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Determination of the Resistance of Tolerant Hybrids of *Buxus* to the Pathogen *Cylindrocladium buxicola* and the Effect of Nutrition and Climatic Conditions on Leaf Color

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Abstract

Boxwood (*Buxus* sp.) plays a key role in historical gardens due to its evergreen foliage and resilience. However, recent outbreaks of disease caused by fungal pathogens such as *Calonectria* spp. (*C. pseudonaviculata*, *C. henricotiae*) and *Pseudonectria* spp. (*P. buxi*, *P. foliicola*), as well as pest pressures from *Cydalima perspectalis*, have led to significant losses. This study examined 100 boxwood plantings across the Czech Republic to evaluate pest and disease occurrence. Further, six modern boxwood cultivars from the groups of BetterBuxus® and NewGen® were tested in field trials under the climatic conditions of the Czech Republic, focusing on their resistance to abiotic stress and foliage color retention throughout the year. Laboratory trials confirmed all cultivars were susceptible to *C. pseudonaviculata*, with 'Renaissance' showing the slowest disease progression. Field assessments under two contrasting management regimes ("Minimalistic" and "Pampered") indicated sporadic boxwood blight incidence but frequent Volutella blight outbreaks, particularly where plants suffered frost stress. Leaf color, an important esthetic trait, was evaluated using Munsell charts and measuring the relative chlorophyll content. 'Skylight' most closely matched *Buxus sempervirens* in the shade of green and winter color.

Keywords: *Calonectria pseudonaviculata*; *Pseudonectria buxi*; susceptibility; evergreen; winter color

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1. Introduction

The evergreen boxwood (*Buxus* sp., *Buxaceae*) is one of the oldest and, until recently, has been the most commonly cultivated woody ornamental, whose popularity has been increased over the centuries by its resistance to pathogens and pests. At first, it provided a consistent appearance of parterres in chateau gardens and parks throughout the year, and later it was also used in private gardens and public green spaces, cemeteries, etc. [1–4]. Since the early 2000s, after the occurrence of both a new disease (boxwood blight) and pest (boxwood moth, *Cydalima perspectalis*), a decline in interest not only in new plantings of boxwood but also in its cultivation in nurseries was recorded.

The Invasive ascomycete fungi *Calonectria pseudonaviculata* [5] and *C. henricotiae* [6] have spread globally over the last two decades, driven by the trade in young asymptomatic plants and nursery stock [7–9], in which symptoms develop only after exposure to weather conditions favorable to the pathogen [10,11]. Boxwood blight occurs not only in Europe, e.g., Germany [9], the Czech Republic [12], Turkey [3], and Italy [13], but also in the USA [14], Canada [15], and New Zealand [16]. Initial efforts in disease control of the plants, based on the knowledge of inoculum characteristics, dispersal, as well as environmental effects and fungicide efficacy, have not reached the desired level. Ref. [9] report the effectiveness of fungicides on *C. pseudonaviculata* in field conditions to be 65–82%. Simultaneously, there has been an effort to find or breed cultivars resistant to boxwood blight, which would also meet the aesthetic requirements for plantings in historic gardens, especially its habitus and unchanging dark green leaf color throughout the year.

Pseudonectria buxi (DC.) Seifert, Gräfenhan & Schroers [17], and *P. foliicola* L. Lombard & Crous [18] are primarily found on *Buxus* sp. [18–20]. While *P. buxi* has been reported in many countries in Europe, Asia, and the Americas, *P. foliicola* has only been recorded in the Czech Republic, New Zealand, and the United States [18,20–22]. Both species penetrate the tissues of stems and leaves [23,24], which usually react to their presence by changing the color of the leaves on individual shoots or large parts of the bush [24].

In addition to pathogens, leaf color is also influenced by abiotic factors, especially low temperatures during the winter months. As a result of the accumulation of carotenoids in the tissues, the color of the leaves changes to orange, red, and various shades of brown [25,26]. The color change is specific for individual species and cultivars, influenced by location, exposure to sunlight, solar intensity, and the amount of available nutrients [27]. Although this is a reversible process, as the green color is restored after winter pigmentation, it is an aesthetically significant element that should be paid attention to when choosing a boxwood cultivar.

The objective of this applied study was to test the resistant and tolerant cultivars to the *C. pseudonaviculata* pathogen under the climatic conditions of the Czech Republic. Laboratory tests evaluating plant resistance to the *C. pseudonaviculata* isolate were conducted under favorable conditions. And an important part of this study was monitoring the color change in the leaves throughout the year in two variants of cultivation. The main aim of this was to provide valuable information for possible plantings and their care in historical gardens.

2. Materials and Methods

2.1. Screening

One hundred locations in the Czech Republic were selected and visited to examine the health status of boxwood plantings (Table 1). The presence of pathogens (*Calonectria* spp. and *Pseudonectria* spp.) and pests (e.g., Cydalima *perspectalis*, *Monarthropalpus flavus*, *Psylla buxi*, and spider mites) was assessed visually at the location. In the case of the occurrence of pathogens, a symptomatic plant sample was taken and placed in a humid chamber and left at laboratory temperature for further microscopical analysis. Molecular analysis was provided only for four isolates, including the isolate further used for artificial inoculation. The collected data were entered into the original map in the QGIS program. The base map shows the average annual temperatures and precipitation in each location in 2023.

Table 1. Description of the experimental variants.

