Genetical Studies with Interspecific Crosses Between Phytophthora cinnamomi and Phytophthora parasitica

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ABSTRACT

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Two interspecific crosses between *Phytophthora parasitica* and *Phytophthora cinnamomi* resulted in the formation of oospores, but only 5% were well formed. About 5% of the normal spores germinated and gave single oospore progeny which were genetically studied. The first cross produced only parental type progeny, but the second cross yielded heterogeneous progeny including parental type cultures and cultures with new associations of phenotypic characters. In both crosses, parental type progeny were interpreted as arising from the germination of self-fertilized oospores from the parents. Progeny with apparent recombinant phenotypes could have resulted either from interspecific hybridization or from self fertilization. Evidence of recombination was obtained in relation to mating type, pathogenicity to citrus and *Persea indica,* and temperature response. Presumed recombinant progeny exhibited a protein pattern similar to *P. parasitica,* indicating that these progeny may have resulted from self fertilization.

Additional key word: ploidy.

RESUMÉ

Deux croisements entre deux isolats de *Phytophthora parasitica* et deux isolats de *Phytophthora cinnamomi* ont produit des populations d'oospores dont 5% seulement étaient bien constituées. Environ 5% de ces oospores normales ont germé produisant des descendants d'origine mono-oospores qui furent génétiquement étudiés. Le premier croisement a donné uniquement des descendants de type parental, le second croisement a produi une descendance hétérogène comprenant des souches de type parental et des souches présentant de nouvelles associations de caractères phénotypiques. Dans les deux croisements, les descendants de type parental sont interpretés comme provenant de la germination d'oospores autofecondées de chacun des parents. Les descendants au phenotype recombiné peuvent provenir soit de l'hybridation entre les espèces parentales, soit de l'autofécondation de chacune d'entre elles. En effet, les phénotypes indiquent des recombinaisons en ce qui concerne le type de compatibilité, le pouvoir pathogène à l'égard des citrus et de *Persea indica,* et la température maximum pour la croissance. Toutes ces souches recombinées présentent cependant un spectre proteïque similaire à celui de *P. parasitica,* ce qui suggére qu'elles peuvent provenir d'oospores autofecondées.

Sexual reproduction may be a source of considerable intraspecific variation in the genus *Phytophthora*. This has been demonstrated in several recent papers (11, 15, 16, 18, 22, 25) which showed the recombination of characters involving mating type, colony morphology, pathogenicity and physiology in the progeny of intraspecific crosses.

The sexual stage is also a potential source of interspecific variability since heterothallic species can form oospores in interspecific crosses involving compatible isolates of A1 and A2 mating types. The production of oospores in interspecific crosses has been the subject of several investigations. Haasis and Nelson (12), exploring the mating possibilities between 18 species, showed that oospores formed in 90% of compatible combinations and they observed abundant production of oospores in crosses between *P. parasitica* and *P. cinnamomi*. Savage et al. (23) obtained similar results with 14 species and demonstrated, by hyphal tracing, the formation of hybrid oospores from interspecific fusion of gametangial hyphae. Similar observations were reported by Zimmerman (28).

Possible exchange of genetic material in such interspecific hybrid oospores may be an important factor in the evolution of natural *Phytophthora* populations, and raises fundamental questions on the validity of the species concept in the genus *Phytophthora*. However, the occurrence of interspecific hybridization remains hypothetical, since viability of hybrid oospores and genetic recombination between two species have not been completely demonstrated. Most attempts to germinate oospores have been unsuccessful and only Apple (1), Zimmerman (28), and Boccas (5) have reported the germination of hybrid oospores. Apple observed in one cross between *P. capsici* and *P. palmivora* a "considerable abundance" of germination, but he was unable to

establish colonies from any of the germinating oospores. With the same species, Zimmerman obtained two germ sporangia from one oospore, but no conclusive indication of genetic recombination was found. More recently, Boccas (5) obtained germination of oospores from a cross of a *P. parasitica* isolate from citrus and a *P. cinnamomi* isolate from avocado. Twenty-two single oospore cultures exhibited recombination of factors for mating type and for pathogenicity to citrus, avocado, and rosella *(Hibiscus sabdariffa).*

The present work was undertaken to confirm these preliminary results with different *Phytophthora* isolates and other genetic characters.

