Exploring Plant-Insect Interactions
Year-Round Through Field Sampling

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Abstract
Teaching ecological concepts and field methods for introductory biology courses can be challenging, especially when class sizes are large and activities or topics depend on environmental conditions that can change dramatically from semester to semester. We provide a laboratory module that can be used throughout the year, where students can explore plant-insect interactions on campus trees in urban environments using nondestructive measures. Students are provided a transparent grid, a measuring tape, and a random selection protocol to estimate gloomy scale (Melanaspis tenebricosa) insect density on preselected Acer rubrum tree trunks. Students are also given a pace-to-plant protocol to evaluate how impervious surfaces around trees in urban environments may be associated with scale insect abundance. This module highlights ecological concepts, such as population dynamics, species interactions, competition, and food webs, among others. Through this module, students also learn skills in ecological field sampling techniques, particularly how to randomly sample, how to estimate insect abundance, and how to use observation skills to inform scientific inquiry.

Key Words: ecology; field techniques; population growth factors; herbivores; trees; research design.

Introduction
Experiential and inquiry-based learning can help students better understand and internalize scientific concepts (Gormally et al., 2009; Minner et al., 2010). Furthermore, experiential and inquiry-based learning in the field, outside the laboratory, is especially important when teaching about concepts in ecology, where several different organisms from different trophic levels may be involved. However, learning outside the laboratory can be challenging with large class sizes and when observations or activities are dependent on seasonal environmental conditions. Destructive sampling methods, such as clipping branches or twigs from trees to estimate arboreal insect abundance, are not realistic for biology programs that may have hundreds of students enrolled each semester. Furthermore, when the weather is cold, most deciduous trees are leafless, insects are in diapause, and many vertebrates are hibernating. Thus teaching about ecological concepts and species interactions in an outdoor experiential setting can be challenging. Our laboratory module overcomes these challenges of large class size and seasonal change. We present a module that focuses on plant-insect interactions that can be observed year-round and encourages students to consider how urban environments affect plant-insect dynamics and how plant-insect relationships fit into larger ecosystem frameworks.

Our module includes low-cost items and an easy-to-use protocol. This module was designed for an introductory biology course for undergraduate science majors at North Carolina State University (NCSU). However, this module can also be used to teach upper-level high school biology students. This module can be used to teach several ecological concepts that are typically covered in an introductory biology course, including but not limited to assessing the role of abiotic and biotic factors in an ecosystem, evaluating how density dependent and density independent factors affect population growth, identifying species interactions, and categorizing producers and consumers in an ecosystem. This module applies to Next Generation Science Standards (NGSS) disciplinary core areas for advanced high school science courses, including “HS-LS2 Ecosystems: Interactions, Energy, and Dynamics” and “HS-LS4 Biological Evolution: Unity and Diversity” (NGSS, 2020). It also applies to NGSS crosscutting concepts, including “Patterns” and “Influence of Science, Engineering, Technology, and Science on Society and the Natural World” (NGSS, 2020). For college-level biology, it aligns with the Core Competencies and Disciplinary Practices as described in Vision and Change in Undergraduate Biology Education (AAAS, 2011).

Ecological Background
Most people on the planet today live in cities (United Nations, 2018). Unfortunately, city environments can present harsh conditions for many biota. One stressful abiotic component of the urban environment is temperature. Impervious surfaces in cities (e.g., roads and buildings) absorb and reradiate heat, making cities typically hotter than the rural areas that surround them, often called the
urban heat island effect (Oke, 1973). These hotter conditions can result in more insect pests, especially sap-feeding herbivore pests, on urban vegetation, like street trees (Parsons & Frank, 2019; Just et al., 2018; Dale & Frank, 2014; Meineke et al., 2013; Raupp et al., 2010; Hankis & Denno, 1993). Recent debate around the “green world hypothesis” (Hairston et al., 1960) has highlighted questions about what drives herbivore populations in cities. Hairston and colleagues (1960) hypothesized that natural enemies (predators and parasitoids) of herbivores play the largest role in controlling herbivore populations. However, many recent urban studies support that abiotic and density-independent factors, such as temperature, can affect herbivore populations (Parsons et al., 2019; Long et al., 2019; Just et al., 2018; Youngsteadt et al., 2017; Dale & Frank, 2014; Meineke et al., 2013). Higher temperatures for some herbivores, including many sap-feeding ones, can mean higher fecundity, faster development, and more rapid population growth (Dale & Frank, 2017; Zvereva & Kozlov, 2006).

