Phytophthora × *pelgrandis* Causes Root and Collar Rot of *Lavandula stoechas* in Italy

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Abstract

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In 2007, *Phytophthora* isolates with atypical morphological and biological characteristics were found associated with root and collar rot of potted plants of *Stoechas* lavender (*Lavandula stoechas*) in an ornamental nursery in Italy. A polyphasic approach, including morphological and cultural observations, sequencing the ITS-rDNA region, the *Pheca* and the mitochondrial *coxI* genes, multiplex PCRs with primers specific for *P. nicotianae* or *P. cactorum*, as well as random amplified polymorphic DNA–polymerase chain reaction, was used to characterize these isolates. On the basis of morpho-cultural and molecular analyses, the isolates from *Stoechas* lavender were identified as *Phytophthora* × *pelgrandis*, a natural hybrid of *P. nicotianae* × *P. cactorum*

previously reported in other European countries, the Americas, and Taiwan, as a pathogen of ornamentals and loquat plants. In pathogenicity tests using potted plants of *Stoechas* lavender, the *P. × pelgrandis* isolates, similarly to the parental species *P. nicotianae*, induced the symptoms observed on plants with natural infections and were reisolated only from artificially inoculated plants. Dispersal of *P. × pelgrandis* on this host could exacerbate the damage caused by *Phytophthora* root and collar rot, of which the main causal agent presently is *P. nicotianae* on lavender in Europe. Application of hygienic measures is important to reduce the proliferation and spread of the *Phytophthora* hybrids.

Lavender (*Lavandula* spp., family Lamiaceae) is widely cultivated in Italy as an ornamental and aromatic plant. It is estimated that lavender plants constitute about 30% of approximately 20 million potted aromatic plants produced each year in Liguria, the leading producing Italian region (28). In Italy, the most common disease of potted lavender in nurseries is *Phytophthora* root and collar rot. *P. nicotianae* is the prevalent species causing this disease. The pathogen was first reported on *Lavandula* spp. in Greece (33) and subsequently on English lavender (*Lavandula angustifolia* Mill.) in several other countries, including the United States (34), Italy (6,27), Spain (1), and Bulgaria (29). *P. palmivora* is another species quite common on lavender; it was recorded both on French lavender (*L. dentata* L.) in Spain (32) and English lavender in southern Italy (10) and Turkey (11). Another species infecting lavender is *P. cinnamomi*, which was found to be the causal agent of shoot and root rot of English lavender in greenhouse beds and container-grown nurseries in Poland (31). Hybrid isolates of *P. nicotianae* and *P. cactorum*, formally described as *Phytophthora* × *pelgrandis* W.F. Gerlach, Nirenberg & Gräfenhan nothosp. nov. (30) from *Pelargonium grandiflorum* (Andr.) Willd., were also recovered from *Lavandula* sp. plants affected by root rot and basal stem necrosis in greenhouse in the Netherlands (4) and more recently in Hungary (35).

In October 2007, symptoms of leaf chlorosis, wilting associated with root and basal stem necrosis, and final collapse of the whole plant were observed on 4- to 6-month-old potted plants of *Stoechas* lavender (*Lavandula stoechas* L.) in an ornamental nursery in Liguria, northern Italy (lat. 44°3'43" N, long. 8°11'5" E). Disease

incidence was about 30%, with 15% dead plants in a nursery stock of 24,000 plants. Isolations from necrotic tissues of roots and basal stem consistently yielded *Phytophthora* colonies exhibiting morphological features resembling those reported for *Phytophthora* × *pelgrandis*. The aim of this study was to identify and characterize the atypical *Phytophthora* isolates from *Stoechas* lavender using a polyphasic approach, comprising morphological and cultural observations, sequencing of nuclear (internal transcribed spacer [ITS]-rDNA and *Pheca*) and mitochondrial (*coxI*) DNA regions as well as two species-specific multiplex polymerase chain reaction (PCR) and random amplified polymorphic DNA (RAPD)-PCR assays. In addition, pathogenicity tests were performed to fulfill Koch's postulates.

