# Effects of Herbicide on the Invasive Biennial *Alliaria petiolata* (Garlic Mustard) and Initial Responses of Native Plants in a Southwestern Ohio Forest

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**Abstract**

**Restoration often includes control of invasive plants, but little is known about how native plant communities respond to this control. The biennial *Alliaria petiolata* (M. Bieb.) Cavara and Grande (garlic mustard) is one of the most prevalent invasive plants in forests of eastern North America. We investigated the effects of the herbicide Round-up (glyphosate) on *Alliaria* and the response of the forest floor plant community to the herbicide and the subsequent decline of *Alliaria*. In an old-growth *Acer–Fagus* stand and a second-growth *Liriodendron*-dominated stand in Hueston Woods State Nature Preserve, Ohio, United States, we spot applied Round-up in November 2000 and 2001 in 25 1×1–m plots and maintained 25 control plots. Herbicide decreased *Alliaria* density in both stands and reduced the density of other species in leaf during treatment (mostly exotic winter annuals) in the old-growth stand. Treatment did not affect the initial density of the *Alliaria* cohort that germinated in the spring of 2001, but decreased the 2002 cohort. Community differences were found in the old-growth stand after *Alliaria* reduction, specifically greater cover of spring ephemerals in the herbicide treatment. In the second-growth stand, herbicide treatment increased reproduction of the late-summer perennial, *Phryma leptostachya*. These results indicate that glyphosate reduces *Alliaria* without negatively impacting native species and that some native species respond positively to a single-year reduction in this invasive biennial.**

**Key words:** **alien species, DCA ordination, exotic species, glyphosate, herbicide, Hueston Woods State Park, invasive species, nonindigenous species, Round-up.**

## Introduction

Invasive plant species have the potential to negatively impact native plant species and communities, including reduction of biodiversity (Lodge 1993; Woods 1997), alteration of community structure, function, and composition (Woods 1997), and changes in dynamic community properties (Huenneke & Mooney 1989). However, direct impacts of invasive plants on native plants have not been well studied (Parker et al. 1999; D'Antonio & Kark 2002), with some exceptions (Miller & Gorchov 2004).

In the United States, nature preserves that have been invaded by exotic species are of special concern, as these preserves are virtually the only remaining representatives of intact communities (Westman 1990). Because control of invasive species is a major concern and cost of management of National Parks and other preserves, as well as a common component of restoration efforts, there is a need for better understanding of the impacts of invaders and the response of plant communities to eradication efforts (D'Antonio & Meyerson 2002).

One of the most exemplary remaining stands of old-growth *Fagus grandifolia* (beech)–*Acer saccharum* (sugar maple) forests in the midwestern United States is in Hueston Woods State Nature Preserve (hereafter, "Hueston Woods") in southwestern Ohio (Runkle et al. 1984). The exotic biennial herb *Alliaria petiolata* (garlic mustard) has established there and is perceived to be a problem by the Ohio Department of Natural Resources (ODNR 1998).

Since its introduction to North America from northern Europe in approximately 1868 for its medicinal and nutritional properties, *Alliaria* has spread into many portions of the northeastern and midwestern United States, including many parts of Ohio (Nuzzo 1993). In these areas, *Alliaria* is most common in damp, semishaded forests (Cavers et al. 1978; Nuzzo 1993; Byers & Quinn 1998). *Alliaria* has higher germination, growth, and reproduction in forest edges and mesic, lowland forests than upland forest interiors, but all of these habitats are susceptible to invasion (Meekins & McCarthy 2001). Although *Alliaria* has been labeled a severe threat to deciduous forests (Nuzzo 1994), there has been little study of the effects of *Alliaria* on native plants. A removal study showed that *Alliaria* had

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negative impacts on forest floor vegetation of a somewhat disturbed lowland forest (McCarthy 1997). *Alliaria petiolata* has been shown to be equal to or more competitive than two of three native understory species from this forest (Meekins & McCarthy 1999). However, *Alliaria* did not significantly affect the growth of any of four herbaceous species via allelopathy (McCarthy & Hanson 1998).

A variety of methods have been suggested and tested for the eradication of *Alliaria* (Auld et al. 1978; Nuzzo 1991, 1993, 1996; Baskin & Baskin 1992; McCarthy 1997). Although dormant-season prescribed fire followed by herbicide application is effective in some areas (Nuzzo 1991), it is not a good choice where fires are not part of the natural disturbance regime, as in Hueston Woods, which lies on the glaciated till plain. Nuzzo (1996) found dormant-season (spring and/or fall) herbicide treatment to be most appropriate for the removal of *Alliaria* in large areas where it grows with native plants that would be vulnerable to herbicide during the growing season. This is the case in Hueston Woods, so fall spot application of herbicide (Round-up; Monsanto, St. Louis, MO, U.S.A.) over many years has been chosen by the Park, in conjunction with the ODNR, to eradicate *Alliaria*.

