Interspecific Crosses Between Closely Related Heterothallic *Phytophthora* Species

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ABSTRACT

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Five crosses between closely related heterothallic *Phytophthora* species produced oospores, a low percentage (1–5%) of which germinated. Colony morphology, optimum and maximum growth temperatures, growth rate, pathogenicity, and composition in soluble porteins among the progeny were studied. Among these single oospore isolates, only one, from a cross between *P. capsici* and *P. palmivora* appeared to be a product of interspecific hybridization. All other crosses resulted in phenotypically heterogeneous progeny that exhibited recombination for some morphological, physiological and pathogenic characters; their protein patterns, however, were of a single parental type. These results suggest that the progeny resulted from the self-fertilization of diploid heterozygous parental strains rather than from hybridization.

Additional key words: P. cambivora, P. cinnamomi, P. megakarya, P. nicotianae var. *parasitica,* genetics, mating types

RESUMÉ

Cinq croisements entre des espèces affines de *Phytophthora* hétérothalliques ont produit des oospores dont un faible pourcentage (de 1 à 5%) a germé. Ces oospores germantes ont donné naissance à des descendants dont la morphologie, le comportement à l'égard de la température, la vitesse de croissance, la pathogénie et la composition en protéines solubles ont été étudiées. Un seul de ces isolats mono-oospores, issu d'un croisement entre *P. capsici* et *P. palmivora*, a révélé un ensemble de caractères recombinés indiquant qu'il pouvait résulter de l'hybridation des deux espèces. Tous les autres croisements ont produit des descendances phénotypiquement hétérogènes et recombinées pour certains caractères morphologiques, physiologiques et pathogéniques, mais de type parental pour la composition en protéines solubles. Selon l'interprétation la plus simple, ces résultats suggèrent que tous ces descendants provenaient de l 'autofécondation des souches parentales diploïdes et hétérozygotes, et non de leur hybridation.

Oospore formation in crosses between sexually compatible isolates of different heterothallic *Phytophthora* species has been reported by several authors (1,2,13,14,15,28,34). Although self-sterile in single culture, heterothallic isolates are bisexual, and it has been shown that two kinds of oospores may be produced in crosses: those from self-fertilization of each parental isolate, and hybrid oospores from pairing of gametangial hyphae of the parents (1,7,10,13,15,23,25). If exchange of genetic material occurs in the hybrid oospores, interspecific crosses might be an important source of variability in the genus *Phytopthora*. However, demonstration of interspecific hybridization is difficult due to the very low percent germination of oospores and the problem of establishing viable colonies from germinated oospores (1,4,34). Boccas and Zentmyer (6) obtained viable progenies from crosses between *P. cinnamomi* and *P. nicotianae* var. *parasitica*, and noted that all the progeny probably arose from selfed oospores. The hybrid oospores were thought to have aborted before germination, so that interspecific hybridization did not occur. However, *P. nicotianae* var. *parasitica* and *P. cinnamomi* belong to group II and VI, respectively, of Waterhouse's key (30,31), and differ markedly in morphology, physiology, pathogenicity, and in chromosome number (2n = 10 in *P. nicotianae* var. *parasitica* (15), and 2n = 18 or 20 in *P. cinnamomi* [10]). In this instance therefore, it would appear that the absence of hybridization may well have resulted from lack of homology between the genomes of the two species, and would not necessarily imply that interspecific hybridization is not feasible in the genus *Phytophthora.*

The purpose of the present work was to determine if hybridization can occur between more closely related species belonging to the same morphological groups (II and IV) as defined in Waterhouse's key (30,31).

MATERIALS AND METHODS

We used 7 isolates of Waterhouse's group II (two of *P. palmivora,* one of *P. megakarya* [9], two of *P. nicotianae* var. *parasitica,* and two of *P. capsici*) and one isolate each of *P. cinnamomi* and *P. cambivora* which belong to Waterhouse's group VI. Their origins and characteristics are shown in Table 1.

