

Patterns in the within-tree distribution of the emerald ash borer *Agrilus planipennis* (Fairmaire) in young, green-ash plantations of south-western Ontario, Canada

Laura L. Timms*, Sandy M. Smith* and Peter de Groot**†

*Faculty of Forestry, University of Toronto, 33 Willcocks Street, Toronto, Ontario, M5S 3B3 Canada and †Canadian Forest Service, Great Lakes Forestry Centre, 1219 Queen St. E., Sault Ste. Marie, Ontario P6A 5M7 Canada

- Abstract**
- 1 The emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is a serious exotic pest of ash trees (*Fraxinus* spp.) in North America, and is responsible for the deaths of millions of trees in Ontario and Michigan. One of the greatest challenges facing the successful management of the pest is the ability to accurately detect its presence in a tree.
 - 2 Observations were made on *A. planipennis* larval feeding galleries found within 65 young, green-ash trees cut from plantations in Essex County, Ontario, Canada. The within-tree distributions of feeding galleries were described in relation to height-above-ground, stem diameter, bark thickness and stem aspect.
 - 3 Galleries were not distributed randomly or evenly; minimum boundaries of stem diameter and bark thickness and a maximum boundary of height-above-ground were detected. Indications of maximum boundaries for stem diameter and bark thickness were also observed. Galleries were found most often on the south-west side of the tree.
 - 4 Using the technique of upper boundary regression, we were able to identify significant quadratic relationships between *A. planipennis* gallery density and stem diameter and bark thickness, as well as a significant negative linear relationship between gallery density and height-above-ground.
 - 5 *Agrilus planipennis* gallery density in newly-infested trees was lower than in previously-infested trees, and was observed to peak at smaller stem diameters and bark thicknesses than in previously-infested trees.
 - 6 Survey teams would increase their probability of detecting new *A. planipennis* infestations by initiating searches for exit holes and feeding galleries in trunk sections and branches of approximately 7 cm in diameter.

Keywords *Agrilus planipennis*, Buprestidae, emerald ash borer (EAB), feeding galleries, within-tree distribution, wood-borers.

Introduction

The emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) (EAB) was first identified from Canada in Windsor, Ontario, in July 2002; however, recent estimates suggest that the beetle may have been established up to 10 years earlier (Haack *et al.*, 2002; McCullough and Katovich, 2004; Cappaert *et al.*, 2005). It is thought that the EAB was originally introduced into Detroit, Michigan,

through untreated solid wood packing material from Asia. Preliminary surveys in 2002 identified millions of affected trees from one county in Canada (Essex, Ontario) and six in the U.S.A. (Michigan: Livingston, Macomb, Monroe, Oakland, Washtenaw and Wayne) (Liu *et al.*, 2003). Subsequently, the beetle has expanded its range, despite intensive suppression efforts by Canadian and American authorities. The EAB is now also found in Chatham-Kent County in Ontario, as well as an additional 15 counties in Michigan, six in Ohio, two in Indiana, and one each in Maryland and Virginia (Liu *et al.*, 2003). The primary means of movement has been human-assisted, via piles of infested firewood and nursery stock (Liu *et al.*, 2003).

Correspondence: Laura Timms, Faculty of Forestry, University of Toronto, 33 Willcocks Street, Toronto, Ontario, M5S 3B3 Canada. Tel.: +1 416 9088962; fax: +1 416 9783834; e-mail: laura.timms@utoronto.ca

The EAB is a member of the Buprestidae, a family commonly known as the flat-headed borers or the metallic wood-boring beetles. Adult buprestids are very active fliers, and the group is one of the most destructive insect families in the forest (Bright, 1987). They kill their hosts by feeding in the soft phloem layer under the outer bark, cutting off the connections between the roots and the shoots and preventing the transport of nutrients and water (Haack and Benjamin, 1982). There are approximately 190 known species of buprestids in Canada, over 120 of which are in the genus *Agrilus* (Bright, 1987). The genus contains two of the best known wood-boring insect pests in North America, the bronze birch borer *Agrilus anxius* Gory and the two-lined chestnut borer *Agrilus bilineatus* (Weber). Despite this, the ecology of buprestid populations in North America has not been well studied (Muzika *et al.*, 2000) and, although it has long been recognized as a pest of ash trees in China, very little is known about the EAB in its native range (Liu *et al.*, 2003).

Aside from the important difference that it is able to attack and kill apparently healthy trees (McCullough and Katovich, 2004), initial observations in North America indicate that the emerald ash borer is similar in ecology and biology to native *Agrilus* species (Barter, 1957; Barter, 1965; Cote and Allen, 1980; McCullough and Katovich, 2004; Cappaert *et al.*, 2005). Eggs are laid in early summer on the trunk and branches of ash trees, under bark scales and flakes. First instars hatch and tunnel through the bark to begin feeding in the cambium underneath. As the larvae pass through the four larval instars over the course of the summer, their feeding creates distinctive S-shaped galleries in the phloem and outer xylem. In late autumn, the fourth instar constructs a pupal chamber in which it overwinters. Pupation occurs in the spring and adults emerge beginning in late May or early June (McCullough and Katovich, 2004). Occasionally, some EAB populations deviate from this cycle and develop over 2 years (Yu, 1992; McCullough and Katovich, 2004); in these populations, larvae overwinter as second or third instars, spend a second summer feeding and then continue the rest of the life cycle as normal.

A number of factors have contributed to the severity of the emerald ash borer problem. Although south-western Ontario is the least-forested area in the province (McLachlan and Bazely, 2003), a large number of both the natural and planted trees in Essex County are *Fraxinus* species (MacFarlane and Meyer, 2005). This enhances the importance of ash in the region as seed-producing wildlife trees, watershed regulators, and as part of the urban forest. To further complicate the problem, the affected area had already been experiencing an unexplained epidemic of ash tree decline (Woodcock *et al.*, 1993; Feeley *et al.*, 2001). In addition to their ecological significance, ash trees are considered valuable for their role in the manufacture of various products; there are many ash plantations and ash-dominated woodlots in south-western Ontario (Paul Giroux, Forester, Essex Region Conservation Authority, Canada, personal communication). Research into the biology and control of the emerald ash borer is necessary to prevent losses to the forest industry and permanent alterations to the urban forest, as well as lasting ecological damage.