MATERIALS AND METHODS

Four *Phytophthora* isolates from California were used (numbers refer to the *Phytophthora* culture collection, Department of Plant Pathology, University of California, Riverside). *Phytophthora cinnamomi* (Pc 40), A2, from avocado roots, was crossed with *P. parasitica* (P 991), A1, from citrus soil. *Phytophthora cinnamomi* (Pc 138), A1, from avocado, was crossed with *P. parasitica* (P 731), A2, from citrus soil. Mating types were related to those of *P. infestans*. Intraspecific crosses were made between *P. parasitica* isolates and *P. cinnamomi* isolates to compare oospore production in both intra- and interspecific crosses. In each cross, the number of oospores was determined with a Levy-Hausser counting chamber on blended cultures.

Certain isolates of *Phytophthora* exhibit phenotypic variation among single zoospore progeny (7). This asexual variability, attributed to cytoplasmically inherited factors, could possibly interfere with genotypic variation resulting from genetic exchange during the sexual stage. Therefore, the phenotypic stability of the isolates studied was examined by comparing a series of single zoospore cultures from each isolate on potato-dextrose agar (PDA), for colony morphology, growth rate, and production of sporangia. In addition, to avoid the possibility of heterokaryosis in parental strains, crosses were made between single zoospore isolates from the stock cultures.

Matings were made by placing disks from agar cultures of opposite mating type 2 cm apart on petri plates of clarified V8 (CV8) agar (27) and incubating them at 25 C in darkness for 15 days, then at room temperature under fluorescent light for 2 weeks. Oospores formed mainly along the zone of contact between the two parental mycelia. Samples of oospores from 1-month-old cultures were removed in agar blocks, blended in deionized sterile water with a commercial Waring Blendor for 45 seconds, then forced with a rubber policeman through a 50-μm pore size nylon mesh. The filtrate was centrifuged for 3 minutes and the supernatant containing agar and most mycelial fragments was discarded. The pellet of oospores was resuspended in sterile deionized water in petri plates. After two days at room temperature (24 $\pm$ 1C) with alternating diffuse daylight and darkness, samples of oospores were microscopically examined to determine the percentage of empty or damaged spores. Single mature oospores, some of which had already germinated in water, were removed with a micropipette, placed on a grid on CV8 agar plates (100 spores per plate) and incubated at room temperature in the dark for 5 days. Germinating oospores were individually transferred to CV8 agar tubes and the colonies formed were used for further genetic studies.

Compatibility types of the single oospore cultures were determined by crossing with the parental strains. Colony and hyphal morphology were examined using 6-day-old cultures on PDA. The same medium was used to determine the maximum temperature for growth. Sporangium production and morphology were observed on CV8 agar and in nonsterile soil extract. To evaluate the pathogenicity of the progeny, 2-month-old seedlings of *Citrus jambhiri* and *Persea indica* grown in U.C. Mix type C (2) were inoculated by adding 25 ml of a water suspension of blended mycelium to each pot (mycelium from two 2-week-old cultures grown in 50 ml of CV8 broth, washed, and blended 10 seconds in 50 ml of sterile deionized water).

The protein patterns of parental strains and single oospore progeny of the Pc 138 $\times$ P 731 cross, were compared using acrylamide gel electrophoresis. Five-day-old mycelia, grown in CV8 broth, were harvested by filtration onto Whatman No. 1 filter paper on a Büchner funnel and washed with deionized water. The buffer-soluble proteins were immediately extracted by grinding 2 g of mycelium in a mortar with 2 ml of buffer (0.1 M potassium monobasic phosphate and sodium dibasic phosphate, pH 7.3) and 4 g of acid-washed sand. The mixture was centrifuged at 34,800 *g* for 1 hour. The supernatant fluid (fungal extract) was immediately used for electrophoresis, using the technique described by Zentmyer et al. (27).