Recent studies from NCSU have investigated the correlation between impervious surface cover (e.g., roads, sidewalks, and buildings), temperature, and herbivore abundance and their effect on tree health (Dale et al., 2016; Just et al., 2018). For example, Dale and colleagues (2016) found that as impervious cover around red maple trees in Raleigh increases, canopy temperature increases, abundance of gloomy scale insects (Melanaspis tenebricosa, Hemiptera: Diaspididae) increases, and tree condition decreases. Gloomy scale is a sap-feeding insect pest that infests tree bark and feeds on parenchyma tissue beneath (Beardsley, 1975; Just et al., 2020). Adult females are sedentary and remain on trunks, limbs, and stems for the duration of their life and can be observed on trees throughout the year (Beardsley, 1975). Since impervious surfaces are fairly constant and easily measured, Dale and colleagues (2016) devised the pace-to-plant method to help practitioners in the field estimate impervious cover around urban trees and identify impervious surface thresholds to determine the best planting location for red maples (Dale et al., 2016). Students in laboratory modules can use the pace-to-plant method to directly observe the associations between urban environments (impervious surfaces), herbivore abundance, and tree health, regardless of the time of year. Our module outlines the pace-to-plant approach and encourages students to explore how urban environments may affect herbivores on campus trees by quantifying scale insect abundance on maple tree trunks.

Counting herbivores, in this case scale insects, on tree trunks provides a nondestructive alternative to removing tree branches and counting herbivores and also helps students develop skills in field sampling and estimation. Although, scale insect abundance on a tree trunk is not the best predictor of scale abundance on a tree (Backe & Frank, 2019), visibly high densities of scales on trunks and limbs is indicative of a severe infestation, especially in places with high impervious surface. Instructors should encourage students to quantitatively observe scale density on branches too. For the most precise estimation of scales, instructors with small class sizes can destructively sample branches and count scales (see Dale et al., 2016; Backe & Frank, 2019).

### Objective

The goal of these modules is to introduce students to field sampling techniques in ecology. Students will learn the basics of research design and sampling. In particular, students will learn the importance of random sampling and sampling replication. Ecological concepts about population growth factors will also be reinforced for students through this module. Students will use observation skills to follow lines of scientific inquiry and ask questions about how characteristics of the urban environment affect plant-insect interactions.

### Setup

Prior to starting the lab, instructors must select red maple trees (Acer rubrum) on campus. Red maples are among the most common urban trees in the eastern United States (Raupp et al., 2006). This protocol works best for Acer rubrum trees, as it was designed for assessing and predicting Acer rubrum tree condition (Dale et al., 2016). For students to see the range of scale insect infestation on trees, instructors should select trees in areas with lots of impervious cover, like a parking lot, as well as those with low amounts of impervious cover, such as a college quad.

This lab module requires three hours to complete. In this module, instructors assign four students to each tree and divide them into pairs. In the laboratory, instructors provide student groups of four with two 1x10 cm transparent grids (made from graphing transparency film), a small resealable plastic bag containing six paper squares (labeled 30 cm, 35 cm, 45 cm, 50 cm, 60 cm, and 75 cm), a Winter Tree Finder booklet (Watts & Watts, 1970) (the iNaturalist app [iNaturalist, 2020] is a good alternative if an identification book is not available), two flexible measuring tapes of 150 cm (or diameter at breast height—DBH—tape), a data sheet (Appendix A, in the Supplementary Material available with the online version of this article), and two small magnifying glasses. The data collection protocol for quantifying gloomy scales on red maple trunks was adapted from methods used by Backe and Frank (2019).