Materials and Methods

Isolation and morpho-cultural characterization. Isolations were made from 20 symptomatic *Stoechas* lavender plants with different disease severity. Basal stems and roots were washed with tap water, rinsed in sterile H₂O, and blotted dry with filter paper before being cut into 5-mm pieces. Stem and root pieces were plated onto *Phytophthora* selective medium PARP (16) in 9-cm-diameter petri dishes (5 pieces per dish). Petri dishes were incubated for 5 days at 24 ± 1°C in the dark. Hyphal tips of colonies resembling the morphology of *Phytophthora* spp. were subcultured on potato dextrose agar (PDA, Oxoid Ltd., Basingstoke, UK) to obtain pure single-hyphae cultures. Isolates were then grown in 9-cm-diameter petri dishes containing 20 ml of either PDA or V8A (Campbell V8 juice agar) at 24 ± 1°C for 14 days in darkness to determine the colony morphology. Temperature–growth relationships of isolates were determined by transferring 5-mm-diameter plugs taken from the margins of 7-day-old actively growing colonies at 24 ± 1°C onto both PDA and V8A and incubating at temperatures from 5 to 35°C with intervals of 5 ± 1°C, in the dark. Colony diameters were measured in two orthogonal directions 5 days after incubation, and the mean daily radial growth rate (mm/day) at each temperature was calculated. Maximum temper-

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ature for growth was tested between 33 and 36°C with $1 \pm 0.2^\circ\text{C}$ increments following the method of Brasier et al. (5). Carrot agar (CA) plates started with three 4-mm actively growing mycelial plugs were immediately transferred to the test temperatures, and the extent of mycelial growth was recorded daily for 3 days. Sporangia production was induced by transferring 5-mm plugs of 7-day-old mycelium grown on V8A into petri dishes containing sterile H_2O . Plates were incubated at room temperature (18 to 24°C) for 5 to 10 days under natural light on the bench, and features of sporangia were recorded for each isolate by performing 70 measurements. Similarly, a total of 50 measurements of oogonia, oospores, and antheridia produced on V8A at $24 \pm 1^\circ\text{C}$ after 14 days of incubation were made for each isolate. A total of five representative isolates (953/07, 956/07, 957/07, and STL78 from roots, and STL11 from stem) were characterized, and each experiment included three replicate dishes per isolate and culture medium. Each experiment was repeated twice. All isolates were deposited at the culture collection of the Department of Agri-food and Environmental Systems Management, Plant Pathology Section, University of Catania.

DNA isolation. Genomic DNA was extracted from two representative isolates (956/07 and 957/07) of the putative hybrid of *P. nicotianae* $\times$ *P. cactorum* recovered from *Stoechas* lavender. Reference isolates of both putative parental species, i.e., *P. nicotianae* isolate IMI 398853 from blue Mediterranean fan palm (13) and *P. cactorum* isolate IRF14 from oregano, were included in this study. DNA was extracted from mycelium of 7-day-old colonies grown in petri dishes on V8A at 24°C by using DNeasy Plant Mini Kit according to the manufacturer's instructions (Qiagen GmbH, Hilden, Germany). DNA concentration and quality were determined using the Quant-it dsDNA BR assay kit and Qubit fluorometer (Invitrogen, Life Technology Corporation, Carlsbad, CA, USA). DNA samples were stored at -20°C before PCR amplification.

ITS-rDNA, *coxI*, and *Pheca* sequencing. ITS regions of the rDNA repeat units of isolates were amplified with the universal primers ITS6 and ITS4 according to the protocol described by Cooke et al. (9). Sequencing of the cytochrome c oxidase subunit I (*coxI*) gene was performed to identify the donor parental species of the mitochondrial genome. Templates of the *coxI* gene cluster were amplified by using the primers FM 85 and the reverse complement of FM 80 (FM80RC) (25). PCR reactions were done in 25 μl and contained 10 ng of DNA, 0.5 μM of each primer, 2.5 μl of 10 $\times$ buffer, 100 μM of each dNTP, 2 mM MgCl_2 , and 1 unit of *Taq* DNA Polymerase (Invitrogen). Thermocycler parameters were 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 90 s, with a final extension at 72°C for 5 min.

