sealed with parafilm (BDH Merk. Ltd. Hunter Boulevard, Magna Park, Lutterworth, Leics. LE17 4XN). Mating cultures were incubated at 18 °C in the dark for a month.

Extraction and germination of oospores: After one month, mating of A1 and A2 isolates, oospores were extracted by blending small agar pieces containing mature oospores in 10 mL of sterile deionised water to form a paste using a glass grinder. A membrane filter holder (Swinnex) was used to hold a 20µm pore nylon filter (Lockertex, LockerWire Weavers Ltd., P.O.Box 161, Warrington, Cheshire, WA1 2SU). The paste was diluted

with distilled deionised water to reduce its viscosity and it was collected into a 50 mL syringe. The syringe was then mounted on one end of the filter holder and diluted paste was passed through the filter holder such that the oospores were collected on the membrane. Approximately 100 mL of sterile deionised water was passed through the filter holder to wash-off excess agar from the oospores. The nylon filter was aseptically removed and placed into a universal bottle containing 10 ml of sterile distilled water and vortexed to release the oospores from the filter. A 5 µ/L mixture of RAN antibiotics and 0.01 g NovoZym 234 (Novo Biolabs, Nova Enzyme Products Ltd. Farnham, Surrey, UK) (Pittis and Shattock, 1994) were added to the oospore suspension. The mixture was incubated at 18°C in the dark for 36 h after which most hyphal fragments and sporangia were lysed by the enzyme. Oospores were again collected on a 20 µm nylon filter, washed with 100 mL of sterile distilled water, resuspended in 10 mL of sterile distilled water. The oospores were left for 30 min. to settle at the bottom after which excess water was removed. The spores were transferred and spread onto Soft Water Agar (SWA) (6 g of agar per L) and excess water was evaporated by removing the lid of the Petri dish for a few minutes under sterile conditions (laminar airflow). The Petri dishes were then sealed with Parafilm (BDH Merk. Ltd. Hunter Boulevard, Magna Park, Lutterworth, Leics. LE17 4XN) and incubated at 18°C in the light and observed daily for germinating oospores.

Establishment of single oospore progeny: Four days into the incubation of the extracted oospores, about 5% of the oospores were germinating, the percentage germination increased to about 50% after 10 days. Germinating oospores were recognised by their size, morphology and colour. Germinating oospores were about 3 to 4 times bigger than non-germinating ones. They appeared granular and had an orange brown colour while non-germinating ones were smooth and clear or sometimes light brown. The germinating oospores also had an emerging germ-tube.

Germinating oospores were aseptically transferred into Petri dishes containing rye B amended with RAN. This was done by looking through a dissecting microscope and a sterile scalpel was used to cut a chunk of agar on which the germinating oospore was situated to avoid bursting of the emerging fragile germ tube. The transferred germinated oospores were monitored

daily and those that continued to grow on the Petri dish were transferred into 24 compartment repliplates (Bibby Sterilin, Staffordshire, UK) containing rye A amended with RAN antibiotics to establish single isolates. Working cultures were then established by inoculating 9 cm Petri dishes containing rye A.

Transferred germinated oospores that were not readily growing were monitored for up to 3 days and were discarded if there was no sign on growth. This process of transferring germinating oospores from the original plates and transferring of transferred growing isolates continued for 3 weeks. The transferring of germinating oospores was then stopped, as it was impossible to identify single isolates as the plates were filling up with germinated isolates that were not picked in time, however, the establishing of single oospore isolates continued for another 4-5 days.

Maintenance of progeny isolates: Mycelial cultures of single oospore progeny were maintained individually in rye A agar in 9 cm Petri dishes at 18°C without illumination. Subcultures were made by inoculating fresh Petri dishes of rye A agar (sometimes amended with RAN to counter contamination) with small mycelial cultures in agar blocks every month.

Medium term storage was either by:

- suspension of agar plugs in 1.5 mL Eppendorf's containing sterile distilled water
- suspension of agar plugs in 2 mL tubes containing sterile mineral oil, prepared by heating at 120°C for 4 h

To maintain viable cultures isolates were taken out of the tubes and grown in Petri dishes every 6-8 months and fresh tubes were prepared from the actively growing region of each isolate (as described above).

