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2 **Using synthetic semiochemicals to train** 3 **canines to detect bark beetle-infested trees**

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28 **Key Message**

- 29 • Detection dogs were rapidly trained to locate release of synthetic bark
30 beetle pheromone components
- 31 • Synthetics allowed dog training off-season both in laboratory and field
- 32 • Dogs trained on synthetics detected naturally target pest insect attacked
33 trees at a distance of more than 100 m.
- 34 • The method allows rapid removal of single, first attacked trees before
35 offspring emergence, thus curbing local pest increase and lowering spread
36 of attacks in the landscape

37 **Abstract**

38 In this proof of concept study, we report the off season training of two detection dogs on a
39 series of synthetic semiochemicals associated with *Ips typographus* pest bark beetle
40 infestations of spruce trees. Scent detection training allowed dogs to discriminate between
41 physiologically-relevant infestation (target) odours, quantified by GC-MS using extracted ion
42 chromatogram to be bio-active at levels of $<10^{-4}$ ng /15 min or lower, and natural non-target
43 odours that might be encountered in the forest. Detection dogs trained to recognize four
44 different synthetic pheromone compounds in the winter time, well before beetle flight, were
45 able to detect natural infested spruce trees unknown to humans the following summer. The
46 trained detection dogs were able to detect an infested spruce tree from the first hour of bark
47 beetle attack until several weeks after the attack. Trained detection dogs appear to be more
48 efficient than humans in detecting early bark beetle infestations because the canines ability to
49 cover a greater area and by olfaction detect infestations from a far greater distance than can
50 humans. Infested spruce trees could be detected by trained detection dogs out to more than
51 100 m.

52 **Key words:** *Ips typographus*, semiochemicals, GC-MS, detection dog, forest protection,
53 Norway spruce

54 **Introduction**

55 Detection dogs are used to locate many objects including humans, explosives, and illicit drugs
56 (see BROWNE et al. 2006 and references therein; LORENZO et al. 2003). Trained canines have
57 also been used to detect invasive organisms (GOODWIN et al. 2010; HOYER-TOMICZEK et al.
58 2016) as well as endangered species (reviewed by BEEBE et al. 2016). Canines have also been
59 trained to detect small or cryptic insects such as termites (BROOKS et al. 2003), palm weevils
60 (NAKASH et al. 2000), and bed bugs (PFIESTER et al. 2008; VAIDYANATHAN AND FELDLAUFER
61 2013) and endangered Coleoptera (MOSCONI et al. 2017). The key benefits of using trained
62 detection dogs are their keen sense of smell (HEPPER AND WELLS 2015), and their ability to
63 cover large areas in a shorter time, when compared to humans (MOSCONI et al. 2017). In most
64 cases, biological material is used for the training (JOHNEN et al. 2013).

65 The European spruce bark beetle – *Ips typographus* (L.) is one of the most destructive forest
66 pests in Europe (GRÉGOIRE AND EVANS 2004). For forest protection, the rapid detection of
67 bark beetle infestations is required to successfully implement a management strategy that
68 relies upon removing recently infested trees within 2 –3 weeks of attack (SVENSSON 2007).
69 However, human detection generally requires close inspection ($\leq 1\text{m}$) of trees, and is
70 therefore time-consuming, costly, and not always practical. Therefore, detection generally
71 occurs 2–3 months after an infestation in N Europe, when tree crown colour fades and bark
72 falls off. By this time, most bark beetles have left the infested tree and may attack other, non-
73 infested stands. Since a rapidly changing, but specific series of beetle pheromone components
74 and other semiochemicals are present for several weeks after an initial attack, the use of
75 detection dogs may prove a better alternative than human inspection. Upon attacking a tree,
76 male bark beetles secrete an aggregation pheromone, consisting of a blend of 2-methyl-3-
77 buten-2-ol and *cis*-verbenol (BIRGERSSON et al. 1984). A few days later, an inhibitory signal
78 (consisting mainly of ipsdienol) is emitted when bark beetle females have begun laying eggs

79 (BIRGERSSON et al. 1984; SCHLYTER et al. 1987). After the first week, an additional chemical
80 cue, indicating that the infested tree is fully utilized and competition is high, is evident. This
81 semiochemical, verbenone, is an oxygenation product by the beetle and by the interaction of
82 fungi and bacteria with damaged tree phloem (LEUFVÉN AND BIRGERSSON 1987; SCHLYTER et
83 al. 1989).

84 In this proof of concept study, we report the laboratory training of two detection dogs on a
85 series of synthetic semiochemicals associated with bark beetle infestations, and the ability of
86 these trained dogs to later detect and locate bark beetle infested trees in the field. Since the
87 semiochemical profile of attacked trees changes rapidly in both the quality and quantity of
88 semiochemicals released, we chose to use several synthetic chemical compounds as
89 representative stimuli in our canine training. We were also interested in determining if dogs
90 trained on synthetic pheromones in the winter months could later locate infested trees in the
91 summer months. Finally, we wanted to determine if a trained dog can detect natural
92 infestations from distances (10 to 100 times) further away than a human.