Sequence analysis of a portion of phenol acid decarboxylase (*Pheca*) gene was performed to establish the allelic state of this gene in the putative *P. nicotianae* $\times$ *cactorum* hybrid isolates. Specific primers (*Pheca*F: 5'-GAAGCCGATCATGGTGGT-3' and *Pheca*R: 5'-TGACCTCCACCTGCTGTACG-3') were designed by aligning available *Pheca* sequences (*P. nicotianae*, FJ459730; *P. cactorum*, FJ459731; *P. nicotianae* $\times$ *cactorum*, FJ459732 and FJ459733) (15) with ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) and chemically synthesized by Invitrogen. Each PCR reaction mix contained 2.5 μl of 10 $\times$ buffer, 2 mM MgCl_2 , 0.2 mM of each dNTP, 0.5 μM of each primer, 10 ng DNA template, and PCR grade water to a total volume of 25 μl . The PCR thermocycling conditions were as follows: 2 min at 95°C, and 35 cycles of 95°C for 20 s, 60°C for 30 s, 72°C for 45 s, followed by a final extension at 72°C for 5 min. The experiment was repeated three times under the same conditions using different DNA extracts.

In all experiments, the presence of PCR products was confirmed by electrophoresis on 1.5% agarose gel and used directly for sequence determination following treatment with ExoSAP-IT (United States Biochemical, Cleveland). DNA sequencing was performed in both directions from PCR products by BMR-Genomics (www.bmr-genomics.it) with the Big Dye Terminator ver. 3.1 (Applied Biosystems, Foster City, CA, USA) on a 96-capillary Applied Biosystems 3730xl DNA Analyzer.

Species-specific multiplex-PCR and RAPD-PCR assays.

Multiplex ITS-PCR assay was performed according to the protocol described by Bonants et al. (4) using the primer pairs NICF1/NICR2.1 and CACTF1/CACTR1, specific for *P. nicotianae* and *P. cactorum*, respectively. In addition, a different multiplex PCR assay (multiplex PN/PC-PCR) was also performed by using the primer pair PNF/PNR designed by Kong et al. (17) on the basis of *P. nicotianae*-specific sequences of the elicitor gene (*parA1*), and the SCAR primer pair PC1/PC2 for the detection of *P. cactorum* (7). The multiplex amplifications were carried out in 25- μl reaction mixtures as described for *Pheca* amplification and subjected to thermal cycling (3 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 62°C, and 1 min at 72°C, and a final step of 10 min at 72°C) with a Perkin-Elmer Cetus (Norwalk, CT, USA) Gene Amp PCR System 9600. A negative control using water instead of template DNA was included in all PCR reactions. Amplicons were analyzed by electrophoresis in 1.5% agarose gel containing SYBR Safe DNA gel stain (Invitrogen) in Tris-acetate-EDTA (TAE) buffer. To confirm the parental origin of the specific PCR products obtained in multiplex ITS-PCR, amplicons were directly sequenced as described above.

For RAPD-PCR, a total of 5 decanucleotide primers (OPB-12, OPB-15, OPB-16, OPE-18, OPE-20) acquired from Operon Technologies Inc. (Alameda, CA, USA) were used. Amplification reactions and post-PCR processes were performed according to the protocol described by Faedda et al. (12).

Pathogenicity tests. Pathogenicity trials were conducted using 6-month-old plants of both *Stoechas* lavender and English lavender 'Rosea' that is known to be very susceptible to *P. nicotianae* root rot (28). *P. nicotianae* reference isolates IMI 398853 and TL8VP from lavender (21), one reference isolate of *P. cactorum* (IRF14), and two representative hybrid isolates (956/07 and 957/07) from *Stoechas* lavender were tested. Inoculum was produced on a mixture of vermiculite and autoclaved oat seeds (10). Plants were transplanted to pots filled with a mixture of soil (sand/lime/peat 1:1:1) and 4% (vol/vol) inoculum. Control plants were grown in pots containing noninfested soil. After transplanting, all pots were flooded for 24 h by plugging the drain hole. Plants were grown in a greenhouse at $24 \pm 4^\circ\text{C}$. The test was performed twice.

Results

Morphological and cultural characterization. Isolates with uniform colony morphology were obtained from both basal stems and roots of all the 20 symptomatic *Stoechas* lavender plants with a mean frequency of 30 and 70%, respectively. On PDA, cultures formed whitish, tender cottony colonies with flattened mycelium and irregular stoloniferous margins. On V8A, colonies showed a chrysanthemum pattern and abundant aerial mycelium (Fig. 1A and B). Optimum growth temperature was $30 \pm 1^\circ\text{C}$, and mean ($\pm$ S.D.) radial growth rates of the 5 isolates were 10 ± 0.5 and 14 ± 1 mm/day on PDA and V8A, respectively. The maximum temperature at which continuous growth occurred was $34 \pm 0.2^\circ\text{C}$. No growth was observed at 5 and 35°C.