Because Round-up is a nondiscriminatory herbicide, other species with leaves at the time of fall spraying (hereafter, "wintergreen species") could potentially be affected. We monitored representative species to quantitatively determine whether or not they were impacted by Round-up.

Our objectives were to determine the extent to which *Alliaria* changes the community on a small scale over several years and whether herbicide application is an effective control mechanism for *Alliaria* in upland mesic deciduous forest—a necessary step before widespread removal attempts are made (Hager & McCoy 1998). In addition, trial removal should reveal other responses to *Alliaria* elimination, such as erosion and invasions of other exotics (Westman 1990; D'Antonio & Meyerson 2002), and thus inform management and restoration decisions. This study addressed the following questions: (1) What is the effect of fall Round-up application on the density of *Alliaria*; (2) What is the effect of fall Round-up application on other wintergreen herbs; and (3) What is the effect of the first year of reduced *Alliaria* density on forest floor vegetation and the demography of native herbs and tree seedlings?

## Methods

### Study Species

In North America, *Alliaria petiolata* (M. Bieb.) Cavara and Grande (Brassicaceae) is a biennial with seeds germinating in February–April (Lhotska 1975; Baskin & Baskin 1992; Anderson et al. 1996; Byers & Quinn 1998). During their first year, plants are rosettes; some leaves are lost following cold spells, but new leaves also expand during the winter (Anderson et al. 1996). Surviving plants bolt the following spring, reaching up to 1.25 m in height, and flowering in April–June (Cavers et al. 1978; Anderson et al. 1996). Flowers self-pollinate (autogamously) but can be pollinated by insects (Cavers et al. 1978; Anderson et al. 1996; Cruden et al. 1996). Seeds are dispersed by water (Nuzzo 1993), humans (Lhotska 1975; Nuzzo 1993), and animals epizoochorously (externally) (Cavers et al. 1978). Most seeds germinate in the next spring, but some remain in the seed bank for up to 5 years (Roberts & Boddrell 1983; Baskin & Baskin 1992; Anderson et al. 1996; Byers & Quinn 1998).

### Study Site

This study took place in the ODNR State Nature Preserve located in Hueston Woods State Park in Butler County, southwestern Ohio. *Alliaria* was reported at this site by 1977 (Runkle et al. 1984) and is currently located throughout the Preserve, but at greater densities along trails, in low, moist areas and tree-fall gaps. Our two sites were a 20-ha portion of an old-growth stand (trees >200 years old) dominated by *Fagus grandifolia* (American beech) and *Acer saccharum* (sugar maple) (39°34′07″ to 39°34′00″ N, 84°45′10″ to 84°45′02″ W) and a 16-ha section of a second-growth stand (approximately 50–100 years old) dominated by *Liriodendron tulipifera* (tulip poplar) (39°34′33″ to 39°34′31″ N, 84°45′41″ to 84°45′37″ W). Silty clay loam, sandy clay loam, and sandy loam soils are found in the uplands, whereas sandy loam and clay loam soils are found in the slope areas (Vankat et al. 1975; Runkle et al. 1984).

### Experimental Design

In mid-May 2000, we established 50 $1 \times 1$–m plots in each stand (100 plots in total); each plot had high abundance of *Alliaria*, was located under a closed canopy, on reasonably level ground, greater than or equal to 8 m away from trails, and greater than or equal to 5 m from neighboring plots. Each plot was randomly assigned to be sprayed or unsprayed (control) to achieve a total of 25 plots per treatment per stand.

On 6 November 2000 and 7 November 2001 we spot applied 1% glyphosate (prepared by dilution of Round-up PRO [41% glyphosate]) to *Alliaria* within 2 m of the center of each treatment plot using backpack sprayers. To prevent repeated spraying, the herbicide contained blue coloring.

### Herbicide Effects on *Alliaria petiolata*

We counted the number of individual *Alliaria* rosettes and adults in each plot in May, June, August, and October 2000, February, May, June, August, and October 2001, and May 2002. To determine the effects of treatment and stand on density of *Alliaria* (adults and rosettes, separately) each May, we carried out two-way ANOVAs using Number

Crunching Statistical Software (NCSS; Hintze 2001), with treatment and stand considered fixed effects. To meet ANOVA assumptions, densities were $\log_{10}(x+1)$ transformed. Density of adults in May 2002 did not meet ANOVA assumptions even following transformation, so treatment effects were tested separately for each stand using Kruskal–Wallis tests.

#### Herbicide Effects on Wintergreen Herbs

Individuals of each wintergreen species (herbs in leaf in October 2000) were counted in each plot in that month, in February, May, and October 2001, and May 2002. Species were pooled because densities for individual species were low. Treatment effects on density before (October 2000) and after (February and October 2001 and February 2002) were analyzed separately for each stand by Kruskal–Wallis tests.

