

SHORT COMMUNICATION

CHARACTERIZATION OF *PHYTOPHTHORA NICOTIANAE* ISOLATES CAUSING COLLAR AND ROOT ROT OF LAVENDER AND ROSEMARY IN SPAIN

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SUMMARY

Since 2004, containerized nursery stocks of rosemary and lavender have been affected by a collar and root rot problem in Valencia (Spain). Nurseries had about 70% of affected plants showing lack of vigour, wilt and dieback symptoms. *Phytophthora* sp. was isolated consistently from symptomatic plants, and eighteen isolates were obtained, ten from lavender and eight from rosemary. The species was identified as *P. nicotianae* based on morphological and physiological features. Some isolates were identified as atypical representatives of *P. nicotianae*. Identification of the isolates was confirmed by the sequence of the nuclear ribosomal internal transcribed spacer (ITS) region. Both morphological characters and DNA analysis supported the species identification for all the isolates. Pathogenicity tests were carried out and Koch's postulates were fulfilled.

Key words: *Phytophthora parasitica*, *Phytophthora nicotianae*, etiology, ornamental plants, root rot, collar rot.

Lavender (*Lavandula angustifolia* Mill.) and rosemary (*Rosmarinus officinalis* L.) are aromatic plants in the family *Lamiaceae* native to the Mediterranean region. Both are widely grown as ornamentals.

Since 2004, several nurseries in Valencia province (eastern Spain) have reported a high mortality (70% of incidence) of lavender and rosemary plants. In the early stages of the disease, symptoms consisted of delayed growth, general lack of vigour, off-colour of the foliage and wilting. Occasionally, apparently healthy plants suddenly collapsed during late spring or summer. Lavender foliage turned grey and severely affected plants wilted permanently. Rosemary foliage scorched along the edges or turned yellow, red, or orange. As the disease progressed, affected plants died. Both hosts showed the entire root system rotted.

A *Phytophthora* sp. was consistently isolated from roots and basal stem lesions; however, some of the isolates showed atypical morphological characteristics. The objectives of this study were to identify *Phytophthora* species present and to confirm Koch's postulates.

Isolations were made from sections of roots, crown and stems of symptomatic plants. Samples were washed under running tap water, surface-disinfested by a 5-10 sec immersion in 70% ethanol, and dried on filter paper. Affected tissues from the edge of the lesions were selected by cutting 2 to 4-mm wide pieces which were placed on PARBPH selective culture medium as described by Jeffers and Martin (1986). Plates were incubated at 24°C in the dark. Pure cultures were obtained by transferring hyphal tips onto potato dextrose agar (PDA) and V8 juice agar (2 g CaCO₃, 200 ml of V8 juice and 15 g agar in 800 ml distilled water).

Isolates obtained were identified on the basis of colony morphology, mycelial characteristics, cardinal growth temperatures, and production, morphology, and dimensions of sporangia, oogonia, and antheridia (Erwin and Ribeiro, 1996). For colony morphology and growth temperature studies, a 5-mm-diameter mycelial plug of each isolate was transferred to three PDA plates, and incubated at 5, 24 and 35°C for 7 days in the dark. Sporangia were produced by cutting 5-mm-diameter disks from the advancing margin of a colony grown on V8, and floating these disks on 10 ml of 1.5% sterile soil extract for 4 - 5 days at 24°C under fluorescent light. Isolates were paired on V8 juice agar with A1 and A2 tester strains of *P. nicotianae* (Breda de Haan) var. *parasitica* Dastur (= *P. parasitica*) and incubated for 2 to 6 weeks at 24°C in the dark. Reference testers were kindly provided by A. Ippolito (University of Bari, Italy).

The ITS region of three representative isolates from lavender (PS-26, PS-28 and PS-35) and three isolates from rosemary (PS-21, PS-23, and PS-30) was amplified with the primers ITS4 and ITS6 (Cooke and Duncan, 1997). PCR products were purified with the High pure PCR product purification kit (Roche Diagnostics GmbH, Mannheim, Germany) and directly sequenced using the Tag DyeDeoxy terminator cycle sequencing Kit (Applied Biosystems, Falmer, Brighton, UK), in an Applied Biosystems automatic DNA sequencer (model

373A). Sequences were compared with *Phytophthora* sequences available in the EMBL/GenBank database.