All seven parental strains were shown to be phenotypically stable by comparison of successive single zoospore cultures from each strain with the original parental strain. Furthermore, to avoid the risk that the parental strains might possibly be heterokaryotic, additional crosses were made between isolates derived from single zoospores of each strain.

Five crosses were studied. The first four involved isolates of Waterhouse's group II: *P. palmivora* 418 × *P. capsici* 375, *P. palmivora* 98 × *P. nicotianae* var. *parasitica* 355, *P. megakarya* 261 × *P. nicotianae* var. *parasitica* 377, and *P. nicotianae* var. *parasitica* 355 × *P. capsici* 376. The fifth involved isolates of group VI: *P. cinnamomi* 156 × *P. cambivora* 426. In some crosses, the number of oospores was determined by using a Malassez counting chamber. These were then compared to the number of oospores produced in intraspecific crosses employing the same species.

Matings were made by placing disks from agar cultures of opposite mating types 2 cm apart on petri plates of pea broth agar (5), incubating them at 26 C in darkness for 15 days, then incubating at room temperature under fluorescent light for 2 wk. After this period of incubation, germinating oospores were harvested as described previously (6).

In all the crosses, the mating types of the progeny were determined by pairing with the original parental strains. Colony morphology was judged by observing 6-day-old cultures on potato-dextrose agar (PDA) incubated at 26 C in darkness. The same medium was used to determine the optimum and maximum temperature for growth. The protein patterns of parental strains and single oospore progeny were compared by using acrylamide gel electrophoresis (33).

The growth rates of the progeny from cross Pn 377 × Pp 261, were evaluated by measuring the dry weight of mycelium formed in 7 days on a synthetic liquid medium (5). The pathogenicity of the progeny of cross 375 × Pp 418 towards *Capsicum annuum* also was evaluated: 1-mo-old seedlings grown in pots of sterile soil were inoculated by adding 25 ml of a water suspension of blended mycelium to each pot (mycelium from 2-wk-old cultures grown in 100 ml of pea broth, washed, and blended 20 sec in 50 ml of deionized water).

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TABLE 1. Characteristics of *Phytophthora* isolates used to study interspecific crosses between closely related heterothallic *Phytophthora* species

Species	Isolates	Source	Mating type
P. palmivora	Pp 98	*Theobroma cacao*; Cameroon	A2
	Pp 418	*Mangifera indica*: Ivory Coast	A2
P. megakarya	Pm 261	*Theobroma cacao*; Sao Thome	A1
P. nicotianae var. *parasitica*	Pn 355	*Citrus*; Ivory Coast	A1
	Pn 377	*Citrus;* California	A2
P. capsici	Pcp 375	*Capsicum annuum*; Mexico	A1
	Pcp 376	*Capsicum annum*; Mexico	A2
P. cinnamomi	Pc 156	*Persea gratissima*; Congo	A2
P. cambivora	Pcm 426	- ; Baarn	A1

RESULTS

Oospore production and germination. Oospore production in intraspecific crosses (Pn 377 × Pn 355 and Pcp 375 × Pcp 376) and in interspecific crosses (Pn 355 × Pcp 376, Pcp 375 × Pp 418, and Pn 377 × Pm 261) was compared. Interspecific crosses produce fewer oospores than the intraspecific crosses (Table 2). However, the rate of oospore production in crosses between closely related species of the same group is about 10 times higher than that in the previously studied crosses between species classified in different groups (5,6). The germination rates are low in both types of interspecific combinations, ranging in the present case from 5% in Pn 377 × Pp 261 to 1% in Pn 355 × Pcp 376.

Distribution of phenotypic characters in the progeny of different crosses. *Pm 261 × Pn 377.* Sixty-five progeny were harvested; 14 were of the A1 mating type, 50 of the A2 mating type, and one was self-fertile. Eighteen of these isolates showed a considerable variation with respect to colony morphology and growth rate (mycelial dry weight). Their characteristics are summarized in Table 3. The table clearly shows the wide heterogeneity for the characters studied. The composition in soluble proteins was the only stable character. The protein patterns of the parental strains differed both in the number of bands and in their relative migration rates in the gels. Each progeny isolate exhibited a protein pattern qualitatively similar to that of one or the other parental strain, although minor differences in the density of some protein bands were observed. Ten progeny had a protein pattern similar to Pm 261, and the eight others were of the Pn 377 type.

