

Population and plant community dynamics involving garlic mustard (*Alliaria petiolata*) in a Minnesota Oak Woodland: a four year study

Author(s): Mark A. Davis, Clare MacMillen, Marta LeFevre-Levy, Casey Dallavalle, Nolan Kriegel, Stephen Tyndel, Yuris Martinez, Michael D. Anderson, and Jerald J. Dosch

Source: The Journal of the Torrey Botanical Society, 141(3):205-216. 2014.

Published By: Torrey Botanical Society

DOI: <http://dx.doi.org/10.3159/TORREY-D-13-00062.1>

URL: <http://www.bioone.org/doi/full/10.3159/TORREY-D-13-00062.1>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

Population and plant community dynamics involving garlic mustard (*Alliaria petiolata*) in a Minnesota Oak Woodland: a four year study¹

Mark A. Davis², Clare MacMillen, Marta LeFevre-Levy, Casey Dallavalle, Nolan Kriegel, Stephen Tyndel, Yuris Martinez, Michael D. Anderson, and Jerald J. Dosch

Department of Biology, Macalester College, 1600 Grand Avenue, St. Paul, MN 55105

DAVIS, M. A., C. MACMILLEN, M. LEFEVRE-LEVY, C. DALLAVALLE, N. KRIEGEL, S. TYNDEL, Y. MARTINEZ, M. D. ANDERSON, AND J. J. DOSCH (Department of Biology, Macalester College, 1600 Grand Avenue, St. Paul, MN 55105). Population and plant community dynamics involving garlic mustard (*Alliaria petiolata*) in a Minnesota Oak Woodland: a four year study. *J. Torrey Bot. Soc.* 141: 205–216. 2014.—Garlic mustard, *Alliaria petiolata* (M. Bieb.) Cavara & Grande, a European biennial, has become common in many Midwestern and eastern North American forests. Researchers have sometimes documented a two-year (two-point) population cycle in which the vegetative and reproductive phases alternate in abundance. However, little research has been conducted on the scale dependence of the two-point cycle and the extent to which the cycle is dependent on the density of *A. petiolata*. In addition to this interest in *A. petiolata*'s population dynamics, researchers have investigated *A. petiolata*'s effects on other plant species, often concluding that it displaces native herbs and inhibits tree seedling growth and survival through the production of allelochemicals. In order to examine both the population dynamics of *A. petiolata* and its possible effects on North American native plants, we conducted a four year study in which we monitored *A. petiolata* in a 6.2 ha portion of a Minnesota oak (*Quercus*) woodland at two spatial scales (0.5 m², 400 m²). We also conducted an indoor seedling pot study to investigate whether any soil inhibitory effects associated with *A. petiolata* may be exacerbated under drought conditions. We found that a two-point cycle was not sustained during the four year study throughout the entire study site. However, the two-point cycle was exhibited in high *A. petiolata* density areas in the study site, providing support for the hypothesis that the cycle is driven by competition between first-year (rosette) and second-year (stem) plants. Both the monitoring study and the pot experiment yielded little evidence that *A. petiolata* is a major agent of ecological change in the woodland. Rather, the data indicate that over the four years of this study *A. petiolata* and the other plant species in the woodland we studied are changing in abundance largely independent of one another.

Key words: invasive plants, nonnative plants, two-point cycle.

Garlic mustard, *Alliaria petiolata* (M. Bieb.) Cavara & Grande (Brassicaceae), is a European biennial now common in many Midwestern and eastern North American forests. It is self-compatible, has abundant and dormant seeds, and can grow in a variety of forest environments (McCarthy 1997). These characteristics allow *A. petiolata* to spread easily. Many consider it to be an invasive species that displaces native herbs (Rodgers et al. 2008) and inhibits tree seedling growth and survival

(Wolfe et al. 2008, Lankau 2011). However, other studies have found *A. petiolata* to have negligible, and sometimes even positive, effects on some native plants (Rogers et al. 2008) and several recent studies have concluded that *A. petiolata* is not a major agent of change in these forests (Nuzzo et al. 2009, Rooney and Rogers 2011).

Some studies have also shown *A. petiolata* to exhibit a two-year (or two-point) reproductive cycle (Van Riper et al. 2010), which is thought to be the result of intraspecific competition between first and second-year plants (Winterer et al. 2005). First year *A. petiolata* plants consist of basal rosettes while second-year plants produce tall, flowering stems, which, if sufficiently dense, are thought to reduce survival of nearby rosettes by outcompeting the rosettes for light. With fewer rosettes then present to become two year plants (and produce flowering stems), the subsequent year is characterized by fewer stems, which in turn means higher survival for new rosettes, and more stems the next year,

¹ Funding for this study was provided by Macalester College, the Louis Daniel Frenzel Jr. Endowed Scholarship, the Duane Roberts Field Biology Study Fund, and the Howard Hughes Medical Institute (HHMI). This study could not have been undertaken without the efforts of Abby Colehour, Jo Daney, Elizabeth Foster, Emily Merrill, Joseph O'Neil, Margaret Pearson, and Megan Whitney, who laid out the grid and collected grid and quadrat data in 2010 and 2011.

² Author for correspondence, E-mail: davis@macalester.edu

Received for publication August 28, 2013, and in revised form April 15, 2014.

etc. (Bauer et al. 2010). The existence of a two-point cycle may have both ecological and management implications. If the magnitude of any effects of *A. petiolata* on other plants is positively correlated with *A. petiolata* abundance (a reasonable assumption), then whatever effects *A. petiolata* might have on other species at a site could fluctuate from year to year if *A. petiolata*'s two-point cycle is associated with a cycling in its overall abundance. On the basis of their simulations of *A. petiolata*'s two-point cycle, Pardini et al. (2009) concluded that management efforts to reduce the abundance of *A. petiolata* at a site through the culling of individuals (e.g., through herbicide application or mechanical removal) will vary in their effectiveness, depending on whether the eradication efforts are undertaken during a peak rosette year (ineffective) or stem year (more effective).

