Ann. For. Res. 66(2): 69-86, 2023
https://doi.org/10.15287/afr.2023.3225

ANNALS OF FOREST RESEARCH
www.afrjournal.org

Valsalnicola oxystoma (Rehm) D.M. Walker & Rossman - primary biotic agent responsible for the dieback and high mortality of green alder in Călimani and Rodnei Mountains (North-Eastern Romania)

Ecaterina Fodor[1], Nicolai Olenici[2]✉, Ovidiu Hâruța[1]

Fodor E., Olenici N., Hâruța O., 2023. *Valsalnicola oxystoma* (Rehm) D.M. Walker & Rossman - primary biotic agent responsible for the dieback and high mortality of green alder in Călimani and Rodnei Mountains (North-Eastern Romania). Ann. For. Res. 66(2): 69-86.

Abstract The primary pathogen responsible for the green alder (*Alnus alnobetula* (Ehrh.) K. Koch subsp. *alnobetula*) decline in two mountain ranges pertaining to Eastern Carpathians and harbouring protected areas, Călimani Mountains National Park and Rodnei Mountains National Park was identified as stem and branch canker and dieback agent, *Valsalnicola oxystoma* (Rehm) D.M. Walker & Rossman (Melanconidaceae, Diaporthales). The identification was based on *in situ* observations and cultivation on PDA medium of diseased inner bark fragments. Field observations performed on transects in both areas showed that the incidence of the disease was high, up to 100% of the inventoried shrubs. The severity of the disease assessed on a five-level scale was variable, reaching high mortality (over 50% of the inventoried shrubs) in the Rodnei Mountains suggesting that the decline was in earlier stages in the Călimani Mountains. Morphometric analysis of ascomata structures showed significant differences compared to measurements reported in the literature (Wilcoxon test) suggesting that there are geographic and ecological differences among the populations of the species, at least at the morphometric level. The confirmation of the canker-inducing agent was supported by Koch's postulate: infected branches of *Alnus glutinosa* (L.) Gaernt., also a host for *V. oxystoma*, were used to assess the symptoms and for the reisolation of the pathogen on PDA medium. Several other species, members of the diseased bark microbiome were identified, among which secondary canker and dieback-inducing pathogens, *Phomopsis alnea* (Sacc.) Höhn. and *Melanconis alni* Tul. & C. Tul.

Keywords: *Valsalnicola oxystoma*, *Alnus alnobetula* subsp. *alnobetula*, Călimani and Rodnei Mountains National Parks, morphometric analysis, Koch postulate, diseased bark microbiome.

Addresses: [1]Faculty of Environmental Protection, University of Oradea, Romania.| [2] SCDEP Campulung Moldovenesc, National Institute for Research and Development in Forestry "Marin Drăcea", Romania.

✉ **Corresponding Author:** Nicolai Olenici (olenicifp@yahoo.com)

Manuscript: received October 16, 2023; revised December 21, 2023; accepted December 21, 2023.

Introduction

When considering woody vegetation, one of the most intriguing and disturbing phenomena of the last decades was the remarkable increase and rapid onset of decline induced by pathogens (viruses, bacteria, and fungi) paralleling climate change (Chapin et al. 2010, Grulke 2011). Drought coupled with pests and epidemics is generally responsible for tree decline events (Hartmann et al. 2018, Caldeira 2019). During this time, there were several reports on the decline of the green alder (*Alnus alnobetula* (Ehrh.) K. Koch subsp. *alnobetula*, syn. *Alnus viridis* (Chaix.) D.C. subsp. *viridis*) populations in Europe, and the phenomenon was put in the context of climate change which facilitates biotic stressors. It was shown that these populations in the European Alps went through a considerable deterioration, mainly as a result of pest damage and fungal disease, where *Valsalnicola oxystoma* (syn. *Cryptodiaportae oxystoma* (Rehm) Z. Urb.) was considered to be the primary pathogen involved (Pisetta et al. 2012). High mortality of *A. alnobetula* due to the infection with *V. oxystoma* was also reported in Bolzano province (Anonymous 2016) but the first reports on green alder decline due to canker and dieback date back to Von Tubeuf (1892), Appel (1904) and Münch (1936). Other examples of green alder decline determined by canker/dieback agents included *Phomopsis alnea* (Sacc.) Höhn. (Maresi & Ambrosi 1999, Moricca 2002, Pisetta et al. 2012) and species of *Phytophthora*, (*P. alni* Brasier & S.A. Kirk, *P. plurivora* T. Jung & T.I. Burgess, and *P. pseudosyringae* T. Jung & Delatour) which were responsible for the decline of green alder in Italian and Austrian Alps (Majek et al. 2019, Cech 1998).

Similar decline events were reported on other *Alnus* species or subspecies such as *A. incana* (L.) Moench subsp. *tenuifolia* (Nutt.) Breitung affected by *Valsa melanodiscus* G.H. Otth in the Rocky Mountains (Worral et al. 2010), affecting also *A. alnobetula* subsp. *fruticosa* (Rupr.) Raus, *A. alnobetula* subsp. *sinuata* (Regel) Raus, and *A. incana* subsp. *tenuifolia* in Alaska (Adams et al. 2012).

Until recently, *V. oxystoma* was included on lists of canker/dieback fungal species residing on *Alnus* spp. in different parts of the world, without specifications on the epidemic behavior of the species (Vleugel 1911, Mann 1987, Cherepanova & Cherepanov 2003, Kodrik et al. 2006, Szabo 2009). Another mentioned host was *Alnus rubra* Bong. (Crous et al. 2012). In this context, the first instance when *V. oxystoma* was mentioned on *A. alnobetula* in Romania, was the study published by Szász (1966) accounting for her inventories on pathogenic fungi in Bâlea Valley from the Făgăraș Mountains (Southern Carpathians), under the name of the synonymous *Valsa oxystoma* Rehm.

A. alnobetula subsp. *alnobetula* is endemic to the mountains of Central Europe and the Balkan Peninsula (Greuter & von Raab-Straube 2011, Hantemirova & Marchuk 2021). It is abundant in the Alps, the Carpathians, the Balkans, and the Dinaric Mountains (Richard 1967). Mauri & Caudullo (2016) also mention its presence in the Pyrenees, Apennines and Norwegian mountains, but at least in Norway the species did not exist naturally, until it was introduced by man (Hegre et al. 2023). It is a multi-stemmed shrub, an early successional species and is growing at medium to high altitudes, under stressful conditions represented by snow cover during several months of the year and avalanche chutes (Richard 1967). This species is found in treeline ecotone characterized by dwarf shrubs intermixed with trees subjected to anthropogenic influences, mainly through pastoralism (Nagy & Grabherr 2009). In the Carpathian Mountain chain, it vegetates at relatively high altitudes: in Northern Carpathians between 1400 m and 1680 m, locally at lower altitudes beginning with 1300 m, in Eastern Carpathians between 1450 m and 1860 m, locally lower, at 1300 m or higher, up to 2004 m, in Southern Carpathians between 1620 m and 1890 m, locally up to 2030 m, and in Romanian Western Carpathians between 1400 m and 1740 m (Georgescu 1952). *A. alnobetula* subsp. *alnobetula* requires particular climate conditions, with winter temperatures between 1 and 10°C, summer temperatures around the average of 15°C, and conspicuous and late-lying winter snow (Richard 1968) and is vegetating mostly in timberline habitats requiring sufficient light and constant water supply. In addition, it is a pioneer nitrogen-fixing species, being in a mutualistic relationship with the actinomycete *Frankia alni* (Woronin, 1866) Von Tubeuf, 1895 (Wiedmer & Senn-Irlet 2006, Dawson 2008). The species is expanding in treeline ecotone zones of the European Alps, mostly in abandoned lands (Oberhuber et al. 2023) not the case in Eastern Carpathians where the areas occupied by *A. alnobetula* scrubs and *Pinus mugo* Turra with *A. alnobetula* scrubs are shrinking, these habitats being considered endangered (Doniță et al. 2005, Iușan 2014).

Green alder plays an important protective role against soil erosion and snowslides (Dakskobler et al. 2013), contributing to soil stabilization, especially on steep slopes and torrential valleys (Șofletea & Curtu 2007) being employed for the rehabilitation of degraded subalpine areas (Ciocârlan 2000, Podrázský & Ulbrichová 2003, Kuneš et al. 2011, Hegre et al. 2023). However, this role is put under discussion by more recent research centered on practical viewpoints, but also from biodiversity impoverishment perspective. At lower altitudes where *A. alnobetula* co-exists with species of *Larix*, *Picea*, and *Pinus* (Bühlmann et al. 2014) it eliminates through competition the seedlings while in montane pastures it determines the decline of subalpine pasture species (Zehder et al. 2020). In Norway it is assessed as having a very high ecological risk, due to a large invasion potential and moderate, but significant, negative ecological effects (Hegre et al. 2023). In Romanian silvicultural practice, green alder remains an important species by delivering significant ecological services, but in terms of montane agriculture, due to the capacity to colonize extensively the alpine pastures, the authors recommend the clearing of already installed green alders (Marușca & Blaj 2019). Regardless of practical and economic interests, from ecological and conservationist viewpoints, the green alder remains an important species.

