ARTICLE

Alternate host screening of *Thekopsora areolata* in Scandinavia: a new record on *Prunus grayana*

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Abstract: The cherry spruce rust caused by *Thekopsora areolata* (Fr.) Magnus results in significant losses in spruce seed production in the forest industry. The pathogen is present in Asia and Europe but absent from North America where it has been considered as a potential threat and listed as a quarantine organism by the United States Department of Agriculture. A comprehensive list and in-depth information regarding the alternate hosts of this pathogen are important for conducting epidemiological studies and for optimal disease control. *Prunus padus* L. is the main alternate host reported for *T. areolata*. In this study, we investigated the susceptibility of domestic and exotic *Prunus* spp. and other potential alternate host-plant species native to Scandinavia to *T. areolata* infection through a field survey and aeciospore inoculation experiments in the greenhouse and laboratory. No new susceptible species were found. In Sweden, a new record of *Prunus grayana* Maxim. with low susceptibility to *T. areolata* was found. In addition, we updated the list of currently confirmed alternate hosts of *T. areolata* according to field observations and inoculation results. *Prunus padus* and *Prunus serotina* Ehrh., as well as their hybrids and subspecies of *Prunus padus*, are highly susceptible, while *Prunus depressa* Pursh, *Prunus grayana*, *Prunus spinosa* L., and *Prunus tenella* Batsch are considered slightly susceptible.

Key words: rust fungi, *Picea abies* (L.) H.Karst, cherry spruce rust, susceptibility, *Prunus*.

Résumé : La rouille de l'épinette causée par *Thekopsora areolata* (Fr.) Magnus entraîne des pertes importantes dans la production de semences d'épinette dans l'industrie forestière. L'agent pathogène est présent en Asie et en Europe, mais il est absent en Amérique du Nord où il a été considéré comme une menace et inscrit comme organisme de quarantaine par le département de l'agriculture des Etats-Unis. Une liste complète et des informations approfondies concernant les hôtes relais de cet agent pathogène sont importantes pour réaliser des études épidémiologiques et pour un contrôle optimal de la maladie. *Prunus padus* L. est le principal hôte relais signalé pour *T. areolata*. Dans cette étude, les auteurs ont étudié la sensibilité de *Prunus* spp. domestiques et exotiques et d'autres espèces végétales hôtes relais potentiels originaires de Scandinavie, à l'infection par *T. areolata*, au moyen d'observations sur le terrain et d'expériences d'inoculation d'écidiospores en serre et en laboratoire. Aucune nouvelle espèce sensible n'a été trouvée. En Suède, un nouveau signalement de *Prunus grayana* Maxim. avec une faible sensibilité à *T. areolata* a été trouvé. De plus, les auteurs ont mis à jour la liste des hôtes relais actuellement confirmés de *T. areolata* selon les observations de terrain et les résultats d'inoculation. *Prunus padus*, *Prunus serotina* Ehrh., ainsi que leurs hybrides et sous-espèces de *Prunus padus*, sont très sensibles, tandis que *Prunus depressa* Pursh, *Prunus grayana*, *Prunus spinosa* L. et *Prunus tenella* Batsch sont considérés comme légèrement sensibles. [Traduit par la Rédaction]

Mots-clés : champignons de rouille, *Picea abies* (L.) H.Karst, rouille de l'épinette, sensibilité, *Prunus*.

Introduction

Norway spruce, *Picea abies* (L.) H.Karst, is a significant coniferous species in natural forests throughout Europe, particularly important in Scandinavia. It has been introduced to and planted in North America, particularly in northeastern United States, southeastern Canada, the Pacific Coast states, and the Rocky Mountain states. It has been widely cultivated in managed forests for timber, pulpwood, and Christmas tree production. The production of Norway spruce in Scandinavia relies on

high-quality seeds produced in seed orchards; however, meeting the demand for improved seeds is challenging due to production shortages (Lundströmer et al. 2020). Furthermore, seed production in Scandinavia has long suffered from insect pests and fungal diseases, of which cherry spruce rust is one of the most destructive diseases (Savonen 2001; Kaitera 2013). Infected cones can be entirely colonized by the fungus and produce a rather low number of seeds with a 10-fold reduction in seed viability (Kaitera and Tillman-Sutela 2014). The seed crop in a seed orchard can be totally lost due to a severe epidemic of this disease (Kaitera 2013).

The currently accepted name of the causal agent of cherry spruce rust is *Thekopsora areolata* (Fr.) Magnus (Magnus 1875), first described as *Pucciniastrum areolatum* (Fr.) G.H. Otth (Otth 1864). This rust fungus is native to and widely distributed in Asia and Europe. It is absent in North America and Australia. The disease is considered a potential threat and *T. areolata* has been listed as a quarantine organism by the United States (Hernández 2005). In Scandinavia, *T. areolata* causes damage to cones of various spruce species including Norway spruce, mountain spruce (*Picea engelmannii* Parry ex Engelm.), Serbian spruce (*Picea omorika* (Pancic) Purk.), and white spruce (*Picea glauca* (Moench) Voss) (Kaitera et al. 2014, 2017). This pathogen is a macrocyclic heteroecious fungus, where both spruce and an alternate host, such as bird cherry (*Prunus padus* L.), are required to complete its life cycle. *Thekopsora areolata* overwinters as teliospores within epidermal cells of *Prunus padus* leaves. In the spring, the teliospores germinate to produce basidia with wind-borne basidiospores, which spread and cause the primary infection on susceptible spruce pistillate cones (Kuprevich and Transchel 1957). Spermogonia are then produced on the outer (abaxial) side of the cone scale, and the sugary fluid containing spermatia is transported by insects between cones (Dennis and Murray 1955) — ensuring mating and fertilization of receptive hyphae. In mid to late summer, aecia with aeciospores are produced on the inner (adaxial) and outer (abaxial) sides of the cone scale. Cones bearing viable aeciospores can remain on the branch for up to 4 years (Kaitera and Tillman-Sutela 2014). From spring in the second year, leaves of the alternate host, *Prunus padus*, can be infected by *T. areolata* aeciospores (Kaitera et al. 2009). Urediniospores produced on the abaxial side of infected leaves can re-infect *Prunus padus* repeatedly during the summer. In autumn, telia and teliospores are produced in epidermal cells on the adaxial side of the *Prunus padus* leaves.