Prior to going in the field, instructors ask students to devise a hypothesis explaining the relationship between impervious surface cover and scale insect abundance (Appendix B, in the Supplementary Material). Students are not given the results of the Dale and colleagues (2016) pace-to-plant research until the end of the lab, so they start the lab module with no prior knowledge of the relationship between impervious surface cover and scale insect abundance. Instructors also review the importance of good research design in science and review the “three Rs” of research design: repetition, randomization, and replication (Appendix B). Furthermore, instructors review the roles of abiotic and biotic elements, as well as density-independent and density-dependent factors, in population growth and regulation of an organism in an ecosystem (Appendix B). Instructors then provide students with information about and pictures of gloomy scales (Figure 1) so that they can train their eye to recognize them in the field. Instructors also teach students how to estimate gloomy scale abundance on trees, how to measure tree DBH, and how to use the pace-to-plant method (Dale et al., 2016) to quantify impervious cover around trees as laid out in the protocols below. While in the field, students record their observations (Appendix A). Students then return to the lab to analyze their data.

### Estimating Gloomy Scale Abundance on Campus Trees

Each pair of students in a group of four randomly selects two heights (30, 35, 45, 50, 60 or 75 cm) to assess gloomy scale populations on red maple tree trunks. Students select heights by randomly pulling labeled paper squares out of the small resealable plastic bag provided. Students do not return labeled paper squares until both pairs have randomly chosen their selected heights. Students measure height starting from the ground at the base of the tree using flexible measuring tape. Pairs of students sample each of their randomly selected
heights on both the east and west sides of the tree by placing the bottom of a 1 × 10 cm$^2$ grid horizontally onto the tree at the randomly determined height. Students will use the compass app on their smartphones to determine East and West directions. The 1 × 10 cm$^2$ grid will help students determine the approximate surface area of scale insect infestation in each square of the 1 × 1 cm$^2$ grid (Figure 1). Students estimate tree trunk surface area covered by scales as follows:

- Full squares (Figure 1) or squares more than half full of scales = 1 cm$^2$
- Half-filled square of scales = 1/2 cm$^2$
- Less than half filled with scales = 0 cm$^2$

For the chosen heights on both the east and west sides of the tree, students record the surface area infested with scale insects for each of the ten 1 × 1 cm$^2$ squares in Appendix A, Table 3, and determine the total area infested for the 1 × 10 cm$^2$ area.

**Measuring Tree DBH of Campus Trees**

Students use the measuring tape to record the circumference of the tree at breast height (approximately 4.5 feet / 137 cm from the ground). A flexible measuring tape could be used to do this. Alternatively, students can use a piece of string that they measure later with the tape measure. Students should record their data in centimeters (Appendix A, Table 2) and then calculate the DBH, using this formula: circumference = πd. They then record their answer in Table 2. If students are provided a DBH tape, they do not have to calculate DBH.

**Using the Pace-to-Plant Method to Quantify Impervious Cover around Trees**

Students use methods adopted from Dale and colleagues (2016) to quantify impervious cover around their focal trees. One student from each group stands with their back to the tree and faces the closest impervious surface edge (like a sidewalk or parking lot). The student turns 45° to the left and then walks 25 paces in a straight line (Figure 2). Students count the number of these 25 paces that land on impervious surfaces and record them (Appendix A, Table 1). Students then turn 90° to the right from their original position and repeat the process, recording the number of paces that land on impervious surfaces.

