



Animal and Plant
Health Inspection
Service

Plant Protection and
Quarantine

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Dear City Forestry Manager or Arborist,

As you may be aware, emerald ash borer (EAB) was discovered in Boulder, Colorado, in the fall of 2013. Since then, the Cooperative Emerald Ash Borer Response Team has worked to delimit the infestation in Boulder, CO. We would now like to broaden the scope of our survey to ensure we understand the extent of EAB presence throughout the state. To ensure a thorough search for this pest within Colorado, we are asking for your participation in surveying your community for emerald ash borer. Colorado was the first state in the U.S. to systematically implement the branch sampling technique developed by Krista Ryall at the Canadian Forest Service. We invite you to participate using this survey technique in an effort to identify any existing populations of EAB throughout our state.

We have developed a packet of information which provides the instructions for implementing the branch sampling technique. This involves taking tree branch samples from identified ash trees, cataloging their locations, and peeling those branches in search of EAB larvae or pupae before flight season April 1st. We have developed a data collection sheet to ensure we are all reporting the same basic information throughout the communities. The Animal Plant Health Inspection Service (APHIS) has created one square mile grid maps that are available upon request for individual cities. These grids can be used to identify the trees sampled, mapping locations for a visual display of areas surveyed as well as results, throughout the state.

The Cooperative EAB Response Team would like to utilize your local knowledge and expertise in conducting the survey while serving as a collection center for the data so we can share with others who are participating and report results to the larger community. This branch sampling survey is in addition to trapping efforts which occur during the flight season. Research trials to test new trapping techniques did occur in Boulder this summer. Results will be evaluated over winter and any new trapping opportunities will be shared when available in the future. In addition, biocontrol agents are being released in Boulder this fall and are expected to continue next spring in an effort to establish parasitic wasps that will parasitize emerald ash borer, reducing their population for long term management.

We ask that you please consider participating in this voluntary survey in whatever capacity your resources allow. The Cooperative EAB Response Team would like to receive your data on the first of every month so as to compile and share among the communities to provide transparent information throughout our larger community. Please contact us with any questions and to indicate your willingness to participate.

With Appreciation,

Jeanne Ring, CDA jeanne.ring@state.co.us

Lisa Peraino, USDA APHIS lisa.j.peraino@aphis.usda.gov

Mike Winks, USDA APHIS PPQ Michael.l.winks@aphis.usda.gov

Emerald Ash Borer (EAB) Branch Sampling Survey Protocol for Colorado

Communities throughout Colorado are being asked to survey for the emerald ash borer and report your findings to the EAB Incident Command (ICS) response team. Please contact Jeanne Ring with Colorado Department of Agriculture (CDA) at (303) 869-9076 or Lisa Peraino with U.S. Department of Agriculture (USDA) at (303) 373-6644 with questions. To survey via the branch sampling method, the following steps are advised:

1. Determine a systematic method for tracking the area to be sampled. For instance, utilize one square mile grids for your community and name the grid with a unique identifier. Community maps may be acquired from the USDA Animal Plant Health Inspection Service (APHIS) office (303) 371-3355.
2. Identify 10 trees within the square mile grids to be sampled. Number the trees within each grid 1-10.
3. Take two branch samples from each tree and label A and B, along with the grid and tree numbers.
4. According to Dr. Krysta Ryall, branches should be taken from the mid-crown of open grown, urban *Fraxinus sp.* trees approximately 6-20 inches diameter at breast height (DBH). Ideally branches should come from the south side of the canopy and be between 2-3" in diameter at the base.
5. Branches should be cut at the base and samples should be approximately 30" in length.
6. Secure the branch in a vice and use a draw knife to strip the bark off in thin strips, searching for galleries, larvae, and or pupae.
7. Record all appropriate information on the "Emerald Ash Borer Branch Sampling Survey Data Sheet" and return completed data sheets to Jeanne Ring Jeanne.Ring@state.co.us or Lisa Peraino at USDA APHIS PPQ lisa.j.peraino@aphis.usda.gov by the 1st of every month.
8. Submit all suspect samples to CDA or APHIS PPQ for identification with a completed sample submission form.
9. Alternatively if a systematic grid of the entire community is not to be sampled, identify with samples with unique sample numbers and follow steps 3-8 for all ash trees sampled while doing routine pruning, removals, or tree maintenance work.
10. Specific details of the branch sampling method may be found in the Technical Note No. 111 "Detection of emerald ash borer in urban environments using branch sampling" by K.L Ryall, J.G. Fridgen, J.J. Turgeon in Frontline Forestry Research Applications which can be found at: <http://cfs.nrcan.gc.ca/pubwarehouse/pdfs/32127.pdf>
11. Branches for sampling should be collected and stripped prior to our flight season in early May or later in the fall beginning in September after summer hatch and larvae have begun to feed in the trees.

