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# Characterization of Parental Traits in Somatic Fusion Progeny of *Phytophthora infestans* and *Phytophthora nicotianae*

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Species hybrids were created via fusion of zoospores of two morphologically distinct species, *P. infestans* and *P. nicotianae.* Sixteen putative hybrid isolates were recovered that expressed differential drug resistance of each parent. Repetitive DNA of *P. nicotiane* was detected readily in all of these isolates by hybridization with the species-specific DNA probe, pPP33A. DNA of *P. infestans* was detected in only two putative hybrid isolates using PCR and primer pair ITS3 and PINF2. The two true hybrids were more similar to *P. nicotianae* than to *P. infestans* on the basis of pathogenic, morphological and molecular evidence. Additionally, hybrids expressed modified host ranges compared to parental species. Fusion of zoospores or hyphae may contribute to formation of such hybrids, particularly in the case of heterothallic species in which the joint occurrence of compatible mating types is rare. Zoospore fusion may prove useful as a tool to study hybridization, pathogenesis, and sources of natural diversity of species.

Keywords: *Phytophthora* spp., interspecific hybrids, zoospore fusion.

There has been increasing interest in hybridization as a process that contributes to genetic variation in fungal species. This is true for *Phytophthora,* an important genus of oomycetous pathogens that cause diseases of hundreds of plant species worldwide.

There have been few reports of interspecific hybridization of *Phytophthora* species in agricultural systems. Sansome et al. (1991) suggested that *P. meadii* may have arisen as a species hybrid in nature. More recently, Man in 't Veld et al. (1998) confirmed the first occurrence of hybrids of natural origin. The hybrids, which carried DNA and isozymes of *P. nicotianae* and *P. cactorum,* were derived from hydroponic production systems in which both pathogen species were present. A "fiendish fungus" (Pain, 1999) destroying alder trees in Europe was more recently proven to be a naturally occurring hybrid of *P. cambivora,* which infects some tree species, and an unknown taxon close to *P. fragariae,* which causes blight of strawberries and raspberries. Neither parental species, however, can infect alder (Brasier et al., 1999).

Interspecific hybrids have been observed more frequently in the laboratory. For example, Boccas (1981) induced sexual crosses among several heterothallic *Phytophthora* species. In 220 progeny tested, one was possibly a hybrid organism. More recently, Goodwin and Fry (1994) characterized hybrids created from sexual crosses of the closely related species, *P. mirabilis* and *P. infestans.*

Brasier (1992) suggested that zoospore fusion may provide a means of natural hybridization in *Phytophthora* species. To examine this possibility, Érsek et al. (1995) created hybrids between the closely related species, *P. capsici* and *P. nicotianae,* by

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inducing zoospore fusion. In addition to carrying genetic and biochemical traits of each parent, some of the hybrid offspring exhibited a broadened host range beyond that exhibited by *P. capsici* or *P. nicotianae*. This observation supported the suggestion (Brasier, 1992) that asexual genetic exchange could generate variability in pathogenicity or virulence within pathogen populations, particularly when complementary mating types needed for sexual reproduction are lacking.

In the present work we created hybrids via zoospore fusion between species from morphologically distinct groups II and IV, *P. infestans* (Mont.) de Bary and *P. nicotianae* Breda de Haan (syn. *P. parasitica*), respectively (Waterhouse, 1963; Stamps et al., 1990). *P. infestans* is the causal agent of late blight of solanaceous plants, and *P. nicotianae* is a pathogen of very broad host range and distribution. Based on the dominance of traits of *P. capsici* over those of *P. nicotianae* in their hybrid progeny (Érsek et al., 1995), it was hypothesized that the fusion offspring of *P. infestans* and *P. nicotianae* would be dominated by the traits of *P. infestans* of narrow host range. Also, it was rational to anticipate that genetic interaction between these two species would create hybrids with modified pathogenicity on host plants susceptible to either parental species.

Consequently, the objectives of this research were to i) confirm the occurrence of species-specific DNA of each parent organism in hybrid isolates and ii) characterize hybrid isolates in relation to pathogenic, morphological and reproductive traits.

