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## Dogs can be trained to detect *Heterobasidion* root rot in the forest

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### ABSTRACT

*Heterobasidion* root rot caused by *Heterobasidion parviporum* is the most destructive disease affecting Norway spruce, while *H. annosum* s.s. is the primary causal agent of root rot in Scots pine. Early diagnosis of these pathogens is essential for effective disease management. Dogs, with their highly developed sense of smell, may have significant potential to detect forest pathogens before they cause severe damage. In this study, we evaluated whether trained volunteer dog-handler teams could identify *Heterobasidion* spp. from wood, fruit bodies, or mycelia *in vitro* and *in vivo*, aiming to enhance early detection of *Heterobasidion* root rot in pine and spruce forests. Dogs were successful both in young and mature pine stands to detect trees infected by *H. annosum* s.s. Seven dogs indicated *H. annosum* s.s. with a mean sensitivity of 72% and mean precision of 84% in young pines. Three dogs indicated infection with a mean sensitivity of 88% (precision 99%) in mature pines. Five of nine dogs achieved sensitivity over 70% in indoors scent discrimination line-up test. The results indicate that dogs can be used as an aid in searching for *Heterobasidion* spp. in Scots pine and Norway spruce stands, but further testing is needed in larger areas.

### ARTICLE HISTORY

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### KEYWORDS

*Pinus sylvestris*; *Picea abies*;  
smell sense; root rot

### Highlights



- Dogs detected *Heterobasidion* root rot in young and mature pine stands in the field
- Dogs detected *Heterobasidion annosum* s.s. and *H. parviporum* in indoors scent discrimination tests
- Dogs were trained to detect the pathogens in both standing trees and dead wood
- Dogs marked *Heterobasidion* fruit bodies in the forest


### Introduction

*Heterobasidion* species are significant forest pathogens, both in terms of the extent of the damage they cause and their wide geographic range in conifer forests across the Northern Hemisphere (Woodward et al. 1998). Two *Heterobasidion* species, *H. parviporum* Niemelä & Korhonen and *H. annosum* sensu stricto (Fr.) Bref., occur in northern Europe (Korhonen et al. (1998). In Finland, the distribution of *H. annosum* s.s. is limited to the southern and central parts of the country, where it mainly affects Scots pines (*Pinus sylvestris* L.). *H. parviporum* has spread throughout Finland, causing severe damage in Norway spruce (*Picea abies* (L.)

Karst.) stands (Korhonen et al. 1998). However, in northern Finland, distribution of *H. parviporum* is so far sporadic (Müller et al. 2018a; Müller et al. 2018b).

In Norway spruce, the fungus decays primarily dead heartwood and the rot can spread up to 12.6 m height in the stem (Gaitnieks et al. 2021). While the rot is confined to the dead heartwood, the tree looks externally healthy making it challenging to identify infected trees in spruce stands without damaging standing trees. Visible signs of disease in an individual spruce tree only become apparent when the rot has advanced to the sapwood. In such cases, typical symptoms are growth retardment and resin flow on the stem. However, these symptoms can also be attributed to several other causes (Asiegbu et al. 2005). An alternative to destructive methods to detect decay in living trees is Rotfinder, a non-destructive decay-sensing instrument that is based on resistant measurements in standing trees. The method is more accurate at the stump level than at breast height (Oliva et al. 2011). When more than 15% of the cross-section was decayed, Rotfinder had an accuracy of 0.86 at the stump level (Oliva et al. 2011). Other similar techniques based on the use of signal detection include acoustic tomography (Axman

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et al. 2004) and infrared thermography based on electromagnetic radiation (Catena 2003).

In contrast to spruce, decay caused by *H. annosum* in pine is usually limited to the root system and the lower part of the stem base. *Heterobasidion* infection appears in pine stands as groups of trees infected in varying degrees (diseased and dead trees) depending on how much time has passed since the infection. Young pines are highly susceptible to *Heterobasidion* infection and may die within one season. Mature pines, instead, are more resistant to infection, and a tree may appear visually healthy even though part of its root system is infected (Rönnerberg et al. 2006). Due to latent infections, it is difficult to assess the extent of the disease in older pine stands (Allikmäe et al. 2017; Piri et al. 2021). Since *H. annosum* has a wide host range, infecting not only pine but also other conifers and deciduous trees, dead junipers (*Juniperus communis* L.) and birches (*Betula pendula* Roth and *B. pubescens* Ehrh.) in pine stand may indicate the presence of *Heterobasidion* root rot (Laine 1976).

Both *Heterobasidion* species spread into the new areas by means of airborne spores. The basidiospores are produced in perennial fruit bodies which are located on old, decayed stumps, on roots of wind-thrown trees, and in stem base of diseased pines. Following spore infection of fresh-cut stumps, or wounds in living tree, the fungus can advance as mycelium to adjacent trees and to the subsequent generation via root contacts (Redfern and Stenlid 1998; Stenlid and Redfern 1998; Piri 2003).