The study described here is part of an ongoing monitoring project of *A. petiolata* interspecific and intraspecific interactions in an eastern Minnesota oak (*Quercus*) woodland begun in 2010. Data from the first two years indicated that the population was exhibiting a two-point cycle (in which rosettes dominate one year and flowering stems the next) and revealed few significant relationships between the abundance of *A. petiolata* and the abundance of other species, and between change in the respective abundances from the first year to the second (Davis et al. 2012). We had several objectives in the study reported here. First, we wanted to determine whether the two-point cycle we observed during the first two years of our study continued in 2012 and 2013. Second, we wanted to assess the extent to which the two-point cycle may be density-dependent. Because the two-point cycle is hypothesized to be driven by competition between first- and second-year plants, the biennial cycling may be exhibited only in areas with high *A. petiolata* abundance. Third, although we documented few negative effects of *A. petiolata* on native plants during the first two years of the study, we wanted to see whether effects might begin to appear by years three or four.

The fourth objective involves the possible effects of *A. petiolata* on tree seedlings. *Alliaria petiolata* has been reported to negatively affect tree seedlings via allelochemical effects on mycorrhizae (Wolfe et al. 2008). A 2011 experimental pot study we conducted found

that the emergence, survival, and growth of first-year bur oak (*Quercus macrocarpa*) seedlings were not affected by whether the seedlings were growing in soil harvested from *A. petiolata* patches ($\geq 25\%$ *A. petiolata* cover) or from areas in the forest without *A. petiolata* (Davis et al. 2012). However, first-year green ash (*Fraxinus pennsylvanica*) seedlings grown in *A. petiolata* soil exhibited a 12% reduction in height (Davis et al. 2012). In the prior experiment, all seedlings were provided abundant light and water. A 2005 study (Davis et al.) showed that growth and survival of *Q. macrocarpa* seedlings differed depending on whether the seedlings were grown in soil 'trained' by native or nonnative grasses, but only when the seedlings were water stressed. Thus, the fourth objective of the present study was to determine if *A. petiolata* soil might affect, or have a greater effect on, tree seedling growth and survival if the seedlings are experiencing another stressor, drought.

Materials and Methods. **STUDY SITE.** We conducted this study at Macalester College's field station, the Katharine Ordway Natural History Study Area, located in Inver Grove Heights in eastern Minnesota (44° 48' 28" N, 93° 01' 31" W). Situated on the bluffs of the Mississippi River, the field station contains a variety of habitats, including grasslands, several wetlands, riparian habitat, a backwater lake, and a woodland, the latter of which was the site of this study. The woodland is dominated by oaks: *Quercus rubra* L., *Quercus ellipsoidalis* E. J. Hill, *Quercus alba* L., and *Quercus macrocarpa* Michx.; however, the absence of fire in most of the woodland during the past half century has resulted in the establishment of a number of fire susceptible tree species that now make up the majority of the understory (in some cases approaching canopy tree size), the most common being *Ulmus americana* L., *U. rubra* Muhl., *Acer negundo* L., *Prunus serotina* Ehrh., *Tilia Americana* L., and *Fraxinus pennsylvanica* Marshall. The two most abundant herb species (based on cover) in the woodland are *Desmodium glutinosum* (Muhl. ex Willd.) A.W. Wood (Fabaceae) and *A. petiolata*, with other common herbs being *Amphicarpaea bracteata* L. Fernald (Fabaceae), *Geranium maculatum* L. (Geraniaceae), *Circaea lutetiana* L. (Onagraceae) and *Ageratina altissima* L. King & H.E. Robins. (Asteraceae). *Ribes missouriense*

Nutt. (Grossulariaceae) and *Rubus* spp. (Rosaceae) are common shrubs, and common fern species include *Athyrium filix-femina* L. Roth ex Mert. var. *angustum* (Willd.) G. Lawson (Dryopteridaceae), *Osmunda claytoniana* L. (Osmundaceae), and *Adiantum pedatum* L. (Pteridaceae). Besides *A. petiolata*, other non-native species in the woodland include *Rhamnus cathartica* L. (Rhamnaceae) and *Lonicera tatarica* L. (Caprifoliaceae). *Alliaria petiolata* was first recorded at the study site in the early 2000s (Karen Schik, personal communication). Earthworms (Megadrilacea) are abundant in the woodland soils and white-tailed deer, *Odocoileus virginianus*, are common in the woodland.

LARGE SCALE DATA COLLECTION. In summer 2010, we set up a 2.84 ha study grid (71 contiguous 20 × 20 m cells with vertices defined by coordinates in the Universal Transverse Mercator (UTM) system). We expanded the grid to 6.08 ha in 2011 (152 total cells). We used belt transects (20 cm wide) to estimate *A. petiolata* cover in the cells in each of the four summers. The cover for a cell was estimated from two parallel transects running north-south along opposite sides of the cell and a third transect running through the middle of the cell parallel to the other two transects. In total, approximately 3 km of transects were sampled in 2010 and approximately 6.6 km of transects were sampled during the subsequent three summers. In 2010 and 2011, no distinction was made between cover by *A. petiolata* rosettes and stems. In 2012 and 2013, cover was recorded separately for rosettes and stems. In addition to cover, the number of stems in each of the three transects of a cell was recorded all four years.

SMALL SCALE DATA COLLECTION. In summer 2010, using the transect data, we identified ten cells that encompassed a wide range of *A. petiolata* abundance. These ten cells included cells in which *A. petiolata* exhibited a range of densities, including being completely absent. In each of these ten cells, we established nine permanent quadrats (1.0 × 0.5 m), one in each corner, one at the midpoint of each of the four sides, and one in the middle. During each of the four summers, the number of *A. petiolata* rosettes and stems were counted and recorded for each quadrat, and the total percent cover of *A. petiolata* was estimated and recorded as

well. In addition, all plant species (including herbs, shrubs, tree seedlings, and ferns) in the quadrats were identified and their percent cover estimated and recorded. These data were used to compare the population dynamics of *A. petiolata* with those of other species and vegetation types over time. (The corner markers for five quadrats either disappeared or were displaced by an unknown agency between the 2011 and 2012 field seasons, and this happened for one additional quadrat between 2012 and 2013. As a result, 2012 and 2013 quadrat data are based on 85 and 84 quadrats, respectively).

SEEDLING POT EXPERIMENT. In order to determine if tree seedlings experiencing water stress may be more likely to be compromised in their growth and survival when grown in *A. petiolata* trained soil than seedlings that were not stressed, we planted bur oak (*Q. macrocarpa*) acorns and green ash (*F. pennsylvanica*) seeds in forty-eight 0.95 liter pots, with half the pots containing soil collected from patches in the woodland where *A. petiolata* was abundant ($\geq 25\%$ cover) and half containing soil from areas where *A. petiolata* was absent (other factors, e.g., tree canopy cover or density of other herbs were not controlled). *Quercus macrocarpa* and *F. pennsylvanica* are two of the most common trees at the study site. The two types of collecting sites were spatially interspersed with one another. The acorns and ash seeds were planted in the pots within 24 hours of the soil being collected. The pots were placed on free standing shelves in the field station laboratory constructed for this purpose, with light provided by fluorescent tubes situated above each shelf. The lights were run on a 15:9 light:dark cycle, approximating the ambient day/night conditions in central Minnesota in June and July. The pots were rotated daily to control for possible varying light conditions along the shelves. Pots were regularly watered until the seedlings emerged and began to leaf out.

