Journal of Visualized Experiments

A Method for Quantifying Foliage-Dwelling Arthropods

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE60110R3
Full Title:	A Method for Quantifying Foliage-Dwelling Arthropods
Section/Category:	JoVE Environment
Keywords:	arthropod abundance, arthropod density, arthropod herbivory, capture, community, diversity, foliage, population, richness
Corresponding Author:	Michael Eichholz Southern Illinois University Carbondale, Illinois UNITED STATES
Corresponding Author's Institution:	Southern Illinois University
Corresponding Author E-Mail:	eichholz@siu.edu
Order of Authors:	Michael Eichholz
	Kevin Sierzega
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Carbondale, IL, Jackson

TITLE:
A Method for Quantifying Foliage-Dwelling Arthropods

AUTHROS AND AFFILIATIONS:
Michael W. Eichholz[1], Kevin P. Sierzega[1]

[1]Cooperative Wildlife Research Laboratory, Center for Ecology, Department of Zoology, Southern Illinois University, Carbondale, IL, USA

Corresponding Author:
Michael W. Eichholz (eichholz@siu.edu)

Email Address of Co-author:
Kevin P. Sierzega (ksierzega2@gmail.com)

KEYWORDS:
arthropod abundance, arthropod density, arthropod herbivory, capture, community, diversity, foliage, population, richness

SUMMARY:
We describe how to quantify leaf dwelling arthropods by sealing the leaves and end of branches in a bag, clipping and freezing the bagged material, and rinsing the previously frozen material in water to separate arthropods from the substrate for quantification.

ABSTRACT:
Terrestrial arthropods play an important role in our environment. Quantifying arthropods in a way that allows for a precise index or estimates of density requires a method with high detection probability and a known sampling area. While most described methods provide a qualitative or semi-quantitative estimate adequate for describing species presence, richness, and diversity, few provide an adequately consistent detection probability and known or consistent sampling areas to provide an index or estimate with adequate precision to detect differences in abundance across environmental, spatial, or temporal variables. We describe how to quantify leaf-dwelling arthropods by sealing the leaves and end of branches in a bag, clipping and freezing the bagged material, and rinsing the previously frozen material in water to separate arthropods from the substrate and quantify them. As we demonstrate, this method can be used at a landscape scale to quantify leaf-dwelling arthropods with adequate precision to test for and describe how spatial, temporal, environmental, and ecological variables influence arthropod richness and abundance. This method allowed us to detect differences in density, richness, and diversity of leaf-dwelling arthropods among 5 genera of trees commonly found in southeastern deciduous forests.

INTRODUCTION:
Terrestrial arthropods play an important role in our ecosystem. In addition to being of scientific interest arthropods can be both detrimental and beneficial to crops, horticultural plants, and natural vegetation as well as provide an important trophic function in food webs. Thus,

understanding the factors that influence arthropod community development and abundance is critical to farmers, pest control managers, plant biologists, entomologists, wildlife ecologists, and conservation biologists that study community dynamics and manage insectivorous organisms. Understanding factors that influence arthropod communities and abundances often requires the capture of individuals. Capture techniques can generally be categorized into qualitative techniques that only detect presence of a species for estimates of species range, richness, and diversity, or semi-quantitative and quantitative techniques that allow for an index or estimate of abundance and density of individuals within a taxonomic group.

Qualitative techniques that only allow inference regarding presence of a species or community structure have an unknown or intrinsically low detection probability or are lacking in providing inference regarding detection probability and size of area sampled. Because detection probability with these techniques is low, variability associated with detection precludes adequate precision for inferring how explanatory variables influence arthropod population metrics. Qualitative techniques used to estimate presence include suction sampling[1], light traps[2], emergence traps[3], feeding patterns on roots[4], brine pipes[5], baits[6], pheromone[3], pitfall traps[7], Malaise traps[8], window traps[9], suction traps[10], beating trays[11], spider webs[12], leaf mines, frass[13], arthropod galls[14], vegetation and root damage[15].

Alternatively, semi-quantitative and quantitative techniques allow researchers to estimate or at least consistently sample a specified sample area and estimate probability of detection or assume detection probability is non-directional and adequate as to not obscure the researcher's ability to detect spatial or temporal variation in abundance. Semi-quantitative and quantitative techniques include sweep nets[16], suction or vacuum sampling[17], systematic counting of visible arthropods[18], sticky traps[19], various pot-type traps[20], entrance or emergent holes[21], chemical knockdown[22], sticky and water filled color traps[23], and branch bagging and clipping[24].

Recent anthropogenic-induced changes to climate and disturbance regimes have led to dramatic changes in plant communities, making interactions between plant-community species composition and arthropod communities an active area of study. Understanding how arthropod communities vary with plant species composition is a critical component for understanding the potential economic and environmental impacts of changes to plant communities. Semi-quantitative or quantitative methods of quantifying arthropod abundance with adequate precision to detect differences among species of plants are needed. In this article, we describe a method for indexing foliage-dwelling arthropods that, with reasonable effort, provided adequate precision to identify differences in individual abundance and biomass, diversity, and richness among 5 taxa of trees commonly found in the southeastern deciduous forests of North America[25]. This approach provided precision adequate for estimating abundance to allow inference as to how changes in species composition of forest plant communities due to anthropic modified disturbance regimes influence composition of arthropods, potentially influencing abundance and distribution of higher trophic insectivorous birds and mammals. More specifically, by using a modified bagging technique first described by Crossley et al.[24], we estimated density of surface, foliage-dwelling arthropods and tested the prediction that we would detect differences in diversity, richness, and abundance of arthropods in the foliage of faster growing more xeric