Primary spore infections can be controlled by avoiding cuttings in summer when basidiospores are released. In summer cuttings, stump surfaces can be treated with urea or suspension of *Phlebiopsis gigantea* (Fr.) Jülich (Rotstop) to prevent *Heterobasidion* spore infections on freshly cut stumps (Pratt et al. 1998; Holdenrieder and Greig 1998). Once the stand is severely affected by *Heterobasidion* root rot, changing to a resistant tree species is the only effective control method to eliminate the disease. In spruce stands infected by *H. parviporum*, the tree species should be changed either to Scots pine or to deciduous species (Garbelotto and Gonthier 2013). In pine stands infected by *H. annosum*, the only sustainable options are pure stands of deciduous trees (Korhonen 1978). If *Heterobasidion* root rot has not spread widely in the previous tree generation, planting of resistant tree species could be directed only to the infested areas, while the healthy parts of the stand could be further regenerated with the same tree species, either as a pure stand or in mixture with other species (Piri 2003; Holmström et al. 2025).

*Heterobasidion* spp. are predicted to spread northwards due to climate warming (Müller et al. 2014; Müller et al. 2015). This is further confirmed by recent

findings of *H. annosum* s.s. in Northern Ostrobothnia (Kaitera et al. 2023). Therefore, especially in northern Finland, early detection of *Heterobasidion* infections is crucial for focusing control measures effectively, both temporally and spatially, thus preventing *Heterobasidion* root rot to gain a foothold in new areas.

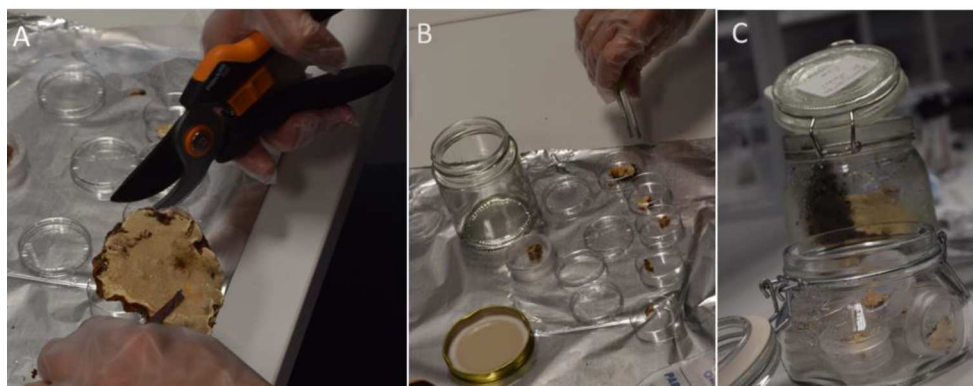
Dogs have an excellent sense of smell and can be trained to detect a wide variety of non-biological and biological scents, ranging from drugs to truffles and rare fungi, as well as human, animal and plant diseases (Browne et al. 2006; Amor et al. 2024; Carter et al. 2023; Cooper et al. 2014; Fischer-Tenhagen et al. 2018; Gottwald et al. 2020; Gustafsson 2022; Vošvrđová et al. 2023; Cejka et al. 2022). Studies have shown that dogs can identify plant pathogens such as *Candidatus Liberibacter asiaticus* Jagoueix, Bove & Gornier and *Uncinula necator* (Schwein.) Burrill, which damage citrus trees (Gottwald et al. 2020; Aviles-Rosa et al. 2025). They can also detect *Raffelea lauricola* T.C. Harr., Fraedrich & Aghayeva, a causal agent of wilt disease of several species in the Lauraceae family (Simon et al. (2017), as well as *Phytophthora* spp., which are highly destructive plant pathogens worldwide (Swiecki et al. 2018). Moreover, promising results indicate that dogs can be trained to recognize fungal spores and wood samples colonized by wood-decaying basidiomycetes including *H. parviporum* and *Ganoderma adspersum* (Schulzer) Donk (Wysocka 2021; Shoon and de Goederen 2024). However, despite of these findings the use of dogs for identifying forest pathogens remains an underutilized resource. To date, there is no available information regarding dogs' ability to identify naturally infected trees in forest environments.

Since detecting *Heterobasidion* infections in living trees – particularly in spruce and older pine stands – is often challenging and usually requires laborious and invasive methods that can damage the trees, we aimed to investigate whether dogs can be trained to detect odor produced by *Heterobasidion* species in different environments, including standing forests. Enhancing the ability to recognize these pathogens at an early stage of infection with the help of trained dogs could facilitate the timely implementation of control measures, thereby helping to prevent *Heterobasidion* root rot from reaching epidemic levels.

