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NEW DISEASE REPORT

First report of *Calonectria henricotiae* causing boxwood blight in Switzerland and Italy

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Calonectria henricotiae is one of two pathogens which cause boxwood blight (Gehesquière *et al*., 2016). Unlike *C. pseudonaviculata*, which is widespread globally (Aiello et al., 2022), *C. henricotiae* has only been found in nine countries, mostly in central Europe (Hong, 2023). Because of its high temperature tolerance, *C. henricotiae* is likely to be present in southern Europe. Following the first discovery of *C. henricotiae* in Spain in 2023 (Kong et al., 2024), we expanded the survey of boxwood gardens and street plantings to include Switzerland, Italy and Greece in the summer of 2024.

Thirty-five boxwood (*Buxus* spp.) twig samples showing either black streaks and/or leaf blight symptoms (Figure 1a) were collected from gardens or lining streets in 12 cities in Greece, Italy and Switzerland (Table 1), and were transported to and processed in the lab under a USDA permit (P526P-21-05960). Samples were surface sterilised with 10% bleach for two minutes and were rinsed three times in deionised water. They were then induced for sporulation in closed plastic containers at 23°C as previously described (Kong et al., 2024). Samples producing white mould were examined under a dissecting microscope. Fruiting bodies from suspected samples were suspended in 15 μL sterile deionised water and transferred to a glass slide for confirmation under a compound microscope. Only three samples from Italy and one from Switzerland produced cylindrical conidia. Fifteen 1-μL drops of the conidia suspensions from each sample were placed on an acidified potato dextrose agar (PDA) plate. Three colonies from each plate were subcultured onto regular PDA.

Species identification was done using PCR with purified culture DNA and *C. henricotiae*- and *C. pseudonaviculata*-specific primers (Guo & Pooler, 2021). All isolates from the four samples which produced cylindrical conidia were positive for *C. henricotiae* and negative for *C. pseudonaviculata*. Their identities were confirmed by sequencing the Hb3 gene (GenBank Accession Nos. PQ464598 - PQ464601) and by conducting thermal tolerance and fungicide sensitivity tests to discriminate between *C. henricotiae* and *C. pseudonaviculata* (Gehesquière *et al*., 2016). The mean growth (mm) of these isolates after 10 days was: 10.3 (± 2.7) at 28°C and 2.1 (± 0.7) at 30°C, and 25.4 (± 7.1), 22.4 (± 8.4) and 24.5 (± 9.6) at 23°C at 1, 10 and 100 ppm of kresoxim-methyl (Sovran®, BASF), respectively. The growth of these isolates was substantially greater than that of a *C. pseudonaviculata* isolate under the same conditions (Figure 2a). A pathogenicity test with *Buxus sempervirens* twigs dipped in the conidial suspension verified these isolates as the causal agent of boxwood blight (Figure 1b).

To our knowledge, this is the first report of *C. henricotiae* causing boxwood blight in Italy and Switzerland. This pathogen has the potential to be more destructive with fewer control options due to its tolerance to certain fungicides. Knowing the distribution of *C. henricotiae* is critical to preventing its spread to other currently unaffected areas.

TABLE 1 Boxwood (*Buxus* spp.) samples collected for *Calonectria henricotiae* detection in Switzerland, Italy and Greece. Samples in bold font produced white fruiting bodies with cylindrical conidia.