Long term storage involved inoculating rye A agar slants in universal bottles. The inoculated slants were incubated at 18°C without illumination and when mycelial growth covered the agar surface of the sterile light mineral oil was added to a level above the agar. The oil slants were then kept at 18°C in light proof boxes.

Morphological characterization: The morphological appearance of the single oospore progeny was characterized. The following criteria were used:

- (-) = no lumps or non-lumpy, when isolate had a fluffy appearance.
- (+) = slightly lumpy, when an isolate had visible but few clusters (lumps) of hyphae, more pronounced on the older part of the colony, i.e. on and around the inoculating plug.

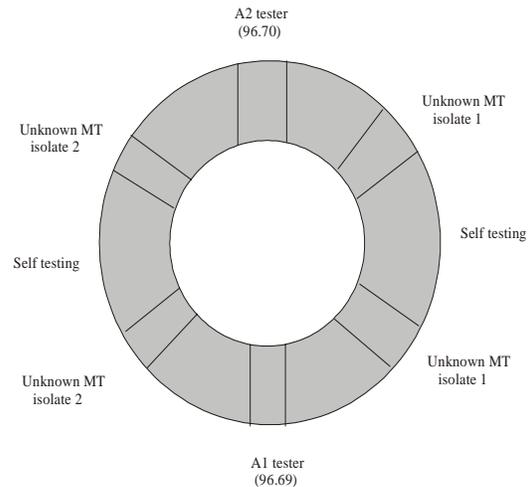


Fig. 1: Diagram showing placement of A1 and A2 mating type tester isolates and tested isolates two isolates of unknown mating type (MT) in a 9mm Petri dish. Each isolate of unknown MT was also tested against itself at the region marked 'self testing'

- (++) = lumpy, when as isolate had visible lumps throughout the colony but with visible sporangiophores and sporangia
- (+++)= very lumpy, when an isolate had no visible sporangiophores nor sporangia but clusters of lumps that would later coalesce to form a thick mat which became water-soaked

Mating type characterization: Rye B agar, which provides a clear see through medium in contrast to the richer, opaque Rye A (Caten and Jinks, 1968), amended with RAN antibiotics at the standard concentration, was used. A central circle of agar of 4 cm diameter was removed from each Petri dish to leave a ring of agar. The antibiotics were used for two purposes, to keep out bacteria that would interfere with the mating type experiment and also to facilitate recognition of zone of interaction between A1 and A2 mating type mycelia (agar turning pink at the interaction zone while remaining yellow where isolates are growing (Fig. 1). Mycelial strips of 96.69 and 96.70 (tester isolates), of same length as the agar ring in the Petri plate, were cut from actively growing isolates and placed at the extreme ends of each plate (Fig. 1). Two strips of mycelial cultures of *P. infestans* isolate were placed next to each tester isolate at about 1cm distance, such that the isolates of unknown mating type were tested against each tester isolate but also against itself (Fig. 1). In each Petri dish two isolates of unknown mating type were tested on either side of each tester isolate.

The dual cultures were incubated at 18°C in the dark. Mating type of the unknown isolates was determined

when oospores were produced opposite one or the other tester isolate. Oospores usually formed after about 7 days after incubation.

Scanning electron microscope of mycelial morphotypes: Three single oospore progeny cultures showing 'lumpy' morphotypes were chosen for SEM studies. The isolates were grown on cellophane overlaid on rye A agar amended with antibiotics (RNA). The isolates formed mycelial lumps on the cellophane as they did on the agar surface but the cellophane could be lifted off the agar and manipulated and cut into small squares to facilitate microscopical examination without disrupting the mycelial architecture. These were fixed in 3% glutaraldehyde in a phosphate buffer (pH 7) for 3 hours at room temperature. The blocks were washed in 6 changes of buffer remaining in the last wash overnight and then dehydrated through an increasing gradient (25, 50, 75, 100%) at 4°C for 30 min. at each concentration. The blocks were placed in 100% acetone over anhydrous copper sulphate for 30 min. at 4°C and left to equilibrate to room temperature. The blocks were critical point dried, mounted on stubs and gold coated using a sputter coater prior to examination using a Hitachi S-520 scanning electron microscope.

