

AN EVALUATION OF MECHANISMS PREVENTING GROWTH AND SURVIVAL OF TWO NATIVE SPECIES IN INVASIVE BOHEMIAN KNOTWEED (*FALLOPIA* \times *BOHEMICA*, POLYGONACEAE)¹

TANIA J. SIEMENS AND BERND BLOSSEY²

Department of Natural Resources, Fernow Hall, Cornell University, Ithaca, New York 14853 USA

Restoring native diversity to habitats dominated by invasive plants requires improved understanding of mechanisms that allow introduced plants to retain dominance. We used a factorial transplant experiment to assess whether light limitation, nutrient limitation, or allelopathic interference by *Fallopia* \times *bohemica* reduces growth or survival of *Eupatorium perfoliatum* or *Acer saccharinum*. Increased light improved *A. saccharinum* performance but had no effect on *E. perfoliatum* growth. Increased light had no effect on *A. saccharinum* survival but improved *E. perfoliatum* survival. Activated carbon addition had short-term benefits allowing *E. perfoliatum* plants to grow for 4 wk and increased *A. saccharinum* leaf width but not biomass. Nutrient addition had no beneficial effect on transplants. These results in combination with the outcome of a cutting experiment suggest that *F.* \times *bohemica* achieves competitive superiority primarily by limiting access to light. Species-specific effects and significant interaction effects particularly of light and activated carbon suggest additional mechanisms. Allelopathic interference or interaction with microbial soil organisms may contribute to the lack of native species in populations of *F.* \times *bohemica*. Restoration efforts should combine selective *F.* \times *bohemica* control with soil amendments (such as activated carbon) to assess their potential to facilitate return of native species to invaded habitats.

Key words: allelopathic interference; carbon addition; central New York; light limitation; plant invasions; Polygonaceae; transplant experiment.

Plant invasions can transform native ecosystems by changing ecosystem processes, disturbance regimes, plant community composition and diversity, nutrient dynamics, and associated food webs (Mack et al., 2000, 2001; National Research Council, 2002). The establishment of an introduced species per se does not necessarily constitute a problem, but negative impacts associated with the usually high abundance of an introduced species do threaten native communities (Levine et al., 2003). Effectively reducing or preventing such impacts is dependent upon our ability to first recognize mechanisms through which introduced species attain their ecological dominance (Levine et al., 2003) and then to develop appropriate management options to reduce invader abundance and impacts.

Recent evidence suggests that invasive species may impact community diversity through reductions in colonization rates of native plants, rather than through competitive replacement of established individuals (Yurkonis and Meiners, 2004). Low colonization rates may be the result of limited arrival of propagules (e.g., through dense canopies), reduced germination rates of available seeds (e.g., through shading, dense litter layers, or allelopathy), or through increased mortality of established seedlings or juvenile plants (e.g., through shading, other resource competition, allelopathy, or interactions with

soil pathogens and mutualists) (Klironomos, 2002; Bais et al., 2003; Ehrenfeld, 2003; Reinhart et al., 2003; Reinhart and Callaway, 2004; Eppinga et al., 2006; Stinson et al., 2006). Facilitating the return of native species into ecosystems currently dominated by non-indigenous species (i.e., restoration) will depend upon the effectiveness of management options to reduce the competitive ability of the invader and elimination of any “legacy effects.” For example, mowing, herbicide treatment, or fire may reduce (aboveground) biomass of an invasive species, but if soils retain allelopathic compounds or pathogens, recruitment of native species may still be prevented. Such “legacy effects” may reduce the success of reseedling or transplanting juvenile native plants into areas currently dominated by invasive species (assuming recruitment limitation due to lack of seeds or propagules), even if the invader is selectively weeded, cut, or treated with herbicide to reduce competition for light, water, or nutrients.

We evaluated potential dominance mechanisms of an invasive species using the clonal herbaceous *Fallopia* \times *bohemica* (Chrtek and Chrtková), Bohemian knotweed (for synonyms and details of plant taxonomy, see Materials and Methods, Experimental organisms). This hybrid and its two parental species (*Fallopia japonica* and *F. sachalinensis*) are invaders of riparian and disturbed areas in North America and Europe where virtual monocultures develop (Shaw and Seiger, 2002; Pysek et al., 2003; Bímová et al., 2004). Successful restoration and the return of native flora and fauna into habitats currently dominated by *F.* \times *bohemica* will be facilitated by a better understanding of the mechanisms allowing *F.* \times *bohemica* to dominate and exclude native species. While light limitation under a tall, dense canopy of *F.* \times *bohemica* appears the most likely mechanism of competitive superiority, the species may also alter physical, chemical, and biological properties of its surroundings as has been documented for other invasive plants (Ehrenfeld, 2003; Levine et al., 2003; Vivanco

¹ Manuscript received 24 February 2006; revision accepted 12 March 2007.

The authors thank E. G. Kraynok (Coastal Forest Resources Company) and T. Hicks (Schuyler County Soil and Water Conservation District) for providing access to the research site. F. Vermeylen (Cornell University) provided invaluable advice on statistical analyses, and members of the Blossey lab at Cornell University helped with fieldwork and experimental design. Comments by V. Nuzzo, A. DiTomasso, L. Weston, and several anonymous reviewers improved earlier versions of this paper. Funding was provided through the USDA Forest Service Health Protection Enterprise Team.

² Author for correspondence (e-mail: bb22@cornell.edu)

et al., 2004). Such changes in the abiotic or biotic environment in *F. ×bohemica* populations may also affect germination and survival of seedlings or juvenile plants of native species and thus prevent their colonization and recruitment. The extensive rhizome system suggests that *F. ×bohemica* is a good competitor for nutrients (Shaw and Seiger, 2002), but the absence of seedlings of other native species in early spring before canopy closure, near complete absence of shade-tolerant species, and an abundance of bare ground among the *F. ×bohemica* stems suggest that allelopathic interference (Inderjit and Callaway, 2003; Weston and Duke, 2003) may play a role in maintaining near monocultures of *F. ×bohemica*.

Riparian areas, which are often invaded by *F. ×bohemica*, are characterized by efficient dispersal of seeds and plant propagules through hydrochory (Rand, 2000; Boedeltje et al., 2004). We therefore do not consider reduced seed/propagule availability an important factor in limiting recruitment of native species in *F. ×bohemica*. Assuming sufficient seed or propagule supply, *F. ×bohemica* can limit recruitment of native species through preventing germination, reducing seedling establishment, or reducing growth and survival of juvenile plants.