Cankers of woody species are among the frequently encountered diseases produced by fungi which are placed among the large functional group of endophytes, a group covering the entire spectrum from mutualist to saprotrophic and, at the end of the spectrum, pathogenic species. Generally, cankers are stress-induced diseases caused by necrotrophic pathogens occurring on stems, branches, and twigs (Marsberg et al. 2017). They infect the hosts following primary stressors such as moisture stress and correlated high temperatures. For instance, *Cytospora* cankers may infect through wounds but also can cause latent infections persisting as endophytes in healthy tissues of the host (Adams et al. 2005). The consequences of canker disease consist of the destruction of phloem, cambium, xylem, and parenchymal cells (Biggs et al. 1983). When cankers evolve and induce stem or branch girdling, the physiology of the tree is impaired in terms of decreased photosynthetic gas exchange, stomatal conductivity, and transpiration rate due mainly to cambial dysfunction (Xing et al. 2020). At the tree population level, the development of epidemic spread of the canker pathogens may trigger decline and at the plant community level, changes in composition and successional trajectory (Hansen & Goheen 2000, Metz et al. 2012).

Trees' decline has also ecosystem-level consequences. From the pathosystem's ecology perspective, the disease is the product of the interaction of the host with a guild of pathogens under particular environmental conditions, the disease being the result of the synergism between members of the pathogenic guild, community or microbiome (Lamichhane & Venturi 2015). Nevertheless, if one pathogen species gains prevalence, the result is the epidemic development and the dominant member of the functional pathogenic guild is responsible for high mortality within the host population. However, the association with other pathogens members of the same functional group is important from the perspective of the pathobiome structure and species interactions, the most significant being the synergistic effect of the co-infection (Fitt et al. 2006). Canker pathogens are facilitators for wood pathogens and sapro-pathogens and develop during a distinct stage of the succession from healthy plant to dead material linking those two processes, pathogenesis and decomposition of the bark and wood.

Current study outcomes are based on observations on green alder decline performed in two mountain ranges, in North-Eastern Romania, pertaining to Carpathians, beginning with 2018. The green alder decline was detected in two protected areas: Călimani Mountains National Park and Rodnei Mountains National Park. The question raised by field observations was how apparently a native, stress-related pathogen became prevalent over a large area comprising two mountain ranges in North-Eastern Romania? Therefore, current study was performed, in order to answer this question, with the aim to identify and characterize the primary pathogen inducing *A. alnobetula* decline detected in the two mountain ranges.

Materials and Methods

Study area

Study sites

The decline of green alder was observed and investigated in two mountain ranges pertaining to Eastern Carpathians: in North-Eastern Romania, Călimani Mountain range, and in the inner area of Eastern Carpathians, Rodnei Mountain Range. Both mountains range harbour protected areas: Călimani Mountains National Park and Rodnei Mountains National Park.

The habitat type in both mountain ranges, according to the classification of the European Redlist of Habitats (Janssen et al. 2016) corresponds to F 2.4 – Subalpine *Pinus mugo* scrub composed of *P. mugo* and *A. alnobetula* in wetter places (Plate 1, a and b). According to habitat classification in Romania (Doniță et al. 2005) green alder establishes subalpine scrubs with *P. mugo* and *Rhododendron myrtifolium* Schott & Kotschy (habitat R 4070) and green alder scrubs (*A. alnobetula*) (R3110). The habitat type R4070 is considered endangered (Iușan 2014). According to EUNIS (2007) classification, the habitat with green alder pertains to F2.3, Subalpine deciduous scrubs. According to Coldea and Cristea (2021), the habitat harbours the association *Rhododendro myrtifollii - Pinetum mugo*, Coldea 1991 which is characteristic to South-Easten Carpathians and included in the alliance *Alnion viridis* Schnyder 1930, class *Betulo carpaticae-Alnetea viridis* Rejmánek in Huml et al. 1979 (Dakskobler et al. 2013).

In Călimani mountains the *P. mugo* scrubs include green alder (*A. alnobetula*), Norway spruce (*Picea abies* (L.) H. Karst), aspen (*Populus tremula* L.), goat willow (*Salix caprea* L.) and birch (*Betula pendula* Roth). The scrub includes also blueberry (*Vaccinium myrtillus* L.), cranberry (*Vaccinium vitis-idaea* L.), bog blueberry (*Vaccinium uliginosum* L.) and rhododendron (*R. myrtifolium*).

The coordinates of Călimani transects (Figure 1) (retrieved from Google Earth) are: transect 1 (47.109401 N, 35.239192 E, altitude 1573 m); transect 2 (47.108939 N, 25.232289 E, altitude 1690 m); transect 3 (47.121568 N, 25.226149 E, altitude 1628 m); transect 4 (47.120479 N, 25.228213 E, altitude 1661 m). The field observations were performed in September 2021.

The similar habitat in the Rodnei Mountains with *A. alnobetula* is a scrub containing *P. mugo*, *R. myrtifolium*, *Pinus cembra* L.,

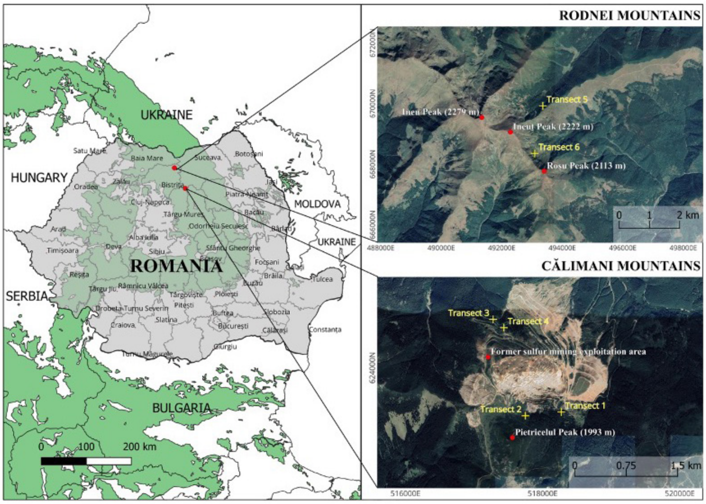

Figure 1 Locations of the sampling areas - Călimani Mountain range: positions of the transects 1 to 4, and Rodnei Mountain range: positions of the transects 5 and 6.

Juniperus communis subsp. *alpina* (Suter) Celak., *P. abies*, *Sorbus aucuparia* L., *V. myrtilus*, *V. vitis-idaea*, and *Ribes petraeum* Wulfen. This type of vegetation characterizes steep slopes as being restricted to altitudes between 1600 and 2000 m (Iușan 2014).

In Rodnei Mountains, the transects were performed in two close locations: Șaua Gajei and Vârful Roșu (Roșu Peak), in October 2022 (Figure 1). The coordinates of the transect 5 in Șaua Gajei are 47.528559 N, 24.910173 E, altitude - 1754 m, and of the transect 6, under the Roșu Peak are 47.51 4410 N, 24.906662 E, altitude - 1799 m.

Climate

The climate characterizing Călimani and Rodnei Mountains is moderately continental and, according to Köppen climate classification - subarctic with cold winters and mild summers. Climatic data show that in Călimani Mountains the average annual temperature varies from 2.4 to ca. 4.0°C while average precipitation varies between 1100 and 1650 mm (Food and Agriculture Organization (FAO) Local Climate Estimator New_LocCLim v. 1). The average annual precipitation in the study area is 1000–1200 mm. The snow cover is present for an average of 180–200 days, the first snowfall occurring in early October. Vegetation season starts in early May and finishes in mid-October (Anonymus 2022a).

In Rodna Mountains National Park observations showed the modification of several climatic parameters such as the decreased snowpack, the annual variation of the relative humidity means, and multiannual precipitation means (at a rate of ≈ -20 mm/decade). The mean annual temperature increased by 0.7°C and the mean summer temperature by 0.9°C during the period 1961-2007 (Dragota & Kucsicsa 2011).

Overall, in the northern part of the Eastern Carpathians, in the period 1961-2010, there was a significant trend of increasing minimum air temperature during winter and of maximum air temperature during spring and summer, as well as a decreasing trend in the number of frost days (Micu et al. 2015). The larger context of the climatic conditions of the two mountain areas is of steadily increasing temperatures since 2000, a general trend reported for Europe (Forzieri et al. 2021).

Field sampling protocol

The incidence and severity of the green alder's decline were assessed by inspecting the trees along transects of 50 m: 4 transects in the Călimani sampling area and 2 transects in the Rodnei Mountains sampling area (Figure 1). Random 10 cm long branch samples were cut from trees with dieback symptoms but still retaining leaves in the upper crown, in areas where transects were performed. Three branch samples/tree were collected showing typical bark discolourations, from 10 trees randomly selected along the transects. We considered the ramets corresponding to sample units.On each 50 m transect, the trees were assigned to one of the dieback severity classes: ranked from no dieback signs with 0% affected crown in symptomless trees, weak for few shoots affected (< 25% of the crown), medium for > 25% of the crown affected, and severe for > 50% affected crown, including also the dead standing stems. The inter-shrub distance was measured for the assessment of green alder thicket's closeness. The incidence of the dieback disease along the transects was quantified by considering symptomless and diseased trees.