Pathogenicity tests were carried out on eight- and six-month-old lavender and rosemary plants, respectively. Ten plants per isolate and host were transplanted into 15-cm-diameter plastic pots (one plant per pot) containing sterilized potting mix (75% peat: 25% sand vol/vol). Lavender plants were inoculated with isolates PS-28 and PS-35; rosemary plants were inoculated with isolates PS-21 and PS-23. Inoculum was prepared by growing each isolate on a medium containing 200 g of oats and 120 ml of V8 juice to 1 litre of distilled water. After inoculation, flasks were incubated in the dark at 20°C for 4 weeks until the mycelium colonized the substrate. The medium colonized by each isolate was buried in the compost around the roots at a rate of 3% (w/v). Control plants were inoculated with similar amounts of sterile medium. Starting from the following day, the pots, including controls, were flooded for 2 days, and then watered twice a week. Plants were maintained in a glasshouse at 24±5°C. Pathogenicity tests were carried out in October 2004 and in April 2005. Incidence of the disease was determined visually as the percentage of affected roots and mortality of the whole plant for each of the ten replicate plants per treatment. Isolations were made onto PARBPH in order to confirm that the disease was caused by the inoculated pathogen.

Phytophthora sp. was isolated from roots and basal stem lesions on 100% of the affected plants. Eighteen fungal isolates were obtained, ten from lavender and eight from rosemary. The phenotypic characteristics of these isolates are summarised in Table 1. On PDA medium at 24°C, all isolates had fluffy cottony mycelium with slightly striated pattern. The growth rate on PDA at 24°C for lavender isolates was (4.6-) 5.1 (-5.7) mm d⁻¹, and for rosemary isolates was (4.0-) 4.8 (-5.3) mm d⁻¹. None of the isolates grew at 5°C but they showed growth at 35°C.

Sporangia were produced abundantly in sterile soil extract; they were papillate, predominantly ovoid or obpyriform. Measurements of sporangia of lavender isolates were (34.7-) 40.2 (-51.4) × (25.9-) 30.6 (-35.7) µm with a length:breadth ratio from 1.31:1 to 1.44:1, while measurements of sporangia of rosemary isolates were (34.4-) 44.2 (-59.1) × (27.8-) 36.0 (-45.9) µm with a length:breadth ratio 1.29:1 to 1.45:1.

Among the ten lavender isolates, five atypical isolates presented sporangia frequently caducous with a short pedicel of 2.1 to 3.8 µm. The caducity in these isolates ranged from 15 to 30%. All isolates obtained from rosemary had persistent sporangia. Chlamydozoospores were formed in all isolates recovered from rosemary, and only in five out of ten from lavender. Isolates that presented caducous sporangia, did not form chlamydozoospores.

All isolates formed sexual structures when paired with the opposite mating type. They showed spherical and smooth oogonia with amphigynous antheridia. In lavender, they were (27.0-) 30.2 (-32.2) µm in diameter and, those obtained from rosemary were (25.3-) 28.7 (-33.0) µm in diameter. All isolates obtained from rosemary plants belonged to mating type A1. However, among the ten isolates obtained from lavender, seven belonged to mating type A1, two to mating type A2 and one isolate was sterile. All isolates showed spherical, aplerotic oospores, which ranged from 27.5 to 28.8 µm in diameter and with an oospore wall of 2.0 to 2.2 µm thick.

Except for the five atypical isolates, the phenotypic characteristics of the isolates obtained from both hosts corresponded to the description of *P. nicotianae* by Erwin and Ribeiro (1996).

According to Erwin and Ribeiro (1996), *P. nicotianae* does not have caducous sporangia. In the original description of *P. nicotianae* by Van Breda de Haan from Java (Indonesia) in 1896 (Shew and Lucas, 1991), and in the description of its synonym, *P. parasitica* by Dastur in 1913, the caducity of sporangia was not considered as

Table 1. Comparative phenotypic characteristics of *Phytophthora nicotianae* isolates obtained from common lavender and rosemary plants.

