Evaluation of Digital Photography for Quantifying Cryptococcus fagisuga (Hemiptera: Eriococcidae) Density on American Beech Trees

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ABSTRACT Beech scale (Cryptococcus fagisuga Lindering) (Hemiptera: Eriococcidae) is an invasive forest insect established in the eastern United States and Canada. It predisposes American beech (Fagus grandifolia Ehrhart) trees to infection by Neonectria spp. Fungi causing beech bark disease. White wax secreted by the diminutive scales obscures individual insects, making it difficult to accurately quantify beech scale density. Our goals were to 1) evaluate the relationship between the area of wax and number of beech scales on bark samples, 2) determine whether digital photos of bark could accurately quantify beech scale density, and 3) compare efficiency and utility of a qualitative visual estimate and using the quantitative digital photo technique to assess beech scale populations. We visually estimated beech scale abundance and photographed designated areas on the trunk of 427 trees in 40 sites across Michigan. Photos were analyzed using a binary threshold technique to quantify the area of beech scale wax on each photo. We also photographed and then collected 104 bark samples from 45 additional beech trees in ten sites. We removed the wax, counted individual scales on each sample using a microscope, and assessed the linear relationship between wax area and scale counts. Area of wax explained ≈50% of the variability in scale density. We could typically quantify beech scale density on 15 photographs per hour. Qualitative visual assessments of beech scale in the field corresponded with estimates derived from photos of bark samples for 79% of trees.

KEY WORDS Cryptococcus fagisuga, beech bark disease, invasive forest pest, Fagus grandifolia

Beech scale (Cryptococcus fagisuga Lindering), an invasive pest affecting American beech (Fagus grandifolia Ehrhart) trees, has spread across much of the North American range of American beech since being introduced into Nova Scotia around 1980 (Ehrlich 1934, Houston and O’Brien 1983). Beech scale insects secrete a white wax as they feed on sap in the sieve cells of the secondary phloem of the trunk and branches of beech trees. This wax obscures the individual scales and heavily infested trees appear to be coated with white ‘wool’ (Ehrlich 1934, Houston and O’Brien 1983). Openings in the bark created by the styles of the scale insects provide entry for the Neonectria faginata (M.L. Lohman, A.M.J. Watson & Ayers) Castl. & Rossman and Neonectria ditissima (Tul.& C.Tul.) Samuels and Rossman fungal pathogens that cause beech bark disease (Castlebury et al. 2006). The fungi kill areas of phloem, which eventually coalesce, usually leading to canopy dieback and eventual tree mortality.

Despite the long-term presence of beech scale in the United States, relatively little is known about its population dynamics and spread within and between stands (Houston et al. 1979, Morin et al. 2007, Garnas et al. 2009, Evans and Finkral 2010, Koch et al. 2010, Kasson and Livingston 2011, Wieferich et al. 2011, Cale et al. 2012, Van Driesche and Japoshvili 2012). This arises in part from the difficulty of quantifying beech scale density. Most studies of beech scale to date relied on visual estimates of wax abundance on infested trees (Wiggins et al. 2004, Houston et al. 2005, Kearny et al. 2005, Petrillo et al. 2005, Kasson and Livingston 2011). While visual estimates are efficient, they are subjective and qualitative, yield coarse results that may fail to capture small changes in populations, and estimates may vary among different observers. Although qualitative measures of scale abundance are useful for monitoring the advance of beech scale at the landscape level, they do not provide the ability to track finer-scale changes in scale density on individual trees. A more precise method to quantify beech scale would be useful for assessing effects of weather events on beech scale mortality, the influence of beech scale abundance on short range dispersal to nearby trees, and variation in beech scale reproductive success that could indicate some degree of host resistance. In a few studies, individual scale insects were counted within defined sample areas on selected trees (Wainhouse 1990, Gardner 2005, Koch et al. 2010). Counting individual scales, which are only 0.5–1 mm in size (Houston and O’Brien 1983, Wainhouse and Gate 1988),...
however, is a slow, tedious process, limiting the number and size of samples that can be examined. Wainhouse (1980), for example, used four samples, each only 1 cm² in area, to represent beech scale density for an entire tree.

