

SHORT COMMUNICATION

BOX BLIGHT, A NEW DISEASE OF *BUXUS* IN ITALY CAUSED BY *CYLINDROCLADIUM BUXICOLA*

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SUMMARY

In spring 2008, in a nursery located in the Como province (Lombardy, northern Italy) leaf blight symptoms were observed on potted box plants (*Buxus sempervirens* 'Suffruticosa') followed by a sudden and severe defoliation. Diseased plants, had been imported from a Belgian nursery. These symptoms were suggestive of box blight, a new disease of the genus *Buxus*. The causal agent was isolated and identified through morphological, cultural and molecular characters as *Cylindrocladium buxicola*. *C. buxicola* was reported as a pathogen of the genus *Buxus* in the 1990s in the UK and New Zealand, and since then has spread throughout northern Europe. To our knowledge this is the first report of *C. buxicola* in Italy.

Key words: new disease report, etiology, fungal plant pathogen, ornamentals.

Box plants of different species are commonly used as ornamentals in Italy, where they are imported by nurseries as cuttings or potted plants from northern Europe, particularly Belgium and The Netherlands. In spring 2008, a leaf blight symptom on potted box plants (*Buxus sempervirens* 'Suffruticosa') was observed in a nursery of the Como province (Lombardy, northern Italy). Blight was quickly followed by severe defoliation, which made the plants unmarketable. Diseased propagating material had been imported as symptomless cuttings from a Belgian nursery, and arrived in Italy in November 2006. Symptoms shown by diseased plants were suggestive of box blight (Henricot *et al.*, 2000), a disease of *Buxus* new to Italy.

Box blight, caused by *Cylindrocladium buxicola*, was reported from Europe and New Zealand in the 1990s (Henricot and Culham 2002). The disease was first found in 1994 in a Hampshire nursery and, by 1997, it

was widespread in the UK (Henricot *et al.*, 2000). In 2000, it was detected in Belgium, first in a private garden, then in other sites throughout the country (Crepel and Inghelbrecht, 2003). In 2004-2005, a record came from Germany (Brand, 2005) and in 2007 the disease was found in a cemetery at Lausanne, Switzerland (Vincent, 2008; www.acw.admin.ch). Box blight causes severe defoliation and dieback of affected plants and, because of its recent epidemics in northern Europe, in 2007 *C. buxicola* was placed on the alert list of the European Plant Protection Organization (www.eppo.org/quarantine/quarantine.htm), with the goal to hamper its spreading.

To our knowledge, box blight has not yet been reported from Italy, although it occurs in neighbouring countries (CABI, 2007) and *C. buxicola* has the potential to spread long distance through conidia, or through trading of symptomless infected plants.

Assuming that diseased box plants found in Italy were infected by *C. buxicola*, the pathogen was isolated and identified using traditional and molecular methods. To this aim, diseased *B. sempervirens* 'Suffruticosa' plants, were first inspected for symptom observation. Diseased boxes showed blighted leaves, often aggregated in variously expanded patches (Fig. 1A). Symptomatic leaf area turned light-brown with dark-green borders, eventually expanding to the petiole and the shoot. Pathogen sporulation covered the abaxial surface of infected leaves (Fig. 1B). Shoot infections resulted in dark-brown to black linear or diamond-shaped lesions, with darker borders, and subsequent blight. New young shoots eventually developed from healthy twigs parts.

Material for scanning electron microscope observations was processed according to Locci (1972). Briefly, about 0.5 cm² of infected leaf area was glued up-side down to an aluminium microscope stub and exposed to 1% OsO₄ vapour overnight. Specimens were then coated under vacuum with a thin gold film and observed with a LEO 438VP electron microscope (Leo Electron Microscopy, UK). For light microscopy, conidia and conidiophores were transferred from sporulating leaves to glass slides to be observed under a Orthoplan Leitz microscope for micro-morphological characters useful for pathogen identification.



Fig. 1. A. *Buxus sempervirens* ‘Suffruticosa’ infected by *C. buxicola* showing an expanding area of curled blighted leaves and incipient defoliation. B. Close-up of an infected leaf. The area colonized by *C. buxicola* is light-brown with dark-green borders. Pathogen propagules cover the abaxial surface.