RESULTS

Establishment of single oospore progeny: The extracted oospores started germinating 4 days after incubation. Initially, the germination percentage was low, about 5%, however, a week after the starting of germination, the germination percentage had increased to about 50% for cross #1 and 2 and over 50% for cross #3. Germinating oospores were recognised by their size as they were 3 or 4 times bigger than non-germinating ones and had a granular appearance with an orange brown colour. Most of the germinating oospores did not give rise to a sporangium rather to multiple germ-tubes. However, few germinating oospores did give rise to a germ sporangium and sometimes 2 or 3 germ sporangia were formed in a chain.

Not all germinated oospores were established into single oospore progeny. At the beginning of the transferring of germinated oospores, care was not taken when lifting them to avoid bursting of the fragile germ-tube. As a result several hundreds of germinated oospores were lost. This problem was rectified by cutting a small block of agar on which the germinating oospore was situated and transferring the block. Keeping the lid of the plate on when looking for germinating oospores also helped reduced bursting of hyphae. Germinating

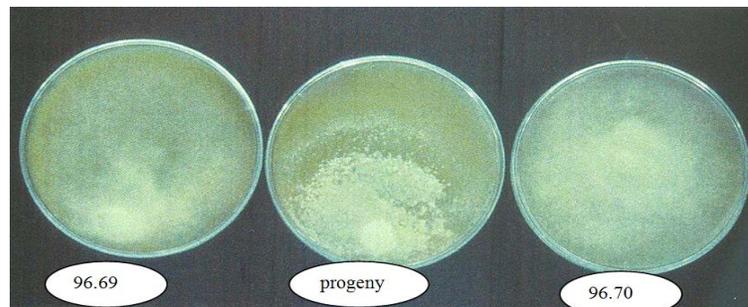


Fig. 2: *In vitro* cultures of *P. infestans* non-lumpy parental isolate 96.69 (A1) and isolate 96.70 (A2) with one of their very lumpy A2 single oospore progeny

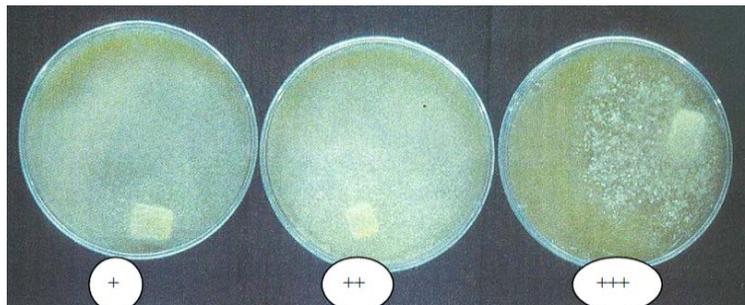


Fig. 3: Three single oospore A2 progeny of *P. infestans* showing the different degrees of lumpiness. Slightly lumpy (+), lumpy (++) and very lumpy (+++)