Prunus padus and its subspecies are known as the most common alternate hosts of *T. areolata*; however, infected cones can still be found from seed-tree stands in remote areas of coniferous forests without any *Prunus padus* in the vicinity (Kaitera 2013). In recent studies, no evidence of autoecism in *T. areolata* was observed as aeciospores from *Picea abies* cones were unable to infect the host

(Kaitera et al. 2019), and inoculations on *Prunus* (Kaitera et al. 2019) as well as population genetic analyses supported a heteroecious life cycle of the fungus (Capador et al. 2020). Therefore, basidiospores are considered the primary inoculum of spruce pistillate cones. The epidemics in remote seed-tree stands and seed orchards may be explained by the significant dissemination distance of *T. areolata* basidiospores, or the existence of an unknown alternate host species close to such seed orchards. Since biotrophic plant pathogens tend to infect closely related species (Gilbert et al. 2012), the phylogenetic distance of the plant taxa can be a useful factor to predict their possible hosts (Morris and Moury 2019). For example, over 90 species and hybrids of *Berberis* spp. and closely related *Mahonia* spp. are susceptible to *Puccinia graminis* Pers. (Roelfs 1985). In the case of *T. areolata*, some other *Prunus* spp. have also been reported as susceptible to this pathogen in Europe and Asia — Japanese bird cherry (*Prunus grayana* Maxim.) and Hokkaido bird cherry (*Prunus ssiori* F.Schmidt) in Japan (Hiratsuka et al. 1992), and *Prunus virginiana* L. and *Prunus spinosa* L. in Scandinavia (Kaitera et al. 2014). Therefore, to continue the screening of potential alternate hosts of *T. areolata*, candidate species can be selected from *Prunus* spp. and from species commonly found near and within seed orchards.

Full information of all possible alternate hosts of *T. areolata* is needed for the management of cherry spruce rust. In the United States Department of Agriculture, Agriculture Research Service Fungus–Host Distributions database (Farr and Rossman 2020), 97 and 80 records from previous literature were listed as the hosts of *T. areolata* and *Pucciniastrum areolatum*, respectively. Some of the information may be outdated due to the updated taxonomic status of the hosts and the pathogen, and some fungus–host associations are questionable due to unreliable methods in old literatures, poor documentation, and altered taxonomy of both the pathogen and the plant species, as well as misinterpretation of references. Therefore, a concise list of *T. areolata* hosts confirmed by field observations and inoculation tests in the laboratory is beneficial for disease management and control of plant imports.

In this study, we investigated the susceptibility of *Prunus* spp. and plants that are native to Scandinavia and can be commonly found within — or in the vicinity of — seed orchards, to screen for additional possible alternate hosts of *T. areolata*. We also reviewed the susceptibility status of previously reported *T. areolata* alternate hosts to provide a practical and comprehensive list of confirmed *T. areolata* alternate hosts.

Materials and methods

Survey of *T. areolata* infections on *Prunus* spp. in a botanical garden

In August 2020, all *Prunus* spp. trees in the Uppsala Botanical Garden were examined to investigate their susceptibility to *T. areolata*. From each tree, 100 leaves

Table 1. Survey of *Thekopsora areolata* infection on leaves of *Prunus* spp. in the Uppsala Botanical Garden in 2020.

Species	Common name	Origin	No. of trees	Plant year	Infected leaves (%)[a]	Disease severity scale[b]
Prunus 'Accolade' (*Prunus serrulata* Franch.)	East Asian cherry	East Asia	1	1992	0	0
Prunus armeniaca Marshall	Apricot	East and central Asia	1	1995	0	0
Prunus avium (L.) L.	Sweet cherry	Europe, West Siberia, Turkey, Pamir, Northwest Africa	4	1945, 1945, 1989, 2013	0	0
Prunus brigantina Vill.	Briançon apricot	France	1	Unknown	0	0
Prunus cerasifera Ehrh.	Cherry plum	Balkan, Southwest and Central Asia	2	1922, 1923	0	0
Prunus cerasifera var. *divaricata* (Ledeb.) L.H.Bailey	Cherry plum	Balkan, Southwest and Central Asia	4	2011	0	0
Prunus cerasus L.	Sour cherry	Europe, West Asia	2	Unknown	0	0
Prunus domestica L.	European plum	Europe	1	1944	0	0
Prunus domestica L. × *Prunus spinosa* L.	European plum × blackthorn	Europe	1	1944	0	0
Prunus grayana Maxim.	Japanese bird cherry	Japan	2	1923, 2017	12%, 0	1
Prunus jamasakura Siebold ex Koidz. (*Prunus serrulata* Lindl.)	East Asian cherry	Japan	1	1985	0	0
Prunus maackii Rupr.	Manchurian cherry	South Amur. Northeast Asia	1	1977	0	0
Prunus mahaleb L.	Mahaleb cherry	Europe, South Siberia, Turkey, Central Asia	3	2005, 2006, Unknown	0	0
Prunus maximowiczii Rupr.	Korean cherry	Northeast Asia	2	1976	0	0
Prunus padus L.	Bird cherry	Europe, Northeast Asia	1	2001	45%	2
Prunus pensylvanica L.f.	Bird cherry	North America	1	2014	0	0
Prunus persica Stokes 'Frost'	Peach	China	1	2016	0	0
Prunus persica Stokes 'Riga'	Peach	China	1	2016	0	0
Prunus sargentii Rehder	North Japanese hill cherry	Japan, Sachalin	3	2004, Unknown	0	0
Prunus × *schmittii* Rehder	Schmitt's cherry	—	1	2017	0	0
Prunus serotina Ehrh.	Black cherry	North America, South America	2	1985, Unknown	0	0
Prunus serrula Franch.	Birch bark cherry	Central and south china	1	2017	0	0
Prunus spinosa L.	Blackthorn	Europe, Turkey, Caucasus, Northwest Africa	2	2002	0	0
Prunus virginiana L.	Bitter-berry	North America	1	1960	0	0
Prunus virginiana var. *demissa* (Nuttall) R.L.Taylor & B.Mac Bryde	Western chokecherry	North America	1	1953	0	0

Note: Species names are according to the botanical garden database.