#### Community Effects

We determined the percent cover of each plant less than or equal to 0.85 m tall (including herbs, vines, tree seedlings, and shrubs) in each plot during mid-May, late June, and mid-August 2000 and 2001 by point frame sampling. Pins were dropped at 50 predetermined equally spaced points. Each separate touch was counted as 2% cover, allowing for total cover per plot to exceed 100%. Nomenclature follows Gleason and Cronquist (1991). Voucher specimens of all species were deposited at the W.S. Turrell Herbarium (Miami University). For each plot, peak percent cover of each species was determined for each year.

For each plot, each year, we determined species richness (total species detected in cover sampling). The effect of herbicide treatment and stand on richness each year was determined by two-way ANOVA. We also compared species diversity between treatments by calculating Shannon–Wiener diversity ($H' = -\Sigma p_i[\ln p_i]$, where $p_i$ = proportion of peak cover accounted for by species $i$) of summed peak covers of the species over all plots of each treatment × stand × year combination.

To examine compositional patterns in the forest floor community, we ordinated plots using detrended correspondence analysis (DCA) of the peak percent cover of each species (excluding *A. petiolata*) using PC-ORD 4.0 (McCune & Mefford 1999). Separate ordinations were done for each year and stand. To determine whether control and sprayed plots differed significantly in species composition, plot scores for each of the first two axes of each ordination were analyzed using one-way ANOVA. Univariate analyses are appropriate because the DCA axes are, by definition, orthogonal and uncorrelated (McCune & Grace 2002).

For one of the four ordinations (old growth, 2001) control and sprayed plots differed significantly on one of the DCA axes. To determine whether this difference was attributable to specific growth forms, we summed cover percentages of all species within each growth form (Appendix), and for each growth form tested the effect of treatment using a Kruskal–Wallis test. Additionally, the total cover of all species (excluding *Alliaria*) in each plot in May 2001 was compared between control and sprayed plots using one-way ANOVA to determine whether or not the overall plot cover was driving the ordination results.

#### Effects on Demography of Native Species

To test whether the reduction in *Alliaria* enhanced the survival, growth, or reproduction of individual plants of native species, we monitored individuals of species representing different growth forms and/or functional groups (Tables 1 & 2), chosen based on high importance percentages reported by Runkle et al. (1984) and abundance in plots early in the growing season of 2000. If a plot had only a single individual of a study species, that individual was marked. If a plot had multiple individuals of a species, we divided the plot into four quadrants and sought the individual closest to the center of each quadrant and the center of the entire plot. Thus, a maximum of five individuals per plot (but usually fewer) of each species were marked.

**Table 1.** Number of individuals and their mean (± SE) size (number of leaves) of selected native species chosen for demographic study, with the stand and year studied.

| | | | | *Control* | | *Sprayed* | |
|---|---|---|---|---|---|---|---|
| *Species* | *Growth Form/Functional Group* | *Stand* | *Year* | *n* | $\bar{X} \pm SE$ | *n* | $\bar{X} \pm SE$ |
| *Impatiens pallida* | Mid- to late-summer annual | OG | 2001 | 33 | 14.5 ± 2.1 | 33 | 14.7 ± 1.5 |
| *Dicentra canadensis* | Spring ephemeral perennial | OG | 2001 | 19 | 1.2 ± 0.1 | 23 | 1.6 ± 0.2 |
| *Osmorhiza longistylis* | Evergreen perennial | SG | 2000 | 21 | 4.0 ± 0.4 | 29 | 4.4 ± 0.6 |
| | | | 2001 | 21 | 2.6 ± 0.3 | 29 | 2.1 ± 0.3 |
| *Phryma leptostachya* | Late-summer perennial | SG | 2000 | 8 | 5.4 ± 0.9 | 12 | 5.4 ± 0.6 |
| | | | 2001 | 8 | 8.9 ± 0.4 | 12 | 9.2 ± 0.5 |
| *Acer saccharum* | Tree seedling | OG | 2000 | 7 | 5.0 ± 2.0 | 18 | 4.4 ± 1.9 |
| | | | 2001 | 7 | 7.7 ± 3.4 | 18 | 7.8 ± 2.9 |

OG, old growth; SG, second growth.
Statistical tests are reported in *Results*.

**Table 2.** Number of individuals and their mean (± SE) reproduction (number of fruits) of selected native species chosen for demographic study, with the stand and year studied.

| | | | | Control | | Sprayed | |
|---|---|---|---|---|---|---|---|
| *Species* | *Growth Form/Functional Group* | *Stand* | *Year* | *n* | $\bar{X} \pm SE$ | *n* | $\bar{X} \pm SE$ |
| *Impatiens pallida* | Mid- to late-summer annual | OG | 2001 | 33 | 5.9 ± 1.3 | 33 | 6.0 ± 1.0 |
| *Claytonia virginica* | Spring ephemeral perennial | OG | 2001 | 40 | 4.5 ± 0.8 | 62 | 4.0 ± 0.6 |
| | | SG | 2001 | 87 | 4.0 ± 0.3 | 87 | 4.3 ± 0.4 |
| *Osmorhiza longistylis* | Evergreen perennial | SG | 2000 | 21 | 0.6 ± 0.6 | 29 | 6.0 ± 2.3 |
| | | | 2001 | 21 | 10.0 ± 3.4 | 29 | 7.1 ± 2.5 |
| *Phryma leptostachya* | Late-summer perennial | SG | 2000 | 8 | 4.8 ± 2.6 | 12 | 0.1 ± 0.1 |
| | | | 2001 | 8 | 2.9 ± 1.9 | 12 | 2.8 ± 2.5 |

OG, old growth; SG, second growth.
Statistical tests are reported in *Results*.

