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## Dogs can detect the rust fungus *Cronartium pini* in the forest

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### Highlights

- Dogs identified *Cronartium pini* spores, fruit bodies and young and old lesions.
- Dogs identified both heteroecious and autoecious *Cronartium pini*.
- Dogs identified *Cronartium pini* at the early epidemical stage of the disease.
- Dogs identified *Cronartium pini* from latent infections in alive shoots.

### Abstract

*Cronartium pini* (Willd.) Jørst. is a major rust pathogen that kills especially Scots pine (*Pinus sylvestris* L.). Early diagnosis of the pathogen would reduce significant losses in managed forest productivity. Dogs (*Canis lupus familiaris* L.) with their accurate sense of smell have potential to detect forest pathogens at an early stage before they cause significant losses in forests. In this study, we tested in northern Finland whether trained volunteer dog-handler teams could identify infected wood, fruit bodies, spores or mycelia of *C. pini* *in vitro* and *in vivo* to facilitate early disease diagnosis. Volunteer dog-handler teams were able to indicate *C. pini* spores, fruit bodies and both fresh and old rust lesions on Scots pine including alive shoots, where the rust was present yet as latent. Five dogs out of five detected *in vitro* *C. pini* (both life-cycle forms), with 51% mean sensitivity and 58% mean precision. Four dogs out of four detected *in vivo* the autoecious life-cycle form of *C. pini*, with 95% mean sensitivity and 89% mean precision. In *in vivo* detection of the heteroecious life cycle form on pine, two dogs out of two performed with 78% mean sensitivity (100% precision). For identifying *C. pini* on alternate hosts *in vivo*, the mean sensitivity was 58% (precision 100%). Trained dog-handler pairs show promise as an aid in searching for *C. pini* especially in Scots pine stands at their early epidemical stage, but further testing is needed.

**Keywords** *Pinus sylvestris*; alternate hosts; canine; resin-top disease; Scots pine blister rust; scent detection

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## 1 Introduction

*Cronartium pini* (Willd.) Jørst is an important rust on *Pinus* spp. in Europe and Asia (CABI 2009). It is a quarantine species in North America (Kim et al. 2022). In Finland, the rust is distributed throughout the country with ca. 2% of infected Scots pines (*Pinus sylvestris* L; Ylikojola and Nevalainen 2006). In single stands ca. 50% of the pines may be infected (Kaitera 2000; Kaitera and Kokko 2023), and in severe cases the stand has to be regenerated before full maturation if the number of healthy trees to be raised becomes too low (Finlex 2013; Metsähallitus 2022). The rust kills all size of trees, but mature trees are most severely infected (Kaitera et al. 1994). Recently, however, severe epidemics have been reported in young pine plantations in nutrient-rich soils (Kaitera 2000; Wulff et al. 2012).

The rust has a heteroecious form that spreads via alternate host plants and an autoecious form that spreads directly from pine to pine (Kaitera 2003; Kaitera and Nuorteva 2008). The *C. pini* populations are genetically close in northern Fennoscandia (Samils et al. 2021). The rust can spread via over 50 susceptible species (Kim et al. 2022) of which hemiparasitic *Melampyrum* spp., *Euphrasia* spp., *Pedicularis* spp. and *Rhinanthus* spp. are highly susceptible (Kaitera et al. 2015). *Vincetoxicum hirundaria* Medicus and *Paeonia* spp. are also important alternate hosts (Kaitera et al. 2005, 2017). *C. pini* has five spore stages: spermogonia and aecia on pines and uredinia, telia and basidia on alternate hosts. Disease symptoms include lesions and aecia on branches and stems, black resinuous wood, dead tops and death of the whole tree on pines (Kaitera et al. 1994). Infected wood contains high amounts of terpenes and resin acids (Kaitera et al. 2021). The rust is recommended to be controlled by removing infected trees during thinnings, and avoiding use of susceptible seed and susceptible sites in pine cultivation (Metsänhoidon suosituksset 2025). Removal of infected trees has been effective to keep the *C. pini* incidence at low level nationally in Finland over decades (Ylikojola and Nevalainen 2006; Hantula et al. 2023). Removal of infected trees during thinnings has also been recommended in Britain (Pawsey 1964) and in southern Europe (Diamandis and Perleron 2003). However, in a stand with high disease incidence and long disease history, thinning did not reduce significantly *C. pini* incidence shortly after thinning (Kaitera 2002). This was probably due to high susceptibility of the pine provenance and high number of latent infections invisible to naked eye on trees during management that progressed to disease symptoms shortly after thinning. Susceptibility of pine to *C. pini* is inherited and known to affect highly *C. pini* incidence (Pawsey 1964; Persson et al. 2024), which information can be utilized in future breeding programs to increase pine resistance to *C. pini* and improve disease control (Persson et al. 2024).