93 **Materials and methods**

94 ***Canines***

95 Two dogs, owned by SnifferDogs Sweden (Hjortsberga, Sweden), were used in this study. Dog
96 A was a nine-year-old female German shepherd that was previously trained as a search and
97 rescue dog for humans. Dog B was a one-year-old female Belgian shepherd (Malinois) that had
98 only basic obedience training, and had no previous formal detection expertise.

99 ***Chemicals***

100 Synthetic bark beetle pheromones used in this study included methylbutenol (2-methyl-3-
101 buten-2-ol; Acros Organics, Gothenburg, Sweden), 4*S*-*cis*-verbenol (Borregard, Sarpsborg,
102 Norway), and (*S*)-ipsdienol (Bedoukian, Danbury Connecticut, USA). Synthetic verbenone, a

103 bark beetle pheromone and a product of the host tree was obtained from Fluka (Sigma-
104 Aldrich, Stockholm, Sweden). Other chemicals used in the study were obtained from our
105 chemical stocks (see ANDERSSON et al. 2012).

106 Each pheromone component was stored separately in separate jars of glass, to avoid cross
107 contamination of odours. In each jar of glass a cotton pad (ICA Basic Bomullsrondeller,
108 Netherlands) was placed in the bottom and a small amount of each semiochemical were
109 dropped on to the cotton pad (10 μ l methylbutenol, \approx 10 mg *cis*-verbenol, 1 μ l ipsdienol, or 10
110 μ l verbenone). The glass jars were then filled with cotton pads, and so molecules in gas phase
111 of each component passed passively by aeration transfer in the closed jar, via adsorption of
112 the odour to the pads placed above (HUDSON-HOLNESS AND FURTON 2010). The glass jars
113 were stored in a freezer (≈ -18 °C).

114 For determination of release rates by GC-MS and dog training response (Fig 1, Table 1) we
115 always used the cotton pads from the top in each glass jar. The last five cotton pads in the
116 glass jars we never used but filled up with new pads when needed. A cotton pad holding the
117 semiochemical (HUDSON-HOLNESS AND FURTON 2010) was placed in a stainless steel tin (5
118 cm dia.) with perforated lids (“Ströare”, Biltema®, Helsingborg, Sweden). For ordinary dog
119 training, we replaced the cotton pads each day. For release rates by GC-MS and dog training
120 response study we prepared 5 steel tins of each synthetic pheromone at the same time. These
121 tins were stored in room temperature (circa + 20 °C). Release rates were determined using
122 odour collections similar to Zhang et al. (2000). An inverted glass funnel (5 cm dia.) was
123 placed above the steel tin and air was drawn through a column packed with Porapak® Q
124 (25 mg mesh 60-80; in a Teflon tube 3 mm i.d.) at 100ml/min at 15 min intervals. Compounds
125 were eluted from the column with 400 μ l pentane (Sigma-Aldrich, Steinheim, Germany) into
126 a 400 μ l insert placed in a 2 ml screw top vial (Agilent Technologies, Böblingen, Germany),
127 and 1 mg heptyl acetate was added as internal standard. Aeration extracts were analysed by
128 gas chromatography- mass spectrometry (GC-MS; Agilent 6890-5975, Agilent Technologies,

129 Santa Clara, CA, USA) with techniques previously reported (BIRGERSSON et al. 1984).
130 Quantifications were based on extracted ion chromatograms of prominent fragments for each
131 tested compound and the internal quantification standard, respectively. The limit of
132 quantification (LOQ) in the analytical procedure was < 0.1 ng/min.

133 **Laboratory tests**

134 Initially, dog A was introduced to the bark beetle pheromones using the synthetic odour from
135 a commercial dispenser, ETOPheron® (Pheronova AG, Switzerland), which is used in bark
136 beetle monitoring traps. Because of the dispensers construction of fabric with a plastic shell it
137 was not 100% sure that the dog learned the scent of the pheromone components as the target
138 odour or if it learned any other odour of the dispenser materials. It is easy to inadvertently
139 train a dog to detect an unexpected or impure source when attempting to train to a pure
140 compound. To be sure that the dog learned the right odours we subsequently trained the dog
141 on pure synthetic semiochemicals applied to cotton pads. Non-target odours, that could
142 disturb search, were also used in the training and consisted of items found in a forest setting
143 such as vegetation odours from spruce needles, cones, resin, bark, moss, and animal odours
144 (i.e. scent from feathers, fur, hoofs and faeces). All non-target (disturbance) odours were
145 collected in the forest, or donated by local hunters (fur and hoofs from moose, deer, and boar).
146 Both target and non-target odours were stored in jars of glass and transferred by aeration to
147 cotton pads to ensure that the background odour of cotton was present in both target and
148 disturbance odours.

149 The training platform used here (2D illustrations in Figure ESM_1 and video in ESM_4_V1),
150 was developed by Stig Meier Berg and Geir Kojedal, Spesialsøk, Selbu, Norway, based on an
151 idea from Hundcampus, Hällefors, Sweden (FISCHER-TENHAGEN et al. 2011). It is designed to
152 let the dog work independently, to minimize the cues from the handler and to be easily
153 manoeuvred by the handler creating a more effective learning situation with a high rate of

154 opportunities to reward the dog for desired behaviour. Disturbance odours were presented
155 together with one or several semiochemical stimuli in a movable tray with 7 positions (Figure
156 in ESM_2).