Marked plants were censused every 2–3 weeks during each growing season for demographic parameters (survival, growth, and reproduction). Multiple measures of size and reproduction were recorded for some species, but only the most widely used measures (number of leaves and number of fruits) are reported.

The effect of treatment on survival of the evergreen perennial *Osmorhiza longistylis* (sweet cicely) from 2000 to 2001 was determined with chi-square tests using Minitab (Minitab Inc. 2000). Treatment effect on number of leaves in the spring ephemeral perennial *Dicentra canadensis* (squirrel corn) was also determined using a chi-square test. We used one-way ANOVA (in NCSS) to analyze the effect of treatment on the spring ephemeral perennial *Claytonia virginica* (spring beauty) fruit with plot number as a nested factor within treatment. All other growth and reproduction data were analyzed by one-way ANOVAs if normal and by Kruskal–Wallis tests if non-normal. Specifically, we tested for treatment effects on changes from 2000 to 2001 in leaf number of the late-summer perennial *Phryma leptostachya* (lopseed), *Osmorhiza*, and seedlings of the tree *A. saccharum* and in number of fruits matured of *Phryma* and *Osmorhiza*. For the mid- to late-summer annual *Impatiens pallida* (yellow touch-me-not), we tested for treatment effects on maximum leaf number and total fruits (calculated as the sum of all fruits and dehisced capsules recorded throughout the growing season) of individuals in 2001.

## Results

### Herbicide Effects on *Alliaria petiolata*

In May 2000, before the first herbicide application, there was no effect of treatment or stand on density of *Alliaria petiolata* rosettes (Table 3) (old-growth stand: treatment mean ± SE 109.5 ± 10.8 vs. control 94.8 ± 9.3, Fig. 1; second-growth stand: 87.1 ± 8.7 vs. 82.5 ± 8.0, Fig. 2). However, after spraying, there was a significant treatment effect and a significant treatment × stand interaction on the density of this 2000 cohort (May 2001 adults; Table 3). In the old-growth stand, sprayed plots averaged 1.3 ± 0.3 *Alliaria* adults in May 2001 whereas control plots averaged 8.3 ± 1.7 (Fig. 1). In the second-growth stand, sprayed plots averaged 3.4 ± 0.6 in May 2001 versus 6.0 ± 1.4 in control plots (Fig. 2).

Density of rosettes (2001 cohort) in May 2001 was not affected by treatment, although there was a significant stand effect (Table 3); old-growth-sprayed plots averaged 85.1 ± 11.8 versus 91.9 ± 17.2 in control plots (Fig. 1), second-growth-sprayed plots averaged 118.2 ± 12.2 versus 100.6 ± 10.9 in controls (Fig. 2). After spraying, when this cohort reached the adult stage (May 2002), densities were extremely low in both treatments in both stands. In the old-growth stand there was a trend for sprayed plots to have less *Alliaria* than controls (0.8 ± 0.2 vs. 1.5 ± 0.9; Fig. 1; Kruskal–Wallis: $H = 3.36$, $p = 0.07$). In the second-growth stand, treatments did not differ in May 2002 adult density (sprayed 2.4 ± 0.6, control 1.7 ± 0.5; Fig. 2; $H = 1.51$).

Density of the 2002 cohort (May 2002 rosettes) was significantly affected by treatment and stand (Table 3). In the old-growth stand, sprayed plots averaged 11.4 ± 2.1 rosettes versus 31.8 ± 6.6 in control plots (Fig. 1); in the second-growth stand, sprayed plots averaged 28.5 ± 4.1 versus 36.4 ± 6.1 in controls (Fig. 2).

### Herbicide Effects on Wintergreen Herbs

Before spraying (October 2000), there was no difference in total density of all wintergreen herb species between treatments in either the old-growth stand or the second-growth stand (Table 4). Lower wintergreen herb density was apparent in sprayed plots in the old-growth stand in February 2001 and February 2002, but not in October 2001 (Table 4). No such treatment effect occurred in the second-growth stand (Table 4).

