GENETICS OF THE GENUS *PHYTOPHTHORA*

B.R. BOCCAS

The genetics of the genus *Phytophthora* is still not entirely understood though considerable information has been published on this topic for the last 15 years.

These publications mainly concern the nature of the sexuality in *Phytophthora* species, the nature of the life cycle, and the possibility of interspecific hybridization throughout the genus.

According to Waterhouse's key, the genus *Phytophthora* includes forty two species. Twenty seven of them are homothallic and form oospores in single culture, whereas twelve are considered as heterothallic since most of their isolates usually produce oospores only when they are paired with a compatible strain. The sexual reproduction of the three last species : *P. oryzae*, *P. lateralis*, and *P. gonapodyides* has not been observed.

Homothallic species may be divided in two groups (SAVAGE & al, 1968) (Table 1) : species with predominantly paragynous antheridia, and species with predominantly amphyginous antheridia.

All heterothallic species form amphyginous antheridia and their isolates are divided into two compatible groups designated as A_1 and A_2. Both A_1 and A_2 compatibility or mating types, first recognised in *P. infestans* (GALLEGLY & GALINDO, 1958), are known to be present in all twelve heterothallic species. Sexual compatibility is independent of the species status : oospore formation occurs in most $A_1 \times A_2$ intra-and interspecific pairings, but never does in $A_1 \times A_1$ or $A_2 \times A_2$ pairings.

Each A_1 or A_2 isolate is bisexual and self incompatible. However, relative degrees of maleness and femaleness have been observed among *P. infestans* A_1 and A_2 isolates (GALINDO & GALLEGLY, 1960). Some isolates of each mating type act as strong male and form principally antheridia, when some isolates behave as strong female and produce mainly oogonia. Some isolates are intermediate in their relative sexual strength and able to form both types of gametangia.

It has been shown that zygote formation in heterothallic species does not always result from the mating of two compatible A_1 and A_2 isolates. Some A_2 isolates can form oospores in single culture by self fertilization, in response to different chemical inductions. In this way, selfing of A_2 isolates of *P. palmivora* and other species may be induced by volatile substances from another fungus : *Trichoderma viride* (BRASIER, 1975). In A_2 *P. cinnamomi*, selfed oospores are produced in presence of avocado roots exudates (ZENTMYER, 1952). In A_2 isolates of *P. capsici*, the fungicide "chloroneb" induces sexual reproduction (NOON & HICKMANN, 1974). None of those stimulating substances is effective on A_1 isolates.

Recently, it has been shown that sexual reproduction in *Phytophthora* is likely under hormonal regulation (KO, 1978). Both A_1 and A_2 isolates of *P. cinnamomi*, *P. parasitica* and *P. palmivora* form oospores by selfing when they are paired with different mating type on opposite side of polycarbonate membrane. In this instance, the membrane prevents direct contact between hyphae of the two compatible isolates,

Table 1 : HOMOTHALLISM, HETEROTHALLISM AND MODE OF FORMATION OF ANTHERIDIA IN THE GENUS *PHYTOPHTHORA*

Homothallic species		Heterothallic species	Species with unknown sexual stage
Antheridia predominantly paragynous	Antheridia predominantly amphyginous	Amphyginous antheridia	
P. cactorum			
	P. heveae	*P. capsici*	
	P. mexicana	*P. palmivora*	
	P. meadii	*P. citrophthora*	
	P. boehmeriae	*P. arecae*	
		P. nicotianae var. nicotianae	
		P. nicotianae var. parasitica	
P. macrospora			
P. inflata			
P. citricola			
P. syringae			
P. primulae			
P. porri			
P. cyperi bulbosi			
P. cyperi			
P. lepironiae			
	P. phaseoli *	*P. infestans f.sp. infestans*	
	P. hibernalis	*P. infestans f.sp. thalictri*	
	P. ilicis		
	P. colocasiae		
	P. verrucosa		
	P. quininea		
	P. fragariae		
	P. megasperma var. megasperma		
	P. megasperma var. sojae		
	P. erythroseptica var. pisi	*P. cinnamomi*	*P. oryzae*
	P. richardiae	*P. cambivora*	*P. lateralis* *
	P. vignae	*P. cryptogea*	*P. gonapodyides*
	P. erythroseptica var.erythroseptica	*P. dreschleri*	