157 For evaluation of the dog detection performance with decreasing amounts of odour molecules
158 over time nine trials were conducted to evaluate the dogs' identification performance with
159 each synthetic semiochemical. For this trials we used 4 of the prepared 5 steel tins containing
160 cotton pads with synthetic semiochemical. Since the trials were conducted over several days
161 (1 hour through 84 hours after the cotton pads being placed in the tins and stored in room
162 temperature) we used a new tin every day. This was done to make sure that the tins weren't
163 contaminated with any other scents such as odour from the dogs. Every trial session lasted for
164 approximately one minute (50-70 seconds).

165 To compare different stimuli linear layouts, mixing target and non-target scent, on the movable
166 tray, the two dogs were tested in three trials with each stimuli layout (ESM_2).

167 ***Outdoor tests***

168 To train the dogs to pinpoint the target odour source outdoors, pieces of the cotton pads
169 containing synthetic pheromone odours as those used for platform training were hidden in
170 cracks of the bark of several species of trees. The cotton pieces were placed in the height of
171 the nose of the dogs and the dogs were shown where to sniff for the target (video
172 ESM_4_V2). When the dog found the cotton piece holding the target odour, it was
173 immediately rewarded by the sound of the clicker and a piece of food delivered between its
174 nose and the odour source. Several pieces of cotton with either target or non-target odours,
175 were put in cracks of the bark in a small area (30x30 cm) to ensure the dogs did not use visual
176 cues for close-range target location. When the dogs were able to consistently (~ 100%) locate
177 the pads, the dogs were gradually sent from longer distances to locate the tree with the cotton

178 pad, allowing the dog to detect decreasing amounts of target molecules and to follow the
179 odour to its source.

180 Dogs were trained in the winter under a variety of weather conditions (*e.g.* rain, snow, sun).

181 Training trials using synthetic odour were conducted on average once a week during 2009 and
182 2010. The temperature ranged from 2 to 28 °C. The handler determined the search strategy to
183 best cover the assigned area based on wind conditions and terrain. These protocols were
184 employed to simulate future practical field survey conditions.

185 A proof-of-concept test, evaluating the detection by dogs of spruces that were known to be
186 recently attacked by bark beetles, was conducted at the Nature Reserve of Nötteryd (near
187 Växjö, Småland, Sweden). The area consisted of wind-felled trees and standing healthy
188 spruces. In the spring of 2009, 95% of all spruces in the reserve were already killed by bark
189 beetles. The remaining spruces that were still alive stood together in clusters of 10-15 trees.
190 We felt these circumstances made this particular Nature Reserve an optimal area to first try
191 the dogs on natural attacks. Another series of tests were conducted at a production-forest in
192 Nottebäck, also near Växjö, Småland, Sweden, with the permission of the owner of the forest.
193 The dog team consisted of one dog (dog A) working off-leash and one handler, and searched
194 three different areas in the production-forest attacked by bark beetles in previous years. The
195 handler had knowledge of the location of former attacks, but no information if there were any
196 new attacks. The dog and handler searched each area with no time-limit. The handler
197 determined their search strategy to best cover the assigned area based on wind and terrain.
198 These protocols were employed to simulate expected future practical field survey conditions.
199 Dog and handler movements were recorded using global positioning systems (GPS) in 5-
200 second intervals in all field trials. These data allowed identification of the point at which the
201 dogs lifted their nose up in the air and made a sudden change in direction of travel and moved
202 directly towards an infested spruce (video of search ESM_4_V3). The GPS units used in the

203 study were Garmin Astro® 220 Nordic handset and Garmin DC30 dog collar (Garmin
204 Corporation, Taiwan). The map used in the handset was Garmin “Friluftskartan Pro V2
205 Götaland”. The data from the GPS-unit were transferred to a PC with Garmin’s software
206 MapSource. Using the measuring tool we could measure the distance from where a track from
207 the dog changed direction to the waypoint where the dog alerted on an infested spruce.

208 **Field trial - detection distance from natural sources**

209 We used 20 different areas, whereof 10 were located in nature reserves and 10 in production
210 forests, with permission of the owners and from The Swedish Forest Agency in Växjö in
211 2010. All areas were 2 – 4 ha and tests were done in three different set-ups; a) 10 search areas
212 with location of infestations known by handler b) 5 areas with location of infestations known
213 by the forest manger, and c) 5 areas with location of infestations unknown, but were
214 considered as risk areas with bark beetles infestations previous seasons.

215 To design the best search strategy for long distance detection, based on wind, terrain and the
216 location of the attacks, the dog handler had prior knowledge of attacks in the 10 first areas. To
217 estimate if the dog handler might involuntarily cue the dog to an odour source (an infested tree),
218 the dog handler was not allowed prior knowledge of attacks in the 10 latter areas.

