Cylindrocladium buxicola is Threatening the Native *Buxus sempervirens* Populations in Turkey – Short Communication

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Abstract

Lehtijärvi A., Doğmuş-Lehtijärvi H.T., Oskay F. (2014): *Cylindrocladium buxicola* **is threatening the native** *Buxus sempervirens* **populations in Turkey – short communication**. Plant Protect Sci., **50**: 227–229.

Cylindrocladium buxicola is a fungal pathogen of *Buxus* spp. in Turkey; the pathogen was first noted in 2011 on the native populations of *B. sempervirens* in forests in Trabzon in the Black Sea region. Surveys conducted in November 2012 revealed a devastating impact of the pathogen on natural *B. sempervirens* populations, in which the trees were either dead or severely defoliated. The epidemic had spread 3–25 km along the river valleys near the Black Sea coast. Similar river valleys further inland were still free of infection or showed only the very first signs of arrival of the epidemic. The disease has recently been found also in nurseries. All isolates tested belonged to the G1 clade.

Keywords: *Cylindrocladium pseudonaviculatum*; boxwood blight; epidemic; Black Sea region; invasive alien species

Cylindrocladium buxicola Henricot (Henricot & Culham 2002) is an invasive alien species of unknown origin. This fungal pathogen is responsible for a disease of *Buxus* spp. called boxwood blight, which emerged in the mid-1990s in the United Kingdom and spread throughout Europe, especially via trade of diseased plants (Henricot & Culham 2002). Since boxwood is one of the most popular ornamental plants in the world, distribution of the disease is not limited to Europe. Boxwood blight has also been known in New Zealand since the late 1990s (Crous *et al*. 2002) and it was first observed in North America in 2011 (Douglas 2011). In Europe, two phylogenetic clades (G1 and G2) of this clonally reproducing pathogen have been identified so far by comparing the sequences of rDNA ITS, β-tubulin, histon H3, and calmodulin genes (Gehesquiere *et al*. 2012). Boxwood blight affects many commercial boxwood species and their varieties, among which *B. sempervirens* and its varieties are the most susceptible ones.

Unlike in many other countries, the disease was first observed in natural forests in Turkey, Georgia, and Iran (Gorgiladze *et al*. 2011; Akilli *et al*. 2012; Mirabolfathy *et al*. 2013). In Turkey, the native populations of *B. sempervirens* usually occur as an understorey in forests, mostly in the Black Sea and the Marmara Sea regions but also as small populations in the Mediterranean region, and cover a total area of approximately 1000 ha. These populations have suffered significant damage over centuries due to harvesting of the valuable wood and unplanned cutting of shoots for floricultural usage.

In Turkey, boxwood blight was first noted in 2011, in the province of Trabzon in the Black Sea region and later in the entire Eastern Black Sea region including forests around the province of Artvin (Akilli *et al*. 2012), next to Georgia, where the fungus was first reported in early 2011 (Gorgiladze *et al*. 2011). It is not known how and when the pathogen was introduced to Turkey.

The aim of the present study was to (*i*) survey the extent of damage in the forest, (*ii*) identify the phylogenetic clade of the pathogen, and (*iii*) evaluate the possibilities to control the disease.

Presented at the IUFRO Meeting "Biosecurity in Naturals Forests, Stands and Plantations, Genomics and Biotechnology for Biosecurity in Forestry", Brno and Cerna Hora, Czech Republic, May 20–25, 2013.

MATERIAL AND METHODS

In November 2012, surveys to determine the extent of the epidemic in the Black Sea region were performed in six locations from Trabzon to Artvin provinces, covering approximately a 180 km wide area along the coastal areas. Infected plant parts were sampled from Galyan Valley near Şimşirli village (Trabzon), Çamlıhemşin *Buxus sempervirens* genetic reserve (Rize), Kamilet Valley in Arhavi (Artvin), Cankurtaran Pass near Çifteköprü village along the highway from Hopa to Borçka (Artvin), Klaskur Valley near Aralık village in Borçka (Artvin), and Hatila Valley National Park (Artvin) (Table 1). In addition, in February 2013, a total of twelve boxwood seedlings grown in three state-owned forest nurseries close to the infected forest areas were sent upon our request to our laboratory for investigation (six healthy looking seedlings from Trabzon and three defoliated seedlings with a few green leaves attached on the top of the shoots and with distinct dark stem streaks from both Tirebolu (Giresun) and Ordu nurseries). Leaves and stem pieces were removed from the samples, incubated on moist filter paper in glass Petri dishes at 18°C to induce sporulation. The Petri dishes were controlled daily and the fungal colonies growing on leaves and stems were examined with a binocular microscope (400× magnification). *C. buxicola* colonies growing on the leaf and stem pieces were isolated by plating on 2% (w/v) malt extract agar (MEA). Phylogenetic clades of ten representative samples were determined by comparing the sequences of rDNA ITS, β-tubulin, histon H3, and calmodulin genes (Gehesquiere *et al.* 2012).