Recent technological advances have resulted in the increased application of digital photography for a variety of uses, including calibrating aerial application equipment (Holownicki et al. 2002), quantifying canopy cover (Englund et al. 2000), and classifying land use (Bruzzone and Fernandez Prieto 2000). Given the relatively high contrast between the gray bark of beech trees and the white wax secreted by immobile scales, digital photography could provide a means to consistently and quantitatively assess beech scale abundance or density (Teale et al. 2009, Cale et al. 2012).

We devised and evaluated a process to quantify beech scale density using digital photography, image processing software, and the linear relationship between wax area and the actual number of beech scales derived from 104 bark samples. We then compared the efficiency and utility of this method with visual estimates of scale abundance taken from 400 beech trees in 40 sites encompassing much of the infested area in Michigan.

**Materials and Methods**

**Digital Photo Collection and Analysis.** We installed a fixed-radius plot (8 m radius) in each of 40 sites encompassing a broad range of beech scale densities in Michigan’s northern Lower Peninsula and eastern Upper Peninsula (Fig. 1) from July to August 2007. Within fixed plots, we encountered and tagged 2–22 beech trees, >6 cm in diameter at breast height (DBH) per plot. When less than eight beech trees were present in a plot, beech trees nearest the plot were included until at least eight trees were tagged. In two sites, beech trees were scarce and we were able to tag only five trees in one site and seven trees in the other. Four trees were severely declining from beech bark disease and were excluded from analyses. In total, 427 trees were tagged and used for this portion of the study, including 319 beech trees in the Lower Peninsula and 108 trees in the Upper Peninsula, with a mean (±SE) of 10 ± 1 trees per site.

Scale abundance on each beech tree was visually ranked by a single observer using a four point qualitative ranking system and reference cards, which helped to ensure consistent rankings (Wieferich 2009). Qualitative ranking was performed by walking around the tree and looking at the trunk, leader and...
large branches to locate the characteristic white wax produced by beech scale insects. Trees were ranked as 0 = absent, if no beech scale wax was visible; 1 = trace, if only a few scattered spots of wax were seen; 2 = patchy, if at least one dense patch of wax was present; and 3 = whitewashed, if wax was abundant on most of the bole and limbs.

Photographs were then taken of three locations on the trunk of each beech tree using a HP Photosmart M425 camera (Hewlett-Packard Development Company, L.P., Alto, CA) mounted on a tripod with a built-in horizontal stabilizer to ensure the camera was the same distance from the tree in every photograph. Each photo captured an area of 18.4 by 24.7 cm. Height and aspects of photos were determined using a stratified random sampling method. Photos were taken at a randomly chosen aspect at 0.9, 1.2, and 1.5 m above the base of each tree. Two photographs were taken at each point to ensure proper focus and contrast within photographs. Presence and general location(s) of beech scale in the photographed area were noted to assist in photo analysis. Presence of lichens, moss, or other organisms that could be misclassified as scale wax was also noted.

Each photograph was analyzed using the image analysis software ImageJ V1.34s (Rasband 1997–2011). We first imported JPEG images into ImageJ and selected the distance scale. The photo was scanned for evidence of beech scale (wax) before converting it to an 8-bit image. Following this conversion, we applied a binary threshold on pixel brightness that classified all pixels into two distinct groups of brightness below and above the defined threshold. Thresholds were set independently for each photo to ensure only pixels containing the white beech scale wax were selected. Thresholds varied, depending on the quality, brightness, and contrast of each photo. The selected pixels were then used to calculate the total area (square centimeter) occupied by scale wax. Density of beech scales (number per square centimeter) was estimated, using the regression equation developed from samples of infested bark (see below). The time required to estimate scale density on photos was monitored.

Relationship Between Area of Wax and Beech Scale Abundance. To evaluate the relationship between the number of beech scales and the area covered by white wax, we collected samples of infested bark from 45 beech trees growing within 100 m of the plots in ten of our 40 sites. In total, 104 samples, each 5.1 cm² in area, were collected using a bark punch between May and October 2008 from the lower 2.5 m of the trunk of trees. Up to four samples were collected from different areas on individual trees. To calibrate an accurate index, we collected similar numbers of samples from areas with sparse (n = 39), intermediate (n = 30) and high (n = 36) amounts of white wax. We used the bark punch (diameter 2.54 cm) to gently delineate the sample area on the tree and then photographed the delineated area. Once the photographs were taken, the bark punch was used to remove the sample. Samples were individually stored in ethanol until they were processed.