219 **Results**

220 ***Laboratory tests***

221 The two dogs were successfully trained to recognize the four different synthetic
222 semiochemical compounds on the educational scent platform. Both dogs learned to recognize
223 a new target scent in just one training trial, similar to Johnston (1999). In that time, the dogs
224 managed to sample the tins for target odour about 30 times on average (video ESM_4_V1).
225 Occasionally, the dogs reacted with an increased interest when a new non-target disturbance
226 odour was presented. When this happened, the handler stood silent and just waited until the

227 dog stopped investigating the new non-target odour and, if the dog did not continue to search
228 by itself, gave the dog a new command to start sampling the other tins again. After a few
229 encounters with the new non-target odour the dogs' interest decreased since they learned that
230 there would not be any reward for that particular odour. Even though the dogs appeared to
231 alert on new, disturbance odours (mostly edible items like cookies and chips or scents from
232 other animals) the handler did not record such behaviour as an alert. When alerting on a target
233 odour, both dogs stopped sampling, and waited for their reward, in contrast to increased
234 sampling a tin in order to investigate a disturbance odour.

235 ***Chemical stimuli strength***

236 Chemical quantification by odour collection and GC-MS was routine with a limit of
237 quantification (LOQ) of < 0.1 ng/min. However, two days later, we found that most stimuli
238 titres, still well biologically active, decreased to below the LOQ. Using estimates based upon
239 linear regression, chemical data indicates that by the third day some compounds were very
240 close to zero (Table 1).

241 The dogs responded to estimated doses of 10^{-4} ng/15min releases or less. The four different
242 semiochemicals were learned equally well, and responses to sub-picogram release rates of
243 stimuli aged up to 3.5 days remained stable (Table in ESM_3).

244 ***Biological responses***

245 The responses of both dogs to target odours are summarized per target scent in Table 2. The
246 dogs achieved a mean of 99% correct indications; 1% of the incorrect indications were either
247 false positive (alerting to a non-target odour; dog A) or false negatives in the beginning of a
248 trial session (dog B) (Table 2). None of the dogs sampled all tins in every repetition. In each
249 repetition four tins were presented, but the trainer could never know where the dog would
250 start searching or in which direction it would continue its search. The only dispenser tin

251 always sampled was the tin holding the target scent. This explains the high success rate of
252 99% for correct positives for the target scent (at which tin the search will stop), but the much
253 lower rate, 55% for the correct negatives with direct sampling of empty tins before finding the
254 target scent.

255 To increase the dogs sampling of all presented tins, we tried the dogs in different kinds of
256 stimuli layouts with zero to three different target odours presented in the same trial, Figure A
257 in ESM_2. The only clear effect was for the layout with no target scent, where response
258 decreased with time, Figure B in ESM_2

259 Interestingly, over the >3 days of testing combined with chemical sampling, the correct
260 responses remained consistently high irrespective of substance (Table 2). The positive
261 responses showed no decline with estimated chemical stimuli levels (Fig 1A), indicating that
262 stimuli levels were above animal detection limit for the period. The correct negative responses
263 (no alert to disturbance odours) declined with the estimated stimuli strength since the dogs
264 learned that these odours weren't going to be rewarded (Fig 1B).

265 ***Outdoor tests***

266 During off-season training, dogs were introduced to cotton pads initially placed at nose height
267 in the cracks of the bark in different kind of trees (Video ESM_4_V2). Dog A, previously
268 trained as a search and rescue dog, just needed to come into contact with one of the new learned
269 target odours to expect a reward hence follow it to the source and pinpoint it to its handler. Dog
270 A also alerted the found target source by barking. Probably because this was the trained alert
271 when locating a hidden human as a search and rescue dog. Dog B, however, which had no
272 previous search training, had to learn how to follow the odour plume to the source. Dog B was
273 not trained to perform any other alert than pinpointing the source of the target odour. This dog
274 did not know any other way to receive its reward but putting its nose on the target source. The
275 target source became a button to push to get its reward.

276 Bark beetle activity usually began at the end of April, when the temperature increased to over
277 20 °C, allowing us to test the dogs' ability to detect natural pheromone from attacking spruce
278 bark beetles. Dog A successfully found the first spruce that was under attack, on the first day.
279 The spruce in question showed no signs of the attack at first sight, but further inspection at
280 close range revealed that the first bark beetles were drilling their way in to the spruce bark and
281 the sound of their drilling could also be heard. This finding was crucial in demonstrating that
282 it is possible to train a dog on a synthetic odour and subsequently showing that it will alert to
283 the natural odour under field conditions. All training and detection beetle in the Nature
284 Reserve was terminated at the end of May when so many spruces were under bark beetle
285 attack that the smell from the attacked trees became obvious even for the human nose.

286 Both dogs were also successful in locating sparser attacks in production forest stands, where
287 attacks were neither known to the dog handler nor the forest manager. In the first area
288 searched, dog A detected and alerted to a single, wind-felled spruce that had been infested by
289 bark beetles. In the second area searched, the same dog found seven infested standing spruces.
290 Five of them stood together in a cluster among old attacks. Two were located in a felling
291 edge.