Dogs (*Canis lupus familiaris* L.) possess highly developed olfactory senses and have been successfully trained to detect various non-biological (e.g., ores, explosives, chemical contaminants, and drugs) and biological scents (e.g., animal and plant species, mushrooms, and several human diseases) (Browne et al. 2006). Dogs have also been used to detect tree pathogens and insects. They can identify trees attacked by bark beetles (Vošvrđová et al. 2023), and pathogenic fungi, *Phytophthora* spp. (*P. ramorum* Werres, De Cock & Man in't Veld), in oaks (*Quercus* spp.; Carter et al. 2023). Although training dogs to detect pathogens offers great potential for forest pathology by detecting latent infections and early disease symptoms, it has not been utilized so far for rust fungi. Recently, dogs were successfully trained to detect *Heterobasidion* spp. in natural forest environment (Kaitera et al. 2025). Dogs are also able to detect *H. parviporum* buried in the soil and water extract from infected wood applied on the ground surface (Swedjemark and Morrison 1987; Wysocka 2021).

Since *C. pini* is difficult to identify especially in young living trees due to long latent period before visible symptoms and on alternate hosts with small size of fruit bodies, we investigated the

potential of dogs' sense of smell for detecting *C. pini* in pine forests. We hypothesised that dogs could be trained to recognise *C. pini* already at an early stage of infection.

The aim of this study was to clarify if dogs can be trained to detect the scent produced by *C. pini* in trees and plants to improve early detection and therefore, forest health. Early detection would enable timely implementation of control measures, preventing *C. pini* epidemics.

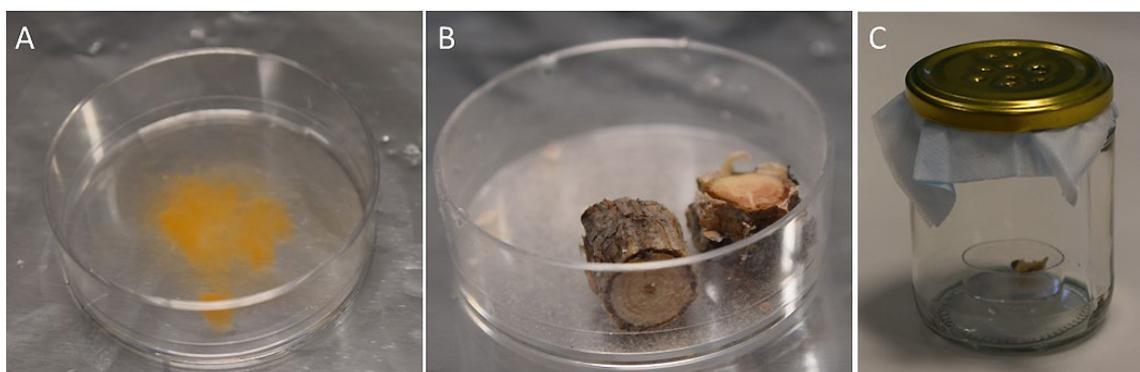
## 2 Materials and methods

### 2.1 Collection and preparation of samples

Aeciospores, young sporulating and old sporulated lesions carrying *C. pini* aecia were collected in June 2021 (Fig. 1), and leaves of *Paeonia lactiflora* Pall., *Impatiens balsamina* L., *Melampyrum sylvaticum* L., *Euphrasia* sp. and *Rhinanthus minor* L. carrying uredinia or telia of *C. pini*, were collected in July-August 2021 and August 2022. Aeciospores were dusted aseptically into glass jars, while lesions with aecia were split into 1–2 cm segments. Leaves with *C. pini* uredinia or telia were separated from rest of the leaf material aseptically directly to glass jars. Rust material was collected in total of 91 glass jars (Table 1).

For the dog training and test trials, fungal material was divided into small petri dishes (Ø 3.3 cm) stored in airtight glass jars at  $-20^{\circ}\text{C}$ . Spores were divided into jars using an artist's brush. Segments of 0.5–1.0 cm were cut from the lesions, while 1.0 cm<sup>2</sup> pieces were cut from big leaves of *Paeonia* and *Impatiens* with scissors and whole leaves of smaller leaves of the other plant species were picked up with tweezers. The surfaces and used equipment were autoclaved or sterilized with alcohol and hands were covered by Akro Eco disposable gloves to minimize addition of human or other contaminating smells to samples and jars. Gloves were changed and the equipment sterilized between different sample types to avoid cross-contamination of scent from one sample to another.