89 species of trees relative to slower growing more mesic species. The goal of this article is to
90 provide detailed instructions of the technique.
91
92 We conducted the study on the Shawnee National Forest (SNF) in southern Illinois. The SNF is a
93 115,738-ha forest located in the Central Hardwoods region of the Ozarks and Shawnee Hills
94 natural divisions[26]. The forest comprises a mosaic of 37% oak/hickory, 25% mixed-upland
95 hardwoods, 16% beech/maple, and 10% bottomland hardwoods. The SNF is dominated by
96 second growth oak/hickory in upland xeric areas and sugar maple, American beech, and tulip tree
97 (*Liriodendron tulipifera*) in sheltered mesic valleys[27,28].
98
99 Site selection for this method will be dependent on the overarching goals of the study. For
100 example, the primarily goal of our original study was to provide insight into how changes in tree
101 community might influence higher trophic organisms by comparing foliage-dwelling arthropod
102 community metrics between mesic and xeric adapted tree communities. Thus, our primary
103 objective was to quantify the arthropod community on individual trees located within the xeric
104 or mesic tree community. We selected 22 study sites along an oak/hickory (xeric) to beech/maple
105 (mesic) dominated gradient using USFS stand cover maps (allveg2008.shp) in ArcGIS 10.1.1. To
106 prevent potential confounding effects, we selected sites using the following criteria: not located
107 in riparian areas, ≥12 ha, and located within contiguous upland-deciduous forest habitat (i.e.,
108 elevation above 120 m). All sites contained mature trees >50 years old in hilly terrain, thus
109 comprised similar slopes and aspects. While beech/maple site boundaries were distinguished
110 based on the transition of tree communities, oak/hickory site boundaries were identified
111 artificially using SNF cover maps and ArcGIS 10.1.1. All sites were large forest blocks within un-
112 glaciated terrain; their differences in tree species composition were not due to differences in
113 location on the landscape but were representative of past land usage (e.g., clear cuts or selective
114 harvest). We ground-truthed the maps by uploading discrete polygon shapefiles of each study
115 site to a handheld Global Positioning System (GPS) and verifying tree species composition. We
116 randomly selected sampling points (n = 5) at each site. At each point, we sampled three trees
117 from 0600−1400 hours during 23 May to 25 June 2014. To locate sample trees, we searched
118 outward to a 30 m radius from vegetation points until mature trees (>20 cm d.b.h.) with branches
119 low enough to sample were found. Typically, the three mature trees that represented three of
120 the five genera (*Acer, Carya, Fagus, Liriodendron,* and *Quercus*) of interest and were closest to
121 the center point were sampled.
122
123 **PROTOCOL:**
124
125 **1. Building the sampling device prior to going to the field**
126
127 1.1. Using bolt cutters, large wire cutters, or an electric grinding disk, remove the bottom 1/3 of
128 the 30 cm wire tomato cage so that it is approximately 55 cm in length.
129
130 1.2. Cut two, 30 cm braces made from aluminum tube or similarly semi-rigid material to use as
131 attachment rods and braces on each side of the largest end of the tomato cage. Attach one end
132 of each of the two attachment rods to opposite sides of the tomato cage with zip ties and duct

or electrical tape ensuring the tape is wrapped around at least 6 cm of the cage and rod. Be certain to wrap the tape around the cage and rod numerous times to ensure the cage is permanently attached to the rod.

1.3. Attach the other end of each of the two attachment rods on the opposite sides of the end of an extendable pole with zip ties and duct or electrical tape. As before, wrap the tape multiple times to affix it permanently ensuring the tape overlaps the pole and rods by at least 6 cm. Be certain the opening of the cage is in contact with the end of the telescoping pole when the cage is attached.

1.4. Attach the cage directly onto the end of the pole using zip ties and electrical or duct tape. Attach hook-and-loop fastener strips at 3 points to the opening of the cage.

NOTE: These strips will be use later to keep the bag open.

2. Enclosing the branch

2.1. Attach 3 pieces of hook-and-loop fastener to the opening of the bag so they align with the hook-and-loop fastener attached to the opening of the cage. These will be used to hold the opening of the bag in place while it is brought over a sample branch. Be certain the hook-and-loop fastener is aligned so when the bag is inserted and attached, the opening to the pull strings of the bag run parallel to the telescoping pole.

2.2. Insert a ~49 L kitchen garbage bag in the wire tomato cage. Place one gator clip on each respective side of the bottom of the bag and attach the clips to both the bag and wire cage to hold the bag against the cage. Repeat the same procedure for the top of the bag with gator clips and attach the opening of the bag to the opening of the cage.

2.3. Orient the gator clips so they are perpendicular to both the telescoping pole and the openings to the draw strings as to run parallel with the telescoping pole. Be certain to leave adequate space near the opening of the bag to allow the bag to close when the draw string is pulled.

2.4. Attach para cord to each of the bag's two draw strings. Cut plastic tubing in one-inch sections and attach with duct or electrical tape at three equidistance locations (i.e., one each along the top, middle and bottom) along the non-extendable outside of the telescoping pole. Run both para cord strings together through the plastic tubing inserts to hold them in place.

2.5. For each sample tree, use a random number generator to select a sample height that is within the height of the extension pole when extended at maximum length. Use a random number generator to select a sample distance from the tree trunk. Identify a branch that will fit in the bag with minimal disturbance to the foliage and is the height and distance from the trunk based on the numbers generated from the random number generator.

2.6. Raise the sampling pole to a height parallel with the desired branch. Quickly slide the bag over the branch then rapidly pull the para cord strings attached to the draw strings on the bag to seal the bag. Practice this a few times prior to the first attempt to become efficient at incorporating the foliage with minimal disturbance to the leaves.

2.7. Have a second person clip the branch at the location adjacent to the bag's opening with the extension pole pruner. Carefully bring the sample bag to the ground and rapidly tie the bag's draw strings closed. Attempt to complete the bagging, cutting, and bag-tying steps as quickly as possible to prevent insects from escaping.

2.8. Store the bagged branch in a freezer until ready to conduct the laboratory arthropod analysis.

3. Arthropod analysis

3.1. Hold the frozen bag and branch upright and shake the sample branch while in the bag to dislodge arthropods into the bag. Carefully remove the branch and rinse in large collection pan to remove remaining arthropods. Empty remaining material from the bag into the collection pan. Remove any non-arthropod debris.

3.2. Separate arthropods into desired taxonomic groups. Note differences between larvae and adults.

3.3. Quantify arthropods as desired. If biomass is of interest, either measure length of arthropods and use published length mass table to estimate biomass, or place arthropods in small drying pans, dry in drying oven for 24 h at 45 °C, and weigh on an electronic balance.