Pn 355 × Pp 98. Eighteen single oospore progeny were obtained (Table 4). These were homogeneous in morphology, mating type, and protein pattern and all were of the A1 mating type. Their colony and protein patterns were similar to that of the parental strain Pn 355. The only differences observed were in the optimum and maximum temperatures for growth.

Pn 355 × Pcp 376. Fifteen single-oospore progeny were harvested. Their chracteristics are shown in Table 5. Thirteen were similar to the Pn 355 parent in colony morphology, mating type, and protein pattern. The other two were identical to the Pcp 376 parent with respect to these characters. As in the Pn 355 × Pp 98 cross, the progeny exhibited considerable heterogeneity in temperature response.

Pcp 375 × Pp 418. Among the 18 single-oospore isolates described in Table 6, 15 were of the A2 mating type and three of the A1 mating type. Variations in optimum and maximum temperature for growth also were observed. Colony morphology was of the parental type among all but three which showed a phenotype differing from either parent. Some progeny could be differentiated from the parental strains by pathogenicity tests.

TABLE 2. Oospore production in intraspecific and interspecific crosses between heterothallic *Phytophthora* species

Crosses	Oospore production (no. per mm^3)	Oospore germination (%)
Intraspecific		
Pn 377 × Pn 355	126	12
Pcp 375 × Pcp 376	100	. . .
Interspecific		
Pn 355 × Pcp 376	24	1
Pcp 375 × Pp 418	18	4
Pn 377 × Pm 261	20	5

TABLE 3. Characteristics of phenotypic recombinants among F_1 progeny of the cross *Phytophthora megakarya* 261 × *Phytophthora nicotianae* var. *parasitica* 377

Parental and progeny isolates	Mating type	Colony type[a]	Optimum temperature (C)	Maximum temperature (C)	Mycelial dry weight (mg)	Protein pattern
Pm 261	A1	261	28	33	16,5	261
Pn 377	A2	377	31	36	103,5	377
F_1 1	A1	R	28	33	23,7	261
2	A1	R	28	36	88,6	261
3	A1	R	28	36	64,0	261
4	A1	R	28	33	97	261
5	A2	R	30	33	40	377
6	A1	R	28	36	67,2	261
7	A2	R	26	30	68	377
8	A1	R	28	36	94	261
9	A1	R	28	< 36	75	261
10	A1	R	26	< 36	40	261
11	A1	R	28	< 36	72	261
12	A2	R	30	. . .[b]	50	377
13	A1	R	28	< 36	79,4	377
14	A1	R	30	< 36	25	377
15	A2	R	26	30	46,2	377
16	A2	R	26	. . .	30,6	377
17	A1	R	28	36	28,9	261
18	A2	R	28	36	24,8	377

[a] 261 = Pm 261 colony type, 377 = Pn 377 colony type, and R = recombinant colony type.
[b] . . . = temperature maximum not tested.

TABLE 4. Characteristics of F_1 progeny of the cross *Phytophthora nicotianae* var. *parasitica* 355 × *Phytophthora palmivora* 98

Parental and progeny isolates	Mating type	Colony type	Optimum temperature (C)	Maximum temperature (C)	Protein pattern
Pn 355	A1	355	30	< 36	355
Pp 98	A2	98	26	29	98
F_1 1	A1	355	28	> 36	355
2	A1	355	30	> 36	355
3	A1	355	24	> 36	355
4	A1	355	30	< 36	355
5	A1	355	30	< 36	355
6	A1	355	30	< 36	355
7	A1	355	30	< 36	355
8	A1	355	30	< 36	355
9	A1	355	30	< 36	355
10	A1	355	28	< 36	355
11	A1	355	30	< 36	355
12	A1	355	30	< 36	355
13	A1	355	26	< 36	355
14	A1	355	30	< 36	355
15	A1	355	26	< 36	355
16	A1	355	30	< 36	355
17	A1	355	28	< 36	355
18	A1	355	28	> 36	355