## Materials and methods

### Collection and preparation of *Heterobasidion* and control samples

Fruit bodies and wood colonized by *H. parviporum* and *H. annosum* s.s. (Figure 1) were collected from infected



**Figure 1.** Preparing of *Heterobasidion* samples for dog training (A–B) and samples in jars (C).

spruce and pine stands in southern Finland (Sipoo 60° 20'53.2"N, 25°10'10.1"E and Läyliäinen 60°36'52.4"N, 24°25'01.3"E), during 2021 and 2022. In addition, control samples, including fruit bodies and spruce wood colonized by *Armillaria* sp., *Fomitopsis pinicola* (Sw.) P. Karst., and *Phlebiopsis gigantea*, were collected in Sipoo and Lapinjärvi (60°40'31.9"N, 26°06'22.8"E). The fruit bodies and wood samples, taken aseptically with an increment borer, were placed in autoclaved glass jars. To confirm the fungal species causing decay, one to two wood samples from each tree were cultured on malt extract agar (2% MEA). After a 5–7-day incubation period, the fungal species was identified based on morphological characterization of the outgrowing mycelia under a dissection microscope (Hallaksela 1984). The distinction between *H. parviporum* and *H. annosum* s.s. was made using mating tests (Korhonen 1978). Mycelium of *H. parviporum* was also isolated from wood discs cut from decayed Norway spruce stumps in Häyryseniemi, Northern Ostrobothnia, following the methodology described by Piri et al. (2021). Outgrowing mycelia of *H. parviporum*, *H. annosum* s.s., *Armillaria* sp., *F. pinicola* and *P. gigantea* were subcultured onto 2% MEA in small Petri dishes ( $\varnothing = 3.3$  cm) to obtain pure fungal cultures.

For scent discrimination training and testing, the fruit bodies and decayed wood were cut in approximately 1 cm<sup>2</sup> pieces, placed on small Petri dishes that were sealed with parafilm and stored in airtight glass jars at –20°C until use. Mycelia growing on agar plates were stored similarly in big airtight glass jars at +4°C (Table 1). Control samples including healthy spruce and pine wood, infected wood and mycelial cultures of *F. pinicola*, *Armillaria* sp. and *P. gigantea*, as well as fruit bodies of *F. pinicola* and *Armillaria* sp., were processed in the same way as the *Heterobasidion* samples. In addition, a number of other biotic and non-biotic control objects were used (Table 1).

### **Heterobasidion field areas**

#### **Norway spruce stand in Häyryseniemi**

In northern Finland, the only suitable area was found in Häyryseniemi, Northern Ostrobothnia (65°14'16"N, 25°17'08"E). This upland area is located next to Gulf of Bothnia in an herb-rich heath forest dominated by middle-aged Norway spruces. The stand was severely infected by *H. parviporum*, with many decayed trees fallen by wind. Eight study plots, each approximately 100 m<sup>2</sup>, containing fallen trees carrying *Heterobasidion* fruit bodies on their roots were marked in the field. Small samples cut from fruit bodies were cultured on 2% MEA, and the outgrowing mycelia was isolated and identified to the species level (Korhonen 1978). Moreover, two to four bore cores were aseptically drilled from each standing spruce within the study plots to evaluate their health status and to isolate the fungal species responsible for decay in diseased trees. A total of 42 trees were sampled: 13 were decayed by *H. parviporum*, 3 by *Armillaria* sp., 6 by other unidentified decay fungi, and 20 were healthy.

#### **Cutting area in Häyryseniemi**

Freshly cut Norway spruce stumps next to the Häyryseniemi experimental stand (65°13'73"N, 25°18'60"E) were used for dog training. Before training, a slice was cut from 21 stumps and were processed following the methodology described by Piri et al. (2021). After that the presence of *Heterobasidion* mycelia with conidiophores was investigated under a dissection microscope. Of these 21 stumps, 18 were decayed by *H. parviporum*, while 3 stumps were healthy. In addition, the dogs marked in the field training two standing trees and two stumps, which were also examined for *Heterobasidion* infection. One standing tree and one stump were found to be decayed by *H. parviporum* whereas the

**Table 1.** Samples of *Heterobasidion* spp. and controls used in scent discrimination tests. Bolded target and non-target samples were used in the final indoors scent discrimination tests.

Sample of <i>Heterobasidion</i> (target)	Control (non-target)
<b><i>H. parviporum</i>, fruit body, frozen</b>	<b><i>Armillaria mellea</i>, fruit body, frozen</b>
<b><i>H. parviporum</i>, fruit body, fresh</b>	<b><i>Fomitopsis pinicola</i>, fruit body, frozen</b>
<b><i>H. parviporum</i>, fruit body, freeze-dried</b>	<b><i>Hypholoma capniids</i>, fruit body, frozen</b>
<b><i>H. parviporum</i>, mycelia, agar, fridge</b>	<b><i>Cantharellula umbonata</i>, fruit body, frozen</b>
<b><i>H. parviporum</i>, wood slice, frozen</b>	<b><i>Kuehneromyces mutabilis</i>, fruit body, frozen</b>
<i>H. parviporum</i> , wood disk, frozen	<b><i>Armillaria mellea</i>, mycelia, agar, fridge</b>
<b><i>H. annosum</i> s.s., fruit body, frozen</b>	<b><i>Phlebiopsis gigantea</i>, mycelia, agar, fridge</b>
<b><i>H. annosum</i> s.s., mycelia, agar, fridge</b>	<b><i>Fomitopsis pinicola</i>, mycelia, agar, fridge</b>
<b><i>H. annosum</i> s.s., wood slice, frozen</b>	<b>Agar, fridge</b>
	Empty agar plate
	<b><i>Fomitopsis pinicola</i>, wood slice, frozen</b>
	<b><i>Armillaria mellea</i>, wood slice, frozen</b>
	Norway spruce, wood disc, frozen
	<b>Norway spruce, sawdust, frozen</b>
	<b>Norway spruce, wood slice, frozen</b>
	<b>Norway spruce, branch, fresh</b>
	<b>Resin, piece, frozen</b>
	<b>Birch bark, room temperature</b>
	<b>Stone, room temperature</b>
	<b>Cone, room temperature</b>
	<b>Soil, room temperature</b>
	Lichen, room temperature
	<b>Empty jar</b>
	<b>Eppendorf vial</b>
	Fiber cloth
	Newspaper
	Rubber glow
	Respiratory mask
	Paper clip
	Foil

others were healthy. The identified samples were further used in dog training.

