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Host susceptibility and microclimatic conditions influencing the development of blight diseases caused by *Calonectria henricotiae*

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Abstract Boxwood blight caused by *Calonectria pseudonaviculata* or *Calonectria henricotiae* is the major disease affecting boxwood (*Buxus* sp.). Other members of the Buxaceae family, *Pachysandra* and *Sarcococca* can also be infected. The trials reported here were conducted to test the susceptibility of *Pachysandra terminalis* 'Compacta' and *Sarcococca confusa* to this pathogen. The susceptibility of both species was compared to three different taxa of boxwood plants, representing three different levels of susceptibility to the pathogen: from the most susceptible *Buxus sempervirens* 'Suffruticosa' via the intermediate *Buxus sempervirens* var. *arborescens* to the most tolerant *Buxus microphylla* 'Herrenhausen'. The experiments took place under in greenhouses and outside in a container field. Plants were artificially inoculated with AT01, further determined as a strain of *Calonectria henricotiae*. The progress of the disease was continuously monitored and parameters such as leafspotting, lesions and leaf drop were evaluated. Results show that the level of susceptibility of *Pachysandra terminalis* 'Compacta' to *Calonectria henricotiae* is low to moderate and comparable to the susceptibility of *Buxus microphylla* 'Herrenhausen' or, depending on the conditions, *Buxus sempervirens* var. *arborescens*. In our trial, *Sarcococca confusa* did not produce any symptoms of a disease caused by *Calonectria henricotiae*.

Keywords *Buxus* · *Pachysandra* · *Sarcococca* · *Calonectria pseudonaviculata* · Environmental conditions

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Introduction

Boxwood blight is the major disease affecting boxwood in ornamental landscapes and a reason for significant losses in production nurseries (Daughtrey 2019). Since the first report and description of this disease in Europe (Henricot et al. 2000) and in New Zealand (Ridley 1998) it has spread throughout Europe (Henricot and Culham 2002; Crepel and Inghelbrecht 2003; Brand 2005; Saracchi et al. 2008; Pintos Varela et al. 2009; Cech et al. 2010; EPPO 2019; Saurat et al. 2012; Šafránková et al. 2012). Further, the occurrence of the disease has been reported from southwestern Asia (Gorgiladze et al. 2011; Akilli et al. 2012; Mirabolfathy et al. 2013), the USA (Ivors et al. 2012), where it is present in 28 states (Daughtrey 2019; Hong 2019), and Canada (Elmhirst and Auxier 2013; LeBlanc et al. 2018).

The disease is caused by *Calonectria pseudonaviculata* (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M.J. Wingf. & Crous (*Cps*) and *Calonectria henricotiae* Gehesquière, Heungens & J.A. Crouch (*Che*). Both pathogens occur in their anamorph stage, *Cylindrocladium pseudonaviculatum* Crous, J.Z. Groenew. & C.F. Hill (syn. *Cylindrocladium buxicola* Henricot and Culham). The teleomorph stage is unknown (Daughtrey 2019).

To date the occurrence of *Che* has been reported in five countries in Europe including Belgium, Germany, the Netherlands, the UK and Slovenia (Gehesquière et al. 2016). Furthermore, it was found in nursery and historical landscape plantings in the Czech Republic in 2017 (Bartíková et al., submitted manuscript).

The host range of *Cps* and *Che* is restricted to the Buxaceae family. Aside from species of *Buxus* (Henricot et al. 2008) *Cps* attacks also plants of the genera *Pachysandra* (LaMondia et al. 2012; LaMondia and Li 2013; Brand and Bartíková 2016) and *Sarcococca* (Malapi-Wight et al. 2016). *Pachysandra*, common name Japanese spurge, is a native evergreen to the Southeast United States. In Europe it is commonly used as ornamental groundcover in landscapes often in shady places, possibly providing conditions favorable for the disease. Therefore, it may serve also as a reservoir of inoculum of *Cps* and as a source for further infections (LaMondia et al. 2012; LaMondia and Li 2013). *Sarcococca* or sweet box, a small evergreen shrub native to the Himalayas and China, is used as an ornamental plant for its evergreen foliage and sweet-scented winterly flowers. It is suitable for shady conditions as well as being insect and disease resistant (Ryan et al. 2018).

Symptoms of boxwood blight on *Buxus* spp. manifest as brown spots, often with a lighter center on the leaves and black lesions on the stems, followed by leaf drop and possibly dieback of the plant. Dark brown, round spots, usually with darker margins and chlorotic halos around the spots appear on leaves of *Pachysandra* (LaMondia and Li 2013; Brand and Bartíková 2016). This is followed by yellowing of the leaves and leaf drop (LaMondia et al. 2012). Dark lesions can also occur on the stems (Brand and Bartíková 2016). Typical symptoms of boxwood blight such as twig dieback, leaf and stem lesions were observed on *Sarcococca* (Malapi-Wight et al. 2016).

The susceptibility to the disease differs between species and cultivars of *Buxus* (Ehsen 2011; Shishkoff et al. 2015). According to Gehesquière et al. (2016) as well as LaMondia and Shishkoff (2017) there is no difference in susceptibility to *Cps* or *Che* in boxwood varieties. As shown by Kong and Hong (2019), detached leaves of boxwood are more susceptible to the disease than those of Japanese spurge and sweet box. Hitherto, the level of susceptibility of the other host species has not been studied on whole plants, which is of major interest, as detached leaves might react differently than whole plants (Orłowska et al. 2013).

Our two main objectives were: a) to test the susceptibility of *Pachysandra* and *Sarcococca* to *Che* in comparison to three boxwood species of known level of susceptibility, namely highly susceptible *Buxus sempervirens* 'Suffruticosa', moderately susceptible *Buxus sempervirens* var. *arborescens* and most tolerant *Buxus microphylla* 'Herrenhausen'; and b) to evaluate the epidemiology of the pathogen and the disease development on selected hosts under different environments.

Materials and methods

Fungal isolate

Infected plants of *Buxus sempervirens* 'Suffruticosa', showing typical symptoms of boxwood blight (leaf spots and stem lesions), were received from a nursery located in South Moravia, Czech Republic, in January 2017. Cuttings of infected plants were placed to large Petri dishes on moist filter paper and kept for a week in these humid chambers on a laboratory bench in order to induce sporulation. It was possible to observe clusters of cylindrical conidia under the binocular on the abaxial side of the leaves and necrotic areas of stem lesions. A single-spore culture (AT01) was isolated from sporulating tissue by separating conidia on a thin layer of PDA medium under the microscope and cultivated on half-strength potato dextrose agar ($PDA_{50\%}$) at 23 °C for two weeks in the dark for further determination.