As controls, healthy Scots pine shoots and leaves of above-mentioned plant species and of *M. pratense* L., were also used. Leaves of *Melampyrum*, *Betula*, *Salix*, *Sorbus* and needles of Norway spruce [*Picea abies* (L.) Karst.] carrying fruiting stages of other rusts, *C. tussilaginis* f.sp. *melampyri* Boerema & Verh., *Chrysomyxa ledi* (Alb. & Schwein) de Bary, *Melampsorium betulinum* (Fr.) Kleb., *Gymnosporangium cornutum* Arthur ex F. Kern and *Melampsora epitea* Thümen coll., were also used. In addition, a number of other biotic and non-biotic control objects were used (Table 1).



**Fig. 1.** Aeciospores (A) and pieces of lesions (B) of *Cronartium pini* on Petri dishes and a jar with a covid mask (C).

**Table 1.** Samples of *Cronartium pini* and controls used in scent discrimination. Bolded target (fungal source) and non-target (control source) samples were used in the final indoors scent discrimination tests. CF = Heteroecious life-cycle form. PP = Autoecious life-cycle form.

Fungal source, type, storing	Control source, type, storing
<b><i>C. pini</i> (CF), aeciospores on Petri dishes, frozen</b>	<b><i>Melampsora epitea</i> coll., leaf of <i>Salix</i>, frozen</b>
<b><i>C. pini</i> (PP), aeciospores on Petri dishes, frozen</b>	<b><i>Melampsoridium betulinum</i>, leaf of <i>Betula</i>, frozen</b>
<b><i>C. pini</i> (CF), piece of fresh branch lesion, frozen</b>	<b><i>Chrysomyxa ledi</i>, needle of Norway spruce, frozen</b>
<b><i>C. pini</i> (CF), piece of old branch lesion, frozen</b>	<b><i>Coleosporium tussilaginis</i>, leaf of <i>Melampyrum</i>, frozen</b>
<b><i>C. pini</i> (PP), piece of fresh branch lesion, frozen</b>	<b>Leaf of <i>M. pratense</i>, frozen</b>
<b><i>C. pini</i> (CF), leaf of <i>M. sylvaticum</i>, frozen</b>	<b>Leaf of <i>M. sylvaticum</i>, frozen</b>
<b><i>C. pini</i> (CF), leaf of <i>Paeonia lactiflora</i>, frozen</b>	<b>Leaf of <i>I. balsamina</i>, frozen</b>
<b><i>C. pini</i> (CF), leaf of <i>Impatiens balsamina</i>, frozen</b>	<b>Leaf of <i>Euphrasia</i> sp., frozen</b>
	<b>Leaf of <i>P. lactiflora</i>, frozen</b>
	<b>Sawdust of Norway spruce, frozen</b>
	<b>Piece of shoot of Norway spruce, frozen</b>
	<b>Piece of resin, frost</b>
	<b>Soil, room temperature</b>
	Lichen, room temperature
	<b>Stone, room temperature</b>
	<b>Cone, lichen, bark, room temperature</b>
	<b>Empty jar</b>
	<b>Eppendorf vial</b>
	Empty agar plate
	Fiber cloth
	Newspaper
	Rubber glow
	Respiratory mask
	Paper clip
	Foil

## 2.2 *Cronartium pini* field areas

### 2.2.1 Scots pine stand damaged by the heteroecious life-cycle form

A young severely injured Scots pine stand infected by the host-alternating life-cycle form in Kolari, Western Lapland (67°20'N, 23°47'E), was selected (Kaitera et al. 2015, 2018). In the stand, eight 100 m<sup>2</sup> groups of infected trees and their rust infections were marked in mid-June 2022 based on sporulating aecia on branches and stems, resinous stem lesions, dead tops or dead trees at a height below 2 m. The Scots pine stand included also susceptible alternate hosts, *M. sylvaticum*, *Euphrasia* sp. and *R. minor*. Ten plant sample plots of 0.25 m<sup>2</sup> of which 8 included infected alternate hosts and two were healthy, were marked in the stand in August 2022.

### 2.2.2 Scots pine stand damaged by the autoecious life-cycle form

A young Scots pine stand severely infected by the autoecious life-cycle form of *C. pini* was selected in Pudasjärvi, Northern Ostrobothnia (65°22'N, 27°00'E; Kaitera and Kokko 2023). The stand was a pine-dominated sub-xeric heath forest, where only a few *M. pratense* grew. Aeciospores had infected Scots pine and inoculations on susceptible alternate hosts, *P. lactiflora* and *Vincetoxicum hirundinaria* Medicus, had been negative confirming the life-cycle of the rust previously (Kaitera 2003; Kaitera and Nuorteva 2008). In the stand, *C. pini* infections were marked in trees in eight groups of infected trees of 1 ha in mid-June 2022 and in four similar size groups in mid-June 2023.