#### Norway spruce stand in Hietasaari

Two stumps of mature Norway spruce trees carrying fruit bodies of *H. parviporum* in Hietasaari in the Oulu city area (65°01'19"N, 25°26'01"E) were used in dog training. The area was selected from the data base of Laji.fi (<https://laji.fi/taxon/MX.205969>) and information from the University of Oulu Botanical Museum.

#### Norway spruce stand in Tuusula

A mature Norway spruce stand located in the research forest of Natural Resources Institute Finland in Tuusula (60°22'10.9"N, 25°00'02.1"E), southern Finland, was also used for dog training. This stand had been previously identified as severely infected by *H. parviporum* (Piri and Korhonen 2008). Within a demarcated area of approximately half a hectare, two to four bore cores were aseptically drilled from each of the 44 trees to identify those trees affected by root rot. To determine the fungi responsible for decay, the bore cores were cultured on 2% MEA, and outgrowing mycelium was identified. Among the 44 trees examined, 18 were infected by *H. parviporum*, 6 by *Armillaria* sp., and 2 were infected by an unknown decay fungus. In addition, the area contained some old, decayed stumps carrying fruit bodies of *H. parviporum*.

#### Scots pine stand in Lyyliäinen

A 17-year-old Scots pine stand infected by *H. annosum* s.s. was selected for dog training in Lyyliäinen (60°36'48.8" N, 24°25'18.9" E), southern Finland. In this 1.2-hectare stand, the infection centers, i.e. groups of infected trees, had been previously mapped to follow the spread of individual *Heterobasidion* genotypes from 2011 to 2019 (Piri et al. 2021). Visible groups of dead and infected trees were frequent throughout the stand. The stand was divided into seven sub-areas of about 1700 m<sup>2</sup> each, allowing the training of dogs up to 1 month before the field test. The same sub-areas were later used in the field tests. In addition, eight infection centers of *H. annosum* s.s. were identified in an adjacent, mature Scots pine stand. One of these infection centers was utilized for training of the dogs, while three areas of about 30 × 50 m, each containing one or more infection centers, were used for the field testing.

#### Scent training

Eight volunteer dogs with their handlers were initially selected for the 1-year-long training in fall 2021. The background of the participating dog-handler teams is presented in Supplementary Table S1. Two dogs dropped out from the training due to various reasons and were replaced by two new dog-handler teams. In addition, two extra dog-handler teams were selected to increase the number of teams, and one extra team

participated to support the technical training process supervision by Vainuvoima Oy, a dog school specialized in scent work. In the end, nine dog-handler teams participated in the last indoors scent discrimination tests. The scent training was planned jointly with Vainuvoima Oy, which supervised the training process. During the first training year, the dog-handler teams practiced in five training weekends, and trained independently with samples given for training at home and in field environments. Indoors scent discrimination tests were conducted to follow the progress of the dog-handlers in 2022. Final indoors scent discrimination tests were conducted in October 2022 – March 2023 on five occasions in two different locations based on dog-handler team availability. Field tests were conducted in August 2023.

For scent training, identified pieces of wood and fruit bodies, mycelia and other control samples were placed in clean (washed and autoclaved) glass jars. Dogs were trained to discriminate the target scents from various control scents (Table 1). Once the initial discrimination and indication training with the training scent was completed, the training progressed to fungal samples. First, the dogs were trained to identify fruit bodies (defrosted samples, preserved as frozen) of *H. parviporum*. After the fruit bodies, dogs were trained to discriminate *H. parviporum* mycelia growing on agar plates and in wood samples from several non-target control samples including empty agar plates, healthy wood samples and other fungal species (Table 1). The number of target and control fungal samples was exceptionally high for scent discrimination training, which challenged the dog-handler teams in the training process and its planned 1-year timetable. *H. annosum* s.s. samples were introduced to the dogs in the last scent discrimination test in the end of 1-year training. *H. annosum* s.s. samples were then included in the training by those dog-handler teams that continued to train towards the field tests.

The scent training in forests started with fungal samples placed in healthy forests. In infested forest stands, dogs were trained to sniff three specific targets for detecting *Heterobasidion* root rot, i.e. windthrows, base of standing trees, and stumps in recent logging areas. Some dog-handler teams also trained to detect *H. parviporum* from fresh wood piles. Additionally, the dogs were trained to focus on fruit bodies at the bases of trees and stumps as well as on root system and underside of wind-thrown trunks. Air currents, weather conditions, and distribution significantly affected the spread of the scent of *H. parviporum* and *H. annosum* s.s. Variations were observed in the intensity and spread distance of the scent in the search areas.