Variant I "Minimalistic"	- no irrigation - no additional fertilization - cut one time per year (end of May)
Variant II "Pampered"	- drop irrigation for 1 h every second day - regular fertilization with water-soluble fertilizer: Universol Green (4×; weekly 15 g/10 L, total 150 L; April) Universol Blue (4×; weekly 15 g/10 L, total 150 L; June) Universol Violet (4×; weekly 15 g/10 L, total 150 L; August) - cut twice a year (mid-June and end of vegetation)

2.2. Molecular Analysis

The identification of the pathogen (*Calonectria* and *Pseudonectria*) was also confirmed by sequencing analyses of two genomic regions, namely the ITS region (ITS1-5,8S DNA-ITS2) and the gene for LSU large subunit 28S RNA, at three monitored sites in the Czech Republic: Český Krumlov, Libochovice, Studenec, and Hodkov. DNA from infected boxwood leaves or derived mycelial cultures was isolated using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the standard protocol. Two DNA regions were sequenced; the coding for the ITS region was sequenced from ITS4/ITS5 primers and the LSU region from LR5/L0R primers. The corresponding ITS area (664 bp) and LSU area (922 bp) were amplified from specific primers, using Taq DNA Polymerase (Qiagen, Hilden, Germany). Reaction mixtures were prepared according to the standard protocol with the addition of 0.5 mM of each primer, 30–50 ng of genomic DNA, and a final concentration of Mg^{2+} of 1.5 mM. The PCR cycling consisted of an initial denaturation of 95 °C/3 min, followed by 40 cycles of 94 °C/15 s, 52 °C/15 s, and 72 °C/50 s for the ITS region and 40 cycles of 94 °C/15 s, 51 °C/15 s, and 72 °C/60 s for the LSU region. The resulting amplicons were separated on 1% agarose gels followed by GelRed staining. The amplified fragments were purified from agarose gel with the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and sequenced from the above-mentioned specific primers by the Sanger sequencing method. The obtained sequences were deposited in GenBank. The obtained sequences of the ITS region were deposited in GenBank under the numbers PV089704, PV089705, PV089706, and PV089707. The obtained sequences of the LSU region were deposited in GenBank under the numbers PV089814, PV089815, PV089816, and PV089817.

2.3. Plant Material

Simultaneously, six boxwood cultivars were selected for testing in the laboratory and open field. Four cultivars from the BetterBuxus® group ('Babylon Beauty', 'Heritage', 'Renaissance' and 'Skylight') and two cultivars from the NewGen® group ('Freedom' and 'Independence'). *Buxus sempervirens*, a highly susceptible species, was selected as a control.

2.4. Laboratory Testing

Eleven plants (replicates) of three-year-old seedlings of the six cultivars were used to test the resistance to the pathogen *Calonectria pseudonaviculata*. An isolate from naturally infected fresh leaves of boxwood plants from Hodkov (GPS 49°47′2.55″ N, 15°8′57.95″ E) covered on the underside with freshly formed conidiophores with conidia was used for artificial inoculation of the seedlings. The seedlings were inoculated by spraying with a suspension of *C. pseudonaviculata* conidia (3×10^6 conidia $\times$ mL^{-1}) from a smear of infected leaves. After inoculation, nine plants per cultivar were each placed in transparent plastic boxes (0.5 m × 0.5 m × 0.4 m) with the bottom covered with sterile hydrophilic foil moistened with 500 mL of distilled water. Until the end of the test, the plants were continuously irrigated with tap water as needed. The plants were incubated for 1 week at 23/18 °C and 94–100% RH, followed by 3 weeks at 20–23/18 °C and 80–90% RH.

2.5. Open Field Testing

Plants were planted in 2021 at three sites: Studenec (49°13′11.12″ N, 16°4′24.42″ E; 435 m a.s.l.; average annual temperature 7–8 °C, average annual precipitation 550–650 mm), Kroměříž (49°18′10.94″ N, 17°23′9.00″ E, 201 m a.s.l.; 8–9 °C, 550–650 mm), and Lednice (48°47′31.79″ N, 16°47′53.75″ E; 176 m a.s.l.; 9.2 °C, 479.7 mm). Two management variants, I "Minimalistic" and II "Pampered" (Table 1), were tested in three replicates. Ten plants for replicate and a total of 432 plants, including border plants, were planted randomly at each experimental site. At each site, a weather station was placed, and meteorological data (air temperature, relative air humidity, and leaf wetness) were automatically collected.

2.6. Pathogen Evaluation

Plants were assessed monthly (March–November) using the modified Horsfall–Barratt scale (Table 2) for pathogen and pest intensity (Table 2). Data were statistically analyzed.

Table 2. Modified Horsfall–Barratt scale—assessment of pathogen occurrence.

Damage Degree	Volutella Blight	Boxwood Blight
1	healthy plants, no symptoms (0%)	healthy leaves and stems, no signs of infestation (0%)
2	loss of the leaf gloss, slight change in color, small spots on the edges of the leaves (1–5%)	individual brown spots on leaves, no leaf drop, no stem lesions (1–5%)
3	spots cover up to half of the leaf blade, leaves turn yellow (6–20%)	spots on leaves cover up to one-half of the blade, do not necrotize, occasional leaf drop, occasional stem lesions (6–20%)
4	leaves turn bronze, bark at the base of the stem cracks, occasionally peels off (21–35%)	drop of primarily infected leaves, formation of spots on new leaves, black lesions on a third of the stem (21–35%)
5	beginning of the dieback of the shoots, necrosis of the wood underneath the bark (36–50%)	leaf spots and stem lesions up to half of the plant, severe leaf drop, especially of the inner parts of the plants (36–50%)
6	dieback of infected shoots or entire plants (51–100%)	new spots occurring on newly grown leaves, stems with numerous long black lesions, almost defoliated plants (51–100%)

2.7. Winter Coloring

The color of the leaves was evaluated using the Munsell Plant Tissue Color Charts, recording hue, brightness, and saturation. From each cultivar, variant, and repetition, two properly developed leaves (fourth leaf from the top of the shoot) were randomly selected and evaluated. A total of six leaves per cultivar and location. Simultaneously, the Normalized Difference Vegetation Index of each leaf was recorded using the PlantPen NDVI300 device (Photon System Instruments, Drásov, Czech Republic).

2.8. Statistical Analysis

The values recorded in 2023 and 2024 were statistically evaluated using TIBCO Statistica 14 software. Descriptive statistics (mean and standard deviation) were calculated for all measured parameters. Homogeneity of variances was tested using Cochran's and Bartlett's tests. Differences among the individual variants were assessed using analysis of variance (ANOVA) at a significance level of $\alpha = 0.05$. When significant effects were found, post hoc comparisons were performed using Tukey's HSD test.

3. Results

3.1. Screening

Survey results are mapped and available on an interactive map (https://www.zdravybuxus.com/mapy, accessed on 15 October 2025). Of the 100 boxwood plantings inspected, 17 cases of boxwood blight and 48 cases *of Pseudonectria* spp. infections (including four cases of *Scolecofusarium ciliatum*) were recorded. *Cydalima perspectalis* was recorded at 59 plantings. Only five stands appeared to be pathogen and pest free (Figure 1).