We were particularly interested in evaluating the impact of *F. ×bohemica* on juvenile plant growth and survival. While we consider germination and early seedling growth a critical and important stage for initial plant establishment, by using well-established native juvenile plants rather than seed addition in our experiments, we avoided complications of microsite availability, seeding density, and potential allelopathic interference with germination processes that also affect local recruitment (Münzbergová, 2004; Vivanco et al., 2004). We hypothesized that, even if seeds or propagules of native species arrive and successfully germinate, establish and initiate growth, *F. ×bohemica* will reduce long-term survival of juvenile plants through interference with their growth requirements. In addition, transplanting juvenile plants is a common practice in wetland and riparian restoration where flooding may wash seeds off the site, and preventing erosion is often an important restoration goal—much easier achieved by using well-established juvenile plants that will grow quickly.

We transplanted juvenile individuals of two native species, silver maple (*Acer saccharinum* L., a deciduous tree) and boneset (*Eupatorium perfoliatum* L., a perennial herb) into a well-established *F. ×bohemica* population growing in a riparian area. Our treatments consisted of manipulations of resource availability (light, nutrients) and potential allelopathic interference (activated carbon addition) to assess mechanisms of invasive plant impacts in a full factorial field experiment. Although resource competition and allelopathy likely act in concert, for example, through root exudation (Callaway and Aschehoug, 2000; Ridenour and Callaway, 2001; Bais et al., 2003; Vivanco et al., 2004), little is known about their relative importance under field conditions (Inderjit and Callaway, 2003). We tested the following hypotheses: (1) Increased access to light will improve survival and growth of transplants. (2) Increased nutrient availability will improve survival and growth of transplants. (3) Activated carbon addition will improve survival and growth of transplants. (4) Transplant survival and growth will be maximized in treatments in which increased light and nutrient availability are combined with activated carbon addition.

MATERIALS AND METHODS

Study site—Our study site is a 40 × 70 m homogeneous stand of *F. ×bohemica* occupying a flood plain of Cayuta Creek in central New York, USA (42°16' N, 76°43' W). The site lacks conspicuous elevation or moisture gradients. Soils were notable for NO₃ limitation (<1 ppm in all samples) as well as low P (5.1 ± 0.78 mg/kg; mean ± 1 SE) and K (47.7 ± 7 mg/kg; mean ± 1 SE) levels (analyzed by Cornell Nutrient Analysis Laboratory). The *F. ×bohemica* stand is bordered by an old field (dominated by goldenrods, *Solidago* spp.) and riparian trees (*Populus* sp.) and has existed at the site for at least 10 yr (B. Blosssey, personal observation). The only other plants occurring within *F. ×bohemica* are the invasive *Alliaria petiolata* (M. Bieb.) Cavara and Grande (garlic mustard) and an onion (*Allium* sp.), and both species occur at low density.

Experimental organisms—There is much taxonomic debate about the correct classification of several Asian taxa commonly referred to as Japanese knotweed, and the species are placed in the genera *Reynoutria*, *Polygonum*, or *Fallopia* by various authors (Shaw and Seiger, 2002; Zika and Jacobson, 2003). The most recent volume of the *Flora of North America* confirms the *Fallopia* designation (Flora of North America Editorial Committee, 2005). Introduced *Fallopia* populations in North America are comprised of Japanese knotweed, *Fallopia japonica* (Houtt.) Ronse Decraene (syn: *Reynoutria japonica* Houtt., *Polygonum cuspidatum* Siebold & Zucc.); giant knotweed, *F. sachalinensis* (F. Schmidt) Ronse Decraene; their common hybrid Bohemian knotweed, *F. ×bohemica* (Chrték and Chrtková), which may be the most widespread *Fallopia* taxa in the United States (Zika and Jacobson, 2003); and Bukhara fleecflower, *F. baldschuanica* (Regel) Holub. We know relatively little about potential differences in ecosystem function between *F. japonica*, *F. sachalinensis*, and *F. ×bohemica*, but all are considered serious economic and environmental threats in Europe and North America (Beerling et al., 1994; Shaw and Seiger, 2002). On the basis of morphological features (Zika and Jacobson, 2003; Flora of North America Editorial Committee, 2005), plants at Cayuta Creek are *F. ×bohemica*. Data from Europe suggest that *F. ×bohemica*, while similar in dispersal and interactions with native species, appears to have increased hybrid vigor and can outcompete both parental species (Pysek et al., 2003; Bímová et al., 2004).

Invasions by Japanese, giant, and Bohemian knotweed create monospecific stands, and native species are competitively excluded as stands expand through growth of an extensive rhizome system (Bímová et al., 2004). The species are particularly abundant along riparian corridors where water can easily transport rhizome or stem fragments and on disturbed grounds. Plants (particularly the roots) contain high concentrations of phenolic compounds (*trans*-resveratrol and others) (Vastano et al., 2000; Vaher and Koel, 2003). Rapid stem growth (up to 8 cm/d) begins in early spring (early to mid-May in central New York), and the slightly arching stems can reach 4 m in height, forming a dense canopy and allowing little light to reach the understory. Plants reach their final height in early summer and flower in late August and September. The species are insect pollinated and, at least for *F. japonica*, seedlings are able to establish in spring and grow (Forman and Kesseli, 2003).

We selected two native plant species expected to thrive at Cayuta Creek in the absence of *F. ×bohemica*. *Acer saccharinum* L. (silver maple) is a native, moderately shade tolerant hardwood deciduous tree that thrives in moist woods and lowlands, including flood plains and stream banks (Burns and Honkala, 1990). The species, often considered important in the restoration of riparian areas, reaches a height of 27 to 36 m at maturity. We obtained bare-root, dormant, 2-yr-old *A. saccharinum* saplings (average height 99 cm, range 74–120 cm) from the Saratoga Tree Nursery (New York State Department of Environmental Conservation, Saratoga Springs, New York, USA).

Eupatorium perfoliatum L. (boneset) is a widespread and common native perennial herb (40–150 cm tall). The species is a facultative wetland plant commonly found in floodplains, swamps, bogs, stream banks, and wet meadows. We chose *E. perfoliatum* because the species occurs in the riparian areas at our field site immediately outside the *F. ×bohemica* stand (28 plants within a 20 m² area adjacent to the creek). We obtained juvenile plants (as 2–3 mo old plugs, average height 12 cm, range 10–18 cm) from JF New native plant nursery (Walkerton, Indiana, USA).