## Materials and Methods

### *Fungal isolates*

*P. infestans* isolate US930287, i.e. clonal lineage US-8 (Goodwin et al., 1995) was obtained from the culture collection of W. E. Fry of the Department of Plant Pathology, Cornell University, Ithaca, NY. This isolate was naturally resistant to at least 100 mg/L metalaxyl (technical grade, Novartis Co., Budapest). Isolate Fpa$^r$10 of *P. nicotianae* was derived from a wild-type isolate as described previously (Érsek et al., 1994a), and it expressed full resistance to 180 mg/L p-fluoro-DL-phenylalanine (Érsek et al., 1994a; 1995). Drug sensitivity was confirmed on pea-broth agar medium (Tuite, 1969) as described by Érsek et al. (1994a). Both isolates were chosen to represent mating type A2 to preclude undesired sexual events in zoospore fusion experiments.

### *Zoospore fusion and selection of fusion progeny*

Zoospores of *P. nicotianae* Fpa$^r$10 were produced as described previously (Érsek et al., 1995). *P. infestans* US930287 was cultivated axenically on potato tuber slices at 20 ºC. Both zoospore release from sporangia and their viability were considerably higher when grown on tubers than on any other common media. Zoospore suspensions ($10^6$ cells/ml) were obtained by allowing thoroughly washed sporangia to discharge zoospores into glass double-distilled water at 10–12 ºC for 2–3 h. Zoospores were separated from sporangia by filtration through Whatman #1 filter paper.

Fusion of zoospores was induced in the presence of LiCl and polyethylene glycol as described previously (Érsek et al., 1991; 1995). Selection of putative hybrids was carried out on pea-broth agar medium that contained metalaxyl and fluorophenylalanine at 15 mg/L each. This concentration is one-half the concentration of either drug that is fully inhibitory to growth of each nonresistant species. The medium was not supplemented with L-sorbose because it inhibited the germination of regenerated spores of *P. infestans.* Growing colonies were transferred to the same medium containing both drugs at 30 mg/L and kept at 20 ºC. Viable colonies on this medium were selected as putative hybrids. These isolates were then maintained on the same double-drug amended medium. In control experiments, zoospores of each parental isolate were treated separately according to the fusion protocol.

*Characterization of growth and oospore formation*

Several characteristics of the putative hybrids were evaluated, including growth and sporulation rates, and sporangial morphologies on pea-broth and V-8 juice (V8C) agar media (Tuite, 1969). These characteristics were compared to those of parental isolates grown under identical conditions. Cultures were initiated in 5-cm plastic Petri plates containing 7 ml media and were incubated in the dark at temperatures ranging from 18 to 38 ºC to determine the optimum and maximum temperatures for growth. Growth ability at each temperature was expressed as the average radial growth of a colony on the basis of three separate experiments, as described elsewhere (English et al., 1999). Growth experiments were performed with two replications of each isolate and incubation temperature. Colony morphology and sporangial characteristics were evaluated under conditions favorable to either *P. infestans* or *P. nicotianae.*

Hybrid isolates were crossed with compatible A1-mating type isolates, *P. infestans* H-4/97, a Hungarian isolate from potato (Bakonyi et al., 1998) and *P. nicotianae* 34-3-9 as described by English et al. (1999). Sexual crosses of the A2 parental isolates (those used for fusion) with the A1 mating type isolates were also made. Mated cultures were incubated at 20 ºC in the dark for up to 3 weeks. Gametangial interaction was visualized under a light microscope. Oospores were counted in 3–5 fields randomly located in the region where two colonies merged, and the sizes of sexual organs were recorded.

*Evaluation of pathogenicity*

Pathogenicity phenotypes of putative hybrids and parental isolates were compared based on symptoms that developed after inoculation of various plant hosts. Plant hosts included potato (*Solanum tuberosum* L., cv. Desirée), tomato (*Lycopersicon esculentum* Mill., cv. Treff) and eggplant (*Solanum melongea* L. cv. Jubileum), hosts common to *P. infestans* and *P. nicotianae;* and pepper (*Capsicum annuum* L. cv. California Red), apple (*Malus domestica* L. Granny Smith) and lemon (*Citrus limon* (L.) Burm. f.), susceptible only to *P. nicotianae.*

To inoculate potato tuber slices, an inoculum plug was placed in the center of the freshly cut, washed and air-dried tuber surface. Wound-inoculations of tomato stems and

lemon fruits were made as described previously (Érsek et al., 1995). To inoculate tomato, eggplant, pepper or apple fruit, an inoculum plug of 5 mm diameter was applied on the fruit surface that had been pierced with a hypodermic needle. Inoculation sites were wrapped in Parafilm. Replicate inoculated plants were maintained in moist chambers at 22 ºC, and symptom development was evaluated daily.

