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Tomodensitometry as a Tool to Detect and Study Two *Agrilus* (Coleoptera: Buprestidae) Species

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Abstract: Exotic insect species are an increasing concern with international trade. Detecting and removing any insect are thus important for any imported/exported product, including wood products. For example, wood transportation is known to be an important pathway for the introduction and dispersal of the Emerald Ash Borer, *Agrilus planipennis* (Coleoptera: Buprestidae). This Asian species is causing high mortality of ash trees in its introduced range because of the weak natural defense of trees and the virtual absence of natural enemies. For similar reasons, there are concerns in Europe that the Bronze Birch Borer, *A. anxius*, native to North America, could be introduced and cause important birch mortality. Having efficient detection methods and phytosanitary measures to prevent introducing it is thus important. In this study, we evaluated tomodensitometry—or CT-scan—as a detection method for detecting these two *Agrilus* spp. using debarking as the method of reference. Using CT-scan, we were also able to precisely measure the depth of insects in ash and birch trees in order to recommend proper phytosanitary measures for exportation and importation of wood products. Both techniques efficiently detect the presence of insects in ash, paper birch, and yellow birch. However, the number of *A. anxius* detected depended on both the technique and the diameter of the sample. The depth of insects depended on tree species, sample diameter, and life-stage. Globally, *A. planipennis* are deeper in ash trees than *A. anxius* in birch trees, and prepupae are deeper than larvae. The maximal depth in the sapwood (excluding bark thickness) for ash, paper birch, and yellow birch was 21.9 mm, 6.30 mm, and 3.22 mm, respectively. While CT-scan is more expensive and requires access to expensive equipment, debarking is more time-consuming, especially if the number of insects needs to be determined.

Keywords: woodborer; detection tool; larval galleries; phytosanitary measures; CT-scan

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1. Introduction

Insect- and disease-free wood is mandatory for exportation and importation purposes, but it is also important when moving wood products between infested and non-infested regions within a country. Many wood-boring insects can be transported in wood products and be accidentally introduced into new habitats, with the potential of becoming invasive. The Emerald Ash Borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a good example of a wood-boring insect that became invasive in Europe and North America and, once established, continued to expand its distribution, among other things because of wood transportation [1,2].

The Bronze Birch Borer (BBB), *A. anxius* Gory, is a wood-boring species, native to North America, that attacks all birch species (*Betula* spp., Betulaceae), native and exotic [3], although native species are more resistant than exotic ones [4]. This common species kills birch trees, mainly after stress events such as droughts or defoliation. As it attacks weakened trees, it acts as a secondary insect. However, if it was to invade a new country, it could become invasive and cause damage to birch trees, just like EAB is a problem in its

invaded range but not in its native range [3]. It is thus a growing concern in Europe that this North American species could be introduced [5].

In their habitats, wood-boring insects can be detected using traps, usually Lindgren or prism traps, of different colors depending on the species targeted and using different baits that are more or less specific. Sex pheromones and kairomones (odors emitted by the stressed host trees) are usually effective, although girdling has also been shown to be very effective in increasing trap captures [6]. However, such traps do not indicate the presence of developing insects underneath the bark. Debarking is a standard method used to detect EAB immatures [7]. By removing thin layers of bark, any galleries or insects can be exposed, allowing us to count and collect them for identification. While this method is effective, it can also be time-consuming and is destructive. Other detection methods, either for living trees or wood products, also include canine detection, electronic noses, or visual inspection for signs and symptoms (reviewed in [8]). Tomodensitometry, or CT-scan, could also be used to detect wood-boring insects. It is a non-destructive and non-invasive imagery technique classically used for medical purposes. Nonetheless, it can be used in different research fields, including oceanography [9,10], paleontology [11], and even museum conservation [12]. CT-scan has been used in forest entomology to study the whitespotted sawyer, *Monochamus scutellatus* (Say) (Coleoptera: Cerambycidae), damage progression in burned logs after a wildfire [13].

