# RELATIONSHIP OF WINTER STARCH LEVELS IN YOUNG ASH TREES AND ATTACK BY THE EMERALD ASH BORER (COLEOPTERA: BUPRESTIDAE)

Ryan E. Morgan[1]*, Peter de Groot[1,2] and Sandy M. Smith[1]

## ABSTRACT

The emerald ash borer, *Agrilus planipennis* (Fairmaire) (Coleoptera: Buprestidae), is native to northeastern Asia. Since its discovery in North America in 2002, the beetle has killed more than 40 million ash trees (*Fraxinus* spp.) and caused serious environmental and economic damage. Understanding factors that may lead to increased tree susceptibility to *A. planipennis* would help to focus detection surveys on higher risk areas and assist in mitigation measures. Winter starch levels in the roots of deciduous tree species have been shown to be a good predictor of a tree's susceptibility to native *Agrilus*, and thus we hypothesized that trees with low starch levels would be associated with larger numbers of *A. planipennis* than those with high reserve levels. We compared winter 2003-04 starch levels with summer 2004 capture rates of *A. planipennis* on 200 ash trees in four plantations [two green ash (*F. pennsylvanica*) and two white ash (*F. americana*)]. Tree stress, as measured by root starch levels, was not significantly correlated with densities of *A. planipennis* adults caught on sticky traps on either previously colonized or uncolonized trees. However, significantly more *A. planipennis* adults were collected on previously colonized trees versus trees that had not yet been attacked.

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The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is native to northeastern Asia, and was first discovered in Canada and the United States in 2002 (Haack et al. 2002, Cappaert et al. 2005). Since its discovery, the beetle has become a very significant pest killing more than 40 million ash (*Fraxinus* spp.) in southwestern Michigan alone, and tens of millions more in Ohio, Illinois, Indiana, Pennsylvania, West Virginia, Missouri, Wisconsin, Virginia, and Ontario and Quebec, Canada (Canadian Food Inspection Agency 2007, Poland 2007, United States Department of Agriculture - Animal and Plant Health Inspection Service 2007, EAB Info 2008). In addition to tree mortality, there will be serious environmental and economic impacts (Cappaert et al. 2005) as ash disappears from the landscape. At least 16 endemic species of ash are threatened in North America with the potential loss of tens of billions of dollars to urban forests alone in the United States (Federal Register, United States 2003). Current estimates indicate that the beetle has been in North America for at least 10 years before its discovery in 2002 (Cappaert et al. 2005, Poland and McCullough 2006). This insect is difficult to detect (Poland and McCullough 2006, de Groot et al. 2006) and understanding the factors that may lead to increased susceptibility of trees to *A. planipennis* would help focus detection surveys to higher risk areas and assist mitigation measures.

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[1]Faculty of Forestry, University of Toronto, 33 Willcocks Street, Toronto, ON, Canada, M5S 3B3. Phone: 416-978-5482. Fax: 416-978-3834. (e-mail: ryan.morgan@utoronto.ca).
[2]Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, 1219 Queen St. E., Sault Ste. Marie, ON, Canada, P6A 2E5, and Faculty of Forestry, University of Toronto, 33 Willcocks Street, Toronto, ON, Canada, M5S 3B3. Phone: 705-541-5640. Fax: 705-541-5700. (e-mail: pdegroot@NRCan.gc.ca).
*Corresponding author.

Tree vitality (*sensu* Shigo 2002) is a significant factor in the determination of a tree's susceptibility to insects and can be an important predictor of a tree's tolerance to stress. Studies on a native North American buprestid, the twolined chestnut borer, *Agrilus bilineatus* (Weber), have demonstrated that this beetle preferentially attacks and kills stressed oak trees (Haack and Benjamin 1982; Dunn et al. 1986, 1987, 1990a, b; Dunn and Potter 1990). In addition, outbreaks of the twolined chestnut borer are most often found in forested areas with histories of drought, defoliation, and natural or human-assisted disturbances (Haack and Benjamin 1982). Another native buprestid, the bronze birch borer, *Agrilus anxius* Gory, also shows a preference for stressed or low vitality birch trees (Loerch and Cameron 1984), and exotic hosts, such as European white birch, *Betula pendula* Roth (Miller et al. 1991) which adds support to the notion that the beetle prefers stressed host trees. It is possible that *A. planipennis* also prefers stressed trees as studies have shown that tree stress induced by the removal of the phloem and a portion of the outer xylem of ash trees increases attraction of the beetle (Poland et al. 2005, McCullough et al. 2006).

