

Progress in the use of detection dogs for emerald ash borer monitoring

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Early detection of infestation by the emerald ash borer (EAB), *Agrilus planipennis* is extremely difficult; hence developing additional methods is desirable. We built on the successful use of canine scent detection for the invasive long-horned beetles *Anoplophora glabripennis* and *Anoplophora chinensis* and trained six dogs in detection of EAB. A first test series was performed to evaluate detection accuracy of five of these dogs. Seven different experimental settings were tested under single blind conditions: (1) forest nursery, (2) piles of firewood, (3) firewood on the ground, (4) ash logs on the ground, (5) old urban ash trees, (6) urban forest with ash trees and (7) natural forest with ash trees. In total, 214 positive samples were presented to the dogs, out of which 20 remained undetected. The experiments ascertained sensitivity (correct positives of all positives) ranging from 73.3 to 100 percent and specificity (correct negatives of all negatives) from 88.9 to 99.8 percent in the tested settings. This initial study demonstrates that trained dogs are able to detect EAB scent from sources such as larval galleries in bark/wood, frass, living or dead larvae or dead dry beetles. The numbers of tested dogs and test series were limited, and further studies are needed to confirm the initial results. However, the preliminary findings demonstrate the potential of the method particularly for inspection of wood or plants at entry points.

Introduction

Surveillance is a key element in management of invasive wood boring insects like the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). This pest that originates from Far East Asia and has caused enormous damage to ash trees and forests in North America has also expanded its range in the European part of Russia since its discovery in Moscow in 2003 (Baranchikov *et al.*, 2008; Orlova-Bienkowskaja, 2014). The westernmost confirmed findings of EAB in Russia are 70 km from the border with Belarus and only 25 km with the border of Ukraine (Baranchikov *et al.*, 2018). In June 2019, EAB was detected for the first time in north-east Ukraine, close to the border with Russia (Drovalenko *et al.*, 2019); a further spread is most likely. The European and Mediterranean Plant Protection Organization considers the likelihood of entry and establishment in other European countries to be high (Petter *et al.*, 2019). A major obstacle for controlling the spread of EAB is the difficulty of early detection. Visual signs and symptoms need time to develop, which makes detection based on visual methods alone inadequate for early detection (Ryall, 2015). Traps and lures for EAB have received considerable attention, and ongoing improvements are being made (e.g. reviewed in Ryall, 2015), and efficient lures and traps are currently commercially available. Nevertheless, additional methods for rapid detection of EAB, particularly at an early stage of infestation or for inspection of imported wood or plants for planting, are desirable.

In order to test one potential method, we built on the successful use of scent detection dogs for *Anoplophora glabripennis* Motschulsky and *Anoplophora chinensis* Forster (Coleoptera: Cerambycidae). For these invasive long-horned beetles, detection of infested trees also depends on effective visual inspection (in this case more suitable than in the case for EAB). As one complementary method, dogs have been trained at the Austrian Federal Research Centre for Forests (BFW) since 2009 and employed for the detection of *A. glabripennis* and *A. chinensis* in outbreak areas, in nurseries and at import controls for wood packaging material and plants in several European countries (Forster and Wermelinger, 2012; Hoyer-Tomiczek and Sauseng, 2013). The method was evaluated in two test series using 10 and 14 dogs, respectively (Hoyer-Tomiczek *et al.*, 2016). Overall sensitivity (correct positives of all positives) was 85–93 percent, and specificity (correct negatives of all negatives) was 79–94 percent for testing different *A. glabripennis* scent material under standardized conditions. Under more realistic but also standardized conditions, the overall sensitivity was 75–88 percent, and specificity was 85–96 percent (Hoyer-Tomiczek *et al.*, 2016). The use of detection dogs or ‘sniffer dogs’ is included as a monitoring method in EPPO Standards PM 9/15 and PM 9/16 on procedures for official control of *A. glabripennis* and *A. chinensis*, respectively (EPPO, 2013a and 2013b), as well as in the German guideline for managing *A. glabripennis* (Julius-Kühn-Institut, 2014). *A. glabripennis* detection dogs were also trained by USDA APHIS/PPQ and employed in the outbreak area in Massachusetts, USA (Errico, 2013).

Table 1 Detection dogs employed in EAB detection training and the test series.

Breed	Initial <i>Anoplophora</i> training	Initial EAB training
Brandlbracke (Austrian black and tan hound) 1	Spring 2009	Autumn 2017
Brandlbracke 2*	Summer 2013	Autumn 2017
Brandlbracke 3	Spring 2014	Autumn 2017
Border Collie**	Summer 2014	Autumn 2017
Petit Bleu de Gascogne	Autumn 2014	Autumn 2017
Short-hair Collie	Summer 2017	Autumn 2017

*Dog was involved in the test series 1 to 4.