Perhaps the greatest challenge facing the successful management of the emerald ash borer is the ability to accurately detect its presence in a tree. Because it spends most of its life cycle under the bark, it has been difficult to assess exactly how widespread and large the EAB population has become. Efforts at developing an operational trapping system, including the use of trap trees and chemical lures, are in the early stages of development (Cappaert *et al.*, 2005), forcing forest managers to rely on individual tree surveys for detection. Survey teams must look for external symptoms of infestation, such as flagging crowns, epicormic shoots, bark cracks, and exit holes, and then destructively remove a section of the bark to confirm EAB presence. Unfortunately, not all of these symptoms are reliably unique indicators of the EAB; those that are, such as exit holes, are often difficult to see and usually appear only once most of the damage has already been done. Data on where within a tree EAB feeding galleries are most likely to be found would allow survey teams to increase their probability of detecting a new infestation, as well as minimizing damage to the tree by reducing the amount of destructive sampling needed.

The aim of the present study was to address the problems with detection by investigating patterns in the within-tree distribution of emerald ash borer feeding galleries. Specifically, we wanted to: (i) describe patterns of within-tree distribution and; (ii) use these patterns to provide recommendations for improved emerald ash borer survey strategies. Literature on the bronze birch borer and the two-lined chestnut borer indicates that their within-tree distributions are influenced by at least one (or a combination) of the variables of height-above-ground, stem diameter, bark thickness, and stem aspect (Ball and Simmons, 1980; Haack and Benjamin, 1982; Loerch and Cameron, 1984; Akers and Nielsen, 1990). Studies on other wood-boring and bark-inhabiting insects also describe several means by which each of these variables might influence insect distribution (Moore *et al.*, 1988; Amezaga and Rodriguez, 1998; Prenzler *et al.*, 1999; Reid and Glubish, 2001). For these reasons, we chose to study the within-tree distribution of EAB feeding galleries in relation to height-above-ground, stem diameter, bark thickness, and stem aspect. Among the literature reviewed, bark thickness is the most commonly indicated parameter of influence; even those studies that do not measure bark thickness directly often use it as an indirect explanation of their results. Thus, we predicted that, among the variables being studied, the within-tree distribution of *A. planipennis* galleries would be most influenced by bark thickness.

Materials and methods

Study sites

Three privately owned, pure green ash (*Fraxinus pennsylvanica* Marsh) plantations (Flood, Stevens and Woodward) in Essex County, Ontario, were selected for use in the study according to the following criteria. Most importantly, positive identification of *A. planipennis* presence was detected at each site in 2003. Second, to reduce the possibility of edge effects, ash

trees on the plantation were required to form a continuous block of at least three hectares. Third, a minimum 10 km separation from the originally detected *A. planipennis* infestation in the city of Windsor was chosen to reduce the sampling of severely infested trees. Finally, trees aged less than 10 years old were not considered for use because the literature suggests that *Agrilus* species will not attack very small diameters. Further site variables are described in Table 1. Infestation by *A. planipennis* was first identified by Canadian Food Inspection Agency surveys at the Flood and Stevens properties in the winter of 2002–03. The presence of *A. planipennis* on the Woodward plantation was first identified during the site surveys for our study. Surveys were conducted by examining trunks for exit holes, bark cracks, and epicormic shoots. When one or more of the symptoms were observed, a small piece of bark was removed to confirm the presence of *A. planipennis*.

Tree selection and measurement of gallery parameters

A survey of the study sites indicated that tree diameters at breast height (d.b.h.) ranged from 4–14 cm. An effort was made to choose an equal number of trees from each 1-cm diameter class between 4.0 and 14.0 cm to provide a representative sample. Trees were examined randomly within the plantations, and a tree was chosen for cutting if there were visible exit holes on the trunk, or if it exhibited other symptoms of *A. planipennis* infestation and was immediately adjacent to a tree with exit holes. On 13 and 14 April 2004, ten trees were removed from the Woodward plantation, 33 from Flood, and 50 from Stevens. Individual tree parameters are detailed by Timms (2005).

Before cutting each tree, its d.b.h. was noted and the North cardinal point marked on the trunk with a wax pencil. Trees were felled and limbed using a chainsaw, then cut into a series of 1-m long sections starting a ground level. In the laboratory, each section was examined for pre-existing exit holes; all exit holes present on the trunk before the 2004 emergence period were classified as old, regardless of the year the beetle had emerged. Exit holes were counted and highlighted on the bark with wax pencil. Four measurements of bark thickness were taken on each bolt; measurements were taken at two locations on opposite sides of the trunk, on both the top and bottom of the bolt. Calipers were used to take measurements from the outer bark to the phloem just outside the sapwood, as seen in cross section at the ends of the bolts. Bark thickness was measured within 1 week of felling to avoid shrinking and deformation due to desiccation.

Sections for each tree were separated into two groups, representing the top and bottom half of the tree. Halves were placed into sealed cardboard tubes of varying diameters (Newark Paperboard Products HandiForms, Home Depot Canada, Canada) to rear larvae through to adults, and to retain all adults, natural enemies, and other associates. Bottom-half tubes contained between the first 2–4 m of the trunk, top-half tubes contained the remaining sections. Insects emerging from the logs were counted daily and collected weekly from alcohol-filled, clear plastic bags (18 oz Whirl-Pak bags, Fisher Scientific, Canada) affixed over a hole cut into the side of the cardboard tube. After approximately 7 weeks, when adult emergence appeared complete, the tubes were opened and each 1-m bolt was examined individually.

A compass rose was drawn onto the bottom of each bolt using the North cardinal mark as a starting point. Measurements of direction were divided into one of eight compass points (north, north-east, east, south-east, south, south-west, west, north-west). Bolt length and diameter at both ends were also recorded. Before debarking, the height from the bottom of the log and aspect of old and new *A. planipennis* exit holes were recorded. New exit holes were defined as those that were not present before the 2004 emergence period, and were created while the logs were sealed within the tubes.