* *P. phaseoli* which is considered as heterothallic in Waterhouse's key was found homothallic by SAVAGE et al.
P. lateralis is given as homothallic by SAVAGE et al.

and oospore formation on both sides demonstrates the production of diffusible substance like plant hormones, as found in related fungi (*Achlya sp.*, RAPER, 1951). At least two different hormones seem to be involved in sexual reproduction of heterothallic species. It has been proposed to designate the first one, produced by A_1 isolates, hormone α_1, and the second, produced by A_2 isolates, hormone α_2. α_1 induces oospores formation in A_2 isolates, and α_2 induces sexual reproduction in A_1 isolates only.

To date, little or nothing is known on the genetical regulation of the mating types, but it seems possible that they could be determined by a complex system of genes. The reason of that uncertainty is mainly that most genetical studies in the recent years have not been specifically undertaken to elucidate the mode of inheritance of mating types, but to provide information on the life cycle of the fungus.

The life cycle of *Phytophthora* species and consequently the ploidy level of vegetative nuclei have long been a matter of controversy. According to the classical concept, the diploid phase is restricted to the oospore which results from the fusion of haploid gametangial nuclei. Meiosis occurs in the oospore, before its germination, resulting in haploid vegetative mycelium. The alternative theory, proposed by SANSOME in 1961 and initially founded on cytological data, describes diploid somatic nuclei, and meiosis in the gametangia. Oospore formation results from the fusion of two haploid gametic nuclei and oospore germination results in diploid vegetative mycelium.

After SANSOME reopened the debate on the position of meiosis in Peronosporales, several cytologists carried out histochemical studies on different *Phytophthora* species, and provided substancial support to the hypothesis of gametangial meiosis. None of these cytological works, however, was entirely convincing and it was necessary to confirm genetically the cytological evidence for diploidy. Such confirmation was difficult to obtain because genetical studies have long been hampered by difficulties in germinating oospores and in obtaining adequate mutants.

Despite these difficulties, several works were carried out on heterothallic species (GALINDO & ZENTMYER, 1967 ; ROMERO & ERWIN, 1969 ; LAVIOLA, 1969 ; SATOUR & BUTLER, 1968 ; TIMMER et al, 1970). They showed that when isolates differing in several characters are crossed, the progeny resulting from germinated oospores includes recombinants. With one exception (LAVIOLA, 1969), the data also showed a lack of segregation among the progeny from individual oospores. This suggested meiosis in gametangia and germination of diploid oospore rather than zygotic meiosis. The latter would imply that only one of the four meiotic products survived in the germinating oospore : a somewhat complicated explanation. But most of the results remained open to interpretation on either hypothesis, gametangial or zygotic meiosis, and these genetical studies were inconclusive.

The first genetical evidence for diploidy was obtained with *P. dreschleri* in 1971 by SHAW and KHAKI. They crossed wild type with mutants resistant to drugs, and obtained segregation ratios consistent with the hypothesis that the somatic nuclei were diploid. It was not possible, however, to draw any definitive conclusion based on their data, since some features remained unexplained : for instance, the non mendelian segregation for the mating type that could be due to selfing of the parental strains.

