Eur J Plant Pathol (2010) 128:149–152
DOI 10.1007/s10658-010-9652-7

Lupinus luteus, a new host of *Phytophthora cinnamomi* in Spanish oak-rangeland ecosystems

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Accepted: 11 June 2010 / Published online: 19 June 2010

Abstract *Phytophthora cinnamomi* is an aggressive pathogen on *Lupinus luteus* (yellow lupin), causing root rot, wilting and death of this crop, common in oak-rangeland ecosystems ('dehesas') in south-western Spain. The oomycete, the main cause of *Quercus* decline in the region, was isolated from roots of wilted lupins in the field. Artificial inoculations on four cultivars of *L. luteus* reproduced the symptoms of the disease, both in pre- and post-emergence stages, recovering the pathogen from necrotic roots. These results suggest the potential of yellow lupin as inoculum reservoir for the infection of *Quercus* roots. This is the first report of *P. cinnamomi* as root pathogen of *L. luteus*.

Keywords Oak decline · *Quercus ilex* ssp. *ballota* · *Quercus rotundifolia* · Root rot

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Introduction

Lupinus luteus L. (yellow lupin) is an annual leguminous plant growing in warm temperate regions. In southern Spain, the culture of yellow lupin is principally concentrated in the western part of the region, being commonly sown in oak-rangeland ecosystems called 'dehesa'. This crop is mainly used for sheep and pig grazing. Additionally, since the early 1990's a severe decline caused by the soilborne pathogen *Phytophthora cinnamomi* Rands is affecting *Quercus* species growing in rangelands in southern Spain, causing defoliation, root rot and death of cork oak (*Q. suber* L.) and holm oak (*Q. ilex* L. subsp. *ballota*) (Brasier 1996; Gallego et al. 1999; Sánchez et al. 2006), killing thousands of trees every year. *Phytophthora cinnamomi* has a large host range, mainly woody species (Erwin and Ribeiro 1996). Among the few herbaceous plants reported as *P. cinnamomi* hosts, there are some belonging to the genus *Lupinus*, such as *L. albus* and *L. angustifolius* (Kirby and Grand 1975; Erwin and Ribeiro 1996). However, no references about *L. luteus* have been found in the literature.

Foci of wilted yellow lupins are frequently observed in 'dehesa' systems suffering oak disease caused by *P. cinnamomi*. Then, the objective of this work was to evaluate the susceptibility of *L. luteus* to *P. cinnamomi* root infection.

Material and methods

Sampling was carried out in spring in four holm oak 'dehesa' ecosystems in south-western Spain. Three open forests were chosen from those previously studied, with known presence of *P. cinnamomi* infecting oak roots (Romero et al. 2007). The fourth site was a recently forested rangeland (10 years-old trees) free of oak root disease. For each site, one focus of diseased lupins was chosen (Table 1). From each focus, 20 plants showing yellowing or wilting were chosen for sampling, those showing complete wilt being excluded from consideration. Lupin roots were carefully washed under running water. Necrotic roots were cut into segments 3–4 mm long with a sterile scalpel, and plated in Petri dishes containing 20 ml of NARPH medium (Nystatin—Ampicillin—Rifamicin—Pentachloronitrobenzene—Hymexazol—Cornmeal agar, selective for the genus *Phytophthora*) (Romero et al. 2007). For each plant, one or two dishes (depending on availability of roots), each containing six root segments, were prepared and incubated at 22°C in the dark for 4 days. Colonies obtained were transferred to CA (Carrot-Agar) medium (Erwin and Ribeiro 1996), incubated in darkness for 4–6 days at 22°C, and identified by direct observation of characteristically clustered hyphal swellings (Erwin and Ribeiro 1996) under the inverted microscope.

For pathogenicity tests, plant material was obtained from seeds of four cultivars of *L. luteus* ('Cardiga, 'Juno, 'Nacional, 'Paris) and one cultivar of *L. angustifolius*, used as tester because of its known high susceptibility to *P. cinnamomi* root infection (Kirby and Grand 1975; Erwin and Ribeiro 1996). Emergence tests were conducted on seeds washed in 10% sodium hypochlorite, rinsed in sterile distilled water and incubated in wet chambers (12 h light per day, 22°C, 3 days) until the radicle just emerged. Pregerminated seeds were placed in plastic trays containing 8 l of sand- peat (1:1 vol) infested with *P. cinnamomi* chlamydospores in sterile water suspension (isolate PE90), prepared as described in Sánchez et al. (2002). Inoculum suspension was adjusted to 2×10^4 chlamydospores ml^{-1} and 800 ml was added to each tray. Three trays (replicates) with 20 pregerminated seeds each were prepared per cultivar, plus their corresponding three control trays with uninfested soil. Trays were incubated in a growth chamber (12 h light per day, 22°C), watered as needed, and seedling emergence evaluated.

Post-emergence tests were performed with plants obtained from seeds similarly washed and pregerminated but sown in plastic trays containing wet vermiculite and incubated in a growth chamber (12 h light per day, 22°C) for 7 additional days. Twenty 10-day old plants from each cultivar were transferred to plastic trays. Three trays with infested soil prepared as described above, were planted with twenty 10-day old plants per cultivar, plus their corresponding three control trays with uninfested soil. Trays were incubated in a growth chamber (12 h light per day, 18–24°C), with watering as needed. Twenty five days after inoculation, root symptoms were assessed for each plant on a 0–4 scale, according to the percentage of root necrosis recorded (0=0% necrotic tissue, 1=1–33%, 2=34–66%, 3=67–99%, 4=dead plant) (Romero et al., 2007). ANOVA was performed for emergence and root symptom values, considering the cultivar, the presence of *P. cinnamomi* in the soil, and their interaction as factors. Mean values were compared by the Tukey´s HSD test for $P<0.05$ (Steel and Torrie 1985). Segments from inoculated and control roots were plated on NARPH medium for re-isolation of the pathogen.