Characterization of AT01

Discriminative physiological tests

To determine whether AT01 is a strain of *Cps* or *Che*, the phenotypic variations of AT01 were tested in vitro in comparison to isolates of *Calonectria pseudonaviculata* (G1) and *Calonectria henricotiae* (G2) by discriminative physiological tests (fungicide susceptibility and

temperature tests) according to Gehesquière et al. (2016). Both strains G1 and G2, serving as standards in comparison, were provided by Flanders Research Institute for agriculture, fisheries and food (ILVO), Merelbeke, Belgium, after reassurance of the identity by means of molecular biological analyses (Heungens, personal communication 2017).

Fungicide susceptibility test: the inhibition of mycelial growth of AT01, G1 and G2 as reaction to the fungicidal active ingredients tetraconazole (Eminent, ISAGRO S.p.A.) and kresoxim-methyl (Discus, Cheminova Deutschland GmbH & Co. KG) was examined in vitro.

Agar plugs (5 mm diameter) from margins of 24-day-old colonies of AT01, G1 and G2 were placed on plates of $PDA_{50\%}$ amended with fungicide after autoclaving, using concentrations of 2 ml l^{-1} of tetraconazole or 10 ml l^{-1} of kresoxim-methyl. As controls, the isolates were grown on $PDA_{50\%}$ without amendment simultaneously. All variants were tested in five replicates. The strains in test were incubated for 14 days at 23 °C in the dark. Following this period, the diameter of the colonies was then measured in two directions and the average was calculated.

Temperature inhibition test: agar plugs (5 mm diameter) from margins of 18-day-old colonies of AT01, G1 and G2 were collected and placed on $PDA_{50\%}$. The plates were incubated in the dark at 25 °C and 28 °C for 14 days. Three replicates for each isolate were assessed for each temperature treatment. The diameter of the colonies was measured in two directions after 14 days. The average of the measured diameters was calculated.

Molecular biological analyses

In order to verify the results of the physiological tests on AT01, the isolate was further examined by biomolecular assays. The analysis of AT01 was conducted as described by Gehesquière (2014) using sequence-based PCR-RFLP assay of *βtub* regions at Flanders Research Institute for agriculture, fisheries and food (ILVO), Merelbeke, Belgium (Heungens, personal communication 2017).

Trials of susceptibility to *Calonectria* sp.

Plant material

For the trials following plants were used:

- *Buxus sempervirens* var. *arborescens* (syn. *B. sempervirens*; 3-year-old, Baumschule Martens GbR, Westerstede-Hoheliet, Germany)
- *Buxus sempervirens* 'Suffruticosa' (1-year-old, LVG Bad Zwischenahn, Germany)
- *Buxus microphylla* 'Herrenhausen' (syn. *B. microphylla* 'Rococo', 'Tide Hill' or *B. sinicica* var. *insularis* 'Nana'; 2-years-old, LVG Bad Zwischenahn, Germany)
- *Pachysandra terminalis* 'Compacta' (1-year-old; Baumschule Broermann, Friesoythe-Kampe, Germany)
- *Sarcococca confusa* (2-years-old; Bunk Pflanzen, Elmshorn, Germany)

All plants were cultivated from cuttings, only *Sarcococca confusa* from seedlings.

Location characteristics and time schedule of the trials

Trials were conducted in Northwest Germany (elevation 0–20 m, coastal climate, annual temperature average 9.5 °C and annual precipitation average 700–800 l m^{-2}) at two locations, Pflanzenschutzamt in Oldenburg (A) and LVG Bad Zwischenahn (B). The locations are about 15 km linear distance apart.

In both locations (A and B) the trial was conducted under two different conditions in order to see if there is a difference in disease development:

A1: Vegetation hall (a highly aerated greenhouse with the south facing wall completely open, providing high ventilation and therefore near outside temperatures but shelter against rain and wind). Plants were broadly spaced with no direct contact.

A2: Greenhouse with shading. Each replicate of 25 plants (see below) was put into frames, covered with gauze, providing shade and low ventilation. Plants stood tightly together in direct contact of their canopy to neighboring plants.

B1: Container field outside, open to weather conditions. Plants were placed to every second position of a pot tray with as little contact as possible.

B2: Greenhouse with good ventilation and soft shade. Plants were spaced with some contact.

Plants were cultivated according to the horticultural practice, with overhead irrigation when necessary, watered and fertilized as needed.

Data loggers (OM-EL-USB-1, Omega Engineering Inc., USA) were employed to record temperature and

humidity every 15 min during the trial, protected from direct sunlight and rainfall or irrigation. From collected data, average hourly temperature, relative frequency of temperature in the range of 18–25 °C (Avenot et al. 2017), average hourly relative humidity (RH), relative frequency of RH ≥ 90% and sum of hours with RH ≥ 90% was calculated (Rowlandson et al. 2015). All trials were conducted simultaneously from end of May until end of August 2017.

General layout of the trials

Plants were arranged completely randomized in five groups each containing 5 plants of each species or variety. The same raster was used for all four trials of inoculated plots as well as for non-inoculated controls. To prevent easy contamination with the pathogen of non-inoculated control plants, plastic screens were placed as a barrier, inhibiting direct splash between the inoculated treatments and the respective control.

Conidia production and inoculation

AT01 was propagated on $PDA_{50\%}$ at 23 °C for the production of conidia. In order to induce sporulation, 18-day-old, fully grown colonies of AT01 were submerged in sterile water (10 ml per plate) and rubbed intensively with a Drigalski spatula. Thereafter, the water was discarded, the plates were let to dry under sterile conditions and then covered with the lid, not sealed. After three days of incubation at 23 °C, sporulation was abundant. Conidia were harvested by abrading with a Drigalski spatula on plates fully submerged in water (10 ml). This process was repeated twice to wash off the conidia thoroughly. The conidia suspension was decanted and filtered through three layers of sterile cheese cloth.

The number of conidia was counted using a counting chamber (Thoma, Marienfeld, Germany) and the suspension of conidia for inoculation was created. Based on the number of conidia, the conidia suspension was diluted to the final inoculum concentration of 6.2×10^4 conidia ml^{-1}.

Plants were inoculated by spraying manually using a hand sprayer to the point of runoff and covered for 36 h with plastic foil and shade cloth in order to preserve optimal humidity for infection. Control plants were sprayed with water only and also covered for the same period of time. The temperatures under the cover during this infection period were 15.0–35.0 °C (A1), 18.0–32.0 °C (A2), 14.5–31.5 °C (B1), 11.5–31.0 °C (B2). Plants remained wet during this period and dried not until removing the cover.

Assessment

Plants were individually examined for typical symptoms of box blight (symptomatic leaf area, lesions on the stem and leaf drop) as well as other disease symptoms. For the evaluation of each symptom, a modified Horsfall-Barratt scale (0–100% with 5% accuracy) was applied (Fig. 1). Evaluation was conducted once before inoculation followed by assessments conducted at weekly intervals, starting on 6th of June and continued until 28th August 2017.