Bolts were debarked using carvers' drawknives (Veritas Carver's Drawknife, Lee Valley Tools, Canada). Height from the bottom of the log and aspect of all observed *A. planipennis* feeding galleries were measured. Gallery parameters were measured only from the gallery start point; irregular and inconsistent gallery patterns meant that measures of the end point of a gallery often did not accurately describe its true length or shape. In addition, because first instar larvae burrow into the phloem directly from the oviposition site on the outer bark, the distribution of gallery start points is likely to be a reasonable estimate of the distribution of oviposition sites.

The age (current or old) and condition (successful or unsuccessful) of each gallery were also noted. Current galleries were defined as those in which the egg had been laid in 2003. The precise age of old galleries was not evaluated, although it is possible to determine gallery age by identifying the growth ring in which it starts. Gallery age was determined visually, based on the amount of callus tissue growth around it and the condition of the frass within it. Old galleries were most often found concealed under a thick layer of callus tissue, and were not completely visible until this layer was removed with a drawknife. In cross-section, it was possible to trace the origin of this callus tissue to within the growth ring of the previous year. Old galleries that were not covered with

Table 1 Descriptive variables for three privately owned green ash (*Fraxinus pennsylvanica* Marsh) plantations in south-western Ontario studied in 2004

Plantation (coordinates)	Size (ha)	Mean d.b.h. (cm)	Mean height (m)	No. of trees	Planting year
Flood (42°10'44"N, 82°50'46.9"W)	10.0	5.78 ± 0.19	5.57 ± 0.14	33	1991
Stevens (42°3'12.5"N, 82°50'45.2"W)	3.2	10.42 ± 0.25	10.17 ± 0.14	50	1983
Woodward (42°11'44.8"N, 82°53'21.7"W)	5.0	7.29 ± 0.32	7.79 ± 0.23	10	1988
All sites mean	–	8.44 ± 0.27	8.29 ± 0.24	93	–

callus tissue appeared worn and contained dark, loosely packed frass. Small amounts of callus tissue were occasionally found growing around the edges of a current gallery; in these cases, both the small amount of tissue and the light-coloured, tightly packed frass were used to identify the gallery as current.

Successful galleries were defined as those in which an adult had completely emerged through an exit hole. Galleries in which the adult had died midway through emergence were classified as unsuccessful, as were all other galleries in which development was not completed. It is possible that some of the galleries in which development was not completed were those in which larvae were engaged in a 2-year life cycle. However, our definition of a successful gallery was chosen to reflect only the population of emerged, potentially reproductive adults for the current year.

The whole tree was used as the sample unit for this study to reflect the ecological level at which a female beetle makes decisions about oviposition. Three of the 93 trees cut contained no feeding galleries and therefore may not have been exposed to selection by *A. planipennis*; these samples were removed from the data set. An additional 25 trees were removed from the data set because they contained sections that were too heavily infested to identify individual galleries. Data from the remaining 65 trees were used to describe the within-tree distributions of galleries and exit holes. Potential differences in gallery distribution between the first and subsequent years of an infestation were addressed by classifying trees into one of two categories of infestation history. Previously-infested trees contained galleries from at least 2 years of infestation ($n = 47$); newly-infested trees contained galleries from only one year ($n = 18$). As survey strategies must be targeted to detect galleries in newly-infested trees, this distinction was considered important.

Description and analysis of patterns in attack density

The density of *A. planipennis* galleries was calculated for each 1-m bolt in terms of galleries per 100 cm² of available surface area of bark. The total available surface area for each bolt was calculated using its length and mean diameter with the formula for the surface area of a cylinder. Density was evaluated per 100 cm² of bark area to reflect the results that would be obtained using a sampling window of 10 × 10 cm in the field. Gallery density was plotted against the mean diameter and bark thickness, as well as the height-above-ground at the midpoint of each bolt. Data from all three sites were combined for analysis; combining the sites permitted us to investigate within-tree patterns in a broader range of tree sizes, and thus allowing us to focus more on general trends than on between-site differences.

The technique of upper boundary regression was used to estimate the relationships between maximum gallery density and each of the parameters of interest. Upper boundary regression has been shown to be a useful technique for estimating relationships between two variables in cases when the independent variable may not be the only cause of variation in the dependant variable, but it is likely to be strongly

related to the maximum value of the dependant variable (Blackburn *et al.*, 1992; Krause-Jensen *et al.*, 2000; Lessin *et al.*, 2001). Upper boundary regression was considered appropriate in this case because although low EAB gallery densities were found at all diameters, bark thicknesses, and heights-above-ground, the highest gallery densities were only found at intermediate values of those parameters. The technique involved dividing the independent variable into a number of classes of even width, and using only the coordinates with the highest gallery densities within each class in simple linear and nonlinear regression models (PROC REG; Sas Institute, 1988). Values for 549 bolts were divided into 32 50-cm wide diameter classes; 12 0.5-mm wide bark thickness classes; and 11 100-cm wide classes of height-above-ground. To reduce variation due to small sample sizes, the values for a class were not used in the regression if it contained fewer than four points (Blackburn *et al.*, 1992).

Compass direction is a nonlinear measurement and cannot be analysed in the same fashion as a continuous linear variable; consequently, the directional within-tree distributions of galleries and exit holes were analysed using circular statistics (Batschelet, 1965). Rose diagrams were used to describe the directional distributions and the Rayleigh test (modified for grouped circular distributions) was used to test for their randomness (Oriana Version 2.02a, Kovach Computing Services, U.K.). Directional distribution means are presented in both degrees and compass bearings.

Probability of *A. planipennis* presence

In delimitation surveys for invasive insects, identifying the pest's presence or absence in an area is more important than quantifying population densities. With this in mind, measures of gallery density in each 1-m bolt were converted to binary values of presence or absence. Presence-absence was then modelled against stem diameter in a logistic regression (PROC LOGISTIC; SAS Institute, 1988) to create a predictive equation for *A. planipennis* occurrence within a tree. Predicted probabilities of *A. planipennis* occurrence were then used to make inferences about which diameter ranges within a tree possess the highest probabilities of containing a gallery. A Pearson correlation matrix (PROC CORR; SAS Institute, 1988) was used to identify correlations between the within-tree variables. High correlation indices between all measured variables justified the use of only one predictor variable in the logistic regression. Stem diameter was chosen as a proxy for the other within-tree variables because it was considered to be the most practical measurement to make in the field.