Without clear and efficient phytosanitary measures allowing us to certify that wood products are free of insects, there is a potential risk when exporting wood products, and this can have important impacts on the access to international markets for the forest industry. These phytosanitary measures include different processes that can be mechanical, chemical, or thermal, among others [14]. One of the mechanical processes that can be applied to remove woodborer insects, such as buprestids, that develop in the phloem is debarking; by removing bark, it mechanically removes developing insects and thus prevents transportation of living insects [15]. To be effective though, all of the bark has to be removed, and sometimes slightly more depending on woodborer biology. It is important to remove enough bark and phloem to remove all insects or at least injure them sufficiently to cause their death, but without removing unnecessary material that would reduce the log's commercial value.

The objectives of this study are to: (1) evaluate tomodensitometry as a detection method, using debarking as a reference; and (2) precisely measure the gallery depth for both EAB and BBB using tomodensitometry to determine the debarking thickness needed to eliminate the risk of transporting live insects.

2. Materials and Methods

2.1. Tree Sampling

2.1.1. Ash Tree

After the discovery of EAB in Quebec City, Canada in 2017, highly infested trees were logged. We collected 134 samples from a total of 22 ash trees in Quebec City (46.820634, −71.232010) in 2017 and 2018, and 9 ash trees from the city of Lévis (in Saint-Romuald 46.743214, −71.223587 and Pintendre 46.76854, −71.138332) on the south shore of Quebec City in 2018. Trees were either white ash (*Fraxinus americana* L.; Oleaceae) or green ash (*F. pennsylvanica* Marsh.). When possible, one or two 1 m sections centered on breast height were taken from the trunk, and up to six 1 m branches (4–10 cm diameter) were sampled according to the method of Ryall et al. [7] from the low, middle, and high canopy depending on the size of the tree. Diameter was measured for all samples using a diameter tape. For larger trees, the trunk sections were squared and reduced into four slabs to facilitate handling and transportation. Samples were transported to the Laurentian Forestry Center in Quebec City in accordance with all the restrictions in force issued by the Canadian Food Inspection Agency (CFIA), including transportation permits when needed. Samples were stored in a log depot, where they were protected from wind and rain but at outdoor air temperature until they were processed.

2.1.2. Birch

Birch trees were selected based on signs of decline, such as dead branches and a thinning crown, in order to increase the probability of finding BBB. A total of 59 samples (branches and logs, ranging from 30 to 180 cm in length) from paper birch (*Betula papyrifera* Marsh.; Betulaceae) and 191 samples (branches and logs, ranging from 45 to 195 cm in length) from yellow birch (*B. alleghaniensis* Britton) were taken in the following sites: Trois-Rivières (46.406077, −72.660097), Valcartier Forestry Research Station of the Canadian Forest Service (46.95218, −71.49720), Canadian Forces Base Valcartier (National Defense), and Seigneurie de Beaupré (47.26034, −70.88442). Birch samples were transported to the Laurentian Forestry Center in Quebec City where they were stored in a log depot, where they were protected from wind and rain but with outdoor temperatures.

2.2. Detection Methods

2.2.1. CT-Scan

All samples were scanned using CT-scan with a radiation energy of 140 kV and 40 mA at the National Institute of Scientific Research laboratory in Quebec City using a Siemens Somatom Volume Access scanner. A transportation permit was obtained from the Canadian Food Inspection Agency when needed for EAB-infested ash samples in 2017. The gantry of the scanner is a round tube with X-ray emissions on one side, while the opposite side has 32 arrays of 600 detectors. The detectors turn around the sample and perceive its variable permeability to X-rays at each 5-degree angle around the sample, thus producing a series of 2D transversal images. The tomographic intensity of each corresponding voxel (volumetric pixel) of an image is then represented on a gray scale of 4096 values with 1 value per pixel. About 1700 sequential transversal sections with a 0.4 × 0.4 mm pixel resolution were obtained for a 100-cm-long sample on a 512 × 512 pixels matrix. Compared with other image formats, the DICOM format, standard for various medical imagery devices, is more precise, has a wider range of gray tones, and integrates several types of data, such as the length of a pixel. The duration for scanning samples was about an hour for ten 1 m samples.

Individual images obtained from the CT-scan were analyzed using the Fiji software [16]. All images from the sequences were observed one by one in a cross-sectional view until a larva or a J-shaped prepupa was detected (Figure 1). When needed, longitudinal views were then analyzed to differentiate between larvae and prepupae. For each larva and pupa found, the gallery depth was measured using the "measure" function by drawing a straight line from the outer edge of the bark to the maximum depth of the gallery or pupal chamber; the gallery depth thus includes the bark. Similarly, the bark thickness was also measured from its outer edge to the beginning of the sapwood. All measures were done three times and a mean was calculated.