292 In the third area, the dog detected five infested spruces, both standing and wind-felled. In this
293 area all the spruces were located near a felling edge by a clear-felled area where felled trap-
294 trees were placed. The dog started its search with detecting and alerting on the synthetic
295 pheromones from the trap-trees. When sent to continue its search the dog detected,
296 recognized, followed, and alerted on the natural pheromones emitted from the bark beetles in
297 standing trees (as shown in video ESM_4_V4).

298 The handler observed by GPS a majority of successfully located sources of natural pheromone
299 to be detected within 50 m, but both dogs located sources in a behavioural sequence over a
300 range of 50 – 100 m (Fig 2A). No differences in detection distance by GPS could be seen

301 among areas with attacks (10) known or unknown (10) to the handler. Later analysis of the
302 GPS-tracks showed several occasions where the more experienced dog A changed direction
303 and was able to detect the pheromones from bark beetle attacked trees at a distance of over
304 100 m from the source and follow it to the source (Fig 2B). In the 20 areas visited, the dogs
305 found in total 193 trees infested by bark beetles, in 77 different groups of attacked trees.

306 **Discussion**

307 Training canines to detect bark beetle-infested trees poses some important limitations,
308 including the relatively short season available for using trees at various stages of attack, as
309 well the risk of inducing a full-blown tree attack by placing pheromone for training purpose
310 on a host tree during the actual beetle flight period. While it is probably possible to train a
311 detection dog to locate spruces that have been attacked by bark beetles by just letting the dog
312 sniff an attacked spruce and reward the dog, such a “natural” method will not teach a dog to
313 recognize the different kinds of semiochemicals the bark beetle releases over the course of an
314 attack. Therefore, we chose to train the dogs to recognize a series of synthetic pheromone
315 compounds and using an indoor training platform. In this study, we demonstrate that canines
316 trained on synthetic bark beetle pheromone compounds at low (sub-picogram) levels, indoors,
317 can later recognize naturally-produced pheromone over long distances, outdoors.

318 Additionally, by using synthetic sources of the bark beetle pheromone in the laboratory, it is
319 possible to train dogs off-season long before the bark beetles start their flight period in the
320 field, and the dog handler has control over which odours the dog learns, one at a time and at
321 very low concentrations. The indoor training of canines also has the benefit in that other
322 environmental distractions are minimized, thereby allowing the dogs to concentrate on and
323 learn the target odours.

324 In the field, detection dogs that work over large areas (“off-leash”) can often be seen lifting
325 their nose up in the air and then make a sudden change in direction of travel. This likely

326 occurs when the dog enters an area with a detectable odour (a plume) that the dog identifies as
327 its trained target odour. While the odour plume structure in a field setting, where the plume
328 shape, size, and persistence is highly dynamic, cannot be easily delineated by chemical means
329 due to the very low titres present in open air (MURLIS et al. 2000; RIFFELL et al. 2008), it can,
330 at least, be observed through olfactory-behavioural responses of dogs to target odour plumes.
331 In our study, a trained detection dog could detect an infested spruce tree from a distance of
332 150 m, which is farther away than that estimated for bark beetles (*Ips typographus*)
333 responding to beetle pheromone dispensers (SCHLYTER 1992).

334 In training dogs to detect bed bugs (*Cimex lectularius* L.), the dog usually searches (either
335 “on-leash” or “off-leash”) the entire room – often several times – before alerting on a bed bug.
336 In this case, the dog-handler interaction is paramount owing to the vastly different scales of
337 indoor room searches (1 – 10 m) compared to free-ranging forest searches (10 – 500 m).
338 Issues surrounding a close interaction between dog and handler have been reported (LIT et al.
339 2011), though during our large scale, forest searches these issues would be minimal, at best.
340 In a study of canines involved in bed bug detection, a high degree of false positives and low
341 true positives were found (COOPER et al. 2014).

342 Little, if any, studies can be found using pure, known synthetic samples for canine detection
343 purposes (JOHNEN et al. 2013). However, it is clear that canines can show a dose-response to
344 relatively low (but quantitatively unknown) doses (KRESTEL et al. 1984; POLGÁR et al. 2016;
345 WALKER et al. 2006). Our levels of correct positives (sensitivity) and correct negatives
346 (specificity) appear high, compared to the seven studies recently reviewed that provided such
347 data (JOHNEN et al. 2013). Hitherto, no quantitative data exist on chemical strength during dog
348 training in the open literature in spite of some early attempts (KRESTEL et al. 1984; WALKER
349 et al. 2006). No doubt, the dearth of chemical data is due to low thresholds for dog response
350 to volatiles. While our data are novel, we must admit that our empirical data spans only a part
351 of the tested range of stimuli diminution over time, mainly the one-day-old dispenser

352 material, and we had to rely on estimates from linear regression for the older material with
353 lower releases. Still, our estimates appear to be the best so far documented.

354 The “search-and-pick” method of detection and removal of bark beetle- infested trees within
355 2–3 weeks of attack (SVENSSON 2007) often fails because of the short time frame involved.