Colonies developed on V8A were homothallic and produced smooth-walled oogonia measuring 33.3 ± 2.8 μm (mean of five isolates $\pm$ S.D.) in diameter (range 26.9 to 38.5 μm); oospores were aplerotic with a mean diameter of 28.5 ± 2.1 μm (range 22.5 to 34.2 μm); antheridia were predominantly amphigynous (Fig. 1E and F). A low proportion of abortive oogonia (less than 20%) were also observed in all isolates. Sporangia produced in water were subglobose with a prominent papilla, both caducous with a short pedicel (2 to 3 μm in length) and persistent, sometimes with a lateral insertion in the sporangiophore. The average sporangium size was $46.9 \pm 4.2 \times 36.5 \pm 3.2$ μm , with a mean length-to-width ratio of 1.28 (Fig. 1C and D). Chlamydospores, both terminal and intercalary, were spherical with a diameter ranging from 26.2 to 30.1 μm .

ITS-rDNA, mitochondrial DNA, and *Pheca* analyses. PCR amplification of ITS regions from 956/07 and 957/07 isolates resulted in a single band of 831 bp. Direct sequencing of this DNA fragment did not reveal any ambiguous nucleotide sites, and

BLASTn search in GenBank showed that sequences of these isolates were identical to those of several *P. nicotianae* reference isolates (e.g., Accession Nos. GU902246 and HG709173).

PCR reactions of *coxI* amplified a DNA fragment of the expected size (654 bp) from all *Phytophthora* isolates examined. The *coxI* sequences obtained from isolates 956/07 (Accession No. KC109828) and 957/07 (Accession No. KC109829) also showed 100% identity with sequences of *P. nicotianae* isolates available in GenBank database (e.g., Accession No. GU945494), including the sequence of the isolate IMI 398853 (Accession No. KC109827).

PCR amplification of a portion of *Pheca* gene of all *Phytophthora* isolates using primers *PhecaF* and *PhecaR* produced DNA fragments of the expected size (271 bp). Consensus sequences of isolates 956/07 (Accession No. KC109825) and 957/07 (Accession No. KC109826) revealed double bases at 12 positions, whereas both *P. nicotianae* IMI 398853 (Accession No. KC109823) and *P. cactorum* IRF14 (Accession No. KC109824) contained whole sequence with single bases, as expected. Multiple sequence alignment showed 10 identical double bases at the same positions between sequences of isolates from *Stoechas* lavender and loquat trees (*P. nicotianae* × *cactorum* 95023: Accession No. FJ459732, and LT2852: Accession No. FJ459733) (15). In addition, *Stoechas* lavender isolates exhibited two additional heterozygous sites at positions 136 and 178 as compared to those of loquat isolates (Table 1). *Pheca* sequences of *P. nicotianae* IMI 398853 and *P. cactorum* IRF14 were 100% identical to those of *P. nicotianae* LT215 (FJ459730) and *P. cactorum* LT198 (FJ459731)

isolates available in GenBank (15) (Table 1). In the hybrids examined in this study, all the nucleotide differences were localized at positions wherein the sequences of *P. nicotianae* and *P. cactorum* diverged.

Multiplex and RAPD PCR analyses. Multiplex ITS-PCR using primers specific for *P. nicotianae* (NICF1 and NICR2.1) and *P. cactorum* (CACTF1 and CACTR1) produced a single DNA fragment of the expected size for *P. nicotianae* IMI 398853 (700 bp) and *P. cactorum* IRF14 (365 bp) reference isolates, while both products were amplified simultaneously for the putative hybrid isolates from *Stoechas* lavender (Fig. 2). BLASTn analysis in GenBank database showed that the 700 bp and 365 bp consensus sequences were identical to those of *P. nicotianae* and *P. cactorum*, respectively. The alignment between these sequences revealed altogether 7 heterozygous bases only at those positions wherein the 365-bp portion of ITS sequences of *P. nicotianae* and *P. cactorum* diverged (Table 2). Therefore, species-specific ITS-PCR assay followed by sequencing highlighted the coexistence of ITS sequences of both *P. nicotianae* and *P. cactorum* in *Stoechas* lavender isolates, unlike the direct sequencing of ITS amplicons with universal primers which did not reveal any heterozygous sites. Likewise, in the multiplex PN/PC-PCR experiments with the primer pairs PNF/PNR and PC1/PC2, both isolates from *Stoechas* lavender showed two bands of 230 bp and 450 bp specific for *P. nicotianae* and *P. cactorum*, respectively. As expected, isolates IMI 398853 of *P. nicotianae* and IRF14 of *P. cactorum* gave a single PCR product of the predicted size for each species (Fig. 3).