Results

Within-tree distribution patterns were discernible in scatterplots of the independent, within-tree variables vs. the density of all observed *A. planipennis* galleries. Approximate maximum and minimum boundaries of gallery distribution were estimated visually from the plots. Minimum boundaries for gallery distribution were observed for stem diameter and bark thickness; galleries were found at very low densities, or not at all, at stem

diameters smaller than 4 cm (Fig. 1) and in bark thinner than 1.5 mm (Fig. 2). No minimum boundary was observed for height-above-ground; high densities of galleries were observed beginning at ground level. However, gallery density was very low at heights-above-ground of 7 m and up (Fig. 3). The density of observed galleries was also lower at stem diameters larger than 13 cm (Fig. 1) and in bark thicker than 5 mm (Fig. 2), although these boundaries are less clearly defined because of fewer samples collected in this range of the data. Some galleries were found at all directions around the trunk. However, more galleries were found on the south and west sides than at any other direction (Fig. 4). Galleries were observed at a mean direction of $226.01 \pm 7.14^\circ$, or south-west.

Significant quadratic relationships were found in regressions of maximum gallery density as a function of stem diameter (Fig. 1) (Table 2) and bark thickness (Fig. 2) (Table 3) for all data, and also when data were split into groups of newly-infested and previously-infested trees. Trees that had experienced only one year of infestation had lower overall gallery densities, and predicted peak densities at smaller stem diameters and in thinner bark, than trees that had been infested for more than 1 year (Tables 2 and 3). Significant negative linear relationships were found in regressions of the maximum gallery density as a function of height-above-ground (Fig. 3) (Table 4) for all data, and also when data were split into groups of newly-infested and previously-infested trees. Gallery density in previously-infested trees decreased with increasing height at a faster rate than in newly-infested trees.

A Pearson correlation matrix (Table 5) revealed a significant positive correlation between diameter and bark thickness. Significant negative correlations were found between

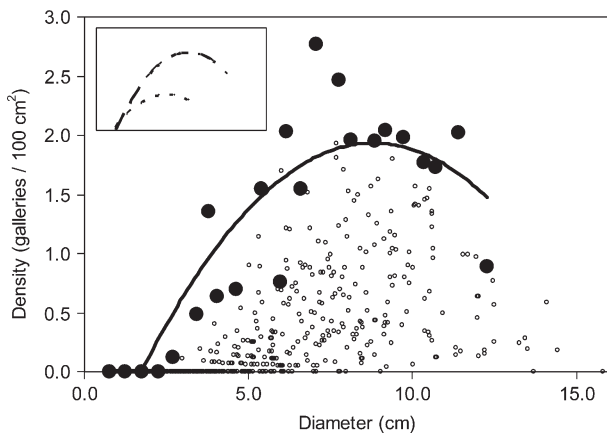


Figure 1 Scatterplot of stem diameter vs. density (number of feeding galleries per 100 cm² of bark) for all observed emerald ash borer (*Agrilus planipennis* Fairmaire) feeding galleries in 65 young, green ash (*Fraxinus pennsylvanica* Marsh) trees cut from plantations in south-western Ontario during April 2004. Solid circles indicate the maximum gallery density within each of 32, 50-cm wide diameter classes; the solid trend line indicates the quadratic relationship between stem diameter and maximum gallery density in all trees. The inset graph shows trend lines for the quadratic relationships between stem diameter and maximum gallery density for galleries in newly-infested trees (dotted line), and previously-infested trees (dashed line).

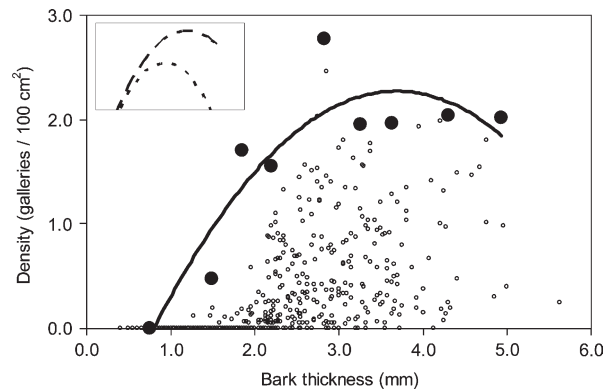


Figure 2 Scatterplot of bark thickness vs. density (number of feeding galleries per 100 cm² of bark) for all observed emerald ash borer (*Agrilus planipennis* Fairmaire) feeding galleries in 65 young, green ash (*Fraxinus pennsylvanica* Marsh) trees cut from plantations in south-western Ontario during April 2004. Solid circles indicate the maximum gallery density within each of 12, 0.5-mm wide bark thickness classes; the solid trend line indicates the quadratic relationship between bark thickness and maximum gallery density in all trees. The inset graph shows trend lines for the quadratic relationships between bark thickness and maximum gallery density for galleries in newly-infested trees (dotted line), and previously-infested trees (dashed line).

height above ground and both diameter and bark thickness. Logistic regression detected a significant relationship between stem diameter and *A. planipennis* presence (Wald $\chi^2 = 145.39$, d.f. = 1, $P < 0.0001$). The presence-absence data agreed with the plotted density distributions; galleries were generally absent at diameters smaller than

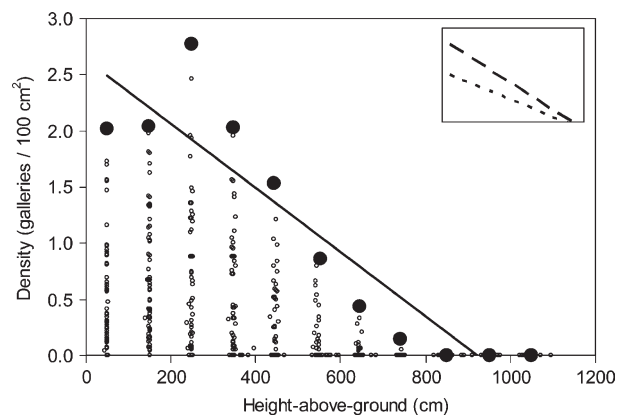


Figure 3 Scatterplot of height-above ground vs. density (number of feeding galleries per 100 cm² of bark) for all observed emerald ash borer (*Agrilus planipennis* Fairmaire) feeding galleries in 65 young, green ash (*Fraxinus pennsylvanica* Marsh) trees cut from plantations in south-western Ontario during April 2004. Solid circles indicate the maximum gallery density within each of 11, 100-cm wide height classes; the solid trend line indicates the linear relationship between height-above-ground and maximum gallery density in all trees. The inset graph shows trend lines for the negative linear relationships between height-above-ground and maximum gallery density for galleries in newly-infested trees (dotted line), and previously-infested trees (dashed line).