Results of these observations showed that the pathogen had cylindrical conidia, rounded at both ends, straight, hyaline, 1-septate, 42–68 μm [average 47.3; s.e. 0.8 ($N = 20$)] \times 4–6 μm [average 3.9; s.e. 0.1 ($N = 20$)] in size. Conidia were formed on conidiophores with penicillate arrangement of fertile branches, and were arranged in cylindrical clusters held together by colourless slime. The stipe of the conidiophores ended with an ellipsoid vesicle 7–11 μm [average 9.2; s.e. 0.4 ($N = 8$)] in diameter (Fig. 2).

The pathogen was isolated by plating 1 μl of a conidial suspension (about 10^3 conidia ml^{-1} water) on the surface of potato dextrose agar (PDA) medium in a Petri dish. After 24 h incubation at 24°C, single hyphae were collected under a microscope (100x) and transferred to a fresh plate. Colony morphology and growth of our reference strain IPV-FW348, were analyzed both on PDA and on 2% malt extract agar, 2% glucose and 0.1% peptone (MEA). After 7 days growth on MEA and PDA at 24°C, colonies of strain IPV-FW348 were approximately 3 and 3.5 cm in diameter, respectively. Colour of colony reverse on MEA after 7 days at 24°C was brown in the centre, fading through sienna with a

pale luteous halo. The growing mycelium at the margin was white-cream. No diffusible pigments were observed. Strain IPV-FW348 produced few aerial hyphae. On PDA the colony reverse, after an incubation of 7 days at 24°C, was characterized by a well defined dark brown central area surrounded by a cream mycelial growth. The colony surface was covered by aerial cottony hyphae.

Experimental inoculation tests were carried out with strain IPV-FW348 grown on PDA for 7 days, by inserting agar plugs, surface down, into wounded twigs of *B. sempervirens* ‘Suffruticosa’. Wounds were wrapped with parafilm and the plants were incubated at 24°C for two weeks. All experimentally infected plants showed blight symptom on twigs and leaves within two weeks. The pathogen was reisolated from surface-sterilized wood chips from symptomatic twigs, thus fulfilling Koch’s postulates.

For molecular analysis, strain IPV-FW348 was grown on cellophane placed onto the surface of PDA in a Petri dish. After 7-day incubation at 24°C in the dark, the mycelium was transferred to an eppendorf tube, frozen at -25°C, lyophilized, ground to a powder and the DNA

extracted using E.Z.N.A. HP Plant DNA Miniprep Kit (Omega Bio-Tek Inc., USA). The universal primers ITS1 and ITS4 were used to amplify the ITS-1, 5.8S and ITS-2 regions of the nuclear rDNA (White *et al.*, 1990). DNA was amplified in a total of 30 µl containing 1x Green Go Taq reaction Buffer (Promega, Italy), 0.1 mM of each dNTP, 1µM of each primer, 0.9 U of GoTaq DNA Polymerase (Promega, Italy) and 1.2 µl DNA. PCR was performed with a Bio-Rad thermocycler at the following conditions: 2 min at 95°C, 25 cycles at 95°C for 30 sec, 55°C for 30 sec and 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were checked by electrophoresis in 1.5% agarose gel in Tris-acetate EDTA buffer stained with ethidium bromide. The DNA was purified by Wizard SW Gel and PCR Clean-Up System (Promega, Italy) and sequenced using the primer ITS1 by Primm (Milano, Italy). The ITS1-5.8S-ITS2 rDNA sequence was compared with those available in the EMBL-EBI GeneBank database using the FASTA program. The 425 bp ITS1-5.8S-ITS2 rDNA sequence obtained was identical to the homologous region of the type strain RHS-9934 of *C. buxicola* (accession No. AY078112) (Henricot and Culham, 2002).

We have successfully isolated from diseased *B. sempervirens* 'Suffruticosa' the causal agent of box blight and fulfilled Koch's postulates. Scanning electron and light microscope measurements of conidiophores, conidia and vesicles coupled with morphological characters of the fungal colony supported the notion that the pathogen was *C. buxicola*, a new species recently described by Henricot and Culham (2002). In addition, the ITS1-5.8S-ITS2 rDNA sequence of our reference strain IPV-FW348 was identical to that of *C. buxicola* type strain RHS-9934 determined by Henricot and Culham (2002).

Based on these results, we can conclude that the disease observed on *B. sempervirens* 'Suffruticosa' in the province of Como is box blight caused by *C. buxicola* which, to our knowledge, represents the first record of this disease in Italy.