### 2.2.3 Other plant stands

Training was performed also around the city of Rovaniemi, southern Lapland (66°30'N, 25°44'E), in June and August 2022. In a public garden in the city area of Rovaniemi dogs were trained on *Paeonia anomala* L., *P. lactiflora* Pall., *P. tenuifolia* L., *Impatiens glandulifera* Royle and *Euphrasia* sp. *Paeonia* spp. were checked for telia prior to training to confirm the presence of *C. pini* on the plants. *Impatiens glandulifera* were also checked before training, but no telia were observed on these plants. After training, the plants marked by the dogs were collected and checked for *C. pini* telia.

## 2.3 Scent training

### 2.3.1 Participating dog-handler teams

Seven (7) volunteer dog-handler teams were selected for the training in fall 2021. As four dog-handler teams dropped out of the training during the first half a year of training, two additional dogs were selected for the training at a later stage. Five dog-handler teams participated in the last indoors scent discrimination testing, and four in young Scots pine stand test in June 2023. The background of the participating dog-handler teams are presented in Supplementary file S1, available at <https://doi.org/10.14214/sf.25036>.

### 2.3.2 Training of dog-handler teams

The scent training was planned jointly with a professional dog training service specialized in scent detection, Vainuvoima Oy. The professional trainers supervised both in face-to-face training and online the training process during one year training, ending with final indoors scent discrimination testing. Open Moodle was used as the learning management system (LMS) that provided information on the training schedule, learning materials, discussion forums, and discussion threads for sharing the videos with the trainers to get feedback during the training process. The one year long supervised training process included five face-to-face training weekends, each lasted 2–3 days between February 2022 and October 2022. In addition, eight online meetings were held to support the training process and discuss training related topics and issues. After the first training year, part of the voluntary dog-handler teams continued training, aiming to participate to the field tests in summer 2023.

The training weekends included both indoor and field training. Indoor training included introducing the trainers to the concept of scent discrimination, building motivation for the dogs for scent work, teaching and practicing indication behaviour, scent discrimination and working on line-ups. Training in field conditions was done first with scent samples and later with infected plants. In addition to face-to-face training weekends, eight online meetings that lasted for about two hours were held. During the entire training year, the dog-handler teams practiced independently with samples given for home training and in field environments.

To follow the progress of the training, indoor scent discrimination tests were conducted during the on-site training weekends in April, June, and October 2022. The last indoors scent discrimination test was conducted between October 2022 and January 2023, depending on dog-handler team availability. Field tests were conducted in June 2023 and August 2023 with those dog-handler teams that continued the training after the first training year.

### 2.3.3 Indoor scent discrimination training

For scent training, fungal spores collected from rust populations identified in earlier experiments (Kaitera and Nuorteva 2008; Kaitera et al. 2015), pieces of wood and fruit bodies, lesions, aecia, leaves, mycelia and other control samples were placed in clean (washed and autoclaved) glass jars. The opening of the jar was covered by a piece of surgical mask fabric to adjust dogs for its smell. Later, the surgical mask was used as protection to prevent spreading, when training outside in natural environment with fungal samples. Metal stands were used as sample stations to hold the sample jars. Dogs were trained to discriminate the target scents from various control scents (Table 1).

Scent training started with a training scent, such as a piece of Kong Classic toy, to learn the alert behavior and scent discrimination in line-ups after which the training progressed to fungal samples. First, the dogs were trained to identify the spores of *C. pini*, which were the purest in scent profile. Initially, the dogs were trained to discriminate these scents from empty jars, then from control scents related to sample handling, such as petri dishes and gloves, and finally from environmental control scents, such as rocks, cones, healthy leaves and shoots (Table 1). Dogs were also trained to discriminate *C. pini*-infected and healthy leaves and to indicate *C. pini* from different host plants. Detection of *C. pini* telia on the small leaves of *Melampyrum* and *Euphrasia* required intensive and focused sniffing for which a precise technique was trained using minute amounts of spores deposited on ca. 0.5 m × 0.5 m surfaces such as doormats. Control fungal samples were introduced to the dogs only after they could discriminate the positive fungal sample from other control samples. The number of target sample variations of *C. pini* and the number of fungal control samples was exceptionally high when considering scent training and discrimination, which challenged and lengthened the training process.