Fig. 1. A and B, Ten-day-old colonies of a *Phytophthora* × *pelgrandis* isolate (956/07) from lavender on PDA and V8A, respectively. C, Persistent papillate sporangium of isolate 956/07. Note the unipodial ramification at the base of the intercalary sporangium. D, Subspherical, prominently papillate, caducous sporangia with a short pedicel of isolate 956/07. E and F, Gametangia and oospores of isolate 956/07 produced in a single culture: paragynous and anphigynous antheridia, respectively. Bar in C, D, E, and F represents 10 μm.

Highly reproducible RAPD profiles were obtained from isolates with all selected random primers. Three primers out of five generated monomorphic bands specific for each isolate of parental species, and some of these species-specific bands were also observed in the patterns of *Stoechas* lavender isolates (Fig. 4). These intermediate profiles were compatible with the hybrid origin of isolates from *Stoechas* lavender with *P. nicotianae* and *P. cactorum* as progenitors.

Pathogenicity tests. Three months after transplanting all plants grown in pots containing soil infested with 956/07 and 957/07 hybrid isolates as well as with IMI 398853 and TL8VP of *P. nicotianae* showed dieback symptoms and extensive root necrosis on both *Lavandula* species tested. Plants grown in pots containing soil infested with the *P. cactorum* isolate showed no aerial symptoms and only a few necrotic roots, suggesting that this species is weakly pathogenic on lavender. Control plants remained healthy. *P. nicotianae* and *P. cactorum* as well as both hybrid isolates were reisolated solely from roots of inoculated plants. The identity of each species was confirmed by both morphological and cultural characteristics and multiplex PCR analyses with species-specific primers, thus fulfilling the postulates of Koch.

Discussion

The putative hybrid nature of the *Phytophthora* isolates recovered from *Stoechas* lavender in Italy was suspected on the basis of morphological traits, namely the stoloniferous colony morphology on PDA peculiar of *P. nicotianae* and homothallism, which is by contrast atypical for the latter, and was confirmed by the molecular analyses. It could be inferred from DNA analysis that *P. nicotianae* and *P. cactorum* are the parental species of these hybrids, as in their genome the *Pheca* gene is in a heterozygous condition and each allelic form corresponds to that of these two species. This conclusion is supported by the results of both multiplex and RAPD PCR analyses showing the DNA of the isolates from *Stoechas* lavender comprised regions from both *P. nicotianae* and *P. cactorum*. Analysis of the *coxI* gene suggests that these interspecific hybrids inherited the mitochondrial genome from *P. nicotianae* as hitherto has been found in all these hybrids to date (15,24). Both morphological characteristics and sequences of *Pheca* gene of the Italian hybrid isolates from *Stoechas* lavender correspond to those of *P. nicotianae* × *cactorum* hybrids recovered from *P. grandiflorum* and formally described by Nirenberg et al. (30) as *Phytophthora* × *pelgrandis*. Natural hybrids between *P. nicotianae* and *P. cactorum* were first reported on various ornamental plants, including *Lavandula* sp., grown in greenhouses in the Netherlands (4,24). Later, two *Phytophthora* isolates from loquat trees (*Eryobotria japonica*) collected in central Taiwan in 1995, which were earlier referred to as atypical strains of *P. nicotianae* (8), were identified as hybrids of *P. nicotianae* and *P. cactorum* (22). Conceivably, the homothallic *Phytophthora* isolates from loquat described by Weltzien and Scwinn (37) in Lebanon were highly likely hybrids of the same species, as their morphological characteristics were very similar to those of isolates from Taiwan. More recently, hybrids originating from *P. nicotianae* and