[a]Percentage of infected leaves determined by evaluating 100 leaves from multiple twigs around the whole tree crown.

[b]Disease severity scale according to Kaitera et al. (2014), where 0 = no uredinia, 1 = a few uredinia on a few leaves, and 2 = abundant uredinia on most leaves.

from multiple twigs around the whole tree crown were examined. A total of 41 trees of 25 species and varieties (Table 1) were evaluated based on the criterion proposed by Kaitera et al. (2014): 0 = no uredinia, 1 = a few uredinia on a few leaves, and 2 = abundant uredinia on many leaves.

Infected leaf samples were collected and examined in the laboratory under a microscope to confirm the rust identity. Leaf spots were sampled with sterilized scalpels. Total DNA from the leaf spots was extracted with NucleoSpin Soil DNA extraction kit (Macherey-Nagel, Germany) according to the manufacturer's manual. The amount of *T. areolata* DNA in each sample was quantified by quantitative polymerase chain reaction (qPCR) with specific primers targeting an 81 bp sequence of the internal transcribed spacer (ITS) region (Hietala et al. 2008). To prepare standard samples, the 81 bp sequence was amplified using *T. areolata* genomic DNA and the same primers. Purified amplicons were quantified with a Nanodrop spectrometer. The concentration was transformed from nanograms per microlitres to ITS copies per microlitres according to the average molecular mass of the 81 bp DNA sequence. Then, the product was serial diluted from 6 to 6×10^6 copies·μL^{-1} to build the standard curve in the qPCR assay. Quantitative PCR was performed in 20 μL reaction volumes (10 μL SsoFast Eva-Green Supermixes (Bio-rad, USA), 5 μL DNA template, 1 μL each of forward and reverse primers (10 $\mu mol·L^{-1}$), 3 μL water). PCR cycling parameters were 95 °C for

Table 2. *Thekopsora areolata* aeciospore sources used for inoculation.

No.	Location	Location type	Collection time	Germination rate (%)	Seedling inoculation	Detached leaf inoculation
1	sv 365, Joutsa, FI	Seed orchard	May 2019	35.3	×	×
2	sv 374, Imatra, FI	Seed orchard	May 2019	17.5	×	×
3	sv 235, Sillanpää, FI	Seed orchard	May 2019	10.0	×	×
4	sv 235, Sillanpää, FI	Seed orchard	May 2019	36.7	×	×
5	sv 235, Sillanpää, FI	Seed orchard	May 2019	56.1	×	×
6	SLU, SE	Forest	March 2019	58.4	×	×
7	SLU, SE	Forest	March 2019	60.6	×	×
8	Östersund, SE	Forest	April 2019	24.6	×	×
9	Gottsunda, SE	Forest	April 2019	22.9	×	×
10	Lillpite, SE	Seed orchard	May 2019	31.4	×	×
11	SLU, SE	Forest	March 2019	39.0	×	
12	SLU, SE	Forest	March 2019	61.0	×	
13	Östersund, SE	Forest	April 2019	35.8	×	
14	Gottsunda, SE	Forest	April 2019	42.6	×	
15	Lillpite, SE	Seed orchard	May 2019	36.2	×	
16	sv175, Vehkasalo, FI	Seed orchard	November 2019	18.9		×
17	sv 365, Joutsa, FI	Seed orchard	November 2019	22.1		×
18	sv235, Sillanpää, FI	Seed orchard	November 2019	23.8		×
19	Hjorten, SE	Seed orchard	October 2019	41.3		×
20	Ålbrunna, SE	Seed orchard	October 2019	33.5		×
21	Östersund, SE	Forest	October 2019	29.2		×

Note: sv, seed orchard number; FI, Finland; SE, Sweden; SLU, Swedish University of Agricultural Sciences.

10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. DNA from symptomatic *Prunus grayana* leaves were loaded as unknown samples, from symptomatic *Prunus padus* leaves as positive controls, and from asymptomatic *Prunus padus* leaves as negative controls in the assays.

Inocula sources and germination rate evaluation

For the inoculation experiment in 2019, 15 aeciospore sources were collected from 10 infected cones from four forest locations and one seed orchard in Sweden from March to May 2019. Five infected cones from three seed orchards were collected from Finland in May 2019 (Table 2). Six aeciospore sources from three infected cones in two seed orchards and one forest in Sweden and from three seed orchards in Finland were collected between October and November 2019 for the inoculation experiment in 2020 (Table 2). All cones were air-dried at room temperature after collection, following which, aecia on individual scales were crushed on the lid of a Petri dish under the dissecting microscope with a scalpel, and the released aeciospores were dusted onto a Petri dish.

To evaluate the quality of aeciospores, all spore sources were dusted onto 1.5% water agar plates. The plates were incubated in the dark for 24 h and then checked under a stereomicroscope with 100× magnification to confirm the spore germination. About 100 aeciospores were examined on each plate to calculate the germination rate (germinated spores/total examined spores × 100%) for each spore source.

Plant seedling and detached leaf inoculation

Before the inoculation experiments, we surveyed two seed orchards in Sweden to determine plant species

Table 3. Plant species inoculated as seedlings with *Thekopsora areolata* aeciospores in greenhouse.

Species[a]	Common name	Uredinia formation[b]
Prunus padus L.	Bird cherry	2
Vaccinium vitis-idaea L.	Lingonberry	0
Vaccinium myrtillus L.	European blueberry	0
Vaccinium uliginosum L.	Bogberry	0
Empetrum nigrum L.	Crowberry	0
Fragaria vesca L.	Wild strawberry	0
Ribes alpinum L.	Mountain currant	0
Achillea millefolium L.	Yarrow	0
Galium boreale L.	Northern bedstraw	0
Galium verum L.	Lady's bedstraw	0
Rubus saxatilis L.	Stone bramble	0
Rubus idaeus L.	Red raspberry	0

[a]All seedlings were obtained from a commercial nursery in Finland and inoculated on 20 June 2019.