Statistical analyses

Nonparametric one-way analysis of variance was applied to analyze the collected data (a nonparametric test was used on the grounds of non-normal distribution of data). A Kruskal-Wallis test was used to determine the presence of statistically significant differences between each species on the level of importance $p = 0.05$. Following this, Conover-Iman test was used to make pairwise comparisons between each species. Statistical tests were conducted separately for five selected assessments: Four assessments from the beginning of the monitoring (week 1, 2, 3 and 4), when the development of the disease was the most dynamic. As a final step, the data from the last week of the monitoring (week 13) were analyzed in order to compare the development of the disease at the end of the testing period. Each monitored parameter (leaf spots, lesions and leaf drop) as well as all four locations (A1, A2, B1, B2) were tested separately. Software RStudio version 1.1.456 (RStudio, Inc.) was used for statistical analyses.

Results

Characterization of AT01

The pathogen was determined as *Calonectria* sp. by morphological characteristics of its anamorph *Cylindrocladium* sp. Microscopic observations revealed that the conidia were straight, colorless, rounded on both ends, two-celled, with an average of (36.9–)51.7(−60.0) × (4.1–)5.8(−7.5) μm ($n = 100$).

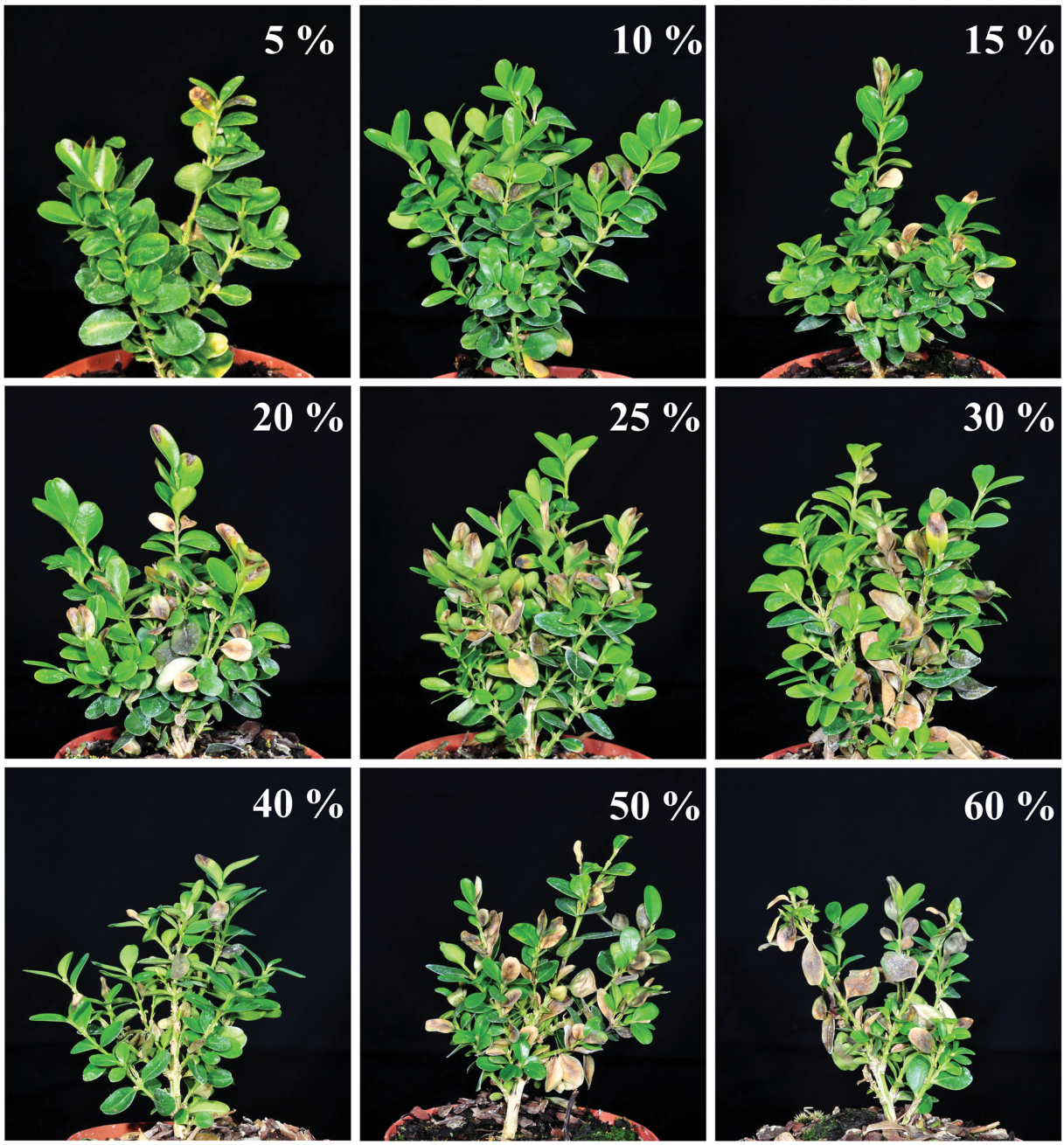

Fig. 1 Horsfall-Barratt scale used for evaluation of leaf spots caused by *Calonectria henricotiae* presented on *Buxus sempervirens* 'Suffruticosa' chart of plants

Discriminative physiological tests

Significant differences between isolates with respect to the susceptibility to active ingredients tetraconazole and kresoxim-methyl were proven (Fig. 2). Averages of controls on $PDA_{50\%}$ were measured after 14 days. Mycelial growth of G1 was completely inhibited by tetraconazole and strongly inhibited by kresoxim-methyl. Higher tolerance to both active ingredients was shown by G2. AT01 showed similar growth pattern as G2 (Table 1). According to the results of fungicide susceptibility tests, AT01 is a strain of *Calonectria henricotiae*.