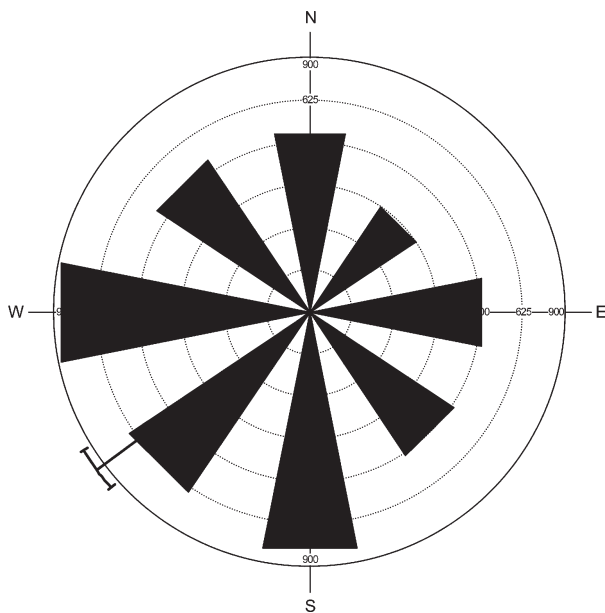


Figure 4 Frequency distribution of the compass directions of observed emerald ash borer (*Agrilus planipennis* Fairmaire) feeding galleries in 65 young, green ash (*Fraxinus pennsylvanica* Marsh) trees cut from plantations in south-western Ontario during April 2004. The line indicates the mean \pm SE direction.

4 cm and present at diameters larger than 7 cm. Output-predicted probabilities of *A. planipennis* occurrence were plotted against stem diameter (Fig. 5). Parameter estimates from the logistic regression were used to solve the logistic equation for the diameters that would result in a high probability of finding a gallery. At a stem diameter of 4.5 cm, the probability of finding a gallery is approximately 0.5; this probability increases to 0.9 at a diameter of 6.75 cm. For a 1-cm increase in diameter, the odds of finding a gallery increases by almost three-fold (Wald 95% confidence interval for adjusted odds ratios = 2.49–3.54).

Discussion

Common patterns of *A. planipennis* gallery distribution were observed among the trees in this study. Although small numbers of feeding galleries were found across the range of available host material, the highest densities of galleries were clustered within certain values of the measured variables.

Directionally, galleries were found most often on the south-west side of the tree. Although occasional exceptions were observed, within-tree gallery distributions were generally bounded by a minimum stem diameter of approximately 4 cm, a minimum bark thickness of approximately 1.5 mm and a maximum height-above-ground of 7 m. The within-tree distributions of feeding galleries were not restricted by a minimum height-above-ground; galleries were often observed at the very base of a tree, but never in the extremes of the canopy. Indications of a maximum stem diameter of approximately 13 cm and a maximum bark thickness of 5 mm were also observed.

Stem height, diameter and bark thickness are all highly correlated. It is likely that one of these variables acts as the main factor in the distribution of galleries within a tree, and that the relationships between gallery distribution and the other variables are a result of their correlation to this factor. It is also likely that the within-tree variables are not the only factors with influence on gallery distributions; host physiology and physical structure, individual *A. planipennis* behaviour, and temporal factors, among others, may play a role. However, we feel that although these other factors may contribute to the observed variation in gallery densities along the gradients of the measured variables, the maximum gallery densities are ultimately influenced by one, or a combination, of stem diameter, bark thickness, or height-above-ground.

Height-above-ground appears to be the least likely variable to have a strong influence on gallery distribution. Although galleries in our study were observed consistently more often in the lower portions of the tree, previous evidence indicates that the vertical distributions of galleries of *Agrilus* species vary depending on the height of the trees being examined. The trees used in this study had a mean d.b.h. of 8.44 ± 0.27 cm and a mean height of 8.29 ± 0.24 m (Table 1). Studies by Loerch and Cameron (1984) and Akers and Nielsen (1990) also used small trees (8-year-old plantation trees and nursery trees, respectively) and found a greater number of galleries at the base. However, Haack and Benjamin (1982) examined larger trees, of at least 15 m in height, and found most galleries in the crown. Ball and Simmons (1980) reported that attack began in the stem, but gave no information on the size of their study trees.

From an ecological perspective, it appears reasonable that bark thickness would be the most important variable affecting the within-tree distribution of feeding galleries. The general condition, nutritional quality and protective value of bark can affect a variety of larval life history traits. Wood-boring insects require bark thick enough to provide adequate

Table 2 Fitted models of emerald ash borer (*Agrilus planipennis* Fairmaire) gallery density as a function of stem diameter, within young, green ash (*Fraxinus pennsylvanica* Marsh) trees, cut from south-western Ontario during April 2004

Data set	Model	P-value	R ²	Vertex (cm)	n
All trees	$y = -0.038x^2 + 0.678x - 1.054$	<0.0001	0.7407	8.92	22
Newly infested	$y = -0.034x^2 + 0.449x - 0.608$	0.0108	0.3800	6.60	18
Previously infested	$y = -0.041x^2 + 0.704x - 1.135$	<0.0001	0.7261	8.59	22

Models were fitted using the maximum gallery densities found within *n* 50-cm wide diameter classes containing more than four data points.