### Indoors scent discrimination tests and field testing

The ability of the dogs to detect and indicate *Heterobasidion* spp. from different sample types was evaluated in indoors scent discrimination tests and search tests in the field at the end of the training. The different sample types used in the training and tests are presented in Table 1, with those target scents bolded that were used in the last indoors scent discrimination tests. In the last indoors scent discrimination tests, *H. annosum* s.s. samples as well as a sample of freeze-dried and a cut piece of fresh *H. parviporum* fruit body were introduced for the first time to the participating dogs. This was done to explore, whether these samples are assessed by dogs to be similar to each other, or would be discriminated from those *H. parviporum* samples (defrosted fruit body, mycelia on agar, and slice of diseased wood) that had been used during the scent discrimination training process.

To assess the practical disease detection capability of the dogs, field test for *H. annosum* s.s. on Scots pine was conducted in both young and old pine stands in Läyliäinen in August 2023. Each dog-handler team was assigned a test area of 1300–1900 m<sup>2</sup> in the young pine stand and a 1500-m<sup>2</sup> area in the mature pine stand. Both stands contained healthy trees as well as diseased trees at various stages of infections. Fruit bodies occurred sparsely at the base of diseased trees. The maximum testing time was 30 min per dog. Findings were recorded as true-positive, false-positive, false-negative identifications.

To determine *H. annosum* s.s. infection in young pine stands, when the fungal expert could not visually confirm the health status of the tree indicated by the dog, the tree was felled for further examination. Sample discs were sawn from the bottom of the stump, just above ground level, and from the main roots near the root collar. The presence of *H. annosum* s.s. infection in the sample discs was examined as described in Section 2.1. In the older pine stand, the fungal experts were not present during the tests. Instead, project workers acted as the evaluators and they marked and numbered the trees and the identifications made by the dog as reported by the handler. These markings were later reviewed visually by a forest pathologist. If necessary, core samples were taken from the base of tree and its main roots to confirm presence or absence of *Heterobasidion* infection.

### Data analysis

For conservation dogs used for scent detection 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 for the participating dogs. We did not calculate the detection performance (ibid.), suggested as a measure of efficiency for benefit per unit effort, and calculated as sensitivity divided by effort, where effort is time spent searching divided by the area searched (e.g. hours per hectare). The 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. For field tests, the real-life environment poses challenges for further statistical analysis beyond performance measures calculated.

## Results

### Performance in indoors scent discrimination tests

A total of 108 line-up tracks with 432 samples, comprising 81 samples of varying types of *Heterobasidion* samples (see Table 1), were included in the last test for scent discrimination of *Heterobasidion* sp. Nine dogs participated in the last test having a total of 48 samples on 108 tracks. Nine target *Heterobasidion* samples were present for each dog, each sample on its track, and the rest were control samples, including other fungal samples. The mean combined sensitivity for participating dogs was 65%, mean specificity was 50%, and mean precision was 62%. The sensitivity of the dogs varied from 43% to 80%, specificity from 14% to 100%, and precision from 22% to 100% (Table 2). The mean accuracy of the nine dogs correctly identifying target

samples was 56% (Table 2). Accuracy varied among the dogs, ranging from 33% to 83%. At the time of the last scent discrimination testing, the sensitivity of 5/9 dogs (56%) was above 0.7, considered an acceptable threshold, and the precision was above 0.7 for three of these dogs. The test included three target sample types of *H. parviporum* that had been trained on, and the rest of the target samples had not been trained on previously (see Table 1). The training of dogs continued after the last scent discrimination test to prepare them for the field tests, including starting the training on the *H. annosum* s.s. samples.

Most dogs (89%, Table 3) discriminated correctly defrosted fruit bodies of *H. parviporum*, with which the scent discrimination training was started on, and which were therefore trained extensively. Mycelia of *H. parviporum* were correctly identified by 56% of the dogs, and a slice of infected wood by *H. parviporum* by 44% of the dogs. Among *H. annosum* s.s. samples, which had not been trained on prior to the test, 67% of the dogs identified correctly slice of infected wood, 56% identified mycelia, and 44% identified defrosted pieces of fruit bodies. Some dogs were able to discriminate the target *Heterobasidion* samples well from the control decay fungi, including *Armillaria* sp., *F. pinicola*, and *P. gigantea*.