We recorded the area covered by scale wax within the area delineated by the bark punch in each photo. We then examined individual bark samples under a dissecting microscope and gently pulled away the wax with forceps to expose the individual scales. Individual scales were removed as they were counted to ensure all individuals were located and counted only once. Plots of the relationship between number of individual scales and the area of waxy coverage were linear, but showed substantial heteroscedasticity. As such, we used a linear regression with a heteroscedastic consistent estimator (White 1980) implemented in SAS 9.2 (SAS Institute 2008). One outlier with a residual value more than 7 SDs from the mean was dropped from this analysis. Dropping the outlier from the regression had relatively little effect on the slope (867.1 ± 85.7 with outlier; 869.7 ± 84.2 without outlier) and intercept (62.6 ± 21.0 with outlier; 45.2 ± 11.4 without outlier) of the equation.

Comparison of Beech Scale Estimates. We applied the regression equation to calculate density (number per square centimeter) of beech scales on the three photos collected from each tree. Density estimates were averaged and used as the per-tree estimate of beech scale density. We compared visual estimates of scale abundance collected in the field with estimates of scale density derived from the photos and assessed the rate at which our digital photographs detected beech scale presence in relation to visual estimates of scale density.

Results

Visual Estimates of Scale Abundance. Qualitatively estimating beech scale abundance as absent, trace, patchy or whitewashed by examining the trunk and major branches of the 427 beech trees was relatively simple and efficient. No beech scale was detected on 98 trees, while 206 trees had trace amounts of wax and 75 trees were ranked as patchy. We classed 48 heavily infested trees as whitewashed, although distribution and abundance of scale wax across the trunks of whitewashed trees was seldom uniform. Visually evaluating scale abundance in the field required ~2 min per tree. Trees that were ranked as patchy or whitewashed generally required relatively little time because of the obvious presence of the scale wax, while identifying trees that either had no beech scale or only a trace infestation required slightly more time.

Scale Density on Bark Samples. We counted up to 1,768 beech scale insects on individual 5.1 cm² samples of bark. As expected, scale density was generally associated with the area covered by wax on the bark samples. There were three samples that had sparse amounts of wax but no live scales, while other samples with similar areas of wax contained up to 59 scales. Samples where wax was abundant usually contained several hundred scales. On a few samples with abundant wax, scales were actually overlapping one another and some appeared to have no direct contact with the bark. We also observed a few samples where dead, desiccated scales were present beneath ample
amounts of wax. Dead scales were not included in the
counts used for our analysis.

Area covered by white wax in the photographs of
bark samples was positively related to the number of
live beech scales in the sample (Fig. 2). The relation-
ship was described by the equation, \( Y = 869.75x + 45.21 \), where \( Y \) = the number of scales per square
centimeter and \( x \) represents the square centimeter of
area covered by white wax, indicating that 1 cm\(^2\) of
white wax in the photo represented \( \approx 870 \) beech
scales. This equation yielded an adjusted \( r^2 \) value of
0.797 (\( P < 0.0001 \)), and a significant \( t \)-statistic for the
coefficient (\( P < 0.0001 \)) and intercept (\( P = 0.0001 \)).

When densities of beech scale were averaged using
the three photos taken of individual trees, mean scale
density (±SE) per tree ranged from 0.0001 ± 0.0002–
14.0511 ± 12.603 scales/cm\(^2\) (\( n = 392 \)). Scale densities
frequently varied among the three photographs on a
given tree (Fig. 3), particularly when trees were moder-
ately to heavily infested. For example, the three
photos taken of the single tree with the greatest
amount of wax scale yielded scale density estimates of
1.06, 14.85, and 26.24 scales/cm\(^2\).