### 2.3.4 Detection training in field conditions

Scent detection in the field was started in healthy forests with *C. pini* spore samples placed in Eppendorf tubes, with the openings covered by surgical mask fabric to prevent fungal spreading to forest. Training continued with infected trees as soon as aeciospores were visible in June, and the handlers able to precisely reinforce the dog's indication behavior to the infected tree. After this, the search and indication training continued in Scots pine and other plant stands where *C. pini* was pre-verified as well as in other areas, where it was detected during the project.

The dogs were trained to detect both life-cycle forms of *C. pini* from pine trunks and branches of various ages. Additionally, the dogs were trained to detect *C. pini* telia from *Melampyrum* spp, *Paeonia* spp., *I. glandulifera* and *Euphrasia* sp. in forest areas and gardens. The dogs searched for infected trees in the forest using air currents and pinpointed the findings to aecia in lesions and old lesions on the trunks or branches. Some dogs indicated higher infections on the tree by looking upwards. For the ground layer vegetation, the dogs were trained to switch to precision searching based on the handler's cue or visual hints provided by the search grid. In the search area, the dogs and handlers systematically covered the area, inspecting potential infected trees and vegetation areas.

### 2.3.5 Indoors scent discrimination tests

The ability of the dogs to detect and indicate *C. pini* from different sample types was tested in scent discrimination tests organized as line-up tests. In these tests, the dogs sniffed 4–6 samples along a prepared line-up, where 0–1 jars contained the target scent (i.e. positive sample) and other

jars either an empty jar or a jar with one of the control samples. The stands were cleaned with an odorless cleaning liquid and ethanol between the runs. The distance between the stations in each line-up row was 60–80 cm depending on the testing environment.

Progress of each dog during the training was followed based on the results of successive tests. Three line-up test sessions were arranged for the last scent discrimination test in an indoors training hall. The final scent detection test consisted of 48 jars distributed into 12 line-up rows. Each line-up row included 0–1 target scents and 3–4 control scents. A total of nine target samples were used in the last test (except seven for dog TR\_D01), each on its own row, to be discriminated from the control samples. The rest of the rows contained only non-target samples.

### 2.3.6 Field tests

Testing of *C. pini* on Scots pine was done in four sample areas of the autoecious life-cycle form in Pudasjärvi (65°22'N, 27°00'E) in late June 2023 and in two sample areas of the heteroecious life-cycle form in Kolari (67°20'N, 23°47'E) in early August 2023. Testing on alternate hosts of *C. pini* was performed in six sample areas in August 2023 in Kolari (67°20'N, 23°47'E). The testing of *C. pini* was performed in 900 m<sup>2</sup> areas on pine for both life-cycle forms and in 2500 cm<sup>2</sup> areas on alternate hosts for the heteroecious life-cycle form.

The maximum testing time was 30 minutes per dog in all pine stands and 15 minutes in plots with alternate host plants. Forest pathologists acted as field test evaluators, recording the identifications by the dogs based on the handlers' reporting the dog's finding verbally. Evaluators checked the findings during or after the test. Findings were recorded as false positive or true positive identifications. Additionally, evaluators made their own notes based on visual inspection during the test, such as the dogs passing by diseased trees (false negatives).

For *C. pini*, all findings on Scots pines reported by the handler were visually inspected and identified on-site during the test. For *C. pini* on alternate hosts, each dog had three plots (0.5 m × 0.5 m) from which all plants were collected and their leaves checked for rust diseases (*C. pini*, *Coleosporium tussilaginis* f.s.p *melampyri*) and powdery mildew using stereomicroscope (Wild) in the laboratory.

## 2.4 Data analysis

For conservation dogs used for scent detection in search tasks in the field, precision (proportion of all alerts targeted toward a true target, positive predictive value), sensitivity (proportion of targets found relative to the total targets available, true positive rate), and effort (time spent searching a unit area or transect) are suggested as the performance measures (Bennett et al. 2020). In addition, for indoors scent discrimination tests we also calculated specificity (true negative rate), and accuracy (true positive and true negative rate). All test results were collected in frequency tables and performance measures were calculated for individual dogs and averaged (mean) over all dogs. The results from scent discrimination and search tests in the field were analyzed for each test separately. Further statistical tests were not calculated as the number of repeated trials per target sample type in indoors scent discrimination testing was low. The field trials were conducted only once for the different *C. pini* life-cycle forms and only two dog-handler teams participated in all three tests.

**Table 2.** Dogs' individual performance and averaged (mean) performance in the last indoor scent discrimination test. TP = True positive, TN = True negative, FN = False negative, FP = False positive.