Sampling units

The diameters of all ramet segments were measured using digital callipers and the collected segments for each tree were placed in a separate polyethylene bag. The sampled branch segments were incubated in outdoor conditions within their initial sampling polyethylene bags for eight months and observations were performed repeatedly every month. We assumed that each bag is a microcosm where organisms developed more or less under natural conditions (except for continuous high humidity), that is under variation of the temperature regime.

Species identification

Identification of *V. oxystoma* was based on observations on incubated material and in vitro isolations from the inner bark fragments. The fragments were cut under *V. oxystoma* ascomata (approximately 2 cm long, placed in two Petri dishes per transect), cleaned and sterilized with commercial bleach and 95% alcohol, repeatedly washed with sterile distilled water and placed on PDA (potato-dextrose agar) medium supplemented with ampicillin. The Petri plates were incubated at 22°C under dark conditions until fungal growth became visible. The obtained colonies were sub-cultured, on the same culture media, in order to obtain pure fungal cultures. Isolations were performed, as described before, also from abundantly sporulating perithecia.

Microscopy observations included measurements of asci, ascospores, length of perithecial necks, and diameters of the perithecia. Macroscopic measurements performed at the stereomicroscope included diameters of stroma, number of converging perithecial necks, and density of the fruiting bodies per mm^2. We employed the optical microscope Zeiss–Primo Star 3 and the stereomicroscope Optika SLX-3 to perform the observations and measurements considered in this paper. Measurements were performed using OPTIKA PROVIEV version x84 software with calibrations using a microscope and stereomicroscope's micrometres.

The species identification was based on the description of Crous et al. (2012). Associated species, developing in diseased *A. alnobetula* bark, were identified in situ and from in vitro cultivated bark fragments on PDA medium in 9 cm in diameter Petri plates.

The nomenclature of the fungi followed Index Fungorum and The Global Biodiversity Information Facility (GBIF), while those of bacteria and plants was done according to GBIF.

Pathogenicity tests

Pathogenicity tests were performed on detached branches of *A. glutinosa* (mentioned as host for *V. oxystoma*) collected from Someș river banks, near the city of Cluj-Napoca, placed in Erlenmeyer flasks with sterilized distilled water containing fragments of active growing colonies of *V. oxystoma* cultivated on PDA medium. We employed 10 branches for the inoculation of *V. oxystoma* and 3 control branches which were placed in flasks with no added *V. oxystoma* inoculum. The openings of the flasks were covered with Parafilm to avoid rapid desiccation and contamination with airborne spores. The branches were kept under room temperature and daylight illumination until the first symptoms were apparent: wilting of leaves and sunken bark areas. Fragments from the diseased bark areas were subsequently cultivated on PDA medium supplemented with ampicillin. The emerging mycelia were sub-cultured on PDA and the Petri dishes were kept at room temperature, in the dark, until colonies developed.

Quantitative data

Calculations for the assessment of the incidence and severity of the disease and the variation of the measured ascocarps' structures, as well as of the branch diameters were performed in PAST (Hammer et al. 2001). The same software was employed to assess the significance of the differences between our measurements of *V. oxystoma* ascocarps' structures and those reported in the literature (Wilcoxon signed-rank test). The graphical representations were performed in R using the package ggplot2 (Wickham 2016, R Core team 2014) and the map represented in Figure 1 was generated in QGIS ver. 3.28.8-Firenze software.

Results

Identification of the canker/ dieback primary pathogen

The species responsible for bark cankers and dieback in *A. alnobetula* populations from Călimani and Rodnei Mountains was identified as *Valsalnicola oxystoma* (Rehm.) D.M. Walker & Rossman (Melanconidaceae, Diaporthales, Diaporthomycetidae, Sordariomycetes, Pezizomycotina, Ascomycota, Fungi).

Synonymy: *Valsa oxystoma* Rehm, Ber. naturhist. Augsburg 26: 70 (1881); *Engizostoma oxystomum* (Rehm) Kuntze, Revis. gen. pl. (Leipzig) 3(3): 475 (1898); *Cryptodiaporthe oxystoma* (Rehm) Z. Urb., Preslia 29: 395 (1957).

The dieback determined by *V. oxystoma* begins in apical and lateral, relatively thin branches and consists in a rapid wilting detected first in leaves which turn brown without dropping, at the beginning of the growing season. The dieback progresses rapidly downward the branches leading to the emergence of elongated, stripe-like areas of sunken bark and cambium discoloration which turns orange-brown (Plate 1, c and d). At the beginning of autumn, the affected bark is already necrotic and covered with pustules of developing *V. oxystoma* perithecia. At the end of the vegetation season, the matured stroma erupts and perithecial necks become visible on the bark (Plate 1, f).

Plate 1 a. Rodnei Mountains National Park - view of the affected by dieback *A. alnobetula* thicket showing wilting of the foliage, habitat with *P. mugo* scrub; b. Călimani Mountains National Park – view with *P. mugo* and *A. alnobetula* scrub, green alder affected by dieback showing brown foliage; c. pustules of *Valsalnicola oxystoma* in and cankers on bark of *A. alnobetula*, in Călimani Mountains; d. discolored cambium in *A. alnobetula* shoot, symptom induced by *Valsalnicola oxystoma*; e. *Peniophora aurantiaca* on *A. alnobetula* stem, in Călimani Mountains; f. and g. errumpent perithecia of *Valsalnicola oxystoma*; h. culture of *Valsalincola oxystoma* on potato-dextrose agar medium, after three weeks incubation. (Photos: a – Stelian Bodnari; b – Nicolai Olenici; c, d, e, f, g – Ovidiu Hâruța; h – Ecaterina Fodor).

The necrotic areas are elongated, stripe-like, progressing toward girdling the shoots which eventually die. *V. oxystoma* induces girdling of the branches during the growing season. Presumably, the initiation of the dieback is not linked to previous wounds.

During the end of the autumn and winter, the ascospores produced in perithecia are abundant, whitish-opalescent, forming watery-sticky masses and are released through the ostioles. As ascospores are released, the bark tissues are invaded by secondary fungi and bacteria. The release of the spores in mucous masses suggests that the dispersion depends on insects and mites. We observed oribatid mites (Acari: Oribatida) feeding on spore masses developed in maturing ascomata, in the incubated material. As perithecia are progressively emptied and the dried, whitish spore masses adhere to the perithecial necks or line the perithecia being released later, as a consequence of the withering process. These dry masses of spores are dispersed by rain splash and probably, wind.

Ascomata are perithecial, aggregated, included in a stroma which may contain one to several perithecia, variable in numbers (Table 1), glossy black, immersed beneath ectostroma, the swelling causing bark rupture. The ectostroma is well-developed, and black, forming a thick disc from which perithecial straight or curved necks emerge (Plate 1, f and g). Does not present conceptaculum.

Perithecia are embedded within bark tissue, partially in wood. Perithecial wall displays textura angularis, brown to black. Perithecia are grouped, globose to subglobose, dimensionally variable (see Table 1), varying also in terms of numbers per fruiting body.

Asci are fusiform or cylindric-clavate, with an acute stipe, rounded or tapering, apex rounded with indistinct ring, ascospores arranged irregularly, multiseriate, reaching maturity at the end of the growing season.

Ascospores are allantoid, hyaline, 1-septate, with median sept, slightly or not constricted at the septum.

Pure fungal cultures on PDA are growing slowly, reaching 16 mm in diameter after four weeks, with low, pale brown to grey-brown mycelium, reverse dark brown (Plate 1, h).

Measurements on ascomatal structures of *V. oxystoma*, performed on the examined material from freshly collected and incubated branches, and measurements data reported by different researchers in their publications (for comparisons) are summarized in Table 1.

The measurements show differences in morphometric values in samples from the Călimani and the Rodnei Mountains compared to values reported by other authors (Table 1). Differences confirmed by the Wilcoxon test are hypothetically due to ecological variations (geographical

Table 1 Measurements of ascomatal structures of *Valsalnicola oxystoma* identified in declining *A. alnobetula*, in Călimani and Rodnei Mountains next to previously reported measurements.