[b]Disease severity scale according to Kaitera et al. (2014), where 0 = no uredinia, 1 = a few uredinia on a few leaves, and 2 = abundant uredinia on most leaves.

found commonly in Swedish seed orchards. According to the survey results, 12 plant species that were available from commercial suppliers (Särkän Perennataimisto, Arkkukari, and Peuraniemen Taimitarha Oy, Kajaani) were included in the seedling inoculation experiment in the greenhouse of the Botanical Garden of the University of Oulu (Table 3).

Plant seedlings were inoculated by brushing the aeciospores onto the abaxial side of all leaves with a paintbrush. Each plant seedling was inoculated with one

Table 4. Plant species inoculated as detached leaves with *Thekopsora areolata* aeciospores in laboratory.

Species	Common name	Source	Inoculation time	Uredinia formation[a]
Prunus armeniaca L.	American plum	UBG	18 June 2019	0
Prunus avium (L.) L.	Sweet cherry	UBG	18 June 2019	0
Prunus brigantina Vill.	Briançon apricot	UBG	18 June 2019	0
Prunus cerasifera Ehrh.	Cherry plum	UBG	18 June 2019	0
Prunus cerasus L. 'Chokoladnaja'	Sour cherry	OBG	2 July 2019	0
Prunus davidiana (Carrière) Franch.	Chinese wild peach	UBG	2 July 2019	0
Prunus dulcis (Mill.) D.A.Webb	Almond	UBG	18 June 2019	0
Prunus grayana Maxim. (2017)	Japanese bird cherry	UBG	18 June 2019	0
Prunus grayana Maxim. (1923)	Japanese bird cherry	UBG	22 July 2020	1
Prunus grayana Maxim. (2017)	Japanese bird cherry	UBG	22 July 2020	0
Prunus humilis Bunge	Bush cherry	UBG	2 July 2019	0
Prunus incana (Pall.) Batsch	Willow leaf cherry	UBG	2 July 2019	0
Prunus laurocerasus L. 'Mano'	Cherry laurel	UBG	2 July 2019	0
Prunus mahaleb L.	Mahaleb cherry	UBG	18 June 2019	0
Prunus maximowiczii Rupr.	Korean cherry	UBG	18 June 2019	0
Prunus mume (Siebold) Siebold & Zucc.	Chinese plum	UBG	18 June 2019	0
Prunus persica (L.) Batsch 'Frost'	Peach	UBG	18 June 2019	0
Prunus padus L.	Bird cherry	OBG	18 June 2019	2
Prunus padus L.	Bird cherry	UBG	22 July 2020	2
Prunus grayana Maxim (1923)	Japanese bird cherry	UBG	22 July 2020	1
Prunus grayana Maxim (2017)	Japanese bird cherry	UBG	22 July 2020	0
Prunus serrula Franch.	Tibetan cherry	UBG	18 June 2019	0
Amelanchier spicata (Lam.) K.Koch	Thicket shadbush	OBG	2 July 2019	0
Crataegus douglasii Lindl.	Black hawthorn	OBG	2 July 2019	0
Epilobium angustifolium L.	Fireweed	OBG	2 July 2019	0
Populus tremula L.	European aspen	OBG	2 July 2019	0
Rhamnus frangula L.	Alder buckthorn	OBG	2 July 2019	0
Rhododendron tomentosum Harmaja	Wild rosemary	OBG	2 July 2019	0
Rosa rugosa Thunb.	Beach rose	OBG	2 July 2019	0
Rubus odoratus L.	Flowering raspberry	OBG	2 July 2019	0
Salix glauca L.	Gray willow	OBG	2 July 2019	0

Note: UBG, Uppsala Botanical Garden; OBG, Botanical Garden of the University of Oulu.

[a]Disease severity scale according to Kaitera et al. (2014), where 0 = no uredinia, 1 = a few uredinia on a few leaves, and 2 = abundant uredinia on most leaves.

spore source (Table 2). In total, 15 seedlings of each plant species were inoculated. One seedling of each species was moistened only with water and incubated in another room as control. The seedlings were covered with a plastic bag for 48 h to keep the moisture in and promote the germination of aeciospores. All seedlings were examined for symptom development and urediniospore production with a dissecting microscope at 2, 4, 6, and 8 weeks post-inoculation.