*Molecular evaluation of hybrids*

The hybrid natures of zoospore fusion offspring were evaluated by DNA and isozyme analyses. Total genomic DNA was extracted from putative species hybrids and parental organisms (Goodwin et al., 1992). To detect DNA of *P. nicotianae,* DNAs were digested with restriction enzymes *Eco*RI and *Xho*I, electrophoresed in a 0.8% agarose gel (Low EEO, Fisher Scientific, St. Louis, MO), using standard protocols (Maniatis et al., 1982; Ausubel et al., 1987), transferred to Hybond™ $N^+$ membrane (Amersham, UK), and hybridized with a $^{32}P$-labeled probe. Labeling using the 'Multiprime DNA Labeling System' (Amersham, UK) was performed as described by Feinberg and Vogelstein (1983). Plasmid pPP33A that contained a 1000-bp *P. nicotianae*-specific sequence (Érsek et al., 1994b) was used as the probe.

To detect DNA of *P. infestans,* polymerase chain reaction (PCR) was used to amplify a 456-bp, *P. infestans*-specific sequence from the internal transcribed spacer (ITS) region 2. The primers for this sequence included ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and PINF2 (5'-CGATTCAAATGCCAAGCTAAAG-3') that derived from ITS2 region of rDNA of *P. infestans* (Tooley et al., 1997). One microliter of each primer (10 µM) was mixed with reaction buffer, $MgSO_4$ (1.5 mM), 1 unit Vent DNA polymerase # 254S (all purchased from Biolabs, New England), dNTPs (2 mM) (Promega Co., Madison, WI), 15 or 100 ng of template DNA, and sterile glass-distilled water in a total volume of 100 µl. Control reactions contained each component except for the template DNA. Reactions were cycled with an automated thermal cycler (Hybaid, model HB-TR1). The thermal cycler was programmed for 30 cycles of 94 ºC for 20 sec, 55 ºC for 40 sec and 72 ºC for 30 sec. These cycles were preceded by one cycle with an extended 3-min denaturation at 94 ºC and were concluded with a final extension for 6 min at 72 ºC. PCR products were visualized by staining gels in ethidium bromide (0.5 mg/L) after electrophoresis on a 1.5% agarose gel (Low EEO, Fisher Scientific, St. Louis, MO) for 2 h at 150 V. To make sure that DNA specific to *P. infestans* was not amplified in *P. nicotianae,* the PCR products were blotted onto nitrocellulose membrane and hybridized to the 456-bp *P. infestans*-specific fragment as described above. This fragment had been purified from an agarose gel by the QIAquick Gel Extraction Kit according to the manufacturer recommendations (QIAGEN Inc., Chastworth, CA) and labeled with $^{32}P$.

Comparisons among hybrids and parent isolates were also made relative to attributes of DNA structure. These comparisons were made using DNA thermal denaturation analysis. Calculations of DNA melting temperature, $T_m$, as well as the $2\sigma$ value and G+C content were carried out as described by English et al. (1999). DNA for these analyses was extracted according to Tooley and Carras (1996).

# Results

*Molecular confirmation of hybridization*

More than 100 colonies with double-drug resistance were recovered at a frequency of 3.4 x $10^{-6}$ following induced fusion of zoospores of metalaxyl-resistant *P. infestans* US930287 and fluorophenylalanine-resistant *P. nicotianae* Fpa$^{r}$10. Sixteen isolates that developed normally and remained stable after repeated transfer onto drug-amended and nonamended media were selected from these colonies and retained for further analyses.

DNA of *P. infestans* was detected in only two isolates, H4 and H11 *(Fig. 1).* Using primer pairs, ITS3 and PINF2 (Tooley and Carras, 1996), a 456-bp amplification product was detected in these isolates but was absent in *P. nicotianae.* This product corresponded to the ITS2 region of *P. infestans.*