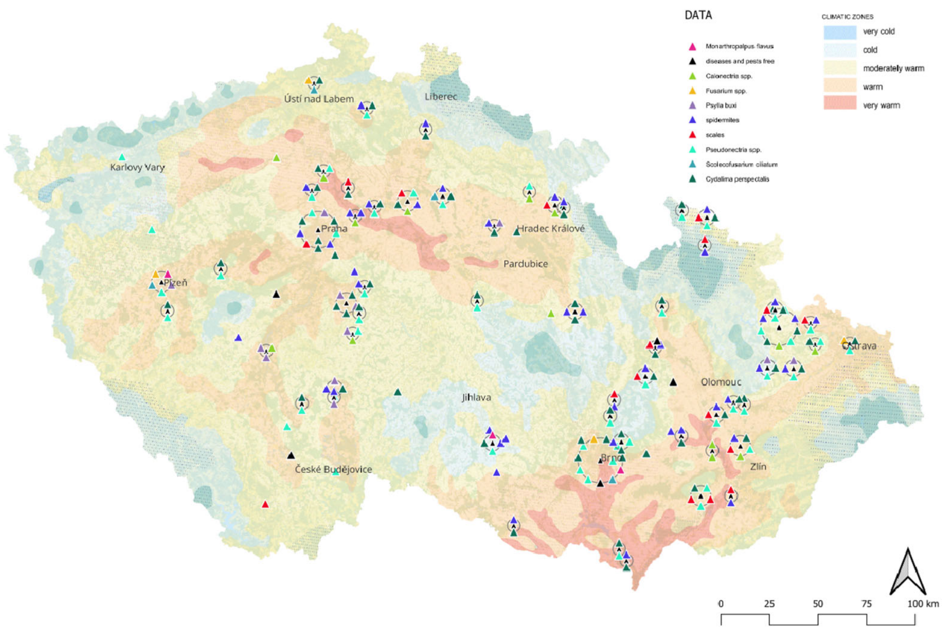

Figure 1. Occurrence of pathogens and pests of boxwood in the Czech Republic in 2023–2024.

3.2. Laboratory Tests

All cultivars were susceptible to *C. pseudonaviculata* under laboratory conditions (Table 3). The first visible symptoms (leaf spots) appeared 4 days post-inoculation. Analysis of variance revealed significant effects of both cultivar (F (7, 225) = 43.15, $p < 0.001$) and time after inoculation (F (2, 225) = 13.43, $p < 0.001$) on disease severity. However, the interaction between cultivar and time was not significant (F (14, 225) = 1.21, $p = 0.269$), indicating that the pattern of symptom development over time was similar among cultivars. The control plants (*Buxus sempervirens*) showed the heaviest infection, statistically similar to 'Freedom' and 'Skylight'. 'Renaissance' was least infected and showed symptoms first on day 18 after inoculation. Infection intensity was statistically comparable to the untreated control along with 'Independence' and 'Heritage'. These findings indicate that, although all tested cultivars were susceptible to *C. pseudonaviculata*, the degree of infection differed significantly among genotypes.

Table 3. Disease severity after artificial inoculation with *C. pseudonaviculata* was evaluated using the modified Horsfall–Barratt scale under controlled conditions.

Cultivar	Time After Inoculation (Days)	The Presence of Symptoms (Score)	Time After Inoculation (Days)	The Presence of Symptoms (Score)	Time After Inoculation (Days)	The Presence of Symptoms (Score)
Buxus sempervirens	4	1.09 ± 0.70^{b}	8	2.36 ± 0.92^{c}	18	2.82 ± 0.60^{d}
'Babylon Beauty'	4	1.18 ± 0.41^{a}	8	1.36 ± 0.51^{ab}	18	2.00 ± 0.63^{bc}
'Skylight'	4	1.82 ± 0.40^{b}	8	1.91 ± 0.54^{bc}	18	2.27 ± 0.47^{bd}

Table 3. *Cont.*

Cultivar	Time After Inoculation (Days)	The Presence of Symptoms (Score)	Time After Inoculation (Days)	The Presence of Symptoms (Score)	Time After Inoculation (Days)	The Presence of Symptoms (Score)
'Renaissance'	4	1.00 ± 0.00^{a}	8	1.00 ± 0.00^{a}	18	1.18 ± 0.40^{a}
'Heritage'	4	1.18 ± 0.40^{a}	8	1.27 ± 0.47^{ab}	18	1.36 ± 0.50^{ac}
'New Gen Independence'	4	1.14 ± 0.38^{a}	8	1.29 ± 0.49^{ab}	18	1.57 ± 0.53^{abc}
'New Gen Freedom'	4	2.20 ± 0.42^{b}	8	2.10 ± 0.32^{bc}	18	2.30 ± 0.48^{bd}
Buxus sempervirens—NC *	4	1.00 ± 0.00^{a}	8	1.00 ± 0.00^{a}	18	1.00 ± 0.00^{a}

*—Negative Control plants. Data are mean ± SD; different letters ^{a,b,c,d} indicate statistically significant differences between cultivars in one-way ANOVA analysis ($p < 0.05$); score 1–6, 1 = no infestation.

3.3. Open Field Tests

Low temperatures were the most common and repetitive cause of damage, particularly at the Sudenec site (Figure 2). Table 4 shows the differences between individual sites based on the average temperatures reached, the lowest temperatures reached in a given month, and the number of days with temperatures below 0 °C (frost days).

Figure 2. Severe frost damage at the Studenec site, spring 2024.

Table 4. Temperature differences between locations.