Sample	Date	Country	City	Location (GPS coordinates)	Symptoms
EB2401	6/20	Switzerland	Zurich	Old Botanic Garden/University of Zurich (47.37° N, 8.53° E)	Stem streaks, leaf spot, blight, leaf spot, blight
EB2402	6/20	Switzerland	Zurich	New Botanic Garden/University of Zurich #1 (47.36° N, 8.56° E)	Stem streaks, leaf spot, blight
EB2403	6/20	Switzerland	Zurich	New Botanic Garden/University of Zurich #2 (47.36° N, 8.56° E)	Stem streaks, leaf spot, blight
EB2404	6/20	Switzerland	Zurich	Street sample near Globus (47.38° N, 8.54° E)	Stem streaks, leaf spot, blight
EB2405	6/22	Switzerland	Zurich	Arboretum (47°.21 N, 8.32° E)	Stem streaks, leaf spot, blight
EB2406	6/22	Switzerland	Zurich	Street sample near AC hotel (47.37° N, 8.54° E)	Stem streaks, leaf spot, blight
EB2407	6/21	Switzerland	Lucerne	Street sample near Chapel Bridge (47.05° N, 8.31° E)	Stem streaks, leaf spot, blight
EB2408	6/22	Switzerland	Thun	Schadau Castle #1-Paronoma museum (46.76° N, 7.63° E)	Stem streaks, leaf spot, blight
EB2409	6/23	Switzerland	Thun	Schadau Castle #2-castle front (46.76° N, 7.63° E)	Stem streaks, leaf spot, blight
EB2410	**6/23**	**Switzerland**	**Thun**	**Seepark hotel #1-front yard with planting in soil (46.75° N, 7.64° E)**	**Stem streaks, leaf spot, blight and defoliation**
EB2411	6/23	Switzerland	Thun	Seepark hotel #2- front yard with planting in in stand (46.75° N, 7.64° E)	Stem streaks, leaf spot, blight
EB2412	6/23	Switzerland	Geneva	Botanic conservatory and gardens #1 (46.23° N, 6.15° E)	Stem streaks, leaf spot, blight
EB2413	6/23	Switzerland	Geneva	Botanic conservatory and gardens #2 (46.23° N, 6.15° E)	Stem streaks, leaf spot, blight
EB2414	6/23	Switzerland	Geneva	Botanic conservatory and gardens #3 (46.23° N, 6.15° E)	Stem streaks, leaf spot, blight
EB2415	6/23	Switzerland	Geneva	Jardin Anglais (English garden) (46.20° N, 6.15° E)	Stem streaks, leaf spot, blight
EB2416	6/24	Switzerland	Montreux	Caux Palace #1 (46.43° N, 6.94° E)	Stem streaks, leaf spot, blight
EB2417	6/24	Switzerland	Montreux	Caux Palace #2 (46.43° N, 6.94° E)	Stem streaks, leaf spot, blight
EB2418	6/24	Switzerland	Montreux	Chillon castle (46.41° N, 6.93° E)	Stem streaks, leaf spot, blight
EB2419	6/24	Switzerland	Montreux	Street sample (46.41° N, 6.93° E)	Stem streaks, leaf spot, blight
EB2420	**6/26**	**Italy**	**Torino**	**Giardini Venaria Reale #1 (45.13 °N, 7.62° E)**	**Stem streaks, leaf spot, blight and defoliation**
EB2421	**6/26**	**Italy**	**Torino**	**Giardini Venaria Reale #2 (45.13 °N, 7.62° E)**	**Stem streaks, leaf spot, and defoliation**
EB2422	6/26	Italy	Torino	Villa Della Regina (45.06° N, 7.71° E)	Stem streaks, leaf spot, blight
EB2423	**6/26**	**Italy**	**Torino**	**University of Torino Botanic Garden #1-near a pond (45.06° N, 7.69° E)**	**Stem streaks, leaf spot, blight**
EB2424	6/26	Italy	Torino	University of Torino Botanic Garden #2 (45.06° N, 7.69° E)	Stem streaks, leaf spot, blight
EB2425	6/26	Italy	Mira	Public garden (45.43° N, 12.12° E)	Stem streaks, leaf spot, blight
EB2426	6/27	Italy	Venice	Public garden (45.43° N, 12.36° E)	Stem streaks, leaf spot, blight
EB2427	6/28	Italy	Rome	Rome-Borgo (41.90° N, 12.46° E)	Stem streaks, leaf spot, blight
EB2428	6/28	Italy	Rome	Vatican (41.90° N, 12.46° E)	Stem streaks, leaf spot, blight
EB2429	6/29	Italy	Tivoli	Villa d'Estes #1 (41.96° N, 12.80° E)	Stem streaks, leaf spot, blight
EB2430	6/29	Italy	Tivoli	Villa d'Estes #2 (41.96° N, 12.80° E)	Stem streaks, leaf spot, blight
EB2431	7/1	Greece	Athens	Hellenic Parliament (37.98° N, 23.74° E)	Stem streaks, leaf spot, blight
EB2432	7/3	Greece	Athens	Temple of Hepharistos (37.98° N, 23,72° E)	Stem streaks, leaf spot, blight
EB2433	7/5	Greece	Nafplio	Waterfront restaurant (37.57° N, 22.80° E)	Stem streaks, leaf spot, blight
EB2434	7/5	Greece	Nafplio	City square #1 (37.57° N, 22.80° E)	Stem streaks, leaf spot, blight
EB2435	7/5	Greece	Nafplio	City square #2 (37.57° N, 22.80° E)	Stem streaks, leaf spot, blight