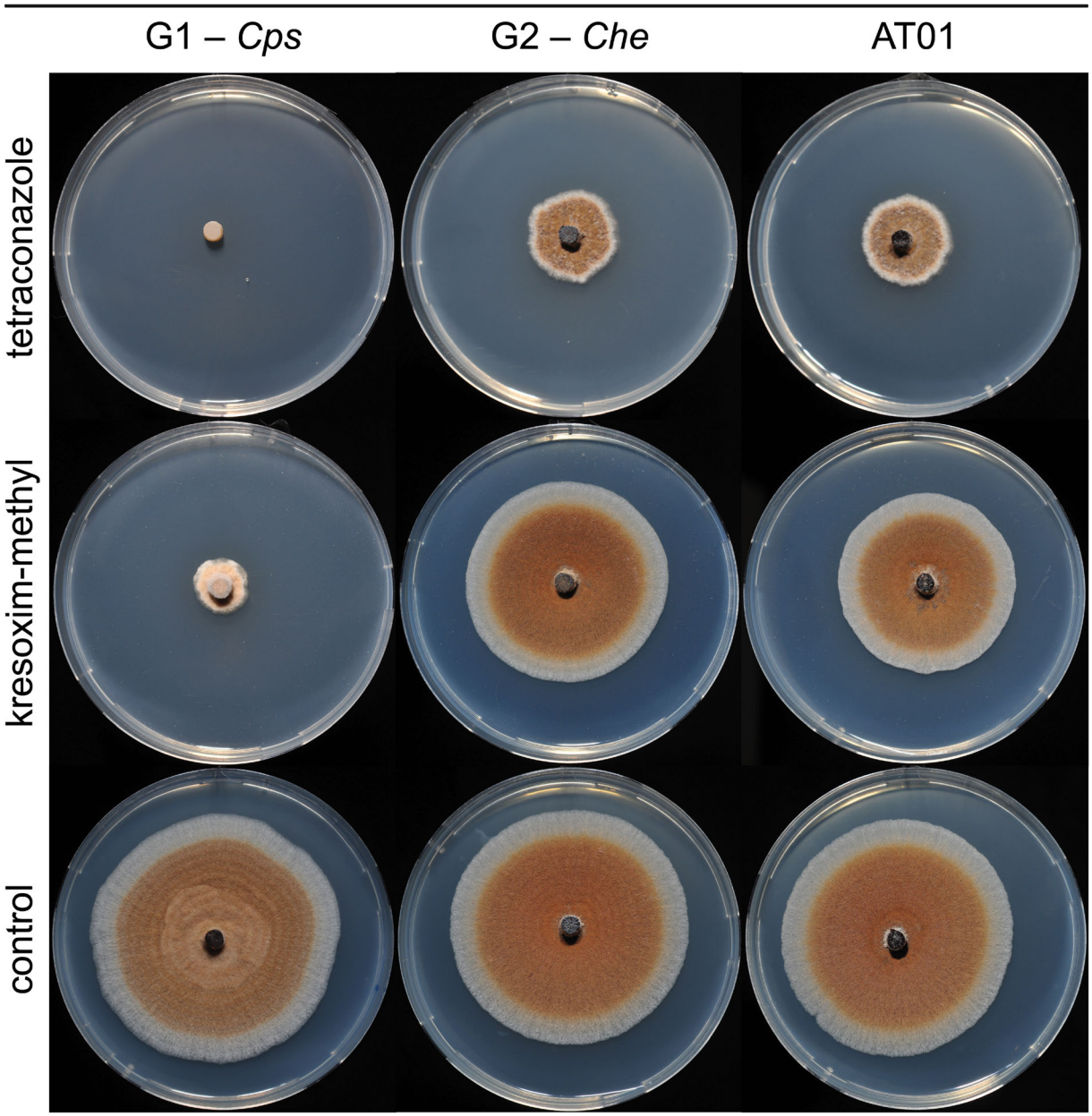

Fig. 2 Influence of active ingredients tetraconazole and kresoxim-methyl on the mycelial growth of *Calonectria pseudonaviculata* and *Calonectria henricotiae* isolates

Temperature test

Differences in mycelial growth after 14 days under different temperatures were obvious (Fig. 3). All three isolates showed similar growth at 25 °C. The average of measured diameters after 14 days was G1 = 57.0 mm; G2 = 61.3 mm; AT01 = 58.0 mm. There was no growth apparent for isolate G1 at 28 °C (G1 = 0.0 mm). The measured diameter of the isolates G2 and AT01 were comparable (G2 = 17.0 mm; AT01 = 17.7 mm). According to the results of temperature tests, AT01 is a strain of *Calonectria henricotiae*.

Table 1 Assessment of in vitro effects of fungicides on mycelial growth of strains of *Calonectria* spp. amended with fungicide active ingredients and compared to the unamended control (after 14 days on $PDA_{50\%}$ at 23 °C)

Active ingredients	AT01		*Calonectria henricotiae* (G2)		*Calonectria pseudonaviculata* (G1)	
	diameter of mycelium (mm)	comparison to control growth (%)	diameter of mycelium (mm)	comparison to control growth (%)	diameter of mycelium (mm)	comparison to control growth (%)
tetraconazole	24.2	38.2	24.6	38.8	0.0	0.0
kresoxim–methyl	46.4	73.2	50.2	79.2	14.0	22.1
control	63.4	–	65.6	–	64.0	–

Molecular biological analyses

The sequence-based PCR-RFLP identified AT01 as *Calonectria henricotiae*.

Epidemiology of AT01 on different hosts

Depending on the host, inoculation with AT01 resulted in a disease outbreak, while the non-inoculated control remained without any symptoms during the trials. The degree of infestation as well as the characteristics of symptoms differed according to host and weather conditions.

Symptoms on different hosts

B. sempervirens 'Suffruticosa' Leaf spots were light brown at first with dark margins and light green to yellow discoloration around the spots, developing to larger dark brown to black areas of the leaf tissue with no restriction (Fig. 4a). Necrosis of the whole leaf as well as quick and heavy leaf drop occurred, reducing the percentage of symptomatic leaf area. Black thin lesions were present on stems within one week after the inoculation. The lesions merged together with the progress of the disease and remained until the end of the trial.