Site		Lednice			Studenec			Kroměříž		
Year	Month	AMT (°C)	AMINT (°C)	NFD (day)	AMT (°C)	AMINT (°C)	NFD (day)	AMT (°C)	AMINT (°C)	NFD (day)
2023	1	2.9	−7.9	15.0	1.4	−10.6	22.0	3.2	−4.6	13.0
	2	2.6	−10.5	19.0	1.0	−12.1	20.0	2.3	−9.3	20.0
	3	6.6	−7.5	16.0	4.6	−7.9	20.0	6.5	−6.4	14.0
	4	8.7	−7.7	4.0	6.6	−8.5	12.0	8.4	−5.1	6.0
	5	14.6	−0.5	1.0	12.6	−1.1	3.0	13.7	0.5	N
	6	19.5	5.2	N	17.7	1.8	N	N	N	N
	7	22.7	8.0	N	20.7	5.8	N	N	N	N
	8	20.7	7.5	N	18.8	3.8	N	20.4	7.0	N
	9	18.4	6.7	N	16.3	3.0	N	17.7	6.9	N
	10	12.8	−4.2	3.0	10.6	−4.1	5.0	12.4	−2.3	4.0
	11	5.6	−6.9	11.0	3.8	−9.8	18.0	5.7	−6.6	12.0
	12	1.9	−18.5	20.0	0.8	−21.4	24.0	2.6	−5.6	17.0

Table 4. *Cont.*

Site		Lednice			Studenec			Kroměříž		
Year	**Month**	**AMT (°C)**	**AMINT (°C)**	**NFD (day)**	**AMT (°C)**	**AMINT (°C)**	**NFD (day)**	**AMT (°C)**	**AMINT (°C)**	**NFD (day)**
2024	1	0.6	−13.2	24.0	−1.0	−14.3	28.0	0.3	−15.1	25.0
	2	7.2	−3.8	8.0	5.3	−5.3	9.0	7.3	−4.3	6.0
	3	9.0	−5.2	9.0	6.8	−7.0	14.0	8.9	−5.0	8.0
	4	12.3	−2.6	6.0	9.7	−6.1	13.0	12.0	−2.1	6.0
	5	16.8	3.0	N	14.5	0.9	N	16.6	2.6	N
	6	20.8	6.6	N	18.5	3.3	N	20.0	6.3	N
	7	23.3	8.4	N	20.6	5.8	N	22.3	9.1	N
	8	23.3	7.6	N	21.3	5.5	N	21.9	9.1	N
	9	16.5	−0.1	1.0	14.9	−1.7	2.0	16.1	1.5	N
	10	10.8	−0.8	1.0	9.2	−1.3	5.0	10.5	−0.1	1.0
	11	3.1	−5.3	19.0	1.8	−7.3	23.0	3.6	−3.6	18.0
	12	1.3	−7.6	22.0	0.0	−8.6	25.0	1.2	−8.3	23.0

AMT—average monthly temperature, AMINT—absolute minimum temperature, NFD—number of frost days.
N = no temperature below 0 was recorded.

The occurrence of boxwood blight was sporadic, detected only on 'Freedom' at Kroměříž in September 2023 and 2024 following rain periods with high humidity. *Pseudonectria buxi* was first recorded at Studenec (March 2023) on winter coloring shoots of 'Heritage'. In May, *Pseudonectria buxi* was observed at the Lednice on various cultivars ('Heritage', 'Babylon Beauty', 'Renaissance' and 'Independence'). Afterwards, *Pseudonectria buxi* was regularly detected at both sites. The highest intensity and frequency of damage were observed at Studenec, where damage to some cultivars ('Heritage', 'Babylon Beauty', and 'Renaissance') led to the dieback of infested shoots (Figure 3). The pathogen infestation preceded severe frost damage.

3.4. Winter Coloring

No tested cultivar matched the rich dark green color year-round of *B. sempervirens*. 'Independence' and 'Skylight' showed the most similar color compared to the control. Leaves of 'Independence' were of the highest intensity and saturation of dark green (Figure 4), and 'Skylight' maintained dark green color throughout the year at Studenec and Kroměříž. 'Babylon Beauty' showed the lightest coloration overall, even in the fertilized variant. 'Heritage' and 'Freedom' showed orange-brown coloration in winter months and persisted until the spring at Studenec (Figure 5). 'Renaissance' showed gradual, homogeneous color changes throughout the year with minimal variant differences. Only a slight difference between variants was observed. Orange-brown coloration correlated with the presence of *Pseudonectria buxi*.

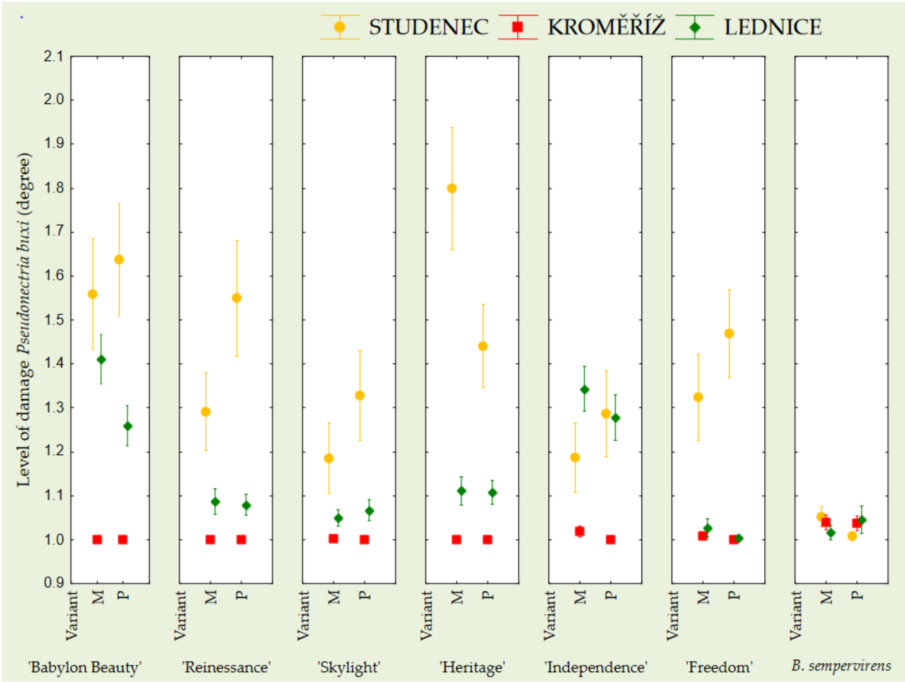

Figure 3. Intensity of *Pseudonectria buxi* infestation expressed using the modified Horsfall–Barratt scale (1–6, 1 = no infestation). M = "Minimalistic" variant, P = "Pampered" variant. Values represent means ± SD; error bars indicate within-treatment variability. Significant effects were found for site and cultivar ($p < 0.001$), while the effect of treatment variant was not significant ($p = 0.67$).