356 Trees were often not cut and removed from the forest until weeks or months later
357 (LÅNGSTRÖM AND BJÖRKLUND 2010), long after beetles had moved to attack other trees.

358 Finding spruces in an early stage of attack is also significant for the timber value, due to a
359 blue stain fungi the beetle introduces into the newly-attacked trees (KIRISITS 2004).

360 Since the pheromone blends used by the bark beetles for intraspecific communication vary in
361 strength and composition over time, we importantly observed that the dogs could detect all of
362 the substances on which they were trained; therefore, it makes no difference which
363 semiochemical composition the bark beetles in an infested tree is currently emitting. Thus, a
364 trained dog will detect and follow any of the odours, alone or in blends, to the source and alert
365 the dog handler. While it is possible that the dog may learn additional odours that may occur
366 when a spruce is under attack (BIRGERSSON AND BERGSTRÖM 1989; SCHIEBE et al. 2012) any
367 conclusions to this effect would be speculation on our part.

368 In our study, searches would often be conducted in colder and wetter periods, in-between the
369 short warm-weather swarming periods of the beetle. It would be interesting and of practical
370 relevance to know more precisely how different weather conditions may affect the dogs’
371 ability to search a larger area.

372 In view of the large number of pheromones identified to date from moths, beetles and other
373 pests (> 1 000) (EL-SAYED 2017), it would seem feasible to start training of detection dogs for
374 many pest management systems.

375 In summary, this is the first report of using synthetic pheromone compounds at known titres
376 to train detection dogs to detect and locate living animals in the field. Dogs could detect and

377 locate the source of pest insect infestations at a distance of over 100 m or more. The use of
378 detection dogs for early detection of bark beetle infestations could contribute to better forest
379 protection. We suggest that, in general, use of stimuli that are biochemically well-defined in
380 both quality and quantity appears to hold promise for both better practise and science in
381 detection dog training to biological objects, such as cryptic pests.

382 **Miscellaneous information**

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384 **Conflict of interest:** The authors declare that they have no conflict of interests.

385 **Ethical approval:** Both dogs participating in this study were private owned working dogs
386 and handled by their owners. According to Swedish legislation no part of this study included
387 abuse to an animal at the time of study.

388 **Author contributions:** AJ and FS designed research; AJ and GB collected data; AJ, GB, and
389 FS analyzed data; all authors contributed to the writing process. All authors read and
390 approved the submitted version.

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487 spruce bark beetle, *Ips typographus* (L.) (Coleoptera: Scolytidae): Chemical and
488 electrophysiological analysis. *Chemoecol.*, 10, 69-80.

489 **Figure captions**

490 **Fig 1. Correct responses in relation to estimated stimuli evaporation rates per 15 min.**

491 **a)** Correct positive responses to stimuli (compounds) with known total release
492 over 3 days of testing. No effect of time for all stimuli joined ($r^2 \approx 0$).
493 **b)** Correct negative responses to stimuli with known total release over 3 days of
494 testing. Weak effect of time for all stimuli joined ($r^2 = 0.18$). Separately, MB
495 shows the strongest effect ($r^2 = 0.85$) together with Vn ($r^2 = 0.48$). Chemical data
496 from Table 1. The responses of the two dogs are pooled here, separate data in
497 Table 2. Semiochemical acronyms: MB) Methylbutenol, cV) 4*S-cis*-Verbenol, Id)
498 Ipsdienol, Vn) (-)-Verbenone.

499 **Fig 2. Field GPS tracks and detection distances.**

500 **a)** Tracks from an example of handler and search dog finding an unknown mass-
501 attacked single tree. GPS-units tracks shown with Google Earth on a background
502 satellite image over the area. Distance from Change of Direction to the attacked
503 trees = 157 m. Maps, aerial photos/satellite images: Copyright/Lantmäteriet,
504 Sweden, consent #: I2011/0096. (See GPS unit with tracks in video ESM_4_V3.)
505 **b)** Detection distances recorded from GPS tracks when locating natural bark
506 beetle mass-attacks unknown to handler.

507 **Supporting Information.**

508 **ESM_1** Fig. Educational scent platform. (PDF)

509 **ESM_2** Fig. Training platform stimuli layout and decline in response to no target scent.
510 (PDF)

511 **ESM_3** Table. Evaluation of the dog detection performance as number of indications with
512 decreasing amounts of scent molecules over time. (PDF)

513 **ESM_4_V1** Video. Educational scent platform in operation. (AVI)

514 **ESM_4_V2** Video. Placement of cotton scent pad and the location of the scent by dog on a
515 pine (a non-host tree of the beetle). (AVI)

516 **ESM_4_V3** Video. The search, GPS tracking, and location of natural attacks. (AVI)

517 **ESM_4_V4** Video. The search, location of two adjacent natural attacks, and rewarding. (AVI)

518 (The four videos can be accessed here as well: [V1 to V4 Suppl material.](#))