4. Estimating density

4.1. To estimate density and control for variation in leaf structure and leaf density between samples within tree species and among tree species either:

4.1.1. Count and measure the surface area of the leaves from each sample.

4.1.2. Dry the leaves in a drying oven for 48 h at 45 °C and weigh the leaves on an electronic balance.

4.1.3. Measure the length of all woody branch within the sample.

NOTE: Diel differences occur in arthropod communities, so sampling should be conducted throughout the entire period of inference.

REPRESENTATIVE RESULTS:

We collected 626 samples from 323 individual trees composing 5 tree groups. For estimates of total arthropod biomass per meter of branch sampled, the standard error ranged from 12% to 18% of the mean for the 5 tree groups (**Table 1**). This level of precision was adequate to detect variation among tree groups and a quadratic change in biomass with date[25]. This technique provided more precision when estimating guild diversity as demonstrated by the standard error of arthropod guild diversity (*H'*) ranging from 3% to 7% of the mean diversity across the 5 tree groups (**Table 1**). Precision at this level was adequate to detect variation across the 5 tree groups[25]. Precision of estimates of richness was also very good as demonstrated by standard errors that ranged from 3% to 6% of the mean richness among the 5 tree groups (**Table 1**). This level of precision was adequate to identify variation among tree groups, a quadratic association with date, decrease in richness with height on the tree, and a positive relationship between arthropod richness and distance from the tree trunk[25].

TABLE LEGENDS:

Table 1: Parameter estimates from most parsimonious model[25]. The mean (X), standard error of the mean (SE), and percentage of the mean of the standard error for each community metric of foliage dwelling-arthropods captured on 5 groups of trees using the described branch clipping method in the Shawnee National Forest in southern Illinois.

DISCUSSION:

Two necessities of accurately quantifying arthropod communities are relatively high detection probabilities and known or consistent sampling areas. When sampling for arthropods, less than 100% detection probability can be attributed to either individual arthropods avoiding traps or some individuals that were trapped being undetected during processing. Interceptor traps that intercept flying arthropods (Malaise/window traps, sticky traps, etc.) appear to be the most frequently used approach to enumerate arthropod communities in the forest canopy[29,30,31]. These types of traps can be placed throughout the canopy, are effective at intercepting flying arthropods, and typically preserve arthropods for long periods (weeks or months) for later identification and quantification[29,30,31], though they are typically limited in their ability to trap crawling arthropods[31]. Interceptor traps that attract arthropods using light or pheromones have additional limitations in that they trap only night flyers and their attractiveness varies with taxon, moonlight, background illumination, and cloud cover impact[32,33]. Additionally, because arthropods captured in interceptor traps are from unknown distances, the area trapped is unknown. As such, although interceptor traps are effective for indexing flying arthropods across an environmental gradient, data produced from interceptor traps cannot be used to estimate arthropod density[25].

An additional method frequently used to monitor foliage arthropods is chemical knockdown[34,35]. Chemical knockdown can be very effective for collecting diverse groups of arthropods providing accurate estimates of taxonomic richness and diversity. However, this method is expensive and time consuming, is non-specific as it samples all arthropods on the tree including those on the bark and branches, may have unintended environmental impacts due to wind drift, and is illegal in some areas[36,37,38,39].

Branch bagging has been demonstrated as an effective method to estimate arthropod density from surface tree foliage with an adequately high capture probability to detect variation across various environmental gradients[24,40]. The wire tomato cages and 49 L garbage bags used in this study allowed researchers to encompass the branch fully with little to no disturbance prior to the closure of the opening of the bag. As such, it is important that researchers are careful to not disturb foliage of the desired branch sample prior to enclosing it with the sampling bag. Thus, a critical step is to bring the sampling bag parallel with the desired sampling bag and swiftly enclose, seal, and tie the bag after each sample is collected. Sample collection is limited to the maximum height the researcher can hold an extended telescoping pole at (8 m in our study), although the same branch-bagging equipment and methodology can be used in other situations such as suspension in the canopy. Some authors have suggested that when using this procedure active, flighted-arthropods are underrepresented[40,41,42]. However, we believe that as long as the foliage remains undisturbed until it is enclosed by the sampling bag, it is unlikely that a substantial number arthropods present in or on the foliage at the time escaped capture. The results of our study support this assertion in that when sampling a reasonable number of trees (323), the standard error was at most 17% of the relative arthropod biomass mean (*Cayra* = 11%, *Acer* = 12%, *Fagus* = 17%, *Liriodendrum* = 15%, and *Quercus* = 11%). Similarly, when considering guild richness and diversity, the most variable estimate was diversity on *Fagus*, with a standard error that was 7% of the mean. Clearly these estimates provided adequate precision to model differences among tree genera groups as well as other ecological or environmental variables. A limitation to our results, however, is that although we are confident that detection probability with this method is high, i.e., likely nearing 100%, we do not have a method of independently verifying this assertion. Thus, while we demonstrated detection probability is adequate to detect variation across an environmental variable, which in this case was tree genera, biomass estimates produced from this methodology have the potential to be biased low by some unknown amount[40].

Most authors have examined the content of the bag in the field[36,42,43,44,45]. We believe a critical step to maximizing detection is to freeze the bag as we did, then examine and quantify the content in the laboratory under controlled conditions. We believe this approach will decrease measurement error by minimizing the number of trapped arthropods that are overlooked or are misidentified.