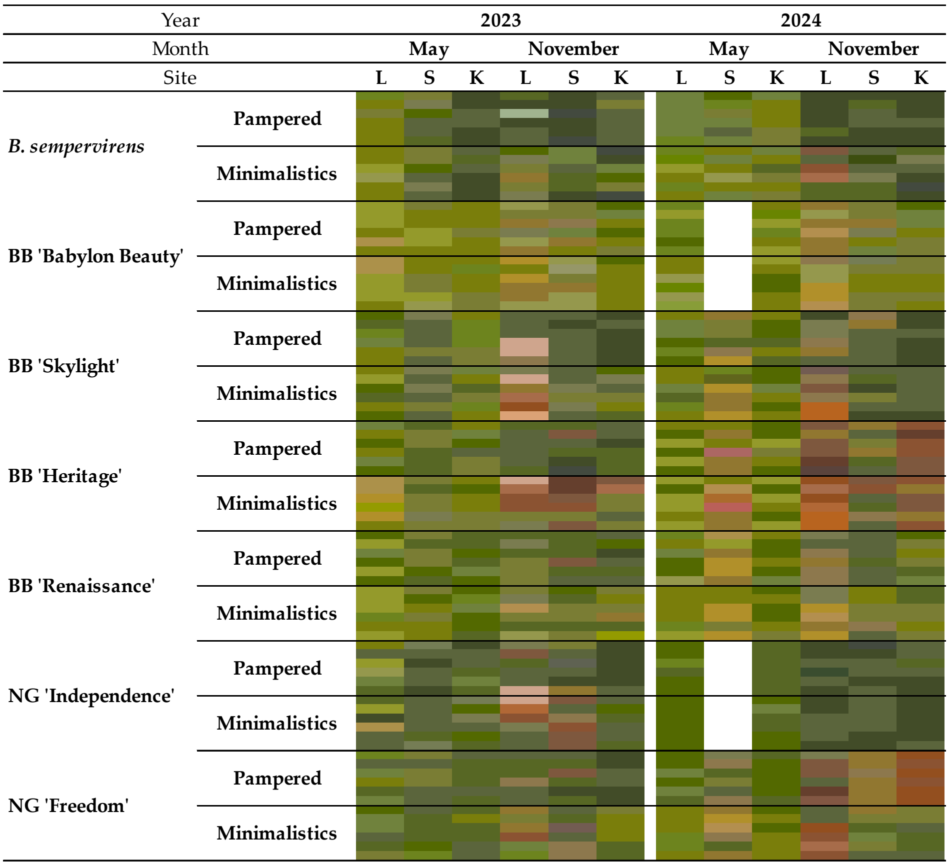

Figure 4. Color shades of the monitored cultivars were recorded in May and November 2023 and 2024. Site: L—Lednice, S—Studenec, K—Kroměříž; blank spots indicate plants where it was not possible to record the color of the leaves due to frost damage.

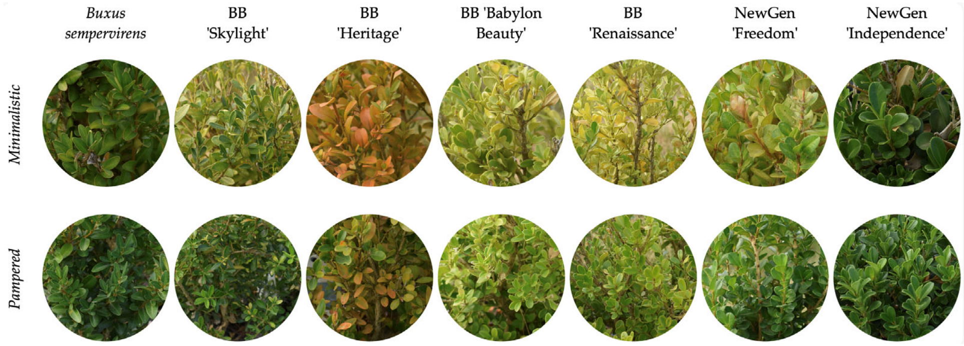

Figure 5. Assessment of color changes in foliage at the Lednice site (5 September 2024).

Statistically significantly lower NDVI average values were achieved for 'Babylon Beauty' in both variants (Table 5). Also, the "Minimalistic" variant (Figure 6) achieved a statistically significant lower NDVI than "Pampered" over the years 2023–2024, which was particularly evident for *B. sempervirens*, reaching an average index of 0.64 ("Pampered" variant), significantly different from other tested cultivars. Only 'Independence' had a 0.01 lower average index in the "Pampered" variant. Cultivars 'Renaissance', 'Skylight', and 'Heritage' in the "Pampered" variant generally achieved higher average NDVI values. The lowest average value of the chlorophyll content was measured in 'Heritage' leaves (index = 0.44, May 2023, "Minimalistic" variant), and the highest in *B. sempervirens* (0.77, November, the "Pampered" variant).

Table 5. The average NDVI values of the cultivars over the testing period (2023–2024).

Variant	Tested Cultivar	NDVI
"Minimalistic"	BetterBuxus® 'Babylon Beauty'	0.54 ± 0.07 [a]
	BetterBuxus® 'Renaissance'	0.58 ± 0.06 [b]
	BetterBuxus® 'Skylight'	0.59 ± 0.07 [bc]
	BetterBuxus® 'Heritage'	0.58 ± 0.08 [bc]
	NewGen® 'Independence'	0.60 ± 0.08 [c]
	NewGen® 'Freedom'	0.59 ± 0.07 [bc]
	Bxuxus sempervirens	0.60 ± 0.08 [c]
"Pampered"	BetterBuxus® 'Babylon Beauty'	0.55 ± 0.10 [a]
	BetterBuxus® 'Renaissance'	0.61 ± 0.06 [bc]
	BetterBuxus® 'Skylight'	0.61 ± 0.05 [bc]
	BetterBuxus®'Heritage'	0.61 ± 0.09 [bc]
	NewGen® 'Independence'	0.59 ± 0.12 [bc]
	NewGen® 'Freedom'	0.60 ± 0.08 [bc]
	Bxuxus sempervirens	0.64 ± 0.05 [d]

Data are presented as mean ± SD. Different letters indicate statistically significant differences between cultivars based on two-way ANOVA analysis followed by Tukey's HSD test ($p < 0.05$). Significant effects were found for variant ($p < 0.0001$), cultivar ($p < 0.0001$), and their interaction ($p < 0.001$).