Experiment 1—Leaving a 5-m buffer from the edge of the stand bordering the old field, and a 10-m buffer from the edge of the creek, we established a regular grid (cell size 2.5 × 2.5 m) inside the *F. ×bohemica* population. We then randomly assigned 160 of the 288 possible transplant locations to receive

either a juvenile *A. saccharinum* or a juvenile *E. perfoliatum* plant. Each location was also assigned to receive one of four treatments: increased light (L), nutrient addition (N), activated carbon addition (C), or a control (no manipulations) in a complete factorial design ($N = 10$ replicates/treatment).

Coinciding with spring plant growth in our region, we transplanted 80 *A. saccharinum* and 80 *E. perfoliatum* plants into the stand of *F. ×bohemica* (stems approximately 1.5 m tall) on 22 and 23 May 2004. We chose the largest, similar-sized individuals available for each species and recorded their initial height (used as a covariate in the statistical analysis). Before transplanting, we pruned *A. saccharinum* roots to a 10 cm radius and measured height on 20 additional reference specimens, which were subsequently dried at 50°C for 1 wk to determine initial stem and root biomass. We transplanted *E. perfoliatum* as plugs but were unable to determine initial biomass due to lack of surplus plants.

To achieve increased access to light, we bent main stems and where absolutely necessary pruned side branches of *F. ×bohemica*, creating a canopy opening of 3–6 m in diameter above our transplants. This treatment, while creating an opening in the *F. ×bohemica* canopy, kept the main stems intact, and we needed to remove only minor amounts of biomass. We maintained the canopy openings as needed (on day 30, then at 14-d intervals) throughout the experiment. We measured actual light levels 10 cm aboveground between 1030 and 1330 hours at each station on a clear day (15 August 2004, 84 d after transplanting) using a light meter (EXTECH Instruments, Model 401025, Waltham, Massachusetts, USA).

To achieve nutrient addition treatments, we inserted one fertilizer spike ($N : P : K = 10 : 10 : 4$, 1.25 g, Jobe's brand, Ithaca Agway, Ithaca, New York, USA) into the root zone of each transplant upon planting. To achieve the carbon addition treatment (allelopathy amelioration), we first removed 1000 mL of soil from each transplant location then added 60 mL of activated powdered carbon (Calgon's BL carbon, product #1246; Calgon Carbon Corp., Pittsburgh, Pennsylvania, USA). We added 60 mL carbon/L of soil, triple the amount of carbon normally used in laboratory experiments, to ensure carbon addition would have an effect in naturally formed soils. Positive growth responses following addition of activated carbon are generally interpreted as the inactivation of plant-derived organic compounds that would otherwise inhibit plant growth (Nilsson, 1994; Callaway and Aschehoug, 2000; Inderjit and Callaway, 2003; Hille and den Ouden, 2005). Soil and carbon was well mixed in a separate container, and we then returned the mix to the transplant location. Roots of transplants were surrounded on all sides with the activated carbon-enriched soil, and the soil/carbon mix occupied all (*E. perfoliatum*) or the vast majority of the rooting zone (*A. saccharinum*) at the end of the experiment. To avoid potential confounding effects of soil disturbance and mixing, we treated soils in all non-carbon addition treatments using the identical procedures but without carbon addition before transplanting seedlings. Activated carbon is suspected to absorb organic compounds with allelopathic properties (Inderjit and Callaway, 2003), but it has recently been demonstrated that activated carbon can also decrease host resistance to pathogens by absorbing protective compounds exuded by plant roots (Bais et al., 2005).

We initially monitored transplants several times per week, and on day 31, 46, 60, 72, and 97, we measured stem height of all transplants and noted any mortality or herbivory. We considered plants as surviving as long as they retained green leaf material. Starting on day 46, we counted all leaves on *A. saccharinum* and measured maximum leaf width (as a nondestructive measure of performance). We terminated the experiment on 28 August 2004 and harvested all *A. saccharinum*. We removed all soil and separated plants into above- and belowground parts. We dried all plant material separately at 50°C for 7 d in a drying oven and subsequently determined biomass. We were unable to determine biomass of *E. perfoliatum* due to mortality.

Experiment 2—Within a 2-mo period after transplanting, we observed near complete mortality of *E. perfoliatum* regardless of treatment in our first experiment. In contrast, a number of initially rejected smaller individuals maintained in a common garden had excellent survival, suggesting that poor field survival was not related to poor plant quality. We therefore conducted a second experiment transplanting *E. perfoliatum* into a different section of the *F. ×bohemica* stand located at least 10 m from original transplants and into the riparian herbaceous community outside the *F. ×bohemica* population. Within the *F. ×bohemica* stand, we used a paired design with two treatments: (1) repeat cutting of all stems in a 7.5×7.5 m area around the transplant, or (2) a control with no clipping. The control treatment was located at least 6 m from the edge of the cutting treatment. In the riparian community we subjected the plant community to no manipulations other than the planting of *E. perfoliatum*.

Cutting of aboveground *F. ×bohemica* is a common control practice to

stress plants and encourage colonization of native species. Cutting of aboveground biomass greatly increases access to light but simultaneously changes the physical and (most likely) chemical (i.e., *F. ×bohemica* root exudate production) and biotic environment transplants will encounter. By transplanting into the unmanipulated surrounding riparian area, we made an attempt to assess whether the overall site conditions prevented growth of our experimental species or whether this was an effect that could be attributed to *F. ×bohemica*. Due to the shortage of remaining individuals from our purchase of *E. perfoliatum* from JF New, we increased the number of plants for our second experiment by excavating local nonflowering juvenile *E. perfoliatum* plants growing adjacent to the *F. ×bohemica* population at Cayuta Creek.

We transplanted 14 juvenile *E. perfoliatum* plants (average height 11 cm) obtained from JF New and eight plants excavated at our field site (average height 33 cm) into the *F. ×bohemica* stand. Half of these transplants (four local and seven from JF New) were assigned to the cutting treatment. We transplanted seven nursery plants and six plants excavated at our field site into the riparian area outside the *F. ×bohemica* stand (where *E. perfoliatum* commonly occurs). We left a ~10 cm diameter clump of soil surrounding the roots of the local *E. perfoliatum* intact to reduce transplant shock. All plantings for experiment 2 occurred on 16 July 2004, and we measured transplant survival weekly for 5 wk and measure light levels at each station on 15 August 2004 (as detailed for experiment 1).