**Dog was involved in the test series 3 and 5 to 7.

Generally, detection dogs have proven their ability to find various insects of medical or phytosanitary importance, such as bed bugs (Pfiester *et al.*, 2008), fire ants (Lin *et al.*, 2011) or the red palm weevil, *Rhynchophorus ferrugineus* (Suma *et al.*, 2014). They may also be a promising additional tool for detecting bark beetle-infested spruce trees. In this case, training is possible with synthetic semiochemicals of the target species as well (Johansson *et al.*, 2019).

Here, we report on the initial progress made in training six dogs in the detection of EAB, which already had previous experience in detection of *Anoplophora* spp. Training followed the method used for *Anoplophora* detection dogs (Hoyer-Tomiczek *et al.*, 2016), and the first test series were performed to evaluate the detection accuracy of the dogs.

Material and methods

Dogs and training method

The initial training of six dogs on *Agrilus planipennis* started in November 2017 at the BFW. All dogs were previously trained for detection of *A. glabripennis* and *A. chinensis*. The dogs were of four breeds and differed in levels of experience in *Anoplophora* detection practice and in the time of their initial training (Table 1).

The scent training method for EAB detection uses the principle of positive reinforcement developed for the training of *Anoplophora* detection (Hoyer-Tomiczek *et al.*, 2016). The dog is rewarded by its handler immediately after correct indication of a finding in order to combine the positive feeling of reward with the target scent (Rebmann *et al.*, 2000; Braun, 2013). The imprinting of the dogs was carried out with living EAB larvae. Therefore, the dogs were immediately rewarded with treats when they got the scent of a larva. Then, the scent pattern of EAB was completed for the dogs by the addition of sawdust with EAB frass and EAB galleries in bark and wood of ash trees. Later, also scent carriers with the scent of living EAB larvae were used for training. The dog training involved inspection of firewood and logs as most likely pathways for introduction of EAB as well as young ash trees in a forestry nursery and old ash trees in urban area. Dogs were additionally trained in small woodlots in urban as well as forest areas to resemble EAB monitoring in potential outbreak areas. After the initial training in November 2017, the

dog handler/dog teams performed individual trainings during the following months before starting the test series.

Scent materials

Living or dead dried EAB larvae, dead dried beetles, dried pieces of ash bark and wood with galleries, dried sawdust and frass originated from EAB-infested *Fraxinus americana* L. trees in Plymouth and Woodbury, Connecticut, USA, and were collected in July 2017, October 2017 and September 2018. Prior to shipment to Austria, the material was stored at 4–6°C in a fridge. Additionally, dead beetles were provided from the USDA APHIS Laboratories in Brighton, MI, USA. Dead dry larvae and beetles as well as dry woody scent samples were stored at room temperature. EAB larvae were cultured in ash wood pieces of European ash (*Fraxinus excelsior* L.) at room temperature or were stored at 6°C for the production of scent carriers (cellulose filters) and for prolonging the pupation under quarantine conditions.

Set-up of the experiments and testing procedure

In June 2018, November 2018 and May 2019, sensitivity and selectivity of the dog detection method towards EAB were quantified with five dogs generally. One setting (no. 3) was performed with six dogs. Seven different experimental set-ups with three repeats each were tested under single blind conditions:

- (1) Wood/bark pieces of *F. americana* with EAB galleries hidden in young *F. excelsior* trees in lane of approximately 20 m length in a forest nursery
- (2) Dead EAB beetle and sawdust of *F. americana* hidden in piles of firewood of ca. 20 m length, 1.5–1.8 m height and 1 m width
- (3) Live EAB larva and EAB sawdust of *F. americana* hidden between firewood on the ground in an area of approximately 15 × 4 m (60 m²)
- (4) Wood/bark pieces of *F. americana* with EAB galleries hidden between wood logs on the ground in an area of approximately 15 × 5 m (75 m²)
- (5) Wood/bark pieces of *F. americana* with EAB galleries and EAB sawdust of *F. americana* hidden in old urban *F. excelsior* trees with several stems per tree on a length of ca. 50 m
- (6) A mixture of dead EAB beetle, scent carriers with scent of alive EAB larva and EAB saw dust and frass on bark of *F. americana* hidden at 1.5–2.0 m height on *F. excelsior* trees in a small urban forest with searching areas of 0.25 ha each
- (7) A mixture of dead EAB beetle, scent carriers with scent of live EAB larva and EAB sawdust and frass on bark of *F. americana* hidden at 1.5–2.0 m height on large *F. excelsior* trees in a natural forest with searching areas of 0.25 ha each

Test set-up 1 consisted of 2 positive and 28 negative samples and set-ups 2 to 7 of 2 positive and 6 negative samples in random order. In set-up 1, young ash trees infested with *Hymenoscyphus fraxineus*, the causal agent of the ash dieback, served as negative samples; non-infested pieces of *F. excelsior* wood were used as negative samples in the set-ups 2, 3 and 4. The firewood and the wood logs served as negative material as well. In the set-ups 5, 6 and 7, six *F. excelsior* trees were used as negative samples, and the other deciduous trees in the searching area represented

Table 2 Sensitivity, specificity and relative accuracy (means \pm standard error; $n = 5$ or 6 dogs) of the detection dogs towards EAB scent materials (combined for three replicates of each test).