Pots in the two soil categories were initially divided into three water treatments: high water, low water (one drought episode), very low water (two drought episodes). Upon leaf out, seedlings in the high water category were watered regularly in order to maintain soil water levels above 10% (by volume). Following leaf out, seedlings in the low water (one drought) category were intended to experience

a 7 day drought during which they would receive no additional water, and after which they would receive the same watering as the high water pots. Seedlings in the low water (two droughts) category were intended to experience the same initial drought as the one drought group of pots then, following a 7 day period of regular watering, they were to experience a second 7 day period of no water. However, due to time constraints (the seedlings took longer to emerge than expected), the two drought groups were lumped into a single group with all drought pots receiving no water following leaf out until the end of the 8 week experiment. Soil water levels (percent soil moisture content) in all pots were measured 3 times per week using a soil moisture meter (Fieldscout TDR300, Spectrum Technologies, Inc.). Soil moisture content in high water pots were maintained between 10 and 15%. Soil moisture content in drought pots began to decline from this level once maintenance watering was stopped following leaf out of the seedlings and dropped to near zero by the end of the experiment. Following leaf out, the vigor of each seedling was assessed 3 times per week on a scale of 1 to 4 based on the degree of wilting of the leaves (1: no sign of wilting; 2: leaves drooping downward, 3: leaves dropping downward, drying out, and beginning to crinkle, 4: leaves very dry and crinkled, often completely folded in on itself, and probably dead). All seedlings were harvested nine weeks following the start of the experiment. Roots and shoots were separated, dried, and weighed. For *Q. macrocarpa*, acorn size (volume) was estimated by measuring the height and width of the acorns and calculating volume using the formula for a cylinder (Davis et al. 2005).

DATA COMPILATION, CALCULATIONS, AND ANALYSIS. We performed several analyses to determine the extent to which *A. petiolata* at our site was exhibiting a two-point cycle over the four years. First we plotted and analyzed the stem count data for each of the 400 m² grid cells (71 cells in 2010 and 152 cells in 2011–13) for the four years using a simple ANOVA, followed by a Tukey's HSD test to compare the four annual means with one another. We also compared the stem and rosette cover in 2012 and 2013 (stem and rosette cover was not recorded separately in 2010 and 2011). We produced similar graphs and conducted

similar analyses on both the stem and rosette counts in the ninety 0.5 m² quadrats (excluding the 28 quadrats that contained no *A. petiolata* during any of the four years). Specifically, we analyzed the quadrat stem and rosette data with a nested ANOVA (nine quadrats nested in each of the ten grid cells) followed by the Tukey's HSD test to compare the four annual means.

In order to assess whether the two-point cycle may only occur in high density areas, we examined how the stem and rosette cover changed from 2012 to 2013 in cells that had stem cover values in 2012 of 5% or greater ($N = 32$) compared to the cover changes (both rosette and stem) in cells with lower stem cover values in 2012 ($N = 120$). We also compared and analyzed stem counts in the same two sets of grid cells over the four year period. All stem counts were log transformed prior to ANOVA analyses.

In order to assess whether *A. petiolata* may be affecting the abundance of other plant species, we compared changes in *A. petiolata* cover from 2010–2013 with the cover change over the same period of time of other species, we analyzed the quadrat data using a nested regression analysis. These analyses were conducted to compare changes in *A. petiolata* cover from 2010–2013 with three vegetation groups (herbs, shrubs, tree seedlings) and of the five most common native herbs (*Desmodium glutinosum*, *Amphicarpaea bracteata*, *Geranium maculatum*, *Circaea lutetiana*, and *Ageratina altissima*), as well as with the species richness of herbs and of all plants. Since it is possible that *A. petiolata* abundance in a given year could be influenced by where it is in the two-point cycle (e.g., stem vs rosette year), we also conducted the same ten *A. petiolata* analyses for the 2011–2013 time period. In order to determine if there was anything unique about how changes in *A. petiolata* cover were associated with the cover and species richness of other vegetation, we conducted the same nested regression analyses using the other five most abundant herb species in place of *A. petiolata*.

Since it is also possible that the effects on other species by *A. petiolata* may be density dependent, e.g., depend on *A. petiolata*'s density and/or the magnitude in the change in *A. petiolata*'s density over time, we also conducted simple regression analyses on the twelve quadrats in which *A. petiolata* exhibited

at least 20% cover in 2010 (range = 20–82%, mean = 37%) and on the fifteen quadrats that exhibited increases in *A. petiolata* cover from 2010 to 2013 of at least five percent (range = 5–30%, mean = 15%).

Due to the many nested regression analyses we conducted (a total of seventy-six), significance levels were adjusted using the Benjamini and Hochberg (BH) correction (Benjamini and Hochberg 1995), which is less conservative than the Bonferroni correction method.

In order to provide a more holistic analysis of how *A. petiolata* may be associated, or not associated, with native herbs, we conducted a principal components analysis (PCA; using the covariance matrix) of all 90 quadrats, using the 2013 cover values for the fifteen most abundant herb species, excluding *A. petiolata* (ranging in cover from 11.2% for *D. glutinosum* to 0.4% for *Geum laciniatum* Murray). We visually examined how *A. petiolata* density was distributed in the PCA plot by marking quadrats according to 2013 *A. petiolata* presence/absence and, among the former, by *A. petiolata* density bin (0 to < 15%, 15 to < 25%, and 25% and above). If the high (or low) density *A. petiolata* quadrats tended to be located in one area of the PCA plot, this would mean that the community composition of herbs in high density *A. petiolata* quadrats (a combination of the specific herb species present and their abundances) was different from the species composition of other quadrats. On the other hand, if the high or low density *A. petiolata* quadrats were distributed randomly throughout the PCA plot, this would mean that herb species compositions in high density *A. petiolata* quadrats did not differ substantially from other quadrats in which *A. petiolata* was absent or present at a low density. We also formalized this process by examining correlations (Spearman's rho) between 2013 *A. petiolata* cover and the first and second principal components (PC1 and PC2, respectively) from the PCA. A significant association between *A. petiolata* and PC1 and/or PC2 would indicate that a significant proportion of the composition of herbs in quadrats was correlated with abundance of *A. petiolata* (Neter et al. 1996).