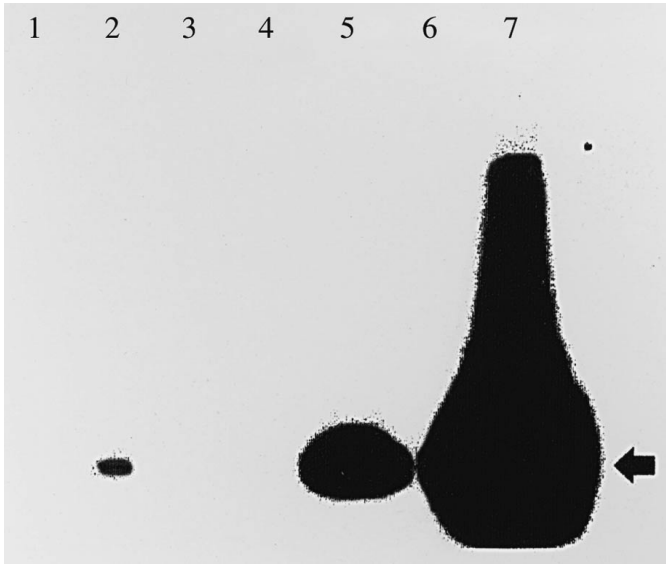


Fig. 1. Confirmation of the occurrence of *P. infestans* DNA sequences in putative species hybrids H4 and H11 by hybridization of the amplified fragment of ITS2 region of *P. infestans* as a radiolabeled probe to products PCR-amplified from total genomic DNAs. Primers ITS3 and PINF2 that derived from ITS2 region of rDNA of *P. infestans* amplified a 456-bp, *P. infestans*-specific product (arrow). Lane 1, *P. nicotianae* Fpa$^{r}$10; lanes 2, 4 and 5, putative hybrid isolates 11, 1, 4; lane 7, *P. infestans* US930287; lanes 3 and 6, none

Hybrids H4 and H11 also carried DNA derived from *P. nicotianae (Fig. 2).* Multiple bands in each hybrid hybridized with pPP33A from the parental isolate. Using the probe, DNA of *P. nicotianae* was also detected in all other putative hybrid isolates (data not shown).

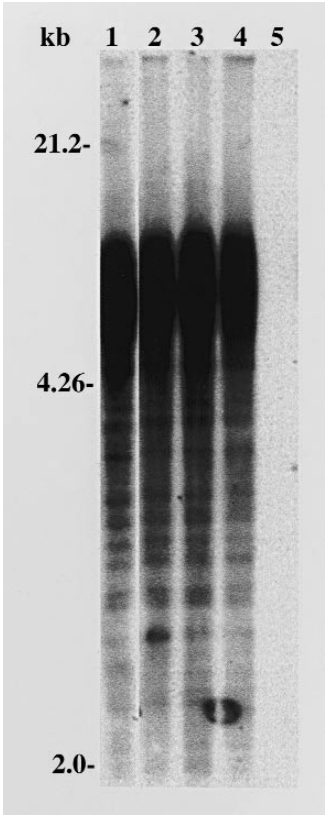


Fig. 2. Detection of *P. nicotianae* DNA sequences in putative species hybrids by hybridization of the *P. nicotianae*-specific probe pPP33A to a Southern blot of *Eco*RI + *Xho*I-digested total genomic DNAs. The plasmid contained a 1,000-bp repetitive sequence from *P. nicotianae.* Lanes 1 to 3, putative hybrids 1, 4, 11; lane 4, *P. nicotianae* Fpa$^r$10; lane 5, *P. infestans* US930287

Thermal denaturation characteristics of DNA from H1, H4 and H11 were similar to those of *P. nicotianae.* For example, melting temperatures ($T_m$) in hybrids varied from 73.98 ºC to 74.27 ºC, as compared to 74.18 ºC for *P. nicotianae* and 74.95 ºC for *P. infestans (Table 1).* Similarly, the hybrid GC contents and values of 2σ were more similar to *P. nicotianae* than to *P. infestans.*

*Morphological characterization and oospore formation*

Colony morphologies and growth patterns of H1, H4 and H11 often were similar to those of *P. nicotianae.* For example, all three isolates and *P. nicotianae* grew most rapidly at 25 ºC, whereas *P. infestans* grew most rapidly at 20 ºC *(Table 2).* When grown on either pea-broth or V8C agar, isolates H1 and H11 developed cottony colonies with dense areal mycelia characteristic of *P. nicotianae.* In contrast, colonies of *P. infestans*

**Table 1**

Thermal denaturation characteristics of DNA from *Phytophthora* spp. and hybrids

| Isolates | Melting point ($T_m$) | % GC content | 2σ value |
|---|---|---|---|
| *P. nicotianae* Fpa$^r$10 | 74.18±0.14 | 47.89 | 7.14±0.08 |
| *P. infestans* US930287 | 74.95±0.16 | 49.49 | 6.26±0.04 |
| H1[a] | 74.27±0.11 | 47.94 | 7.03±0.04 |
| H4 | 73.98±0.22 | 47.48 | 7.22±0.06 |
| H11 | 74.15±0.18 | 47.83 | 7.14±0.10 |