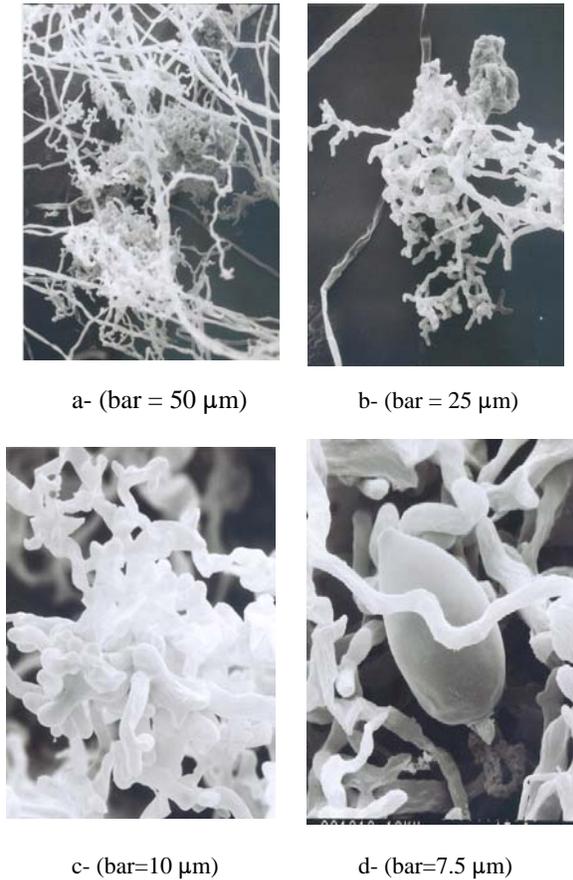


Fig. 4: Scanning electron micrographs showing lumps from an A₂ mating type single oospore of *Phytophthora infestans* which was rated as very lumpy (+++).
 a- showing the curling hyphae which give rise to the lumps (bar = 50 µm).
 b- showing the profuse branching of short hyphae (bar = 25 µm).
 c- a close-up of the profuse branching short hyphae (bar=10 µm).
 d- another close-up of the curled short hyphae with a sporangium (bar=7.5 µm)

oospores were first transferred into a Petri dish, containing rye B amended with RAN about 20 to 25 per plate and later transferred into a 24 compartment Repliplate, containing rye A amended with RAN, if they were growing. Oospores that germinated to give rise to one or more sporangia were slow to start growing as the sporangium had to germinate first. These usually took 48 hours or more to start growing while those oospores that germinated to give rise to one a more germ-tube grew more quickly, and sometimes, had to be transferred into Repliplates a few hours after their transfer from the germination plate. Another factor that contributed to failure to capture more germinated oospores as single oospore isolates was the number of plates that had to be dealt with each day. As more and more oospores

germinated, more and more plates had to be transferred and as a result some germinated oospores were not transferred (into Repliplates) in time before 2 or more single oospore cultures joined: such isolates were discarded.

Nonetheless, a total of 545 single oospore progeny (SOP) (cross #1, 190; #2, 170; and #3, 185) were established from the three matings (Table 2). These were characterised for mating type and morphological characteristics.

Mating type: Across the three crosses, the ratio of A1:A2 mating type of the SOP was approximately 2:1 and percentage, about 63% were A1, 28% were A2, 5% were selfs and 4% were undetermined. However, the A1:A2 the ratio differed for each cross with 3:1 for cross #1; 1:1 for cross #2; and 3:1 for cross #3 (percentage of A1 to A2, was 69:21 for cross #1, 52:42 for cross #2, and 65:23 for cross #3). A total of 22 SOP from all three matings (see Table 2) had their mating type undetermined. Eight of these progeny when paired with tester isolates (A1 and A2) did not produce any reaction zone, i.e., no sexual gametangia were formed by either isolates (tester and tested) at the interaction zone.

Thirteen isolates when paired with the tester isolates did not grow to the mating zone even when they were placed a few millimetres from the tester isolate. When viewed under the microscope, their advancing hyphae were observed to have burst. This would appear like there was some incompatibility reaction taking place. Most of these isolates became casualties and were not used for further studies. One progeny from this group of non-determined isolates, also did not produce any gametangia when paired with the tester but the medium where it was growing was always pink, an indication of oospores (or at least sexual gametangia) formation. This was unique to this isolate as all other isolates only caused the change in colour of the medium when fertilisation was taking place (D.S. Shaw, pers. comm.).

Morphology of single oospore progeny: While all A1 single oospore progeny and 7% (11 of 154) of the A2 single oospore progeny had non-lumpy (-) morphology, the rest (93%) of the A2 progeny exhibited lumpiness of varying degrees. Lumpiness varied from slight lumpy (+) (17%), lumpy (++) (56%), to being very lumpy (+++) (20%) (Table 3) (Fig. 3). There were no visible sporangiophores among the very lumpy progeny, however, sporulation (i.e., number of sporangia per unit area) was not determined. The A2 SOP were lumpy as opposed to their non-lumpy A2 parent, which was a fluffy isolate just like the A1 parents (Fig. 2). Scanning Electron Microscope pictures revealed that the lumpiness was an aggregate of short branching hyphae (Fig. 4).