TABLE 5. Characteristics of F_1 progeny of the cross *Phytophthora nicotianae* var. *parasitica* 355 × *Phytophthora capsici* 376

Parental and progeny isolates	Mating type	Colony type	Optimum temperature (C)	Maximum temperature (C)	Protein pattern
Pp 355	A1	355	30	< 36	355
Pcp 376	A2	376	28	< 36	376
F_1 1	A1	355	28	> 36	355
2	A1	355	32	< 36	355
3	A1	355	31	> 36	355
4	A2	376	32	> 36	376
5	A1	355	30	< 36	355
6	A1	355	30	> 36	355
7	A1	355	30	< 36	355
8	A1	355	28	< 36	355
9	A1	355	30	> 36	355
10	A1	355	30	> 36	355
11	A2	376	32	< 36	376
12	A1	355	30	> 36	355
13	A1	355	28	< 36	355
14	A1	355	28	> 36	355
15	A1	355	28	> 36	355

TABLE 6. Characteristics of F_1 progeny of the cross *Phytophthora capsici* 375 × *Phytophthora palmivora* 418

Parental and progeny isolates	Mating type	Colony type[a]	Optimum temperature (C)	Maximum temperature (C)	Pathogenicity to pepper[b]	Protein pattern[c]
Pcp 375	A1	375	28	> 36	P (20/20)	375
Pp 418	A2	418	28	33	NP	418
F_1 1	A2	418	28	33	NP	418
2	A2	418	28	33	P (10/10)	418
3	A2	418	28	33	NP	418
4	A2	418	26	33	NP	418
5	A1	I	26	33	P (20/20)	I
6	A2	418	26	33	NP	418
7	A2	I	26	> 36	P (6/6)	418
8	A2	418	28	33	NP	418
9	A2	418	28	33	P (9/18)	418
10	A2	418	28	33	P (8/8)	418
11	A2	418	28	33	P (2/10)	418
12	A2	418	26	33	NP	418
13	A2	I	26	33	NP	418
14	A2	418	30	33	NP	418
15	A2	418	28	33	NP	418
16	A2	418	26	33	NP	418
17	A1	418	28	33	NP	418
18	A1	375	26	33	NP	375

[a] 375 = colony type of Pcp 375, 418 = colony type of Pp 418, and I = intermediate colony type.
[b] NP = nonpathogenic, P = pathogenic, numerals separated by a slash indicate the number of plants killed over the number of plants attacked of 20 plants inoculated.
[c] 375 = Pcp 375 parental protein pattern, 418 = Pp 418 parental protein, and I = intermediate protein pattern.

When young pepper seedlings were inoculated, parent Pcp 375 attacked the collar region, whereas Pp 418 was not pathogenic. Progeny isolates 2,7,10, and 11 were less aggressive; the inoculated plants were not always infected and infection did not always result in the death of the plant (Fig. 1). The protein pattern of most of the progeny was of the parental type, with few variations in the density of protein bands (Fig. 2). However, isolate 5 exhibited a protein pattern which was quite different from those of the two parental strains.

Pcm 426 × Pc 156. The fertility of the cross was low; few oospores were formed and less than 1% of these germinated. Only six progeny were obtained and the mating types, colony morphologies and protein patterns of all of them resembled that of Pc 156. The only character for which some variation was obtained was the maximum temperature for growth.

DISCUSSION

The progeny of the five crosses showed some phenotypic variability in quantitative characters. In all crosses the progeny varied in optimum and maximum temperatures for mycelial growth. Comparable variability was observed in colony morphology in the cross Pp 261 × Pn 277 and in pathogenicity to *Capsicum annuum* in the cross Pcp 375 × Pp 418.