[a] H1, H4 and H11 are putative hybrid isolates

**Table 2**

Temperature-dependent growth and sporangial dimensions of parental and hybrid isolates of *Phytophthora* on pea-broth agar

| Isolates | Growth temp. (opt./max. ºC) | Radial growth (mm±SE/day) at 20 ºC/25 ºC | Mean (±SE) sporangial length x breadth (µm) |
|---|---|---|---|
| *P. nicotianae* Fpa$^r$10 | 25 / 37 | 7.06±0.45 / 9.75±1.00 | 30.33±1.27 x 26.03±1.03 |
| *P. infestans* US930287 | 20 / 27 | 3.78±0.10 / 2.44±0.08 | 21.65±0.77 x 12.45±0.42 |
| H1[a] | 25 / 34 | 4.00±0.00 / 6.25±0.10 | 30.87±0.71 x 28.12±1.00 |
| H4 | 25 / 34 | 6.80±0.53 / 10.50±0.50 | 15.48±0.54 x 14.00±0.44 |
| H11 | 25 / 35 | 7.43±0.23 / 10.50±1.00 | 27.42±0.66 x 25.00±0.41 |

[a] H1, H4 and H11 are putative hybrid isolates

developed densely floccose myeclium. Isolate H4 differed from all other isolates in its colony morphology; it developed heavily cottony colony. Isolates H1, H4 and H11 produced papillate and noncaducous sporangia characteristic of *P. nicotianae;* however, the sporangial dimensions of isolate H4 were less than either parental isolate *(Table 2).*

Isolates H1 and H11 and the parental A2 isolate produced oospores following pairings with compatible A1 mating type isolates of *P. infestans* 4/97 and *P. nicotianae* 34-3-9 *(Table 3, Fig. 3).* Oospores did not form when these isolates were paired with parental A2 isolates, *P. infestans* US930287 and *P. nicotianae* Fpa$^r$10. Isolate H4 did not form oospores in pairings with any A1 or A2 isolates.

For isolates H1 and H11 and each parental isolate, oospores formed more abundantly when crossed with *P. nicotiane* 34-3-9 (A1) than with *P. infestans* 4/97 (A1). However, the mean diameters of oogonia and oospores formed in crosses of all isolates with *P. infestans* 4/97 were greater than those formed in crosses with *P. nicotianae* 34-3-9 *(Table 3).* Although antheridial widths were similar among all isolate pairings, antheridial lengths varied considerably. For example, the average length of antheridia formed in matings of *P. infestans* US930287 with either *P. infestans* 4/97 or *P. nicotianae* 34-3-9 was 22.7 and 20 µm, respectively *(Table 3).* When *P. nicotianae* Fpa$^r$10 was mated with *P.*

**Table 3**

Features of gametangial pairings of interspecific somatic hybrids (H1 and H11) or their parental A2 isolates with A1 isolates of *P. infestans* or *P. nicotianae*

| Cross or backcross | Oospores (±SE/cm²) | Oogonium/oospore diameter (µm±SE) | Length/breadth (µm±SE) of antheridia |
|---|---|---|---|
| *Pi* 4/97[a] x *Pi* US930287 | 2500±165 | 26.0±0.22 / 20.7±0.18 | 22.7±0.31 / 12.7±0.21 |
| *Pi* 4/97 x *Pn* Fpa$^r$10 | 2031± 86 | 25.4±0.18 / 20.3±0.10 | 11.0±0.09 / 11.7±0.12 |
| *Pi* 4/97 x H1 | 1563±215 | 22.6±0.38 / 19.2±0.08 | 14.7±0.59 / 11.4±0.10 |
| *Pi* 4/97 x H11 | 2344±102 | 24.7±0.31 / 20.0±0.24 | 15.0±0.65 / 10.8±0.08 |
| *Pn* 34-3-9 x *Pn* Fpa$^r$10 | 6094±320 | 17.3±0.12 / 14.7±0.07 | 9.3±0.06 / 10.7±0.08 |
| *Pn* 34-3-9 x *Pi* US930287 | 2813±120 | 20.0±0.16 / 17.3±0.09 | 20.0±0.21 / 10.7±0.10 |
| *Pn* 34-3-9 x H1 | 4842±333 | 16.9±0.14 / 14.1±0.08 | 11.2±0.19 / 10.2±0.09 |
| *Pn* 34-3-9 x H11 | 5000±204 | 16.7±0.11 / 14.4±0.07 | 10.9±0.31 / 8.9±0.08 |