Ascoma structures	Mean±SE	Min	Max	CV	N	Reported (mean)	Wilcoxon Test[4]
Stroma diam. (mm)	1.85±0.16	0.7	3.34	39.82	21	*0.81*	**
No. of perithecia/stroma	5.43±0.70	1	19	73.85	32	*13-23*	**
Ascomata density/sqrt.mm	22.28±12.51	11	45	56.14	7	-	
Perithecia diameter (mm)	0.47±0.03	0.1	1.38	60.04	75	*0.28*	**
Perithecial necks (mm)	1.49±0.08	0.94	1.98	19.51	12	*0.47*	**
Asci length (μm)	29.02±2.12	22.08	40	21.99	9	*44*[1] *40*[2]	**
Asci - largest width (μm)	5.18±0.18	4.35	6.2	10.99	9	*11*[1] *7*[3]	**
Ascospores length (μm)	9.05±0.18	6	11.66	12.69	39	*11*[1] *12*[3]	**
Ascospores width (μm)	1.78±0.04	1.35	2.5	15.02	39	*2*[1] *1.2-1.5*[2]	**
Asci stipe length (μm)	7.167±1.09	3.44	12	46..03	9	-	

Note: [1]Reported measurements from Crous et al. (2012), [2]Senn-Irlet et al. (2012), [3]Szabó (2009) [4]Compared to reported values at $P<0.005$.

variation) of the pathogens' populations.

Pathogenicity test using thin branches of *A. glutinosa* and cultured *V. oxystoma* confirmed the observed and then isolated pathogen as being responsible for the developing bark cankers, signalled by the orange-brown discolouration of the cambium and the re-isolation of the pathogen from diseased tissues. Eight of ten incubated branches in the presence of the pathogen were found to be infected. Consequently, Koch's postulate was verified and the results confirmed the hypothesis of *V. oxystoma* being responsible for the *A. alnobetula* epidemic in the investigated areas.

We deposited a voucher specimen at the Herbarium of the Babeș-Bolyai University - Cluj-Napoca, Romania under the registration number 672117.

Associated canker species

Associated canker species on diseased branches which developed on incubated branch fragments and in vitro cultivated inner bark fragments included: *Melanconis alni* Tul. & C. Tul., *Hysterium angustatum* Alb. & Schw. ex. Mér, *Phomopsis alnea* (Sacc.) Höhn., *Cryptosporella suffusa* (Fr.) L.C. Mejia & Castl. and *Phoma* spp. Sapro-pathogenic species such as *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not. or opportunistic saprotrophic invaders of the cankers included cosmopolitan generalists such as *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., *Epicoccum nigrum* Link, *Cladosporium* spp., *Fusarium* spp., *Trichothecium roseum* (Pers) Link and *Alternaria alternata* (Fr.) Keissl. Advancing mycelial strands of *Peniophora aurantiaca* (Bres.) Höhn. & Litsch (Plate 1, e) which develop fruitbodies during the next vegetation season, mainly on dead branches, cover the lesions produced by canker pathogens in a short time. The species is a secondary bark canker/dieback pathogen in the Călimani and Rodnei Mountains.

Incidence and severity of *Alnus alnobetula* decline

Incidence of the disease induced by *V. oxystoma* in sampling areas along the transects is depicted in Figure 2. Except for transect 1 (Călimani), with a lower proportion of diseased trees, other transects display high severity suggesting epidemic spread. In terms of severity of the disease rated on 5 levels scale, transect 1 (Călimani) (36%) and 6 (Rodnei) (61%) display the highest proportion of symptomless trees, while transect 5 (Rodnei) with 100% incidence and transects 2 and 3 from Călimani with 95% incidence show the highest proportion of diseased trees.

Figure 3 refers to the severity of the disease induced by *V. oxystoma* in *A. alnobetula*, in the investigated sampling areas. The highest severity in terms of diseased and dead trees, corresponding to categories 4 and 5 of the scale displayed on transect 6 (Rodnei) and transect 1 (Călimani), while the highest proportion of symptomless trees was encountered on transect 2 (Călimani).

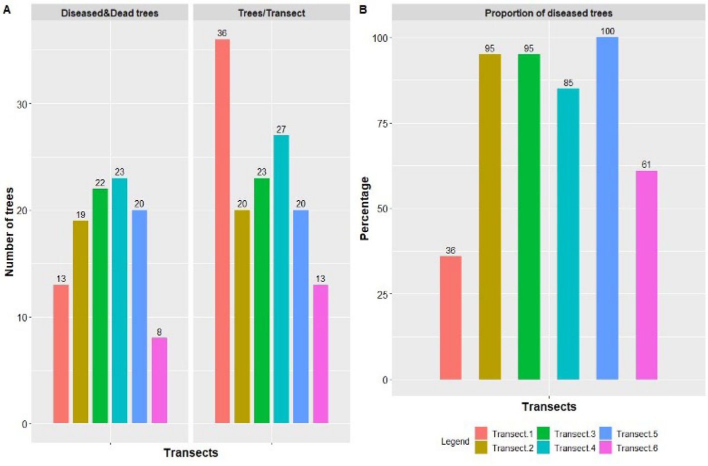

Figure 2 a. The incidence of *A. alnobetula* dieback – number of diseased trees and number of trees per transect (transects 1 to 4 from Călimani and transects 5 and 6 from Rodnei Mountains); b. proportional incidence.

Characteristic for the Rodnei area, the severity of the disease was high and the shrubs pertained mostly to severity categories 4 and 5. An explanation might be a different, earlier stage in the development of the *V. oxystoma* epidemics in Călimani Mountains, the distance between the shrubs being similar in the two areas (between 1.3 ± 0.14 m and 3.9 ± 0.06 m in Călimani; between 2.4 ± 0.14 m and 3.76 ± 0.1 m in Rodnei Mountains).

Box-plot representation in Figure 4 indicates a greater variability of shoot diameters in samples collected from the Rodnei mountains. However, the median values are close in both locations varying in the range of 10 to 15 mm. The relative dimensional homogeneity of the fragments justifies considering them valid sampling units.

The data confirm the initial observation that the disease progresses from thin apical and lateral shoots which harbour the most extended lesions and later the most abundant fruiting bodies of the canker agent. *V. oxystoma* covers from 5% to 100% (average and SE = 30.9 ± 7.39) the sampled branch segments, high proportions being linked to complete girdling of the ramet.

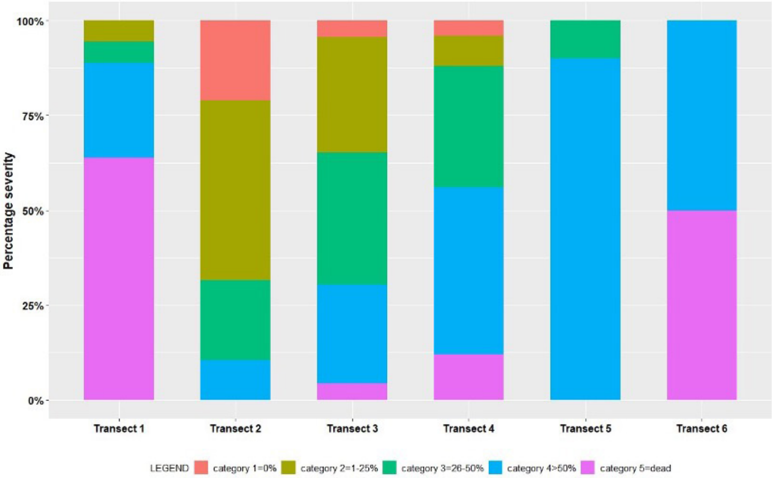

Figure 3 Severity of the *A. alnobetula* dieback (transects 1 to 4 from Călimani Mountains and transects 5 and 6 from Rodnei Mountains).

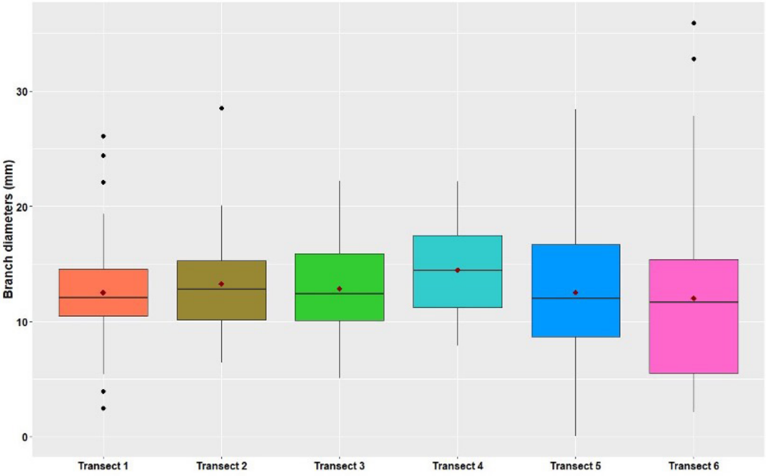

Figure 4 Box-plot representation of diameters (in mm) variation in the diseased sampled branches of *A. alnobetula* in Călimani (transects 1to 4) and Rodnei Mountains (transects 5 and 6).

Discussion

V. oxystoma was identified as the primary pathogen responsible for green alder decline based on the presence of the aggregated perithecia abundantly covering the bark of the declining shrubs of *A. alnobetula* in the investigated areas from Călimani and Rodnei Mountains, and confirmed by isolation and cultivation on PDA medium.

We did not find the anamorphic stage of *V. oxystoma* in our material but it is worth mentioning the information provided by Crous et al. (2012)

on this topic showing that conidiomata are pycnidial, exuding masses of brown conidia which are 3-5 euseptate, verruculose, cylindrical.