RESULTS

Phenotypic stability of parental strains.—Forty single zoospore cultures from each parental isolate were compared for colony morphology, growth rate, and sporangium production. All parental strains produced homogenous asexual progeny which did not exhibit significant variation among single zoospore cultures. This stability reasonably excludes the possibility of confusion between asexual phenotypic variability, as described by Caten and Jinks (7), and genetic recombination expected from the sexual stage.

TABLE 1. Oospore production and germination in intra- and interspecific crosses of *Phytophthora cinnamomi* and *P. parasitica*

Crosses: Isolate mating types A2 $\times$ A1	Oogonia produced (no. per mm^3)	Well-formed oospores (%)	Germination of well-formed oospores (%)
Pc[a] 40 $\times$ Pc 138	32	78	. . .[c]
P[b] 731 $\times$ P 991	91	68	. . .
Pc 40 $\times$ P 991	2	5	4
P 731 $\times$ Pc 138	3	4	5

[a]Pc = *P. cinnamomi.*
[b]P = *P. parasitica.*
[c]. . . = germination not tested.

TABLE 2. Characteristics of the phenotypically recombined F_1 progeny of the cross *Phytophthora cinnamomi* (Pc 138) × *Phytophthora parasitica* (P 731)

Isolate	Mating type[a]	Colony type[b]	Sporangia type[b]	Maximum temperature	Pathogenicity to *P. indica*[c]	Pathogenicity to *C. jambhiri*[c]	Protein pattern[b]
Pc 138	A1	C	C	< 33	++	0	C
P 731	A2	P	P	> 36	0	+	P
F_1 2	A2	I	P	> 33 < 36	0	0	P
3	A1	I	P	36	0	0	P
6	A1†	I	P	36	+	+	P
7	A1A2	I	P	> 36	0	0	P
8	A1	I	P	36	0	0	P
9	A2	I	P	> 33 < 36	0	0	P
11	A2	P	P	> 36	++	+	P
12	A2	P	P	> 36	++	0	P
13	A1A2	P	P	> 36	+	0	P
16	A2	I	P	> 33 < 36	0	0	P
18	A1	I	P	> 36	0	0	P
20	† A2	I	P	> 36	0	0	P
21	A1†	I	P	> 36	0	+	P
22	A1A2	P	P	> 36	0	0	P
24	A2	I	P	> 33 < 36	0	0	P
25	A1†	I	P	36	0	0	P
29	† A2	I	P	> 33 < 36	0	0	P
30	† A2	I	P	> 36	0	0	P
31	A1	I	P	> 36	+	0	P
32	A1†	I	P	> 36	0	0	P
33	† A2	I	P	36	+	0	P
34	A1	I	P	> 33 < 36	0	0	P
36	A1†	I	P	> 36	0	0	P
37	A2	I	P	< 33	0	0	P
39	A2	I	P	> 33 < 36	0	0	P
40	A2	I	P	> 36	0	0	P
41	A2	I	P	> 36	0	0	P
42	A1	I	P	> 36	0	0	P
44	A2	I	P	> 33 < 36	+	0	P

[a] † = isolates producing a few oospores after 4 weeks in single culture.
[b] C = *P. cinnamomi* type, P = *P. parasitica* type, I = intermediate type.
[c] 0 = nonpathogenic, + = reduction of plant growth, ++ = canker and death of plant.

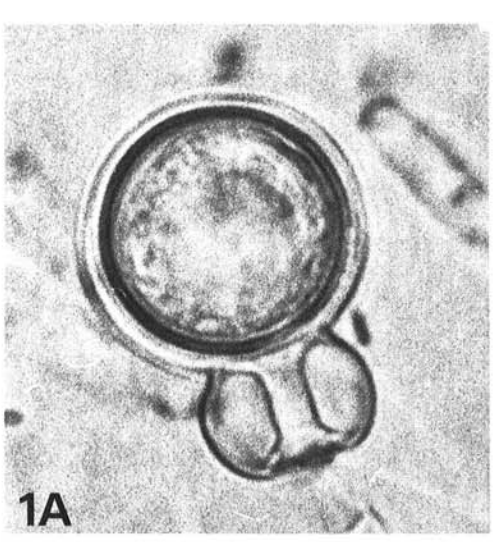
Oospore production and germination.—Since oospore production reflects the mating affinity between two isolates and the fertility of their cross, oospore production in interspecific crosses (Pc 40 × P 991, Pc 138 × P 731) and in intraspecific crosses (Pc 40 × Pc 138, P 731 × P 991) was compared. Oospore production in the two types of crosses was quantitatively and qualitatively different (Table 1).