Dog code	TP	TN	FN	FP	Sensitivity TP / (TP + FN)	Specificity TN / (FP + TN)	Precision TP / (TP + FP)	Accuracy (TP + TN) / (TP + FP + TN + FN)
TR_D01	3	3	3	3	0.50	0.50	0.50	0.50
TR_D02	6	3	3	0	0.67	1.00	1.00	0.75
TR_D03	4	2	1	5	0.80	0.29	0.44	0.50
TR_D04	3	3	4	2	0.43	0.60	0.60	0.50
TR_D05	1	3	6	2	0.14	0.60	0.33	0.33
Total of 60 tracks	17	14	17	12	Mean 51%	Mean 60%	Mean 58%	Mean 52%

### 3 Results

#### 3.1 Indoors scent discrimination test

A total of 60 tracks with 240 samples of which 44 were of *C. pini* were included for scent detection of *C. pini* in the last indoors scent discrimination test. Three of 12 tracks contained only non-target samples. Five dogs participated in the final test. The dogs identified the samples with a mean accuracy of 52% (Table 2). The accuracy varied between 33% and 75% among the dogs. The mean sensitivity was 51%, mean specificity 60% and mean precision 58%. The participating dogs' sensitivity varied between 14% and 80%, specificity between 29% and 100%, and precision between 33% and 100% (Table 2). One dog (TR\_D3) performed well with a sensitivity (0.8) over a threshold value of 0.7 considered acceptable, but the precision of the dog was relatively low (0.44), due to the number of false positive alerts (5) by the dog. Another dog (TR\_D2) had a slightly lower sensitivity (0.67), but the precision of the dog was 1.0, as the dog made no false positive alerts, and the accuracy of the dog was 0.75 (dog did not identify three target scents from nine, i.e., 3 False Negatives). The results of the rest of dogs were less promising at the time of the last indoors discrimination test. However, training continued for about half a year after the last indoors testing and aimed for real-life scent detection in the forest.

Among *C. pini* samples, aeciospores of the autoecious life-cycle form were more difficult to identify than aeciospores of the heteroecious life-cycle form, as only 20% of the dogs identified spores of the autoecious life-cycle form compared to 40% of the heteroecious life-cycle form (see Table 3). On the other hand, 100% of the dogs identified correctly sporulating, young lesions of the autoecious life-cycle form, while 60% identified correctly lesions of the heteroecious life-cycle form.

**Table 3.** Results of five dogs in the final indoor scent discrimination tests by sample type. CF = Heteroecious life-cycle form. PP = Autoecious life-cycle form. TP = True positive, FN = False negative.

Dog code	CF1, aecio- spores on Petri dishes*	CF2, aecio- spores on Petri dishes*	PP aecio- spores on Petri dishes	PP, piece of fresh branch lesion	CF, piece of fresh branch lesion	CF, piece of old branch lesion	CF, leaf of <i>Paeonia</i> <i>lactiflora</i>	CF, leaf of <i>Impatiens</i> <i>balsamina</i>	CF, leaf of <i>Melampyrum</i> <i>sylvaticum</i>
TR_D01	FN	TP	FP	TP	FN	sample not tested	TP	sample not tested	FN
TR_D02	TP	TP	TP	TP	TP	FN	FN	FN	TP
TR_D03	TP	FP	FP	TP	TP	FP	TP	FN	FP
TR_D04	FP	FN	FP	TP	TP	FN	FN	FN	TP
TR_D05	FN	FP	FN	TP	FP	FN	FN	FN	FN
	40%	40%	20%	100%	60%	0%	40%	0%	40%

\*The CF sample (aeciospores on Petri dishes) appeared twice in the test tracks. The results for each occurrence have been reported separately as CF1 and CF2.

**Table 4.** Scent detection performance in field testing at the time of aeciospore dissemination of the autoecious life-cycle form of *Cronartium pini* on Scots pine in Pudasjärvi. TP = True positive, FN = False negative, FP = False positive.

Dog code	TP	FN	FP	Sensitivity TP / (TP + FN)	Precision TP / (TP + FP)	Used time 0–30 min
TR_D02	7	1	3	0.88	0.70	30
TR_D03	4	0	0	1.00	1.00	25
TR_D04	8	0	0	1.00	1.00	20
TR_D05	12	1	2	0.92	0.86	25
Total	3	2	5	Mean 95%	Mean 89%	Mean 25 min

cle form of *C. pini*. None of the dogs indicated old lesions of *C. pini*. The occurrence of telia on *Melampyrum* and *Paeonia* were identified by 40% of the dogs while none indicated telia on *Impatiens* leaves (Table 3).

### 3.2 Field tests

Four dogs with their handlers participated in the *C. pini* field test for the autoecious life-cycle form in Pudasjärvi. Out of these four dogs, two continued to participate in the test conducted for the identification of *C. pini* lesions of the heteroecious life-cycle form on pines and *C. pini* telia on alternate hosts in Kolari.