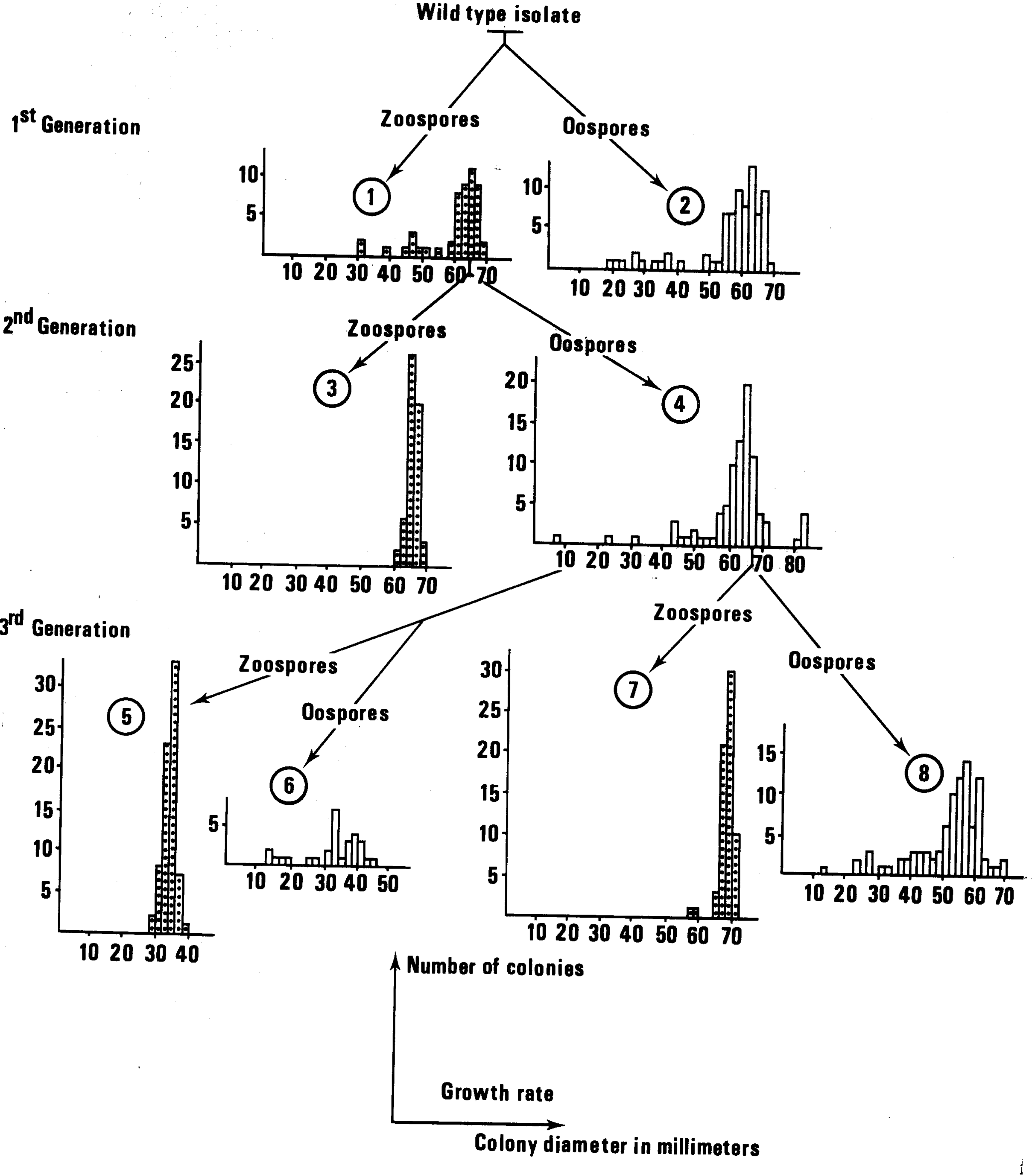
Further informations were obtained from the study of homothallic species.

In homothallic species, populations of progeny from sexual and asexual origin, derived from oospores and zoospores of the same clone, can be compared.

Table 1 : HOMOTHALLISM, HETEROTHALLISM AND MODE OF FORMATION

PHYTOPHTHORA SYRINGAE

Variation in growth rate among sexual and asexual progeny



Variation among both types of populations will be caused by mutation, by heterokaryosis of the parent clone, by cytoplasmic factors, and by mitotic recombination if the parent clone is diploid. In that case, and if the parent clone is also heterozygous for some loci, the variation among sexual progeny will be greater than that among asexual progeny, since oospores result from fusion of different meiotic products, when zoospores only derive from mitotic division of somatic nuclei.