**Figure 1.** Gloomy scales. (A) a close-up image of a female gloomy scale insect with her armored covering removed. Photo courtesy of Adam Dale. (B) A picture of a heavily infested red maple trunk, where most 1 × 1 cm$^2$ grid squares are 100% full of gloomy scales. In this picture, scale insects are the black soot-like substance on the trees. Thus gloomy scale coverage in this instance is 10 cm$^2$. Many scales on tree trunks may be dead. The armored covering of scales still remains even if they are dead. For the purposes of this module all scales, alive or dead, are counted in the grid squares. Students do not have to make the distinction between alive and dead scales.

**Figure 2.** The pace-to-plant method. Students walk four transects around the tree for a total of 100 paces from the tree, 25 paces in each direction. The total number of paces that land on impervious surface (pavement, road surface, or buildings) corresponds to the percentage of impervious cover around the tree. White footprints in the image land on impervious surfaces and total to 52 paces, equating to an estimate of 52% impervious surface within a 25m radius around the tree. Graphic courtesy of Adam Dale.
surface (Figure 2). Students repeat this process two more times, such that students create four 25-pace transects originating from the tree in four directions for a total of 100 paces. The sum of paces that landed on impervious surface equals the estimated percent cover of impervious surfaces around the tree. One student serves as a spotter to watch for cars to ensure everyone’s safety.

○ Analyzing & Understanding the Results

Once back in the laboratory, students calculate the total surface area of gloomy scale infestation on the east and west sides for each height of each tree. They then calculate the average percentage of coverage of scale insects on trunks for each tree for the whole group. Students also record the estimated percentage of impervious surface around each tree. All students share their data with the instructor or in a document that can be viewed by all students, and the instructor and/or the students use the data to construct a scatter plot with percentage of impervious surface data on the x axis and percentage of scale insect coverage on the y axis (Figure 3). Students also make bar graphs comparing the percentage of scale infestation and the percentage of impervious cover around trees in highly impervious areas to those in areas with less impervious surface (Figure 4). Once students construct plots, they visually assess if a relationship exists between percentage of coverage of scale insect infestation on tree trunks and percentage of coverage of impervious surfaces around trees. Once students complete graphs, then the instructor can run correlation tests in Excel, R, or other statistical software to see if a relationship exists (as shown previously).

Figure 3. Sample graph from a class illustrating the relationship between impervious cover around trees and gloomy scale abundance for all trees sampled by the class. Students assess the relationship between impervious cover around trees and scale insect abundance by sharing with the class the data collected for the two trees they sampled and creating a scatter plot of the class data to visually assess if a trend exists. In this case, six trees were sampled in Area A and six trees in Area B. Each student group sampled one tree from each area. Trees from Area A were surrounded by more impervious cover and had more scale insects. Trees from Area B were surrounded by less impervious cover and had fewer scale insects. Letters by points in the graph indicate the sample area of the tree. In more advanced classes, students can run correlation tests in Excel, R, or other statistical software to see if a relationship exists (as shown previously).

Figure 4. Sample graph from a student group highlighting the relationship between impervious cover around trees and scale insect abundance for the two trees they sampled. Students assess the relationship between categorical impervious cover and scale insect abundance on trees with bar graphs. Area A is the area with trees that have high amounts of surrounding impervious cover, and Area B has trees with low impervious cover. Trees with more than 62% impervious cover around trees are most likely to be heavily infested with scale insects and in poor condition (Dale et al., 2016). Each group makes a bar graph for the tree they sampled in each area. They later share their results with the class, and make a scatter plot of the class data for all trees sampled by all groups (Figure 3).