Estimating the area sampled for comparison of density among tree species may be problematic if leaf structure varies considerably among tree species, as was the case in our study. In past studies, when authors were interested in quantifying foliage-dwelling arthropods, they often estimated sampling area by weighing leaves to estimate the amount of substrate available for arthropods[46,47,48]. The various species of oak trees, however, tend to have thicker waxy leaf cuticles than other tree species. Thus, the mass to surface area ratio for oaks is greater than other species[49]. Because the mass to surface area ratio is greater in oaks, using the mass of leaves as an estimate of substrate for foliage dwelling arthropods would overestimate sampling area and underestimate arthropod density for oak trees relative to tree species with less thick leaf cuticles. Additionally, if the ability to support arthropods varies among tree species, the surface area of

the landscape covered by a given tree species will dictate the level of substrate supported within a specified landscape. Because the amount of surface area a given tree occupies is determined by crown spread (i.e., branch spread outwards from the trunk) and leaf density varies among trees, we believe when quantifying arthropods for consumption by insectivores, total branch length sampled is more appropriate than leaf biomass when estimating total area sampled. Our results again appear to support this assertion in that we detected differences among tree groups consistent with the predicted pattern based on previous studies[25]. We believe arthropod abundance or biomass per measure of branch length is most appropriate when the primary objective is to compare resources provided for insectivores among tree species. If, however, individuals are comparing tree species that produce leaves with similar leaf cuticle thickness, using leaf biomass as an estimate of sampling area may be more appropriate. Regardless of whether researchers use actual leaf area, leaf area as estimated by leaf biomass, or total branch length as a quantifiable metric, by using the bagging technique, a measurable quantity of arthropods at a specific point in time on a measurable surface area is captured per sample. This allows researchers to use leaf surface area, leaf area as estimated by leaf biomass, or total branch length as a quantifiable metric. This method provides a consistent estimate for comparing quantified arthropods among spatial or temporal variables and an estimate of arthropod density[25].

In general, the sampling method described in this article appears to be effective at allowing for spatial or temporal comparisons of foliage-dwelling arthropod metrics. This approach is affordable and feasible at the landscape scale. Furthermore, although freezing the entire branch requires substantial freezer space, freezing the branch then rinsing the branch in water is an effective way to separate arthropods from foliage with minimal effort, therefore providing a cost-efficient approach to obtaining arthropod metrics. Finally, because the primary objective of our original study was to better understand how mesophication of southeastern deciduous forests is likely to impact forest-dwelling insectivorous birds and mammals we grouped arthropods into guilds based on diagnostic morphological features. However, we do not see a reason why these capture techniques cannot be used to quantify arthropods at the species or any other taxonomic level.

ACKNOWLEDGMENTS:
The authors would like to thank the U.S. Department of Agriculture Forest Service for funding this project through USFS Agreement 13-CS-11090800-022. We would like to thank J. Suda, W. Holland, and others for laboratory assistance, and R. Richards for field assistance.

DISCLOSURES:
The authors have nothing to disclose.

REFERENCES:
1. Arnold, A. J. Insect sampling without nets, bags, or filters. *Crop Protection*. **13**, 73-76 (1994).
2. Roberts, R. J., Campbell, A. J., Porter, M. R., Sawtell, N. L. Funturations in the abundance of pasture scarbs in relation to Eucalyptus trees. In *Proceeding of the 3rd Australian Conference on*

Grassland Invertebrate Ecology. Edited by Lee, K. E., 75-79, SA Government Printer. Adelaide (1982).
3. Southwood, T. R. E., Henderson, P. A. *Ecological Methods*. Blackwell Science. Oxford (2000).
4. Masters, G. J. Insect herbivory above- and belowground: individual and joint effect on plant fitness. *Ecology*. **79**, 1281-1293 (1995).
5. Stewart, R. M., Kozicki, K. R. DIY assessment of leatherjacket numbers in grassland. In *Proceedings of the crop protection in North Britain Conference*. 349-353, Scottish Crop Research Institute. Dundee (1987).
6. Ward, R. H., Keaster, A. J. Wireworm baiting: use of solar energy to enhance early detection of *Melanotus depressus*, *M*. *verberans*, and *M*. *mellillus* in Midwest cornfields. *Journal of Economic Entomology*. **70**, 403-406 (1977).
7. Barber, H. S. Traps for cave inhabiting insects. *Journal of the Elisha Michell Scientific Society*. **46**, 259-266 (1931).
8. Malaise, R. A new insect trap. *Entomologisk Tidskrift*. **58**, 148-160 (1937).
9. Peck, S. B., Davis, A. E. Collecting small beetles with large-area "window traps". *Coleopterists Bulletin*. 34, 237-239 (1980).
10. Taylor, L. R. An improved suction trap for insects. *Annals of Applied Biology*. **38**, 582-591 (1951).
11. White, T. C. R. A quantitative method of beating for sampling larvae of *Selidosema suavis* (Lepidoptera: Geometridae) in plantations in New Zealand. *Canadian Entomologist*. **107**, 403-412 (1975).
12. Ozanne, C. M. Techniques and methods for sampling canopy insects. In *Insect Sampling in Forest Ecosystems*. Edited by Leather, S. R., 146-167, Blackwell Publishing. Malden, MA (2005).
13. Sterling, P. H., Hambler, C. Coppicing for conservation: do hazel communities benefit? In *Woodland conservation and research in the Clay Veil of Oxfordshire and Buckinghamshire*. Edited by Kirby, K., Wright, F. J., 69-80, Research and Survey in Nature Conservation 15. NCC, Peterborough (1988).
14. Fidgen, J. G., Teerling, C. R., McKinnon, M. L. Intra- and inter-crown distribution of eastern spruce gall adelgid*, Adelges abietis* (L.), on young white spruce. *Canadian Entomologist*. **126**, 1105-1110 (1994).
15. Prueitt, S. C., Ross, D. W. Effect of environment and host genetics on *Eucosma sonomana* (Lepidopter; Tortricidae) infestation levels. *Environmental Entomology*. **27**, 1469-1472 (1998).
16. Gray, H., Treloar, A. On the enumeration of insect populations by the method of net collection. *Ecology*. **14**, 356-367 (1933).
17. Dietrick, E. J. An improved backpack motor fan for suction sampling of insect populations. *Journal of Economic Entomology*. **54**, 394-395 (1961).
18. Speight, M. R. Reproductive capacity of the horse chestnut scale insect, *Pulvinaria regalis* Canard (Hom., Coccidae). *Journal of Applied Entomology*. **118**, 59-67 (1994).
19. Webb, R. E., White, G. B., Thorpe, K. W. Response of gypsy moth (Lepidoptera: Lymantriidae) larvae to sticky barrier bands on simulated trees. *Proceeding of the Entomological Society of Washington*. **97**, 695-700 (1995).
20. Agassiz, D., Gradwell, G. A trap for wingless moths. *Proceedings and Transactions of the British Entomological and Natural History Society*. **10**, 69-70 (1977).