Measurements on ascomatal structures of *V. oxystoma* show differences in morphometric values compared to values reported by other authors. So, we assume that it is due to geographical variation of the pathogens' populations, such variability being encountered and reported in fungal populations by several authors (Swart et al. 1993, Iqbal and Mukhtar 2014, Fodor et al. 2015).

Easily confounded with *Valsa melanodiscus* G.H. Otth. which affects *Alnus* spp. and causes similar symptoms, *V. oxystoma* lacks the black lines surrounding stromata in the ascomatal cavity (Crous et al. 2012) and the dimensions of asci and ascospores differ being smaller in *V. melanodiscus* (Spielman 1985). Also, the slow progression of cultures' growth confirms the identity of *V. oxystoma* since *V. melanodiscus* displays a rapid growth rate on media (Walker et al. 2012).

The pathogen is specific for *A. incana*, *A. rubra*, *A. glutinosa*, *A. alnobetula*, *A. alnobetula* ssp. *fruticosa* and *A. incana* ssp. *tenuifolia* being consequently considered monophagous or oligophagous, responsible for stem cankers and branch dieback. The accepted name is *Valsalnicola oxystoma* according to Index Fungorum and GBIF checklist (2022) but the most often used name in publications is *Valsa oxystoma* as indicate the occurrences in GBIF.

Species distribution includes Japan (Kobayashi 2007), Europe where it was reported from Italy (Pisetta et al. 2012), Switzerland, Austria (Urban 1960), Belgium, Sweden (Vleugel 1911), United Kingdom (Cannon et al. 1995), Hungary (Szabo 2009), Czech Republic, in Tatra Mountains (Urban 1960), Slovenia (Anonymous 2022b) as well as Canada (British Columbia) (Lee et al. 2023) and USA (Alaska) (Winton 2013). The presence of *V. oxystoma* is mentioned for the third time in Romania, the first identification being made by Urban (1960) in the Făgăraș Mountains, on *A. alnobetula* in 1956. However, that mention in Romania is under question as Urban (1960) used the term presumably for the presence of the pathogen, after consulting old herborized material. The first valid mention of the species under the synonymous *Valsa oxystoma*, based on field observations, also in Făgăraș Mountains, across Bâlea Valley was due to Szász (1966). The author observed *V. oxystoma*'s high incidence in October 1963 and June 1965 but did not consider the event as being epidemic.

Generally, being a latent pathogen, a member of the canker-dieback inducing pathosystem, since 2000 *V. oxystoma* has become an emergent, epidemic pathogen under the steadily rising annual temperatures and recurring droughts (Pisseta et al. 2012). Other abiotic and biotic disturbances in European forests, including insect outbreaks are linked to the same environmental stress (Forzieri et al. 2021). The situation is similar to that presented by (Maresi & Ambrosi 1999, Moricca 2002; Pisetta et al., 2012) for Italy corresponding to the mid-1990s. Since then, the decline spread to other parts of Europe such as the Eastern Carpathians, as our data show.

The dominance of *V. oxystoma* is linked to the invasion of the cambium and the capacity to spread downward the branches while the other canker species remain restricted. Sporulation and dispersion of the ascospores take place at the end of the vegetation season, end of September-beginning of November, and probably also, during the winter season if temperatures are mild (a situation mimicked by the long duration of the microcosm experiment in our study). The long and narrow cankers develop during the fall and winter and tend to induce girdling of the affected branches in a similar way to *V. melanodiscus* as reported by Worral et al (2010) in *A. incana* ssp. *tenuifolia*, in Alaska. The artificially infected ramets of *A. glutinosa* employed to demonstrate Koch's postulate permitted the isolation of *V. oxystoma* from cankered cambium, thus confirming that *V. oxystoma* was responsible for the decline. The experiment confirmed the data provided by Pisetta et al. (2012) in Italy. One explanation for the infection progress in the absence of wounds or stubs might be the early installation of the pathogen in a similar manner as the situation reported for *V. melanodiscus* on *A. incana* ssp. *tenuifolia* (Worrall et al. 2010).

The dominating pathogen in terms of occupied bark areas of the host and in terms of prevalence was *V. oxystoma*. However, the bark areas contained fruiting bodies of other canker-inducing species or opportunistic saprotrophs which establish the diseased bark microbiome.

The stromae are invaded in a short time by saprobic fungi, yeasts, and bacteria, a phenomenon reported previously from declining green alder in Italy, infected with *V. oxystoma* (Pisetta et al. 2012). In particular, Maresi and Ambrosi (1999) identified *V. oxystoma* and *Melanconis alni* as canker-inducing weak pathogens or even saprotrophs of *A. alnobetula*, in Northern Italy. Moricca identified as frequent canker pathogens *Phomopsis alnea*, *Melanconium apiocarpium* Link, and a species of *Hymenopsis* (Moricca 2002). He enumerated other species isolated from diseased shoots of *A. alnobetula* such as *Nectria* spp., *Ophiovals*a spp., *Penicillium* spp., *Pezicula* spp., *Phloeosporella* spp., *Phoma* spp. The dominant and perennial canker-inducing pathogen was *Phomopsis alnea* linked also to water stress. *Hymenopsis* and *Melanconium* were considered secondary opportunistic invaders. The microbiome associated with stem and branch cankers in our study included also, *Phomopsis alnea*, *Melanconis alni, Hysterium angustatum*, *Phoma* spp., *Cryptosporella suffusa*, and several other species of weak canker pathogens, and saprobic species colonizing the cankered areas after spore release of the primary canker pathogens. The dead and dying branches and stems were quickly colonized by wood-degrading *Peniophora aurantiaca*, the same species, and the colonization pattern being reported by Pisetta et al. (2012) on declining *A. alnobetula* in Italy. The disease appears to be the result of the synergistic action of the diseased bark microbiome (Lamichhane & Venturi 2015), not solely of the primary and most conspicuous pathogen.

Apparently, the decline of green alder is a climate-driven event caused by bark and dieback agents among which, the dominant species is *V. oxystoma*. The statement is sustained by the steady annual average temperature increase in Călimani and Rodnei Mountains (Micu et al. 2015, Forzieri et al. 2021) and an increasing trend in the frequency and duration of periods of hot and dry weather in Romania, including in the studied areas (Markonis et al. 2021, Nagavciuc et al. 2022a, 2022b) as we consider that these trends are responsible for the emergence of epidemics and the decline of *A. alnobetula* infected with *V. oxystoma*. However, considering the severity and incidence of the disease, the green alder decline onset was later in the Călimani Mountains than in Rodnei, where the Norway spruce specimens dispersed in the mountain pine thickets, located downstream of transect 6, were observed in the spring of 2022 to be recently dead (Dr. Eng. Ionel Popa, personal communication). It can be assumed that it is not a mere coincidence that in 2018, when the decline of green alder was first observed in Călimani, in the same area there was also an outbreak, in a relatively limited area, of the species *Lasiocampa quercus quercus* f. *alpina* (Olenici 2019).

Like other species of *Alnus*, green alder appears not to tolerate water stress (Giordano et al. 1993). It can be hypothesized that the behavior of *V. oxystoma* epidemic follows the same pattern as *V. melanodiscus* in the Rocky Mountains where warmer periods of 20 years facilitated the epidemics affecting *A. incana* and alternated with cooler periods which led to the remission of epidemics (Worrall et al. 2010): during the 20th century these oscillations were followed by alternations of endemic and epidemic grows patterns of pathogen's populations.

Studies have shown that the infection produced before drought inception spreads within the host developing specific canker lesions in a short time (Hossain et al. 2018), the mechanism involved being the lower production of non-structural metabolites (polyphenols) responsible for the host's defense response (Pilotti et al. 2014). The consequence of the hydraulic failure induced by drought in *A. alnobetula* subsp. *fruticosa*, a shrub vegetating in the Alaskan circumpolar North, was the decrease of photosynthesis and stomatal conductance (Rohrs-Richey et al. 2010), a mechanism we suppose to occur in *A. alnobetula* subsp. *alnobetula*, too.

Dispersion of the pathogen with sticky, mucilaginous spore masses is facilitated locally by dense thickets of *A. alnobetula* and their multi-stemmed architecture. The high density of the stems and the relatively short height of individuals (Caviezel et al. 2017) are facilitating the rapid spread of stem and branch infections, an explanation for the high incidence and severity of the disease. Our field measurements indicated a high density of shrubs, an important factor facilitating the disease spread in both areas. The biotic vectors for *V. oxystoma* spores might be insects and mites. In our observations, mites fed on spore masses released through ostioles, this fact suggesting that biotic vectors are responsible for short-range dispersion of the pathogen. A similar mechanism was mentioned in relation to other bark pathogens such as *Valsa* spp., *Nectria* and *Neonectria* spp., or *Phomopsis* (Houston 1994, Mihál et al. 2014).