2.2.2. Debarking

After CT-scan, all samples (branches, trunk sections, or slabs) were debarked to count the number of larvae and prepupae. Samples were placed in a portable clamping system, and bark was slowly and progressively removed in thin chips of 1–2 mm using a plane in order to expose the sapwood and any galleries or insects developing. Once the sample was completely debarked, the numbers of larvae and prepupae were counted. The time required to debark each sample was noted. Although we tried to debark the samples shortly after scanning, it was performed on average 15 d later, depending on the staff availability, and in the worst case several weeks. However, all processing was done during fall or winter, i.e., when little to no insect development could occur.

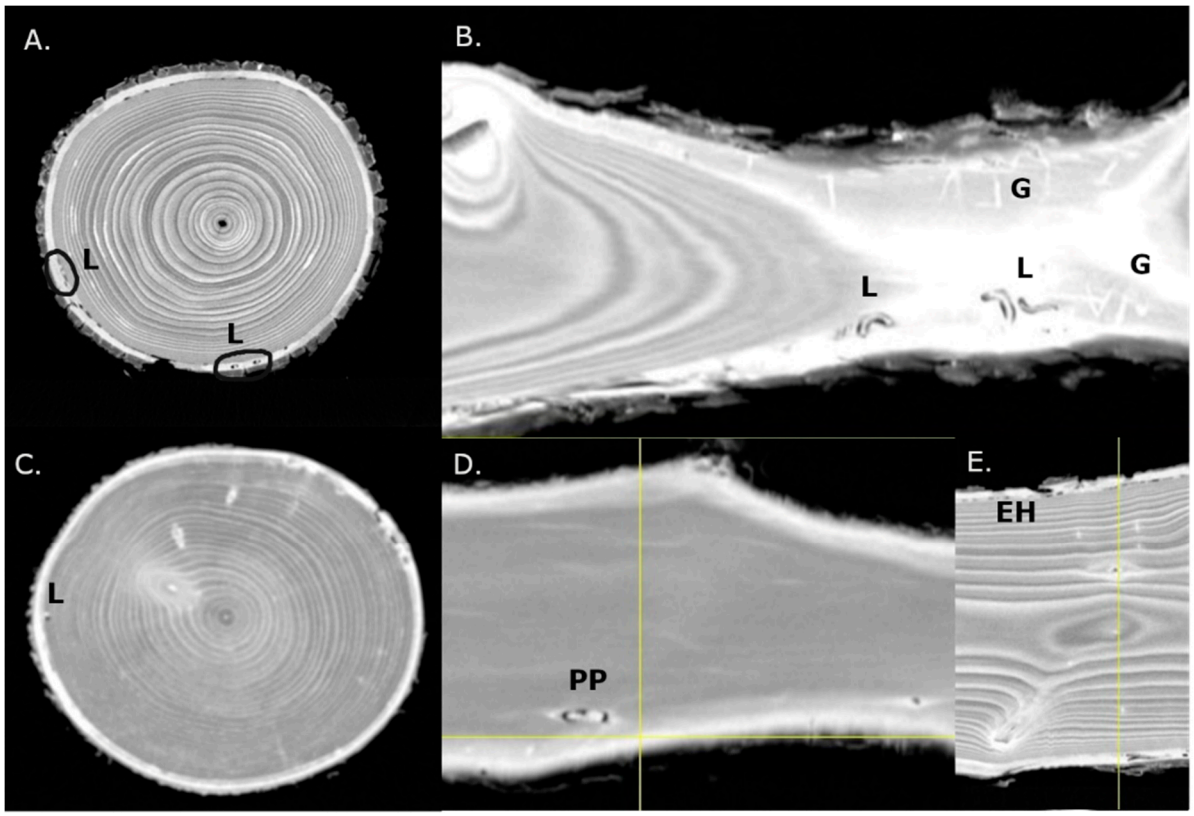

Figure 1. Images obtained from CT-scan showing the developing larvae (L) and prepupae (PP), larval galleries (G), and emergence holes (EH) in (**A**) a transversal section and (**B**) a longitudinal section of an ash sample and in (**C**) a transversal section and (**D**,**E**) longitudinal sections of a white birch sample.