Table 2: Number of Single Oospore Progeny (SOP) from three matings of *Phytophthora infestans* and their mating types

Parents (A1×A2)	Cross UMBER	A1	A2	Self Fertile	Undetermined	Total
96.89.43×96.70	1	132(69) ^a	40(21)	8	10	190
96.69×96.70	2	88(52)	72(42)	4	6	170
96.69×96.70	2	88(52)	72(42)	4	6	170
Total		341	154	28	22	545

a: Number in parenthesis is the percentage of A1 and A2 mating type

DISCUSSION

In the past, many researchers have not been able to obtain large numbers of single oospore progeny (Smoot *et al.*, 1958; Shattock, 1988; Turkensteen *et al.*, 2000; Lee *et al.*, 2002). In anticipation of such a problem, large pieces of agar were cut from the mating plates to increase the number of oospores to be extracted. However, when blended, the agar posed a problem as it clogged the filter membrane that was used to separate the oospores. As a result of not all the agar being washed off, the oospores were incubated with pieces of agar. The presence of the agar may have proved to be beneficial as it provided nutrients for the oospores. This could have been the reason why there was a high germination percentage and also why the germinating oospores gave rise to multiple germ-tube as opposed to a germ sporangium. Providing nutrients to extracted oospores was found by Ann and Ko (1988), to stimulate germination in oospores of *P. parasitica*. This was also observed by Harrison (1991), she observed that addition of clarified rye A broth to oospores increased the rate and final percentage of germination.

The majority of the germinating oospores were preceded by a swelling followed by production of multiple germ-tubes. This has been reported before (Gallegly, 1968; Romeo and Erwin, 1969; Harrison, 1991). Harrison (1991) attributed the swelling of germinating oospores to be a characteristic of germinating oospores in liquid medium. However, in this current study, the oospores were not in a liquid medium yet they were still swollen and also produced multiple germ-tubes, suggesting that the presence of nutrients was responsible for the swelling and production of multiple germ-tubes. Very few of the oospores germinated without a swelling and these gave rise to a germ sporangium.

A total 545 single oospore progeny were obtained from 3 matings. The number of single oospore progeny per mating were 190, 170 and 185 for crosses #1, #2 and #3, respectively. Only 523 were characterised for mating type and the ratio of A1:A2 was 3:1 for crosses #1 and #3, and 1:1 for cross #2. Although mating type is governed by a single locus (Judelson, 1996), aberrant segregation of A1s and A2s is always observed. Romeo and Erwin (1969), reported a ratio of 3:1 (A1: A2) from a total of 34 single oospore progeny. Twenty years later, Shattock (1988) reported a ratio of 2:1, and Al-Kherb (1988) reported several different ratios of A1: A2, with the A1 ratio predominantly higher than the A2. However, sometimes more A2 mating type SOP than A1s have been

obtained (Shattock *et al.*, 1986a; Smith, 1993; Al-Kherb *et al.*, 1995). In most cases, when the number of progeny is above a hundred the number of A1 progeny is equal or higher than that of A2. In this study, in cross #2 there were approximately equal numbers of A1s and A2s and this has been observed also by Shaw *et al.* (1985), Shattock *et al.* (1986a), Turkensteen *et al.* (2000) and Knapova *et al.* (2002). Whether or not the ratio of mating type in sexual progeny is determined by parental phenotype e.g. degree of maleness/femaleness, genetical basis of A1/A2 mating type loci e.g., gene dosage, inhibitor genes, lethal factors, remains to be investigated.

Although in this study all three crosses yielded less than 200 single oospore progeny per cross, it is worth reporting that even though the germination rate was not measured, it was observed to have been quite high ($\geq 50\%$) in all three matings. As a result of the high germination rate, less than half of the germinated oospores were recovered before the Petri dishes were filled with mycelium of germinated oospores. Combinations and placement of isolates have been reported to have a significant effect on oospore formation (Ko and Kunimoto, 1981; Kemitt, 1993). This means isolates vary in their ability to both detect and produce α pheromones. Kemitt (1993) observed that pheromone production was influenced by a number of parameters, e.g., age of isolate. He observed that cultures of *P. infestans* older than 12 days were superior α pheromone producers and he suggested 15 day old cultures to be the best age.