Emerald Ash Borer Branch Sampling Survey Data Sheet

(Individual Tree Data)

Location Information:

| Number (Grid + Tree #) | City | County | Latitude | Longitude |
|------------------------|------|--------|----------|-----------|
| | | | | |

Property Type: Government-Federal Government-State Government-County Government-Municipal
 Commercial Private Other: _____

Field Collection:

| Surveyor | Date | DBH of Tree | D-Shaped Holes | S-Shaped Galleries | Woodpecker Damage |
|----------|------|-------------|----------------|--------------------|-------------------|
| | | | | | |

Branch Peeling:

| Surveyor | Date |
|----------|------|
| | |

Written or Sketched Location Details (Optional)

| Branch | Branch Diameter | S-Shaped Galleries | Lifestage (Larvae/Pupae #) |
|--------|-----------------|--------------------|----------------------------|
| A | | | |
| B | | | |

*See back for instructions

Instructions

This data sheet is designed to capture all data for one sampled tree.

Location Information:

-Number (Grid + Tree #): This needs to be a unique number for the tree sampled. You can use the APHIS grid number from the APHIS generated maps and assign a tree number after the grid name (ex. E3-11). Or you can use an existing number if you already have a tree inventory.

-Latitude/Longitude- The preferred format is in decimal degrees (ex. 46.458256, -104.569635). If a GPS unit is not available to capture GPS coordinates, sketch a map or write directions with enough detail to obtain coordinates. A street address with details on tree location or a google map with the tree location plotted is also sufficient.

Field Collection:

-DBH of tree: in inches

-D-Shaped Holes/ S-Shaped Galleries /Woodpecker Damage: Yes or No

Branch Peeling:

-Branch Diameter: in inches

- S-Shaped Galleries: Yes or No and number of galleries

- Lifestage: Enter lifestage (larvae or pupae) and number collected

Emerald Ash Borer How to Submit Samples

If you suspect you have larvae or adults of *Agrilus planipennis* (emerald ash borer) submit them using the instructions below to the nearest Colorado State University-Extension office. A directory of offices can be found here <http://www.ext.colostate.edu/cedirectory/countylist.cfm>

Digital Pictures

Digital pictures can be used to document the condition of an ash tree and help determine if further investigation or a site visit is warranted. Good quality photos (non-blurry) can be emailed to the Colorado Department of Agriculture at CAPS.Program@state.co.us

Provide good quality photos of one or more of the following:

- The whole tree or trees showing symptoms (canopy dieback, epicormic shoots, bark splits)
- Adult insects resembling EAB
- Larvae found underneath the bark of an ash tree
- Serpentine galleries and or D-shaped exit holes

For pictures of larvae, adult insects, or exit-holes, use a ruler or coin to demonstrate the size of the subject.

Submitting Samples

Do not ship live specimens. Adults and or Larvae should be placed in a vial or some other leak proof container and placed in alcohol (rubbing alcohol or hand sanitizer). Specimen condition affects the accuracy and speed of identification. Specimens cannot be identified if they arrive crushed, broken or moldy. Use bubble wrap or some other material for cushioning. Submitted specimens will not be returned.

Label samples with the following information

Your Name _____
Your Phone _____
Your Email _____

Address of Collection Site

City _____ Zip _____
County _____

To be identified (number by)
__ Larvae
__ Pupae
__ Adults

Type of Property (check)

- Residential
- Agricultural
- Park
- Commercial
- Other _____

Signs and Symptoms Observed (check)

- Canopy Dieback
- D-shaped exit holes
- Serpentine Galleries
- Bark Splits
- Epicormic Sprouting
- Woodpecker activity

For Official Use Only

| Identification | Date | By |
|----------------|------|----|
| | | |

Instructions for Extension agents:

If you suspect that the larvae or adult is an emerald ash borer, *Agilus planipennis*, and you are in a non-infested county (all counties other than Boulder County), submit the specimen to the Plant Diagnostic Clinic (for Dr. Boris Kondratieff). If you submit an EAB suspect, please notify the Colorado Department of Agriculture (CDA) OR the United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA APHIS PPQ).