Quercus macrocarpa seedling data were analyzed using ANCOVA, with soil type (*A. petiolata* vs non-*A. petiolata*) and water regime (drought vs no drought) being treatments and acorn size being the covariate. *Fraxinus*

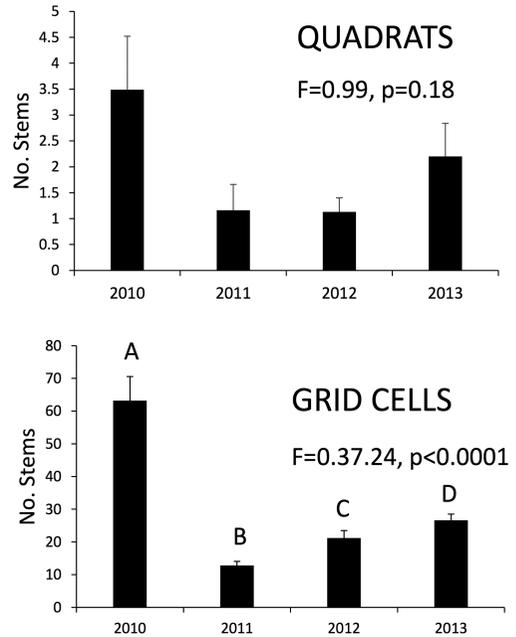


FIG. 1. Mean number of stems recorded in the quadrats (1.0 × 0.5 m) and in the grid cells (20 × 20 m) during each of the four years (Quadrats $N = 62$; Grid Cells $N = 71$ in 2010, $N = 152$ in 2011–2013). The number of stems did not differ statistically among years in the quadrats, but did in the grid cells. Grid cell annual totals that were distinguished from one another by the Tukey's HSD test are indicated with different letters.

pennsylvanica seedling data were analyzed using a 2-way ANOVA. Dependent metrics were root mass, shoot mass, total mass, and amount of time it took for a seedling to reach index level 4. Seed size was used a covariate for *Q. macrocarpa* due to the significant variation in size of the acorns.

All analyses were performed using JMP 10.0 (SAS Institute, Cary, NC). Errors presented are standard errors.

Results. TWO-POINT CYCLE. The stem counts we recorded in the grid cells and the small quadrats during the four years exhibited the same temporal pattern (Fig. 1). Year 2010 was a bonanza reproductive year for *A. petiolata* at the study site, followed by a dramatic reduction in stem production in 2011. However, rather than continuing a biennial alternation in abundance, overall stem production during the subsequent two years remained modest, generally exhibiting a gradual increase in stem production over the two years (Fig. 1).

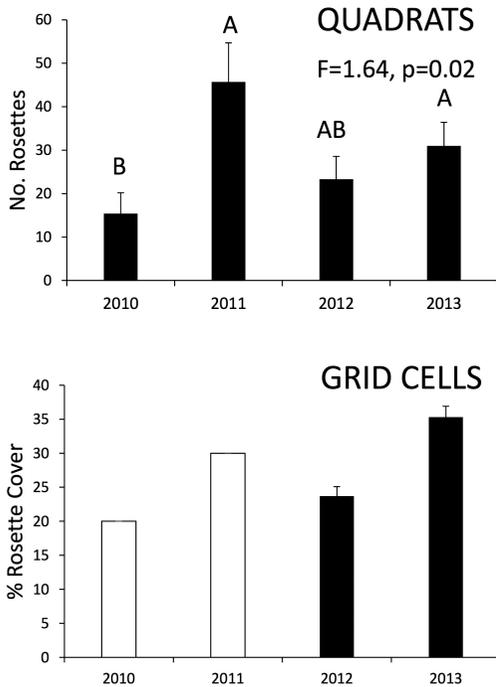


FIG. 2. Number of rosettes in the quadrats and the percent cover by rosettes in the grid cells during each of the four years. Note: the percent rosette cover in the grid cells in 2010 and 2011 could only be estimated (see text for more information).

We did find more evidence for biennial cycling in abundance with the rosettes over the four years. The quadrat data showed that the annual number of rosettes was consistent with an alternate year cycle (Fig 2). The rosette cover data from the grid cells also showed a general biennial cycle of abundance over the four years (Fig. 2). Since we did not record rosette and stem cover separately when we sampled the grid cells in 2010 and 2011 (as we did in 2012 and 2013), we cannot provide exact values for rosette cover in the grid cells during 2010 and 2011. However, using the total *A. petiolata* cover data, along with the stem count data, collected in the grid cells during 2010 and 2011, it is possible to provide a rough estimate of mean rosette cover in the grid cells during the first two years. For example, we know that rosette cover must have increased substantially from 2010 to 2011. This must be true because even though the number of stems per cell declined by 80% from 2010 to 2011 (Fig. 1), and hence stem cover would have declined substantially, total cover by *A. petiolata* cover increased from 2010 to 2011 (Fig 3.). Similarly,

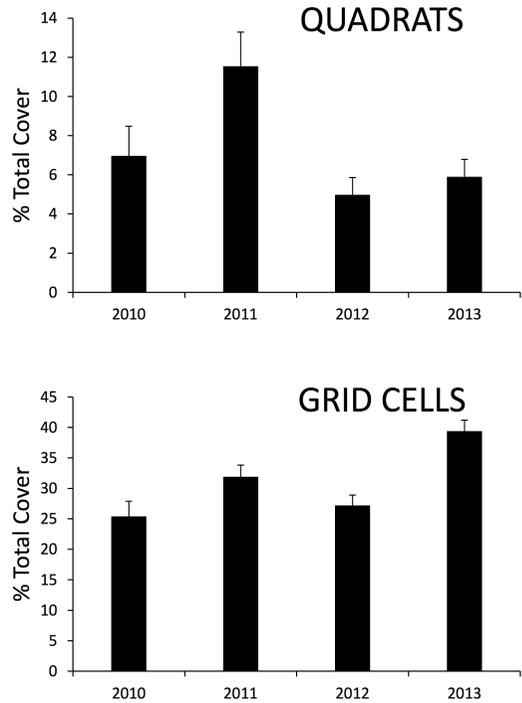


FIG. 3. Mean percent total cover of *A. petiolata* in the quadrats and the grid cells during each of the four years.

given that the overall *A. petiolata* cover in 2012 cells was less than in 2011 (Fig. 3) despite a 70% increase in the number of stems (Fig. 1), the cover of rosettes in 2011 must have been greater than it was in 2012, although again we cannot know by how much. While the absence of specific data for rosette cover at the intermediate scale in 2010 and 2011 prevented us from analyzing the data more formally, the general pattern of rosette abundance in the grid cells over the four years (constructed partly from data and partly from deduction) does exhibit an alternating annual abundance, one which is very similar to the pattern of rosette abundance documented from the quadrats.