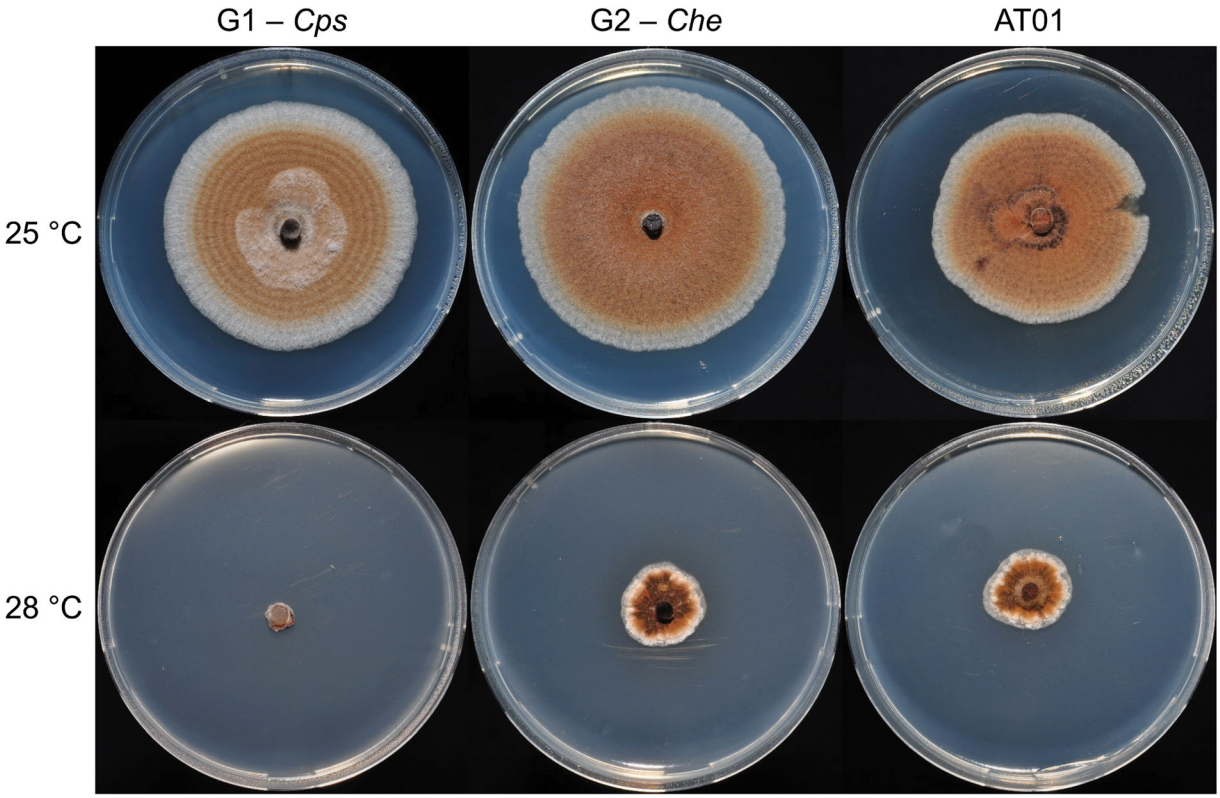

Fig. 3 Influence of temperature on the mycelial growth of *Calonectria pseudonaviculata* and *Calonectria henricotiae* isolates

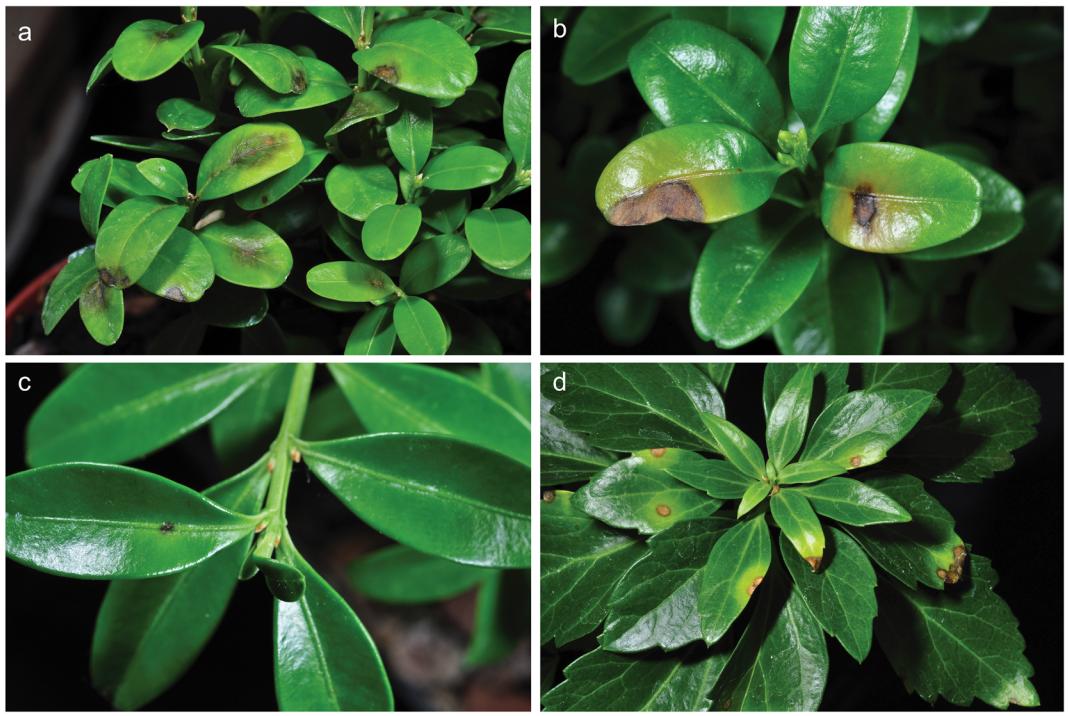

Fig. 4 Characteristic spots caused by *Calonectria henricotiae* on leaves of tested species **a** *Buxus sempervirens* 'Suffruticosa', **b** *Buxus sempervirens* var. *arborescens*, **c** *Buxus microphylla* 'Herrenhausen', **d** *Pachysandra terminalis* 'Compacta'

B. sempervirens var. arborescens Leaf spots were light brown with dark margins (Fig. 4b) and a yellow halo around the spots remaining restricted with progression of the disease. Leaf drop was moderate. On the stems single lesions occurred often below fallen leaves. In general, disease incidence as well as intensity of symptoms were less pronounced in *B. sempervirens* var. *arborescens* compared to 'Suffruticosa'.

B. microphylla 'Herrenhausen' Considerably smaller irregular black spots appeared often on the main venation of the leaves (Fig. 4c). 'Herrenhausen' was very sensitive to leaf-dropping as the leaves dropped within one week after the occurrence of the first spot on the leaf, however the overall severity of the leaf-dropping was low to moderate. Stem lesions were short, singular and quickly suberized, thus not visible anymore. Compared to the other boxwood varieties, 'Herrenhausen' showed the lowest frequency of infestation and the weakest symptoms.

Pachysandra terminalis 'Compacta' Most often leaf spots occurred at the tips of the leaves, starting as a discoloration of a small leaf area and then developing a round spot with brown center, dark margins and chlorotic halo around (Fig. 4d), very similar to those of *B. sempervirens* var. *arborescens*. With time, the chlorosis continued to enlarge until leaf drop, which remained low to moderate, statistically comparable to *B. microphylla* 'Herrenhausen' or lower. No stem lesions occurred on *Pachysandra terminalis* 'Compacta'.

Sarcococca confusa No symptoms such as leaf spots, leaf drop or stem lesions were observed at any time (data not shown).

Disease development

Symptomatic leaf area

First leaf spots appeared on *B. sempervirens* 'Suffruticosa' already one week after inoculation (a.i.). The symptomatic leaf area culminated one week later (2 weeks a.i.). At this point, the symptomatic leaf area was significantly higher on *B. sempervirens* 'Suffruticosa' in comparison to other varieties and reaching its maximum of the testing period in locations A1, A2, B2. The most severe leaf symptoms of all locations were in average 28.3% (5–70%) in A2 (Fig. 5a).