Table 3 Fitted models of emerald ash borer (*Agilus planipennis* Fairmaire) gallery density as a function of bark thickness, within young, green ash (*Fraxinus pennsylvanica* Marsh) trees, cut from south-western Ontario during April 2004

Data set	Model	P-value	R ²	Vertex (mm)	n
All trees	$y = -0.273x^2 + 2.010x - 1.431$	0.0062	0.7556	3.68	9
Newly infested	$y = -0.406x^2 + 2.269x - 1.816$	0.0261	0.6745	2.79	8
Previously infested	$y = -0.283x^2 + 2.094x - 1.594$	0.0076	0.7381	3.70	9

Models were fitted using the maximum gallery densities found within *n* 0.5-mm wide bark thickness classes.

nutrition (Haack *et al.*, 1987; Hanks *et al.*, 1993; Manville *et al.*, 2002; Smith *et al.*, 2002); protection from desiccation and extreme temperatures (Dutt, 1969; Cote and Allen, 1980; Akers and Nielsen, 1986); and avoidance of predation or parasitism (Ohmart, 1979; Paine *et al.*, 2000; Wermelinger, 2002). Even studies that report a correlation between stem height or diameter and insect distribution often speculate that the biological reason behind the relationship is bark thickness (Hespenheide, 1969; Simandl, 1993; Jactel *et al.*, 1996).

Bark thickness may be the biological mechanism for some within-tree insect distributions; however, other characteristics such as stem diameter may act as the functional mechanism by which females make oviposition decisions. Reid and Glubish (2001) suggest that Douglas-fir beetles (*Dendroctonus pseudotsugae* Hopkins) orientate themselves towards certain sizes of trunk silhouettes when flying. Buprestids are capable and strong fliers with large eyes (Bright, 1987); it is likely that they use visual cues for orientation. Preliminary evidence by Francese *et al.* (2005) suggests that *A. planipennis* are significantly more attracted to purple objects than other colours, and that they may use visual cues for mate-finding. It is also possible that bark type and texture play an important role. Barter (1957) describes how a female *A. anxius* will crawl along the sunny side of the trunk with her ovipositor extended, using it to probe into crevices in the bark until a suitable location for oviposition is detected. However, details of how these factors affect the oviposition decisions of *A. planipennis* females remain unknown.

Barter (1957) also suggests that oviposition site selection in *A. anxius* is based on temperature and an olfactory response to tree stress. Haack and Benjamin (1982) and Dunn *et al.* (1986) have documented attraction to both stressed oak trees and their volatiles in *A. bilineatus*. Preliminary electroantennogram tests by Cappaert *et al.* (2005) indicate that *A. planipennis* may be more attracted to volatiles from stressed ash trees than from healthy ash. Akers and Nielsen (1990) suggested that there are important changes in host physiology which occur once a tree has begun to be attacked

by *A. anxius*. A tree stressed by previous infestation would therefore be more likely to attract future infestation; furthermore, areas within a tree containing old feeding galleries may also be more attractive for oviposition.

We attempted to address these temporal issues by considering the gallery densities in newly-infested and previously-infested trees separately. Some important differences were detected; gallery densities in newly-infested trees were low at both very small (4 cm, 1.5 mm) and very large (10 cm, 4 mm) stem diameters and bark thicknesses, and exhibited peak densities at medium values. Gallery densities in previously-infested trees were also low at small stem diameters and bark thicknesses, but exhibited slower decreases in density at the larger ends of the ranges. As a result, peak gallery densities in previously-infested trees were observed at larger stem diameters and bark thicknesses than in newly-infested trees. In addition, gallery densities were consistently lower in newly-infested trees than in those that had experienced multiple years of attack. Although thick bark is important for larval survival for a variety of reasons, bark that is too thick or vigorous can kill larvae (Cote and Allen, 1980; Haack *et al.*, 1983; Hanks *et al.*, 1993) and hinder development (Barter, 1957; Ohmart, 1979; Haack and Benjamin, 1982). The data from the present study suggest that the negative aspects of thick bark affect galleries in healthy trees that have not yet experienced attack by *A. planipennis*, but are less important once a tree has been attacked for more than one generation. This must be taken into account when considering where in a tree the first attacks occur, and therefore also when developing survey strategies.

Finally, although *A. planipennis* feeding galleries were observed at all directions around the trunk, a distinct preference for the south-west side was observed. This result confirms previous descriptions of *Agilus* species distributions. Barter (1957, 1965) observed that female *A. anxius* and *A. liragus* preferred the sunny side of the trunk for oviposition. Reports also indicate that adult *A. planipennis* prefer to be in sunlit areas and are more active on sunny days (Yu, 1992). It is

Table 4 Fitted models of emerald ash borer (*Agilus planipennis* Fairmaire) gallery density as a function of height-above-ground, within young, green ash (*Fraxinus pennsylvanica* Marsh) trees, cut from south-western Ontario during April 2004

Data set	Model	P-value	R ²	n
All trees	$y = -0.003x + 2.638$	<0.0001	0.8277	11
Newly infested	$y = -0.002x + 1.761$	<0.0001	0.9351	9
Previously infested	$y = -0.003x + 2.638$	<0.0001	0.8277	11

Models were fitted using the maximum gallery densities found within *n* 100-cm wide height classes.

Table 5 Pearson correlation matrix of the characteristics of emerald ash borer (*Agrilus planipennis* Fairmaire) infested sections of young, green ash (*Fraxinus pennsylvanica*) trees, cut from plantations in south-western Ontario during April 2004

	Gallery density	Height	Diameter	Bark thickness
Gallery density	1.00	–	–	–
Height	–0.48	1.00	–	–
Diameter	0.59	–0.72	1.00	–
Bark thickness	0.58	–0.77	0.86	1.00

All correlation indices are significant at greater than the 99% level.

possible that *A. planipennis* females prefer the sunny side of the tree for oviposition because the warmer temperatures at this direction benefit larval survival and development (Haack and Benjamin, 1982) or because they themselves prefer to be in the sun as they lay their eggs (Barter, 1957).

The results of the present study indicate that delimitation survey teams will realize the highest probability of detecting an *A. planipennis* exit hole or gallery if they begin looking in sections of the trunk or branches with diameters of 7 cm and continue looking at thicker diameters. Starting at 7 cm and then moving to thicker sections will ensure that a surveyor has a good chance of finding a gallery regardless of how many generations of attack the tree has experienced. Although galleries in previously-attacked trees are not likely to be restricted by very large diameters, newly-attacked trees may have fewer galleries present in sections of very large diameter with thick bark. Therefore, when surveying large trees for the purposes of delimitation, climbing into the canopy and examining trunk sections of smaller diameter will probably be more effective for detecting galleries than examining the bark on the trunk at eye level.