Among all the progeny except isolate 5 of cross Pcp 375 × Pp 418, the protein pattern was always of the parental type. The patterns of parental types differ widely: Fig. 3 illustrates the differences between protein patterns of *P. nicotianae* var. *parasitica, P. palmivora, P. capsici,* and *P. cinnamomi.* In crosses Pm 261 × Pn 377, Pn 355 × Pcp 376, and Pcp 375 × Pp 418, each of the two parental types was represented among the progeny, whereas in Pn 355 × Pm 98 and Pc 156 × Pcm 426 the protein pattern of only one of the parental strains (Pn 355 and Pcp 156, respectively) was observed in the progeny. Occasionally, however, densitometer recordings (Fig. 2) showed slight variations in either the relative density of some protein bands or in the distance between two adjacent bands; these differences may reflect quantitative differences in the soluble protein composition of the progeny.

Isolate 5 of cross Pcp 375 × Pp 418 was the only progeny isolate to exhibit a protein pattern qualitatively and quantitavely different from that of the parents (Fig. 2). This would seem to imply important modifications in the genome of this isolate. Since it also differs from the parents in other characters (Table 6), it can be concluded that this progeny is probably an interspecific hybrid, the only one to be obtained from an in vitro cross between *P. capsici* and *P. palmivora.* This interpretation is supported by the fact that there is a similarity in the reported chromosome number of the two parent species: n = 8 to 10 in *P. capsici* (14) and n = 9 to 12 in *P. palmivora* (9). However, isolate 5 is a single isolated example. In all other crosses we are faced with the same situation as in the study of crosses between *P. cinnamomi* and *P. nicotianae* var. *parasitica* (6): all the progeny show rather wide morphological, physiological and pathogenic variation. These characters are likely to be polygenically controlled, and the variation probably reflects recombinations between the controlling loci. It contrasts, however, with the qualitative homogeneity of the protein patterns. The latter would tend to indicate that no genetic exchange occurred between the parents. Thus, true hybridization between the parents cannot be considered to be the source of the observed variation in the progeny.

Since *Phytophthora* species are diploid throughout their vegetative state (3,5,11,16,17,18,22,24,26,29), it is concluded, according to the simplest interpretation, that the parental strains were heterozygous for loci controlling those characters showing variation, and that all the progeny, except isolate 5 of the cross Pcp 375 × Pp 418, resulted from self-fertilization of the parental strains.

Self-fertilization among heterothallic *Phytophthora* species is a consequence of the potential bisexuality of these organisms. Although less common than hybrid oospores (24,27), selfed oospores are formed in both intra- and interspecific crosses, after a normal meiosis in the paired gametangia (10). However, in crosses between two different species, whether closely related or morphologically distant, hybrid oospores appear unable to germinate, probably because the resultant genomes lack homology. Only selfed oospores may produce viable progeny. Thus, the mating of compatible strains of different species does not generally produce hybrid progeny, but appears to lead to a reciprocal induction of self-fertilization which may result in phenotypic variation of the progeny if the parental strains are heterozygous.

On this assumption it is interesting to note that the variability resulting from interspecific crosses is, in fact, narrower than that obtained from intraspecific crosses. In the first case, variations would be due only to the recombinations resulting from the self-fertilization of the parents and from chromosomal exchange between them.

A further important characteristic of the selfing induced in

Fig. 1. Symptoms induced on *Capsicum annuum* seedlings by parental strains *Phytophthora capsici* 375 and *P. palmivora* 418 and some recombinant progeny.