In the scent discrimination tests, *H. annosum* s.s. samples as well as the freeze-dried sample of *H. parviporum* fruit body were introduced to see if the dogs would identify them as similar to *H. parviporum*. This was done to assess whether the dogs trained on cut samples of fruit bodies that were preserved as frozen assess these species as having similar scent profiles. Most dogs did not alert on fresh and freeze-dried fruit body samples of *H. parviporum* (Table 4) in the last scent discrimination test. However, as freshly cut *H. parviporum* fruit bodies were alerted on only by one dog that also alerted on a sample of frozen fruit body, it is possible that freezing the cut pieces of fruit bodies and using them as defrozen

**Table 2.** Dogs' individual performance and averaged (mean) performance for all participating dogs in last indoors 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)
JK_D21	6	3	2	1	0.75	0.75	0.86	0.75
JK_D22	4	1	1	6	0.80	0.14	0.40	0.42
JK_D23	6	2	2	2	0.75	0.50	0.75	0.67
JK_D24	5	2	2	3	0.71	0.40	0.63	0.58
JK_D25	7	3	2	0	0.78	1.00	1.00	0.83
JK_D26	3	2	4	3	0.43	0.40	0.50	0.42
JK_D27	3	3	4	2	0.43	0.60	0.60	0.50
JK_D28	4	2	4	2	0.50	0.50	0.67	0.50
JK_D29	2	2	1	7	0.67	0.22	0.22	0.33
Total of 108 tracks	40	20	22	26	Mean 65%	Mean 50%	Mean 62%	Mean 56%

**Table 3.** Comparison of dogs' performance for detecting target scent samples of *H. parviporum* (HP) and *H. annosum* s.s. (HA) in last indoors scent discrimination test. TP = True positive, TN = True negative, FN = False negative, FP = False positive.

Dog code	HP fruit body, defrosted	HA fruit body, defrosted	Both defrosted HP and HA fruit body, correct	HP mycelia, fridge	HA mycelia, fridge	Both HP and HA mycelia correct	HP wood slice, defrosted	HA wood slice, defrosted	Both defrosted HP and HA wood slice correct
JK_D21	TP	TP	x	TP	TP	x	FP	FN	
JK_D22	TP	FP		TP	TP	x	TP	FP	
JK_D23	TP	TP	x	FP	TP		FN	TP	
JK_D24	TP	TP	x	FN	FN		TP	TP	x
JK_D25	TP	TP	x	TP	TP	x	TP	TP	x
JK_D26	TP	FN		FN	FN		TP	TP	x
JK_D27	TP	FN		TP	FN		FN	TP	
JK_D28	FN	FP		TP	FN		FN	TP	
JK_D29	TP	FP		FN	TP		FP	FP	
TP, Total	8	4	4	5	5	3	4	6	3
TP %	89%	44%	44%	56%	56%	33%	44%	67%	33%

**Table 4.** Comparison of participating dogs' performance in detecting defrosted, fresh, and freeze-dried samples of *H. parviporum* (HP) in final indoors scent discrimination test. Participating dogs had no prior training with freeze-dried HP fruit body samples. TP = True positive, TN = True negative, FN = False negative, FP = False positive.

Dog code	HP fruit body, defrosted	HP fruit body, fresh	HP fruit body, freeze-dried	Both defrosted and fresh HP correct	Both defrosted and freeze-dried HP correct	Both fresh and freeze-dried HP correct	All 3 HP sample types correct
JK_D21	TP	FN	TP		x		
JK_D22	TP	FP	FP				
JK_D23	TP	FN	TP		x		
JK_D24	TP	FP	FP				
JK_D25	TP	TP	FN	x			
JK_D26	TP	FN	FP				
JK_D27	TP	FP	FP				
JK_D28	FN	TP	FN				
JK_D29	TP	FP	FP				
TP, Total	8	2	2	1	2	0	0
TP %	89%	22%	22%	11%	22%	0%	0%

samples may affect the scent profile and dogs' performance in the last test. Therefore, further studies are needed whether using defrosted samples is recommendable in the training process, and whether using fresh samples would be more effective. Further testing whether freeze-dried fruit bodies would have a similar scent profile to fresh fruit body samples would be needed in the future, as training requires year-round keeping up the training and skills and would benefit from using samples that can be preserved in long-term.

Among all target sample types 17 *H. parviporum* and 15 *H. annosum* s.s. samples were correctly identified. This suggests that the scent profiles of the species are likely to be relatively similar. A comparison of the identification of *H. parviporum* and *H. annosum* s.s. samples among the dogs is described in Table 3. There is difference between the performance of the dogs, and further more extensive studies are needed in the future.

### Field tests

Seven dogs with their handlers continued the training process for about a half year after the last indoors scent discrimination test. They participated in the

*H. annosum* s.s. field test organized in the young Scots pine forest. Out of the seven dogs, three continued to participate in the field test conducted in the mature Scots pine forest. The dogs that participated in the field tests recognized well *H. annosum* s.s. both in young and old Scots pine forests.