Students share the results of the Dale and colleagues (2016) paper, so students can compare their results to previous research, assess whether they support or refute their initial hypothesis, and answer post-lab questions. This activity applies to NGSS science and engineering practices for “Analyzing and Interpreting Data.” Students answer post-lab questions to reinforce ecological concepts learned in the lab, primarily how different “factors affect populations in ecosystems at different scales” (NGSS HS-LS2-2) (Appendix B). More specifically, students are encouraged to consider what abiotic and biotic elements, as well as density independent and density dependent factors, may be acting on populations of scale insects (Appendix B). Students also are asked to apply what they have learned to help devise “a solution to mitigate adverse impacts of human activity on biodiversity” (NGSS HS-LS4-6). Students are encouraged to consider where red maples should be planted to ensure tree health and promote biodiversity on campus, an area in which human impact is high. Instructors can also include post-lab questions about species interactions, food webs, competition, and trophic levels to reinforce other key ecological concepts covered in course curriculum and address other NGSS standards. Lastly, post-lab questions also prompt students to review good practices in research design.

○ Conclusions

Our module facilitates student inquiry about plant-insect interactions in urban spaces while teaching students valuable skills in research design. Students devise and test a hypothesis as it relates to impervious surface cover and scale insect abundance on urban red maple trees. Students also identify elements of good research design (repetition, randomization, and replication) and apply them to the observational study they conduct during the lab. Lastly, this module reinforces...
several ecological concepts about population growth factors taught in introductory biology courses. Instructors can add other pre- and post-lab questions to reinforce concepts about trophic levels, food webs, and species interactions, among other ecological concepts.

Students find this module engaging and appealing. Students expressed enthusiasm when surveyed after participating in our module, describing that they liked being a part of current research in the field. As one teaching assistant described, “Overall I think they [the students] enjoyed it. They were definitely more interested since it was connected to actual research being done at NCSU.” We think this module provides an excellent path forward for teaching students about key ecological concepts and equipping them with valuable skills in research design that will serve them well as they advance in their STEM education.

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References

American Association for the Advancement of Science (AAAS). (2011). Vision and Change in Undergraduate Biology Education: A Call to Action.

Backe, K.M. & Frank, S.D. (2019). Chronology of gloomy scale (Hemiptera: Diaspididae) infestations on urban trees. *Environmental Entomology*, 48(5), 1113–20. https://doi.org/10.1093/ee/nvz094

Beardsley, J.W. & Gonzalez, R.H. (1975). The biology and ecology of armored scales. *Annual Review of Entomology*, 20, 47–73.

Dale, A.G. & Frank, S.D. (2014). The effects of urban warming on herbivore abundance and street tree condition. *PLOS ONE*, 9, 1–10. https://doi.org/10.1371/journal.pone.0102996

Dale, A.G. & Frank, S.D. (2017). Warming and drought combine to increase pest insect fitness on urban trees. *PLOS ONE*, 12, 1–14. https://doi.org/10.1371/journal.pone.0173844.

Dale, A.G. & Frank, S.D. (2018). Urban plants and climate drive unique arthropod interactions with unpredictable consequences. *Current Opinion Insect Science*, 29, 27–33.

Dale, A.G., Youngsteadt, E. & Frank, S.D. (2016). Forecasting the effects of heat and pests on urban trees: Impervious surface thresholds and the ‘pace- to-plant’ technique. *Arboriculture & Urban Forestry*, 42(3), 181–91.

Gormally, C., Brickman, P., Hallar, B. & Armstrong, N. (2009). Effects of inquiry-based learning on students’ science literacy skills and confidence. *International Journal for the Scholarship of Teaching and Learning*, 3(2). https://doi.org/10.20429/ijsotl.2009.030216.

Hanks, L.M. & Denno, R.F. (1993). Natural enemies and plant water relations influence the distribution of an armored scale insect. *Ecology*, 74, 1081–91.

Hairston, N.E., Smith, F.E. & Slobodkin, L.B. (1960) Community structure, population control, and competition. *American Naturalist*, 94, 421–25.

Huberty, A.F. & Denno, R.F. (2004). Plant water stress and its consequences for herbivorous insects: A new synthesis. *Ecology*, 85, 1383–98. iNaturalist. https://www.inaturalist.org.