2.3. Statistical Analyses

All statistical analyses were done using the R Software [17]. The ability of CT-scan to detect the presence or absence of a developing *Agrilus* was first analyzed. Yellow birch was excluded because of the low number of samples containing developing insects (i.e., 3 logs). All sites where no insects were detected in any of the samples were also excluded for ash and paper birch, as the model could not converge with such an excess of zeros. The model was thus developed with 87 samples of ash trees belonging to 19 trees and 53 samples of paper birch belonging to 6 trees. The qualitative detection (presence or absence) of developing *Agrilus* was tested using a generalized linear mixed model with a binomial distribution with the detection method (CT-scan vs. debarking), the tree species, the sample diameter, and all two-term interactions as fixed effects and the sample number as a random effect to account for the fact that each method was used on the same samples (*glmer* function). For samples with insects detected by at least one of the methods (130 ash and 36 paper birch samples), the ability of both methods to quantify the number of immatures in the samples was analyzed. The number of insects detected was tested using a generalized linear mixed model with a Poisson distribution with the detection method, the sample diameter, and their interactions as fixed effects and the sample number as a random effect to account for the fact that each method was used on the same samples (*glmer* function). Because the number of insects is highly different for each *Agrilus* species, EAB being invasive and BBB being native, one model was made for each tree species, excluding yellow birch because of the very few data available. The duration needed to debark the samples was analyzed using a general linear model with a negative binomial distribution, with tree species, sample diameter, number of insects found, and all two-term interactions as fixed factors. The likelihood ratio was calculated using the 'anova' function.

The depth of the galleries was compared separately for each insect species, as the goal was to determine the phloem thickness that needs to be removed in each tree in a context of phytosanitary measures, and not with the objective of comparing the two insect species. A linear mixed model was made with a Gaussian distribution with the insect stage (larval or prepupal), the sample diameter, and their interaction as fixed effects and the tree number as a random effect. In the case of *A. anxius*, once again only the data from paper birch were used because of the few data available in yellow birch (7 immature insects). The likelihood ratio was calculated using the 'anova' function (package lmertest).

3. Results

3.1. CT-Scan as a Detection Method

The probability to detect insects was the same with CT-scan and debarking, independently of the diameter of the sample. Tree species was the only factor having a significant effect on the probability to detect immatures, with a higher probability to find insects in ash trees (Table 1).

Table 1. Statistical values from the generalized linear mixed model (binomial distribution) for the presence or absence of immatures of *Agrilus* spp. detected in ash and paper birch with CT-scan and debarking. *p* values considered significant (<0.05) are shown in bold.

Factors	z	DF	*p*
Method	0.009	1	0.993
Tree species	4.644	2	**<0.0001**
Diameter	0.202	1	0.840
Method: Tree species	−1.274	1	0.203
Method: Diameter	−0.011	1	0.991
Tree species: Diameter	−0.124	1	0.901

Diameter significantly affected the number of insects detected in ash samples, but neither the detection method nor the interaction between both factors had an effect (Table 2); more insects were detected in samples of larger diameters. For *A. anxius* in paper birch, the detection method, diameter, and their interactions had a significant effect on the number of insects detected (Table 2); CT-scan detected more insects than debarking in small diameters, while it detected fewer in large diameters.

Table 2. Statistical values from the generalized linear mixed models (Poisson distribution) for the number of *Agrilus* immatures detected with CT-scan and debarking. *p* values considered significant (<0.05) are shown in bold.

Species and Factors	z	DF	*p*
A. planipennis			
Method	0.101	1	0.91933
Diameter	3.084	1	**0.00204**
Method: Diameter	−1.296	1	0.19491
A. anxius in paper birch			
Method	2.758	1	**0.00582**
Diameter	2.258	1	**0.02398**
Method: Diameter	−2.855	1	**0.00430**

While the duration for scanning the samples is simply dependent on their length (about an hour for ten 1 m samples), all interactions significantly affected the duration of debarking (Table 3, Figure 2); larger logs and ash samples took longer to debark, and this increase was higher when more insects were present.