Test	Setting	Scent material	No. of dogs	No. of samples (no. of positives)	Sensitivity	Specificity	Accuracy
1	Nursery	Wood/bark pieces	5	450 (30)	96.7 \pm 3.3	99.8 \pm 0.2	99.6 \pm 0.3
2	Firewood piles	Beetle and sawdust	5	120 (30)	83.3 \pm 7.5	88.9 \pm 2.5	87.5 \pm 0.0
3	Firewood on ground	Larva and sawdust	6	144 (36)	91.7 \pm 8.3	98.1 \pm 1.9	96.5 \pm 3.5
4	Logs	Wood/bark pieces	5	120 (30)	100.0 \pm 0.0	98.9 \pm 1.1	99.2 \pm 0.8
5	Old urban trees	Wood/bark pieces, sawdust	5	112 (28)	100.0 \pm 0.0	96.7 \pm 2.2	97.5 \pm 1.7
6	Urban forest	Beetle/larva, bark/sawdust	5	120 (30)	90.0 \pm 4.1	96.7 \pm 1.4	95.0 \pm 2.0
7	Natural forest	Beetle/larva, bark/sawdust	5	120 (30)	73.3 \pm 8.5	93.3 \pm 3.2	88.3 \pm 3.6

additional negative background. The dead EAB beetle and the EAB sawdust used as positive scent material in set-up 2 were wrapped in scent-neutral filter paper; live EAB larva and EAB scent carrier were placed in a small metal tea filter to prevent loss of the material. Bark and wood pieces of *F. americana* with EAB galleries and EAB sawdust were placed in branch forks or holes of the ash trees or among the wood logs on the ground.

To guarantee blind testing, positions of the samples were unknown to dog handlers and dogs. The experimenter took great care to hide scent materials very well, and scent contamination was avoided by wearing gloves. All tests were carried out outdoors.

At the beginning of each run, ambient air temperature, air humidity, wind speed and direction were recorded. Air temperatures ranged from 11.0 to 22.0°C and the wind from 9 to 19 km/h, and it was intensively turning during the third run of set-up 4 (wood logs on the ground). During set-up 3 (firewood on the ground), the wind speed was only 0–2.5 km/h. Also during the first two runs of set-up 7 (natural forest), the wind speed ranged from 2 to 4 km/h and increased during the third run to 8 km/h. The relative air humidity ranged from 48 to 97 percent, mainly from 45 to 65 percent, with the highest air humidity during the searches among the firewood on the ground (set-up 3) followed by the searches among the young ash trees in the nursery.

At the beginning of each test, dog handlers were instructed about the number of positive samples, the type of scent material deployed and the area or number of trees to be inspected. The maximum allowed searching time per test was 8 min. Any indication by the dog had to be clearly communicated by the dog handler to the experimenter. The experimenter immediately confirmed whether the indication was correct. In the case of correct positives, the dog handler rewarded the dog with its usual reward, typically with food, a toy or a combination of both. Then the search was resumed until all samples had been examined and the two positive ones identified or time had run out. Moreover, the dog handler could abandon the test run in exceptional cases when the dog became too distracted to continue to work.

Data analysis

For each test run and dog, the numbers of correct positive, correct negative, false-positive and false-negative indications were counted. The following values were computed to characterize searching success:

- (1) Sensitivity = number of correctly identified positives/total number of positive samples \times 100
- (2) Specificity = number of correctly identified negatives/total number of negative samples \times 100
- (3) Relative accuracy = number of correct identifications/total number of samples \times 100

For each test, the values per dog were based on three runs, i.e. a total of 6 positive and 84 negative samples examined in test set-up 1 as well as a total of 6 positive and 18 negative samples examined in the test set-ups 2–7.

Results

In total, 214 positive samples were presented in the tests, out of which 20 remained undetected. The dogs were most successful at detecting wood/bark pieces of *F. americana* with EAB galleries hidden between wood logs on the ground and at detecting wood/bark pieces with EAB galleries and EAB sawdust of *F. americana* in old urban *F. excelsior* trees; overall sensitivity was 100 percent in both tests and specificity 98.9 and 96.7 percent, respectively. The values were slightly lower for the sensitivity but slightly higher for the specificity when the same EAB scent material was hidden in young ash trees in a forestry nursery; overall sensitivity was 96.7 percent and specificity 99.8 percent. It was more difficult for the dogs to detect EAB scent material consisting of live EAB larva and EAB sawdust of *F. americana* hidden between firewood on the ground (overall sensitivity 91.7 percent and specificity 98.1 percent) but easier than to detect dead EAB beetle and sawdust hidden in piles of firewood of poplar and ash (overall sensitivity 83.3 percent and specificity 88.9 percent). The detection of EAB scent material hidden at 1.5–2.0 m height on *F. excelsior* trees was easier for the dogs in the urban forest than in the natural forest (Table 2).