Detached leaf inoculations were carried out in 2019 and 2020. Healthy leaves of 17 *Prunus* spp. and nine other plant species common or native to Scandinavia (Table 4) were collected from the Botanical Garden of the University of Oulu and the Uppsala Botanical Garden. One or two leaves (with the abaxial side facing up) were placed in a Petri dish lined with filter paper and filled with 5–10 mL of distilled water. The leaves were inoculated by spreading aeciospores onto the abaxial side with a paintbrush. Two leaves of each species were left uninoculated as control. Four leaves of each species were inoculated with each spore source. Ten and six spore sources were inoculated on each plant species in 2019 and 2020, respectively. All
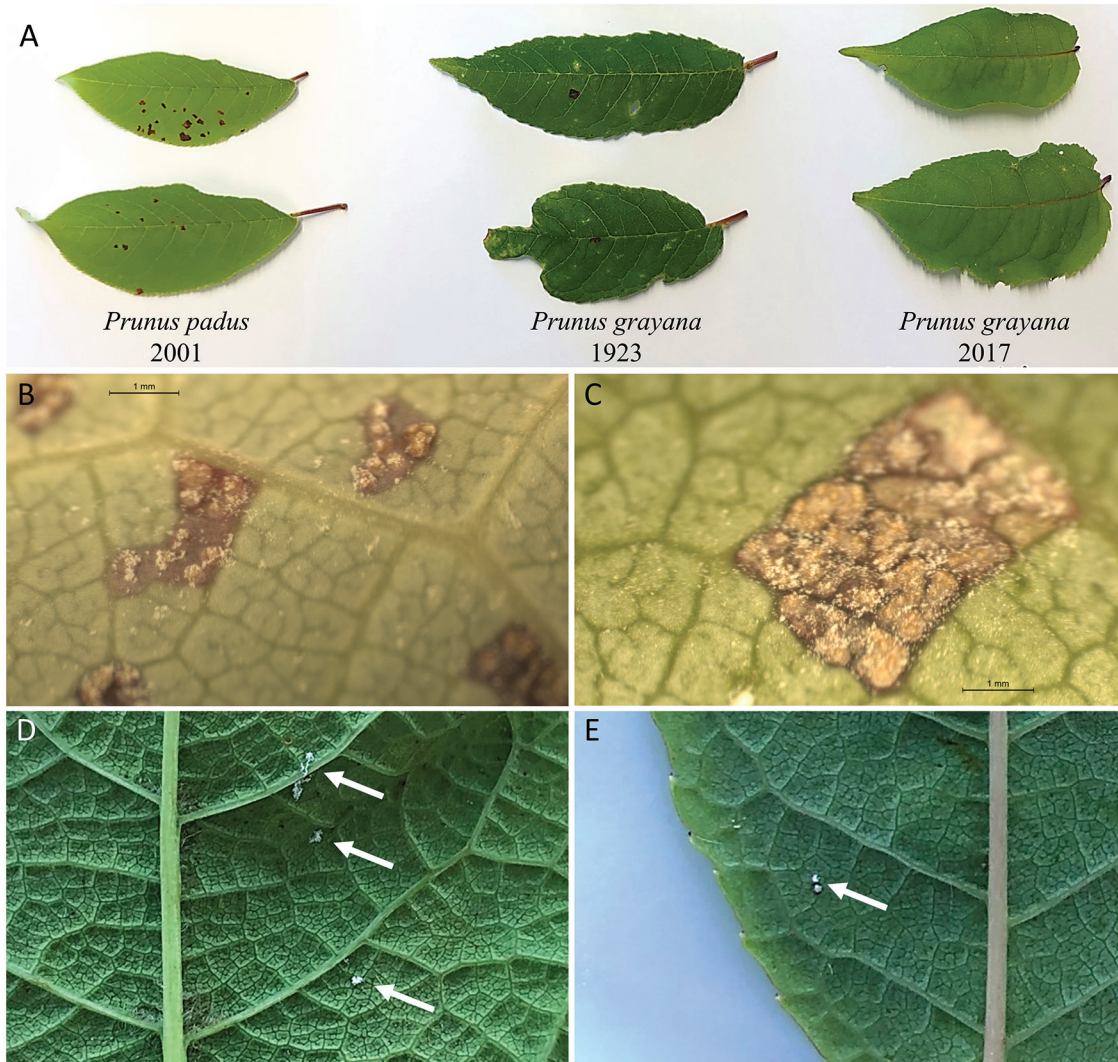
leaves were incubated in growth chambers at 20 °C, and a 12 h light : 12 h dark cycle. Leaf samples were examined with a stereo microscope, and symptom development and the production of urediniospores were recorded at 2 and 4 weeks post-inoculation.

Urediniospores produced from detached leaf inoculations were collected with a pipette and 30 μL of sterilized water, then transferred into a 1.5 mL centrifuge tube. Molecular identification of urediniospores was confirmed by qPCR as described above.

Re-inventory of *T. areolata* alternate hosts in database and literature

A list of the alternate hosts of *T. areolata* and its synonymy *Pucciniastrum areolatum* was obtained from the Fungus–Host Distributions database (https://nt.ars-grin.gov/fungaldatabases/fungushost/FungusHost.cfm; retrieved 10 November 2020). Current taxonomy and accepted names of the host species were confirmed according to Tropicos (https://www.tropicos.org/home) database and the Global Biodiversity Information Facility (https://www.gbif.org/) database. The susceptibility of each

Fig. 1. *Thekopsora areolata* infections on *Prunus padus* and *Prunus grayana* leaves in the Uppsala Botanical Garden and in detached leaf inoculation test. (A) Symptomatic *Prunus padus* leaves, symptomatic *Prunus grayana* (1923) leaves, and asymptomatic *Prunus grayana* (2017) leaves collected from the botanical garden. (B) Abaxial side of a *Prunus padus* leaf with uredinia under a dissecting microscope. (C) Abaxial side of a *Prunus grayana* leaf with uredinia under a dissecting microscope. (D) Urediniospore production on an aeciospore inoculated *Prunus padus* leaf; the arrows point to urediniospore clusters. (E) Urediniospore production on an aeciospore inoculated *Prunus grayana* leaf; the arrow points to urediniospore clusters. [Colour online.]

species was determined according to previous literature and recent studies (Kaitera et al. 2014, 2019) including field surveys and laboratory inoculations of this study. The names of the *Prunus* spp. in previous studies were based on original information of varieties and cultivars of the plant material in literature and databases of botanical gardens. The determination criteria were as follows: susceptible, both literature and recent studies suggested moderate to high disease severity in the field and inoculation experiments; slightly susceptible, species that had or had not been reported in previous studies and recent literature suggested low disease severity in the field or inoculation experiments; resistant, literature and recent studies suggested full resistance; and to be determined, previous literature suggested susceptibility but recent studies suggested resistance, and thus, additional information is required for confirmation.

Results

Survey of *T. areolata* infections on *Prunus* spp. in the Uppsala Botanical Garden

The survey results of *T. areolata* infection on *Prunus* spp. in the Uppsala Botanical Garden are listed in Table 1. *Thekopsora areolata* infection and urediniospore production were observed in *Prunus padus* and *Prunus grayana* leaves in 2020. Forty-five percent of the leaves on *Prunus padus* trees displayed cherry spruce rust symptoms: violet or reddish-brown leaf spots on the adaxial side and uredinia with urediniospores production on the abaxial side (Fig. 1A). Two *Prunus grayana* trees with different

origins were examined. *Prunus grayana* (1923) had been obtained as seeds from the Plant Research Institute, Central Experimental Farm in Canada in 1923, and the seedling of *Prunus grayana* (2017) was purchased from a Swedish commercial nursery. No *T. areolata* infection was found on *Prunus grayana* (2017) (Fig. 1A), while 12% of the leaves from *Prunus grayana* (1923) were infected by *T. areolata* (Fig. 1A). Besides a higher percentage of infected leaves, multiple leaf spots were usually observed on each *Prunus padus* leaf, while each infected *Prunus grayana* leaf had only one leaf spot (Fig. 1E). No other domestic or exotic *Prunus* spp. showed cherry spruce rust symptoms (Table 1).