Figure 6. Winter coloring of model plant leaves, "Minimalistic" variant, Lednice, autumn 2023.

4. Discussion

Since the last survey in the Czech Republic [28], the pests and diseases have spread significantly. Molecular analysis of symptomatic plant samples was not part of the project, however the genera of relevant pathogens (*Calonectria* and *Pseudonectria*) were confirmed by microscopic observation and their distribution demonstrated.

The primary objective of the project was to promote sustainable production by using resistant cultivars, thereby reducing pesticide use and minimizing their impact on the environment. While breeding and selection for resistant or tolerant varieties has long been a key part of integrated pest management (IPM) in agriculture and fruit and vegetable production, the focus in the breeding of ornamental plants and especially in woody plant production is primarily on aesthetic value. Only recently have initiatives and breeding programs by ornamental nurseries in Europe and the United States demonstrated progress towards selecting tolerant boxwood species [29,30]. Ref. [29] also recommend a two-stage selection process to effectively breed resistant boxwood to boxwood blight. They suggest eliminating highly susceptible cultivars by artificially inoculating and then field testing the more resistant seedlings under natural infection for several years. Such field trials can also be used to determine other important traits, such as growth habit, winter coloring, and winter hardiness.

Boxwood is unquestionably one of the most economically important ornamental crops. Its future likely depends on a combination of conventional and molecular breeding methods, alongside genetic engineering approaches [31–34]. As the laboratory tests demonstrated that none of the tested cultivars exhibited resistance to the pathogen *Calonectria pseudonaviculata*, which underscores the urgent need for continued breeding efforts and possibly biotechnological interventions. Moreover, field tests revealed a high incidence and severity of Volutella blight, highlighting its persistent threat to boxwood health. However, it is considered to be a secondary pathogen [35], occurring on senescent and damaged plant parts. It is possible that *Pseudonectria* species act as endophytes or latent pathogens in boxwood plants, with their outbreaks often triggered by physical damage or abiotic stress [11,36,37]. It is also important to note that pest infestations, such as those caused by *Cydalima perspectalis*, can increase a plant's susceptibility to disease. Defoliation caused by caterpillars of this invasive moth damages leaf blades and weakens boxwood plants, and therefore increases their susceptibility to secondary fungal infections, including the pathogen *Calonectria pseudonaviculata*. This underscores the necessity of integrating disease resistance strategies and pest monitoring into breeding and cultivation programs [38–40].

This pattern aligns with the observed prevalence of *Pseudonectria*-infected plants at Lednice and Studenec, as well as the occurrence of Volutella blight on winter-discolored shoots of 'Heritage' at Studenec. Furthermore, [11] microclimatic conditions, primarily temperature extremes and humidity fluctuations, can significantly influence the susceptibility of boxwood to fungal infections.

Low temperatures were the most common cause of damage, which significantly limits the success of the introduction of any plant species [41]. Although no significant differences in abiotic damage intensity were found between "Minimalistic" and "Pampered" variants across 2023–2024. In the "Minimalistic" variant, all cultivars showed significantly higher damage than the *B. sempervirens* control. In the "Pampered" variant, only 'Renaissance' and 'Skylight' achieved low damage levels, comparable to *B. sempervirens*. 'Babylon Beauty' and 'Independence' showed the highest damage in both variants.

An important aspect for the possible use of new boxwood cultivars in garden and landscape architecture is the dark green color of the foliage throughout the year and the growth habit [29]. Winter coloring, also referred to as "winter bronzing", can be undesirable and can change the aesthetic value of architectural work, especially of historical gardens. The visual assessment of leaf color change (Munsell charts evaluation) in boxwood was minimally affected by the variant, i.e., fertilization. The results are consistent with [42] presenting that fertilization had no effect on the results of their experiment and the leaf color change in the new cultivars ('Freedom' and 'Independence'), where 'Independence' had the lowest level of winter color change.

On the contrary, differences between variants were observed by measuring the NDVI. The higher the NDVI, the less stressed the plants are [43]. In 2023, the monitored cultivars achieved higher average NDVI values than in 2024, most likely due to low temperatures in winter months, particularly at Studenec and Lednice. Also, plants of the "Minimalistic" variant often had lower NDVI values than in the "Pampered" one, which was mainly due to different plant care management. Based on these findings, it is advisable to provide the plants with sufficient nutrients in order to lower the level of winter color change.

These findings reinforce the importance of integrating abiotic stress and disease and pest resistance into breeding programs for boxwood to enhance their resilience and ensure sustainability in ornamental horticulture.

5. Conclusions

Testing of newly bred resistant and tolerant cultivars of boxwood (*Buxus* sp.) took place during the monitored period in 2023–2024. This is a very short period of time, which was taken into account when evaluating the results. And given the longevity of the plants, it would be advisable to continue the experiments. None of the tested cultivars bred for resistance or tolerance to boxwood blight proved to be fully resistant to *Calonectria pseudonaviculata*. This was demonstrated under laboratory conditions optimal for pathogen infection. Furthermore, in field conditions where no inoculation was performed, isolated occurrences of the pathogen were observed on 'Freedom'. Based on the findings, the cultivar 'Renaissance' can be a relatively better option for planting in the climatic conditions of the Czech Republic. In laboratory experiments, it showed the highest resistance to the pathogen *Calonectria pseudonaviculata*. However, in field conditions, infestation with the pathogen *Pseudonectria buxi* was observed, especially in combination with abiotic stress caused by frost damage. In comparison to the commonly planted B. sempervirens, the cultivar does not achieve the same intensity of leaf color, and the shade changes throughout the year. Frost damage was observed on the cultivar, mainly due to insufficiently mature tissue in warm autumn. However, the damage was not deep, mainly affecting the tops of the shoots, and with sufficient nutrition the plants regenerated relatively well. Continued monitoring and implementation of advanced IPM are recommended to support sustainable use of boxwood in historic and modern gardens.