Table 3. Statistical values from the general linear model (negative binomial distribution) for the duration required to debark samples. *p* values considered significant (<0.05) are shown in bold.

Factors	χ^2	DF	*p*
Tree species	900.83	2	**<0.0001**
Diameter	574.12	1	**<0.0001**
Number of insects	456.57	1	**<0.0001**
Tree species: Diameter	434.15	2	**<0.0001**
Tree species: Number of insects	409.49	2	**<0.0001**
Diameter: Number of insects	372.72	1	**<0.0001**

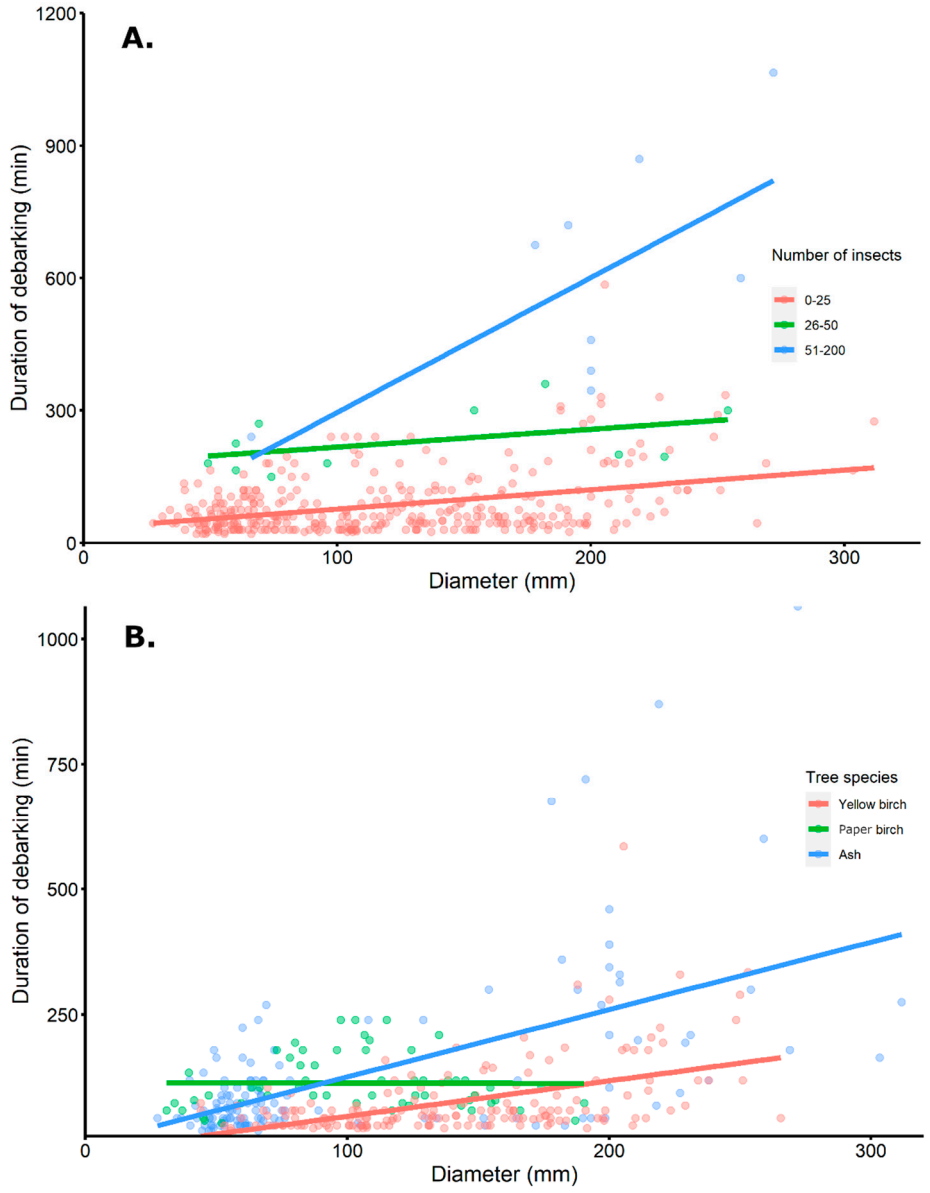

Figure 2. Duration (mean ± SD) of debarking for each *Agrilus* species depending on the diameter of the sample, the number of insects found (**A**), and the tree species (**B**).