The level of starch stored during the winter in the roots of deciduous trees reflects the net photosynthetic capacity of the tree in previous growing seasons, and is a useful measure of tree vitality (Wargo 1975, 1978). Wargo (1975) developed a simple staining technique for estimating the level of stored starch in the roots of deciduous trees. This technique has been used to evaluate the role of sugar maple vitality on the fecundity of pear thrips (Carey et al. 1992) and to examine the effect of tree vitality on the susceptibility of oak trees to attack by *A. bilineatus* (Haack and Benjamin 1982, Dunn et al. 1987). Higher numbers of *A. bilineatus* captures and attacks were found on oak trees low in root starch levels compared to trees with higher levels of stored starch (Dunn et al. 1987).

In this study, we examined the relationship between winter starch levels of ash trees and attack rates the following year by *A. planipennis*. We hypothesized that ash trees with low starch levels would have a higher number of *A. planipennis* attracted to them.

## MATERIALS AND METHODS

The study was conducted in four ash plantations within 5-18 km of each other in Essex County, Ontario, Canada. Two plantations contained white ash, *Fraxinus americana* L. (Oleaceae) and two had green ash, *F. pennsylvanica* Marsh. Young plantations were used to ensure the accuracy of detecting attacks and colonization by *A. planipennis* because mature trees often have attacks high up in the crown that remain undetected until the tree shows signs of decline or until the tree has been cut down (Poland and McCullough 2006). In addition, young trees were ideal because the bark is smoother than that of mature trees, which made it easier to detect exit holes. The plantations were situated on poorly drained and predominately clay soils, which is common in the area of the infestation in Essex County. An examination of the annual rings of the callus tissue around *A. planipennis* exit holes suggested that the beetle had been present in the plantations for 1-2 years before 2003 (personal observations, PdG). All plantations were less than 20 years old, and trees ranged from 6.1 - 9.5 m tall with a 3.5-13.3 cm diameter at breast height (DBH). Additional characteristics of the plantations are summarized in Table 1.

During September and October 2003, 50 ash trees were selected throughout each plantation. Within each plantation, a randomly located starting point was selected at a minimum distance of 10 m from any edge and from this starting point, a 150 m U-shaped transect (50 m long on each side) was positioned. The nearest ash tree with no sign of borer colonization (e.g., exit holes) was selected at 3-m intervals along each transect. Ash trees that had been attacked and colonized by *A. planipennis* were identified by the presence of cracks in the bark,

Table 1. Summary statistics of site and tree characteristics, starch levels, and captures of *Agrilus planipennis* in four ash plantations surveyed for root starch levels in Essex County, Ontario, Canada.

| | Plantation[1] | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Species of ash | Green | White | Green | White |
| Year trees were planted | 1991 | 1985 | 1993 | 1988 |
| No. of ash trees planted | 70,000 | 7,000 | 8,000 | 8,200 |
| Area (ha) | 30.0 | 2.8 | 3.5 | 3.5 |
| Mean diameter at breast height (cm) ± stdev.[2] | 5.70 ± 1.01 | 7.34 ± 1.65 | 9.00 ± 1.87 | 6.82 ± 1.79 |
| Mean height of ash (m) ± stdev. | 6.15 ± 0.91 | 7.72 ± 1.13 | 9.47 ± 1.51 | 7.49 ± 1.48 |
| Mean crown length (m) ± stdev. | 3.69 ± 0.76 | 4.32 ± 0.97 | 4.29 ± 0.84 | 4.16 ± 0.77 |
| Mean crown width (m) ± stdev. | 2.13 ± 0.43 | 1.96 ± 0.42 | 2.66 ± 0.48 | 2.60 ± 0.6 |
| Starch Levels (n=50)[3] | | | | |
| No. of trees rated as High (15-30%) | 12 | 15 | 14 | 17 |
| Mean no. of *A. planipennis* trapped /$m^2$ (high)[4] | 110.6 ± 16.7 | 107.3 ± 16.9 | 68.1 ± 14.5 | 10.5 ± 3.4 |
| No. of trees rated as Medium (7-12%) | 29 | 29 | 27 | 30 |
| Mean no. of *A. planipennis* trapped /$m^2$ (med.) | 172.6 ± 31.8 | 81.8 ± 10.8 | 44.8 ± 10.5 | 12.1 ± 2.89 |
| No. of trees rated as Low (3-6%) | 9 | 6 | 9 | 3 |
| Mean no. of *A. planipennis* trapped /$m^2$ (low) | 122.5 ± 27.0 | 38.4 ± 12.4 | 50.4 ± 20.4 | 5.75 ± 5.75 |
| Total no. of *A. planipennis* trapped | 603 | 443 | 354 | 54 |
| Mean[5] no. of *A. planipennis* trapped /$m^2$ | 149 a ± 19.7 | 84 b ± 8.6 | 52 bc ± 7.85 | 11 c ± 2.09 |
| Trees previously colonized by *A. planipennis* out of the 50 study trees per plantation | 40 | 6 | 19 | 4 |
| Main *A. planipennis* activity period in 2004 | June 9-15 | June 23-29 | June 30-July 6 | June 23-29 |