The number of oospores was considerably lower in interspecific- than in intraspecific crosses. In interspecific crosses, most oogonia were small (20 μm in diameter), empty, or contained aborted oospores with fragmented cytoplasm and malformed walls (Fig. 1-C). Antheridia were in general elongated (average 20 μm long) in both interspecific crosses, and similar in appearance to *P. cinnamomi* antheridia. Most well-formed oospores were small in size (average 20 μm) and resembled those of *P. parasitica* (Fig. 1-A); others (40-45 μm) were like *P. cinnamomi* oospores (Fig. 1-B). None of the well-formed spores produced germ sporangia, but about 5% of them germinated directly and established colonies. Thirty-three single germinated oospores from the Pc 40 × P 991 cross and forty three from Pc 138 × P 731 were harvested and used for further genetic studies.

Distribution of phenotypic characters in F_1 progeny.—Colony and hyphal morphology, maximum temperature for growth, sporangial morphology, mating type, and pathogenicity, of all F_1 progeny from the Pc 40 × P 991 cross were of parental types; nine were like Pc 40 and 24 were like P 991. Progeny from the Pc 138 × P 731 cross were heterogeneous. Fourteen isolates were of parental types, three similar to Pc 138 and 11 to P 731; but 29 single oospore cultures exhibited new associations of characters. These isolates are described below and their characteristics are summarized in Table 2.

1) *Colony and hyphal morphology.*—On PDA at 25 C, *P. cinnamomi* P 138 completely colonizes a 100-mm diameter petri dish in 6 days, forming a "camellioid" pattern with a sparse aerial mycelium. Hyphae show numerous clusters of terminal spherical swellings. Growth of *P. parasitica* P 731 is slower, and the colony has an irregular rosette pattern with sparse, short, aerial hyphae. Hyphae of P 731 are thick, sometimes irregularly branched, but do not exhibit any vesicular swellings.