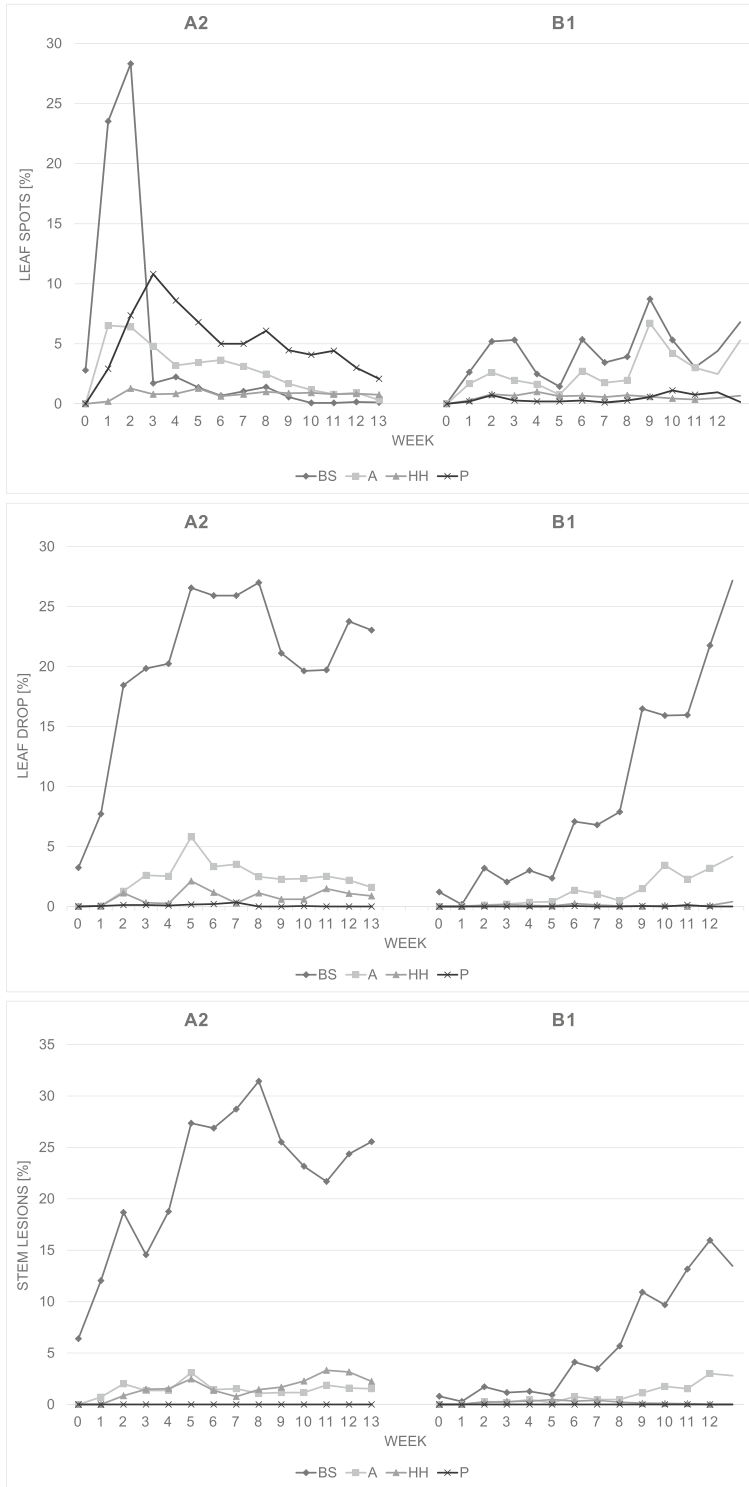

Fig. 5 Development of symptoms caused by *Calonectria henricotiae* on tested species from the inoculation (0) until the end of the trial in location A2 and B1; **a** leaf spots; **b** leaf drop; **c** stem lesions; (BS = *Buxus sempervirens* 'Suffruticosa'; A = *Buxus sempervirens* var. *arborescens*; HH = *Buxus microphylla* 'Herrenhausen'; P = *Pachysandra terminalis* 'Compacta')

Similar progression with lower incidence and intensity of infestation was apparent on the canopy of *B. sempervirens* var. *arborescens*. This course was particularly evident in A1, A2 and B2, while the disease

developed very slowly in B1, with a renewed increase at the end of the experiment.

Estimated symptomatic leaf area of *B. microphylla* 'Herrenhausen' was low over the whole testing period at all locations.

The estimated percentage of symptomatic leaf area remained low in *Pachysandra* over the whole trial course in A1, B1 and B2, with a maximum estimation of 2.96% in average in A1 four weeks after inoculation. In A2, however, the development of symptomatic leaf area of *Pachysandra* was distinct, peaking 3 weeks a.i. (10.8%). The majority of the data for *P. terminalis* 'Compacta' shows no significant difference to *B. microphylla* 'Herrenhausen' regarding the percentage of symptomatic leaf area. Although especially under the controlled condition A2, where the disease was the most severe and its progress was the most expressive on all tested species, there was no significant difference between *P. terminalis* 'Compacta' and *B. sempervirens* var. *arborescens* two and three weeks a.i. From this point on, in comparison to boxwood plants, Pachysandra showed the highest percentage of symptomatic leaf area in A2, which slowly decreased until the end of the testing period. By week 13 a.i., there was no significant difference between *P. terminalis* 'Compacta' and *B. microphylla* 'Herrenhausen' in location A1 and between *P. terminalis* 'Compacta' and all three tested *Buxus* spp. in location B2.

Leaf drop

The percentage of symptomatic leaf area decreased rapidly in correlation with leaf drop, which occurred shortly after the appearance of symptomatic leaf areas. The leaf drop of *B. sempervirens* 'Suffruticosa' occurred immediately and had gradually increasing tendency until the third week a.i. in A1 and B2, until the fifth week a.i in A2 reaching in average 26.6% and remaining around 26% for the next three weeks, followed by a decline due to new shoot growth (Fig. 5b). In B1 was the leaf drop increasing over the whole trial and culminated at the end of the testing period.

Leaf drop of *B. sempervirens* var. *arborescens* was moderate, peaking 5 weeks a.i. reaching a maximum for all locations of 5.80% in A2 and towards the end of the trial in location B1 (4.16%).

B. microphylla 'Herrenhausen' was very sensitive to leaf-dropping and the leaves dropped within one week after the occurrence of the first spot on the leaf or even before spots were visible, however the overall intensity of leaf-dropping was low with a maximum of 2.12% on average.

Leaf drop of *P. terminalis* 'Compacta' was very low, with again A2 being the location with highest estimations (maximum of 0.33% on average). Only entirely chlorotic leaves dropped. Statistically there was mostly no significant difference in leaf drop between *P. terminalis* 'Compacta' and *B. microphylla* 'Herrenhausen' at locations A1 and A2 and between *P. terminalis* 'Compacta' and *B. microphylla* 'Herrenhausen' as well as *B. sempervirens* var. *arborescens* at locations B1 and B2.