Leaf spots of *Prunus grayana* (1923) in Fig. 1A were confirmed as *T. areolata* using species-specific qPCR. DNA was extracted from *Prunus grayana* leaf spots; a healthy *Prunus padus* leaf section was the negative control, and *Prunus padus* leaf spots were the positive control. The qPCR assay detected 8.09×10^5 and 3.18×10^6 ITS copies from symptomatic *Prunus padus* samples, and 4.35×10^5 and 4.48×10^5 ITS copies from symptomatic *Prunus grayana* samples. These results confirm that the disease symptoms observed on *Prunus grayana* were caused by *T. areolata* infection.

Plant seedling inoculation

A total of 15 spore sources were inoculated on seedlings of 12 potential plant hosts (Tables 2 and 3). All inoculated plant seedlings were incubated in the greenhouse for 8 weeks. After 2 weeks, abundant uredinia with mature urediniospores were produced on most leaves of all 15 *Prunus padus* seedlings inoculated with the aeciospore sources. No symptoms of *T. areolata* infection were found on any other species inoculated with any of the aeciospore sources (Table 3). None of the seedlings in the control group showed symptoms of *T. areolata* infection.

Detached leaf inoculation

During the 2019 detached leaf inoculation experiment in Finland, 17 *Prunus* spp. and nine native Scandinavian species were tested with 10 spore sources. The positive controls (*Prunus padus* leaves inoculated with all 10 aeciospore sources), produced leaf lesions with multiple uredinia with mature urediniospores within 2 weeks. None of the other *Prunus* spp. or other tested plant species produced any *T. areolata* uredinia during the 4 weeks of incubation (Table 4).

In the 2020 detached leaf inoculation in Sweden, all the *Prunus padus* leaves inoculated with each spore source showed severe disease symptoms with multiple uredinia producing mature urediniospores (Fig. 1D). None of the *Prunus grayana* (2017) leaves developed uredinia, but one *Prunus grayana* (1923) leaf, inoculated with spore source 17 (sv 365, Joutsa, Finland), produced one leaf lesion with multiple uredinia and mature urediniospores (Fig. 1E).

Upon identification of urediniospores produced on *Prunus grayana* (1923) in Fig. 1E by qPCR, they were confirmed as *T. areolata*, as were urediniospores from *Prunus padus* in Fig. 1D, the positive control. The qPCR assay detected 1.82×10^7 and 2.38×10^6 ITS copies from two urediniospore samples collected from inoculated *Prunus padus* samples, and 1.02×10^6 ITS copies from urediniospores collected from inoculated *Prunus grayana* samples. This confirms the origin of the urediniospores as *T. areolata*.

Thekopsora areolata alternate host inventory

Besides *Picea* spp. as the aecial host, 31 and 14 species were listed as telial and (or) alternate hosts of *T. areolata* and *Pucciniastrum areolatum* (syn.), respectively, in the Fungus–Host Distributions database. Table 5 is a summary of the distribution and literature record of 37 species after combining overlapped host species records. Seven species records were considered as resistant due to lack of sporulation of these plants during a rust outbreak in 2012 in Finland (Kaitera et al. 2014); these species were assigned accordingly. Due to changes in the taxonomy of both the pathogen and the plant over time, older literature is not fully reliable. For 12 species records that are to be determined, negative laboratory inoculations and field observations were reported in Finland (Kaitera et al. 2014, 2019) and in this study. Previous literature records usually only listed species names as the alternate hosts of *T. areolata* without description and detailed location information in the Fungus–Host Distribution database. Therefore, it is impractical for us to confirm or reject the identification. Thirteen species records are classified as susceptible and five as slightly susceptible. These species were either confirmed by inoculation experiments or because they were subspecies of *Prunus padus*. After verifying the current taxonomy of *Prunus* spp., the confirmed alternate hosts of *T. areolata* are as follows: *Prunus padus* and its subspecies; *Prunus serotina* Ehrh., *Prunus virginiana*, and their hybrids (being highly to moderately susceptible); *Prunus depressa* Pursh, *Prunus grayana*, *Prunus spinosa,* and *Prunus tenella* Batsch (being slightly susceptible).

Discussion

For obligate parasites like the heteroecious rust *T. areolata*, information about the alternate host range is valuable to control the occurrence of epidemics. This study aimed to add information about the alternate host range of *T. areolata* based on field and laboratory evidence in combination with results from other recent studies, previous literature, and database records. This study provides an update on the current knowledge of the *T. areolata* alternate host range.

Based on evidence from a survey in the Uppsala Botanical Garden and inoculation experiments in the laboratory and greenhouse, we report *Prunus grayana* as a new alternate host record of *T. areolata* in Sweden. However,

Table 5. *Thekopsora areolata* alternate host inventory.