P. cactorum were recovered from loquat in Peru (2). After natural hybrids of *P. nicotianae* and *P. cactorum* were recovered from *P. grandiflorum* in Germany and formally described as *Phytophthora* × *pelgrandis* (30), they were also reported with the same name in the United States (20) as well as on common box, lavender, and Port-Orford-cedar in different ornamental nurseries in Hungary (35). In this study, *Phytophthora* × *pelgrandis* is reported for the first time in Italy. *Phytophthora* hybrids generally show numerous abortive oospores, heterozygous profiles for *Mdh-2*, *Mdhp*, or *Gpi* isoenzymes, and ITS sequences of both parental species (23); however, in some instances a molecular polyphasic approach is required to ascertain the hybrid nature of the isolates, as direct sequencing of ITS-rDNA does not always reveal intra-isolate heterogeneity. In fact, ITS sequencing of PCR products obtained

Fig. 2. Internal transcribed spacer (ITS)-rDNA products amplified in multiplex polymerase chain reaction, using primer-pairs NICF1/NICR2.1 and CACTF1/CACTR1, from *Phytophthora nicotianae* IMI 398853, *P. cactorum* IRF14 and one (956/07) of the two putative hybrid isolates from *Stoechas* lavender. Size of marker bands is indicated in bp.

Table 1. Comparison of variable nucleotide positions identified in a 271-bp fragment of the Phenol acid decarboxylase (*Pheca*) gene between *Phytophthora* hybrids and their parental species^a

Accession number	Isolate	Species	Nucleotide position											
			19	34	40	46	49	91	100	136	178	220	229	235
KC109825	956/07	<i>P. × pelgrandis</i>	R	R	R	R	S	S	S	R	R	R	R	R
KC109826	957/07	<i>P. × pelgrandis</i>	R	R	R	R	S	S	S	R	R	R	R	R
FJ459733	95023	<i>P. × pelgrandis</i>	R	R	R	R	S	S	S	A	A	R	R	R
FJ459732	LT2852	<i>P. × pelgrandis</i>	R	R	R	R	S	S	S	A	A	R	R	R
KC109823	IMI 398853	<i>P. nicotianae</i>	G	G	A	G	C	C	C	A	A	A	A	G
FJ459730	LT215	<i>P. nicotianae</i>	G	G	A	G	C	C	C	A	A	A	A	G
KC109824	IRF14	<i>P. cactorum</i>	A	A	G	A	G	G	G	G	G	G	G	A
FJ459731	LT198	<i>P. cactorum</i>	A	A	G	A	G	G	G	G	G	G	G	A

^a R = G/A; S = C/G. *Pheca* sequences FJ459733, FJ459732, FJ459730, and FJ459731 deposited in GenBank by Hurtado-Gonzales et al. (15) were used as references.

with universal primers ITS6 and ITS4 failed to discriminate the *Stoechas* lavender isolates of *P. × pelgrandis* from *P. nicotianae* in this study, and similar results were already reported by Nirenberg et al. (30) for *P. grandiflorum* hybrids from Germany. However, amplification of ITS region with specific primer pairs NICF1/NICR2.1 and CACTF1/CACTR1 revealed the heterozygous condition of this region in the lavender *Stoechas* isolates. The inability of generic primers to reveal the heterozygosity within the ITS-rDNA with direct sequencing may be explained assuming that they contain a number of copies from the parental *P. nicotianae* species larger than those of the other donor species. Moreover, it can be supposed that the high proportion of G-C in *P. cactorum* ITS sequences might have favored the amplification of the allele of the other parental species *P. nicotianae* as demonstrated by Walsh et al. (36) for other genes in humans. The heterozygous condition of ITS regions in lavender *Stoechas* isolates was confirmed by the multiplex ITS-PCR assay. Likewise, multiplex PN/PC-PCR provides an additional molecular tool to further demonstrate the hybrid nature of the Italian *Stoechas* lavender isolates. Interestingly, the nucleotide differences at two positions in the sequence of the *Pheca* gene between Italian isolates from *Stoechas* lavender and isolates from loquat trees found in Taiwan and Peru (2) suggest these hybrids originated from independent hybridization events involving parental species with intraspecific mutations.