In the young pine stand, the mean sensitivity of correct *H. annosum* s.s. identification was 72% and it varied between 50% and 93% among the seven participating dogs (Table 5). The mean precision of

**Table 5.** Dogs' individual performance and averaged (mean) performance for participating dogs in target scent search field tests of *H. annosum* s.s. in a young Scots pine stand in Lälliäinen, southern Finland. 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
JK_D22	13	1	4	0.93	0.76	20
JK_D23	10	4	2	0.71	0.83	29
JK_D24	5	4	4	0.56	0.83	23
JK_D25	4	4	0	0.50	1.00	16
JK_D26	6	1	3	0.86	0.67	22
JK_D27	8	3	0	0.73	1.00	27
JK_D29	7	2	2	0.78	0.78	22
Total	53	19	15	Mean 72%	Mean 84%	Mean 23 min

identification was 84% and it varied between 67% and 100% among the dogs. Overall, the dogs alerted on a total of 53 infected trees (True Positive). Dogs passed without identification 19 infected trees (False Negative) within a half-meter radius of dog's search trail. These trees exhibited typical disease symptoms (light green to brown needles and/or a thin crown) as assessed by a forest pathologist. There were 15 false alerts (False Positive) as assessed in the test situation in the field visually by the forest pathologist. The health status of these asymptomatic trees marked as infected by the dogs was assessed after the test by cutting down the trees and taking wood disks from the stem base and collar of the main roots. The presence or absence of the *Heterobasidion* infection in the samples was analyzed by a forest pathologist. Seven of these trees had incipient *Heterobasidion* infection and were correctly identified by the dogs as infected.

In the mature Scots pine stand, the mean sensitivity of correct identification of *H. annosum* s.s. was 88% and it varied between 78% and 95% among the three participating dogs (Table 6). The mean precision was 97%, and it varied between 90% and 100% among dogs. Overall, the dogs alerted on a total of 35 infected trees (True Positive), and missed only four trees with visible disease symptoms within a half-meter radius (False Negative). There were two false alerts (False Positive).

## Discussion

Our results show that trained volunteer dog-handler teams were successful in searching for and identifying trees infected by *H. annosum* s.s. both in young and mature pine stands. There was some difference between the dogs in the effort, i.e. time spent searching a unit area or transect. The number of true-positive alerts varied among the dog-handler teams and in the sensitivity of the dogs. In case the detection performance would be calculated, there would be difference between the dog-handler teams in their efficiency. It should be noted that because the presence of the target species in field tests in real-life environments varies, comparing the dogs' detection performance based solely on the number of alerts may not be

meaningful. Moreover, teamwork influences accuracy: some handlers verify alerts by inspecting the tree base and root collars, while others rely only on the dog's alert. Indoor tests also revealed differences in dogs' ability to detect target samples and to distinguish them from control samples, likely due to variations in training duration and intensity.

The dogs' ability to detect the pathogen in asymptomatic trees may be underestimated due to the uncertainty in determining the health status of living trees based on the collected bore samples. Incipient, small decay at the base of the tree or in the root system remain easily undetected even if several core samples are collected (Stenlid and Wåsterlund 1986). This means that some trees correctly identified as infected by the dog may have been incorrectly marked as false positives. In the young pine stand, where the trees could be cut down afterwards, half of the asymptomatic trees were correctly identified by dogs as infected, suggesting a higher proportion of true positives than reported. Overall, detecting disease in visually healthy-looking stands is challenging, especially at the early training phase. Old stumps with *Heterobasidion* fruit bodies enhance disease detection.

For dog-handlers in real-life context, the fruit bodies are a visible sign and the most reliable indicator of the presence of *Heterobasidion* root rot in the stand. The development of fruit bodies requires moist conditions, and therefore, they are usually hidden in the root cavities covered by ground vegetation and litter, making their detection challenging (Greig 1998; Gaitnieks et al. 2021). Thus dogs being able to identify hidden fruit bodies are a significant help, especially in Norway spruce stands where the previous generation has been affected by *Heterobasidion* root rot, but the current tree generation does not yet show any visible signs of *Heterobasidion* infection. As the disease progresses, fallen trees and decayed stumps, in addition to fruit bodies, can provide important information about the occurrence of *Heterobasidion* root rot.

Often, the information about whether *Heterobasidion* root rot is present or not on a site (regardless of the extent of the disease) is valuable for forest owners, particularly when considering the establishment of continuous cover forestry (CCF) systems with species such as

**Table 6.** Dogs' individual performance and averaged (mean) performance for participating dogs in scent search field tests of *H. annosum* s.s. in an old Scots pine stand in LÄyliäinen, southern Finland. 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
JK_D22	18	1	2	0.95	0.90	17.5
JK_D23	10	1	0	0.91	1.00	20.0
JK_D29	7	2	0	0.78	1.00	20.0
Total	35	4	2	Mean 88%	Mean 97%	Mean 20.0

Norway spruce. *Heterobasidion* infections can significantly impact forest health and timber yield in CCF, where the management practices do not allow for clear-cutting and changing to more resistant tree species. On a site infested with *Heterobasidion* root rot, the mycelium can spread both between and among different tree classes, leading to a chronic state of infection. This not only poses an immediate risk to the health of the forest but also affects long-term timber production, as chronic infections can reduce the quality and quantity of timber wood over time (Piri and Korhonen 2001; Piri and Valkonen 2013). Therefore, the ability to detect the presence of *Heterobasidion* infections before making decision regarding CCF, using trained dogs, can help forest owners ensure sustainable forestry practices.