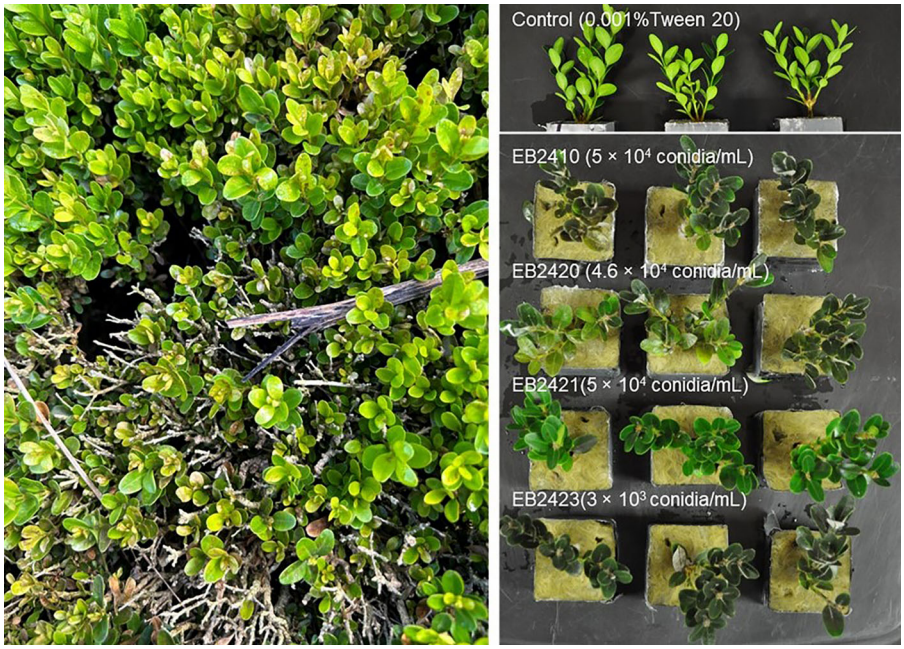

FIGURE 1 Symptoms of boxwood blight (a) at a sample (EB2410) collection site in Turino, Italy and (b) on *Buxus sempervirens* twigs inoculated with isolates EB2410, 20, 21 and 23 of *Calonectria henricotiae* at >3×10^3 conidia/ml.

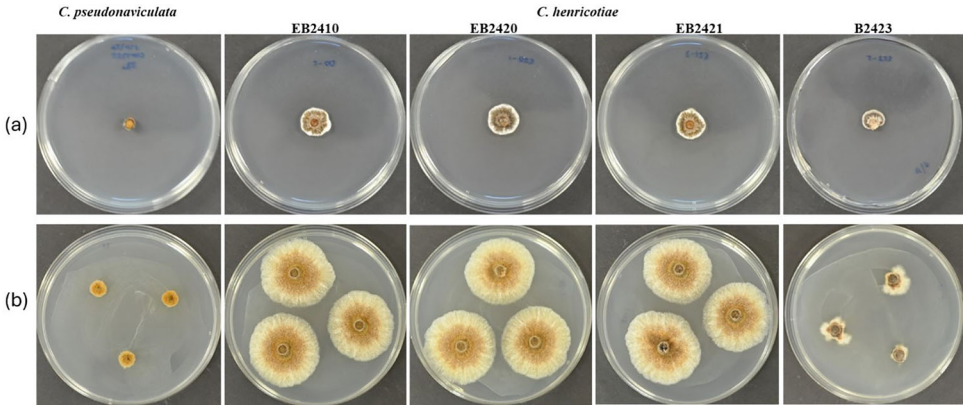

FIGURE 2 Growth of *Calonectria henricotiae* isolates from Switzerland and Italy and a control isolate of *C. pseudonaviculata* on (a) potato dextrose agar (PDA) for 10 days at 28°C and (b) on PDA amended with kresoxim-methyl at 100 ppm.

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