Stem lesions

The highest percentage of stem lesions of *B. sempervirens* 'Suffruticosa' was reached eight weeks a.i. in greenhouse (A2), where it increased gradually from initial 6.40% and culminated at 31.44% followed by a decrease due to new shoot growth and a stable phase at about 22–25% until the end of the trial (Fig. 5c). In the container field (B1) the onset of stem lesions was much slower than in other locations with the percentage of stem lesions gradually increasing from the week 5 a.i.

The development of stem lesions on *B. sempervirens* var. *arborescens* was in low numbers copying the development of *B. sempervirens* 'Suffruticosa' in all location except B2 (data not shown). In the conditions of A1 and B2 was the presence of lesions low in general for all boxwood species.

Microclimatic conditions

Although the differences in mean temperature and mean relative humidity (RH) over the whole period of the trials were marginal (data not shown), there were notable differences between the four localities (A1 – B2) concerning temperatures and RH, being important for disease development: The hourly average temperatures as well as the daily number of hours of RH ≥ 90% are presented in Fig. 6 for A2 (locality with the most favorable conditions).

Differences in relative frequency of temperatures favorable for the pathogen (i.e. the proportion of times with temperatures between 18 and 25 °C of the total duration of the tests) were observed between all localities (A1 – B2): 53.3% in vegetation hall (A1), 72.7% in

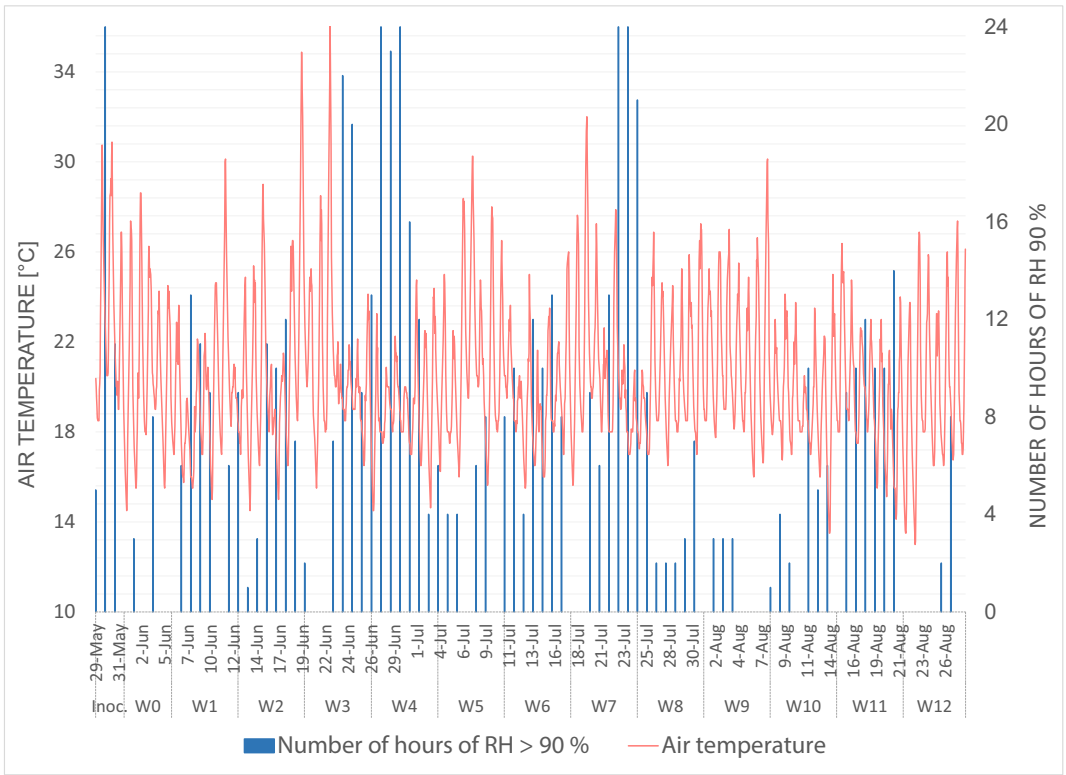

Fig. 6 Microclimate measured in the level of canopy of tested plants in locality A2, presenting hourly average air temperature (°C) and summery of hours per day with relative humidity (RH) ≥ 90% (hours)

greenhouse (A2), 34.5% in outside container field (B1, data missing week 4–8) and 43.3% in greenhouse (B2).

The relative frequency of RH lower 70% as parameter for unfavorable conditions for infection and disease development showed differences between the localities: 23.6% in vegetation hall (A1), 13.0% in greenhouse (A2), 29.9% in outside container field (B1, data missing week 4–8) and 28.2% in greenhouse (B2).

During the initial 36-h phase after inoculation, when plants were covered by plastic foil, RH continuously ranged between 90 and 100% at A1, A2 and B2. At B1 this phase with high humidity was interrupted after 21 h by a 12 h lasting phase with lower RH (77.5–89.5% RH) probably due to opening of the cover by wind.

These conditions promoted successful infection, shown by rapid and distinct development of symptoms (see above). Within the first week, the disease symptoms occurred most rapidly and most distinctly in A2, while the disease was far less pronounced in B1.

During the following period, stagnation or slight decrease of monitored symptoms development were visible between the second and third week a.i., when high average hourly temperature during the day with corresponding low RH prevailed. This phase preceded a period of high RH towards the end of the third week a.i. and in the beginning of the fourth week a.i. in all localities except B2.

Long-lasting RH in the fourth week a.i. in A2 influenced the development of symptoms expression in the fifth week a.i. of the testing period, when percentage of symptomatic area decreased, and percentage of leaf drop and stem lesions peak suddenly on all boxwood species.

Looking at the first three weeks of August, there is a clear difference between the values for the relative frequency of favorable humidity (≥ 90% RH): 31.2% in vegetation hall (A1), 20.8% in greenhouse (A2), 43.6% in outside container field (B1) and 14.4% in greenhouse (B2). The disease development during August reflected the conditions most favorable for the pathogen in B1, where a dynamic increase of symptoms was observed especially for *B. sempervirens* 'Suffruticosa' and *B. sempervirens* var. *arborescens*. In contrast, the development of symptoms stagnated in the other localities.

Discussion

Based on the results of the discriminative physiological tests as well as molecular analysis, AT01 is a strain of *Calonectria henricotiae*. This is therefore the first report of *Calonectria henricotiae* in Czech Republic. The occurrence in nurseries might be due to import of boxwood from countries where *Che* is already known. Regarding the active international trade with nursery stock in general and *Buxus* sp. in particular this is no surprise. From nurseries, further distributing to plantings such as historical gardens is likely (Bartíková et al., submitted manuscript).