As mentioned above, the Italian nursery imported symptomless box cuttings in November 2006 and the first leaf blight symptoms appeared on a few plants in spring 2007. However, that spring was particularly hot, so the grower attributed the blight symptom to drought and destroyed the damaged plants. Spring 2008, however, was particularly rainy and cool, so the blight was severe and widespread, but only within the same lot of *B. sempervirens* 'Suffruticosa'. In the absence of chemical treatments, the epidemic caused severe defoliation on most plants and the nurseryman decided to destroy the whole lot, which fortunately was small. To date, the search for box blight on other species of *Buxus* (*B. sempervirens*, *B. sempervirens* 'Baluer Heinz', *B. microphylla* 'Winter Gem', 'Golden Triumph' and 'Rocodò') within the same nursery has been negative. *B. sempervirens*

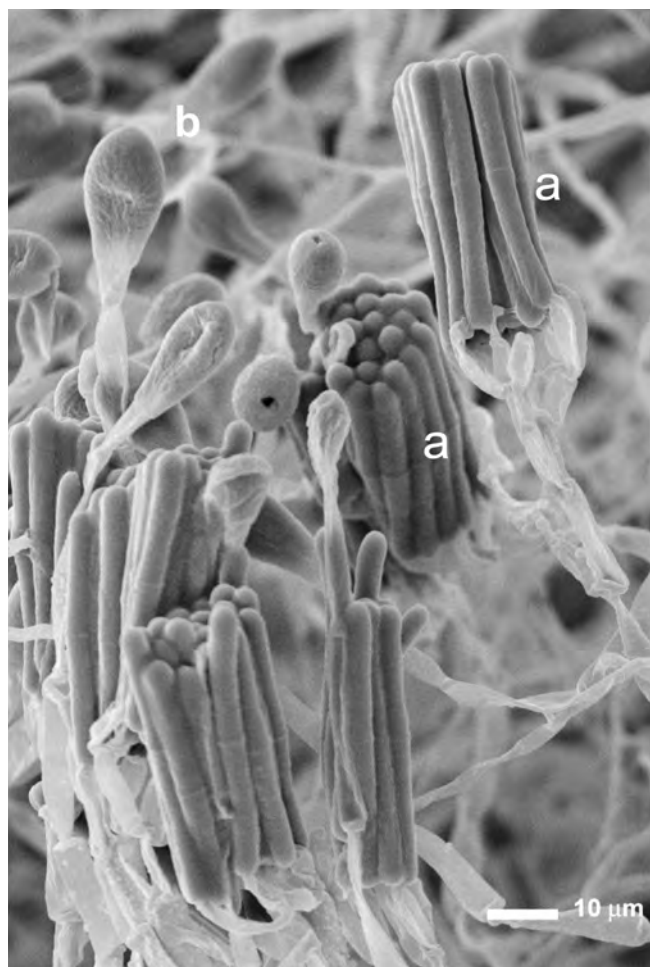


Fig. 2. Scanning electron micrograph of *C. buxicola* efflorescence on the abaxial leaf surface of *Buxus sempervirens* 'Suffruticosa', showing clusters of conidia (a), conidiophores and vesicles (b).

'Suffruticosa' seems to be less tolerant to the disease than other species within the genus *Buxus* (Henricot *et al.*, 2000), although this claim is based on anecdotal field observations rather than experimental data, whereas *B. balearica* and the genus *Sarcococca* are the most tolerant (Henricot *et al.*, 2008).

In Italy, the genus *Buxus* is widespread in private gardens and public areas, as well as in nurseries, which provide planting material often imported from northern Europe. To date, sound epidemiological data are not available, but we can infer that the disease spreads long distance through trade in box plants and locally because of the abundant production of conidia, when climatic conditions are favourable and disease control strategies are absent (at least for Italy). Therefore, the finding of the disease in our country constitutes a risk for the extensive use of *Buxus* which, so far, has not suffered serious pest and disease damages.

Disease management of box blight should be based on epidemiological knowledge and information on which fungicides are most effective against the

pathogen. Preliminary laboratory essays showed that only a few fungicides could be operatively effective (Brand, 2006; Henricot *et al.*, 2008). Therefore, highly efficient quarantine measures must be implemented to prevent any further movement of the pathogen with shipments of box plants.

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