The 2012 and 2013 rosette and stem cover data from the grid cells supported the hypothesis that the two-point cycle is density dependent. In the 32 cells with at least 5% stem cover in 2012, stem cover declined in 2013, while rosette cover increased (Fig. 4). A plotting of the stem counts in these 32 cells over the four year period were consistent with a sustained pattern of biennial cycling from 2010–2013 (Fig. 5). In contrast, in the 120 cells with less than 5% stem cover in 2012, both

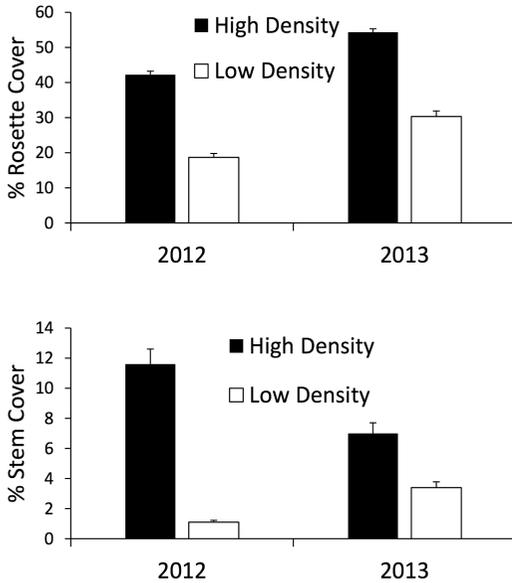


FIG. 4. Mean percent rosette and stem cover in high and low *A. petiolata* density grid cells in 2012 and 2013. High density cells were defined as cells with stem cover exceeding 5%.

stem and rosette cover increased from 2012 to 2013 (Fig. 4). Similarly, stem counts in these 120 cells did not exhibit a biennial cycling pattern during the four years (Fig. 5).

It should be noted that, due to the field sampling protocol used, *A. petiolata* cover values presented at the cell levels are higher than the actual cover. When recording garlic mustard presence along the belt transects, a patch of *A. petiolata* was considered to be continuous as long as there was less than a 10 cm gap between plants. While the belt transect data are useful for comparative purposes at the scale of the 20 × 20 m cells, the quadrat data provide a more accurate characterization of actual *A. petiolata* cover in the sampled area. For example, while mean total *A. petiolata* cover estimates for grid cells over the four years ranged from roughly 25–35%, mean total cover estimates for quadrats ranged from 5–11%, the latter a more accurate characterization of the abundance of *A. petiolata* in the study area.

LONGITUDINAL SPECIES ASSOCIATIONS. The comparisons of the change in *A. petiolata* cover from 2010 to 2013 with the change in cover of other species and vegetation types and of plant species richness within quadrats over the same period yielded only one near

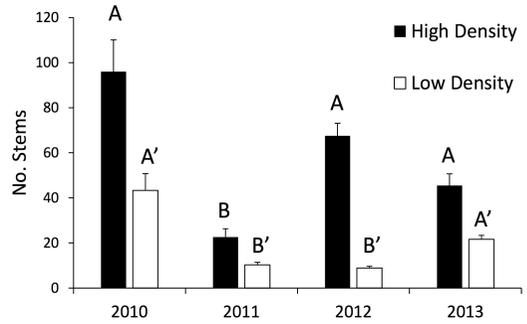


FIG. 5. Mean number of stems in high and low *A. petiolata* density grid cells during all four years. The number of stems varied among years in both the high and low density cells (high density $F = 13.86$, $P < 0.0001$; low density $F = 19.99$, $P < 0.0001$). Annual means that differed from one another are indicated with different letters, with one set of letters (A, B) applied to high density cells and a second set of letters (A', B') applied to the low density cells.

statistically significant relationship (of the 9 tested): *A. petiolata* vs *Ageratina altissima* ($F = 3.49$, $P = 0.0014$, BH correction = 0.079), and in this case, the relationship varied among the ten grid cells that contained the quadrats. Specifically, in two of the ten cells, change in *A. petiolata* cover among the nine quadrats in the cell was significantly negatively associated with change in *A. altissima* cover, while in one cell, the changes in the two groups were significantly positively correlated. The quadrats in the remaining seven cells did not exhibit any significant correlation between change in *A. petiolata* cover and change in the cover of *A. altissima*. The same comparisons between *A. petiolata* and the same nine vegetation metrics done for 2011–2013 yielded no statistically significant relationships between change in *A. petiolata* cover and change in any of the same nine vegetation variables (BH corrected P values > 0.20 for all analyses).

In only two of the forty analyses did any of the other five most common herbs (*Desmodium glutinosum*, *Amphicarpaea bracteata*, *Geranium maculatum*, *Circaea lutetiana*, and *Ageratina altissima*) exhibit a change in cover from 2010–2013 that was associated with changes in any of the eight vegetation metrics examined: herbaceous cover (including *A. petiolata* but excluding the cover of the target species), shrub cover, tree seedling cover, species richness of herbs, and the cover of the other four common herbs. The change in cover from 2010–2013 of *C. lutetiana* was