3.2. Gallery Depth

For *A. planipennis*, lifestage, diameter, and their interaction significantly affected the gallery depth (Tables 4 and 5); the galleries were deeper for prepupae, and it increased with an increase in the diameter of the sample, but this increase was faster for larvae (Figure 3).

A total of 27.4% of the insects were found in the bark: 26.9% of the larvae and 29.3% of the prepupae. For insects found in the wood, the maximal depth was 21.9 mm in the sapwood, excluding bark. Raw data on gallery depths in the sapwood, i.e., excluding bark, can be found in Figure S1A.

Table 4. Statistical values from the models for the gallery depths of *A. planipennis* and *A. anxius*. *p* values considered significant (<0.05) are shown in bold.

Species	F	DF	*p*
A. planipennis in ash			
Lifestage	37.698	1	**<0.0001**
Diameter	321.867	1	**<0.0001**
Stage: Diameter	11.800	1	**0.0006218**
A. anxius in paper birch			
Lifestage	4.1388	1	**0.04387**
Diameter	5.7028	1	**0.01832**
Stage: Diameter	2.3841	1	0.12492

Table 5. Mean gallery depths (including bark) and bark thickness of *A. planipennis* and *A. anxius* immatures measured using CT-scan imagery.

Species	Bark Thickness (mm; Mean ± SD)	Larval Gallery Depth (mm)			Pupal Chamber Depth (mm)		
		Min	Max	Mean ± SD (*n*)	Min	Max	Mean ± SD (*n*)
A. planipennis in ash	9.21 ± 3.71	3.93	22.10	10.60 ± 4.15 (1067)	5.33	28.64	12.64 ± 4.43 (270)
A. anxius in paper birch	4.18 ± 1.02	4.12	11.01	6.60 ± 1.38 (12)	4.12	11.01	7.43 ± 1.17 (127)
A. anxius in yellow birch	8.85 ± 1.23	na	na	10.72 (1)	5.40	14.16	9.67 ± 3.67 (7)

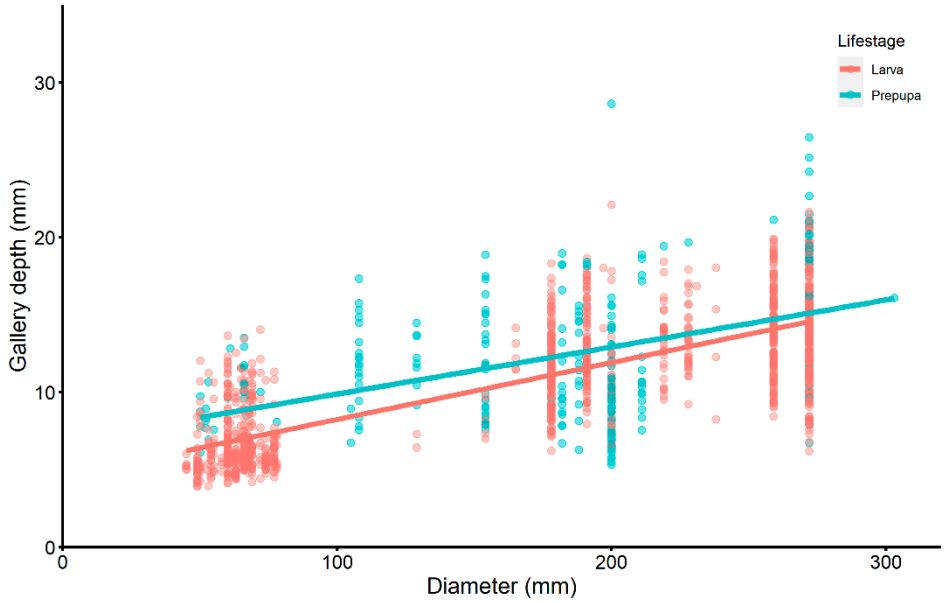

Figure 3. Gallery depths (mean ± SD) of immature *Agrilus planipennis* depending on the diameter of the sample and the lifestage.