[a] *Pi* or *Pn* indicate isolates of *P. infestans* or *P. nicotianae,* respectively, as defined in text

*infestans* 4/97 or *P. nicotianae* 34-3-9, antheridial length was 11 and 9.3 µm, respectively. When crossed with either *P. infestans* 4/97 or *P. nicotianae* 34-3-9, antherdial lengths of isolates H1 and H11 were intermediate or more similiar to those of *P. nicotianae* Fpa$^r$10 than to *P. infestans* US930287. However, in each pairing of A1 isolates with putative hybrids, ca. one fourth (22 to 28%) of the mating structures had elongated antheridia the length of which clearly exceeded the average *(Fig. 3).*

*Evaluation of pathogenicity*

*P. nicotianae* and *P. infestans* produced distinctly different symptoms over a range of host plants but on leaves of potato and tomato symptoms could not be differentiated. *P. nicotianae* typically induced a soft rot on fruit of apple, pepper, lemon and tomato. Similarly, on tomato stems, infection usually resulted in water-soaked lesion. *P. infestans* produced lesions on tomato and eggplant fruit; however, lesion tissues remained firm and did not become water-soaked. In the case of tomato, lesion formation on stems was often followed by leaf drop. Symptoms characteristic of *P. nicotianae* developed two to three times faster than those induced by *P. infestans* on any test plant.

Symptoms induced by zoospore-fusion isolates varied among host plants *(Table 4).* For example, all isolates induced disease symptoms typical of *P. nicotianae* on either pepper or apple, both of which are susceptible only to that species. All isolates also induced symptoms typical of *P. nicotianae* when inoculated to eggplant or tomato, hosts susceptible to both *P. nicotianae* and *P. infestans.* The isolates rarely induced symptoms typical only of *P. infestans,* and all of them elicited a hypersensitive reaction on tuber slices of potato.

Of the hosts in common to both parental species, eggplant fruit was the most suitable for differentiation of symptoms *(Fig. 4). P. nicotianae* caused yellow lesions that spread rapidly over the skin and into the inner tissues, softening the infected area. In contrast, *P.*

