

Reciprocally beneficial interactions between introduced plants and ants are induced by the presence of a third introduced species

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Interspecific interactions play an important role in the success of introduced species. For example, the ‘enemy release’ hypothesis posits that introduced species become invasive because they escape top–down regulation by natural enemies while the ‘invasional meltdown’ hypothesis posits that invasions may be facilitated by synergistic interactions between introduced species. Here, we explore how facilitation and enemy release interact to moderate the potential effect of a large category of positive interactions – protection mutualisms. We use the interactions between an introduced plant (Japanese knotweed *Fallopia japonica*), an introduced herbivore (Japanese beetle *Popillia japonica*), an introduced ant (European red ant *Myrmica rubra*), and native ants and herbivores in riparian zones of the northeastern United States as a model system. Japanese knotweed produces sugary extrafloral nectar that is attractive to ants, and we show that both sugar reward production and ant attendance increase when plants experience a level of leaf damage that is typical in the plants’ native range. Using manipulative experiments at six sites, we demonstrate low levels of ant patrolling, little effect of ants on herbivory rates, and low herbivore pressure during midsummer. Herbivory rates and the capacity of ants to protect plants (as evidenced by effects of ant exclusion) increased significantly when plants were exposed to introduced Japanese beetles that attack plants in the late summer. Beetles were also associated with greater on-plant foraging by ants, and among-plant differences in ant-foraging were correlated with the magnitude of damage inflicted on plants by the beetles. Last, we found that sites occupied by introduced *M. rubra* ants almost invariably included Japanese knotweed. Thus, underlying variation in the spatiotemporal distribution of the introduced herbivore influences the provision of benefits to the introduced plant and to the introduced ant. More specifically, the presence of the introduced herbivore converts an otherwise weak interaction between two introduced species into a reciprocally beneficial mutualism. Because the prospects for facilitation are linked to the prospects for enemy release in protection mutualisms, species introductions can have complex effects on existing species interactions, between both native and introduced species.

Interspecific interactions play an important role in the success of introduced species. For example, Darwin (1859) proposed that introduced species may become successful (‘invasive’) because they escape regulation by natural enemies, an idea now termed the ‘enemy release’ hypothesis. More recently, the ‘invasional meltdown’ hypothesis posits that invasions are facilitated by synergistic interactions between pairs of introduced species (Simberloff and Von Holle 1999), with more general versions of this hypothesis emphasizing the role of facilitation for species invasions (Simberloff 2006). Ultimately, both positive and negative interactions combine to determine the success of introduced species (Levin et al. 2002, Mitchell et al. 2006, Preisser and Elkinton 2008, Johnson et al. 2009), and the significance of these interactions can change as community composition changes over time (Grosholz 2005). Below, we suggest that enemy release reduces the potential for facilitation of introduced species via enemy protection in the case of

protection mutualism. Furthermore, where reward production is strongly induced, enemy release may limit the benefit received by the protecting partner, including introduced ones. Thus, protection mutualisms involving introduced species provide a unique opportunity to study the importance of both positive and negative species interactions for species introductions.

The ‘enemy release’ hypothesis predicts that introduced species become invasive due to escape from a subset of their natural enemies during immigration. The enemy-release hypothesis is supported by recent meta-analyses showing that herbivore pressure is reduced for species in introduced relative to native ranges (Liu and Stiling 2006). Moreover, differences in the degree of escape from natural enemies often distinguishes invasive species from native species or innocuous introduced species (Mitchell and Power 2003, Cappuccino and Carpenter 2005). Interestingly, enemy release may be most important early on in species

introductions – over time introduced species incrementally acquire natural enemies in their new environs (Grabenweger et al. 2010, Mitchell et al. 2010) and this guild can include a combination of natural enemies that are native to the environment as well as those that are themselves introduced (Parker et al. 2006). In some cases, these acquisitions can diminish or mute among-species differences in either the incidence or consequences of attacks by natural enemies (Parker and Gilbert 2007, Van Kleunen and Fischer 2009). Thus, spatial and temporal variation in community composition should have important consequences for the population dynamics of introduced species.

Facilitation can also play an important role in successful invasions. For example, the competitive dominance of invasive species can result from their ability to deprive native species with access to their mutualists (e.g. by distracting or poisoning the allies; Chittka and Schurkens 2001, Stinson et al. 2006). More directly, invading populations can benefit from the presence of other species (native or introduced) that act as seed dispersers (Bourgeois et al. 2005, Best 2008, Best and Arcese 2009, Heimpel et al. 2010), alternative hosts (Heimpel et al. 2010), natural enemies of native competitors (Grosholz 2005, Adams et al. 2003) or that provide new or augmented resources (Best 2008, Madritch and Lindroth 2009, Heimpel et al. 2010).