519 **Video stills**

Video stills



V1



V2



V3



V4

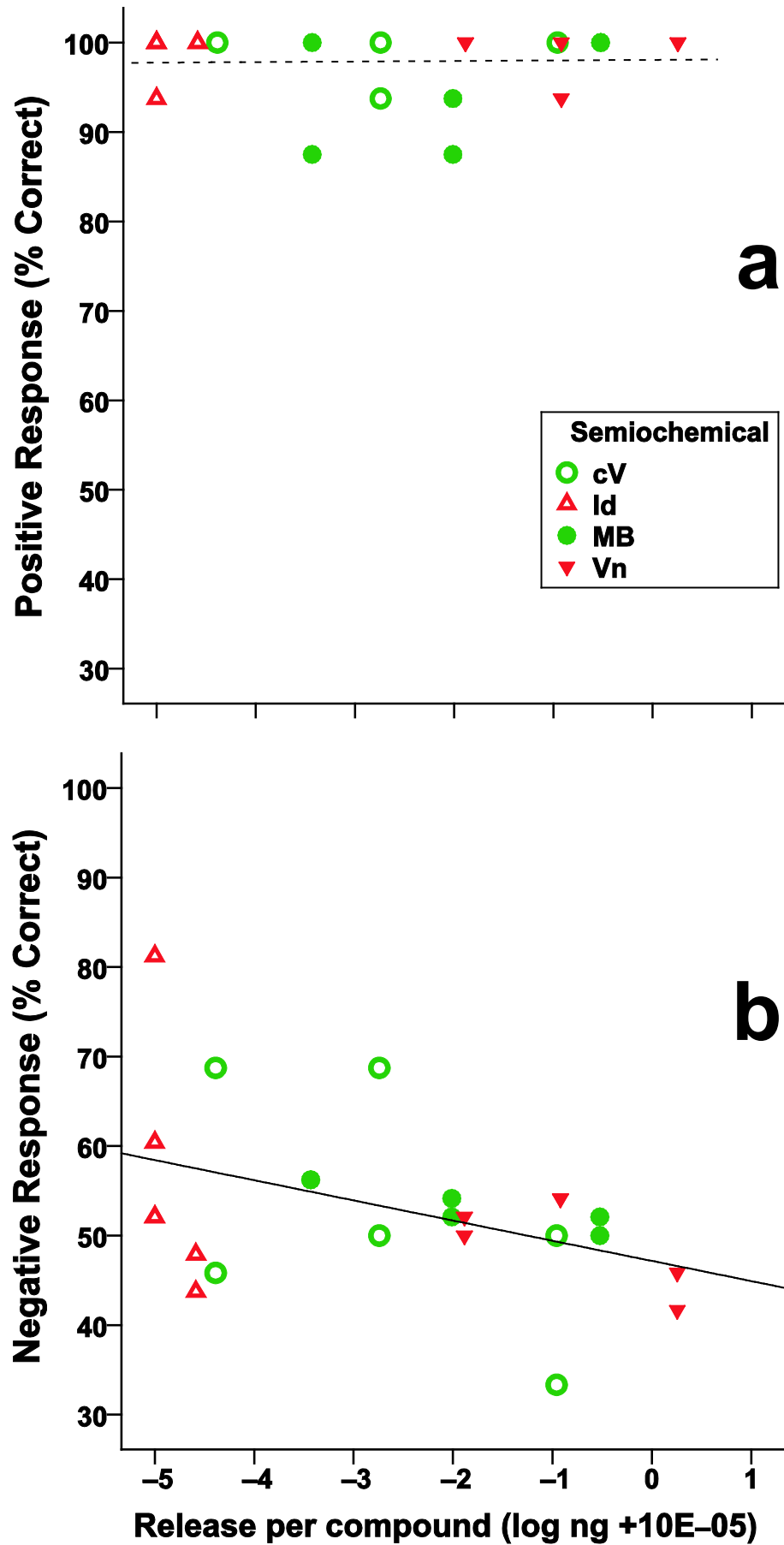
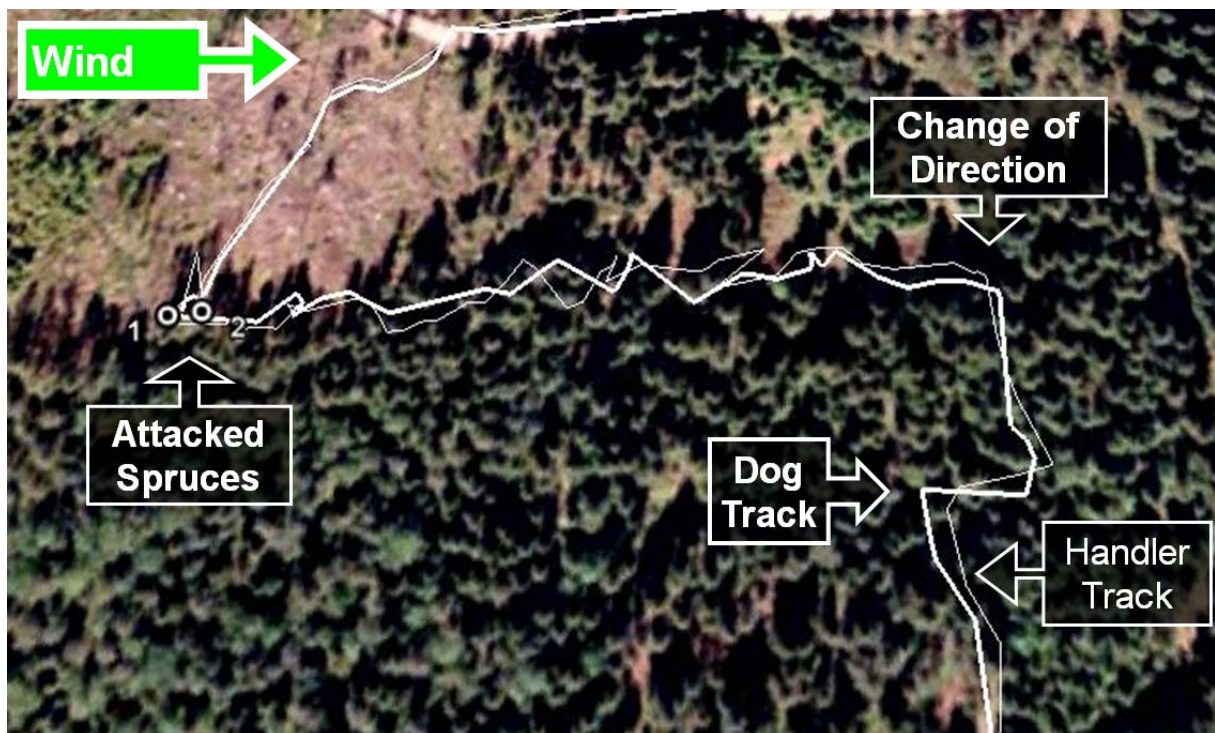