21. Lozano, C., Kidd, N. A. C., Jervis, M. A., Campos, M. Effects of parasitoid spatial hererogeneity, sex ratio and mutual interaction between the olive bark beetle *Phloeotribus scarabaeoides* (Col. Scolytidae) and the pteromalid parasitoid *Cheiropachus quardrum* (Hum. Pteromalidae). *Journal of Applied Entomology*. **121**, 521-528 (1997).
22. Roberts, H. R. Arboreal Orthoptera in the rain forest of Costa Rica collected woith insecticide: a report on grasshoppers (Acrididae) including new species. *Proceedings of the Academy of Natural Sciences, Philadelphia*. **125**, 46-66 (1973).
23. Disney, R. H. L. et al. Collecting methods and the adequacy of attempted fauna surveys, with reference to the Diptera. *Field Studies*. **5**, 607-621 (1982).
24. Crossley, D. A. Jr., Callahan, J. T., Gist, C. S., Maudsley, J. R., Waide, J. B. Compartmentalization of arthropod communities in forest canopies at Coweeta. *Journal of the Georgia Entomological Society*. **11**, 44-49 (1976).
25. Sierzega, K. P., Eichholz, M. W. Understanding the potential biological impacts of modifying disturbance regimes in deciduous forests. *Oecologia*. **189**, 267-277 (2019).
26. Schwegman, J. The natural divisions of Illinois. In *Guide to the vascular flora of Illinois*. Edited by Mohlenbrock, R. H., 1-47, Southern Illinois University Press. Carbondale, IL (1975).
27. Fralish, J. S., McArdle, T. G. Forest dynamics across three century-length disturbance regimes in the Illinois Ozark hills. *American Midland Naturalist*. **162**, 418-449 (2009).
28. Thompson, F. R. The Hoosier-Shawnee Ecological Assessment 3rd Edition. General Technical Report. NC-244. U.S. Department of Agriculture, Forest Service, North Central Research Station. St. Paul, MN (2004).
29. Townes, H. A light-weight Malaise trap. *Entomological News*. **83**, 239-247 (1972).
30. Wilkening, J., Foltz, J. L., Atkonson T. H., Connor, M. D. An omnidirectional flight trap for ascending and descending insects. *Canadian Entomologist*. **113,** 453-455 (1981).
31. Basset, Y. A composite interception trap for sampling arthropods in tree canopies. *Australian Journal of Entomology*. **27**, 213-219 (1988).
32. Bowden, J. An analysis of factors affecting catches of insects in light traps. *Bulletin of Entomological Research*. **72**, 535-556 (1982).
33. Müller, J. et al. Airborne LiDAR reveals context dependence in the effects of canopy architecture on arthropod diversity. *Forest Ecology and Management*. **312**, 129-137 (2014).
34. Southwood, T. R. E. The components of diversity. In *Diversity of Insect Faunas*. Edited by Mound, L. A., Waloff, N., 19-40, Symposia of the Royal Entomological Society of London No. 9 (1978).
35. Southwood, T. R. E., Moran, V. C., Kennedy, C. E. J. The assessment of arboreal insect fauna-comparisons of knockdown sampling and faunal lists. *Ecological Entomology*. **7**, 331-340 (1982).
36. Majer J. D., Recher H. F. Invertebrate communities on Western Australian eucalypts: a comparison of branch clipping and chemical knockdown. *Australian Journal of Ecology*. **13**, 269-278 (1988).
37. Basset Y. The arboreal fauna of the rainforest tree *Argyrodendron actinophyllum* as sampled with restricted canopy fogging: composition of the fauna. *Entomologist*. **109**, 173-183 (1990).
38. Majer, J. D., Recher, H. Keals, N. Branchlet shaking: a method for sampling tree canopy arthropods under windy conditions. *Australian Journal of Ecology*. **21**, 229-234 (1996).

436 39. Moir, M. L., Brennan, K. E. C., Majer J. D., Fletcher, M. J., Koch, J. M. Toward an optimal
437 sampling protocol for Hemiptera on understorey plants. *Journal of Insect Conservation*. **9**, 3-20
438 (2005).
439 40. Johnson, M. D. Evaluation of arthropod sampling technique for measuring food availability
440 for forest insectivorous birds. *Journal of Field Ornithology*. **71**, 88-109 (2000).
441 41. Cooper, R. J., Whitmore, R. C. Arthropod sampling methods in ornithology. *Studies in Avian*
442 *Biology*. **13**, 29-37 (1990).
443 42. Cooper, N. W., Thomas, M. A., Garfinkel, M. B., Schneider, K. L., Marra, P. P. Comparing the
444 precision, accuracy, and efficiency of branch clipping and sweep netting for sampling arthropods
445 in two Jamaican forest types. *Journal of Field Ornithology*. **83**, 381-390 (2012).
446 43. Schowalter, T. D., Webb, J. W., Crossley, Jr. D. A. Community structure and nutrient content
447 of canopy arthropod in clearcut and uncut forest ecosystems. *Ecology*. **62**, 1010-1019 (1981).
448 44. Majer, J. D., Recher, H. F., Perriman, W. S., Achuthan, N. Spatial variation of invertebrate
449 abundance within the canopies of two Australian eucalypt forests. *Studies in Avian Biology*. **13**,
450 65-72 (1990).
451 45. Beltran. W., Wunderle, Jr. J. M. Temporal dynamics of arthropods on six tree species in dry
452 woodlands on the Caribbean Island of Puerto Rico. *Journal of Insect Science*. **14**, 1-14 (2014).
453 46. Schowalter, T. D., Crossley, Jr. D. A., Hargrove, W. Herbivory in forest ecosystems. *Annual*
454 *Review of Entomology*. **31**, 177-196 (1986).
455 47. Summerville, K. S., Crist, T. O. Effects of timber harvest on Lepidoptera: community, guild,
456 and species responses. *Ecological Applications*. **12**, 820-835 (2002).
457 48. Barbosa, P. et al. Associational resistance and associational susceptibility: having right or
458 wrong neighbors. *Annual Review of Ecology, Evolution, and Systematics*. **40**, 1-20 (2009).
459 49. Burns, R. M., Honkala, B. H. Silvics of North America: Vol 2. Hardwoods. Agriculture Handbook
460 654. U.S. Department of Agriculture, Forest Service, Washington, D.C. (1990).