Of the 523 SOP that were characterised for mating type, 29 were self-fertile (Table 2). Although self-fertile isolates have a characteristic feature of having appressed hyphae and a shiny waxy appearance (Shattock *et al.*, 1987; Spielman *et al.*, 1990; Shaw and Shattock, 1991), only 13 of self-fertile progeny showed the typical self-fertile isolate appearance. The remaining 16 appeared fluffy, had sporangia and few oospores that were characteristically light brown in colour. When these isolates were paired with tester isolates, they produced more oospores with both testers. Not all the self-fertile progeny remained self-fertile. Only 7 of them were still self-fertile after 24 months. All these were from the shiny waxy group. The remaining shiny waxy group (6 of them) segregated to become lumpy, a characteristic that was observed to be unique to A2 mating type progeny. Segregation of self-fertile isolates has been reported before (Fyfe and Shaw, 1992; Kemitt, 1993). Fyfe and Shaw (1992) concluded that self-fertile isolates of *P. infestans* appeared to be intimate mixtures of A1 and

Table 3: Morphological characteristics of A2 mating type single oospore progeny of the three matings of *Phytophthora infestans*

Mating(A1×A2)	Non-Lumpy (-)	Slightly Lumpy (+)	Lumpy (++)	Very Lumpy (+++)	Total
96.89.43×96.70	2	12	23	3	40
96.69×96.70	7	10	35	20	72
P9463×96.70	2	4	29	7	42
Total	11	26	87	30	154

A2 hyphae within which rare mating type heterokaryons may occur. Most of the shiny waxy group of self-fertile progeny produced very small (in size) oospores compared to those produced by two opposite mating type isolates. Their viability was not tested and efforts to germinate them were unsuccessful. Only one progeny from Cross #3, the Californian and UK cross [P9463 (A1 & Calif.) x 96.70 (A2 & UK)] progeny #70 had normal size oospores, although not very many. Very few oospores were extracted and efforts to germinate them was also not so successful. Only one putative progeny (SF₁) was recovered and it was of the A1 mating type. The other self-fertile progeny (16) that had sporangia with few oospores, when checked after 24 months, were still producing oospores when paired with either tester. It was very difficult to extract their oospores because of the small numbers. All these oospores were tested for pathogenicity and for sensitivity to fungicides.

A total of 22 SOP from all three matings did not produce any sexual gametangia when paired with tester isolates. This would mean that these progeny were not able to produce any pheromones which would have stimulated the tester isolate to produce sexual gametangia, and as a result that the tester isolates neither produced nor received any pheromones.

Characterising mycelial cultures of single oospore progeny has been reported before. Romeo and Erwin (1969) described the colony characterisation of 34 putative hybrid single oospore progeny of *P. infestans*. They observed that morphologically, there was a remarkable difference in colony type among the SOPs, and none closely resembled either parent. They observed a difference in growth rate in clarified V-8 agar, where some progeny hardly grew on the medium. This phenomenon has been observed in wild type isolates (Shattock *et al.*, 1990), where an A2 isolate (88/24/6) failed to grow on 10% V8 agar and was termed 'V8 shy'. None of the progeny from cross #1, #2 and #3 were grown on V-8 agar and so their V8-shyness (if any) was not determined. Romeo and Erwin (1969) also reported differences in colour and texture of mycelium of some progeny to that of parents. Some progeny had white and cottony mycelium as opposed to grey and appressed mycelium of parents. In this study, there were notable differences in the morphology of the progeny compared to their parents. While some progeny had colony morphology similar to their parents, i.e. white fluffy mycelium [non-lumpy (-)], some had smooth appressed

mycelium while others had clusters of lumps within their colony (Fig. 3). When mating type of the progeny was established, it was noted that the smooth progeny with appressed mycelium were self-fertile isolates.