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Abbreviations

The following abbreviations are used in this manuscript:

BB	BetterBuxus®
NG	NewGen®
NDVI	Normalized Difference Vegetation Index
IPM	Integrated Pest Management

References

1. Batdorf, L.R. *Boxwood Handbook—A Practical Guide to Knowing and Growing Boxwood*, 3rd ed.; The American Boxwood Society, Greater Valley Publications Inc.: Winchester, VA, USA, 2005; 123p, ISBN 978-1886833012.
2. Kramer, M.; Guo, Y.; Pooler, M. Ranking resistance of Buxus cultivars to boxwood blight—An integrated analysis. *J. Environ. Hortic.* **2020**, *38*, 50–55. [CrossRef]
3. Lehtijärvi, A.; Doğmuş-Lehtijärvi, H.T.; Oskay, F. Boxwood blight in Turkey: Impact on natural boxwood populations and management challenges. *Balt. For.* **2017**, *23*, 274–278.
4. Shishkoff, N.; Miller, M.E.; Cubeta, M.A. Rooting response of boxwood cultivars to hot water treatment and thermal sensitivity of *Calonectria henricotiae* and *C. pseudonaviculata* in diseased boxwood (*Buxus* spp.). *J. Environ. Hortic.* **2021**, *39*, 1–10. [CrossRef]
5. Lombard, L.; Crous, P.W.; Wingfield, B.D.; Wingfield, M.J. Systematics of *Calonectria*: A genus of root, shoot and foliar pathogens. *Stud. Mycol.* **2010**, *66*, 1–71. [CrossRef] [PubMed]
6. Gehesquière, B.; Crouch, J.A.; Marra, R.E.; Van Poucke, K.; Rys, F.; Maes, M.; Gobin, B.; Höfte, M.; Heungens, K. Characterization and taxonomic reassessment of the box blight pathogen *Calonectria pseudonaviculata*, introducing *Calonectria henricotiae* sp. nov. *Plant Pathol.* **2016**, *65*, 37–52. [CrossRef]
7. Daughtrey, M.L. Boxwood blight: Threat to ornamentals. *Annu. Rev. Phytopathol.* **2019**, *57*, 189–209. [CrossRef]
8. Gehesquière, B. *Cylindrocladium buxicola* Nom. Cons. Prop. (syn. *Calonectria pseudonaviculata*) on Buxus: Molecular Characterization, Epidemiology, Host Resistance and Fungicide Control. Ph.D. Thesis, Ghent University, Ghent, Belgium, 2014.
9. Brand, T.; Beltz, H.; Adhikari, U.; Daughtrey, M.L.; Luster, D.G.; Kong, P.; Hong, C. Evaluation of fungicides for management of boxwood blight caused by *Calonectria* spp. under field conditions in Northern Germany. *J. Plant Dis. Prot.* **2023**, *130*, 325–335. [CrossRef]
10. LeBlanc, N.; Salgado-Salazar, C.; Crouch, J.A. Boxwood blight: An ongoing threat to ornamental and native boxwood. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 4371–4380. [CrossRef]
11. Khaliq, I.; Brand, T.; Weiland, J.E.; Hong, C.; Daughtrey, M.L.; Shishkoff, N. Investigating Weather Variables Driving Boxwood Blight Epidemics. *Plant Pathol.* **2024**, *73*, e13969. [CrossRef]
12. Šafránková, I.; Holková, L.; Kmoch, M. First report of *Cylindrocladium buxicola* on box in the Czech Republic. *New Dis. Rep.* **2012**, *25*, 5. [CrossRef]