I found it to be so in *P. syringae* (BOCCAS, 1972) (Figure 1). Over three generations, I compared colony diameter in populations of sexual and asexual progeny and noted highly significant difference in variation between the two kinds of populations. The variation in growth rate among colonies from sexual origin was always greater than that in colonies derived from zoospores. If *P. syringae* were haploid, variations among sexual progeny should not then differ significantly from that among asexual progeny derived from the same parent clone. Therefore, the significant difference in variation between the two kinds of populations was most simply explained by genetic recombination in the oospores, following meiosis in gametangia.

In 1973, ELLIOT and MAC INTYRE treated zoospores of the homothallic *P. cactorum* with a mutagen and showed that auxotrophic mutant characters, induced in heterozygous condition, segregated in mendelian fashion in subsequent generations. The patterns of inheritance obtained were entirely consistent with the hypothesis of diploid mycelium and meiosis in the gametangia.

More recently, LONG and KEEN (1977) dealing with another homothallic species, *P. megasperma var. sojae*, reached a similar conclusion. They constructed heterokaryons from two different auxotrophic mutants. By self fertilization, these heterokaryons produced parental type auxotrophic progeny and prototrophic progeny, but no double auxotrophic offspring. The selfing of these F_1 monokaryotic prototrophs segregated in F_2 four classes of progeny, i.e prototrophs, two classes of single auxotrophs and double auxotrophs, as it was expected in a vegetatively diploid organism.

Today, cytological and genetical evidences for diploidy have been accumulated in more than forty species of biflagellate Oomycetes, and in particular in ten homothallic and heterothallic *Phytophthora* species : *P. erythroseptica* (SANSOME, 1965), *P. cactorum* (SANSOME, 1965 ; ELLIOT & MAC INTYRE, 1973), *P. parasitica* (HUGUENIN & BOCCAS, 1970), *P. dreschleri* (SHAW & KHAKI, 1971), *P. syringae* (BOCCAS, 1972), *P. cinnamomi*, *P. infestans* (BRASIER & SANSOME, 1975), *P. capsici* (MAIA, 1976 ; SANSOME, 1976), *P. palmivora* (LEGRAND-PERNOT & PELLEGRIN, 1976) and *P. megasperma* (LONG & KEEN, 1977). Therefore, it seems reasonable to end the controversy and to conclude that *Phytophthora*, like other Oomycetes, are diploid in their vegetative state, with meiosis occuring in oogonia and antheridia, prior to oospore formation.

Since heterothallic species can form oospores in interspecific crosses, the sexual stage is a potential source of variability in the genus *Phytophthora*. Possible exchange of genetic material in interspecific crosses might be an important factor in the evolution of natural *Phytophthora* populations and would raise fundamental questions on the validity of the species concept in the genus. However, until recently, the occurence of interspecific hybridization remained hypothetical due to the difficulty to germinate oospores formed in interspecific crosses and to establish viable colonies from such germinations.

I have studied two kinds of interspecific crosses (BOCCAS, 1976, 1980) : crosses between *P. parasitica* and *P. cinnamomi*, which differ markedly in morphology, physiology and pathogenicity, and crosses between more closely related species belonging to the same morphological group of Waterhouse's key. (Table 2).

Table 2 : INTERSPECIFIC CROSSES STUDIED

- CROSSES BETWEEN SPECIES OF DIFFERENT MORPHOLOGICAL GROUPS

P. cinnamomi A_1 x *P. parasitica* A_2	43 progeny
P. cinnamomi A_2 x *P. parasitica* A_1	33 progeny

- CROSSES BETWEEN CLOSELY RELATED SPECIES

P. palmivora A_2 x *P. capsici* A_2	18 progeny
P. palmivora A_2 x *P. parasitica* A_2	18 progeny
P. megakarya A_1 x *P. parasitica* A_2	65 progeny
P. parasitica A_1 x *P. capsici* A_2	15 progeny
P. cinnamomi A_2 x *P. cambivora* A_1	6 progeny