Most of the progeny with white fluffy mycelium (non-lumpy) were found to be of the A1 mating type. Few of the fluffy progeny were found to be of A2 mating type. However, when these were viewed under a microscope, some were found to contain lumps which were very small and these were rated as being slightly lumpy (+) (Table 3). Such observation was also made by Shattock *et al.* (1990), whereby they observed that isolates of the A2 mating type had less obvious lumps when grown on pea agar than in rye A agar, where the lumps were more pronounced. However, Shattock *et al.* (1990) focussed only on characteristics of field isolates *in vitro*.

Lumpiness among the A2 SOP from the 3 matings was divided into 3-levels (Fig. 3): (1) slightly lumpy (+) - these were the SOP which although appeared non-lumpy when observed by naked-eye, were found to contain small lumps when viewed under the microscope. The second level was the lumpy (++) - these were SOP that had visible lumps but still produced visible sporangia. The third level was the very lumpy (+++) - these were SOP that had lumps instead of mycelium and there were no visible sporangia (sporangia were not quantified) and lumps were filled with fluids. When these isolates were grown in liquid medium, they did not grown horizontally, i.e., to fill the Petri dish, but instead they formed vertical clusters around the mycelial discs used as inoculum. The accumulation of liquids by very lumpy isolates has been reported before (Turner, 1988, unpublished data), who showed that fluid that accumulated in the network of mycelium did not react with Sudan IV reagent and Steinmetz's reagent. This indicated that it was neither oil nor mucilage and moreover it had the same pH as the condensation in the Petri dish lid, i.e. pH5. She concluded that the fluid was more likely to be condensation than an exudate. This then raises the question why some isolates retain the fluid within their mycelium and some do not and why only certain isolates of the A2 mating type have this ability.

The very lumpy SOP when viewed under the microscope had short stubby hyphae and even when paired with A1 isolates, the mycelium did not seem to straighten, as would be expected when two different mating types are grown side by side as a result of

pheromone induced accelerated growth of A2 towards A1 isolates in paired cultures (Kemitt, 1993). Similar morphological characteristics have been reported before (Shattock *et al.*, 1990) observed that the A2 isolates exhibited distinctive colony morphology. They observed an aggregation of dichotomously branched aerial hyphae. When Shaw *et al.* (1985) crossed Egyptian A2 isolate (E13a) with a UK A1 isolate (83/3), they also observed that some of the single oospore progeny exhibited dichotomously branching hyphae. Morphology of the very lumpy isolates was further studied using a scanning electron microscope, the lumps were observed to be short stubby highly branched aggregates of hyphae (Fig. 4).

Oospores can be produced not only through fertilization of two compatible mating types (Ko, 1978), but also through stimulation by other species of *Phytophthora* e.g. *P. drechsleri* (Skidmore *et al.*, 1984; Shattock *et al.*, 1986a), through other non related fungi e.g., *Trichoderma* (Brasier, 1975; as cited in Campbell and Duncan, 1985; Brasier, 1978).

Quite a number of researchers have been able to produce and study oospores in the laboratory. In most of the studies, two strips of agar containing A1 and another containing A2 were placed side by side, providing a 1:1 ratio of A1 to A2. However, inoculum of A1 and A2 need not be present at equal proportions for oospores to be produced, as the ratio between A1/A2 sporangia has a minor effect on the number of oospores produced (Cohen *et al.*, 1996). The authors reported extreme proportions of A1/A2 statistically possible for oospore formation to be 1 sporangium of A1 and 19 sporangia of A2 and vice versa, i.e., ratio of 5:95 to 95:5. With such extreme proportions, they were able to obtain between 175 to 232 and 272 and 207 oospores per 8 mm² leaf disc on potato and tomato, respectively. Such high numbers of oospore were probably produced through selfing of each isolate, as the few sporangia of the opposite mating type would produce either α^1 (by A1) or α^2 (by A2) sex pheromones (Ko, 1978 1988) which would stimulate formation of gametangia and then fertilization (Smoot *et al.*, 1958).

In conclusion, the 3 matings produced large numbers of SOPs contrary to the majority of mating studies previously published. The phenotypic characteristics of the 3 sets of progeny were not atypical of previous reports and like those that did not shed light on reasons of uneven mating type ratios, lumpiness of A2 isolates. The data do show that large progeny sets can be obtained but whether this is determined by choice of parents, techniques or chance still cannot be resolved.

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