13. Garibaldi, A.; Bertetti, D.; Ortu, G.; Gullino, M.L. First report of Volutella blight caused by *Pseudonectria buxi* on Japanese boxwood (*Buxus microphylla*) in Italy. *Plant Dis.* **2016**, *100*, 1499. [CrossRef]
14. Douglas, S.M. *Boxwood Blight—A New Disease for Connecticut and the U.S*; The Connecticut Agricultural Experiment Station: New Haven, CT, USA, 2011.
15. Elmhirst, J.F.; Auxier, B.E.; Wegener, L.A. First report of box blight caused by *Cylindrocladium pseudonaviculatum* (*C. buxicola*) in British Columbia, Canada. *Plant Dis.* **2013**, *97*, 559. [CrossRef] [PubMed]
16. Henricot, B.; Culham, A. *Cylindrocladium buxicola*, a new species affecting *Buxus* spp., and its phylogenetic status. *Mycologia* **2002**, *94*, 980–997. [CrossRef] [PubMed]
17. Gräfenhan, T.; Schroers, H.J.; Nirenberg, H.I.; Seifert, K.A. An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in Cosmospora, Acremonium, Fusarium, Stilbella, and Volutella. *Stud. Mycol.* **2011**, *68*, 79–113. [CrossRef]
18. Lombard, L.; van der Merwe, N.A.; Groenewald, J.Z.; Crous, P.W. Generic concepts in *Nectriaceae*. *Stud. Mycol.* **2015**, *80*, 189–245. [CrossRef]
19. Castroagudín, V.L.J.; Weiland, E.; Baysal-Gurel, F.; Cubeta, M.A.; Daughtrey, M.L.; Gauthier, N.W.; LaMondia, J.; Luster, D.G.; Hand, F.P.; Shishkoff, N. One clonal lineage of *Calonectria pseudonaviculata* is primarily responsible for the boxwood blight epidemic in the United States. *Phytopathology* **2020**, *110*, 1845–1853. [CrossRef]
20. Salgado-Salazar, C.; Shiskoff, N.; LeBlanc, N.; Ismaiel, A.A.; Collins, M.; Cubeta, M.A.; Crouch, J.A. *Coccinonectria pachysandricola*, causal agent of a new foliar blight disease of *Sarcococca hookeriana*. *Plant Dis.* **2019**, *103*, 1337–1346. [CrossRef]
21. Špetík, M.; Berraf-Tebbal, A.; Cechova, J.; Eichmeier, A. Occurrence of *Pseudonectria foliicola* causing Volutella blight on boxwood in Czech Republic. *Plant Dis.* **2020**, *104*, 1547. [CrossRef]
22. Baysal-Gurel, F.; Bika, R.; Avin, F.A.; Jennings, C.; Simmons, T. Occurrence of Volutella blight caused by *Pseudonectria foliicola* on boxwood in Tennessee. *Plant Dis.* **2021**, *105*, 2014. [CrossRef]
23. Rivera, Y.; Salgado-Salazar, C.; Veltri, D.; Malapi-Wight, M.; Crouch, J.A. Genome analysis of the ubiquitous boxwood pathogen *Pseudonectria foliicola*. *PeerJ* **2018**, *6*, e5401. [CrossRef]
24. Shi, F.; Hsiang, T. First report of *Pseudonectria buxi* causing Volutella blight on boxwood (*Buxus* sp.) in Beijing, China. *Plant Dis.* **2014**, *98*, 1282. [CrossRef]
25. Brand, T.; Grüner, J. *Gehölzkrankheiten*; Eugen Ulmer KG: Stuttgart, Germany, 2024; 195p, ISBN 978-3-8186-2052-3.
26. Hormaetxe, K.; Hernández, A.; Becerril, J.M.; García-Plazaola, J.I. Role of red carotenoids in photoprotection during winter acclimation in *Buxus sempervirens* leaves. *Plant Biol.* **2004**, *6*, 325–332. [CrossRef]
27. Hughes, N.M. Winter leaf reddening in 'evergreen' species. *New Phytol.* **2011**, *190*, 573–581. [CrossRef]
28. Bartíková, M.; Holková, L.; Šafránková, I. Occurrence of boxwood blight (*Calonectria pseudonaviculata* and *C. henricotiae*) in historical gardens in the Czech Republic. *Eur. J. Plant Pathol.* **2020**, *158*, 1007–1019. [CrossRef]
29. Van Laere, K.; Heungens, K.; Gehesquière, B.; Leus, L.; Hermans, D.; Van Huylenbroeck, J. Breeding and selection of *Buxus* for resistance to *Calonectria pseudonaviculata*. *J. Phytopathol.* **2019**, *167*, 363–370. [CrossRef]
30. Omolehin, O.; Keller, J.; Gouker, F.; Daughtrey, M.; Luster, D.; Pscheidt, J.; Hong, C. Combating an invasive boxwood pathogen—*Calonectria pseudonaviculata*—In the United States by shifting production to less-susceptible cultivars. *Plant Dis.* **2023**, *107*, 2185–2196. [CrossRef] [PubMed]
31. Leus, L. Breeding for disease resistance in ornamentals. In *Ornamental Crops*; Handbook of Plant Breeding; Van Huylenbroeck, J., Ed.; Springer: Cham, Switzerland, 2018; Volume 11, pp. 225–246. [CrossRef]
32. Mekapogu, M.; Jung, J.-A.; Kwon, O.-K.; Ahn, M.-S.; Song, H.-Y.; Jang, S. Recent progress in enhancing fungal disease resistance in ornamental plants. *Int. J. Mol. Sci.* **2021**, *22*, 7956. [CrossRef]
33. Van Huylenbroeck, J.; Bhattarai, K. Ornamental plant breeding: Entering a new era? *Ornam. Hortic.* **2022**, *28*, 297–305. [CrossRef]
34. Van Laere, K.; Hokanson, S.C.; Contreras, R.; Van Huylenbroeck, J. Woody ornamentals of the temperate zone. In *Ornamental Crops*; Handbook of Plant Breeding; Van Huylenbroeck, J., Ed.; Springer: Cham, Switzerland, 2018; Volume 11. [CrossRef]
35. Dhakal, K.; Bika, R.; Ghimire, B.; Parajuli, M.; Neupane, S.; Neupane, K.; Addesso, K.; Baysal-Gurel, F. Arthropod and disease management in boxwood production. *J. Integr. Pest Manag.* **2022**, *13*, 18. [CrossRef]
36. Yang, X.; Castroagudín, V.L.; Daughtrey, M.L.; Loyd, A.L.; Weiland, J.E.; Shishkoff, N.; Baysal-Gurel, F.; Santamaria, L.; Salgado-Salazar, C.; LaMondia, J.A.; et al. A diagnostic guide for Volutella blight affecting *Buxaceae*. *Plant Health Prog.* **2021**, *22*, 578–590. [CrossRef]
37. Shi, F.; Hsiang, T. Volutella blight of boxwood. *Landsc. Trades* **2012**, *34*, 16–17.
38. Budziszewska, M.; Bereś, P.K. The box tree moth *Cydalima perspectalis*: A review of biology, invasiveness, management practices, and future perspectives of control strategy in Europe. *Plant Prot.* **2024**, *64*, 275–286. [CrossRef]
39. Barker, B.S.; Coop, L.; Hong, C. Potential distribution of invasive boxwood blight pathogen *Calonectria pseudonaviculata* as Predicted by Process-Based and Correlative Models. *Biology* **2022**, *11*, 849. [CrossRef]
40. Likins, M.; Davis, B.; Anderson, P.; Gillis, B.; Hong, C.X. Flutriafol drench provides season-long protection of boxwood plantings pre-infected by *Calonectria pseudonaviculata* in the Mid-Atlantic. *Plant Dis.* **2025**, *109*, 2185–2196. [CrossRef]

41. Weiland, J.E.; Beck, B.R.; Brand, T.; Hong, C.; Daughtrey, M.L. Cool Temperatures Favor Growth of Oregon Isolates of Boxwood Blight Pathogen. *Plant Dis.* **2022**, *106*, 3421–3430. [CrossRef]
42. Loyd, L.; Zwart, D. Why is my boxwood orange? Factors that influence winter color change in boxwood foliage in Charlotte, North Carolina, USA. *Arboric. Urban For.* **2023**, *49*, 213–229. [CrossRef]
43. Kureel, N.; Sarup, J.; Matin, S.; Goswami, S.; Kureel, K. Modelling vegetation health and stress using hyperspectral remote sensing data. *Model. Earth Syst. Environ.* **2021**, *8*, 733–748. [CrossRef]