Table 3 : OOSPORE PRODUCTION AND GERMINATION IN INTERSPECIFIC CROSSES

Crosses	Oospore production (no. per mm3 of culture medium)	Oospore germination %
P. cinnamomi A_2 x *P. parasitica* A_1	2	0,2
P. cinnamomi A_1 x *P. parasitica* A_2	3	0,2
P. palmivora A_2 x *P. capsici* A_1	18	0,4
P. palmivora A_2 x *P. parasitica* A_1	62	0,5
P. megakarya A_2 x *P. parasitica* A_2	20	0,5
P. parasitica A_1 x *P. capsici* A_2	24	0,1

All the crosses formed oospores, but the rate of oospore production in crosses between closely related species was about ten time higher than that in crosses between species of different morphological groups. In both kinds of crosses, the percentages of oospore germination were extremely low (from 0,1 to 0,5 %) (Table 3), nevertheless 198 single oospores isolates from seven crosses were harvested and genetically analysed.

Among that progeny, colony morphology, optimum and maximum temperature for growth, growth rate, pathogenicity and composition in soluble proteins, determined by acrylamide gel electrophoresis, were studied.

Most of the crosses resulted in phenotypically heterogeneous progeny which exhibited recombinations for morphological, physiological and pathogenic characters. This was first interpreted as an indication of interspecific hybridization between the species crossed.

That interpretation, however, was confirmed by the study of protein patterns for only one of the progeny isolates. This single oospore isolate from a cross between *P. capsici* and *P. palmivora* was the only one to exhibit a protein pattern qualitatively and quantitatively different from that of its parents. As it also differed from the parental strains by other characters, it was interpreted as an interspecific hybrid.

But all other phenotypically recombined progeny produced protein patterns of parental types, and did not show any indication of recombination for that character, as it was expected in the hypothesis of true hybridization.

Since the composition in soluble proteins of an isolate is assumed to be a significant expression of its genome, the lack of any indication of recombination among the progeny patterns, strongly suggested that no exchange of genetic material occurred between the different species. Therefore, it was concluded, according to the simplest interpretation, that the parental strains were heterozygous for loci controlling those characters showing variation, and that all the progeny, but one, resulted from the self fertilization of the parental strains.

Self fertilization among heterothallic species of *Phytophthora* is a consequence of the potential bisexuality of these organisms. It has been shown that, although less common than hybrid oospores, selfed oospores are formed in both intra-and interspecific crosses. However, in interspecific crosses, either between closely related or morphologically distant species most hybrid oospores appear unable to germinate and probably abort because they associate genomes which lack homology. Only selfed oospores may produce viable progeny. Thus, the mating of compatible strains of different species does not generally result in hybrid progeny, but leads to a reciprocal induction of selfing which may result in phenotypic variation of the progeny if the parental strains are heterozygous. From a more practical point of view, it is of particular interest to note that the crossing of two different species, if occuring in nature, may produce a wide range of progeny, greatly variable in morphology, physiology and in pathogenic aggressiveness. This might well contribute to the evolution of *Phytophthora* populations and especially to their pathogenic adaptation.

LITERATURE CITED

BOCCAS, B. 1972. Contribution à l'étude du cycle chez les *Phytophthora* C.R. Acad. Sci. (Paris) 275 D : 663-666.

BOCCAS, B. and ZENTMYER, G.A. 1976. Genetical studies with interspecific crosses between *Phytophthora cinnamomi* and *P. parasitica*. Phytopathology 66 : 477-484.

BOCCAS, B. 1980. Interspecific crosses between closely related heterothallic *Phytophthora* species. Accepted for publication in Phytopathology.

BRASIER, C.M. 1975. Stimulation of sex organ formation in *Phytophthora* by antagonistic species of Trichoderma. New Phytologist, 74, 183-194.

BRASIER, C.M. and SANSOME, E.R. 1975. Diploidy and gametangial meiosis in *P.cinnamomi*, *P. infestans*, *P. dreschleri*. Trans. Br. Mycol. Soc., 65 (1) : 49-65.