Like the parental species *P. nicotianae*, *P. × pelgrandis* seems a quite common pathogen of lavender, as it was recovered from lavender plants affected by root rot and basal stem necrosis in ornamental nurseries in several European countries, including the Netherlands (4), Hungary (35), and now Italy. By contrast, *P. cactorum*, the other parental species, is an occasional and weak root pathogen on this host. Pathogenicity tests indicate that natural hybrids of these two species are as aggressive as the parental *P. nicotianae*, from which they also inherited other ecological traits, such as a high optimum temperature and partly amphigynous antheridia and noncaducous sporangia. Conversely, these hybrids share with *P. cactorum* homothallism, partly paragynous antheridia and sporangium caducity. It can be hypothesized that this novel combination of biological and ecological characteristics may improve the fitness of the interspecific hybrids of *P. nicotianae* and *P. cactorum* and their adaptability to the environmental conditions in nurseries of ornamentals. A high optimum temperature, comparable to the optimum of the thermophilic parental species *P. nicotianae*, conceivably favors their activity in the greenhouse and during summer months, while a minimum temperature higher than the minimum of some isolates of *P. nicotianae* (30) may be conducive to early infections in late winter, thus widening the favorable period for the infections by hybrids. In addition, these hybrids can produce resting structures such as chlamydozoospores that allow them to survive during winter or dry periods in soil or within infected host tissues. Moreover, the rapid spread of these hybrids in nurseries is favored by the production of caducous sporangia which are disseminated more easily than persistent sporangia by irrigation water. So far, only natural hybrids of a few *Phytophthora* species have been identified and described (19,23). Recently, *Phytophthora andina* has also been identified as a natural hybrid of *P. infestans* and a hitherto unknown other parent (14). The wide diffusion of natural hybrids of *P. nicotianae* and *P. cactorum* and the differences in the sequences of the *Pheca* gene between isolates,

recovered from *Stoechas* lavender in Italy and isolates from loquat trees found in Taiwan and Peru, indicate that breeding events between these two *Phytophthora* species occur frequently, even though *P. nicotianae* and *P. cactorum* are in different ITS subclades (3,9,18), thus suggesting a higher affinity than inferred on the basis of ITS sequences alone. Both parents evolved allopatric and hence may not have developed premating barriers. By contrast, it can be supposed that the close similarity between *P. × pelgrandis* isolates recovered from lavender in Hungary, Italy, and the Netherlands would suggest these hybrids propagate clonally and are spreading in nursery ornamentals through global trade following commercial routes. Horticultural centers are known to be the main propagators of pathogen dispersion (26). High-resolution whole genome DNA profiling analysis (e.g., AFLP or microsatellites) would be required to determine whether the lavender strains are really identical. As demonstrated by AFLP analysis, the three hybrid strains from lavender in the Netherlands originated from at least two independent hybridization events (4). The diffusion of *P. × pelgrandis* pathogenic isolates in cultivations of lavender could exacerbate the damage caused by *Phytophthora* root and collar rot whose main causal agent in Europe presently is *P. nicotianae*, one of the parental strains of these hybrids. Hygienic measures such as presymp-

Fig. 3. Multiplex polymerase chain reaction with primer pairs PNF/PNR and PC1/PC2 specific for *Phytophthora nicotianae* and *P. cactorum*, respectively, of DNA from *P. nicotianae* IMI 398853, *P. cactorum* IRF14, and putative hybrid isolates 957/07 and 956/07 from *Stoechas* lavender. Size of marker bands is indicated in bp.

Table 2. Comparison of variable nucleotide positions identified in a 365-bp fragment of the ITS1-5.8S-ITS2 regions between *Phytophthora* hybrids and their parental species^a

Isolate	Species	Nucleotide position						
		1	15	27	47	48	55	363
956/07	<i>P. × pelgrandis</i>	R	W	R	K	Y	Y	Y
957/07	<i>P. × pelgrandis</i>	R	W	R	K	Y	Y	Y
IMI 398853	<i>P. nicotianae</i>	A	A	A	T	T	T	C
IRF14	<i>P. cactorum</i>	G	T	G	G	C	C	T

^a R = G/A; W = A/T; K = G/T; Y = C/T.

Fig. 4. Random amplified polymorphic DNA profiles of *Phytophthora nicotianae* IMI 398853, *P. cactorum* IRF14, and one putative hybrid isolate 956/07 from *Stoechas* lavender obtained using two universal decamer primers OPB-15 and OPB-16.

tomatic testing of plants may reduce the proliferation and spread of the *Phytophthora* hybrids.

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