Recorded host species name in database[a]	Location of record	Literature	Synonym of[b]	Susceptibility[c]
Amygdalus nana L.	USSR	Kuprevich and Transchel 1957	*Prunus tenella* Batsch	Slightly susceptible
Cerasus fruticosa (Pall.) G. Woron.	USSR	Kuprevich and Transchel 1957	*Prunus avium* (L.) L.	TBD
Cerasus mahaleb (L.) Miller	Poland	Mulenko et al. 2008	*Prunus mahaleb* L.	TBD
Cerasus vulgaris Mill.	USSR	Kuprevich and Transchel 1957	*Prunus cerasus* (L.) L.	TBD
Padus asiatica Kom.	USSR, Mongolia	Kuprevich and Transchel 1957; Braun 1999	*Prunus padus* var. *asiatica* (Kom.) T. C. Ku & B. M. Barthol.	Susceptible
Padus avium Mill.	Czech Republic, Poland, Belarus, Germany	Dietrich 2005; Majewski 1971; Girilovich et al. 2003	*Prunus padus* subsp. *padus* L.	Susceptible
Padus maackii (Rupr.) Kom.	USSR	Kuprevich and Transchel 1957	*Prunus maackii* Rupr.	TBD
Padus petraea (Tausch) M.Roem	Poland	Mulenko et al. 2008	*Prunus padus* subsp. *borealis* (A.Blytt) Nyman	Susceptible
Padus racemosa (Lam.) Gilib.	China, USSR	Zhuang 2005; Kuprevich and Transchel 1957	*Prunus padus* subsp. *padus* L.	Susceptible
Padus ssiori Fr. Schm.	USSR	Kuprevich and Transchel 1957	*Prunus ssiori* F. Schmidt	TBD
Prunus americana Marsh	Finland	Kaitera et al. 2014, 2019	—	Resistant
Prunus avium (L.) L.	Norway, Sweden	Jorstad 1962; Hylander et al. 1953	—	TBD
Prunus cerasus L.	Norway, Finland	Gjaerum 1974; Kaitera et al. 2014, 2019	*Prunus avium* (L.) L.	TBD
Prunus domestica L.	Norway, Sweden	Gjaerum 1974; Hylander et al. 1953	—	TBD
Prunus domestica L. subsp. *insititia*	Finland	Kaitera et al. 2014	*Prunus domestica* subsp. *insititia* (L.) Bonnier & Layens	Resistant
Prunus grayana Maxim.	Norway, Japan, Sweden	Gjaerum 1974; Hiratsuka et al. 1992	—	Slightly susceptible
Prunus maackii Rupr.	Russia, Finland	Gjaerum 1996; Kaitera et al. 2014, 2019	—	TBD
Prunus mahaleb L.	Norway	Gjaerum 1974	—	TBD
Prunus maritima Marsh.	Finland	Kaitera et al. 2014, 2019	—	Resistant
Prunus padus L.	China, Japan, Finland, Sweden, Norway, Czech Republic, Belgium, Germany, Russia, UK	Kaitera et al. 2014, 2019; Zhuang 1989; Hiratsuka et al. 1992; Gjaerum 1974; Muller 2010; Kuprevich and Transchel 1957; Henderson 2000	—	Susceptible
Prunus padus subsp. *borealis* (Schneb. Ex A. Blytt)	Finland	Kaitera et al. 2014, 2019	—	Susceptible
Prunus padus var. *pubescens* Regel & Tiling	China	Zhuang 1989	—	Susceptible
Prunus pennsylvanica L.	Finland	Kaitera et al. 2014, 2019	—	Resistant
Prunus pumila L.	Finland	Kaitera et al. 2014	—	Resistant
Prunus pumila var. *bessey* (Bailey) Gleason	Finland	Kaitera et al. 2014, 2019	—	Resistant
Prunus pumila var. *depressa* (Pursh) Gleason	Finland	Kaitera et al. 2014, 2019	*Prunus depressa* Pursh	Slightly susceptible
Prunus racemosa Lam.	China, Russia	Zhuang 1989; Benua and Karpova-Benua 1973	*Prunus padus* subsp. *padus* L.	Susceptible
Prunus sargentii Rehder	Finland	Kaitera et al. 2014, 2019	—	Resistant
Prunus serotina Ehrh.	Finland	Kaitera et al. 2014, 2019	—	Slightly susceptible
Prunus spinosa L.	Norway, Sweden, Finland	Jorstad 1962; Hylander et al. 1953; Kaitera et al. 2014, 2019	—	Slightly susceptible

Table 5 (*concluded*).

Recorded host species name in database[a]	Location of record	Literature	Synonym of[b]	Susceptibility[c]
Prunus ssiori F. Schmidt	Japan	Hiratsuka et al. 1992	—	TBD
Prunus tenella Batsch	Finland	Kaitera et al. 2019	—	Slightly susceptible
Prunus virginiana L.	Norway, Poland, Finland	Jorstad 1962; Mulenko et al. 2008; Kaitera et al. 2014	*Prunus serotina* Ehrh.	Susceptible
Prunus virginiana var. *demissa* (Nutt.) Torr.	Finland	Kaitera et al. 2014, 2019	*Prunus serotina* Ehrh.	Susceptible
Prunus virginiana var. *melanocarpa* (A. Nelson) Sarg.	Finland	Kaitera et al. 2014, 2019	*Prunus serotina* Ehrh.	Susceptible
Prunus virginiana × *Prunus padus*	Finland	Kaitera et al. 2014	—	Susceptible
Pyrus pashia Buch.-Ham. ex D. Don	Pakistan	Afshan and Khalid 2009	—	TBD

[a]Species name of the alternate host of *T. areolata* or its synonymy *Pucciniastrum areolatum* in the Fungus–Host Distributions database.
[b]Current accepted name of the alternate host based on Tropicos and the Global Biodiversity Information Facility database.
[c]TBD, to be determined. For instances when previous literature suggested susceptibility, but recent observations suggested resistance.

Prunus grayana has previously been recorded as an alternate host in Norway in 1974 and in Japan in 1992 (Table 5). When compared to *Prunus padus*, *Prunus grayana* is less susceptible because fewer leaves were infected in the botanical garden and fewer leaf spots developed in the laboratory inoculations. Other tested *Prunus* spp., as well as native plant species commonly found within seed orchards in Sweden, were unaffected by *T. areolata*. All the aeciospore sources collected from Sweden and Finland were pathogenic on *Prunus padus*, suggesting that there is low variation in pathogenicity of *Prunus padus* among Scandinavian *T. areolata* populations.

The results of inoculations and field observations of the susceptibility of the same host species are not always consistent within one study or between different studies. Although *Prunus padus* and its subspecies are usually reliable positive controls to confirm the virulence of *T. areolata* spores, not all inoculated leaves produced uredinia in this study nor in a previous study (Kaitera et al. 2019). This may partly be due to variation in age of the test leaves, which can never be fully standardized in inoculation studies. Therefore, multiple leaves need to be tested when screening for alternate hosts of *T. areolata*. In this study, leaves from two *Prunus grayana* trees with different origins showed different levels of susceptibility and resistance. Since the leaves of the two trees were subjected to similar inoculum density, inoculum source, and environmental conditions in both the detached leaf inoculation experiment and the field, the variance of susceptibility between the two may be explained by the different genetic backgrounds of the two trees, which can result in different levels of host–parasite recognition.