At a larger spatial scale, the pathogenic spread is facilitated by the steadily increasing tourism in both protected areas where *A. alnobetula* vegetates, with the correlated increase of roads' and hiking trails' density.

Main stressors linked to *A. alnobetula* decline in the investigated areas and also mentioned elsewhere (Pisetta et al. 2012) are linked to global climate change: rising annual average temperatures, drought, insufficient snow cover during the winter, the edaphic conditions such as shallow soils. It was shown in similar situations in the case of *Alnus cordata* (Loisel.) Duby that insufficient snow cover determined cavitation and embolism in stems and branches (Tognetti & Borghetti 1994). Also, a reduction in transpiration attributable to stem cankers was observed (Rorhs-Ritchey et al. 2010), presumably the same mechanism affecting *A. alnobetula* attacked by *V. oxystoma.*

In Călimani Mountains other stressors affecting the phytocoenoses where *A. alnobetula* vegetates are long-term effects of previous sulfur exploitations in the area and tourism. Worth mentioning that the current area of this habitat type in Romania, of 500 km^2, is continuously decreasing (Sanda et al. 2000). In the Călimani Mountains, the green alder scrub covers 63.48 ha (Anonymous 2022). Important disturbances affected the scrub in the past: fires, deforestation, and sheep grazing but the most important historical disturbance consisted of abandoned open sulfur exploitation quarries slowly recovering where scrubs with *A. alnobetula* scrubs vegetated (Popa & Popa 2021). The exploitation leading to vegetation destruction beginning in 1969, and the date of abandonment being 1997, the presence of *P. mugo* scrub with *A. alnobetula* corresponds to relatively early secondary succession in this area.

One of the future major threats is overtourism affecting increasing mountain areas (González-Domingo 2021). The steady increase of tourists visiting the Rodnei and Călimani National Parks and the increasing density of hiking trails are factors facilitating the dispersion of plant pathogens to which one must add the vegetation damage due to trampling, factors to be considered in the future for sustainable management of both protected areas. Similar paths and causes of the pathogenic spread were reported in the case of *Phytophthora* *ramorum* Werres, De Cock & Man in Northern California (Cushman et al. 2008).

Incidence of the disease reported for Italy in areas with *V. oxystoma* inducing green alder decline was 75% compared to our data showing higher proportions: up to 100% diseased trees in transect 5 (Rodnei) and 95% in transect 3 (Călimani). However, shrubs vegetating isolated, in the Rodnei Mountains were symptomless, apparently healthy (Hâruța, personal observation), a fact suggesting that high shrub density is facilitating the high severity and incidence of the disease elsewhere in the investigated area. Also in Slovenia, the disease was reported recently with medium severity (Anonymous 2022b). It is unclear if the phenomenon is determined by the early stage of the epidemic onset in Slovenia or other factors not mentioned in the report as far as in Romania and Italy the disease induced by *V. oxystoma* developed into severe epidemics.

It is difficult to make predictions on the evolution of green alder populations affected at present by the epidemic spread of *V. oxystoma* and the associated canker guild if the climate trend will be the same. Based on the high seed production and strong colonization ability (Caviezel et al. 2017) which were already invoked as facilitating the encroachment of green alder in the Alps (detrimental successional trend partially due to abandonment of montane agriculture) we consider that there are chances to maintain the rare Carpathians habitats with *P. mugo* and *A. alnobetula*, given no other major stressors of anthropogenic origin. One must consider also the fact that the mountain environment, at medium and high altitudes, is particularly sensitive to climate change doubled by anthropogenic and biotic stressors (phytophagous insects and pathogens) which impose conservation measures for rare and diverse mountain ecosystems characterized by high endemism (Körner 2019, Scarano 2019, Payne et al. 2020, Schmeller et al. 2022).

A provisional conclusion should stress the phenomenon of declining green alder populations as of major concern but supplementary data should be collected from other areas in Romania where *A. alnobetula* vegetates to confirm the hypothesis of climate change facilitating epidemics in the Carpathian Mountain chain.

Acknowledgment

We express our gratitude for the invaluable fieldwork performed by Mr. Stelian Bodnari who provided us with the necessary samples and made measurements and photos in both the investigated areas. We also wish to transmit our appreciation to the administrations of both protected areas, Călimani Mountains National Park and Rodnei Mountains National Park for the permission to make observations and collect samples. N.O. was supported by the projects PN 23090102 “Scientific foundations for the development of forest protection methods”, and 34PFE./30.12.2021 “Increasing the institutional capacity and performance of INCDS ‘Marin Drăcea’ in the activity of RDI – CresPerfInst” funded by the Ministry of Research, Innovation and Digitalization of Romania.

References

Adams G., Winton L., Mulvey R., 2012. Alder canker. In: Mulvey R., Lamb M. (eds), Forest Health Conditions in Alaska – 2011. FHP Protection Report R10-PR-25. U.S.D.A. Forest Service, Alaska Region, Anchorage, Alaska, pp. 27–28.

Adams G.C., Wingfield M.J., Common R., Roux J., 2005. Phylogenetic relationships and morphology of *Cytospora* species and related teleomorphs (Ascomycota, Diaporthales, Valsaceae) from *Eucalyptus*. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Anonymous, 2016. Tutela boschiva & danni boschivi. Administrazione provincia Bolzano. https://www.provincia.bz.it/agricoltura-foreste/bosco-legno-malghe/difesa-boschiva/tutela-boschiva-2016.asp. (accessed on 2023-07-24)

Anonymus, 2022a. Planul de management al Parcului Național Călimani, al sitului de importanță comunitară ROSCI0019 Călimani-Gurghiu (partea care se suprapune cu Parcul Național Călimani), al ariei de protecție specială avifaunistică ROSPA0133 Munții Călimani și al ariilor naturale protejate de interes național care se suprapun acestuia. Ministerul Mediului, Agenția Națională pentru Protecția Mediului, București.

Anonymous, 2022b. Poročilo Zavoda za Gozdove Slovenije o gozdovih za leto 2021 [Report of the Forest Institute of Slovenia on forests for the year 2021]. Ljubljana, Slovenia. 123 p.

Appel O., 1904. Über bestandweises Absterben von Roterlen. Naturw. Zeitschr. Land- u. Forstw. 2: 311–320.

Biggs A.R., Davis D.D., Merrill W., 1983. Histology of cankers on *Populus* caused by *Cytospora chrysosperma*. Can. J. Bot. 61: 563–574. https://doi.org/10.1139/b83-064

Bühlmann T., Hiltbrunner E., Körner Ch., 2014. *Alnus viridis* expansion contributes to excess reactive nitrogen release, reduces biodiversity and constrains forest succession in the Alps. Alp Botany 124: 187–191. https://doi.org/10.1007/s00035-014-0134-y

Caldeira M.C., 2019. The timing of drought coupled with pathogens may boost tree mortality. Tree Physiol. 39(1): 1–5. doi: 10.1093/treephys/tpy141. PMID: 30615167.

Cannon P.F., Hawksworth D.L., Sherwood-Pike M.A., 1985. The British Ascomycotina. An annotated checklist. Commonwealth Mycological Institute, Kew, Surrey, England, 302 p.

Caviezel C., Hunzinker M., Khun N., 2017. Green alder encroachment in the European Alps. The need for analyzing the spread of a native-invasion species across spatial data. Catena 159: 149–158. https://doi.org/10.1016/j. catena.2017.08.006

Cech T., 1998. *Phytophthora* decline of alder (*Alnus* spp.) in Europe. J. Arboric. 24(6): 339–343. https://doi.org/10.48044/jauf.1998.043

Chapin F.S. III, McGuire A.D., Ruess R.W., Hollingsworth T.N., Mack M.C., Johnstone J.F. et al., 2010. Resilience of Alaska's boreal forest to climatic change. Can. J. For. Res. 40: 1360–1370. https://doi.org/10.1139/X10-074

Cherepanova N.P., Cherepanov P.S., 2003. Ascomycetes on Betulaceae species on North-West of European Russia. Biological Communications 48(1): 20–25 (in Russian)

Ciocârlan V., 2000. Flora ilustrată a României – Pteridophyta et Spermatophyta. Ediția a doua revăzută și adăugită. Editura Ceres, București, 1138 p.

Coldea G., Cristea V., 2021. Syntaxonomic characterization of the vegetation belts from the Southeastern Carpathians (Romanian Carpathians). Flora Mediterr. 31 (Special Issue): 361–369. https://doi.org/10.7320/FlMedit31SI.361

Crous P.W., Shivas R.G., Wingfield M.J, Summerel B.A., Rossman A.Y., Alves J.L. et al., 2012. Fungal Planet description sheets: 128–153. Persoonia 29: 146–201. http://dx.doi.org/10.3767/003158512X661589

Cushman Hall J., Cooper M., Meentemeyer R.K., Benson S., 2008. Human activity and the spread of *Phytophthora ramorum*. In: Frankel S.J.; Kliejunas, John T., Palmieri K.M., tech. coords., 2008. Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW-GTR-214. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. pp. 179–180.