Data analyses—We used SAS 9.1 (SAS Institute, Cary, North Carolina, USA) for all analyses. In experiment 1, we analyzed responses for *A. saccharinum* and *E. perfoliatum* separately. Due to early mortality, our analysis of *E. perfoliatum* growth was limited to the first time interval (22 May to 23 June). We evaluated the relative change in total *E. perfoliatum* stem length (%) with a three-way ANOVA using PROC MIXED with L (light), N (nutrient), and C (carbon) as fixed, and individual plant as a random effect because each plant could have multiple stems. We evaluated the significance of any *E. perfoliatum* growth by comparing mean growth (lsmeans) to zero using *t* tests. We determined and compared *E. perfoliatum* survivorship curves using PROC LIFETEST, the Kaplan-Meier product-limit method.

We used three-way mixed model ANOVAs to analyze *A. saccharinum* root and aboveground biomass production. We estimated aboveground biomass production by subtracting initial stem biomass (determined by a regression equation of the height – stem biomass relationship in our unplanted references; initial biomass = $-5.27 + (0.1542 \times \text{initial stem height})$; SE = 1.69; $r^2 = 0.6153$; $P = 0.000026$) from the final aboveground stem and new aboveground growth biomass values. We analyzed *A. saccharinum* average leaf width over time using a repeated measures three-way mixed model ANOVA. We used initial stem height as a continuous covariate to account for initial variation in our analysis of average leaf width and root biomass. We used PROC MIXED for all analyses of *A. saccharinum* growth responses and designated L, N, and C as fixed and individual plant and day as random factors. Differences among treatments and unplanted references were analyzed using Tukey's HSD test and degrees of freedom were Kenward and Roger adjusted (Kenward and Roger, 1997). We excluded 10 saplings with evidence of deer herbivory casewise from all analyses.

In experiment 2, we used a binary logistic regression model (PROC GENMOD) to evaluate whether *E. perfoliatum* mortality differed by growing location (within the *F. ×bohemica* stand or outside), treatment (clipped or control), or plant origin (nursery or local). We used survivorship data from day 28 because full mortality in the shade treatments at the end of the experiment (day 38) violated model assumptions.

RESULTS

Experiment 1—Our bending and slight pruning achieved the desired results and increased light levels from 1011 ± 77.25 lux in the control to 8162 ± 469.8 lux (mean ± 1 SE) in the increased light treatments. The juvenile *E. perfoliatum* survived transplanting well, but only plants in the LXC and the LXC treatments had significant positive growth compared to plants in the control treatment (LXC, $t = 2.94$, $P = 0.004$; LXC, $t = 3.38$, $P = 0.0013$, Fig. 1). Carbon addition was the only treatment with a significant effect on *E. perfoliatum* height ($F_{1,61} = 14.36$, $P = 0.0003$). After day 31, we observed rapid

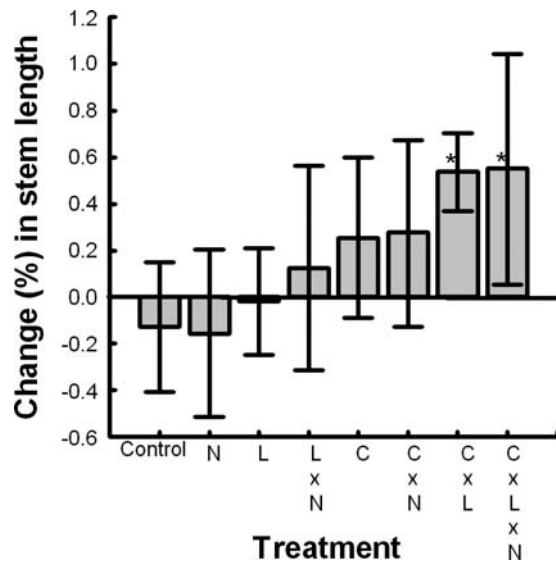


Fig. 1. Relative change (%) in *Eupatorium perfoliatum* stem length (25 May to 23 June 2004) across treatments (C, carbon addition; L, light addition; N, nutrient addition; control, no manipulations). Data are means \pm 2 SE of 10 replicates/treatment. (*) indicates response significantly greater than zero.

mortality preventing further growth analyses, and all but two of the *E. perfoliatum* plants had died by the end of the experiment (Fig. 2). Differences in survivorship among treatments were significant ($\chi^2 = 14.87$, $P = 0.0377$) with plants in the LxC ($\chi^2 = 5.63$, $P = 0.0176$) and LxNxC ($\chi^2 = 4.46$, $P = 0.0347$) treatments surviving significantly longer than control plants (Fig. 2). Across the experiment, *E. perfoliatum* transplants with increased access to light survived significantly longer ($\chi^2 = 10.06$, $P = 0.0015$), while carbon addition marginally improved survival ($\chi^2 = 3.24$, $P = 0.0719$) and nutrient addition had no effect ($\chi^2 = 0.073$, $P = 0.8954$).

All *A. saccharinum* transplants (except for a single individual) survived to the end of the experiment and had positive growth. However, plants in the control treatment had blackening of branches and eventual premature senescence and leaf loss, a typical symptom for light stress in plants. Increased

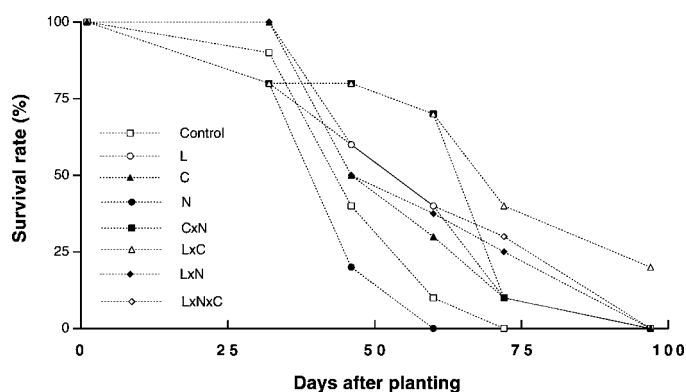


Fig. 2. Survival rate (%) of *Eupatorium perfoliatum* ($N = 10$ plants/treatment) across treatments (C, carbon addition; L, light addition; N, nutrient addition; control, no manipulations).