The allowed maximum time for searching of 8 min was required 10 times in these tests; mean searching time ranged from 3.9 to 6.3 min. The fastest nine correct searches were finished within 2 min for the set-up with the ash wood/bark pieces with EAB galleries hidden in young ash trees (test 1) and hidden between wood logs on the ground (test 4) as well as for the set-up with the live EAB larva and EAB sawdust hidden between firewood on the ground (test 3) and for the set-up with ash wood/bark pieces with EAB galleries and EAB sawdust hidden in the old urban *F. excelsior* trees (test 5, three times). In two

cases the search was abandoned by the dog handler because the dog was too distracted from work.

Discussion

This initial study demonstrates that after training dogs of various breeds, they are capable of detecting *A. planipennis* scent of different origin, such as larval galleries, frass or dead dry beetles. The mean percentage of correct positive indications (i.e. sensitivity) ranged from 73.3 to 100 percent. These values are within the range of results reported for dogs trained to detect other insects. The sensitivity of dogs searching for *Anoplophora glabripennis* ranged from 75 to 93 percent (Hoyer-Tomiczek *et al.*, 2016), and dogs searching for red palm weevil, *Rhynchophorus ferrugineus*, scent material in potted palm trees gave correct positive indications of 78 percent (Suma *et al.*, 2014). Similarly, reported positive indications (i.e. sensitivity) towards bed bugs are 97.5 percent (Pfiester *et al.*, 2008), and towards fire ants 98 percent (Lin *et al.*, 2011), which indicates that detector dogs can be trained for use for a variety of invasive insect pests. Scent detection dogs have also been used for detection of fungal diseases of plants, such as laurel wilt disease, *Raffaelea lauricola* (Mendel *et al.*, 2018). The USDA Animal and Plant Health Inspection Service has also increased the use of canine scent detection and performed pilot studies showing the great potential of dogs in detecting pests, such as *Oryctes rhinoceros* or *Ceratitis capitata* (Rosenthal, 2017). Detection dogs are well established in customs and border control to detect restricted agricultural imports; the USDA PPQ trained 67 dogs and 91 handler for use by US plant protection and quarantine survey teams in 2016 alone (Rosenthal, 2017).

The experimental trials in settings 2 (firewood piles) and 7 (natural forest) were potentially influenced by air currents and wind. Within a firewood pile, the air circulates in different, and partially even opposed, directions. This along with the partial warming of the pile by sunshine causes different internal thermal currents, which make it very difficult for the dogs to localize the positive samples exactly. Therefore, the sensitivity and the specificity of 83.3 and 88.9 percent, respectively, in setting 2 trial are lower than, e.g. in the setting with the firewood or the wood logs on the ground. In the setting 7 trial, which was conducted in the natural forest, a lower sensitivity of 73.3 percent was achieved, which perhaps reflected the influence of wind or still air on the capacity of scent detection in a complex environment. During the first two runs, the wind speed ranged from 2 to 4 km/h and a wind speed of almost zero inside the stand with understory vegetation. Therefore, the dogs may have struggled to find the EAB scent because the scent needed much more time to distribute in the surrounding area and to build up a scent cloud around its immediate location, with the understory vegetation also perhaps additionally inhibiting the dispersal of the scent. During the third run of this setting, the wind increased slightly to 8 km/h that would have allowed a wider distribution of the scent of the EAB samples and consequently a better detection by the dogs.

Although the number of dogs used and number of test series and tested settings was limited in this initial study, the first results are very encouraging. The tested settings demonstrated that the method has potential for inspection of transported wood or

plants, and further experiments will be carried out for confirmation along with further testing in other settings. In particular, the placement of samples at different heights above ground will be tested more intensively to evaluate the potential of the method for the inspection of standing trees. Hence, we believe that surveys of EAB with detection dogs could provide one promising method to help prevent the introduction of EAB into the European Union. Dogs could be efficiently employed at import points of wood (including firewood) or plants for planting, along routes of movement, or at final destination points where they would likely increase the chance of intercepting EAB introductions. Additionally, dogs could also be used for surveys of host trees in high-risk areas and thereby complement visual inspection methods and trap surveys. An incorporation of canine scent detection into EU and national surveillance regimes could thereby further increase the chance of interception or early detection of the pest.

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Conflict of interest statement

None declared.

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