Twenty-five single oospore isolates showed a large range of morphological variation differing from the parental types with respect to colony appearance, relative amount of aerial mycelium, and growth rate. These phenotypic modifications were stable over 8 months and several transfers. Hyphae of most isolates exhibited an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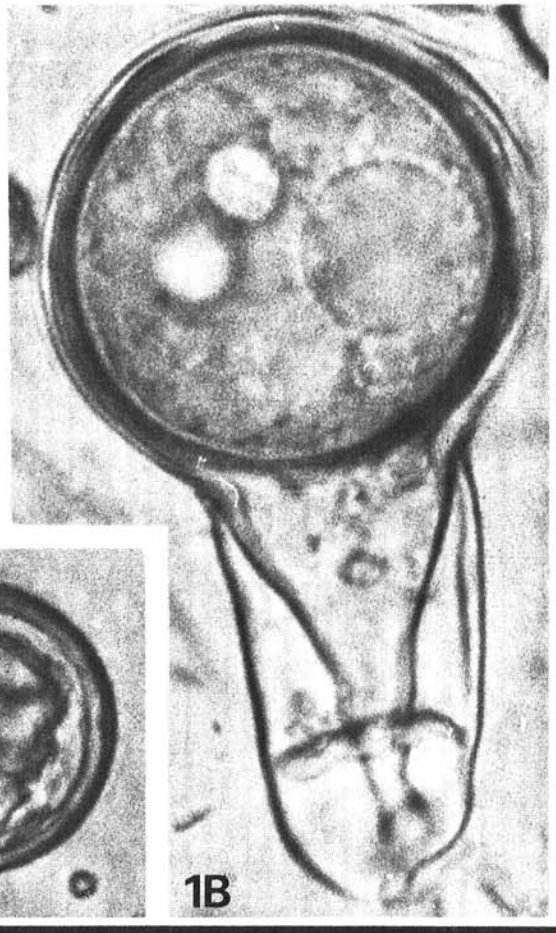
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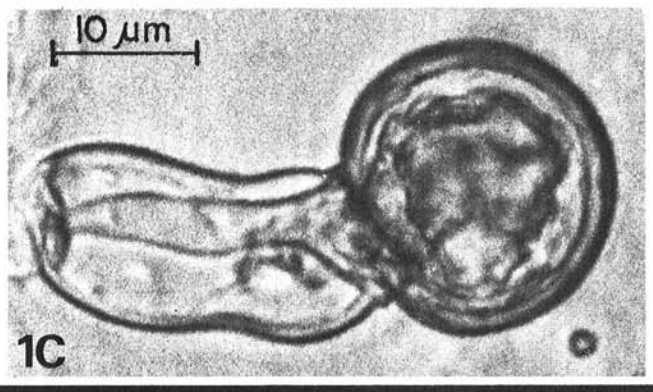
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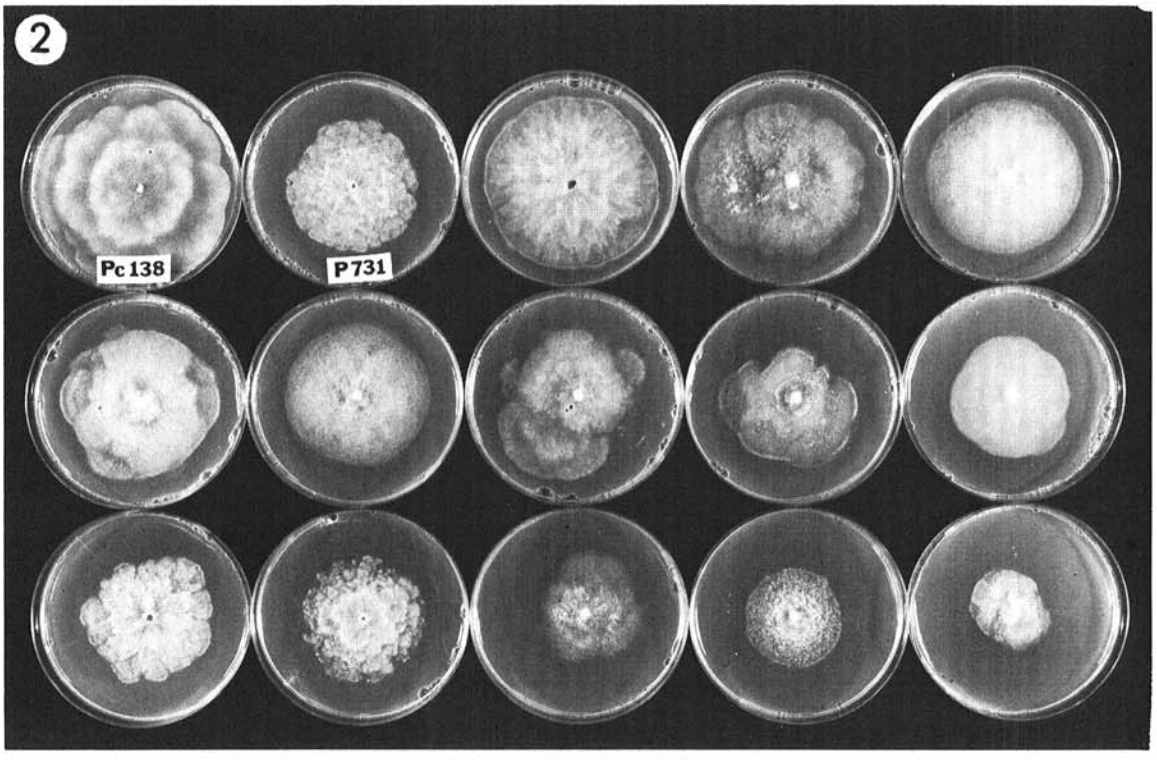
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Pc 138
P731

intermediate phenotype with chains of intercalary subspherical swellings. A sample of the different colony types is presented in Fig. 2.