Table 1.

Tree species	Richness			Biomass			Shannon Diversity		
	X	SE	% of mean	X	SE	% of mean	X	SE	% of mean
Maple spp. N = 140	3.54	0.17	5%	0.003	0.0004	13%	0.86	0.05	6%
Hickory spp. N = 141	4.62	0.20	4%	0.013	0.002	15%	1.10	0.04	4%
Tulip Poplar N = 70	4.32	0.20	5%	0.011	0.002	18%	1.12	0.05	4%
American Beach N = 67	3.23	0.22	7%	0.002	0.0003	15%	0.81	0.06	7%
Oak spp. N = 208	4.77	0.15	3%	0.006	0.0007	12%	1.10	0.03	3%

Table

	Richness			Biomass			Shannon Diversity		
Tree species	X	SE	% of mean	X	SE	% of mean	X	SE	% of mean
Maple spp. (N = 140)	3.54	0.17	5%	0.003	0.0004	13%	0.86	0.05	6%
Hickory spp. (N = 141)	4.62	0.20	4%	0.013	0.002	15%	1.10	0.04	4%
Tulip Poplar (N = 70)	4.32	0.20	5%	0.011	0.002	18%	1.12	0.05	4%
American Beach (N = 67)	3.23	0.22	7%	0.002	0.0003	15%	0.81	0.06	7%
Oak spp. (N = 208)	4.77	0.15	3%	0.006	0.0007	12%	1.10	0.03	3%

Name of Material/Equipment	Company	Catalog Number	Comments/Description
13 gallon garbage bags	Glad	78374	
Aluminum rod	Grainger	48ku20	
Pruner	Bartlet arborist supply	pp-125b-2stick	
Telescoping pole	BES	TPF620	
Tomato Cage	Gilbert and Bennet	42 inch galvanized	

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: A method of quantifying foliage-dwelling arthropods

Author(s): Eichholz & Sierzega

Item 1: The Author elects to have the Materials be made available (as described at http://www.jove.com/publish) via:

☒ Standard Access ☐ Open Access

Item 2: Please select one of the following items:

☒ The Author is **NOT** a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of The Journal of Visualized Experiments; "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and(c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. **Grant of Rights in Video – Standard Access.** This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. **Grant of Rights in Video – Open Access.** This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this **Section 6** is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. **Government Employees.** If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. **Protection of the Work.** The Author(s) authorize JoVE to take steps in the Author(s) name and on their behalf if JoVE believes some third party could be infringing or might infringe the copyright of either the Author's Article and/or Video.

9. **Likeness, Privacy, Personality.** The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

10. **Author Warranties.** The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

11. **JoVE Discretion.** If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

12. **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

13. **Fees.** To cover the cost incurred for publication, JoVE must receive payment before production and publication of the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

14. **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement is required per submission.

CORRESPONDING AUTHOR

Name:	Mike Eichholz
Department:	CWRL, Center for Ecology, Dept Zoology
Institution:	Southern Illinois Univ. Carbondale
Title:	Assoc. Prof.
Signature:	
Date:	4-8-19

Please submit a **signed** and **dated** copy of this license by one of the following three methods:

1. Upload an electronic version on the JoVE submission site
2. Fax the document to +1.866.381.2236
3. Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02140

Thank you for allowing us the opportunity to revise our manuscript. Our responses can be found following the comments below.

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Done

2. Please revise lines 64-67 and 73-86 to avoid textual overlap with previously published work.

Text on lines 64-67 revised from:

"We conducted our study in the forested, un-glaciated landscape of southern Illinois throughout the Shawnee National Forest (SNF). The 286,000ac SNF is located in the Central Hardwoods region within the Ozarks and Shawnee Hills natural divisions[26]. Current forest composition is a mosaic of 37% oak/hickory, 25% mixed-upland hardwoods, 16% beech/maple, and 10% bottomland hardwoods and is dominated by second growth oak/hickory in the uplands and sugar maple, American beech, and tulip tree (*Liriodendron tulipifera*), in sheltered mesic valleys[27,28]."

To:

We conducted the study on the Shawnee National Forest (SNF) in southern Illinois. The SNF is a 115,738 ha forest located in the Central Hardwoods region of the Ozarks and Shawnee Hills natural divisions[26]. The forest comprises a mosaic of 37% oak/hickory, 25% mixed-upland hardwoods, 16% beech/maple, and 10% bottomland hardwoods and is dominated by second growth oak/hickory in upland xeric areas and sugar maple, American beech, and tulip tree (*Liriodendron tulipifera*), in sheltered mesic valleys[27,28]."

Lines 71-86 have been revised from:
"To ensure the factors influencing the arthropod community described on an individual tree was limited to those within our scope of interest, we selected 22 study sites along an oak/hickory dominance gradient by identifying either oak/hickory or beech/maple dominated sites with the USFS stand cover map for the SNF (allveg2008.shp) in ArcGIS 10.1.1 (ESRI, Redlands, CA, USA). We used the following criteria for site selection to prevent potential confounding effects: located within contiguous upland-deciduous forest habitat (i.e., elevation above 120 m), $\geq$ 12 ha, and not located in riparian areas. Both oak/hickory and beech/maple sites contained mature trees > 50 years old and were situated in hilly terrain, thus comprised similar slopes and aspects. Boundaries of beech/maple sites were distinguished based on the transition of tree communities while boundaries of oak/hickory sites were identified artificially using SNF cover maps and ArcGIS 10.1.1 (ESRI, Redlands, CA, USA). As the SNF is primarily second growth timber, differences in tree species composition among our sites were representative of past land usage (e.g., clear cuts or selective harvest), not due to differences in location on the landscape; our sites

were large forest blocks within un-glaciated terrain. Our study sites have not experienced landscape level disturbance (i.e., logging) for > 50 years. We uploaded discrete polygon shapefiles of each study site to a handheld Global Positioning System (GPS) for ground-truthing purposes. We sampled three trees from 0600-1400 hours during 23 May to 25 June 2014. We located trees by searching outward to a 30 m radius around each vegetation point until a mature tree > 20 cm d.b.h. with branches low enough to sample was found. In general, the three mature trees closest to the center point that represented three of the five genera (*Acer, Carya, Fagus, Liriodendron,* and *Quercus*) of interest were sampled. "