For *A. anxius*, tree species and the interaction between tree species and diameter were significant (Table 4). For the model with *A. anxius* in paper birch, there was a significant impact of both lifestage and diameter, but not their interaction (Table 4); prepupae were deeper in the wood than larvae, and insects were deeper in samples of larger diameter. While all insects found in paper birch were found in the sapwood, in yellow birch 37.5% of the insects were found in the bark, all of them prepupae, representing three individuals.

For insects found in the wood, the maximal depth was 6.30 mm and 3.22 mm in the sapwood for paper birch and yellow birch, respectively. Raw data on gallery depths in the sapwood, i.e., excluding bark, can be found in Figure S1B,C.

4. Discussion

Tomodensitometry allowed for the detection of immature *Agrilus* spp. in ash and birch. Both methods had a similar ability to detect the presence of developing insects in both ash and birch, and they detected similar numbers of insects in yellow birch and ash samples, but not in paper birch. In paper birch, the number of insects detected with each method depended on the diameter of the sample; while CT-scan detected more insects in small-diameter samples, the opposite was true for large-diameter samples, although the difference was smaller for larger samples. Although this could be inherent to the method, the delays between scanning and debarking were sometimes long because of limited staff availability. This caused the samples to dry and made debarking more difficult. In addition, the smaller larvae found by debarking in paper birch were often dried and dead, making them more difficult to detect. This drying effect was more important in smaller samples than in larger ones, which could partly explain the difference between these two methods in small samples. Paper birch samples were also the most decayed ones, with other xylophagous species, such as Scolytinae or Cerambycidae, often found, and some of these larvae could have been mistaken for *A. anxius* larvae on CT-scan images. Some of the galleries or pupal chambers in six paper birch samples were found to contain predatory larvae from the Cleridae family instead of *Agrilus* spp. larvae when debarking. It is thus possible that in the delay between scanning and debarking, some of the *Agrilus* immatures could have been preyed upon, thus changing the final count of insects. Finally, on CT-scan images, if the wood was decayed and rotten, detection of insects was almost impossible because of the lack of contrast on the resulting image, while debarking for these samples allowed us to find insects.

The duration and costs of both methods are also very different. Scanning wood logs is very quick compared with debarking samples. However, it requires access to specialized and expensive equipment that may need to be rented at an hourly rate, making it more expensive than debarking. Debarking takes longer when numerous insects are present if precise counts are needed, as the galleries must be carefully followed in order to not miss any insects or section them. The duration is also longer with larger diameters because the area to remove is larger. In our case, debarking took longer for large diameters of ash samples compared with birch samples, probably because the number of insects was much higher in ash than birch samples. In addition, even though scanning is much faster, the images still need to be examined in order to detect insects. For both methods, the time necessary to process logs also depended on the data requirement; counting all insects took much longer than determining the presence of insects.

The precise depth of insects, either larvae in galleries or prepupae in pupal chambers, could be measured using the tomodensitometry images for each tree species. For *A. planipennis*, the depth increases with the sample diameter, meaning that insects would be deeper in large trees or in logs than in branches. Considering that the bark is thicker in large trees, and that our measures include the bark, this finding is not surprising. This increase in depth with diameter was faster for larvae than for prepupae. In paper birch, *A. anxius* prepupae were found deeper than larvae, and they both were deeper in larger samples. It could be expected that the pupal chamber would go deeper, especially considering that the larvae collected could be from different stages and that larvae in the first larval stage hatch from eggs that are laid on the bark and eat their way into the wood. Smaller larvae are thus closer to the inner bark than larger larvae. In addition, although the prepupae are positioned to be ready to emerge out of the tree once they become adults, the bottom of the pupal chamber is still deeper than larval galleries. Although it was not specifically tested, we can see from the mean gallery depths that *A. planipennis* is deeper in the tree than *A. anxius*, and that the latter is deeper in yellow birch than in white birch, although