Contact Information:**Plant Diagnostic Clinic**

E215 Plant Sciences Bldg. 1177 Campus Delivery
Colorado State University Fort Collins, CO 80523-1177
Ph. 970-491-6950
Fax 970-491-3862
Email: plantlab@lamar.colostate.edu

Colorado Department of Agriculture

Jeanne Ring, jeanne.ring@state.co.us
Ph. 303-869-9076
305 Interlocken Pkwy
Broomfield, CO 80021

USDA APHIS PPQ

Lisa Peraino, lisa.j.peraino@aphis.usda.gov
Ph. 303-371-3355
3950 N. Lewiston St. Suite 104
Aurora, CO 8001



Frontline

Forestry Research Applications

Canadian Forest Service - Sault Ste. Marie

Technical Note No. 111

Detection of emerald ash borer in urban environments using branch sampling

K. L. Ryall, J. G. Fidgen, J.J. Turgeon

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Fig. 1), a non-native insect pest of Asian origin, presently infests large numbers of ash (*Fraxinus* spp.) trees in Ontario and Québec and could soon spread to other provinces.



Fig. 1. Adult emerald ash borer.

One of the many requirements for effective management of EAB is early detection of infestations, when densities are still low and before signs and symptoms are obvious. *Visual surveys* rely on external signs and symptoms (e.g., exit holes, larval tunnels seen through cracks in the bark, feeding by woodpeckers or squirrels) that may not be noticeable for 2 to 3 or more years after the arrival of the population, particularly if the infestation begins in the upper part of the tree. *Sticky traps* baited with an attractant have the potential to detect EAB adults in an area before signs or symptoms become visible, but may not necessarily provide information on the infestation status of individual trees.

Ryall et al. (2010) sampled many ash trees with **no** obvious sign or symptom of EAB attack (Fig. 2) and showed that *branch sampling* was an effective method of detecting EAB-infested trees; indeed, 74% of the infested trees would have been discovered if the method described below had been used. The purpose of this note is to describe this basic sampling technique.



Fig. 2. Healthy-looking ash trees with no visible sign or symptom, but determined to be infested with EAB using branch sampling.

DESCRIPTION OF THE BRANCH SAMPLING METHOD

This method is suitable for sampling open-grown ash in any landscape, but it is of particular value in urban areas with high-value ash trees (Fig. 2). Branch sampling can be performed at any time between September and May; however, because larvae continue to feed and grow in size in early fall, their galleries are easiest to see if branches are sampled after October. This technique can be performed using the following steps:

1. Select an open grown black, red, green, European or white ash, 19.7-59 feet (6-18 m) tall and 5.9-19.7 inches (15-50) cm DBH (diameter at breast height) with large open crown;
2. Identify **two** live branches in the mid-crown preferably 2-2.8 inches (5-7 cm) in diameter at the base ((minimum 1.2 inches (3 cm); maximum 3.9 inches (10 cm)) ideally from the south side of the tree. **NOTE:** Be sure to follow appropriate safety procedures and to cut branches using proper tree pruning methods.

3. Cut each branch at its base using a pole, chain or pruning saw (see Fig. 3a);
4. Measure off 29.5 inches (75 cm) from the base and cut the branch again at this point. Remove any lateral branches from this piece (Fig. 3b);
5. Secure the 29.5 inch (75 cm) piece in a vise (Fig. 3c);
6. Peel (whittle) the bark in thin strips (.04 -.08 inch (1-2 mm) thickness) from the basal 19.7 inches (50 cm) of the branch using a good quality draw- or paring-knife (Fig. 3d);
7. Examine the branch carefully, looking for EAB galleries and/or larvae. Remember that gallery length varies from about 1 inch (a few millimetres) to (Fig. 4a) approximately 8 inches (several centimetres) (Fig. 4b).

If the objective is only to detect EAB, then sampling can stop when the first gallery is found. If the objective is to assess densities, then it is important to count all EAB galleries and living larvae on the sample. Counts take 2-3 times longer to carry out than presence/absence sampling.

The branch sampling technique can be done concurrently with other tree management activities, such as pruning. Samples from ash trees could be rerouted to a centre where whittling is performed. Because ash tree material can contain live EAB, **it must not be moved outside of regulated areas established by the Canadian Food Inspection Agency (CFIA). In non-regulated areas, discovery of EAB galleries or of a live specimen must be reported to the CFIA.** Procedures for **movement and disposal** of ash wood are available at: <http://www.inspection.gc.ca/english/plaveg/pestrava/agrpla/regrestrice.shtml>.



