Identification of *Phytophthora cinnamomi* as the cause of decline and death in *Taxus baccata* in Greece

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Abstract In June 2012, a heterothallic *Phytophthora* was isolated from soil and roots of *Taxus baccata* (English yew) plants growing in the field near Thessaloniki, Greece. The pathogen was identified as *P. cinnamomi* on the basis of morphology and ITS sequencing. A pathogenicity test (soil infestation method) was performed and *P. cinnamomi* was re-isolated, confirming Koch's postulates. This is the first identification of *P. cinnamomi* as the cause of decline and death of the important ornamental English yew in Greece.

Keywords Soil samples

The English yew (*Taxus baccata*) is a conifer of the yew family (Taxaceae) used widely in landscaping and ornamental horticulture. Due to its dense, dark green foliage this ever-green tree is often found in private gardens and public parks.

In June 2012, several declining 4-year-old *Taxus baccata* plants were observed in a 0.5 ha field in Lagkadas County near the city of Thessaloniki (Northern Greece, Central Macedonia). The disease was severe and widespread resulting in 25–30 % mortality of approximately 500 plants. The whole root system was affected; few feeder roots were present (Fig. 1) while vascular tissue on the lower stem exhibited brown discoloration (Fig. 2). Crown symptoms involved yellowing and chlorosis eventually leading to decline of plants (Fig. 3).

For pathogen isolation and identification, root and soil samples containing the rhizosphere and fine roots of declining *T. baccata* plants were collected from the field and immediately transported to the laboratory. Roots from diseased plants

were rinsed in running water over night. Tissue fragments from these roots were plated directly onto PARBHy selective medium (10 mg pimarin, 250 mg ampicillin, 10 mg rifampicin, 15 mg benomyl, 50 mg hymexazol, 20 g agar, 200 ml V8 juice and 800 ml H₂O). Soil samples were subjected to rhododendron leaf baiting (Themann and Werres 1998). New, well developed leaves of *Rhododendron catawbiense* cv. Cunningham's White were floated over flooded soil and after 2 to 8 days tissue pieces from around the developed necrotic spots on the leaves were again plated onto PARBHy medium. Petri dishes with PARBHy medium were then incubated at 20 °C in the dark in both cases (isolation from roots and isolation using baiting method). Petri dishes were examined daily under the microscope and *Phytophthora* structures were sub-cultured on V8 agar. For the identification of the pathogen, single hyphal cultures of the isolates were used. On V8 agar colonies were white and characterized by coraloid-type mycelium and thin-walled chlamydospores. Sporangia were produced in a 1.0 % non-sterilized soil extract solution according to the method described by Erwin and Ribeiro (1996). Sporangia were ovoid to ellipsoid in shape, persistent, non-papillate and 42 to 75 µm long and 25 to 38 µm wide at the broadest point. The pathogen was heterothallic and all isolates were determined as A2 mating type by crossing them with known A1 and A2 tester strains and following the sandwich method (Erwin and Ribeiro 1996). Oogonia did not form in single culture, antheridia were amphigynous while oospores were plerotic 35 µm in average diameter. One isolate was deposited at the Benaki Phytopathological Institute Culture Collection (Athens, Greece) as BPIC 2690.

The morphological descriptions and measurements of the pathogen were similar to *Phytophthora cinnamomi* Rands (Erwin and Ribeiro 1996; Gallegly and Hong 2008). Mycelial DNA was extracted using the Qiagen DNeasy Plant Mini Kit according to the manufacturer's instructions. The ITS region

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Fig. 1 Rotted root system of *T. baccata* caused by *P. cinnamomi*

of nuclear rDNA of three isolates was amplified with primers ITS4 and ITS6 and the amplicons were directly sequenced and deposited in GenBank (Accession Nos. KF484730, KF484734, KF484732). A BLAST search of the GenBank revealed 100 % homology with the sequences of various *P. cinnamomi* isolates (e.g. KC170729). Therefore, the pathogen was identified as *P. cinnamomi* on the basis of its morphological characteristics and ITS sequencing.

For pathogenicity tests, two isolates obtained from soil and one isolate from diseased roots were used. Pathogenicity of *P. cinnamomi* was evaluated using the soil infestation method

Fig. 2 Brown discoloration on the lower stem of an English yew plant infected by *P. cinnamomi*

Fig. 3 Declining *T. baccata* plant in a nursery

developed by Vettriano et al. (2001). *P. cinnamomi* inoculum was prepared by growing the above three cultures for 4 weeks at 20 °C on sterilized millet seeds which were moistened with V8 broth (200 ml V8 juice, 3 g CaCO₃ and 800 ml H₂O). Pathogenicity tests were carried out in a greenhouse under controlled conditions, at 22 °C using five, 4-year-old *T. baccata* plants potted in containers for each isolate, and five non-inoculated plants as controls. The inoculum was rinsed with sterile water in order to remove unassimilated nutrients and then it was added to the potting mixture (30 ml inoculum/

Fig. 4 Crown wilting of *T. baccata* plants inoculated with *P. cinnamomi* (left), and non-inoculated plants (right)

1000 ml potting mixture). In non-inoculated plants, sterilized millet seeds were added to the potting mixture. All plants were flooded for 24 h twice at 2 weeks intervals. After 6 weeks, all inoculated plants showed chlorosis and crown wilting, while control plants remained without symptoms (Fig. 4). *P. cinnamomi* was re-isolated from artificially inoculated plants confirming Koch's postulates.

P. cinnamomi has been reported as the causal agent of *Taxus baccata* decline in Australia, Canada, The Netherlands, New Zealand, Oregon and the Southeastern United States (Farr and Rossman 2013). To our knowledge, this is the first identification of *P. cinnamomi* as the cause of decline and death on *T. baccata* in Greece. The economic importance of the disease is considerable because of the popularity of *T. baccata* and its high market value. Furthermore, as *P. cinnamomi* has a wide host range, it remains a potential threat to plants with economic and aesthetic importance such as ornamental trees and shrubs, as well as to forest trees.

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