To:

"We selected 22 study sites along an oak/hickory (xeric) to beech/maple (mesic) dominated gradient using USFS stand cover maps (allveg2008.shp) in ArcGIS 10.1.1 (ESRI, Redlands, CA, USA). We selected sites using the following criteria to prevent potential confounding effects: located within contiguous upland-deciduous forest habitat (i.e., elevation above 120 m), $\geq$ 12 ha, and not located in riparian areas. All sites contained mature trees > 50 years old in hilly terrain, thus comprised similar slopes and aspects. Beech/maple site boundaries were distinguished based on the transition of tree communities while oak/hickory site boundaries were identified artificially using SNF cover maps and ArcGIS 10.1.1 (ESRI, Redlands, CA, USA). Differences composition of tree species among our sites were a function of past land usage (e.g., clear cuts or selective harvest), not differences in location on the landscape; all sites were large forest blocks within un-glaciated terrain. The study sites have not experienced disturbance at a landscape level (i.e., logging) for > 50 years. We ground-truthed the maps by uploading discrete polygon shapefiles of each study site to a handheld Global Positioning System (GPS) and verifying tree species composition. We sampled three trees from 0600-1400 hours during 23 May to 25 June 2014. To locate sample trees, we searched outward to a 30 m radius from vegetation points until mature trees (> 20 cm d.b.h.) with branches low enough to sample were found. Typically, the three mature trees that represented three of the five genera (*Acer, Carya, Fagus, Liriodendron,* and *Quercus*) of interest and were closest to the center point were sampled."

3. 1.1: What tool is used here?

Changed text from:

" 1.1 remove the bottom 1/3 of the 30 cm wire tomato cage so that it is approximately 55 cm in length

To:

"1.1 Using bolt cutters, large wire cutters, or an electric grinding disk, remove the bottom 1/3 of the 30 cm wire tomato cage so that it is approximately 55 cm in length"

4. Please combine some of the shorter Protocol steps so that individual steps contain 2-3 actions and maximum of 4 sentences per step.

Modified text from:

1.1 Using bolt cutters, large wire cutters, or an electric grinding disk, remove the bottom 1/3 of the 30 cm wire tomato cage so that it is approximately 55 cm in length

1.2 Cut two, 30 cm braces made from aluminum tube or similarly semi-rigid material to use as attachment rods and braces on each side of the largest end of the tomato cage

1.3 Attach one end of each of the two attachment rods to opposite sides of the tomato cage with duct or electrical tape ensuring the tape is wrapped around at least 6 cm of the cage and rod. Be certain to rap the tape around the cage and rod numerous times to ensure the cage is permanently attached to the rod.

1.4 Attach the other end of each of the two attachment rods on the opposite sides of the end of an extendable pole with duct or electrical tape. As before wrap the tape multiple times to affix it permanently ensuring the tape overlaps the pole and rods by at least 6 centimeters. Be certain the opening of the cage is in contact with the end of the telescoping pole when the cage is attached

1.5 Attach the cage directly onto the end of the pole using the electrical or duct tape.

1.6 Attach Velcro strips at 3 points to the opening of the cage. These Velcro strips will be use later to hold the opening of the bag open.

2 Enclosing the branch

2.1 Attach 3 pieces of Velcro to the opening of the bag so they align with the Velcro attached to the opening of the cage. These will be used to hold the opening of the bag in place while it is being placed of the branch. Be certain the Velcro is aligned so when the bag is inserted and attached, the opening to the pull strings of the bag run parallel to the telescoping pole.

2.2 Insert a 13-gallon kitchen garbage bag in the wire tomato cage and, using 2 gator clips, one on each side of the bottom of the bag, attach the clips to both the bag and wire cage to hold the bag against the cage.

2.3 Using 2 gator clips, attach the opening of the bag to the opening of the cage. Orient the gator clips so they are perpendicular to the telescoping pole and the openings to the draw strings so the run parallel to the telescoping pole. Be certain to leave adequate space near the opening of the bag to allow the bag to close when the draw string is pulled.

2.4 Attach para cord to each side of the bags draw string.

2.5 Use a random number generator to randomly select a height of the tree within the height of the extension pole when it is extended to its maximum length.

2.6 Use a random number generator to randomly select a distance from the tree trunk of the tree.

2.7 Identify a branch that will fit in the bag with minimal disturbance to the foliage and is the height and distance from the trunk based on the numbers generated from the random number generator.

2.8 Quickly slide the bag over the branch and pull the cords attached to the draw string on the bag to seal the bag. Practice this a few time prior to the first attempt to become efficient at incorporating the foliage with minima disturbance to the leaves.

2.9 Clip the branch adjacent to the bag opening with the extension pole pruner

2.10 Store the bagged branch in a freezer until you are ready to conduct the laboratory arthropod analysis.

3. Arthropod analysis

3.1 Hold frozen bag and branch upright and shake branch while in the bag to dislodge arthropods into the bag.

3.2 Carefully remove the branch and rinse in large collection pan to remove remaining arthropods.

3.3 Empty remaining material from the bag into the collection pan.

3.4 Remove any non-arthropod debris

3.5 Separate arthropods into desired taxonomic groups recording larvae and adults

3.6 Quantify arthropods as desired. If biomass is of interest either:

3.6a Measure length of arthropods and use published length mass table to estimate biomass.

3.6b Place arthropods in small drying pans, dry in drying oven for 24 hours at 45˚ C, and weigh on an electronic balance.

4 Estimating density

4.1 To estimate density and control for variation in leaf structure and leaf density between samples within tree species and among tree species either:

4.1a Count and measure the surface area of the leaves from each sample.

4.1b Dry the leaves in a drying oven for 48 hours at 45˚ C and weigh the leaves on an electronic balance.

4.1c Measure the length of all woody branch within the sample.

Diel differences occur in arthropod communities so sampling should be conducted throughout the entire period of inference.