2) *Maximum temperature for growth.*—The maximum temperature for growth is below 33 C for Pc 138, and above 36 C for P 731. Nine of the recombinant progeny failed to grow at 36 C, five grew weakly, and 15 formed a normal thallus. With one exception, all isolates with suppressed or reduced growth at 36 C, were able to grow at 33 C.

3) *Production and morphology of sporangia.*—Clarified V8 (CV8) agar cultures of P 731 incubated at room temperature under fluorescent light (daylight type) produced abundant sporangia with marked papillae and hemispherical apical thickening. As is typical of *P. cinnamomi,* Pc 138 formed sporangia only under special conditions in liquid culture (8). In this study, sporangia produced in nonsterile soil extract were nonpapillate and had no apical thickening. All the F_1 recombinant progeny formed papillate sporangia, like those of *P. parasitica.*

4) *Mating type.*—Among the recombinant isolates, three behaved like homothallic strains, readily producing in single culture a large number of well-formed oospores typical of *P. parasitica.* These isolates were in fact heterokaryotic since their single zoospore progeny segregated for mating type. One produced 95% A1 and 5% A2 single-zoospore cultures, but a second isolate produced 4% A1 and 96% A2. All these asexual progeny, either A1 or A2, were identical in morphology and growth rate to their respective F_1 parent.

All other recombinant cultures were heterothallic. Eleven were A1 and 15 were A2 mating type. However, nine of these isolates (five of A1 and four of A2) formed a few oospores, of *P. parasitica* type, in single culture after several weeks; but none exhibited segregation of A1 and A2 mating types among single zoospore progeny.

5) *Pathogenicity.*—Isolate Pc 138 was not pathogenic to *C. jambhiri,* but was highly aggressive to *P. indica.* Two weeks after inoculation, *P. indica* seedlings showed extensive stem cankers and died a few days later. Isolate P 731 did not produce symptoms on *P. indica,* but was mildly aggressive to *C. jambhiri,* which exhibited significantly reduced growth after inoculation.

Most of the F_1 recombinant progeny were nonpathogenic to *C. jambhiri.* Only three isolates produced the same symptoms as P 731; i.e., reduced growth of inoculated seedlings. Seven cultures showed different levels of aggressiveness on *P. indica.* Two of them induced canker formation followed by the death of the plant. Five others caused more or less extensive root damage resulting in a reduction of plant growth. Two of these pathogenic isolates were aggressive to both test plants. Symptoms induced by some of the progeny are shown in Fig. 3 and 4.

6) *Protein pattern.*—The electrophoretic protein patterns of Pc 138 and P 731 differed in the total number of protein bands (24 bands for Pc 138, 18 bands for P 731) and their migration in the gels. Dominant bands were at R_f 0.40, 0.52, 0.39, 0.91 for Pc 138, and at R_f 0.35, 0.45, 0.57, 0.66 for P 731. These protein patterns were typical of several *P. cinnamomi* and *P. parasitica* isolates from California.

All phenotypically recombined F_1 progeny produced protein patterns similar to P 731. Number and position of protein bands, including dominant bands, were identical for *P. parasitica* parental isolate and the progeny. Only minor differences in the relative density of the fastest-moving bands were observed on densitometer recording. Protein patterns of the parental isolates and some of the progeny are shown in Fig. 5.

Second-generation progeny F_2.—Two second-generation crosses (F_2) and two back crosses were studied. Second-generation (F_2) crosses, involving F_1 isolates with recombined phenotype (F_1 41 × F_1 42, F_1 8 × F_1 41) and one back cross (F_1 42 × P 731), produced a large number of oospores resembling those of *P. parasitica.* About 65% of these oospores were well formed and 15% germinated in the experimental conditions previously described. Oospore production of the F_1 41 × Pc 138 back cross was poor and more than 90% of the sexual spores aborted. Among the remaining well-formed spores, about 5% germinated. Eighty-six F_2 single-oospore progeny from both crosses presented a large variation in morphological colony types, all producing papillate sporangia of *P. parasitica* type.