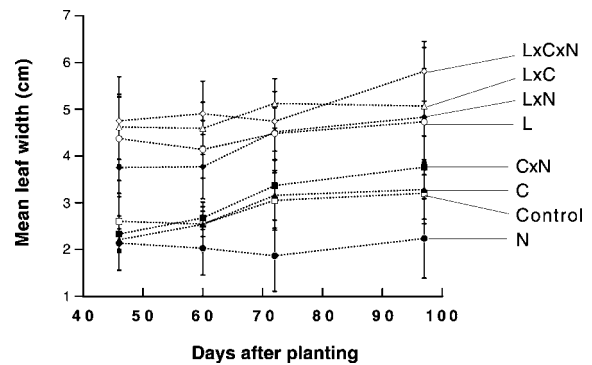


Fig. 3. Mean leaf width (cm) of *Acer saccharinum* saplings during the 2004 growing season across treatments (C, carbon addition; L, light addition; N, nutrient addition; control, no manipulations). Data are means \pm 2 SE of 8–10 replicates/treatment.

access to light ($F_{1,71.1} = 143.3$, $P < 0.0001$, Fig. 3) and carbon addition ($F_{1,71.3} = 7.51$, $P < 0.0077$) produced significant increases in mean leaf width of *A. saccharinum*, but nutrient addition had no significant effect ($F_{1,71.3} = 0.97$, $P = 0.328$). Leaf width was also significantly affected by day ($F_{3,203} = 37.27$, $P < 0.0001$), interaction of CxN ($F_{1,71.4} = 3.91$, $P = 0.0519$), Lxday ($F_{3,203} = 3.83$, $P = 0.0107$), and LxCxday ($F_{3,203} = 4.52$, $P = 0.0043$).

Initial stem height was a significant predictor of *A. saccharinum* stem biomass at the end of the experiment ($r^2 = 0.6153$, $P < 0.0001$). After accounting for the influence of initial stem height, greater light availability increased root ($F_{1,72} = 41.36$, $P < 0.0001$) and aboveground biomass ($F_{1,86} = 55.27$, $P < 0.0001$, Fig. 4), but neither nutrient nor carbon addition showed any significant effect on *A. saccharinum* biomass ($P > 0.05$). We also found a significant interaction of CxN for aboveground biomass ($F_{1,86} = 5.1$, $P = 0.027$).

Experiment 2—In our second experiment, plants in the unmanipulated control treatments within *F. xbohemica* showed significantly lower survivorship ($\chi^2 = 7.49$, $P = 0.006$) with only 11 (30%) survivors compared to the cutting treatment and plants transplanted into the riparian area where 24 (80%) survived to day 28. By day 38, none of the plants in the control treatment were alive compared to 81% survivorship in the cutting treatment and in the riparian area. There was no significant difference in survival of *E. perfoliatum* transplants ($\chi^2 = 0.01$, $P = 0.928$) between the cutting treatment inside the *F. xbohemica* stand (82%, $N = 11$) and in the unmanipulated riparian area (85%, $N = 13$). There was no significant difference ($\chi^2 = 0.66$, $P = 0.415$) in survival of nursery plants (65%, $N = 20$) and local *E. perfoliatum* (71%, $N = 14$). Interestingly, midday light levels (at the height of our transplants) in the riparian area and in the *F. xbohemica* cutting treatment were similar (9000 to 10000 lux).

DISCUSSION

A lack of well-designed field experiments and bioassays currently limits our understanding of the role of allelopathy and its interaction with biotic and abiotic factors and resource availability in shaping natural plant communities (Inderjit and

Callaway, 2003). We used a full factorial field experiment to simultaneously evaluate contributions of resource availability and potential allelopathic interference that may facilitate the lasting dominance of an invasive over native species by reducing colonization rates (Yurkonis and Meiners, 2004). To the best of our knowledge, this is the first field experiment to simultaneously vary resource availability and potential allelopathic interference.

Our transplanting worked well, and we had extremely little immediate mortality, probably aided by frequent rain events eliminating the need to water experimental plants. We found strong support for our first hypothesis that increased access to light improves survival of transplanted juvenile native plant species. However, this increased survival was temporary and only realized for *E. perfoliatum*. Regardless of treatment, catastrophic mortality over the next 2 mo eliminated all but two individuals in the L×C and L×N×C treatments. In contrast, all transplanted *A. saccharinum* individuals survived to the end of our experiment, although plants growing in unmanipulated control treatments had symptoms of light stress and performance decreases. Their long-term survival under a *F. ×bohemica* canopy is questionable. Increased access to light improved *A. saccharinum* performance (measured as leaf width and biomass production) supporting the second part of our first hypothesis. However, no performance increase as a function of light increase was evident for *E. perfoliatum*. Clearly, increased light availability had species-specific effects.

Nutrient addition in combination with carbon or light improved growth of juvenile *E. perfoliatum* or *A. saccharinum* transplants, but we found no support for our second hypothesis that increased nutrient availability alone will improve survival and growth. We found limited support for our third hypothesis that carbon addition will improve survival and growth of juvenile transplants. In the first 4 wk, carbon addition allowed *E. perfoliatum* plants to grow, and carbon addition increased *A. saccharinum* leaf width (but not overall biomass). We found no support for our fourth hypothesis that combinations of increased light, nutrient, and carbon addition will maximize survival and growth of native juvenile plants growing in *F. ×bohemica*, although *E. perfoliatum* plants in L×C treatments survived longest.

It is not surprising that light limitation may reduce the ability of native plant species to colonize *F. ×bohemica* populations. This hybrid and its two parental species are known for their dense, closed canopies in their introduced ranges (Beerling et al., 1994). In contrast, in its native range in Japan, *F. japonica* is a pioneer species colonizing new soils, for example, after eruptions of Mt. Fuji (Adachi et al., 1996). Relatively short-lived *F. japonica* patches act as nurseries for later successional species unable to colonize bare ground (Adachi et al., 1996). These important differences in growth and community impacts of *Fallopia* spp. between the native (facilitation of diversity) and introduced ranges (suppression of colonization and reduction in diversity) suggest that either (1) recipient communities and its members are ill adapted to co-exist with the new arrival or (2) through introduction and selection/hybridization *Fallopia* spp. have obtained traits allowing them to exclude native species.