Compared to standing forest, there are better opportunities to obtain a reliable picture of the frequency of *Heterobasidion* infections on cutting areas, where stumps and piles of timber and pulpwood logs prove good material for dogs to identify *Heterobasidion* root rot and distinguishing it from decay caused by other fungi. Currently, the spatial distribution of decayed stumps in a clear-cut area can be determined using data collected by harvesters. This information allows for the strategic placement of tree species resistant to *Heterobasidion* root rot in affected parts of the stand, without requiring a change the tree species across the entire regeneration area (Aza et al. 2022; Holmström et al. 2025). However, visually identifying the fungal species responsible for decay is challenging, which may lead to misunderstanding about the extent and distribution of root rot caused by *Heterobasidion* infection (Lara et al. 2024). Therefore, trained scent-detection dogs could play a crucial role in distinguishing stumps decayed by *Heterobasidion* spp. from those decayed by other fungi, helping to target resistant tree species more precisely.

To our knowledge, the present study is the first to train dogs to use olfactory sense to detect *Heterobasidion* spp. in their natural forest environment and test their performance in real-life forest stands. Previous Swedish studies have shown that dogs are able to detect spruce wood infected by *H. parviporum* buried in the soil as well as water extract from infected wood applied on the ground surface (Swedjemark and Morrison 1987; Wysocka 2021). Thus the results of these Swedish studies support the findings obtained in this study about the usefulness of dogs' sense of smell in recognizing *Heterobasidion* spp.

Dogs have been successfully used in similar studies to detect some other fungi, bacteria and viruses. For example, dogs outperformed human surveyors in identifying the endangered fungus,

*Hylocreopsis amplexans* T.W. May & P.R. Johns, in natural habitat (Amor et al. 2024). They have also shown 100% accuracy in detecting *Phytophthora* spp. from water and soil batches (Swiecki et al. 2018). Early detection of *L. asiaticus*, weeks to years before visual surveys and molecular methods, has proven effective in agricultural systems (Gottwald et al. 2020). Odor detection using dogs also offers a promising diagnostic tool for identifying *Staphylococcus aureus* Rosenbach and differentiating it from other mastitis-causing pathogens (Fischer-Tenhagen et al. 2018). Additionally, dogs have been able to distinguish BVDV from BHV1 in cell cultures in the laboratory (Angle et al. 2016). An olfactometer has also been successfully used in laboratory experiments to assess dogs' ability to detect *U. necator* on *Vitis vinifera* L. leaves (Aviles-Rosa et al. 2025).

The detection of odors in complex environments like forests presents several challenges for odor dogs. As noted by Caldicott et al. (2024), the odor released from a target substance may vary over time due to aging, interactions with extraneous odors, and environmental factors, complicating detection. Additionally, variables such as pre-exposure to certain odors or odor enrichment may affect generalization of results of dog training (Caldicott et al. 2024). Studies on truffle dogs have also demonstrated the importance of interdisciplinary approaches that combine insights from dog behavior, veterinary science, and comparative psychology, along with practical experiences from truffle hunters and farmers to improve quantity and quality of truffle harvest (Cejka et al. 2022).

During the training period, it became clear that the learning process of volunteer dog-handler teams, involving multiple target and control scent variations together with discrimination and search training, is a lengthy, complex, extensive process. It requires time, motivation, and dedication from the handlers. Prior experience in scent training both of the dogs, but especially of the handlers, can affect the overall length of the training process. For first time dog-handler teams, learning first the basics of scent training extends the duration of the training. This needs to be taken into account, when planning and training volunteer dog-handler teams for this type of scent detection and discrimination tasks of searching for pathogens in forests. In addition, real-life use of the acquired scent detection and discrimination skills after the training process needs to be ensured, including developing the processes to certify and include use of dog-handler teams in forestry, also to further motivate the participating dog-handler teams during the extensive training process.

## Conclusion

The use of trained dogs can be recommended for detecting *Heterobasidion* infections in Scots pine and Norway spruce stands. The dogs were able to identify infections caused by *H. annosum* s.s. in both young and mature Scots pine stands. During the training process, the dogs also trained and detected *H. parviporum* in standing trees, stumps, wood piles, and wind-thrown trees in Norway spruce. Dogs can be a significant help in detecting *Heterobasidion* fruit bodies which are often hidden and difficult to find. Multiple scent sources can provide additional information for increasing the reliability of scent detection. Furthermore, the dog-handler's familiarity with *Heterobasidion* root rot and handler's seamless cooperation with the dog are essential for successful scent work.

Due to the clear benefits of using dogs for the early detection of *Heterobasidion* root rot, there is a need to expand the training of scent-detection dogs for detecting pathogens in the forest. Large-scale training and practical work require continuous learning and practice for both the dogs and their handlers. This can only be achieved through close cooperation between forest owners, dog-handlers, research organizations, and forest experts. Processes need to be developed for certifying dog-handler teams and including them into the experts working in forestry.

## CRedit authorship contribution statement

**Juha Kaitera:** Writing – original draft, Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Supervision, Resources, Project administration. **Tuula Piri:** Writing – original draft, Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Supervision, Resources. **Minna Männistö:** Writing – original draft, Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources. **Sanna Vinblad:** Writing – original draft, Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources. **Heli Väättäjä:** Writing – original draft, Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources. **Kari Mäkitalo:** Writing – review and editing, Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources, Project Administration.

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## Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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