Morphology	Common lavender	Rosemary
Growth rate (mm d ⁻¹) ^Z	(4.6-) 5.1 (-5.7)	(4.0-) 4.8 (-5.3)
Sporangia		
Type	Persistent/Caducous	Persistent
Length (µm)	(34.7-) 40.2 (-51.4)	(34.4-) 44.2 (-59.1)
Breadth (µm)	(25.9-) 30.6 (-35.7)	(27.8-) 36.0 (-45.9)
Length/Breadth ratio	1.31:1	1.44:1
Chlamydozoospores	Present/Absent	Present
Oogonia diameter (µm)	(27.0-) 30.2 (-32.2)	(25.3-) 28.7 (-33.0)
Oospores		
Diameter (µm)	(27.5-) 27.7 (-28.3)	(28.0-) 28.5 (-28.8)
Wall thickness (µm)	2.0 - 2.2	2.0 - 2.2
Mating types	A1/A2/Sterile	A1

^ZGrowth rate at 24°C in PDA.

Table 2. Percentage of root rot and mortality in lavender and rosemary plants 12 to 16 weeks after inoculation with *Phytophthora nicotianae* (10 plants were inoculated per host and isolate).

Isolates	Experiment 1 ^a		Experiment 2 ^b	
	Root rot %	Dead plants %	Root rot %	Dead plants %
Rosemary				
Ps - 21	80	60	70	80
Ps - 23	100	100	100	80
Lavender				
Ps - 28	90	100	100	80
Ps - 35	90	70	100	100

^aExperiment conducted in October to January 2004.

^bExperiment conducted in April to July 2005.

a distinctive character. Hall (1993), in a re-description of *P. nicotianae*, considered the caducity of sporangia as a characteristic of this species. According to this statement, *P. nicotianae* can display caducous sporangia with a short pedicel (5 µm). Cacciola *et al.* (1994) found that isolates identified as *P. nicotianae* obtained from affected *Forsythia* plants had caducous sporangia with a very short pedicel (mean length less than 5 µm). This description is in agreement with our observations on atypical isolates of *P. nicotianae* from lavender.

The PCR products obtained using primers ITS4 and ITS6 were of about 900 bp. Their sequences were compared with ITS sequences of *Phytophthora* species available in GenBank using BLAST searches, and were identical to those of *P. nicotianae*. Atypical isolates (PS-26 and PS-35) were also confirmed as *P. nicotianae* by sequence data.

Pathogenicity tests showed that the isolates were pathogenic on their respective hosts (Table 2), including the atypical isolate PS-35 from lavender. The percentage of affected roots in diseased plants ranged from 80 to 100% in both hosts. Most of the lavender plants showed symptoms ten weeks after inoculation and they died approximately twelve weeks after inoculation. In rosemary plants, severe wilting of lower leaves and death were observed between 14 to 16 weeks after inoculation. No symptoms were observed on the control plants. *P. nicotianae* was re-isolated from affected lavender and rosemary plants, completing Koch's postulates.

P. nicotianae affects a wide range of predominantly dicotyledonous crops and ornamental plants, including the family *Lamiaceae* (Erwin and Ribeiro, 1996). This species has already been reported on lavender and rosemary. Pappas (1978) first reported *P. nicotianae* as the cause of collar rot of *Lavandula* spp in Greece, and Putnam (1991) recorded this pathogen causing root rot of *L. angustifolia* in Maryland, USA. Recently, Minuto *et al.* (1999) and Tsay *et al.* (2002) encountered *P. nicotianae* causing root and stem rot of lavender in Italy and Taiwan respectively, causing significant losses in both countries.

Wheeler and Boyle (1971) in the USA and Cacciola *et al.* (1994) in Italy isolated *P. nicotianae* from rosemary plants with collar and root rot.

P. nicotianae is a destructive pathogen widely isolated in tropical and warm-temperate regions (Hall, 1993; Erwin and Ribeiro, 1996). Sporulation of *Phytophthora* on lesions is favoured by high-humidity and high-temperature. Consequently, the symptoms on lavender and rosemary were more noticeable during summer months. Increased and continuous moisture on the plant substrate during the dry period (July to September), due to the use of sprinklers and drip irrigation would enhance survival and dispersal of the pathogen. This has been observed previously in other diseases caused by *Phytophthora* (Brasier *et al.*, 1993).

Although the disease was encountered in some nurseries in Valencia province, there is no information on its distribution in the region. It appears to have great potential to become a severe problem to the production of lavender and rosemary. This is the first report on its presence in lavender and rosemary plants in Spain.

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