The enemy release hypothesis (Keane and Crawley, 2002), the evolution of increased competitive ability (EICA) hypothesis (Blossey and Nötzold, 1995) and the novel weapons hypothesis (Callaway and Ridenour, 2004) all may offer explanations for these observations. With the exception of

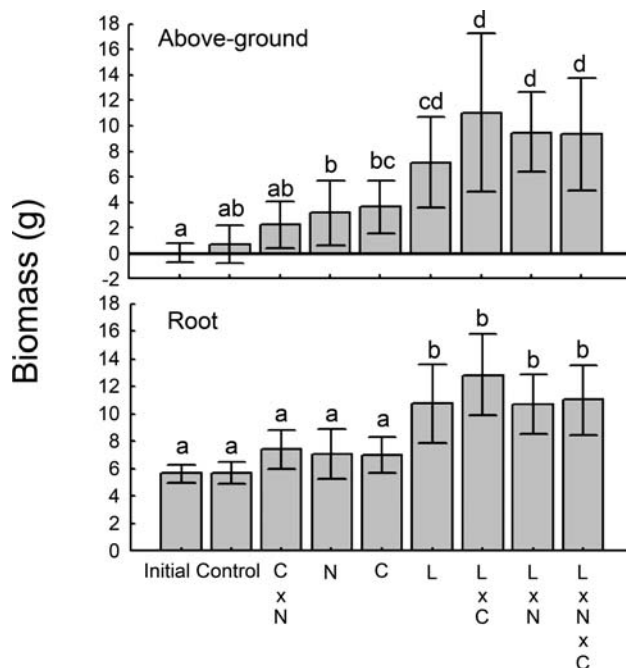


Fig. 4. Aboveground and root biomass (g) of *Acer saccharinum* saplings before (initial, before planting) and after the 2004 growing season across treatments (C, carbon addition; L, light addition; N, nutrient addition; control, no manipulations). Aboveground biomass was calculated as an increase during the 2004 season by subtracting predicted initial stem biomass (see Materials and Methods, *Experiment 1* for details). Data are means \pm 2 SE of 6–10 replicates/treatment. A different letter above the column indicates significant differences ($P < 0.05$) among treatment groups (Tukey's pairwise comparisons).

introduced Japanese beetles (*Popilia japonica* Newman) *F. ×bohemica* is essentially herbivore-free in North America (B. Blossey, personal observation), while native species are attacked by multiple herbivores and pathogens, thus giving a competitive advantage to *F. ×bohemica*. We continuously surveyed all transplants for aboveground pathogens and herbivores in our experiment. A few *A. saccharinum* individuals were browsed by mammals (most likely by white-tailed deer, *Odocoileus virginianus*), which we excluded from our analyses. We noticed that *E. perfoliatum* stems were attacked by a pathogen (identified as a generalist *Fusarium* sp. by the Plant Disease Diagnostic Clinic at Cornell University), but only plants inside *F. ×bohemica* were attacked, turned black, and died. While the fungus may have contributed to mortality of *E. perfoliatum*, we consider it unlikely that it was the cause of the poor plant performance because this opportunistic fungus colonized already weakened individuals. We also consider it unlikely that aboveground herbivores or pathogens contributed to poor survival and growth of *E. perfoliatum* or *A. saccharinum* at our site, weakening the role of natural enemies, at least in our experimental setup. However, we are unable to exclude the possibility that soil pathogens facilitated by *F. ×bohemica* may have caused the observed catastrophic mortality of *E. perfoliatum*. Such facilitation of soil pathogens by invasive plants to the detriment of native plant species has been recently reported for *A. petiolata* (Stinson et al., 2006).

The apparent lack of specialized natural enemies of *F. ×bohemica*, which can be quite damaging in Japan (Shaw and

Seiger, 2002), or hybridization (Birchler et al., 2006) may have allowed selection to favor genotypes with improved competitive abilities (e.g., increased growth, reproductive output, according to EICA). Alternatively, the lack of co-evolutionary history with invader-specific secondary chemistry may allow introduced plant species to attain long-term dominance in the introduced range through allelopathic effects (the novel weapons hypothesis) (Callaway and Ridenour, 2004). In the native range, members of a plant community are assumed to have adaptations to the secondary chemistry of other members of the community (Callaway and Ridenour, 2004; Hierro et al., 2005). The genus *Fallopia* is well known for its assortment of secondary compounds, which have been evaluated for their medicinal properties including cancer prevention (Yang et al., 2001; Gao et al., 2002; Park et al., 2004), antifungal and antimicrobial activity (Filip et al., 2003), inhibition of seedling growth (Inoue et al., 1992), and effect on soil inorganic ion availability (Inderjit and Nishimura, 1999). The main compounds found in *F. japonica* and *F. sachalinensis*, the two parental species of *F. ×bohemica*, are polyphenolics (resveratrol, emodin, and physicon) with concentrations of some biologically active compounds twice as high in *F. japonica* from New Jersey compared with concentrations of the same compounds found in China (Vastano et al., 2000). We did not test for differential responses of North American and Asian species to *F. ×bohemica*, but the ability of activated carbon to extend survival of *E. perfoliatum* and contribute to performance improvements of *A. saccharinum* fit certain predictions of the novel weapons hypothesis (Bais et al., 2003; Callaway and Ridenour, 2004). Positive growth responses following addition of activated carbon are generally interpreted as inactivation of plant-derived organic compounds that would otherwise inhibit plant growth (Nilsson, 1994; Callaway and Aschehoug, 2000; Inderjit and Callaway, 2003; Hille and den Ouden, 2005). However, in our experiment these responses were species specific, temporary, and weak, and we still lack a full understanding of the multiple ways of how activated carbon may interact with resource availability, soil organisms, root exudates, and plant growth (Inderjit and Callaway, 2003; Inderjit and Weston, 2003). For example, addition of activated carbon may eliminate the ability of root exudates to suppress colonization by soil pathogens leading to increased disease symptoms and increased plant mortality (Bais et al., 2005).

Poor *E. perfoliatum* survival in our first experiment and limited beneficial effects of carbon addition suggest that additional mechanisms may interfere with survival and growth of native species in *F. ×bohemica*. We cannot exclude the possibility that the amount of carbon we added to our seedlings was insufficient and quickly saturated nor that soil microbial communities contribute to the success of *F. ×bohemica*. Soil microbial communities may either suppress native plants or facilitate invaders through beneficial associations (Klironomos, 2002; Reinhart et al., 2003; Callaway et al., 2004a, b; Reinhart and Callaway, 2004; Eppinga et al., 2006; Stinson et al., 2006).