ELLIOT, G.C. and MC INTYRE, D. 1973. Genetical evidence on the life history of *Phytophthora*. Trans. Br. Mycol. Soc., 60 : 311-316.

GALINDO, J., and GALLEGLY, M.E. 1960. The nature of sexuality in *Phytophthora infestans*. Phytopathology 50 : 123-128.

GALINDO, J., and ZENTMYER, G.A. 1967. Genetical and cytological studies of *Phytophthora* strains pathogenic to pepper plants. Phytopathology, 57, 1300-1304.

GALLEGLY, M.E and GALINDO, J. 1958. Mating types and oospores of *Phytophthora infestans* in nature in Mexico. Phytopathology 48, 274-277.

HUGUENIN, B. and BOCCAS, B. 1970. Etude de la caryocinèse chez le *Phytophthora parasitica*. C.R. Acad. Sci. (Paris) 271 : 660-663.

KO, W.H. 1978. Heterothallic *Phytophthora* : Evidence for hormonal regulation of sexual reproduction. J. Gen. Micro., 107, 15-18.

LAVIOLA, C. 1969. Studies on the genetics of *Phytophtora infestans*. Diss. Abstr. 29, 35-73.

LEGRAND-PERNOT, F. et PELLEGRIN, F., 1976. Nature du cycle végétatif des *Phytophthora parasitica* et *palmivora*. Ann. Phytopathol., 8 (4) : 379-388.

LONG, M. and KEEN, N.T. 1977. Genetic evidence for diploidy in *P. megasperma var. sojae*. Phytopathology, 67 : 675-677.

MAIA, N., VENARD, P. et LAVRUT, F., 1976 - Etude des divisions mitotiques et méiotiques du cycle de *P. capsici*. Ann. Phytopathol., 8, 141-146.

NOON, J.P. and HICKMAN, C.J. 1974. Oospore production by a single isolate of *Phytophthora capsici* in the presence of chloroneb. Can. J. Bot., 52, 1591-1595.

RAPER, J.R. 1951. Sexual hormones in *Achlya*. Amer. Sci., 39 : 110-121.

ROMERO, S. and ERWIN, D.C. 1969. Variation in pathogenicity of progeny from germinated oospores of *P. infestans*.
Phytopathology, 59, 1310-1317.

SANSOME, E.R., 1961. Meiosis in the oogonium and antheridium of *Pythium debaryanum*. Nature, Lond., 911, 827-28.

SANSOME, E.R., 1965. Meiosis in diploid and polyploid sex organs of *Phytophthora* and *Achlya*. Cytologia 30 : 103-107.

SANSOME, E.R., 1976. Gametangial meiosis in *Phytophthora capsici*. Can. J. Bot. 54 (13) : 1535-1545.

SATOUR, M.H. and BUTLER, E.E., 1968. Comparative morphological and physiological studies on the progenies from intraspecific matings of *Phytophthora capsici*. Phytopathology, 58, 183-192.

SAVAGE, E.J., CLAYTON, C.W., HUNTER, J.H., BRENNEMAN, J.A., LAVIOLA, C. and GALLEGLY, M.E., 1968. Homothallism, heterothallism and interspecific hybridization in the genus *Phytophthora*. Phytopathology, 58 : 1004-1021.

SHAW, D.S., and KHAKI, I.A., 1971. Genetical evidence for diploidy in *Phytophthora*. Genet. Res. Camb. 17 : 165-167.

TIMMER, L.W., CASTRO, J., ERWIN, D.C., BELSER, W.L., and ZENTMYER, G.A., 1970 Genetic evidence for zygotic meiosis in *Phytophthora capsici*. Amer. J. Bot. 57, 1211-1218.

WATERHOUSE, G.M., 1963. Key to the species of *Phytophthora* de Bary. Mycological papers, 92, C.M.I. Kew, Surrey, England.

ZENTMYER, G.A. 1952. A substance stimulating sexual reproduction in *Phytophthora cinnamomi*. Phytopathology, 42 : 24.