Because protection mutualisms inherently link facilitation with natural enemy attack, these interactions are uniquely suited for understanding the importance of positive and negative species interactions for species introductions. Ant-protection mutualisms are a well-studied example of protection mutualism in which a host partner, such as a plant or hemipteran aggregation, provides a resource reward to ants in exchange for protection from enemies or competitors. There is considerable evidence that these mutualisms can mediate, and be influenced by, invasion of introduced species. For example, access to mutualist-provided food rewards can enhance the success of introduced ant species (Hoffman et al. 1999, Holway et al. 2002, Ness and Bronstein 2004, Styrsky and Eubanks 2007, Lach et al. 2009, Savage et al. 2009, Helms et al. 2011) and can magnify the effect of introduced ants on the recipient community (Kaplan and Eubanks 2005, Brightwell and Silverman 2010). Further, because the quantity and quality of mutualistic interactions (e.g. numbers or per capita effects) can depend on ant community composition, the benefits received by protected partners in invaded and non-invaded communities can differ substantially – for better (Savage et al. 2009) or for worse (Bond and Slingsby 1984, Christian 2001, Ness 2004, Ness et al. 2004, Lach and Hoffmann 2011). Finally, introduced ants sometimes engage in protection mutualisms with reward producers that are themselves introduced (Koptur 1979, Bach 1991, Abbott and Green 2007, Lach et al. 2010, Helms et al. 2011) – an interaction that could promote invasional meltdown.

Notably, the outcome of protection mutualisms for both partners may depend on natural enemy abundance. Protected partners only benefit from protection when the presence of natural enemies makes them vulnerable (Cushman and Whitham 1989). Further, because protected partners often increase reward production in response to

attacks (Agrawal 1998a, Ness 2003a, Rogers et al. 2003, Lach et al. 2009), the magnitude of benefit received by protectors can be mediated by the natural enemy community. Specifically, in the absence of substantial enemy pressure, non-native protected partners will not need protection, and may not provide rewards for their protectors, including non-native protectors. In contrast, in the presence of substantial enemy pressure, non-native protected partners stand to benefit substantially from protection, and to the extent that reward production is induced by enemy attack, will increase reward protection to protectors, including non-native ones. In this tri-partite context, the extent to which protections mutualisms contribute to invasiveness may depend upon the overall composition of communities and corresponding patterns of enemy release.

Here, we use the tri-partite interactions in the northeastern USA between a reward-producing introduced plant (Japanese knotweed *Fallopia japonica*), native and introduced ants (European red ant *Myrmica rubra*), and native and introduced herbivores (Japanese beetle *Popillia japonica*) to explore how protection mutualism and enemy release interact. In particular, we explore how community composition modifies the consequences of pairwise interactions between these prospective mutualists: 1) to evaluate the extent of enemy release in Japanese knotweed we compare the leaf damage experienced by introduced populations of Japanese knotweed with that described from populations in the native range. 2) to evaluate the potential for facilitation of Japanese knotweed by native and introduced ants, we test the hypothesis that ants protect the plants from herbivores. 3) to evaluate how enemy abundance on knotweed may affect benefits received by ants and potentially facilitation of the introduced ant *M. rubra*, we quantify the extent to which reward production by plants is contingent upon damage experienced by the plants and quantify the change in ant foraging intensity following leaf damage in sites with and without introduced *M. rubra* ants. 4) to evaluate the possibility of reciprocal facilitation between the introduced species *M. rubra* and Japanese knotweed, we evaluate the extent to which these species are disproportionately found together. 5) finally, we use spatiotemporal variation in Japanese Beetle herbivory on knotweed to evaluate the extent to which enemy release (1 above) affects the potential benefit received by both plants (2 above) and ants (3 above). Ultimately, we argue that the spatiotemporal distribution of the introduced beetles creates substantial variation in the benefits experienced by the plants and in the rewards the plants provide to the nectar-collecting ant community.

Study system

Fallopia japonica (= *Polygonum cuspidatum* = *Reynoutria japonica*; Japanese knotweed) is native to eastern Asia and is an introduced invasive plant in the United States and Europe (Weston et al. 2005). In its introduced range, Japanese knotweed colonizes highly disturbed areas, often at the sides of roads and in riparian corridors (Weston et al. 2005). In the area of our study site where it is firmly established, the presence of *F. japonica* is associated with a decline in the number of native plant species (Aguilera et al.