Dakskobler I., Rozman A., Seliškar A., 2013. Forest and scrub communities with green alder (*Alnus viridis*) in Slovenia. Hacquetia 12(2): 95–185. https://doi.org/10.2478/hacq-2013-0012

Dawson J.O., 2008. Ecology of actinorhizal plants. In: Pawlowski K., Newton W.E. (eds.), Nitrogen–fixing actinorhizal symbioses. Springer, Berlin, pp. 199–234.

Doniță N., Popescu A., Paucă-Comănescu M., Mihăilescu S., Biriş I.A., 2005. Habitatele din România. Ed. Tehnică Silvică, București, 496 p.

Dragota C., Kucsicsa Gh., 2011. Global climate change–related particularities in the Rodnei Mountains National Park. Carpathian J. Earth Environ. Sci. 6(1): 43–50. https://doi.org/10.13140/RG.2.1.4342.1283.

EUNIS, 2007. EUNIS Habitat Classification. http://www.eea.europa.eu/themes/biodiversity/eunis/eunis-habitat-classification (accessed on 2023-07-23)

Fitt B.D.L., Huang Y.J., Bosch F.V.D., West J.S., 2006. Coexistence of related pathogen species on arable crops in space and time. Annu. Rev. Phytopathol. 44: 163–182. https://doi.org/10.1146/annurev.phyto.44.070505.143417.

Fodor E., Hâruța O., Milenković I., Lyubenova A., Tziros G., Keča N., Slavov S.B., Diamandis S., Kostov K.V., 2015. Geometric morphometry of *Phytophthora plurivora* sporangia. Ann. For. Res. 58: 275–294. https://doi.org/ 10.15287/afr.2015.411

Forzieri G., Girardello M., Ceccherini G., Spinoni J., Feyen L., Hartmann H. et al., 2021. Emergent vulnerability to climate-driven disturbances in European forests. Nat. Commun. 12, 1081. https://doi.org/10.1038/s41467-021-21399-7

GBIF Backbone Taxonomy, 2022. Checklist dataset https://doi.org/10.15468/39omei. *Valsalnicola oxystoma* (Rehm) D.M.Walker & Rossman in GBIF Secretariat (accessed via GBIF.org on 2023-08-03)

Georgescu C.C., 1952. Genul *Alnus* Gaertn. In: Săvulescu, T. (ed.), Flora Republicii Populare Române. Vol. I. Ed. Academiei RP Române, București, pp. 210–217.

Giordano P.A., Hibbs D.E., 1993. Morphological response to competition in red alder: the role of water. Funct. Ecol. 7: 462–468.

González-Domingo A., Fosse J., Costa-Salavedra C., 2021. Managing (over)tourism in natural protected areas: Learnings from national parks in Spain and in Europe. NATUR Project: Conserving and Escaping Overtourism. Ed. eco-union. Barcelona. Proyecto NATUR: Conservar y Evitar la Masificación. Ed. eco-union. Barcelona. https://doi.org/10.5281/zenodo.5532456.

Grulke N., 2011. The nexus of host and pathogen phenology: understanding the disease triangle with climate change. New Phytol. 189: 8–11. https://doi.org/10.1111/j.1469-8137.2010.03568.x

Hammer Ø., Harper D.T., Ryan P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Paleontol. Electron. http://palaeo-electronica.org.

Hansen E.M., Goheen E.M., 2000. *Phellinus weirii* and other native root pathogens as determinants of forest structure and process in western North America. Annu. Rev. Phytopathol. 38: 515–539. https://doi.org/10.1146/annurev.phyto.38.1.515

Hantemirova E.V., Marchuk E.A., 2021. Phylogeography and genetic structure of a subarctic-alpine shrub species, *Alnus alnobetula* (Ehrh.) K. Koch s. l., inferred from chloroplast DNA markers. Tree Genet. Genomes (2021) 17: 18. https://doi.org/10.1007/s11295-021-01503-0

Hartmann H., Moura C.F., Anderegg W.R.L., Ruehr N.K., Salmon Y., Allen C.D. et al., 2018. Research frontiers for improving our understanding of drought-induced tree and forest mortality. New Phytol. 218: 15–28. https://doi.org/10.1111/nph.15048

Hegre H., Solstad H., Alm T., Fløistad I.S., Pedersen O., Schei F.H., Vandvik V., Vollering J., Westergaard K.B., Skarpaas O. 2023. Karplanter: Vurdering av grønnor *Alnus alnobetula* for Fastlands-Norge med havområder [Vascular plants: Assessment of green alder *Alnus alnobetula* for mainland Norway with marine areas]. Fremmedartslista 2023. Artsdatabanken. https://www.artsdatabanken.no/lister/fremmedartslista/2023/1285

Hossain M., Veneklaas E.J., Hardy G.E.S.J., Poot P., 2018. Tree host-pathogen interactions as influenced by drought timing: Linking physiological performance, biochemical defence and disease severity. Tree Physiol. 39(1): 6-18. https://doi.org/10.1093/treephys/tpy113

Houston D.R., 1994. Major new tree disease epidemics: beech bark disease. Annu. Rev. Phytopathol. 32: 75–89. http://doi.org/10.1146/annurev.py.32.090194.000451

Iqbal U., Mukhtar T., 2014. Morphological and pathogenic variability among *Macrophomina phaseolina* isolates associated with mungbean *Vigna radiata* (L.) Wilczek from Pakistan. Sci. World J., vol. 2014, Article ID 950175, 9 p. https://doi.org/10.1155/2014/950175.

Iușan C., 2014. Strategia de conservare a biodiversității din Parcul Național Munții Rodnei. Analele Parcul Național Munții Rodnei vol. III. Administrația Parcul Național Munții Rodnei. Consiliul Județean Maramureș, 101 p.

Janssen J.A.M., Rodwell J.S., García Criado M., Gubbay S., Haynes T., et al., 2016. European Red List of Habitats. Part 2. Terrestrial and freshwater habitats. Publications Office of the European Union, Luxembourg, 38 p.

Kobayashi T., 2007. Index of fungi inhabiting woody plants in Japan. Host, distribution and literature. Zenkoku-Noson-Kyoiku Kyokai Publishing Co., Ltd., Japan, 1227 p.

Kodrík J., Kodrik M., Hlaváč P., 2006. The occurrence of fungal and insect pests in riparian stands of the central Hron and Slatina rivers. J. For. Sci. 52: 22–29. https://doi.org/10.17221/4483-JFS.

Körner Ch., 2019. Mountain biodiversity, its causes and function: an overview. In: Körner Ch., Spehn E.M. (eds.), Mountain biodiversity. Routledge, London, pp. 3–20.

Kuneš I., Koňasová T., Balcar V., Baláš M., Zahradník D., Kacálek D., Vítámvás J., Jakl M., Jaklová Dytrtová J., 2011. Growth response of *Alnus viridis* to application of crushed limestone and amphibolite and forestry potential of the species in harsh acidic mountain sites. J. For. Sci., 2011, 57(5): 200–209. https://doi.org/10.17221/72/2010-JFS

Lamichhane J.R., Venturi V., 2015. Synergisms between microbial pathogens in plant disease complexes: a growing trend. Front. Plant. Sci. 6:385. https://doi.org/10.3389/fpls.2015.00385.

Lee G.Y.S., Wertman D.L., Carroll A.L., Hamelin R.C., 2023. Filamentous fungal associates of the alder bark beetle, *Alniphagus aspericollis*, including an undescribed species of *Neonectria*. PloS ONE 18(5): e0284393. https://doi.org/ 10.1371/journal.pone.0284393.

Majek J., Schwanda K., Cech T., 2019. Decline of green alder (*Alnus viridis*) in relation to *Phytophthora* species, preliminary results. 9th Meeting of the IUFRO Working Party 7.02.09: *Phytophthora* in Forests and Natural Ecosystems; La Maddalena, Sardinia, Italy; 17-25 October 2019. https://zenodo.org/record/3564233

Mann H.J., 1987. Unsere Baumarten 16. Die beiden Erlen. Die Waldarbeit 38: 159–160.

Markonis Y., Kumar R., Hanel M., Rakovec O., Máca P., AghaKouchak A., 2021. The rise of compound warm-season droughts in Europe. Sci. Adv. 2021; 7: eabb9668. https://doi.org/10.1126/sciadv.abb9668

Maresi G., Ambrosi P., 1999. Nuovi disseccamenti di ontano verde: prime osservazioni in Trentino. Sherwood 47: 39–42.

Marsberg A., Kemler M., Jami F., Nagel J.H., Postma-Smidt A., Naidoo S., Wingfield M.J., Crous P.W., Spatafora J.W., Hesse C.N., Robbertse B., Slippers B., 2017. *Botryosphaeria dothidea*: a latent pathogen of global importance to woody plant health. Mol. Plant Pathol. 18(4): 477–488. https://doi.org/10.1111/mpp.12495

Marușca T., Blaj V.A., 2019. Mulceritul și cositul pășunilor, lucrări obligatorii pentru acordarea subvențiilor [Mulching and mowing of pastures, mandatory works for granting subsidies]. Profitul agricol, nr. 23, 12 iunie 2019. (in Romanian)

Mauri A., Caudullo G., 2016. *Alnus viridis* in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayanz J., de Rigo, D., Caudullo G., Houston Durrant T., Mauri A. (eds.), European Atlas of Forest Tree Species. Publ. Off. EU, Luxembourg, pp. 68.