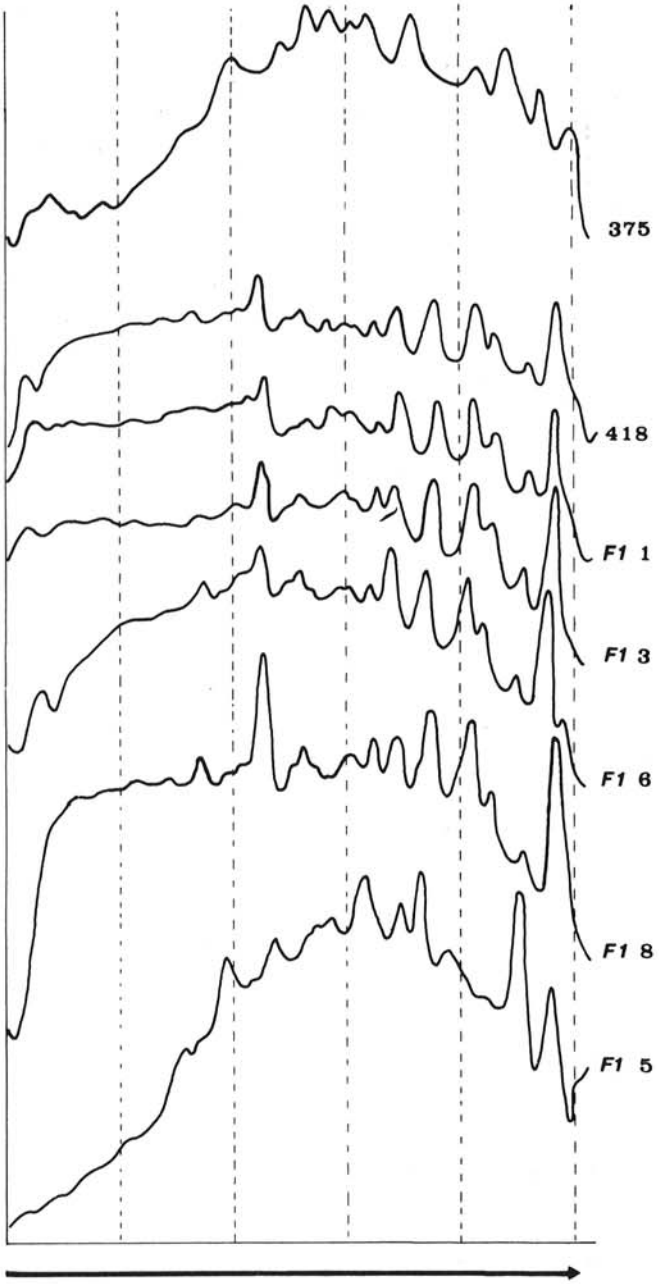

Fig. 2. Densitometer recordings of protein patterns of Pcp 375, Pp 418, and some F_1 progeny, including isolate 5.

interspecific crosses is its reciprocity. In these crosses, self-fertilization was reciprocally induced in both A1 and A2 isolates, whereas the selfing induced by chemical or physical stimulation (8,21,32) occurs only in some A2 isolates. Moreover, self-fertilization has different consequences in A1 and A2 isolates. The results given here and those obtained from crosses between *P. cinnamomi* and *P. nicotianae* var. *parasitica* (6) indicate that the selfing of the A1 type always produces progeny of the same compatibility type, whereas selfed A2 isolates segregate A1 and A2 progeny. If the stimulation of sexual reproduction and the subsequent differentiation of gametangia in the genus *Phytophthora* are determined by a series of chemical substances, like those reported in some other Oomycetes of Zygomycetes (12,19,20), it is possible that the potential bisexuality of heterothallic *Phytophthora* spp. is inhibited at different levels in the two compatibility types. The block in the metabolic pathway leading to the differentiation of gametangia may be less efficient and more easily reversible in A2 strains than in A1. A2 isolates appear to control functions involved in oogenesis, since oogenesis can be induced by A1 isolates or by less specific stimuli (8,21,32). On the other hand, A1 isolates are self-