2010). Japanese knotweed produces a carbohydrate-rich solution at extrafloral nectaries on the petioles and nodes of stems that can attract ants and other insects (Kawano et al. 1999). In its native range, knotweed interacts with a variety of ants (nine species in six genera reported in Kawano et al. 1999) and an assemblage of natural enemies that often defoliate > 30% of total leaf area (Kawano et al. 1999).

Myrmica rubra is a Palearctic ant species first reported in the United States by Wheeler (1908) in the Arnold Arboretum, Forest Hills, MA. Additional populations have since been discovered primarily along coastal locations of the northeastern United States (Creighton 1950). Nevertheless, a few inland populations are widely distributed (including the one in our study system) and evidence supports the hypothesis that the ant has been introduced several times (Grodén et al. 2005). Although *M. rubra* can spread by colony budding (Elmes 1980) or nuptial flights, budding is the dominant mode of expansion in its introduced range (Grodén et al. 2005). *Myrmica rubra* can achieve much higher densities in its introduced range relative to its native range (Grodén et al. 2005), and in the area of our study site the presence of *M. rubra* is associated with a decline in the number of native ant species (Morales et al. unpubl.).

Popillia japonica (Japanese beetle) is a univoltine insect that feeds on plant roots and leaves (as larvae and adults, respectively). Adults emerge in July in the area of our study sites at which point they feed, mate, and oviposit for approximately one month (Vittum 1986). Japanese beetles are extreme generalist herbivores and feed on > 300 species in at least 79 families (Potter and Held 2002), including *Fallopia* in their shared native range in Japan (Kawano et al. 1999). Because adult beetles are attracted to a suite of host plant volatiles that can be released by insect-damaged leaves (Loughrin et al. 1998), aggregations coalesce to defoliate individual plants (Potter and Held 2002). Among-year and among-site variation in population densities of adults and larvae is substantial (Dalthorp et al. 2000).

Field sites were located in the Hoosic, Kayaderosseras and Battenkill watersheds, all of which drain into the Hudson River in New York, USA. Japanese knotweed is common in all three watersheds, and experiments were conducted in monoculture stands of Japanese knotweed within 1 km of the main branch of the Hoosic and Kayaderosseras rivers as well as on the campus of Skidmore College (in the Kayaderosseras watershed). Baiting trials at 40 sites in the three watersheds in 2008 found no evidence of *M. rubra* in the Kayaderosseras and Battenkill watersheds, and *M. rubra* was the only ant species observed at Japanese knotweed nectaries at the Hoosic experimental site. At the Kayaderosseras and Battenkill sites, the plant-tending ant guild included *Tapinoma sessile*, *Camponotus* sp. and *Formica* spp. (Ness unpubl.), although species identifications were limited to noting the absence of *M. rubra*. Based on the pronounced among-site differences in the ant tending communities, we hereafter refer to sites in the Hoosic and Kayaderosseras watersheds as invaded and non-invaded, respectively (referencing the distribution of *M. rubra* rather than knotweed, as all the experimental sites include the latter by necessity).

Among-site variation in the genetic composition of knotweed is extremely modest within the invaded range

(Hollingsworth and Bailey 2000, Grimsby et al. 2007), supporting the interpretation that among-site differences in ant-plant interactions are driven by among-site differences in the ant communities. That said, our intent here is not to provide a rigorous comparison of whether and how *M. rubra* may differ from any one other species in particular. Rather we explore whether and how plant-insect interactions differ in sites invaded by *M. rubra* in comparison to non-invaded sites, and in the presence or absence of introduced herbivores.

Methods

Enemy-release and facilitation of Japanese knotweed by ants

We quantified leaf damage on control and ant excluded plants at six sites from May–June 2007 and repeated the experiment in the invaded watershed from July–August, 2010 to coincide with the period of Japanese beetle emergence and herbivory. We used this information to contrast the magnitude of leaf damage experienced at our sites with that reported in the native range, to test the hypothesis that leaf damage is increased when ants are excluded from plants, and to explore whether these patterns differ with site or season.

In the May–June experiment, three sites were chosen along each of the invaded and non-invaded watersheds. Initially, 20 plants at each watershed-site were selected. After excluding plants that had been knocked down by flooding, 111 plants were available for analysis. Treatments began between 18 and 23 May at which point ants were excluded from half of all plants using Tangletrap applied to the base of stems. Plants were monitored at least once per week to maintain ant exclusions (thirteen plants were excluded from the analysis because exclusions failed). After approximately one month, the level of herbivory on each plant was estimated as the percent of leaf area missing for each leaf rounded to the nearest fifth percentile. Average percent herbivory, calculated both for all leaves and damaged leaves only, was logit transformed (with 0.01 added to the numerator and denominator, Warton and Hui 2011) to correct for normality. Probability of herbivory was analyzed using logistic regression on a per-plant basis as the number of leaves damaged relative to the total number of leaves.