### Community Effects

There was no significant treatment effect on species richness either before (2000) or after spraying (2001) (Table 5; 2000: $F_{1,96} = 3.61$; 2001: $F_{1,96} = 0.30$). Species richness was

**Table 3.** *F*-ratios from two-factor analyses of variance (ANOVAs) for log(x + 1)-transformed densities of *Alliaria petiolata.*

| Alliaria *Cohort* | *Stage, Date* | *Treatment* | *Stand* | *Stand × Treatment* |
|---|---|---|---|---|
| 2000 | Rosette, May 2000 | 0.91 | 1.74 | 0.31 |
| | Adult, May 2001 | 18.73*** | 1.52 | 5.11* |
| 2001 | Rosette, May 2001 | 0.58 | 7.41* | 0.04 |
| 2002 | Rosette, May 2002 | 5.22* | 10.05** | 3.07 |

Stand refers to old growth or second growth; treatment refers to sprayed or control. For each *F*, $df = 1, 96$.
*$p < 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

higher in the second-growth stand each year (Table 5; stand effect 2000: $F_{1,96} = 48.29$, $p < 0.001$; 2001: $F_{1,96} = 20.80$, $p < 0.001$), but there was no stand × treatment interaction (2000: $F_{1,96} = 0.00$, 2001: $F_{1,96} = 1.33$). Similarly, Shannon–Wiener diversity indices of the two treatments were similar for each stand in each year of the study (old-growth 2000 control: 2.05 vs. sprayed 2.23; 2001 control: 2.40 vs. sprayed 2.72; second-growth 2000 control: 2.81 vs. sprayed 2.85; 2001 control: 3.06 vs. sprayed 3.05).

Control and sprayed plots did not differ in community composition before spraying (2000 growing season) in either stand, based on ordinations (Carlson 2002). This finding was confirmed by ANOVAs, which revealed no treatment effects on plot scores on axis 1 or 2 generated by the detrended correspondence analysis ordination (Carlson 2002). In 2001, however, the control and sprayed plots tended to group separately in the ordination in the old-growth stand, but not in the second-growth stand (Fig. 3). A significant difference was found between control and sprayed plot scores for axis 1 in the old-growth stand (Fig. 3), but no differences were found in the second-growth stand (Carlson 2002).

When these peak cover data from the old-growth stand in 2001 were grouped by growth form, only one of the six growth forms was significantly affected by treatment: sprayed plots had higher cover of spring perennials (Table 6). However, there was no treatment effect on total plot cover (excluding *Alliaria*) in May 2001 in the old-growth stand ($F_{1,48} = 0.56$).

#### Effects on Demography of Native Species

In the old-growth stand, the annual *Impatiens pallida* was not significantly impacted by spray treatment in terms of either growth (log-transformed leaf number; Table 1; $F_{1,64} = 0.68$) or reproduction (fruit number; Table 2; $H = 0.07$). Similarly, no treatment effect was found for either of the spring ephemerals: *Claytonia virginica* fruit number (Table 2) in the old-growth stand (nested ANOVA, $F_{1,34} = 0.13$) or second-growth stand ($F_{1,40} = 0.41$); proportion of *Dicentra canadensis* individuals with more than one leaf in the old-growth stand (Table 1; $\chi^2 = 1.23$). However, fruit production of the late-summer perennial herb *Phryma leptostachya* in the second-growth stand tended to increase (from 2000 to 2001) in the spray treatment

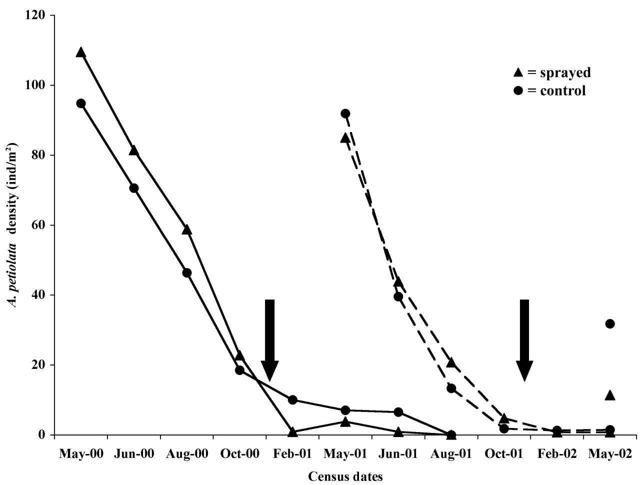


Figure 1. Change in mean density of 2000 (solid line), 2001 (dashed line), and 2002 (no line) *Alliaria petiolata* cohorts in sprayed and control plots in the old-growth stand. Herbicide was applied in November 2000 and 2001, indicated by arrows. Treatment differences are reported in the text and in Table 3.