Results and discussion

Symptoms were similar for diseased lupins in the field and artificially inoculated: yellowing and wilting of leaves leading to general wilt and death. Roots showed extensive necrosis. Colonies obtained from necrotic lupin roots were white coloured, showing a flat or slight petaloid pattern after 4 days growing on CA medium, with abundant, cottony aerial mycelium after 6 days of growth. Subspherical, clustered hyphal swellings, with

Table 1 Size of yellow lupin foci and isolation frequencies of *P. cinnamomi* from necrotic roots

Site	Radius (m) of diseased lupin foci	Isolation of *P. cinnamomi* from lupin roots[a]
1	1	6.5
2	12	7.2
3	7	18.2
4	30	2.8

[a] Percentage of root segments yielding a colony when platted on NARPH medium

Table 2 Percentage of emergence of *L. luteus* and *L. angustifolius* seeds sowed in soil infested or uninfested with *P. cinnamomi*

Plant material	Soil	Emergence (%)±SE	Homogeneous group*		
L. luteus cv. Paris	Uninfested	96.7±3.3	a		
	Infested	31.7±3.3		b	
L. luteus cv. Cardiga	Uninfested	93.3±1.7	a		
	Infested	1.7±1.6			c
L. luteus cv. Juno	Uninfested	80.0±5.8	a		
	Infested	5.0±2.9			c
L. luteus cv. Nacional	Uninfested	75.0±7.6	a		
	Infested	1.7±1.6			c
L. angustifolius	Uninfested	90.0±10.0	a		
	Infested	16.7±6.0		b	c

*According to Tukey´s HSD test ($P<0.05$) (Steel and Torrie 1985)

abundant smooth walled chlamydospores where observed under the inverted microscope. Chlamydospores appeared spherical shape, formed both terminally and intercalary, slightly thick-walled, and 32–40 μm in diameter. These distinctive characteristics correspond with the species *P. cinnamomi* (Erwin and Ribeiro 1996). Percentages of isolation (Table 1) were low, but this is common for *P. cinnamomi* root infections (Brasier 1996; Sánchez et al. 2006).

The results of pathogenicity tests in pre-emergence stage are in Table 2. All seeds sown in infested soil exhibited emergence levels significantly lower ($P< 0.05$) than those sown in the absence of *P. cinnamomi*. For seeds sown in infested soil, the cultivar Paris showed a significantly higher emergence value ($P < 0.05$) than the other cultivars.

Post-emergence results are in Table 3. For each cultivar tested, mean values of root symptoms recorded in inoculated soils were significantly higher ($P<0.05$) than those recorded in their respective controls. Root symptoms obtained for inoculated plants were significantly higher than those recorded for blue lupin for three up the four yellow lupin cultivars tested. Twenty two days after transferring the plants to infested soil, near 20% of yellow lupin plants of each cultivar were dead, rising to more than 80% (root necrosis value=4) at the end of the experiment, 25 days after inoculation. Mortality was lower for blue lupin, rising to 10% (root necrosis value = 4) 25 days after inoculation. *Phytophthora cinnamomi* was consistently re-isolated from necrotic roots of each lupin cultivar growing in infested soil and never from roots of control plants.

Yellow lupin resulted highly susceptible to *P. cinnamomi*, even more than blue lupin, used as tester because of its known high susceptibility to the pathogen (Kirby and Grand 1975; Erwin and Ribeiro 1996). These results suggest the potential of yellow lupin to acts as inoculum reservoir and consequently, to favour the infection of *Quercus* roots, as previously reported about weeds for other soilborne pathogens, such as *Verticillium dahliae*-olive (Trapero and

Table 3 Average values of root symptoms recorded for *L. luteus* and *L. angustifolius* seedlings growing in soil infested or uninfested with *P. cinnamomi*

Plant material	Soil	Root symptoms±SE	Homogeneous group*				
L. luteus cv. Paris	Uninfested	0.6±0.1					e
	Infested	3.7±0.7	a	b			
L. luteus cv. Cardiga	Uninfested	1.0±0.1			c	d	e
	Infested	3.9±0.1	a				
L. luteus cv. Juno	Uninfested	1.2±0.2			c	d	
	Infested	3.8±0.1	a				
L. luteus cv. Nacional	Uninfested	1.2±0.1			c		
	Infested	3.8±0.1	a				
L. angustifolius	Uninfested	0.7±0.1				d	e
	Infested	3.2±0.1		b			

*According to Tukey´s HSD test ($P<0.05$) (Steel and Torrie 1985)

Blanco 2004) or *Phytophthora capsici*-vegetables (French-Monar et al. 2006). This is the first report of *P. cinnamomi* as root pathogen of yellow lupin.

Acknowledgements The authors want to thanks D.R. Rose (The Forest Authority, UK) for reviewing the English. This work has been supported by the project AGL2009-12243-C02-01 and Consejería de Agricultura y Pesca, Junta de Andalucía.

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