Kaitera et al. (2014) observed *T. areolata* infection in three *Prunus virginiana* varieties and confirmed this by detached leaf inoculation (Kaitera et al. 2019). These

Prunus virginiana varieties are synonyms of *Prunus serotina* (Brummitt 2011; Applequist 2013). In this study, no *T. areolata* infection was found in trees identified as *Prunus serotina* or *Prunus virginiana* in 2020 in the Uppsala Botanical Garden. This indicates that different varieties, subspecies, or cultivars within a broad taxonomic species may show variation in susceptibility to *T. areolata*. Similarly, a low level of *T. areolata* infection was found in *Prunus spinosa* in detached leaf inoculations (Kaitera et al. 2019), while no *T. areolata* infection was observed in this study. The variance in susceptibility may be caused by the different genetic backgrounds of both the trees and the pathogen, different levels of inoculum pressure, and different environmental conditions. Even though some early reported species such as *Prunus maackii* Rupr. and *Prunus mahaleb* L. (Kuprevich and Transchel 1957) have never shown any susceptibility in recent studies or this one (Kaitera et al. 2014, 2019), we could not completely reject their probability of being alternate hosts of *T. areolata*. More information about previous identifications and new inoculation tests — especially regarding local plant and aeciospore material from the initially reported geographic locations — are required to clarify the extent of susceptibility of these hosts. These species might be resistant to Scandinavian spores, but susceptible to local ones.

Since the first description of *Pucciniastrum areolatum* (Otth 1864) and *T. areolata* (Magnus 1875) on *Prunus* spp., the taxonomies of both the plant and the pathogen have changed gradually. Cummins and Hiratsuka (2003) classified *Thekopsora* as distinct from *Pucciniastrum* based on morphological characteristics, and the separation of the two genera has been confirmed by phylogenetic analysis based on the small subunit gene of the rRNA (Wingfield et al. 2004). Hence, the current accepted name of the pathogen is *T. areolata*. Various databases still have

separate and confusing records of the alternate host of *Pucciniastrum areolatum* based on information from early literature and even recent publications. For instance, Afshan and Khalid (2009) reported *Pucciniastrum areolatum* from *Pyrus pashia* Buch.-Ham. ex D.Don in Pakistan based on morphological characteristics of only urediniospores (Table 5). Due to the lack of molecular evidence, lack of description of disease symptoms, and lack of teliospores within epidermal cells, we consider this record as being *Pucciniastrum* sp. rather than *T. areolata*. The current accepted names of some *Prunus* spp. have also changed. For example, *Prunus serotina*, rather than *Prunus virginiana*, has been conserved according to the List of Symbols Committee for Vascular Plants (Brummitt 2011; Applequist 2013). In view of this, we summarized and updated the current accepted names and susceptibility of *T. areolata* alternate host records listed in the Fungus–Host Distributions database to remove redundant information.

Prunus padus and *Prunus spinosa* are native to and widely distributed in Scandinavia (GBIF 2019a, 2019b). Because of their high susceptibility, *Prunus padus* and its subspecies are the most commonly reported alternate hosts of *T. areolata*. *Prunus spinosa* has seldom been reported (Kaitera et al. 2019) and is less significant in the spreading of cherry spruce rust because of its low susceptibility. *Prunus serotina* is an invasive species in southern Scandinavia. It has been identified as one of the most problematic alien vascular plant species in Sweden (Tyler et al. 2015). Due to its susceptibility in previous inoculation tests (Kaitera et al. 2019), and susceptibility of its varieties (*Prunus virginiana*) under natural inocula (Kaitera et al. 2014), seed orchard owners must pay attention to the role *Prunus serotina*, *Prunus padus*, and their respective varieties play in spreading the rust disease to seed orchards, where the rust poses a threat to the production of high quality seeds. *Prunus tenella*, *Prunus depressa*, and *Prunus grayana* have limited distribution in Scandinavia (Wu et al. 2003; Rohrer 2014; GBIF 2019c). These species are slightly susceptible to *T. areolata*, but they currently do not pose a high risk to the Scandinavian forest industry because of their limited distribution. However, the importance of each species may need to be re-evaluated as the distribution, cultivation, and use of domestic and exotic *Prunus* spp. expanded due to increasing trade and climate change. Today, *T. areolata* is absent in North America, but the two most susceptible alternate host species, *Prunus serotina* and *Prunus padus*, are native and introduced, respectively. Moreover, *Picea* spp., both native and (or) introduced, exist in North America, and have the potential of being the primary hosts of *T. areolata*. Therefore, accidental introduction of this pathogen may pose a critical risk to the natural regeneration of *Picea* spp. forests and the forest industry. Some *Prunus* spp. mentioned above are available for purchase from commercial nurseries in Scandinavia and North America. Phytosanitary practices need to be followed during the trading of plant materials.

According to recent phylogenetic studies (Shi et al. 2013; Chin et al. 2014), *Prunus padus*, *Prunus grayana*, and *Prunus serotina* (syn. *Prunus virginiana*) belong to subgenus *Laurocerasus*. These susceptible species are closely related and belong to the same monophyletic clade, while other *Prunus* spp. in this clade have not yet been tested for their susceptibility to *T. areolata*. *Prunus spinosa* and *Prunus tenella* belong to subgenus *Prunus,* and they are not closely related to each other or to the above three susceptible species. Based on these data, we hypothesize that *T. areolata* coevolved with the ancestral species of *Prunus padus*, *Prunus grayana,* and *Prunus serotina*. The susceptibility of other species within this clade, such as dog cherry (*Prunus buergeriana* Miq.) and Taiwan bird cherry (*Prunus obtusata* Koehne), should be tested to support this hypothesis.

In conclusion, from the aeciospore inoculation experiment and field survey results conducted in this study, we found *Prunus grayana* to be a new alternate host record for *T. areolata* in Sweden. Its susceptibility was confirmed by inoculation tests and the identity of *T. areolata* was confirmed by qPCR analysis. Furthermore, we summarized *T. areolata* alternate host records from a database and literature and provided a shortlist of alternate hosts that have been confirmed by recent laboratory and field studies. This list of susceptible host plants will be of practical importance for the management of cherry spruce rust for the industry and policymakers. The susceptibility status of some earlier reported species remains to be determined and requires more field observations and inoculation tests. This study did not discover additional alternate host species that could explain the disease epidemics in remote seed orchards where *Prunus* species were absent, and therefore, epidemiological studies focused on basidiospore dispersal are needed to understand the cause of disease outbreaks in these locations.

Competing interests

The authors declare there are no competing interests.

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