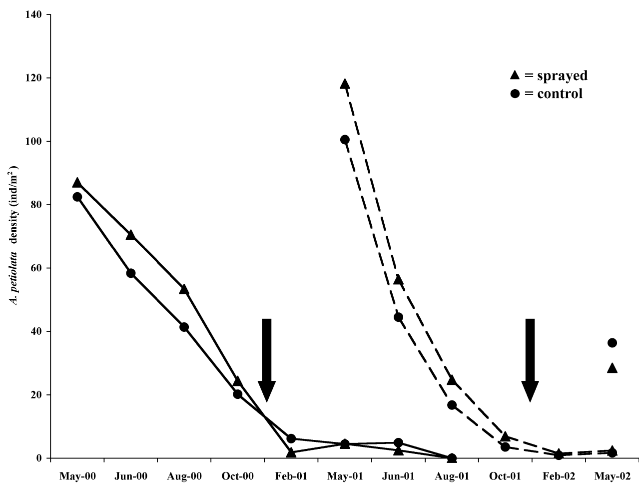


Figure 2. Change in mean density of 2000 (solid line), 2001 (dashed line), and 2002 (no line) *Alliaria petiolata* cohorts in sprayed and control plots in the second-growth stand. Herbicide was applied in November 2000 and 2001, indicated by arrows. Treatment differences are reported in the text and in Table 3.

**Table 4.** Densities (mean ± SE) of wintergreen herbs per 1-m$^2$ plot in October 2000 (before the first herbicide application) and at three subsequent dates.

| *Stand* | *Treatment* | *October 2000* | *February 2001* | *October 2001* | *February 2002* |
|---|---|---|---|---|---|
| Old growth | Control | 47.6 ± 14.7 | 10.7 ± 6.1 | 22.3 ± 6.0 | 9.1 ± 2.4 |
| | Sprayed | 38.0 ± 7.6 | 2.7 ± 1.0 | 12.1 ± 3.3 | 4.0 ± 1.2 |
| | | $H = 0.30$ | $H = 4.05$ | $H = 2.12$ | $H = 6.03$ |
| | | $p = 0.587$ | $p = 0.044$ | $p = 0.145$ | $p = 0.014$ |
| Second growth | Control | 10.5 ± 1.3 | 2.3 ± 0.4 | 9.8 ± 1.6 | 6.4 ± 1.1 |
| | Sprayed | 15.9 ± 5.8 | 3.3 ± 1.3 | 9.7 ± 1.5 | 6.0 ± 0.9 |
| | | $H = 0.01$ | $H = 0.71$ | $H = 0.01$ | $H = 0.02$ |
| | | $p = 0.907$ | $p = 0.398$ | $p = 0.938$ | $p = 0.899$ |

For each date, the treatments are compared with the Kruskal–Wallis statistic ($H$).

compared to a decrease in the control (Table 2; $H = 3.19$, $p = 0.074$), although there was no significant effect on the change in leaf number (Table 1; $F_{1,18} = 0.59$). Treatment did not affect survival of *Osmorhiza longistylis* in the second-growth stand (78% vs. 62%, $\chi^2 = 1.27$), nor the change in leaf number (Table 1; $H = 1.18$) for the survivors. However, plants in control plots tended to have greater increases in fruit number than those on sprayed plots (Table 2; $H = 3.51$, $p = 0.061$). There was no effect of treatment on change in the number of leaves of seedlings of the tree *Acer saccharum* in the old-growth stand (Table 1; $F_{1,24} = 0.19$).

## Discussion

In comparison to control plots, spot application of Roundup in the fall reduced the density of the 2000 cohort of *Alliaria* by 85% in the old-growth stand and 44% in the second-growth stand. These reductions are comparable to the 58 to 100% difference between control plots and those sprayed with glyphosate fall and spring in a *Quercus alba* (white oak)-dominated forest in Illinois (Nuzzo 1991).

In contrast, application of herbicide in the fall of 2001 only reduced *Alliaria* in the old-growth stand, and this effect was only marginally significant. This weak effect on the 2001 cohort appears due to the extremely low density of *Alliaria* adults in both treatments in May 2002. This low density of the 2001 cohort might be explained by yearly density fluctuation associated with the biennial life cycle, a phenomenon apparent in some other U.S. populations (McCarthy 1997; Winterer et al. 2001; Meekins & McCarthy 2002). However, according to this hypothesis, one would expect to see the high rosette densities in plots established in 2000 to be followed by low rosette densities in 2001 and high again in 2002—a pattern not observed in this study. Treatment effect can be ruled out because the same trend occurred in both control and sprayed plots. More likely, the low density of adults on control plots in May 2002 was due to mortality caused by the low precipitation in June 2001 (both fall rosette and spring adult densities correlate strongly with precipitation the previous June; Slaughter & Gorchov 2004).

After removal efforts, reestablishment of *Alliaria* populations by seeds from the seed bank or immigration is likely due to newly available space and resources (Baskin & Baskin 1992; Dhillion & Anderson 1999). McCarthy (1997), in fact, reported a higher density of seedlings in plots after eradication was attempted through inflorescence stalk removal. However, we found no effect of the fall 2000 herbicide treatment on the 2001 cohort of *Alliaria* seedlings, similar to Nuzzo's findings for seedling density after fall (Nuzzo 1991) or fall-and-spring (Nuzzo 1996) herbicide application on the cohort of the previous year. Furthermore, after the second herbicide application (2001), we found lower density of seedlings (2002 cohort) in sprayed plots. We attribute this decline to the herbicide-induced reduction of the 2000 cohort, which presumably produced the majority of the seeds that germinated in 2002.