The dogs identified well both the autoecious and heteroecious life-cycle forms of *C. pini* in young Scots pines as well as the heteroecious life-cycle form on alternate hosts. The mean sensitivity of correct identification of fresh sporulating lesions of the autoecious life-cycle form of *C. pini* was 95%, and it varied between 88% and 100% among dogs (Table 4). The mean precision of identification was 89%, and it varied between 70% and 100%. Overall, the dogs found a total of 31 infected trees (True Positive) and missed two infected trees within a half-meter radius of the dog's path (False Negative). There were five false detections as visually inspected by the fungal expert (False Positive).

The mean sensitivity of correct identification of old sporulated lesions of the heteroecious life-cycle form was 78%, and it varied between 67% and 90% (Table 5). The mean precision of identification was 100%, i.e. it was 100% for both dogs. The two dogs found a total of 11 infected trees (True Positive) and missed two infected trees within a half-meter radius of the dog's path (False Negative). There were no false detections as identified by visual inspection of the fungal expert (False Positive). In the field test area on alternate hosts, the mean sensitivity of correct identification of *C. pini* telia on *M. sylvaticum* and *Euphrasia* sp. was 58%, and it varied between 50% and 67% (Table 6). The mean precision of identification was 100%. Neither of the dogs made false positive identifications, but both did not alert on one of three infected sample plots.

**Table 5.** Scent detection performance in field testing with old lesions of *Cronartium pini* on Scots pine in Kolari. TP = True positive, FN = False negative, FP = False positive.

Dog code	TP	FN	FP	Sensitivity TP / (TP + FN)	Precision TP / (TP + FP)	Used time 0–30 min
TR_D02	9	1	0	0.90	1.00	27
TR_D03	2	1	0	0.67	1.00	17
Total	11	2	0	Mean 78%	Mean 100%	Mean 22 min

**Table 6.** Smell detection performance in field testings of *Cronartium pini* on leaves of alternate hosts in plant sample plots in Kolari. TP = True positive, TN = True negative, FN = False negative, FP = False positive.

Dog code	TP	TN	FN	FP	Sensitivity TP / (TP + FN)	Precision TP / (TP + FP)	Used time 0–15 min
TR_D02	2	0	1	0	0.67	1.00	8.5
TR_D03	1	1	1	0	0.50	1.00	5.6
Total	3	1	2	0	Mean 58%	Mean 100%	Mean 7.1 min

The dogs performance varied in discriminating and identifying *C. pini* from spores, lesions, aecia, and telia on large plant leaves already in the last indoor test. In the field, the dogs identified *C. pini* spores, branches with aecia, fresh and old lesions, and even latent infections without disease symptoms in branches and stems from mycelia inside wood in standing trees. Identification succeeded well for both the heteroecious and autoecious life-cycle forms. Identification was accurate at heights below 1.5 meters, which the dogs' noses could reach, but the dogs could detect single infections even higher. Both life-cycle forms of *C. pini* were identified equally well.

## 4 Discussion

All four tested dogs succeeded well in detecting *C. pini* in the field test in young Scots pine stand. Two of the five tested dogs identified *C. pini* well also in the final indoor scent discrimination test. The indoor scent discrimination test was arranged 6–8 month before the field tests and thus the training was not as complete and likely explained partly the lower success in the indoor trial. In addition, the training required detecting an exceptionally high number (eight) of target fungal sample types and also discrimination from a number of fungal control samples, which takes time in the training process. For example, while none of the dogs identified old branch lesions in the indoor test, two dogs identified old lesions in the field test by mean sensitivity of 78% and 100% precision. In the field tests, aeciospores of both life-cycle forms were identified similarly although the indoor scent discrimination test indicated that the autoecious form was more challenging. Detection of odors in various environments is complicated. According to Caldicott et al. (2024) ageing, interaction with extraneous odors and environmental factors cause variation in odors over time. Generalization of results of dog training may be affected by pre-exposure to certain odors or odor enrichment (Caldicott et al. 2024). Besides training method and environment, dog breed and personality as well as the handler may affect the results (McKeague et al. 2024). As an example interdisciplinary approaches combining insights from dog behavior, veterinary science, comparative psychology and practical experience from truffle hunters and farmers have been demonstrated to be important for truffle dogs (Cejka et al. 2022).