Our results clearly establish the importance of light limitation in preventing survival and growth of native species, even if they were to successfully germinate in areas invaded by *F. ×bohemica*. Beneficial effects of carbon addition and combinations of light and carbon, at least for *E. perfoliatum*, suggest the potential for additional allelopathic effects of *F. ×bohemica* in maintaining dominance over native species. Secondary chemicals in *F. ×bohemica* may have even stronger effects on germination or seedlings. However, even carbon

addition could not prevent major *E. perfoliatum* mortality, suggesting the existence of other important mechanisms (such as facilitation or suppression by soil microbial communities) that contribute to the success of *F. ×bohemica* in the introduced range.

Overall, our data support findings (Yurkonis and Meiners, 2004) that recruitment limitation by invasive species (through light limitation, potential allelopathic interference, and other mechanisms) may facilitate the long-term dominance and development of near monocultures of *F. ×bohemica*. Manipulation of light availability appears an obvious choice (for example, by repeated cutting) to restore diverse communities. Our second experiment clearly established that cutting of *F. ×bohemica* alone allowed *E. perfoliatum* to survive. It also established that conditions at Cayuta Creek were suitable for *E. perfoliatum* to grow and that *F. ×bohemica* invasion has an important negative impact on site conditions. However, while the improved *E. perfoliatum* survival rates in our second experiment offer some hope for restoration efforts using repeated selective cuttings, we cannot exclude the possibility that cutting of large areas changed more than access to light. The removal of a substantial amount of *F. ×bohemica* biomass and the redirection of resources to regenerate aboveground tissues is likely to have affected plant chemistry or the ability to produce root exudates and their potential impact on *E. perfoliatum* survival and growth. In addition, cutting would have to be maintained for extended periods, and our experiment was terminated after 5 wk. Although transplants appeared healthy, our experiment was comparatively short (for a field experiment) and long-term survival of transplants uncertain (particularly if *F. ×bohemica* recovers). Canopy gaps to improve access to light for *E. perfoliatum* seedlings also had to be substantial to prevent mortality (7.5×7.5 m clearing in our second experiment), and bending and light pruning were not sufficient to prevent near total mortality, even under improved light conditions (Fig. 2).

Most importantly, our field experiment established that no single mechanism appears solely responsible for the competitive exclusion of colonization of native species by *F. ×bohemica*. While light limitation appeared clearly to have dramatic impacts for juvenile plants, many newly germinated seedlings may not even make it this far in a dense *F. ×bohemica* population. Our field experiment also demonstrated species-specific responses of native species to conditions created by an invader and showed that different performance measures (survival vs. growth) may give different answers. Ultimately, the goal of restoration of invaded riparian areas is the return of native diversity (fauna and flora). In light of our results, it appears that experiments using different integrated restoration techniques (cutting or selective herbicide treatments or biological control plus potential soil amendments and seeding and planting of different native species) as attempted for *Phalaris arundinacea* (Perry et al., 2004) may be most productive in developing successful long-term management of *F. ×bohemica* and its parental species.

LITERATURE CITED

- ADACHI, N., I. TERASHIMA, AND M. TAKAHASHI. 1996. Central die-back of monoclonal stands of *Reynoutria japonica* in an early stage of primary succession on Mount Fuji. *Annals of Botany* 77: 477–486.
- BAIS, H. P., B. PRITHIVIRAJ, A. K. JHA, F. M. AUSUBEL, AND J. M. VIVANCO.