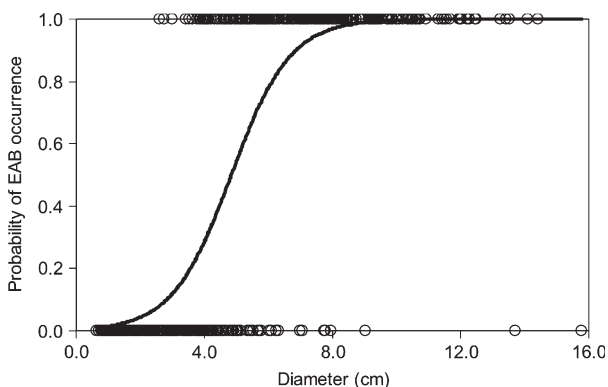


Figure 5 Observed presence and absence (open circles) and probabilities of emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) presence (line) as a function of stem diameter (probability of emerald ash borer occurrence = $\frac{e^{1.0877 \text{diameter} - 5.2656}}{1 + e^{1.0877 \text{diameter} - 5.2656}}$), using predictions from the output of a logistic regression of emerald ash borer presence against stem diameter in 65 young, green ash (*Fraxinus pennsylvanica* Marsh) trees cut from plantations in south-western Ontario during April 2004.

Although it is important to remember that these results are based solely on observational data from three plantation sites, they provide an interesting starting point for further studies. More work will be necessary to see whether these results are applicable in larger trees, in natural stands, and with different species of ash. It is also important to remember that the within-tree distributions described in this research are for typical *A. planipennis* galleries. Exceptions to the rule will always occur; for example, larval feeding galleries have been reported from branches as small as 1 cm in diameter (personal communication, Barry Lyons, Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario). One of the characteristics of a successful invasive species is the ability to be phenotypically plastic in a new environment. Care should be taken to keep track of these exceptions to the rule in case they represent a permanent shift in behaviour.

Acknowledgements

We thank James Dennis, Rosie Page, Nadia Qureshi, Jenni Skinner, Agnes Sztaba, Reg Nott and Morgan Hall-Boenke for technical assistance in the field and laboratory. We are grateful to Mr and Mrs Dave Creery, Mr and Mrs Kevin Flood, Roger and Maryanne Stevens, and Steve and Judy Woodward for the use of their properties. This work was funded by the Canadian Forest Service, Natural Resources Canada and the Faculty of Forestry, University of Toronto, as part of the senior author's Master's thesis: their support is gratefully acknowledged.

References

- Akers, R.C. & Nielsen, D.G. (1986) Influence of post-felling treatment of birch logs on emergence success of bronze birch borer, *Agrilus anxius*, adults (Coleoptera: Buprestidae). *Journal of Entomological Science*, **21**, 63–67.
- Akers, R.C. & Nielsen, D.G. (1990) Spatial emergence pattern of bronze birch borer (Coleoptera: Buprestidae) from European white birch. *Journal of Entomological Science*, **25**, 150–157.
- Amezaga, I. & Rodriguez, M.A. (1998) Resource partitioning of four sympatric bark beetles depending on swarming dates and tree species. *Forest Ecology and Management*, **109**, 127–135.
- Ball, J. & Simmons, G. (1980) The relationship between bronze birch borer and birch dieback. *Journal of Arboriculture*, **6**, 309–314.
- Barter, G.W. (1957) Studies of the bronze birch borer, *Agrilus anxius* Gory, in New Brunswick. *Canadian Entomologist*, **89**, 12–36.
- Barter, G.W. (1965) Survival and development of the bronze poplar borer *Agrilus liragus* Barter & Brown (Coleoptera: Buprestidae). *Canadian Entomologist*, **97**, 1063–1068.
- Batschelet, E. (1965) *Statistical Methods for the Analysis of Problems in Animal Orientation and Certain Biological Rhythms*. American Institute of Biological Sciences, Washington, District of Columbia.
- Blackburn, T.M., Lawton, J.H. & Perry, J.N. (1992) A method of estimating the slope of upper bounds of plots of body size and abundance in natural animal assemblages. *Oikos*, **65**, 107–112.
- Bright, D.E. (1987) *The Metallic Wood-Boring Beetles of Canada and Alaska. Coleoptera: Buprestidae*. Research Branch, Agriculture Canada, Canada.