The pathogen *Calonectria* is the causal agent of blight diseases on *Buxus*, *Pachysandra* and *Sarcococca*, all members of the Buxaceae family (Daughtrey 2019). The sister species of *Cps*, *Che*, is known to cause blight on *Buxus* (Gehesquière et al. 2016; LaMondia and Shishkoff 2017; Daughtrey 2019), but was not observed as pathogen on other plants, including *Pachysandra* or *Sarcococca*. To our knowledge, our experiment was the first of *Che* on potted plants of *Buxus* spp. and above all on *Pachysandra* and *Sarcococca*, at all. Beside known hosts of Buxaceae, Richardson et al. (2020) found under experimental conditions, several plant species from different families of ground covers plants to be susceptible to *Cps*, however there is no evidence for relevance under natural conditions.

Multiple tests in Europe and the USA have shown, that the susceptibility to the disease differs between species and cultivars of *Buxus* (Henricot et al. 2008; Ehsen 2011; Ganci et al. 2013a; Guo et al. 2015, 2016; LaMondia and Shishkoff 2017; Van Laere et al. 2019). Despite of slightly different results of the experiments done on whole plants (Ehsen 2011; Ganci et al. 2013a; Guo et al. 2016), mainly in consequence of the variety of tested species and cultivars of boxwood and different evaluation methods, they correspond in view of the fact that *B. sempervirens* 'Suffruticosa' being the most susceptible and in general *B. microphylla* cultivars being less susceptible. Our results support the assumption of a graduating level of susceptibility of boxwood species, stated by Ehsen (2011), Shishkoff et al. (2015) and Guo et al. (2016), from the most susceptible *B. sempervirens* 'Suffruticosa', via *B. sempervirens* var. *arborescens* to the least susceptible variety in tests, *B. microphylla* 'Herrenhausen'. The different degrees of susceptibility are generally also observed in production and in landscape plantings. However, there are some observations of serious infections of less sensitive varieties such as 'Herrenhausen' in shady and humid microclimates. According to the detached leaf assays conducted by LaMondia and Shishkoff (2017) as well as whole plant trials of Gehesquière (2014) the susceptibility of *Buxus* cultivars do not differ for the two *Calonectria* species.

As shown by LaMondia et al. (2012), LaMondia and Li (2013) as well as Brand and Bartíková (2016), *Pachysandra* spp. are susceptible hosts of *Cps*. Furthermore, LaMondia (2017) documents differences in the level of susceptibility to *Cps* among species and cultivars of *Pachysandra*. To our knowledge, this is the first evidence of *Pachysandra* being susceptible to *Che*. With regard to *Che*, *Pachysandra terminalis* 'Compacta' could be characterized as moderately susceptible to tolerant as it is approximately as susceptible as *B. microphylla* 'Herrenhausen' or, probably due to the prevailing conditions, *B. sempervirens* var. *arborescens*. It is possible that the susceptibility of *Pachysandra* differs for the pathogen species or isolate, however, hitherto there are no comparative results on the virulence of *Cps* or *Che* on different hosts tested on whole plants available. Natural infections with *Cps* of *Pachysandra* are known and reported only from the USA (Douglas 2012; Kong et al. 2017), where *Che* is not present yet (Daughtrey 2019). And even though *Pachysandra* spp. is of great importance in horticulture also in Europe and often planted in vicinity to boxwood, no reports about naturally infected plants of this or other *Pachysandra* species from Europe are available.

The same applies to *Sarcococca* spp., though much less frequently used than *Pachysandra*. Malapi-Wight et al. (2016) as well as Kong et al. (2017) report on natural infections with *Cps* of *Sarcococca* in landscapes in the USA. According to the experiments conducted on detached shoots by Ryan et al. (2018) *Sarcococca confusa* shows significantly higher susceptibility to *Cps* in comparison to other *Sarcococca* species. Based on the trials reported here, *S. confusa* could be rated as resistant to *Che* as no symptoms developed. However, after inoculation of detached shoots with *Che*, leaf spots and leaf drop occurred in vitro (Brand, unpublished data), which is in accordance with Henricot et al. (2008) and Ryan et al. (2018). Although Guo et al. (2016) found good agreement between detached leaf assays and tests on whole plants in comparative studies, evaluations of susceptibility in vitro need to be carefully interpreted as detached plant parts might show different results compared to whole plants (Orłowska et al. 2013; Shishkoff et al. 2015; LaMondia and Shishkoff 2017).

Our comparative field trials revealed that *Pachysandra* and *Sarcococca* are less suitable hosts for *Che*, which is in good accordance with the results of Kong and Hong (2019) for *Cps* in vitro. In terms of leaf infection and lesion size non-boxwood hosts showed reduced susceptibility. Knowledge of differences in susceptibility is important in disease management. Especially with regard to breeding efforts, less susceptible cultivars are of great importance and crucial for integrated disease management (Van Laere et al. 2019). However, such less susceptible, but still infected plants may possibly serve as an undetected reservoir of inoculum and source for a new infection in landscape plantings (LaMondia et al. 2012; Kong and Hong 2019).

Differences in microclimatic conditions between the locations, where the pathogen has occurred in the USA and Europe, might be causal for the varying findings. The importance of temperature and humidity is well known for box blight (Ridley 1998; Avenot et al. 2017; LeBlanc et al. 2018), which is also shown by the weather data and assessments of disease development in the presented trials. Favorable conditions for infection prevailed after moistening and covering the plants as described by Avenot (2017,), LaMondia (2017) and LaMondia and Shishkoff (2017). Furthermore, at the locality with highest humidity and temperature (A2) developed most severe blight disease for all boxwood species and *Pachysandra terminalis* 'Compacta'. Also, tight stand and overhead irrigation together with favorable conditions in A2 might have promoted the rapid spread of conidia, most likely with water splash, which is in accordance with Ganci et al. (2013b) and Gehesquière (2014). It seems that humidity is more important for disease development than temperature as the disease was on low level in the outdoor field (B1) until the humidity increased due to a rain period at the end of the trial and high RH often preceded stronger symptom expression. In contrast, periods of lower relative air humidity with high air temperature led to stagnation or decrease of symptomatic leave area development, which corresponds with experiments of dry interruptions of Avenot et al. (2017).

Conclusion

Susceptibility of *P. terminalis* 'Compacta' to *C. henricotiae* was proven, although the intensity of infestation was low and dependent on weather conditions. The epidemiology of the pathogen was closely related with the microclimatic conditions also on tested boxwood species, most importantly longer periods of high or low relative air humidity were apparent on disease development.

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Compliance with ethical standards

Conflict of interest Author M. Bartíková declares, that she has no conflict of interest. Author T. Brand declares, that he has no conflict of interest. Author H. Beltz declares, that he has no conflict of interest. Author I. Šafránková declares, that she has also no conflict of interest.

Ethical approval This research does not involve studies with human participants neither studies with animals.

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