Just, M., Frank, S. & Dale, A. (2018). Impervious surface thresholds for urban tree site selection. *Urban Forestry & Urban Greening*, 34, 191–46. https://doi.org/10.1016/j.ufug.2018.06.008.

Just, M.G., Dale, A.G. & Frank, S.D. (2020). Gloomy scale (Hemiptera: Diaspididae) ecology and management on landscape trees. *Journal of Integrated Pest Management*.

Long, L.C., D’Amico, V. & Frank, S.D. (2019). Urban forest fragments buffer trees from warming and pests. *Science of the Total Environment*, 658, 1523–30.

Meineke, E.K., Dunn, R.R., Sexton, J.O. & Frank, S.D. (2013). Urban warming drives insect pest abundance on street trees. *PLOS ONE*, 8, 1–7. https://doi.org/10.1371/journal.pone.0059687.

Minner, D.D., Levy, A.J. & Century, J. (2010). Inquiry-based science instruction—What is it and does it matter? Results from a research synthesis years 1984–2002. *Journal of Research in Science Teaching*, 47(4), 794–796.

Next Generation Science Standards (NGSS). https://www.nextgenscience.org.

Oke, T.R. (1973). City size and the urban heat island. *Atmospheric Environment*, 7, 769–79. https://doi.org/10.1016/0004-6981(73)90140-6.

Parsons, S.E. & Frank, S.D. (2019). Urban tree pests and natural enemies respond to habitat at different spatial scales. *Journal of Urban Ecology*, 1–15. https://doi.org/10.1093/jue/juz010.

Parsons, S.E., Sozanski, K.S., Wilson, A.A. & Frank, S.D. (2019). Effects of temperature and habitat complexity on an urban tree pest (Tinocallis kahalalani), natural enemies, and predation services in the city. *Urban Ecosystems*. https://doi.org/10.1007/s11252-019-00900-7.

Rauupp, M. J., Cumming, A.B. & Rauupp, E.C. (2006). Street tree diversity in eastern North America and its potential for tree loss to exotic borers. *Arboriculture Urban Forestry*, 32, 297–304.

Rauupp, M.J., Shrewsbury, P.M. & Herms, D.A. (2010). Ecology of herbivorous arthropods in urban landscapes. *Annual Review of Entomology*, 55, 19–38. https://doi.org/10.1146/annurev-ento-112008-085351.

Shrewsbury, P.M. & Rauupp, M.J. (2000). Evaluation of components of vegetational texture for predicting azalea lace bug, *Stephanitis pyrioides* (Heteroptera: Tingidae), abundance in managed landscapes. *Environmental Entomology*, 29, 919–26.

Tooker, J.F. & Hanks, L.M. (2000). Influence of plant community structure on natural enemies of pine needle scale (Homoptera: Diaspididae) in urban landscapes. *Environmental Entomology*, 29, 1305–11. https://doi.org/10.1603/0046-225X-29.6.1305.

United Nations. (2018). *World Urbanization Prospects: The 2018 Revision*. Department of Economic and Social Affairs. https://population.un.org/wup/Publications/Files/WUP2018-Report.pdf.

Watts, M.T. & Watts, T. (1970). *Winter Tree Finder: A Manual for Identifying Deciduous Trees in Winter*. Nature Study Guild.

Youngsteadt, E., Ernst, A.F., Dunn, R.R. & Frank, S.D. (2017). Responses of arthropod populations to warming depend on latitude: Evidence from urban heat islands. *Global Change Biology*, 23(4), 1436–47. https://doi.org/10.1111/gcb.13550.

Zvereva, E.L. & Kozlov, M.V. (2006). Consequences of simultaneous elevation of carbon dioxide and temperature for plant-herbivore interactions: A metanalysis. *Global Change Biology*, 12, 27–41. https://doi.org/10.1111/j.1365-2486.2005.0086.x.

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