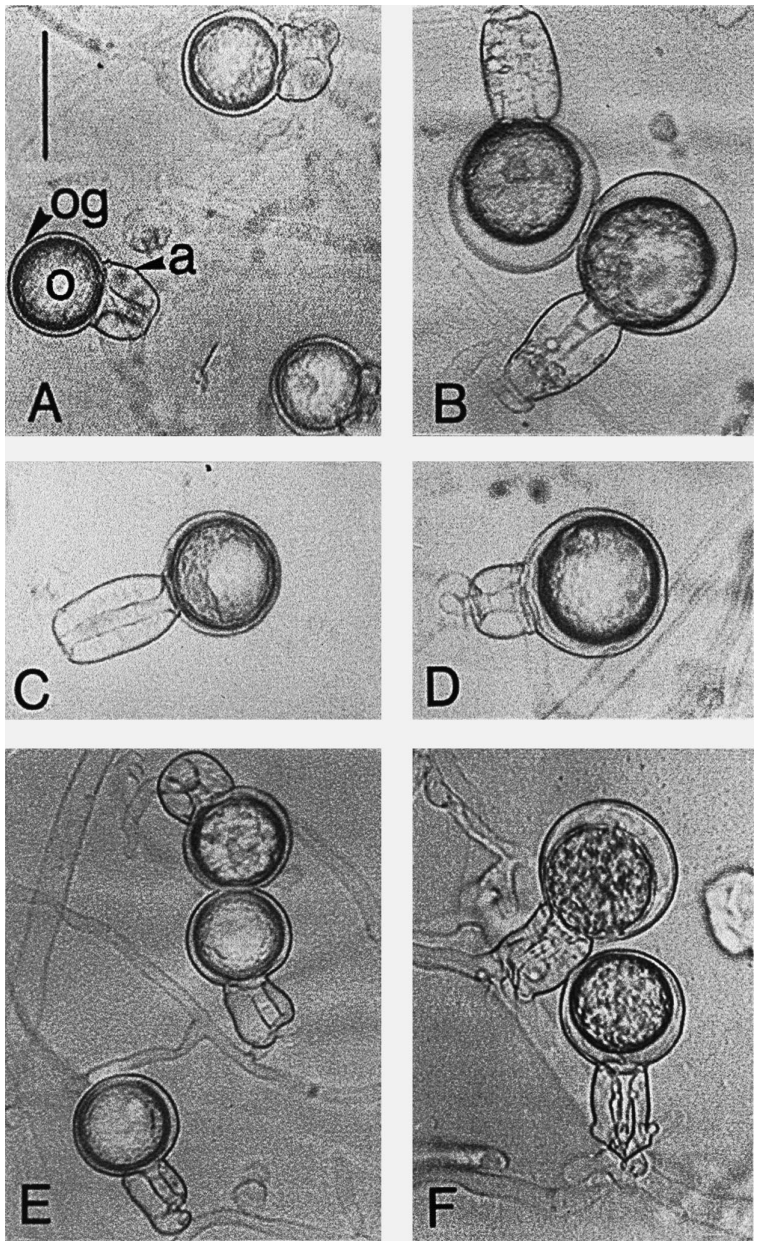


Fig. 3. Gametangial matings following crosses of parental (A2 mating type) and hybrid (H11) isolates with compatible A1 mating-type isolates of *P. infestans* (Pi) 4/97 and *P. nicotianae* (Pn) 34-3-9. Crosses are as follows: **A,** Pn A1 x Pn A2; **B,** Pi A1 x Pi A2; **C,** Pn A1 x Pi A2; **D,** Pi A1 x Pn A2; **E,** Pn A1 x H11; **F,** Pi A1 x H11. Bar: 20 µm, og: oogonium, o: oospore, a: antheridium. Note elongated antheridia in **B, C, E** and **F**

**Table 4**

Pathogenicity of parental and putative hybrid isolates (H1, H4 and H11) of *Phytophthora*

| Isolate | Plant component | | | | | |
|---|---|---|---|---|---|---|
| | Eggplant fruit | Tomato plant/fruit | Potato tuber | Pepper fruit | Apple fruit | Lemon fruit |
| *P. nicotianae* Fpa$^r$10 | N[a] | N/N | N | N | N | N |
| *P. infestans* US930287 | I | I/I | I | — | — | — |
| H1 | NI | N/N | HR | HR | N | — |
| H4 | I | N/N | HR | N | N | — |
| H11 | I | I/N | HR | N | HR | — |

[a] Symbols N and I depict symptoms characteristic of *P. nicotianae* and *P. infestans,* respectively; NI depicts intermediate symptom; HR: hypersensitive reaction; —: no symptom

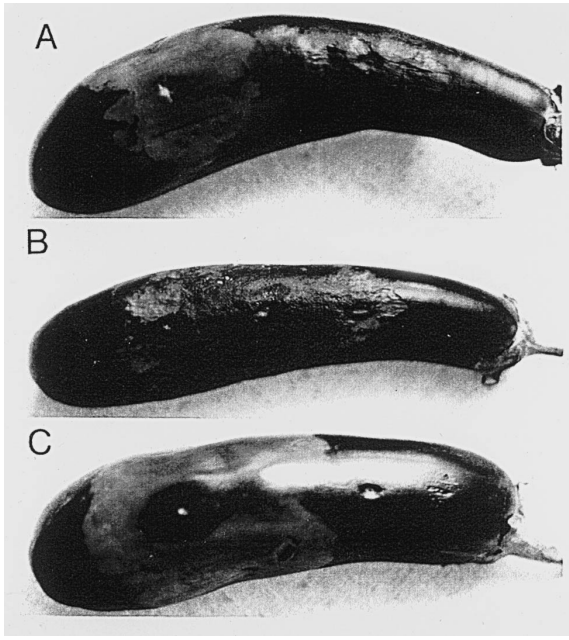


Fig. 4. Pathogenicity phenotypes of parental and selected hybrid isolates on eggplant fruit. From left to right, **A,** *P. nicotianae* (left) and *P. infestans* (right); **B,** left to right: hybrid isolates H11, H4 and H11; **C,** H1 (left) and a nonpathogenic hybrid (right). Photographs were taken 6 days post-inoculation with *P. infestans* and hybrid isolates, and 3 days after inoculation with *P. nicotianae*

*infestans* induced darkish and wrinkled, sunken lesions that did not spread to deeper tissue layers. Isolates H4 and H11 induced symptoms that were similar to *P. infestans.* Isolate H1 induced intermediate symptoms that initially looked like those of *P. infestans,* but the dark lesion later was surrounded by yellowish halo.