Fig. 1

a



b

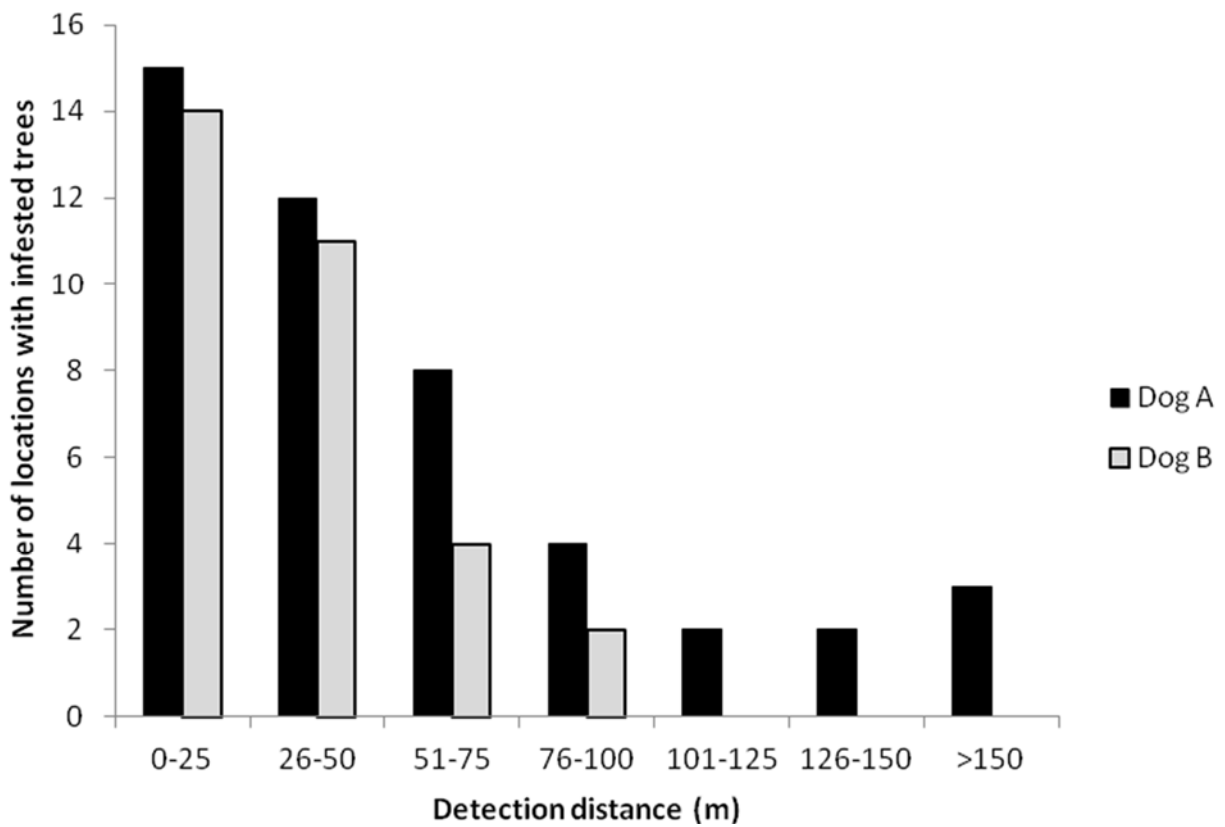


Fig. 2