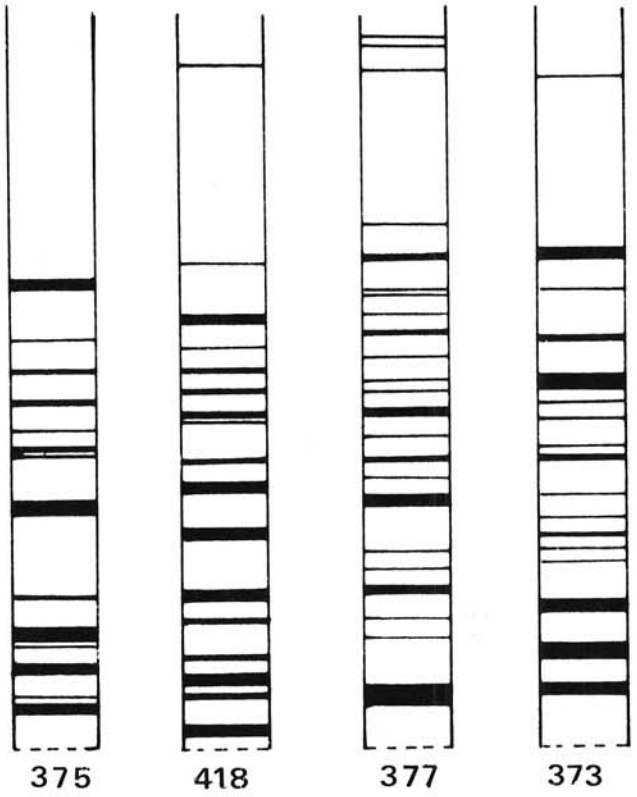

Fig. 3. Diagrams of parental type electrophoretic protein patterns of *Phytophthora* spp. used in a study of interspecific crosses: 375, *P. capsici*; 418, *P. palmivora*; 377, *P. parasitica*; and 373, *P. cinnamomi*.

fertile only when specifically induced by A2 isolates, and thus evidently lack functions directly involved in oogenesis.

In conclusion, with one exception, 220 single oospore isolates from eight different crosses between closely related or morphologically more distant *Phytophthora* species (6), appear to be of uniparental origin. Therefore, it seems that in almost all cases there is little likelihood of genetic exchange between the species examined in this study and consequently interspecific hybridization is unlikely to be an important source of variability in the genus *Phytophthora*.

LITERATURE CITED

1. APPLE, J. L. 1959. Sexuality of *Phytophthora parasitica* var. *nicotianae*. Phytopathology 49:37-43.
2. BARRET, J. T. 1948. Induced oospore formation in the genus *Phytophthora*. (Abstr.) Phytopathology 38:2.
3. BOCCAS, B. 1972. Contribution à l'étude du cycle chez les *Phytophthora*. C. R. Acad. Sci. (Paris) Sér. D 275:663-666.
4. BOCCAS, B. 1973. Observation sur un cas d'hybridation interspécifique entre *Phytophthora parasitica* et *Phytophthora cinnamomi*. Fruits 28:445-451.
5. BOCCAS, B. 1979. La reproduction sexuelle chez les *Phytophthora*. Travaux et Documents de l'ORSTOM N° 100:1-188.
6. BOCCAS, B., and G. A. ZENTMYER. 1976. Genetical studies with interspecific crosses between *Phytophthora cinnamomi* and *P. parasitica*. Phytopathology 66:477-484.
7. BRASIER, C. M. 1972. Observations on the sexual mechanism in *Phytophthora palmivora* and related species. Trans. Br. Mycol. Soc. 58:237-251.
8. BRASIER, C. M. 1975. Stimulation of sex organ formation in *Phytophthora* by antagonistic species of *Trichoderma*. New Phytol. 74:183-194.
9. BRASIER, C. M., and M. J. GRIFFIN. 1979. Taxonomy of *Phytophthora palmivora* on cocoa. Trans. Br. Mycol. Soc. 72:111-143.
10. BRASIER, C. M., and E. R. SANSOME. 1975. Diploidy and gametangial meiosis in *Phytophthora cinnamomi, P. infestans, P. drechsleri*. Trans. Br. Mycol. Soc. 65:49-65.
11. ELLIOT, G. C., and D. McINTYRE. 1973. Genetical evidence on the life history of *Phytophthora*. Trans. Br. Mycol. Soc. 60:311-316.
12. ESSER, K., and R. KUENEN. 1967. Genetics of fungi. Springer-