To:

1 Building the sampling device - prior to going to the field

1.1 Using bolt cutters, large wire cutters, or an electric grinding disk, remove the bottom 1/3 of the 30 cm wire tomato cage so that it is approximately 55 cm in length

1.2 Cut two, 30 cm braces made from aluminum tube or similarly semi-rigid material to use as attachment rods and braces on each side of the largest end of the tomato cage. Attach one end of each of the two attachment rods to opposite sides of the tomato cage with duct or electrical tape ensuring the tape is wrapped around at least 6 cm of the cage and rod. Be certain to rap the tape around the cage and rod numerous times to ensure the cage is permanently attached to the rod.

1.3 Attach the other end of each of the two attachment rods on the opposite sides of the end of an extendable pole with duct or electrical tape. As before wrap the tape multiple times to affix it permanently ensuring the tape overlaps the pole and rods by at least 6 centimeters. Be certain the opening of the cage is in contact with the end of the telescoping pole when the cage is attached

1.4 Attach the cage directly onto the end of the pole using the electrical or duct tape. Attach Velcro strips at 3 points to the opening of the cage. These Velcro strips will be use later to hold the opening of the bag open.

2 Enclosing the branch

2.1 Attach 3 pieces of Velcro to the opening of the bag so they align with the Velcro attached to the opening of the cage. These will be used to hold the opening of the bag in place while it is being placed of the branch. Be certain the Velcro is aligned so when the bag is inserted and attached, the opening to the pull strings of the bag run parallel to the telescoping pole.

2.2 Insert a 13-gallon kitchen garbage bag in the wire tomato cage and, using 2 gator clips, one on each side of the bottom of the bag, attach the clips to both the bag and wire cage to hold the bag against the cage. Using 2 gator clips, attach the opening of the bag to the opening of the cage. Orient the gator clips so they are perpendicular to the telescoping pole and the openings to the draw strings so the run parallel to the telescoping pole. Be certain to leave adequate space near the opening of the bag to allow the bag to close when the draw string is pulled. Attach para cord to each side of the bags draw string.

2.3 Use a random number generator to randomly select a height of the tree within the height of the extension pole when it is extended to its maximum length. Use a random number generator to randomly select a distance from the tree trunk of the tree. Identify a branch that will fit in the bag with minimal disturbance to the foliage and is the height and distance from the trunk based on the numbers generated from the random number generator.

2.4 Quickly slide the bag over the branch and pull the cords attached to the draw string on the bag to seal the bag. Practice this a few time prior to the first attempt to become efficient at incorporating the foliage with minima disturbance to the leaves. Clip the branch adjacent to the bag opening with the extension pole pruner. Store the bagged branch in a freezer until you are ready to conduct the laboratory arthropod analysis.

3. Arthropod analysis

3.1 Hold frozen bag and branch upright and shake branch while in the bag to dislodge arthropods into the bag. Carefully remove the branch and rinse in large collection pan to remove remaining arthropods. Empty remaining material from the bag into the collection pan. Remove any non-arthropod debris.

3.2 Separate arthropods into desired taxonomic groups recording larvae and adults.

3.3 Quantify arthropods as desired. If biomass is of interest either:

3.3a Measure length of arthropods and use published length mass table to estimate biomass.

3.3b Place arthropods in small drying pans, dry in drying oven for 24 hours at 45˚ C, and weigh on an electronic balance.

4 Estimating density

4.1 To estimate density and control for variation in leaf structure and leaf density between samples within tree species and among tree species either:

4.1a Count and measure the surface area of the leaves from each sample.

4.1b Dry the leaves in a drying oven for 48 hours at 45˚ C and weigh the leaves on an electronic balance.

4.1c Measure the length of all woody branch within the sample.

Diel differences occur in arthropod communities so sampling should be conducted throughout the entire period of inference.

5. Please include single line spacing between each numbered step or note in the protocol.

Done

6. After you have made all the recommended changes to your protocol section (listed above), please highlight in yellow up to 2.75 pages (no less than 1 page) of protocol text (including headers and spacing) to be featured in the video. Bear in mind the goal of the protocol and highlight the critical steps to be filmed. Our scriptwriters will derive the video script directly from the highlighted text.

Done

7. Discussion: As we are a methods-based journal, please discuss critical steps in the protocol, modifications and troubleshooting of the method, and limitations of the method.

Modified the text on line 162-168 to make it more clear a limitation to this method is active or flighted-arthropods may be under-represented. We then caution researchers a critical step of this procedure is to ensure foliage remains undisturbed until it is enclosed.

Lines 177-180: We identify assessing samples in the field as a critical issue when trying to maximize detection probability and recommend a procedure we devised for doing so in the lab. We modified the text to better emphasize this.

Lines 181-199: Discuss the issue difficulty and limitation of using this procedure to estimate arthropod density and provide recommended approaches.

8. Table 1: Please upload it to your Editorial Manager account as an .xlsx file. Avoid any coloring or formatting in the tables.

Done

9. Table of Materials: Please ensure that it has information on all relevant supplies, reagents, equipment and software used, especially those mentioned in the Protocol. Please sort the materials alphabetically by material name.

Done

Reviewers' comments:
Reviewer #2:
Manuscript Summary:
The revision of the manuscript entitled "A method for quantifying foliage-dwelling arthropods" has been improved greatly. Thus, I suggest that it can be accepted with the present version.

Major Concerns:
No.

Minor Concerns:
No.

Reviewer #5:
The ms describe the benefits of the branch bagging methodology to quantify density, richness and diversity of foliage dwelling arthropods in 5 groups of tree species in Shawnee National Forest, Illinois. The freezing of the sample branch followed by it rinse with water can reduce the time spent looking for individuals, as well decrease the chances of lost some of them.
The estimating means and standard errors were used to know the level of precision from the samplings. The results showed that this method can be precise to test the influence of environmental variables on richness, diversity and density of species, since the values of percentage of mean of the standard errors for community metrics were low.
The method procedure was clear and described in detail. The method is sound, I believe that the ms will benefit field researchers providing a substantive way to estimate differences in arthropods abundance and biomass, diversity and richness of foliage-dwelling arthropods communities.