Fig. 3. Cutting (a), measuring and trimming (b) ash branches. Branches, cut to a length of 29.5 inches (75 cm), are placed in a vise and bark is whittled off the basal 19.7 inches (50 cm) (c) (1.5 m piece shown here). Whittling removes bark in thin .04-.08 inch (1-2 mm)

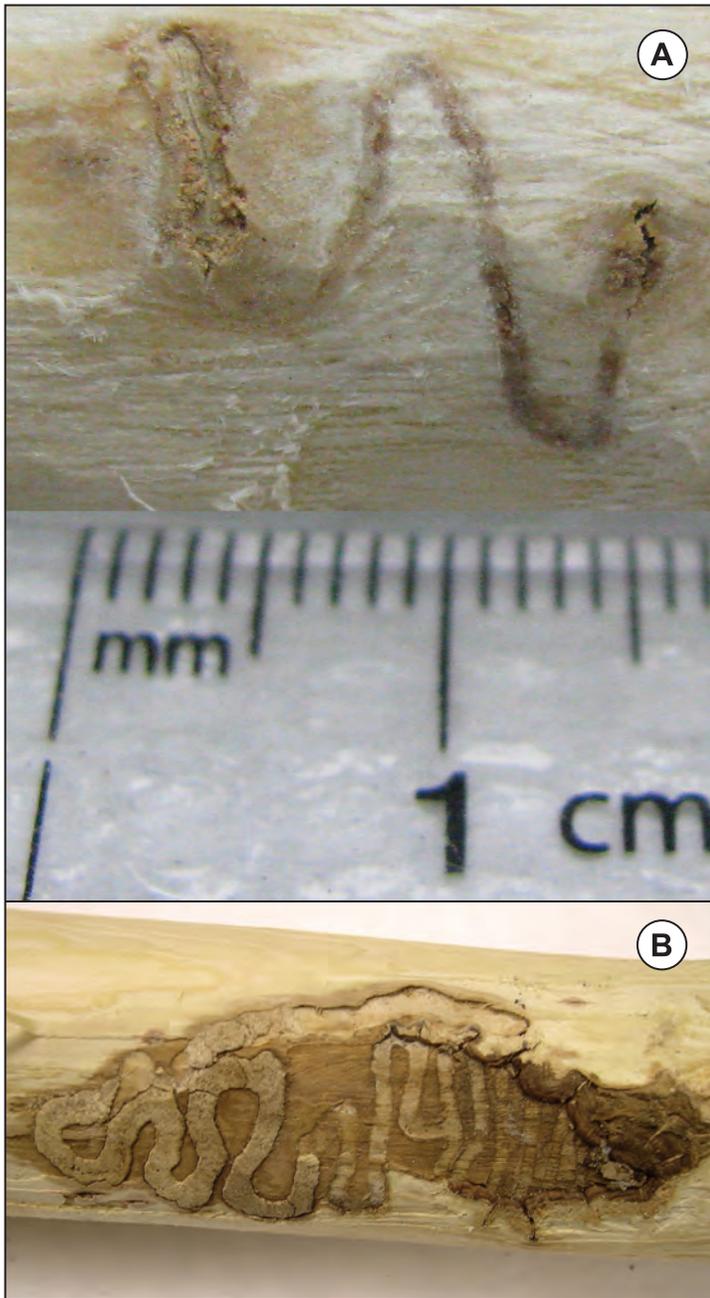


Fig. 4. Early (a) and late (b) stage serpentine galleries made by EAB larvae, found by branch sampling.

CAVEATS

This technique was developed using open-grown urban trees. Its efficacy for use in woodlots has not been tested. Similarly, sampling of much larger or smaller branches and trees than those recommended herein may result in lower detectability of EAB infestations.

CONCLUSIONS

Branch sampling is a highly effective tool for detection of incipient EAB populations, before outward signs or symptoms become apparent. Early detection of EAB populations can provide managers with additional time to identify and implement management options before unacceptable ash mortality occurs. This technique can be used for early detection of incipient EAB populations; to provide estimates of EAB density on infested trees and to delimit the extent of outbreaks. Ongoing research is developing area-wide detection and delimitation

survey protocols, is relating EAB density to severity of visual signs and symptoms, and is calibrating effectiveness of baited traps as another early detection tool.

CONTACT INFORMATION

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ADDITIONAL READING

de Groot, P.; Biggs, W.D.; Lyons, D.B.; Scarr, T.; Czerwinski, E.; Evans, H.J.; Ingram, W.; Marchant, K. 2006. A visual guide to detecting emerald ash borer damage. Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre. 16 pages.

Lyons, D.B.; Caister, C.; de Groot, P.; Hamilton, B.; Marchant, K.; Scarr, T.; Turgeon, J.J. 2007. Survey guide for detection of emerald ash borer. Natural Resources Canada – Canadian Forest Service and Canadian Food Inspection Agency, Sault Ste. Marie, Ontario, Canada.

Ryall, K.L.; Fidge, J.G.; Turgeon, J.J. 2010. Development of a sampling unit for early detection of the emerald ash borer, *Agrilus planipennis*, in individual urban trees. Unpublished.

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