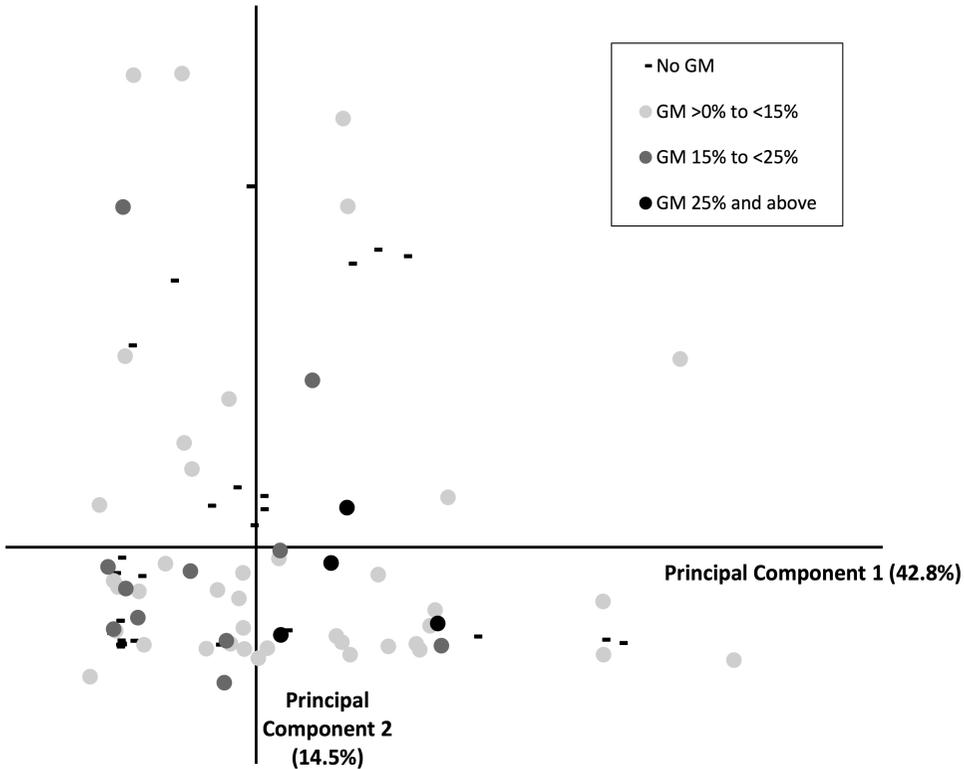


FIG. 6. PCA plot of the 90 quadrats, based on the cover of the fifteen most common herb species (*A. petiolata* not included). The distribution of quadrats containing different abundances of *A. petiolata* are shown by the different markers (see legend). Principal component 1 (PC1) accounted for 42.8% of the variation in the data and was strongly associated with density of *Desmodium glutinosum* cover (loading value (LV) > 0.99); PC2 accounted for 14.5% of the total variation and was strongly associated with *Amphicarpaea bracteata* cover (LV = 0.96). Both high and low cover *A. petiolata* quadrats are distributed mostly throughout the plot, indicating minimal association between *A. petiolata* presence and cover and the rest of the herbaceous community, except for a slight trend of very high cover *A. petiolata* quadrats having positive PCA1 values. *A. petiolata* cover was significantly correlated with PC1 (Spearman's rho (ρ) = 0.22, P = 0.039), but was not correlated with PC2 (ρ = 0.05, P = 0.66).

associated with the change in cover of other herbs (F = 3.09, P = 0.0029, BH correction = 0.079). However, similar to the case above, the relationship was positive in one cell and negative in another (with the quadrats in each of the other eight cells not exhibiting any relationship between the change in cover of these two species). The change in cover from 2010–2013 of *C. lutetiana* was also associated with the change in cover of *Geranium maculatum* (F = 3.06, P = 0.0031, BH correction = 0.079). Once again the nature of the relationship varied among cells, with the change in cover of the two species being positively correlated in one cell and negatively correlated in another (and not correlated at all in the other eight cells). None of the other thirty eight nested regression analyses produced

Benjamini and Hochberg (1995) corrected P values lower than 0.16.

When we restricted simple regression analyses to the twelve high *A. petiolata* cover (20–80%) quadrats, we found no significant associations between the change of *A. petiolata* cover from 2010–2013 and any of the nine vegetation metrics (P > 0.16 in all instances). Similarly, when we confined the simple regression analyses to the fifteen quadrats that exhibited the greatest increase in *A. petiolata* cover (5–30%) from 2010 to 2013, we also found no significant associations (P > 0.34 in all cases).

Ordination of the 2013 herbaceous cover data via PCA indicated that most of the variation in presence and abundance of native herbs was accounted for by the first two

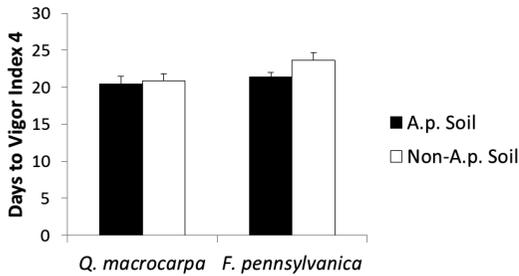


FIG. 7. Days to leaf vigor index 4 (leaves very dry and crinkled, often completely folded in on themselves, and probably dead) for *Q. macrocarpa* and *F. pennsylvanica* grown in soil collected from areas in the woodland where *A. petiolata* was abundant (*A. p.* soil) and in soil from areas where *A. petiolata* was absent (Non-*A. p.* soil). Analyzed separately, *Q. macrocarpa* showed no difference in time to complete wilting between the two soil type ($F = 1.51, P = 0.23$) while *F. pennsylvanica* did show a difference ($F = 9.85, P = 0.02$), with seedlings growing in *A. petiolata* soil declining to the vigor 4 index approximately 10% faster than seedlings grown in non-*A. petiolata* soil.

principal components: PC1 accounted for 42.8%, and PC2 14.5% (Fig. 6). PC1 was strongly associated with density of *Desmodium glutinosum* cover (loading value (LV) > 0.99), and weakly associated with *Galeopsis* spp. (LV = 0.35) and *Viola* spp. (LV = 0.29). PC2 was strongly associated with *Amphicarpaea bracteata* cover (LV = 0.96) and weakly associated with *Geranium maculatum* (LV = 0.36), *Ageratina altissima* (LV = 0.33), and *Gallium triflorum* (LV = 0.28). *Alliaria petiolata* cover in 2013 was significantly correlated with PC1 (Spearman's rho (ρ) = 0.22, $P = 0.039$), which is also evident from the distribution of *A. petiolata* cover among quadrats in Figure 6; however, *A. petiolata* cover was more strongly correlated with *Desmodium glutinosum* directly ($\rho = 0.25, P = 0.018$), suggesting this correlation underlies the relationship with PC1. *Alliaria petiolata* cover was not correlated with PC2 ($\rho = 0.05, P = 0.66$).