[1] Means were calculated using 50 trees per plantation, with the exception of means calculated for starch levels.
[2] stdev. = Standard Deviation.
[3] Root tissues used for rating starch levels were extracted from Dec. 2003 - Jan. 2004
[4] Means for the number of *A. planipennis* trapped /$m^2$ are presented with standard errors (± SE) for each starch rating. *Agrilus planipennis* were trapped from May 24 - Aug. 20, 2004
[5] Means ±SE within a row followed by the same letter are not significantly different at $\alpha = 0.05$ (Tukey's test).

exposed portions of larval galleries or 'D-shaped' holes made by exiting adults (de Groot et al. 2006). During the following spring and summer (May-September 2004), trees were re-examined (second sample) and attack by *A. planipennis* in the previous year was recorded again.

During December 2003 and January 2004, we exposed one primary root of each of these 200 trees by excavating distally from the root-collar to approximately 1 m from the stem. Samples of root wood (4 × 3 × 2 cm deep) were collected using a hammer and chisel and kept frozen until returned to the laboratory. We used the histochemical techniques of Wargo (1975) to categorize the starch content in the roots as high (15-30%), medium (7-12%), low (3-6%), or depleted (0-1%). Although chemical extraction of starch from root tissue has the advantage of being quantitative, Wargo (1975) and Dunn et al. (1987) found a close agreement in root starch ratings between the colorimetric and histochemical methods. Few trees were found to have depleted levels of starch in our study; therefore, trees with depleted levels of starch were categorized as having low levels for the analysis. Dunn et al. (1987) found that variability in starch content among roots of the same tree existed in 23% of the trees sampled using the Wargo (1975) staining technique. Therefore, we sampled two primary roots from 30% of all trees to determine if differences among roots were present. Within-tree comparisons of starch levels were based on the visual assessment of the root samples.

Beetles attracted to the sample trees were trapped on 45-cm wide polyethylene bands coated with Tangletrap® (Great Lakes IPM Inc., Vestaburg, Michigan, USA). The trap bands were placed around the trunk of each tree just before beetle emergence (unpublished data, PdG), with the mid-point of the trap at 1.3 m above ground (diameter breast height or DBH). Collections were made weekly from 24 May - 20 August 2004 by removing adults from the traps. The number of *A. planipennis* adult beetles caught on the traps were totalled per tree and standardized (= no. per $m^2$ of trapping surface) to account for differences in the trap surface area on trees of varying diameters.

A one-way ANOVA (PROC GLM, SAS Institute 1985) was used to compare differences in the mean number of *A. planipennis* caught per $m^2$ on ash trees with varying levels of winter starch levels. The same analysis was used to compare the differences in beetle captures between plantations. The possibility that starch levels were affected by colonizing *A. planipennis* did exist; therefore, we further separated the analysis to explore the relationship between starch levels and beetle captures on trees previously colonized by *A. planipennis* and trees not previously colonized, separately. A significant difference in total beetle captures was detected between plantations (Table 1). Therefore, we further analyzed the relationship between starch levels and beetle captures separately for each plantation. When the ANOVA results were significant ($p \leq 0.05$), a Tukey's multiple comparisons test was used to determine which treatments differed significantly. Homogeneity of variance was tested with Levene's test and the requirements of the ANOVA were met.