Complete pixel analysis of the digital photographs
produced more precise and less subjective estimates
of scale density than qualitatively ranking the degree
of infestation in the field, but not surprisingly, re-
quired more time. In the field, we needed \( \sim 2 \) min to
set up and focus the camera, and take the two pho-
tographs of each area. A subset of 20 photographs of
infested bark required an average of 4.0 ± 1.41 min

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**Fig. 2.** Linear relationship between the area (square centimeter) of white wax produced by beech scale and the number of live beech scale insects on samples of bark, each 5.1 cm\(^2\), collected from the trunk of 45 American beech trees between May and October 2008 from ten sites in Michigan. The outlier represented by the triangle was dropped from the analysis.

**Fig. 3.** SD of beech scale density (number of scales per square centimeter) plotted against the mean beech scale density derived using three photographs of the tree trunk (\( n = 292 \) trees).
Table 1. Comparison of beech scale abundance from visual estimates in the field and beech scale density derived from the avg. of three digital photographs per tree (n = 292 trees)

<table>
<thead>
<tr>
<th>Visual estimate</th>
<th>Digital photograph estimates (scales/cm²)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trace</td>
<td></td>
<td>0.001</td>
<td>0.2672</td>
<td>0.02</td>
<td>0.0344</td>
</tr>
<tr>
<td>Patchy</td>
<td></td>
<td>0.0093</td>
<td>1.1006</td>
<td>0.2013</td>
<td>0.2317</td>
</tr>
<tr>
<td>Whitewashed</td>
<td></td>
<td>0.035</td>
<td>14.0512</td>
<td>1.9323</td>
<td>3.028</td>
</tr>
</tbody>
</table>

each for binary threshold processing. Photographs of bark on uninfested trees, which were also noted in the field, typically required less than a minute to visually scan to verify our initial assessment.

While binary threshold measurements successfully quantified beech scale density, we encountered some minor issues. One challenge was the lack of consistency in contrast within and between photos of individual trees when portions of the photo were covered by shadows, making threshold application more difficult. We discovered we could minimize this problem in the field by simply using a clipboard to shade the entire photo frame. The most common contrast issues across all photos included variation in bark roughness, overall photo brightness, or bark coloration. These differences did not allow us to set a common threshold across photographs, which increased processing time. In addition, the use of binary thresholds resulted in an occasional misclassification of snow, spider eggs, moss, lichens, and other light colored subjects. Compared with beech scale, however, the surface area of the misidentified objects was minimal. In most cases, the errors were identified and corrected before analysis. When misclassification was apparent, corrections were made using the paint options within ImageJ to increase contrast between the wax and bark before analysis. This process usually resolved the issues of threshold sensitivity, but required up to 10 min of additional time for analysis of individual photos. Despite additional efforts, 40 photographs from 35 trees were deemed unusable because of moss, photo clarity, or poor contrast.

Visual Estimates Versus Digital Photo Assessments. Beech scale density per tree, estimated using the photos and regression equation, generally corresponded with the visually estimated ranks of scale abundance (Table 1). We acquired at least one good photo of the trunk from 392 of the 427 trees that were evaluated visually in the field. We found no evidence of beech scale wax on any photos taken of 119 trees. We classified beech scale as absent on 100 of these 119 trees during the visual examination in the field. Of the remaining 19 trees, beech scale infestation levels were ranked either as trace (n = 17) or patchy (n = 2) and therefore, if only the photos were used, false negatives would have been generated. Of the 1241 individual photos collected from the 427 trees, beech scale was not present in 178 photos (14.3%) when other areas of the tree had at least a trace level of infestation.

Both the field examination and the digital photos detected beech scale on 273 trees. In the field, we ranked 168 of these trees as having a trace of beech scale wax, while 40 trees were ranked as whitewashed with abundant scale wax. We created a list of scale densities estimated from the photos, then sorted the list in ascending order. We found 151 of the expected 168 trees were visually ranked as trace in the field, while 16 were ranked as patchy and one was ranked as whitewashed in the field. At the other end of the list, the 40 trees with highest scale densities based on the photos included 28 trees ranked as whitewashed in the field. For 12 of the 40 trees, however, scale wax was abundant in the photos but we ranked the infestation as patchy in the field. Overall, scale density estimated from photos corresponded to the rank recorded in the field for 79% of the trees.