RESULTS AND DISCUSSION

Totally 18 *C. buxicola* isolates were obtained from the field (forest) samples (Table 1). In addition, some other species of fungi, including *Pseudonectria* (*Volutella*) *buxi* (DC.) Seifert, Gräfenhan & Schroers, *Puccinia buxi* Sowerby, *Rosellinia buxi* Fabre, Sacc. Syll., *Fusarium* sp. Link, *Verticillium* sp. Nees, *Acre-*

Table 1. Surveyed locations, number of obtained isolates and observations of the symptoms and damage on boxwood

Location	Coordinates	Altitude (m)	Length of observation track	No. of isolates	Clade	Observations
Galyan Valley, along Galyan river (Trabzon, Şimşirli Village)	40°48'33"N 39°42'32"E	500–700	1 km	4	G1	Boxwood lost all foliage with a few exceptions at the bottom of the valley; adventive shoots formed mostly from the lower part of the stem; a few healthier boxwood individuals spotted higher up on the slopes, distinct dark streaks on the shoots
Boxwood Gene Protection Forest (Rize, Çamlıhemşin)	40°54'25"N 40°56'53"E	1000	200 m	1	nd	Trees lost 90-100% of their foliage; some trees in more open spots had 30–50% of foliage left
Kamilet Valley (Arhavi, Artvin)	41°15'34"N 41°21'35"E	350–500	2 km	–	–	Boxwood next to the river lost all foliage with an exception of individuals that were growing under a rock cliff (obviously escaped from infection, e.g. for being out of easy reach for air currents carrying spores)
Klaskur Valley (Artvin, Borçka, Aralık Village)	41°24'03"N 41°42'18"E	200–300	1 km	10	G1	No obvious loss of foliage, dark lesions observed on some leaves indicating a recent arrival of the disease, sporadic infections caused by *Puccinia buxi*
Hatila Valley National Park (Artvin)	41°09'34"N 41°42'05"E	450–650	9 km	–	–	Loss of foliage not detected, sporadic infections caused by *Puccinia buxi*
Hopa-Atrvin, Cankurtaran	41°23'40"N 41°31'59"E	700	–	3	nd	Loss of foliage in general 60–100%; some escaped infection or only initial infection signs; adventive shoots; distinct dark streaks on the shoots
Total				18	10	

nd – not determined

monium sp. Link were recognised on the leaves and stems incubated in humid conditions in the laboratory. *V. buxi* is a common, wound pathogen of boxwood, associated with wilt and canker. *C. buxicola* was occasionally found together with this fungus (Henricot *et al.* 2000; Šafránková *et al.* 2012, 2013).

C. buxicola was detected in samples originating from each of the nurseries. In samples from Tirebolu forest nursery *C. buxicola* developed in humid conditions on the black streaks. In the samples from Ordu nursery *C. buxicola* developed both on the black streaks and on green attached leaves. Despite the green and healthy appearance of the seedlings from the nursery, they turned out to be infected by *C. buxicola,* too.

The surveys conducted in November 2012 revealed a devastating impact of the pathogen on natural *B. sempervirens* populations, in which boxwood bushes and exceptional trees were found to be killed or severely defoliated. The epidemic had spread 3 to 25 km along the steep, moist river valleys near the Black Sea coast. Similar river valleys further inland were still free of infection or showed only the very first signs of arrival of the epidemic. On drier sites no symptoms were observed. All so far tested isolates belong to the G1 clade, which is more sensitive to fungicides.

Due to obvious lack of resistance against the blight among the *B. sempervirens* plants (Henricot *et al.* 2008), control of the disease with fungicides seems to be the only alternative to save the boxwood populations. However, there are only limited possibilities to use fungicides in the infected areas, due to the risk of the chemicals ending up in the rivers and therefrom in households. Moreover, the climatic conditions in the river valleys during the growing season (temperature range 15–25°C, precipitation occurring on average every second day) are optimal for the fungus. In such a climate *C. buxicola* can complete its life cycle within one week (Henricot *et al.* 2008), making repeated fungicide treatments necessary.

Acknowledgements. The authors would like to thank Bjorn Gehesquiere and Kurt Heungens for their help in identification of the clade of *C. buxicola*.

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Received for publication February 12, 2014
Accepted after corrections May 21, 2014

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Indicators of tree vitality
CZ.1.07/2.3.00/20.0265

Subsidy title: Education for Competitiveness Operational Programme
Priority axis 2.3 Human resources in research and development
Solution period: 1 May 2012–30 April 2015
Beneficiary: Mendel University in Brno, Czech Republic
Project coordinator: Prof Dr Ing. Libor Jankovský

The project objective is to boost the quality and excellence, to engage in international projects and publishing teams, establish the target group members mainly of young scientists and students in international science and to stabilise them. Engagement of top-quality foreign scientists in the field of complex diseases of tree species result in the team being linked – in terms of research – to institutions in Austria, Norway, Italy, and Slovenia. The team connection improves professional and organisational competences, excellence of members of the target group, intensification of communication and team relations to foreign institutions.
Complex assessment of forest health status including vitality and stress assessment of individual trees is crucial for understanding the climate extremes and environmental change. A team capable of analysis and synthesis of knowledge is necessary for the interpretation of vitality indicators in order to propose measures in adaptation management and for the elimination of impacts of partial stress factors; the measures are to be proposed in relation to excellent European teams.

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