Metz M.R., Frangioso K.M., Wickland A.C., Meentemeyer R.K., Rizzo D.M., 2012. An emergent disease causes directional changes in forest species composition in coastal California. Ecosphere 3(10):86. http://dx.doi.org/10. 1890/ES12-00107.1.

Micu D.M., Dumitrescu A., Cheval S., Birsan M.-V., 2015. Climate of the Romanian Carpathians - Variability and Trends. Springer, Cham, Switzerland, 213 p.

Mihál I., Cicák A., Tsakov H., 2014. Selected biotic vectors transmitting beech bark necrotic disease in Central and South-Eastern Europe. Folia Oecol. 41: 62–74.

Moricca S., 2002. *Phomopsis alnea*, the cause of dieback of black alder in Italy. Plant Pathol. 51: 755–764. https://doi.org/10.1046/j.1365-3059.2002.00749.x

Münch E., 1936. Das Erlensterben. Forstwiss. Cent.bl. 58: 173–194, 230–248.

Nagavciuc V., Ioniță M., Kern Z., McCarroll D., Popa I., 2022b. A ~700 years perspective on the 21st century drying in the eastern part of Europe based on δ18O in tree ring cellulose. Commun. Earth Environ. (2022) 3: 277. https://doi.org/10.1038/s43247-022-00605-4

Nagavciuc V., Patrick Scholz P., Ioniță M., 2022. Hotspots for warm and dry summers in Romania. Nat. Hazards Earth Syst. Sci. 22: 1347–1369. https://doi.org/10.5194/nhess-22-1347-2022

Nagy L., Grabherr G., 2009. The Biology of Alpine Habitats. Oxford University Press, New York, 142–143.

Oberhuber W., Salino E., Obexer L., Wieser G., Gruber A., 2023. Is growth of Swiss stone pine at the alpine treeline impaired or enhanced by competition with N2-fixing green alder? EGU General Assembly 2023, Vienna, Austria, 24–28 Apr, EGU23-2212, https://doi.org/10.5194/egusphere-egu23-2212, 2023.

Olenici N., 2019. Un caz de mortalitate în masă a larvelor de *Lasiocampa quercus quercus* f. *alpina* în Munții Călimani (România) [A case of mass mortality of *Lasiocampa quercus quercus* f. *alpina* caterpillars in Călimani Mountains (Romania)]. Bucov. For. 19(2): 123–130. https://doi.org/10.4316/bf.2019.017

Payne D., Spehn E.M., Prescott G.W., Geschke J., Snethlage M.A., Fischer M., 2020. Mountain biodiversity is central to sustainable development in mountains and beyond. One Earth 3: 530–533. https://doi.org/10.1016/j.oneear.2020.10.013

Pisetta M., Montecchio L., Oliveira Longa C.M., Salvadori C., Zottele F., Maresi G., 2012. Green alder decline in the Italian Alps. For. Ecol. Manag. 281: 75–83. https://doi.org/10.1016/j.foreco.2012.06.024

Podrázský V.V., Ulbrichová I., 2003. Soil chemistry changes in green alder [*Alnus alnobetula* (Ehrh.) C. Koch] stands in mountain areas. J. For. Sci. 49(3): 104–107. https://doi.org/10.17221/4685-JFS

Popa A., Popa I., 2021. Photosynthesis traits of pioneer broadleaves species from tailing dumps in Călimani Mountains (Eastern Carpathians). Forests 2021, 12, 658. https://doi.org/10.3390/f12060658.

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Richard L., 1967. L'aire de repartition de l'aune vert (*Alnus viridis* Chaix). Documents pour la carte de la vegetation des Alpes 5, 81–111.

Richard L., 1968. Ecologie de l'Aune vert (*Alnus viridis* Chaix). Documents pour la carte de la vegetation des Alpes 6, 107–157.

Rohrs-Richey J.K., Mulder C.P.H., Winton L.M., Stanosz G., 2010. Physiological performance of an Alaskan shrub (*Alnus fruticosa*) in response to disease (*Valsa melanodiscus*) and water stress. New Phytol. 189: 295–307. https://doi.org/10.1111/j.1469-8137.2010.03472.x

Sanda V., Barabas N., Bita-Nicolae C., 2005. Breviar privind parametrii structurali si caracteristicile ecologice ale fitocenozelor din Romania. Part I. Editura "Ion Borcea", Bacău, Romania, 255 p.

Scarano F.R., 2019. Biodiversity sector: Risks of temperature increase to biodiversity and ecosystems. In: Nobre C.A., Marengo J.A., Soares W.R. (eds.), Climate Change Risks in Brazil. Springer International Publishing, Cham, pp. 131–141.

Schmeller D.S., Urbach D., Bates K., Catalan J., Cogălniceanu D., Fisher M.C. et al. 2022. Scientists' warning of threats to mountains. Sci. Total Environ. 853: 158611. https://doi.org/10.1016/j.scitotenv.2022.158611

Senn-Irlet B., Mürner R., Küffer N., Bieri G., Martini E., 2012. Saprobic fungi on wood and litter of *Alnus alnobetula* in the Swiss Alps. Mycotaxon 120: 506, checklist p. 1–32. https://doi.or/10.5248/119.505

Șofletea N., Curtu L., 2007. Dendrologie. Editura Universității Transilvania, Brașov, 418 p. + 122 pl.

Spielman L.J., 1985. A monograph of *Valsa* on hardwoods in North America. Can. J. Bot. 63: 1355–1378. https://doi.org/10.1139/b85-190

Swart W.J., Wingfield M.J., Van Wyk, P., 1993. Variation in conidial morphology among geographic isolates of Sphaeropsis sapinea. Mycol. Res. 97(7): 832–838. https://doi.org/10.1016/S0953-7562(09)81158-1

Szabó I., 2009. A mézgás éger korokozói [Pathogens of gummy alder]. Erdesz. Lapok 144(6): 182–183.

Szász E., 1966. Ciuperci parazite și saprofite pe *Alnus viridis* (Chaix.) Lam.et DC. [Parasitic and saprophyte fungi on *Alnus viridis* (Chaix.) Lam.et DC.]. Contrib. Bot. II: 27-33.

Tognetti R., Borghetti M., 1994. Formation and seasonal occurrence of xylem embolism in *Alnus cordata*. Tree Physiol. 14: 241–250. https://doi.org/10.1093/treephys/14.3.241

Urban Z.,1960. Micromycetes new for Czechoslovakia. Acta Mus. Nat. Pragae 16B (1-2): 1–16.

Vleugel J. 1911. Zweiter Beitrag zur Kenntnis der Pilzflora in der Umgegend von Umeä. Sven. Bot. Tidskr. 5(3): 325–350. https://www.biodiversitylibrary.org/partpdf/247077

von Tubeuf C., 1892. Feinde der Alpenerle *Alnus viridis* D.C. Forstl.-nat.wiss. Z. 1: 387–390.

Walker D.M., Rossman A.Y., Adams G.C., Longa C.M., Maresi G., 2012. *Valsalnicola* D. Walker & Rossman, gen. nov. Pers: Mol. Phylogeny Evol. Fungi 29: 148–149. https://doi.org/10.3767/003158512X661589

Wickham H., 2016. Ggplot2: Elegant graphics for data analysis (2nd ed.) [PDF]. Springer International Publishing.

Wiedmer E., Senn-Irlet B., 2006. Biomass and primary productivity of an *Alnus viridis* stand – a case study from the Schächental valley, Switzerland. Bot. Helv. 116(1): 55–64. https://doi.org/10.1007/s00035-006-0758-7

Winton L., 2013. Building an index for alder canker pathogens in Alaska. Forest Health Condition in Alaska: 28–32. https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/fseprd904030.pdf

Worral J.J., Adam G.C., Tharp S.C., 2010. Summer heat and epidemic of *Cytospora* canker on *Alnus*. Can. J. Plant Pathol. 32(2): 376–386. https://doi.org/10.1081/07060661.2021.499265

Xing J., Li P., Zhang Y., Li J., Liu Y., Lachenbruch B., Su X., Zhao J., 2020. Fungal pathogens of canker disease trigger canopy dieback in poplar saplings by inducing functional failure of the phloem and cambium and carbon starvation in the xylem. Physiol. Mol. Plant Pathol. 112: 101523. https://doi.org/10.1016/j.pmpp.2020.101523

Zehnder T., Lüscher A., Ritzmann C., Pauler C.M., Berard J., Kreuzer M., et al., 2020. Dominant shrub species are a strong predictor of plant species diversity along subalpine pasture-shrub transects. Alp. Bot. 130: 141–156. https://doi.org/ 10.1007/s00035-020-00241-8