The results indicate, however, that with adequate training, dogs' sense of smell is well-suited for the detection of fruit bodies, lesions and even latent infections of *C. pini* on Scots pines. There are no identification methods available to identify *C. pini* from wood and lesions or latent infections currently, and therefore, dogs' scent detection method offers a good additional tool for rust identification, which can intensify management of stands by removing infected trees. The rust can only be identified from aeciospores using genetic markers based on heterozygosity of *C. pini* (Samils et al. 2021) or from telia on leaves of alternate hosts (Kaitera et al. 2015). Besides genetic identification, different life-cycle forms of *C. pini* can be distinguished with tedious alternate host or pine inoculations (Kaitera 2003; Kaitera and Nuorteva 2008; Kaitera et al. 2015), for which the scent detection method offers a rapid alternative.

Identification of *C. pini* from old lesions is also suitable for the detection of both the heteroecious and autoecious life-cycle forms of the rust. Best results seem to be achieved in young Scots pine stands, where the infections occur on pine below a height of 1–2 m from the ground. This height will enable effective scent detection work for dogs. Recently, severe damage of *C. pini* was reported on young Scots pines in northern Sweden (Wulff et al. 2012). Disease symptoms in northern Sweden include sporulating lesions at the basal part of the stem of the trees close to the ground, which are difficult to recognize at the young stage of the trees. Therefore, scent-detection trained dogs could offer a tool to find infected trees at the early stage in young pine plantations. Identification from taller part of pine stem is also possible depending on size of the dog and accuracy of its nose for the smell detection. Using scent detection dogs is most effective on Scots pines during aecial sporulation between mid-June and mid-July, but it can be used throughout the growing season efficiently from old lesions as was shown in this study. Biologically, there is no limitation for the size of the forest for scent detection dogs. In practice, however, tree density, mixture of deciduous trees and size of the forest area affects detection efficiency of dogs. In dense stands an optimal size may be less than 1 ha, but in sparse stands the size can be higher. This, however, depends also on wind, moisture and temperature as well as the motivation of the dog (McKeague et al. 2024).

Detection of *C. pini* on alternate hosts is more difficult and affected by the degree of the infection as well as the diversity and strength of background odors. Detection of *C. pini* from large leaves such as *Paeonia* sp. was reliable indicating that dogs could be used efficiently for detecting *C. pini* on garden plants especially in dense plant communities. Opposite to plants with larger leaves, hemiparasite plant species with small leaves like *Melampyrum* spp. and *Euphrasia* spp., identification is more challenging due to small size of the leaves and sporadic occurrence of infected leaves within the plants. Despite of that the dogs succeeded rather well (average sensitivity 58% and precision 100%) in detecting *C. pini* on hemiparasite plant species in the field test. Very precise sniffing is required from the dog, but detection of infected fresh plants with small leaves is more effective using dogs compared to human identification in the field. Although telia on leaves are recognizable by naked eye, usually plants must be collected, transported to laboratory and rust identification confirmed using microscopy. Identification by humans succeeds also only in August-September, when telia are formed on alternate hosts' leaves (Kaitera et al. 2023, 2024). Both on alternate hosts and pine, identification of the rust at an early stage enables early control actions against the rust especially in young plantations.

Some challenges arose during dogs' training process regarding *C. pini*. Differences in the level of experience of the dogs and handlers as well as the possibility to commit to the relatively long training process resulted in several drop outs as well as variation in the results. The high number of target scent variations and host plants, the high number of control scents, variation between scents of indoor and outdoor samples, and the short time of training in real-life environment with live plants during the growth season and at the time of rust sporulation, challenged the learning process of dogs.

## 5 Conclusions

Findings of the study show that dog-handler team can be used to detect *C. pini* in young pine plantations, and detection is appropriate up to the height of trees below 2–3 meters. Dog-handler team are a reliable approach for detecting both life-cycle forms of *C. pini*. On alternate hosts, dog-handler teams are effective in detecting *C. pini* especially on species with large leaves, such as *Paeonia*, but detection succeeds also on species with small leaves, such as *Melampyrum* and *Euphrasia*. Therefore, in *C. pini*-susceptible nutrient-rich sites with *M. sylvaticum*, identification of *C. pini* should be done on both pines and alternate hosts for best detection sensitivity.

## Supplementary files

S1.pdf,  
Metadata of research data.pdf,  
available at <https://doi.org/10.14214/sf.25036>.

## Authors' contributions

Conceptualisation (JK, TP, MM, SV, HV, KM), data acquisition, curation and analysis (JK, TP, MM, SV, HV, KM), methodology (JK, TP, MM, SV, HV, KM), writing – original draft preparation and visualization (JK, TP, MM, SV, HV, KM), funding acquisition (JK, TP, MM, SV, HV, KM) and supervision (JK, TP). All authors commented on the manuscript.

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## Declaration of openness of research materials and data

The data that support the findings of this study is available openly in Zenodo <https://doi.org/10.5281/zenodo.17145211>.

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