more insects were found in the yellow birch bark compared with paper birch bark. These results are consistent with the bark thickness for each of these tree species, which is thicker in ash, followed by yellow birch and then paper birch. However, as it was very difficult to find *A. anxius* in yellow birch, these measures come from only one larva and seven prepupae, and may thus be considered preliminary. The obtained average measure for the depth of *A. planipennis* pupal chambers in this study was higher than in a previous study (12.64 mm vs. 10.67 mm in [18]). However, while our measures include bark, the authors of that study did not specify how the depth was measured (e.g., the top, middle, or bottom of the pupal chamber, including bark or not), and they conducted their study in China on velvet ash (*F. velutina* Torr.) samples with a diameter of 5–10 cm. That could thus explain our higher depth measures and wider variation in measures as our diameter range was from 4 to 30 cm. Our measure of pupal chamber depth in birch is smaller than the depth reported in the literature (about half an inch, i.e., 12.7 mm), although it is not clear how this was obtained, on which tree species, and for which diameter [19].

The number of *Agrilus* detected increased with larger diameters. This can be explained by the fact that large samples can support the development of more insects than smaller ones, and it has been shown that the distribution of *A. anxius* within a tree is correlated with the available surface area, thus translating into more insects being present in the lower part (and larger part) of the tree [20]. In addition, the female oviposition preference for bark thickness or roughness will also likely influence the abundance of insects, but this was not tested in this study [21,22].

Although finding *A. planipennis* is unfortunately easy in heavily infested areas in North America, it is more challenging to find *A. anxius*, the native species. Finding stressed paper birch was easier than finding stressed yellow birch, and the resulting sample size for *A. anxius* in paper birch is thus higher. When stressed yellow birch was found, there were very few *A. anxius* immatures—only three samples containing a total of only eight immatures. In both birch species, many galleries found simply aborted; they seemed to disappear, as we did not find any insects, pupal chambers, or adult emergence holes, suggesting that the immatures were unable to develop as they faced tree defenses. Such an absence of insects in aborted galleries could also be caused by natural enemies, especially predators such as Coleoptera from the Cleridae family. This suggests that these two native birch trees, but especially yellow birch, are able to better defend themselves against this native insect species and that natural enemies are able to localize and attack them. This result also converges with a study showing that *A. anxius* causes more mortality in exotic than native birch species [4]. Finally, it also suggests that transportation of *A. anxius* in yellow birch is less likely than in paper birch given its very low occurrence.

5. Conclusions

In conclusion, CT-scan is as effective as debarking to find immature *Agrilus* spp. The quantification slightly differs depending on log diameter, but both methods were as efficient in identifying samples in which the insect was present, which is what is needed in an import/export context. CT-scan was identified as "one of the most useful non-destructive techniques providing qualitative and quantitative results" for hidden-lifestyle arthropods [23]. However, it requires access to expensive and fragile equipment and some training in order to assign the insect to the species, if needed. It should be noted that Artificial Intelligence could be used to develop algorithms recognizing a few target species (under development), thus speeding-up insect detection using the CT-scan method.

Although the use of CT-scan to detect insects might not be currently entirely compatible in a wood industry environment, for example because of the acquisition and processing speed that slows down log processing, there have been developments towards a CT-scan method adapted to wood industry environments (reviewed by [24]). CT-scan is a promising tool that can increase a log's value by providing detailed information on its quality prior to sawing [25]. For example, CT-scan coupled with algorithms has been shown to effectively detect knots [26]; a similar automatic insect detection algorithm could be developed and

tested to speed up this technique and support both sawmills and researchers. It is likely that it will become more important in the future as the technology develops and the added value for the industry increases, although expensive equipment and more research and development will be needed. The present study will help adjust the recommendation on the bark and phloem removal for each tree species in order to eliminate the presence of any developing insects. These results show that debarking is not sufficient as most insects are in the sapwood. However, considering the high variability in gallery depths, to remove all living insects, the maximum depth at which insects were found in the sapwood should be removed, in addition to the bark, from logs of any size, i.e., 21.9 mm, 6.30 mm, and 3.22 mm in ash, paper birch, and yellow birch, respectively. However, more data would be needed on yellow birch and proper tests with a system approach (e.g., [27]) would be relevant.

Supplementary Materials: The following is available online at https://www.mdpi.com/article/10.3390/f13071092/s1, Figure S1: Gallery depth in the sapwood (excluding bark) for (A) *A. planipennis* in ash and *A. anxius* in (B) paper birch and (C) yellow birch.

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