Herbicide treatment negatively affected native species in leaf (wintergreen herbs) in the old-growth stand but not the second-growth stand. However, the most common wintergreen herbs in this stand were the winter annuals *Stellaria media* (common chickweed) and *Galium aparine* (cleavers) (Carlson 2002), species that are considered weeds in North America (*Stellaria* is non-native, Turkington et al. 1980; *Galium* has both native and exotic populations, Malik & Vanden Born 1988). This direct effect of the herbicide probably explains the lower May 2001 cover of *Stellaria* in sprayed versus control plots of the old-growth stand (Carlson 2002). Other herbs in both stands occurred at very low percent cover before treatment, so could generally be avoided during herbicide application. In

**Table 5.** Mean (± SE) species richness per 1-m$^2$ plot for control and herbicide-treated plots in the old- and second-growth stands before (2000 growing season) and after treatment (2001 growing season).

| *Stand* | *Treatment* | *2000 Richness* | *2001 Richness* |
|---|---|---|---|
| Old-growth stand | Control | 5.4 ± 0.5 | 7.7 ± 0.6 |
| | Sprayed | 6.6 ± 0.5 | 8.8 ± 0.5 |
| Second-growth stand | Control | 9.6 ± 0.6 | 11.4 ± 0.8 |
| | Sprayed | 10.8 ± 0.8 | 11.0 ± 0.7 |

$n = 25$ plots per treatment per stand. ANOVA statistics are reported in *Results*.

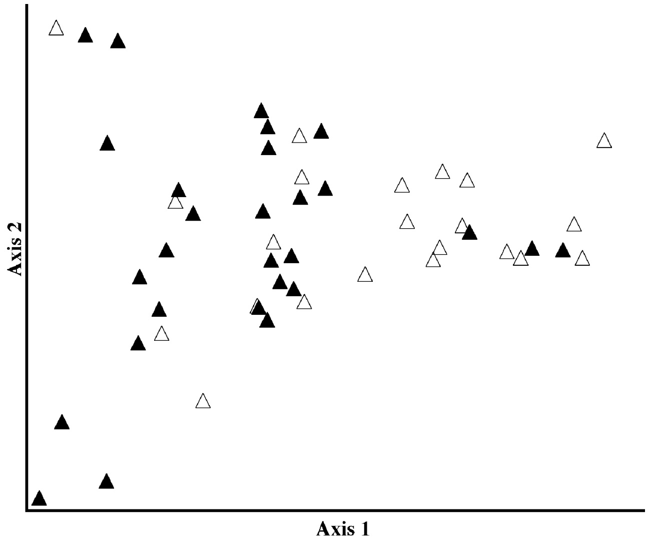


Figure 3. Detrended correspondence analysis (DCA) ordination of sprayed (filled triangles) and control plots (open triangles) in the old-growth stand based on peak 2001 percent covers of all species except *Alliaria petiolata*. Eigenvalues: axis 1 = 0.52, axis 2 = 0.40. Control and sprayed plots differed significantly in their scores for axis 1 (ANOVA $F_{1,48} = 5.68$, $p = 0.021$).

addition, leaf litter probably buried and protected many green native herbs from Round-up. Nuzzo (1996) found no significant differences in cover of wintergreen herbs after treatment in three lightly to moderately disturbed forest stands in Illinois, although she reported some "sensitivity" of *Galium aparine* and *Geum canadense* (white avens) to fall glyphosate application. We recognize that fall application of herbicide could impact native wintergreen herbs if application is less discriminating or if species composition at the time of spray differs due to interyear differences in weather and phenology.

Although some community effects were identified in this study, McCarthy (1997) speculated that in general, *Alliaria* removal experiments require 3–5 years of treatment before community changes are statistically evident, due to the wide variety of species life-history patterns. Our findings for these communities therefore should not yet be regarded as definitive.

**Table 6.** Mean percent cover of each growth form/functional group and for garlic mustard (*Alliaria petiolata*) in the old-growth stand in 2001 with Kruskal–Wallis statistic for treatment difference.

| *Growth Form* | *Control* | *Sprayed* | *H* |
|---|---|---|---|
| Annuals | 23.9 | 18.7 | 1.11 |
| Graminoids | 3.6 | 1.7 | 0.02 |
| Summer perennials | 2.6 | 2.5 | 0.14 |
| Spring perennials | 26.8 | 45.8 | 4.01* |
| Trees | 8.6 | 10.4 | 1.56 |
| Shrubs | 2.6 | 1.4 | 0.19 |
| *Alliaria petiolata* | 71.0 | 32.3 | |

Mean cover refers to the average cover per plot of all species categorized in each growth form group. For each species, we used peak cover (maximum of cover measured May, June, and August 2001). *Alliaria petiolata* cover data were not included in the ordination and therefore were not analyzed by Kruskal–Wallis test.
*$p < 0.05$.