# Discussion

This report extends the application of zoospore fusion to hybridization of *P. nicotianae* and *P. infestans.* Prior to these experiments and previous studies (Érsek et al., 1995; 1997), interspecific hybridization had only been achieved by sexual crosses (Boccas, 1981; Vorob'eva and Gridnev, 1984; Goodwin and Fry, 1994) or by transfer of isolated nuclei (Gu and Ko, 2000).

Pathogenicity of zoospore-fusion offspring varied over a range of host plants. In our previous study of species hybrids, we observed an expanded host range compared to the parent organisms (Érsek et al., 1995). Results of this study differ in that isolates H1, H4 and H11 produced symptoms that resembled predominantly those induced by one of the parents, *P. nicotianae.* In the case of potato tubers, the isolates induced only an HR response indicative of a loss of virulence compared to either parent. These observations reinforce the difficulty of predicting the effects of species hybridization on pathogen survival and dominance among populations in nature.

The expression of parental morphological and molecular traits in hybrids of *P. infestans* and *P. nicotianae* also differred from previous hybridizations between *P. capsici* and *P. nicotianae* (Érsek et al., 1995; English et al., 1999). In the earlier study, hybrids exhibited traits that were predominantly characteristic of *P. capsici.* Consequently, we expected that hybrids constructed from *P. nicotianae* and *P. infestans* would exhibit traits that resemble *P. infestans* rather than *P. nicotianae.* Surprisingly, fusion progeny exhibited traits more in common with *P. nicotianae.* Additional zoospore fusions will need to be made between related species to assess the potential for predicting species dominance in hybrid offspring.

Numerous putative hybrid isolates were recovered from zoospore fusions based on expression of differential-drug resistance and modified pathogenicity or gametangial morphologies. Crosses of the parental A2-mating type isolate of *P. infestans* or putative hybrids with compatible A1 isolates indicated that the A2 type acting as predominant male (Judelson, 1997) expresses traits, such as elongated antheridia, of *P. infestans* in fusion offspring.

Isozyme analyses by cellulose acetate electrophoresis (Hebert and Beaton, 1993) of a series of enzymes including e.g. glucose-6-phosphate isomerase for which the US-8 genotype has a characteristic pattern revealed polymorphisms between parental isolates. Isozyme patterns of putative hybrids, with one exception, however, resembled the patterns of *P. nicotianae.* Isolate H1 was the only fusion offspring that expressed a unique isozyme, i.e. isocitrate dehydrogenase phenotype, but its pattern was not that which would be expected in normal recombination of a dimeric enzyme (data not shown). The significance of this intermediate phenotype is uncertain.

Repetitive DNA of *P. nicotianae* was detected readily in all of the selected isolates when blots of total genomic DNAs were probed with the species-specific pPP33A. However, preliminary experiments in which a probe, RG57, that contains a 1.2-kb, moderately repetitive *P. infestans* sequence (Goodwin et al., 1992), did not reveal *P. infestans*-specific bands in blots of hybrid DNAs (data not shown).

Only two isolates, H4 and H11, were confirmed as true hybrids based on molecular traits. DNA of *P. infestans* was also detected by PCR using primers based on the ITS2 region of *P. infestans* (Tooley et al., 1997).

Although hybrids H4 and H11 expressed resistance to metalaxyl, in preliminary studies we were not able to detect any specific PCR products using primer sets W4 or AL14 that normally is linked to a major metalaxyl-resistance gene in *P. infestan*s (Fabritius et al., 1997; Judelson and Roberts, 1999). It is difficult to explain this contradiction. It may be that our gene for metalaxyl resistance does not correspond to resistance linked to W4 and AL14. Complementation studies may assist in clarifying the relationships between these genes.

With the development of molecular and biochemical markers, there have been more frequent reports of naturally occurring *Phytophthora* species hybrids (Man in 't Veld et al., 1998; Brasier et al., 1999; Brasier, 2000; Bonants et al., 2000). Fusion of zoospores or hyphae may contribute to formation of such hybrids, particularly in the case of heterothallic species in which the joint occurrence of compatible mating types is rare. Unfortunately, it is difficult to assess the mechanisms of hybridization using field-based studies. However, zoospore fusion may prove useful as a tool to study hybridization, pathogenesis, and sources of natural diversity of species even if recombinational patterns typical of sexual hybrids cannot be expected.

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