Verlag, Berlin, Heidelberg, and New York.
13. GALINDO, J., and M. E. GALLEGLY. 1960. The nature of sexuality in *Phytophthora infestans*. Phytopathology 50:123-128.
14. HAASIS, F. A., and R. R. NELSON. 1963. Studies on the biological relationship of species of *Phytophthora* as measured by oospore formation in intra- and interspecific crosses. Plant Dis. Rep. 47:705-709.
15. HUGUENIN, B. 1973. Application de la technique du marquage fluorescent à la détermination du comportement sexuel du *P. palmivora*. Cah. ORSTOM, Sér. Biol. 20:59-61.
16. HUGUENIN, B., and B. BOCCAS. 1970. Etude de la caryocinèse chez le *Phytophthora parasitica*. C. R. Acad. Sci. (Paris) 271:660-663.
17. LEGRAND-PERNOT, F., and F. PELLEGRIN. 1976. Nature du cycle végétatif des *Phytophthora parasitica* et *palmivora*. Ann. Phytopathol. 8:379-388.
18. LONG, M., and N. T. KEEN. 1977. Genetic evidence for diploidy in *P. megasperma* var. *sojae*. Phytopathology 67:675-677.
19. PLEMPEL, M. 1963. Die chemischen Grundlagen der Sexual reaktion bei Zygomyceten. Planta (Berl.) 59:492-508.
20. RAPER, J. R. 1951. Sexual hormones in *Achlya*. Am. Sci. 39:110-121.
21. REEVES, R. J., and R. M. JACKSON. 1974. Stimulation of sexual reproduction in *Phytophthora* by damage. J. Gen. Microbiol., 84:303-310.
22. SANSOME, E. 1965. Meiosis in diploid and polyploid sex organs of *Phytophthora* and *Achlya*. Cytologia 30:103-107.
23. SANSOME, E. 1970. Selfing as a possible cause of disturbed ratios in *Phytophthora* crosses. Trans. Br. Mycol. Soc., 54:101-107.
24. SANSOME, E. 1976. Gametangial meiosis in *Phytophthora capsici*. Can. J. Bot. 54:1535-1545.
25. SANSOME, E. 1977. Polyploidy and induced gametangial formation in British isolates of *Phytophthora infestans*. J. Gen. Microbiol. 99:311-316.
26. SANSOME, E., and C. M. BRASIER. 1974. Diploidy and chromosomal structural hybridity in *Phytophthora infestans*. Nature 24:344-345.
27. SANSOME, E., C. M. BRASIER, and F. W. SANSOME. 1979. Further cytological studies on the "L" and "S" types of *Phytophthora* from cocoa. Trans. Br. Mycol. Soc. 73:293-302.
28. SAVAGE, E. J., C. W. CLAYTON, J. H. HUNTER, J. A. BRENNEMAN, C. LAVIOLA, and M. E. GALLEGLY. 1968. Homothallism, heterothallism and interspecific hybridization in the genus *Phytopathora*. Phytopathology 58:1004-1021.
29. SHAW, D. S., and I. A. KHAKI. 1971. Genetical evidence for diploidy in *Phytophthora*. Genet. Res. Camb. 17:165-167.
30. WATERHOUSE, G. M. 1963. Key to the species of *Phytophthora* de Bary. Mycol. Pap. 92. Commonw. Mycol. Inst., Assoc. Applied Biologists, Kew, Surrey, England.
31. WATERHOUSE, G. M. 1970. The genus *Phytophthora* de Bary. Mycol. Pap. 122, Commonw. Mycol. Inst., Assoc. Applied Biologists, Kew, Surrey, England.
32. ZENTMYER, G. A. 1952. A substance stimulating sexual reproduction in *Phytophthora cinnamomi*. Phytopathology 42:24.
33. ZENTMYER, G. A., L. JEFFERSON, C. J. HICKMAN, and Y. CHANG-HO. 1974. Studies of *Phytophthora citricola*, isolated on *Persea americana*. Mycologia 66:830-845.
34. ZIMMERMAN, R. K. 1973. Problems in the establishment of interspecific hybrids in the genus *Phytophthora* de Bary. Ph.D. dissertation. West Virginia University, Morgantown. 71 pp.