Eight progeny of the F_1 41 × Pc 138 back cross were of the Pc 138 morphological type and formed nonpapillate sporangia only in soil extract. Twenty-five progeny from the same cross were morphologically heterogeneous, but produced papillate sporangia on CV8 agar medium.

Protein patterns were determined for twelve F_2 single oospore cultures (six from each cross) which were phenotypically distinct from both parental strains Pc 138 and P 731. They all were similar to *P. parasitica* P 731 protein band patterns.

DISCUSSION

Heterothallic species of the genus *Phytophthora,* though normally self sterile, are potentially bisexual and able to form both types of gametangia: oogonia and antheridia (3, 6, 10, 13, 23). Certain A2 isolates of heterothallic species produce oospores in single culture in response to different chemical induction (6, 17, 26). Another consequence of the potential bisexuality is that any cross involving compatible isolates may theoretically produce two kinds of sexual spores: (i) hybrid oospores from pairings of gametangial hyphae from the two parents, and (ii) oospores from the self fertilization of each parental isolate. Huguenin (13) found that in intraspecific crosses of *P. palmivora* the percentage of self-fertilized oospores may sometimes be higher than 50% (13).

In the present case, the frequent association in F_1 crosses of *P. cinnamomi*-type antheridia with *P.*

Fig. 1-2. 1-A) Oospore of *Phytophthora parasitica* type. **1-B)** Oospore of *P. cinnamomi* type. **1-C)** Oospore formed in interspecific crosses (magnification × 2,000); scale applied also to 1-A and 1-B. **2)** Colony morphology of the parental strains (Pc 138 and P 731) and several progeny: top row, left to right–Pc 138, P 731, progeny 2, 6, 18; second row; progeny 13, 22, 16, 20, and 36; and third row; progeny 9, 7, 24, 44, and 37 (see Table 2 for description of progeny).

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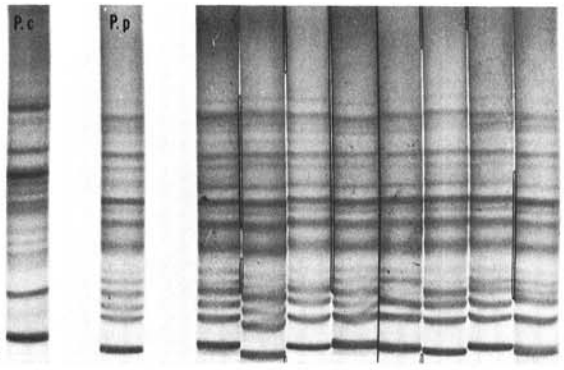

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Fig. 3-5. 3) Symptoms induced by parental strains of *Phytophthora cinnamomi* (Pc 138) and *Phytophthora parasitica* (P 731) and some isolates on *Citrus jambhiri*. **4)** Symptoms induced on *Persea indica*. **5)** Electrophoresis protein band patterns of Pc 138 (P.c), P 731 (P.p) and F_1 progeny.

parasitica-sized oogonia, indicated that most pairs of gametangia probably arose from interspecific fusion. However, the large number of aborted oospores reflected a lack of genetic compatibility between the species, and raised questions on the nature of well-formed oospores, especially those that germinated. The possibility exists that at least some of these spores were self-fertilized. This seems to have occurred in the Pc 40 × P 991 cross where homogeneity of the progeny, which exhibited only parental types suggests that they arose from self-fertilized oospores of each parental strain.

The same interpretation can be proposed for the parental type progeny of the *P. cinnamomi* (Pc 138) × *P. parasitica* (P 731) cross. However, this cross also produced a large number of progeny with new associations of characters. The stability of these new phenotypes indicated the occurrence of true recombination and genetical reassortment, rather than cytoplasmically inherited factors.

The wide range of variation among those cultures with respect to colony morphology, and temperature maximum for growth and pathogenicity to citrus, clearly indicated that all these characters were polygenically controlled.

In the first instance, the emergence of single-oospore isolates with intermediate characters and those combining the pathogenicity of both parental strains (isolates F_1 6 and F_1 11) suggests that exchanges of genetical material could have occurred between the species crossed. This conclusion seems to be consistent with the observed repartition of sporangial morphology and mating type, two markers that segregated in the F_1 independently from other characters. All progeny inherited the capacity to form papillate sporangia of the *P. parasitica* type, and the various morphological characters were associated randomly with either the A1 or A2 mating types.