DROUGHT AND SOIL EFFECTS ON TREE SEEDLINGS. *Quercus macrocarpa*. Root, shoot, and total biomass were all positively correlated with acorn volume ($P < 0.0001$ in the shoot and total biomass analyses, $P < 0.01$ in the root analysis). Root biomass was significantly reduced in seedlings exposed to drought ($F = 9.71, P = 0.004$) and total biomass was borderline significantly smaller in drought seedlings ($F = 3.72, P = 0.06$). Shoot mass did not differ between water levels ($F = 0.00,$

$P = 0.98$) or soil types ($F = 0.48, P = 0.49$). There was no significant interaction between water treatment and soil type for any of the three mass metrics ($P > 0.30$ in all three analyses). While none of the seedlings that received water throughout the study exhibited any wilting, all of the drought seedlings did. Considering just the seedlings exposed to drought, neither acorn size nor soil type affected time to vigor index 4 (acorn $F = 1.18, P = 0.29$; soil type $F = 1.51, P = 0.23$; Fig. 6).

Fraxinus pennsylvanica. Both shoot and total seedling biomass were reduced under drought conditions (shoot $F = 36.46, P < 0.0001$; total $F = 31.74, P < 0.0001$), but were not affected by soil type (shoot $F = 2.68, P = 0.12$; total $F = 1.98, P = 0.18$). There was no significant interaction between soil type and water regime in either analysis. Root mass was unaffected by either soil type ($F = 0.00, P = 0.95$) or water regime ($F = 2.64, P = 0.12$). As was the case with *Q. macrocarpa*, none of the seedlings that received water throughout the study exhibited any wilting. Unlike *Q. macrocarpa*, however, *F. pennsylvanica* seedlings growing in drought conditions declined in vigor more quickly when grown in *A. petiolata* soil ($F = 9.85, P = 0.02$). Specifically, seedlings grown in *A. petiolata* soil reached the vigor 4 index approximately 10% faster than seedlings grown in non-*A. petiolata* soil (*A. petiolata* soil = 21.4 ± 0.40 days; non *A. petiolata* soil = 23.7 ± 0.67 days; $F = 9.85, P = 0.02$; Fig. 7).

Discussion. **TWO-POINT CYCLE.** We did not find evidence of a sustained two-point cycle throughout the study site as a whole during the four year study. The varying abundance of first-year rosettes were consistent with a two-point cycle, but this was not the case for the second-year stems. The boom stem production year of 2010 was followed by three subsequent years of low stem abundance. Given that the theory behind the two-point cycle is based on competition between the first and second-year plants, it is likely that the two-point cycle would only be exhibited in high *A. petiolata* density areas (Winterer et al. 2005, Pardini et al. 2009, Van Riper et al. 2010). The results of our study support this prediction of a patchy spatial distribution of smaller scale areas that do and do not follow the two-point

cycle. At a large scale, stem numbers did not cycle when all grid cells were considered, but at a smaller scale, when just high *A. petiolata* density cells were considered, stem production over the four years was consistent with a two-point cycling. That the two-point cycle is likely density dependent may explain why it has been documented at some sites and not others (Van Riper et al. 2010).

Our findings also show the value of longer term studies. During the first two years of the study we documented a clear two-point cycle throughout the study site (Davis et al. 2012); however, the two-point cycle was not sustained at this scale during the subsequent two years. It was sustained in smaller areas in the study site that contained high densities of *A. petiolata*, or at least sufficiently high densities to likely cause significant competition.

SPECIES ASSOCIATIONS. Based on our four-year monitoring of *A. petiolata* and the other plant species in the 1.0×0.5 m quadrats, *A. petiolata* appears to be acting similarly to other species, with *A. petiolata* and the other common native herbs changing in abundance largely independent of one another and of herbaceous species richness. Since it has been more than ten years since *A. petiolata* colonized our study site, it is possible that it had already excluded the sensitive species when we began our study in 2010 and that the species remaining are ones that are able to cope with its presence. Although *A. petiolata* is one of the most abundant herbs in the woodland, and even though it does grow in some dense patches, it does not inhabit the majority of the woodland area. Also, since it undoubtedly took several years following its introduction (most likely in the late 1990s) to reach the coverage documented in 2010, the population has probably been persisting at its current level for no more than ten years. Given the combination of its modest abundance and its short period of occupancy, it is difficult to imagine that *A. petiolata* would have been able to competitively exclude all the sensitive native herbs from the woodland in this short amount of time.

Another possible explanation for why we have not yet documented consistent negative associations between *A. petiolata* and any of the native species is that the negative effects are density dependent. If measureable negative effects occur only when *A. petiolata* is

abundant, given that approximately a third of the ninety quadrats in this study contained no *A. petiolata* during any of the four years, it might not be surprising that no negative associations were documented. However, when we restricted our analyses to quadrats in which *A. petiolata* was abundant in 2010 (20–82% cover), we still found no negative association between *A. petiolata* and any of the other vegetation metrics ($P > 0.16$ in all cases). Similarly, when we restricted our analyses to quadrats in which *A. petiolata* increased markedly in abundance from 2010 to 2013, we also did not find any negative relationships ($P > 0.37$ in all cases). We do note that restricting our analyses to the high density quadrats inevitably reduced the power of the analysis.

Results of the PCA are consistent with these findings: i.e., the fact that the density of the dominant herbaceous species in the woodland is positively correlated with density of *A. petiolata* (the second most dominant herb) suggests that both species are responding similarly to environmental variation. If *D. glutinosum* and *A. petiolata* are competing with one another, or are potential competitors, then apparently the respective densities are too low to produce the intensity of competition required to yield an inverse relationship between the abundances of the two species.

EFFECTS ON TREE SEEDLINGS. We found no evidence of *A. petiolata* soil inhibition of the *Q. macrocarpa* seedlings, whether water stressed or not. Given that seedling growth was strongly positively associated with acorn size, a relationship commonly documented and believed to be due to the additional resources provided by larger acorns (Villar-Salvador et al. 2010), it is possible that young *Q. macrocarpa* seedlings may be buffered against soil inhibitory effects by the resources provided by the acorns, at least until acorn resources are depleted and seedlings become reliant on mycorrhizal communities.