Species richness was not significantly affected by treatment of *Alliaria*, similar to results from McCarthy's (1997) 3-year study, which indicated little difference in species diversity with or without reduced *Alliaria* biomass. He proposed therefore that, in general, diversity is reduced by the presence of *Alliaria*, so will not change until *Alliaria* is completely eliminated. Future years of data are needed to test this hypothesis in this system.

We attribute the separation of control versus sprayed plots in the ordination of the 2001 peak cover data from the old-growth stand to community changes resulting from the herbicide treatment, because these treatments did not segregate in ordinations based on 2000 peak cover. Although cumulative effects of cover changes of all species were responsible for the plot separation in the ordinations, specific responses of growth form groups and species are also important in understanding how *Alliaria* affects native communities. McCarthy (1997) found that the removal of *Alliaria* increased cover of species with seed banks (e.g., annuals), high seed input (e.g., trees), or high vegetative growth (e.g., vines). We did not find effects on growth of seedlings of the tree *Acer saccharum* or cover of tree seedlings or annuals, and vines were too sparse in our plots for analysis. For the one annual whose demography we investigated, the native *Impatiens pallida*, we found no treatment effect on growth or reproduction in the old-growth stand, but we did find a trend toward higher cover in sprayed plots (Carlson 2002), suggesting higher recruitment.

Our finding that in the old-growth stand, sprayed plots had higher cover of spring perennials indicates that some of these herbs do respond within 1 year to reduction of *Alliaria*. Of the spring perennials, only *Erythronium americanum* (trout-lily) had significantly higher cover in sprayed versus control plots after, but not before, spraying (Carlson 2002), suggesting this clonal herb may respond more quickly than other spring perennials. The trend toward greater fruit production of *Phryma leptostachya* in sprayed plots was paralleled by significantly greater height and inflorescence production (Carlson 2002). However, percent cover of this summer perennial was not affected (Carlson 2002), suggesting that demographic parameters of perennials are sometimes more sensitive than their cover values to short-term changes in *Alliaria* density and thus are useful for rapid assessment of chronic effects of invasive species.

In conclusion, a single fall application of herbicide significantly reduced density of *Alliaria* but resulted in only modest responses of the native community and representative species. It is possible that in mature forest stands such as these, *Alliaria* does not have a large competitive effect on native plants. Within the interior of these stands, it appears that *Alliaria* does not persist in high densities in the same places over time (as documented by Nuzzo 1999), so individuals of native perennial species may be exposed to only intermittent periods of competition with this invasive. Alternatively, the modest effects may be due to the short duration of this study, so we are investigating whether continued *Alliaria* reduction via annual herbicide application results in greater responses of native plants.

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**Appendix.** All species other than *Alliaria* recorded in old-growth plots in May 2001, categorized into growth forms based on Gleason and Cronquist (1991) and Snyder and Vankat (1984).

| *Growth Form* | *Species Included* |
|---|---|
| Annuals | **Galium aparine, Impatiens pallida, Pilea pumila,* **Stellaria media* |
| Graminoids | *Carex albursina, Carex digitalis, Carex gracilescens, Carex jamesii, Carex laxiculmis, Elymus riparius, Festuca subertcillata* |
| Summer perennial herbs: conspicuous in May, then flower, fruit, and senesce by August or September | *Actaea alba, Agrimonia pubescens, Arisaema triphyllum, Aster divaricatus, Circaea lutetiana, Eupatorium rugosum, Galium circaezans, Galium triflorum,* **Geum canadense, Laportea canadensis, Panax quinquefolia, Phryma leptostachya, Phytolacca americana, Polygonum virginianum, Sanicula canadensis, Sanicula trifoliata* |
| Spring perennial herbs: conspicuous in March or April, then flower, fruit, and senesce by late May or early June | *Allium tricoccum, Cardamine concatenata, Claytonia virginica, Dicentra canadensis, Dicentra cucullaria, Erigenia bulbosa, Erythronium americanum, Hydrophyllum appendiculatum,* **Osmorhiza longistylis, Podophyllum peltatum, Polygonatum biflorum Sanguinaria canadensis, Senecio obovatus, Trillium sessile, Viola pubescens, Viola sororia, Viola striata* |
| Trees | *Acer saccharum, Acer rubra, Asimina triloba, Carya cordiformis, Celtis occidentalis, Cornus florida, Fagus grandifolia, Fraxinus americana, Liriodendron tulipifera, Prunus serotina, Quercus muelenbergii, Ulmus rubra* |
| Shrubs | *Lindera benzoin* |
| Vines | *Parthenocissus quinquefolia, Smilax hispida, Toxicodendron radicans* |

*"Wintergreen" species (herbaceous species with leaves in October). In addition, some graminoids were "wintergreen."