It was not our initial intent to compare the number of beetles captured on trees previously colonized by *A. planipennis* with those captured on uncolonized trees; however, because many of our study trees were subsequently found to be attacked by *A. planipennis* during our second period of sampling (May-September 2004), a two sample t-test on the mean number of beetles captured/$m^2$ per tree was used to explore this relationship.

Main *A. planipennis* activity periods for each plantation were determined by selecting the weeks in which the highest percentages of total beetles were captured.

## RESULTS AND DISCUSSION

The number of beetles captured per $m^2$ of trapping surface (hereafter referred to as captured) did not differ between ash trees with different levels of stored starch when all plantations were pooled together ($F = 0.22$; df = 2, 197; $p = 0.81$, n = 200) and when each plantation was analyzed separately (Plantation 1: $F = 1.04$, df = 2, 47; $p = 0.36$, n = 50; Plantation 2: $F = 3.04$, df = 2, 47; $p = 0.06$, n = 50; Plantation 3: $F = 0.82$, df = 2, 47; $p = 0.45$, n = 50; Plantation 4: $F = 0.27$, df = 2, 47; $p = 0.77$, n = 50). Plantations were analyzed separately because the number of beetles captured differed significantly between them ($F = 25.52$; df = 3, 196; $p < 0.0001$; $R^2 = 0.28$ (Table 1). Based on visual assessments of the root samples, it was determined that the starch ratings of all 60 trees sampled twice (two primary roots from the same tree) were consistent with each other (both roots contained the same level of stored starch). The number of beetles captured was not related to the amount of winter starch levels within trees previously colonized trees by *A. planipennis* ($F = 0.91$; df = 2, 66; $p = 0.410$; n = 69), nor within uncolonized trees ($F = 1.08$; df = 2, 128; $p = 0.34$; n = 131). The 2004 main *A. planipennis* activity periods for each plantation studied were: 9-15 June, 23-29 June, 30 June - 6 July, and 23-29 June (Table 1).

Our results suggest winter starch levels in roots are not a predictor of attack by *A. planipennis* in young green and white ash grown in plantations. MacFarlane and Meyer (2005) reviewed the ecology of ash trees and the biology of *A. planipennis* to assess the relative risk to the beetle and noted that a relationship between tree vitality and colonization by *A. planipennis* had not been established. A preliminary analysis by Witter and Storer (2005) of over 400 site and visual assessments conducted in infested Michigan stands during 2003 indicated that mean ash vitality was generally high, with only 5% of sites having poor vitality. Early results from a study by Herms et al. (2005) suggest that *A. planipennis* prefers trees fertilized with nitrogen, however this finding has not yet been correlated to starch levels in the roots.

Trees previously colonized by *A. planipennis* had a significantly higher number of beetles per $m^2$ of trap surface [133 ± 1.53 (mean ± SE); n = 69] than trees that had not yet been colonized (43 ± 0.437;; n = 131). These previously attacked ash trees captured over 300% as many beetles as uncolonized trees. Timms et al. (2006) in their study of the spatial distribution and attack dynamics of *A. planipennis* on young ash trees noted that previously attacked trees had a higher incidence of beetles the year after attack than trees that were uninfested. Similarly, Haack and Benjamin (1982) and Haack et al. (1983) also found higher incidence of *A. bilineatus* beetles in oak trees attacked previously than in uncolonized trees. It is likely that some of the newly emerging beetles were captured on the previously attacked trees.

Our results may indicate that *A. planipennis* attacks all ash trees regardless of low or high stress levels, albeit as currently measured by starch levels. Studies cited in Poland (2007) indicate that *A. planipennis* shows a preference for girdled trees in an area, but “stress” has never been specifically measured and compared between girdled and ungirdled trees. The apparent preference by *A. planipennis* for girdled trees may also be a result of qualitative and quantitative differences in host volatiles that it may use in host location and selection. Although our work suggests that winter starch levels are not a good predictor of attack by *A. planipennis*, other measurements of tree vitality, such as electrical resistance may be useful, as it has been for *A. anxius* (Ball and Simmons 1984). Further work is needed to discover appropriate measures of tree vitality that might best predict locations and trees more likely to be attacked by *A. planipennis* when it first arrives in an area, and in so doing, improve early detection and pest mitigation measures to deal with this highly destructive invasive forest insect.

## ACKNOWLEDGEMENTS

The University of Toronto and a Science & Technology Internship through the Canadian Forest Service supported this work. We thank the reviewers for their constructive comments on a previous version of this paper.

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