As was the case for the *Q. macrocarpa* seedlings, drought stress did not affect root and shoot mass of *F. pennsylvanica* seedlings growing in *A. petiolata* soil compared to seedlings growing in non-*A. petiolata* soil. Drought-stressed *F. pennsylvanica* seedlings did exhibit a faster decline to complete desiccation when grown in *A. petiolata* soil, specifically two days faster over a three week

period; however, the differences that were documented between *F. pennsylvanica* seedlings grown in *A. petiolata* and non-*A. petiolata* soil were very modest, as was the case in the 2011 study (Davis et al. 2012). Even assuming that the 10% accelerated rate of desiccation we found would occur in the field, it is not known at this time whether this small difference would have any significant impact on the recruitment of *F. pennsylvanica* in the woodland. Providing further evidence that *A. petiolata* is not having a significant ecological effect on tree seedlings is the fact that we did not find any correlation in the quadrat study between the change in the cover of *A. petiolata* and the cover of tree seedlings from 2010 to 2013.

Conclusions. If a two-point cycle exists only when both stems and rosettes are exhibiting a biennial cycle in abundance in which each is offset from the other by a year, then we did not document a two-point cycle at the scale of our entire several ha study site. At a finer scale, we did document two-point cycling occurring in smaller areas of the study where *A. petiolata* occurred in dense patches. The fact that we documented two-point cycling in areas where *A. petiolata* was dense and not in other areas supports the hypothesis that the two-point cycle is driven by competition between first (rosette) and second-year (stem) plants.

While, strictly speaking, a two-point cycle of both plant growth forms may not be occurring at a landscape scale (e.g., because stem production may not be cycling in a biennial pattern), our study showed that rosette cover may still be exhibiting biennial cycling in abundance. Since in our study, total *A. petiolata* cover was strongly correlated with rosette cover (but not stem cover; Fig. 2, 3), total *A. petiolata* cover may be cycling in this way as well. Whether or not a population is exhibiting a two-point cycle may have management implications. Based on a computer model, Pardini et al. (2009) concluded that removal efforts (whether by mechanically removing plants or killing them with an herbicide) will be more effective if management is directed at the stems during a stem peak year. Biennial cycling in overall abundance in an *A. petiolata* population, whether a strict two-point cycle is being exhibited or not, may also have ecological implications. To the extent that *A. petiolata* is affecting other plants, and that the magnitude of its effects

are associated with its abundance in a given year (e.g., through its demands for nutrients, water, and light), the degree of its influence may also fluctuate from year to year along with its abundance.

In four years of study, we have not been able to document any substantial effects by *A. petiolata* on other plant species. Overall, our findings are not consistent with the common claim that *A. petiolata* is a noxious invasive species responsible for the decline of many North American native forest herbs (Rodgers et al. 2008). Rather, our findings are more consistent with other recent studies and reviews that have concluded that *A. petiolata* is primarily responding to ecological changes in North American forests and that it is not a significant driver of change (Nuzzo et al. 2009, Rooney and Rogers 2011).

Literature Cited

- BAUER, J. T., R. C. ANDERSON, AND M. R. ANDERSON. 2010. Competitive interactions among first-year and second-year plants of the invasive, biennial garlic mustard (*Alliaria petiolata*) and native ground layer vegetation. *Restor. Ecol.* 18: 720–728.
- BENJAMINI, Y. AND Y. HOCHBERG. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. Royal Stat. Soc., Series B (Methodological)* 57: 289–300.
- DAVIS, M. A., L. BIER, E. BUSHELLE, C. DIEGEL, A. JOHNSON, AND B. KUJALA. 2005. Non-indigenous grasses impede woody succession. *Plant Ecol.* 178: 249–264.
- DAVIS, M. A., A. COLEHOUR, J. DANAY, E. FOSTER, C. MACMILLAN, E. MERRILL, J. O'NEIL, M. PEARSON, M. WHITNEY, M. A. ANDERSON, AND J. J. DOSCH. 2012. The population dynamics and ecological effects of garlic mustard, *Alliaria petiolata*, in a Minnesota oak woodland. *Am. Midl. Nat.* 168: 364–374.
- LANKAU, R. A. 2011. Intraspecific variation in allelochemistry determines an invasive species' impact on soil microbial communities. *Oecologia* 165: 453–463.
- MCCARTHY, B. 1997. Response of a forest understory community to experimental removal of an invasive non-indigenous plant (*Alliaria petiolata*, Brassicaceae), pp. 117–130. *In* J. Luken and J. Thieret [eds.], *Assessment and Management of Plant Invasions*. Springer-Verlag, New York, NY.
- NETTER, J., M. H. KUTNER, W. WASSERMAN, AND C. J. NACHTSHEIM, eds. 1996. *Applied Linear Regression Models*, Third edition. McGraw-Hill, New York. 1184 p.
- NUZZO, V. A., J. C. MAERZ, AND B. BLOSSEY. 2009. Earthworm invasion as the driving force behind plant invasion and community change in north-eastern North American forests. *Conserv. Biol.* 23: 966–974.

- PARDINI, E. A., J. M. DRAKE, J. M. CHASE, AND T. M. KNIGHT. 2009. Complex population dynamics and control of the invasive biennial *Alliaria petiolata* (garlic mustard). *Ecol. Appl.* 19: 387–397.
- RODGERS, V. L., K. A. STINSON, AND A. C. FINZI. 2008. Ready or not, garlic mustard is moving in: *Alliaria petiolata* as a member of eastern North American forests. *Bioscience* 58: 426–436.
- ROONEY, T. P. AND D. A. ROGERS. 2011. Colonization and effects of garlic mustard (*Alliaria petiolata*), European buckthorn (*Rhamnus cathartica*), and Bell's Honeysuckle (*Lonicera × bella*) on understory plants after five decades in southern Wisconsin forests. *Invas. Plant Sci. Manage.* 4: 317–325.
- VAN RIPER, L. C., R. L. BECKER, AND L. C. SKINNER. 2010. Population biology of garlic mustard (*Alliaria petiolata*) in Minnesota hardwood forests. *Invas. Plant Sci. Manage.* 3: 48–59.
- VILLAR-SALVADOR, P., N. HEREDIA, AND P. MILLARD. 2010. Remobilization of acorn nitrogen for seedling growth in holm oak (*Quercus ilex*), cultivated with contrasting nutrient availability. *Tree Physiol.* 30: 257–263.
- WINTERER, J., M. C. WALSH, M. PODDAR, J. W. BRENNAN, AND S. M. PRIMAK. 2005. Spatial and temporal segregation of juvenile and mature garlic mustard plants (*Alliaria petiolata*) in a central Pennsylvania woodland. *Am. Midl. Nat.* 153: 209–216.
- WOLFE, B. E., V. L. RODGERS, K. A. STINSON, AND A. PRINGLE. 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. *J. Ecol.* 96: 777–783.