- Cappaert, D., McCullough, D.G., Poland, T.M. & Siegert, N.W. (2005) Emerald ash borer in North America: a research and regulatory challenge. *American Entomologist*, **51**, 152–165.
- Cote, W.A.I. & Allen, D.C. (1980) Biology of two-lined chestnut borer, *Agrilus bilineatus*, in Pennsylvania and New York. *Annals of the Entomological Society of America*, **73**, 409–413.
- Dunn, J.P., Kimmerer, T.W. & Nordin, G.L. (1986) Attraction of the twolined chestnut borer, *Agrilus bilineatus* (Weber) (Coleoptera: Buprestidae), and associated borers to volatiles of stressed white oak. *Canadian Entomologist*, **118**, 503–509.
- Dutt, D.K. (1969) Bionomics of *Agrilus acutus* (Thnb.) (Col. Buprestidae) on Mesta (*Hibiscus cannabinus*) in India. *Bulletin of Entomological Research*, **58**, 421–429.
- Feeley, C.J., Hart, E.R., Thompson, J.R. & Harrington, T.C. (2001) Occurrence, associated symptoms, and potential insect vectors of the ash yellows phytoplasma in Iowa, U.S. *Journal of Arboriculture*, **27**, 331–340.
- Francese, J.A., Mastro, V.C., Oliver, J.B., Lance, D.R., Youssef, N. & Lavalley, S.G. (2005) Evaluation of colors for trapping *Agrilus planipennis* (Coleoptera: Buprestidae). *Journal of Entomological Science*, **40**, 93–95.
- Haack, R.A. & Benjamin, D.A. (1982) The biology and ecology of the twolined chestnut borer, *Agrilus bilineatus* (Coleoptera: Buprestidae), on oaks, *Quercus* spp., in Wisconsin. *Canadian Entomologist*, **114**, 385–396.
- Haack, R.A., Benjamin, D.A. & Haack, K.D. (1983) Buprestidae, Cerambycidae, and Scolytidae associated with successive stages of *Agrilus bilineatus* (Coleoptera: Buprestidae) infestation of oaks in Wisconsin. *Great Lakes Entomologist*, **16**, 47–55.
- Haack, R.A., Jendek, E., Liu, H., Marchant, K.R., Petrice, T.R., Poland, T.M. & Ye, H. (2002) The emerald ash borer: a new exotic pest in North America. *Newsletter of the Michigan Entomological Society*, **47**, 1–5.
- Haack, R.A., Wilkinson, R.C. & Foltz, J.L. (1987) Plasticity in life-history traits of the bark beetle *Ips calligraphus* as influenced by phloem thickness. *Oecologia*, **72**, 32–38.
- Hanks, L.M., Paine, T.D. & Millar, J.G. (1993) Host species preference and larval performance in the wood-boring beetle, *Phoracantha semipunctata* F. *Oecologia*, **95**, 22–29.
- Hespenheide, H.A. (1969) Larval feeding site of species of *Agrilus* (Coleoptera) using a common host plant. *Oikos*, **20**, 558–561.
- Jactel, H., Perthuisot, N., Menassieu, P., Raise, G. & Burban, C. (1996) A sampling design for within-tree larval populations of the maritime pine scale, *Matsucoccus feytaudi* Duc. (Homoptera: Margarodidae), and the relationship between larval population estimates and male catch in pheromone traps. *Canadian Entomologist*, **128**, 1143–1156.
- Krause-Jensen, D., Middelboe, A.L., Sand-Jensen, K. & Christensen, P.B. (2000) Eelgrass, *Zostera marina*, growth along depth gradients: upper boundaries of the variation as a powerful predictive tool. *Oikos*, **91**, 233–244.
- Lessin, L.M., Dyer, A.R. & Goldberg, D.E. (2001) Using upper boundary constraints to quantify competitive response of desert annuals. *Oikos*, **92**, 153–159.
- Liu, H., Bauer, L.S., Gao, R., Zhao, T., Petrice, T.R. & Haack, R.A. (2003) Exploratory survey for the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae) in China. *Great Lakes Entomologist*, **36**, 191–203.
- Loerch, C.R. & Cameron, E.A. (1984) Within-tree distributions and seasonality of immature stages of the bronze birch borer, *Agrilus anxius* (Coleoptera: Buprestidae). *Canadian Entomologist*, **116**, 147–152.
- MacFarlane, D.W. & Meyer, S.P. (2005) Characteristics and distribution of potential ash tree hosts for emerald ash borer. *Forest Ecology and Management*, **213**, 15–24.
- Manville, J.F., Sahota, T.S., Hollmann, J. & Ibaraki, A.I. (2002) Primary cortex thickness influences the location of ovarian maturation feeding and oviposition of *Pissodes strobi* (Coleoptera: Curculionidae) within a tree. *Environmental Entomology*, **31**, 198–207.
- McCullough, D.G. & Katovich, S.A. (2004) *Emerald Ash Borer*. USDA Forest Service, Northeastern Area, State and Private Forestry, Pest Alert NA-PR-02–04. USDA Forest Service: Newtown Square, Pennsylvania.
- McLachlan, S.M. & Bazely, D.R. (2003) Outcomes of longterm deciduous forest restoration in southwestern Ontario, Canada. *Biological Conservation*, **113**, 159–169.
- Moore, L.V., Myers, J.H. & Eng, R. (1988) Western tent caterpillars prefer the sunny side of the tree, but why? *Oikos*, **51**, 321–326.
- Muzika, R.M., Liebhold, A.M. & Twery, M.J. (2000) Dynamics of twolined chestnut borer *Agrilus bilineatus* as influenced by defoliation and selection thinning. *Agricultural and Forest Entomology*, **2**, 283–289.
- Ohmart, C.P. (1979) The within-tree distributions of *Ips paraconfusus* (Coleoptera: Scolytidae) and its insect associates in Monterey pine (*Pinus radiata*). *Annals of the Entomological Society of America*, **72**, 607–609.
- Paine, T.D., Paine, E.O., Hanks, L.M. & Millar, J.G. (2000) Resource partitioning among parasitoids (Hymenoptera: Braconidae) of *Phoracantha semipunctata* in their native range. *Biological Control*, **19**, 223–231.
- Prenzel, B.G., Laidlaw, W.G. & Wieser, H. (1999) Within-tree dynamics of mass attack by *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae) on its host. *Canadian Entomologist*, **131**, 635–643.
- Reid, M.L. & Glubish, S.S. (2001) Tree size and growth history predict breeding densities of Douglas-fir beetles in fallen trees. *Canadian Entomologist*, **133**, 697–704.
- SAS Institute Inc. (1988) *SAS/STAT User's Guide: Statistics*, Version 6.03. SAS Institute Inc, Cary, North Carolina.
- Simandl, J. (1993) The spatial pattern, diversity and niche partitioning in xylophagous beetles (Coleoptera) associated with *Frangula alnus* Mill. *Acta Oecologica*, **14**, 161–171.
- Smith, M.T., Bancroft, J. & Tropp, J. (2002) Age-specific fecundity of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) on three tree species infested in the United States. *Environmental Entomology*, **31**, 76–83.
- Timms, L.L. (2005) *Within-tree distribution of the emerald ash borer (Agrilus planipennis Fairmaire)*. *Coleoptera: Buprestidae in young, green ash plantations of southwestern Ontario*. MSc Thesis, University of Toronto.
- Wermelinger, B. (2002) Development and distribution of predators and parasitoids during two consecutive years of an *Ips typographus* (Col., Scolytidae) infestation. *Journal of Applied Entomology*, **126**, 521–527.
- Woodcock, H., Patterson III, W.A. & Davies, K.M. Jr (1993) The relationship between site factors and white ash (*Fraxinus americana* L.) decline in Massachusetts. *Forest Ecology and Management*, **60**, 271–290.
- Yu, C. (1992) *Agrilus marcopoli* Obenberger (Coleoptera: Buprestidae). *Forest Insects of China*, pp. 400–401. China Forestry Publishing House, China.

Accepted 19 July 2006