The occurrence of hybridization in the Pc 138 × P 731 cross was not confirmed by analysis of protein patterns, since the putative recombinants did not exhibit intermediate protein patterns from the parents. This lack of indication of genetic recombination reflected in protein composition of progeny, leads to two alternative hypotheses.

The first proceeds from a methodological uncertainty. The significance of soluble proteins from a fungal crude extract analyzed on gel electrophoresis is not clearly defined. These proteins represent only a fraction of the total proteins and may express a narrow and nonsignificant part of the genome. Therefore absence of intermediate patterns among the progeny may be accidental and it does not necessarily modify the previous conclusion on hybridization.

On the other hand, protein patterns of the progeny might significantly express genomes which were basically similar to *P. parasitica* P 731. Then, the lack of any indication of recombination in F_1 and F_2 protein patterns, suggests that F_1 progeny arose from self-fertilized oospores from the P 731 parent. This interpretation is enhanced by the high fertility of F_2 crosses and of the back cross with P 731 contrasting with the low fertility and poor oospore production of the back cross with Pc 138. Such a contrast seems to indicate a certain similarity if not a homology of the F_1 and P 731 genomes and lack of genetical compatibility between the F_1 and the Pc 138 genomes, as in the F_1 cross between P 731 and Pc 138.

In this second hypothesis of a selfed origin of the progeny, we have to consider the nuclear cycle of the fungus to explain the variability of F_1 progeny for phenotypic characters. The nuclear cycle of *Phytophthora,* and consequently the ploidy level of vegetative nuclei, remains controversial. According to the classical concept, still supported by several authors (16, 24, 25), 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, initially proposed by Sansome (19, 20, 21), and now accepted by many investigators (4, 9, 14, 15), describes diploid vegetative nuclei and meiosis in the gametangia, preceding oospore formation which results from the fusion of two haploid meiotic nuclei. Oospore germination results in diploid vegetative mycelium.

Considering our results, if recombinant F_1 progeny developed from self fertilization of the P 731 parent, as suggested by their protein patterns, phenotypic variation among these cultures may be simply explained only if the fungus is diploid (or eventually polyploid) in its vegetative stage. The self fertilization of a homokaryotic (P 731 was a single zoospore culture) diploid isolate, may produce sexual progeny variable for polygenic characters if the parental clone is heterozygous for loci controlling these characters. In this hypothesis, the germination of oospores formed by fusion of different meiotic products may lead to various phenotypes reflecting genetic reassortment, while asexual progeny resulting from mitotic divisions should not differ significantly from the parental phenotype. Therefore, in a diploid organism, phenotypic variation should be greater in sexual progeny than in asexual progeny, whereas both types should not differ in a haploid organism. Significant differences, already observed in a previous study on a homothallic species (4), may also be found in the present case since asexual progeny of P 731 did not show any phenotypic variation for colony and hyphal morphology, growth rate, or temperature maximum for growth.

If all the F_1 progeny were a result of self fertilization, the segregation of the A1 and A2 mating types from the A2 parent and the emergence of cultures pathogenic to both test plants could only occur if the homokaryotic P 731 parent was heterogametic and was carrying factors for A1 compatibility type and pathogenicity to *P. indica* in its genome as a heterozygote. However, the other parent *(P. cinnamomi* Pc 138) is also a possible source of A1 compatibility type and pathogenicity to *P. indica.*

In conclusion, although indication of hybridization is provided by genetical recombination involving different phenotypic characters in the Pc 138 × P 731 cross, it is not possible to eliminate the possibility that only the self-fertilized oospores germinated. Despite this uncertainty, from a more practical point of view, it is important to note that the crossing of two different species may produce, either by hybridization or by reciprocally induced self fertilization, a wide range of progeny greatly variable in morphology, in physiology, and in pathogenic aggressiveness. Such interspecific crosses, if occurring in nature could contribute to the evolution of *Phytophthora*

populations, and especially to their pathogenic adaptation.

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