2005. Mediation of pathogen resistance by exudation of antimicrobials from roots. *Nature* 434: 217–221.
- BAIS, H. P., R. VEPACHEDU, S. GILROY, R. M. CALLAWAY, AND J. M. VIVANCO. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* 301: 1377–1380.
- BEERLING, D. J., J. P. BAILEY, AND A. P. CONOLLY. 1994. *Fallopia japonica* (Houtt.) Ronse Decraene (*Reynoutria japonica* Houtt.; *Polygonum cuspidatum* Sieb. & Zucc.). *Journal of Ecology* 82: 959–979.
- BÍMOVÁ, K., M. BOHUMIL, AND I. KASPAROVÁ. 2004. How does *Reynoutria* invasion fit the various theories of invasibility? *Journal of Vegetation Science* 15: 495–504.
- BIRCHLER, J. A., H. YAO, AND S. CHUDALAYANDI. 2006. Unraveling the genetic basis of hybrid vigor. *Proceedings of the National Academy of Sciences, USA* 103: 12957–12958.
- BLOSSEY, B., AND R. NÖTZOLD. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology* 83: 887–889.
- BOEDELTIJE, G., J. P. BAKKER, A. T. BRINKE, J. M. VAN GROENENDAEL, AND M. SOESBERGEN. 2004. Dispersal phenology of hydrochorous plants in relation to discharge, seed release time and buoyancy of seeds: the flood pulse concept supported. *Journal of Ecology* 92: 786–796.
- BURNS, R. M., AND B. H. HONKALA. 1990. Silvics of North America. U.S. Department of Agriculture, Forest Service, Washington, D.C., USA.
- CALLAWAY, R. M., AND E. T. ASCHENHOU. 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* 290: 521–523.
- CALLAWAY, R. M., AND W. M. RIDENOUR. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2: 436–443.
- CALLAWAY, R. M., G. C. THELEN, S. BARTH, P. W. RAMSEY, AND J. E. GANNON. 2004a. Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. *Ecology* 85: 1062–1071.
- CALLAWAY, R. M., G. C. THELEN, A. RODRIGUEZ, AND W. E. HOLBEN. 2004b. Soil biota and exotic plant invasion. *Nature* 427: 731–733.
- EHRENFELD, J. G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6: 503–523.
- EPPINGA, M., M. RIETKERK, S. C. DEKKER, AND P. C. DE RUITER. 2006. Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. *Oikos* 114: 168–176.
- FILIP, V., M. PLOCKOVÁ, J. SMIDRKA, Z. SPICKOVÁ, K. MELZICH, AND S. SCHMIDT. 2003. Resveratrol and its antioxidant and antimicrobial effectiveness. *Food Chemistry* 83: 585–593.
- FLORA OF NORTH AMERICA EDITORIAL COMMITTEE. 2005. Flora of North America. Oxford University Press, New York, New York, USA.
- FORMAN, J., AND R. V. KESSELL. 2003. Sexual reproduction in the invasive species *Fallopia japonica* (Polygonaceae). *American Journal of Botany* 90: 586–592.
- GAO, L., Q. CHU, AND J. YE. 2002. Determination of *trans*-Resveratrol in wines, herbs, and health food by capillary electrophoresis with electrochemical detection. *Food Chemistry* 78: 255–260.
- HIERRO, J. L., J. L. MARON, AND R. M. CALLAWAY. 2005. A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of Ecology* 93: 5–15.
- HILLE, M., AND J. DEN OUDEN. 2005. Charcoal and activated carbon as adsorbate of phytotoxic compounds—a comparative study. *Oikos* 108: 202–207.
- INDERJIT, AND R. M. CALLAWAY. 2003. Experimental designs for the study of allelopathy. *Plant and Soil* 256: 1–11.
- INDERJIT, AND H. NISHIMURA. 1999. Effect of anthraquinones emodin and physcion on availability of selected inorganic ions. *Annals of Applied Biology* 135: 425–429.
- INDERJIT, AND L. A. WESTON. 2003. Root exudates: an overview. In H. de Kroon and E. J. W. Visser [eds.], *Root ecology*, 235–255. Springer-Verlag, Berlin, Germany.
- INOUE, M., H. NISHIMURA, H.-H. LI, AND J. MIZUTANI. 1992. Allelochemicals from *Polygonum sachalinense* Fr. Schm. (Polygonaceae). *Journal of Chemical Ecology* 18: 1833–1840.
- KEANE, R. M., AND M. J. CRAWLEY. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution* 17: 164–170.
- KENWARD, M., AND J. ROGER. 1997. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 53: 983–997.
- KLIRONOMOS, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417: 67–70.
- LEVINE, J. M., M. VILA, C. M. D'ANTONIO, J. S. DUKES, K. GRIGULIS, AND S. LAVOREL. 2003. Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London, B, Biological Sciences* 270: 775–781.
- MACK, M. C., C. M. D'ANTONIO, AND R. E. LEY. 2001. Alteration of ecosystem nitrogen dynamics by exotic plants: a case study of C₄ grasses in Hawaii. *Ecological Applications* 11: 1323–1335.
- MACK, R. N., D. SIMBERLOFF, W. M. LONSDALE, H. EVANS, M. CLOUT, AND F. A. BAZZAZ. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689–710.
- MÜNZZBERGOVÁ, Z. 2004. Effect of spatial scale on factors limiting species distributions in dry grassland fragments. *Journal of Ecology* 92: 854–867.
- NATIONAL RESEARCH COUNCIL. 2002. Predicting invasions of nonindigenous plants and plant pests. National Academy Press, Washington, D.C., USA.
- NILSSON, M. C. 1994. Separation of allelopathy and resource competition by the boreal dwarf-shrub *Empetrum hermaphroditum* Hagerup. *Oecologia* 98: 1–7.
- PARK, C.-S., Y.-C. LEE, J.-D. KIM, H.-M. KIM, AND C.-H. KIM. 2004. Inhibitory effects of *Polygonum cuspidatum* water extract (PCWE) and its component resveratrol on acyl-coenzyme A-cholesterol acyltransferase activity for cholesteryl ester synthesis in HepG2 cells. *Vascular Pharmacology* 40: 279–284.
- PERRY, L. G., S. M. GALATOWITSCH, AND C. J. ROSEN. 2004. Competitive control of invasive vegetation: a native wetland sedge suppresses *Phalaris arundinacea* in carbon-enriched soil. *Journal of Applied Ecology* 41: 151–162.
- PYSEK, P., J. H. BROCK, K. BÍMOVÁ, B. MANDÁK, V. JAROSIK, I. KOUKOLÍKOVÁ, J. PERGL, AND J. STĚPÁNEK. 2003. Vegetative regeneration in invasive *Reynoutria* (Polygonaceae) taxa: the determinant of invasibility at the genotype level. *American Journal of Botany* 90: 1487–1495.
- RAND, T. A. 2000. Seed dispersal, habitat suitability and the distribution of halophytes across a salt marsh tidal gradient. *Journal of Ecology* 88: 608–621.
- REINHART, K. O., AND R. M. CALLAWAY. 2004. Soil biota facilitate exotic *Acer* invasion in Europe and North America. *Ecological Applications* 14: 1737–1745.
- REINHART, K. O., A. PACKER, W. H. VAN DER PUTTEN, AND K. CLAY. 2003. Plant–soil biota interactions and spatial distribution of black cherry in its native and invasive range. *Ecology Letters* 6: 1046–1050.
- RIDENOUR, W. M., AND R. M. CALLAWAY. 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia* 126: 444–450.
- SHAW, R. H., AND L. A. SEIGER. 2002. Japanese knotweed. In R. van Driesche, B. Blossey, M. Hoddle, S. Lyon, and R. Reardon [eds.], *Biological control of invasive plants in the eastern United States*, 159–166. USDA, Forest Health Technology Enterprise Team, Morgantown, West Virginia, USA.
- STINSON, K. A., S. A. CAMPBELL, J. R. POWELL, B. E. WOLFE, R. M. CALLAWAY, G. C. THELEN, S. G. HALLETT, D. PRATI, AND J. N. KLIRONOMOS. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *Public Library of Science Biology* 4(5):e140 [DOI:10.1371/journal.pbio.0040140].
- VAHER, M., AND M. KOEL. 2003. Separation of polyphenolic compounds extracted from plant matrices using capillary electrophoresis. *Journal of Chromatography* 990: 225–230.
- VASTANO, B. C., Y. CHEN, N. ZHU, C. T. HO, Z. ZHOU, AND R. T. ROSEN. 2000. Isolation and identification of stilbenes in two varieties of *Polygonum cuspidatum*. *Journal of Agricultural and Food Chemistry* 48: 253–256.
- VIVANCO, J. M., H. P. BAIS, F. R. STERMITZ, G. C. THELEN, AND R. M.

- CALLAWAY. 2004. Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. *Ecology Letters* 7: 285–292.
- WESTON, L. A., AND S. O. DUKE. 2003. Weed and crop allelopathy. *Critical Reviews in Plant Sciences* 22: 367–389.
- YANG, F., T. ZHANG, AND Y. ITO. 2001. Large-scale separation of resveratrol, anthraglycoside A and anthraglycoside B from *Polygonum cuspidatum* Sieb. et Zucc by high speed counter-current chromatography. *Journal of Chromatography A* 919: 443–448.
- YURKONIS, K. A., AND S. J. MEINERS. 2004. Invasion impacts local species turnover in a successional system. *Ecology Letters* 7: 764–769.
- ZIKA, P. F., AND A. L. JACOBSON. 2003. An overlooked hybrid Japanese knotweed (*Polygonum cuspidatum* × *sachalinense*; Polygonaceae) in North America. *Rhodora* 105: 143–152.