Discussion

Digital photography is increasingly used as a means to quantify abundance or area occupied by an organism or material of interest. Advantages of photograph analysis include reduced variability among observers and the ability to quantitatively rather than qualitatively assess differences among samples or over time. Digital photos also provide versatile and lasting records (Englund et al. 2000). Techniques similar to ours are routinely used to assess spray droplets during calibration of aerial application equipment, quantify forest canopy cover, and measure light availability in forests (Bruzzone and Fernandez Prieto 2000, Englund et al. 2000, Holownicki et al. 2002, Nobis and Hunziker 2005). Use of digital photography can likely be expanded to quantify densities of other insects with characteristics similar to beech scale. Hemlock woolly adelgid (Adelges tsugae Annand) and balsam woolly adelgid (Adelges piceae Ratzeburg), for example, are immobile for much of their life cycle and produce wax that would similarly contrast with the foliage or bark of their host trees.

Our results, along with those of Teale et al. (2009), demonstrate digital photography can be an effective means to quantify beech scale density. Biological and physical traits such as the immobility of beech scale insects (once feeding begins) and the high contrast between the scale wax and tree bark, facilitate accurate and efficient identification of beech scale wax in photographs, particularly when a threshold technique is applied. Digital photos provide a means to capture lasting records of beech scale abundance at a given time and location, and to produce quantitative and reproducible results. These qualities enable detailed analyses and comparisons of beech scale densities to be made over time, presumably facilitating evaluation of scale mortality or population growth.

Our results, however, illustrate the need to collect photos from multiple locations to accurately assess scale infestation on an individual tree. We originally expected scale density would vary most often among photos taken of trees with trace or patchy levels of scale abundance. However, even on trees that appeared to be covered by scale wax in the field, scale density consistently varied considerably among the three areas of the trunk we photographed. At least
three photographs, each capturing 400 cm² of bark surface, should be collected per tree to ensure beech scale presence will be detected if infestations are low and to generate a reasonably accurate estimate of scale density when scale wax is patchy or abundant.

Our samples of infested bark, like those evaluated by Teale et al. (2009), showed a strong positive relationship between the area of wax on the bark of infested trees and the number of scales. In our samples, there were ≈8.7 scales/mm² of bark while Teale et al. (2009) estimated 11.9 scales/mm². This discrepancy is likely attributable to differences in the number of samples evaluated, the time of year that samples were collected and the photo assessment techniques used. For example, we collected bark samples between May and early October, when adult scales were actively feeding and generating wax, but before the new cohort of scale crawlers would have settled and begun feeding. Although we occasionally encountered dead adult scales in our samples, scale wax appeared to persist throughout the summer (Wieferich 2009). In contrast, Teale et al. (2009) collected bark samples in January and April, when scales would have been inactive. Presumably the amount of wax per individual scale remaining on trees may have been reduced by harsh winter weather.

In addition, our individual bark samples (5.1 cm²) were more than five times larger than the samples (≤100 mm²) collected by Teale et al. (2009), which enabled us to evaluate a relatively wide range of wax abundance and scales density. We also found photographing large areas of bark (400 cm²) reduced the risk of missing beech scale on lightly infested trees and using binary thresholds allowed us to efficiently analyze the large photos of beech bark to quantify the area covered by scale wax. Teale et al. (2009) did not use the binary threshold method because it caused problems with subjects of low contrast. We found similar contrast issues, but used the paint options in the software to quickly manipulate these areas before implementing a binary threshold.

While digital photos provided several advantages, visual assessments of beech scale abundance, using our reference cards to ensure consistency, were efficient and yielded estimates that will be adequate for operational projects and many research scenarios. Visual assessments generally produced comparable results to those of digital photography in a fraction of the time. In addition, our digital photos captured a small proportion of the tree trunk while visual assessments encompassed the entire trunk and lower branches. When beech scale densities were low, the photographs occasionally missed areas of beech scale by chance alone. On other trees, scales appeared to be relatively abundant in areas of the upper trunk or on large branches, but were sparse on the lower three meters of the trunk, where photos were taken. This situation generated relatively high estimates of scale infestation in the field, but relatively low density estimates on the corresponding photos. These issues illustrate the importance of using the technique most suitable for specific project goals. A double-sampling approach, using both qualitative rankings and quantitative estimates with photos, may be particularly appropriate for monitoring changes in beech scale density and distribution over time.

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