To quantify the potential impact of the introduced Japanese beetle on the amount of herbivory experienced by Japanese knotweed, we repeated this exclusion experiment in the invaded experimental site during July–August 2010 to coincide with the period of adult beetle emergence. To ensure that Japanese beetles were at sufficiently high densities, approximately 5 beetles were released around each plant ($n = 60$) and sections of pheromone lures were attached to stakes adjacent to focal plants. As in 2007, Tanglefoot was used to exclude ants but beginning on 22 July. Censuses of ant abundance were taken twice daily and estimates of leaf herbivory (calculated as in 2007) were made on 9 August. Control plants were excluded from analysis if ants were not observed at any of the 19 censuses ($n = 2$) and treatment plants were excluded if ant density

exceeded two on any given census ($n = 3$). Otherwise, data were analyzed as for the 2007 herbivory experiment.

Nectar production in response to leaf damage

To evaluate whether leaf damage was associated with an increase in reward production by plants, we conducted an experiment during early June 2011. Forty plants were randomly assigned to damage and control categories ($n = 20$ per plant) and we used scissors to remove approximately one-third of the area on each leaf for treatment plants on 3 June. This level of simulated herbivory was based on reports of herbivory on Japanese knotweed in its native range (Kawano et al. 1999). Manufactured damage (e.g. by scissors, pins) is capable of inducing responses by ant-tended plants in this and other systems (Kawano et al. 1999, Agrawal 1998a, b). On 6 June, Tanglefoot was applied to plants to exclude ants and to allow nectar to accumulate. On 7 June, we collected nectary secretions from the 2nd and 4th fully expanded leaf of the distal-most stem. Because sugars had crystallized in the absence of ant foraging, water was added to the nectaries to dissolve and better collect sugars, and sugar content for the resulting solutions was measured with a refractometer using the Brix scale. As a result, we can describe total standing sugar for each leaf as the product of the sampled volumes and sugar content of each sampled volume, but cannot independently describe any changes in nectar volume and/or sugar concentration that could occur when plants are damaged and nectar does not crystallize (i.e. in the presence of ant foragers). This experiment was performed at the Hoosic site.

To confirm that treatment and control plants did not differ in ant attendance prior to the experiment, we censused all plants for ants three times in the 36 h prior to the initiation of the damage treatment, and used a t-test to compare mean attendance among the two groups.

Ant responses to leaf damage

To evaluate whether ant attendance increases in response to leaf damage and whether any response differed among watersheds with native ants versus those dominated by *M. rubra*, we conducted identical experiments during June 2008 in the invaded and non-invaded watersheds ($n = 70$ and 80 plants, respectively). At each site, plants were randomly assigned to damage and control categories. We used scissors to remove approximately one-third of the area on each leaf for damage-treatment plants. Censuses of ant abundance on individual plants were performed for several days prior to the initiation of the experiment, and twice daily for at least one week after the damage treatments. Preliminary analysis indicated that our data were consistent with previous studies showing an increase and subsequent decrease in ant abundance following leaf damage (Agrawal 1998b). To account for this pattern, we included a quadratic term to model the pattern of ant abundance over time following the initiation of treatments.

Ant responses to herbivory by Japanese beetles

To test the hypothesis that folivory by Japanese beetles induces changes in ant tending of plants, we selected

65 plants and introduced five beetles onto approximately half of these plants beginning 22 July 2008. Beetles were confined to plants using an insect rearing bag with the bottom of the bag open to allow access by ants. A large rain event on 23 July resulted in heavy flooding and decreased the number of available plants to 21 treatment plants and 20 beetle-control plants (i.e. bagged plants without beetles). We monitored ant attendance on the plants at morning and afternoon censuses from 25 July to 1 August for a total of 15 censuses. The experiment was repeated in 2009 with ant counts beginning on 23 July for a total of 13 censuses ($n = 30$ plants per treatment). Because the effect of beetles did not vary between years (Year \times Beetles + Year \times Beetles \times Day | Year + Beetles + Day + Beetles \times Day, $\chi^2_{DF=3} = 2.22$, $p = 0.53$), data were combined for analysis. This experiment was performed at the Hoosic site.

To describe the relationship between the amount of beetle herbivory and the magnitude of ant induction, we monitored 50 plants beginning 4 July 2008. We introduced five beetles onto each treatment plant ($n = 12$). Beetles were confined to treatment plants using an insect rearing bag with the bottom of the bag open to allow access by ants. We also established two sets of controls: beetle-control plants ($n = 6$ bagged plants without beetles) and bag + beetle-control plants ($n = 32$ non-bagged plants without beetles). We monitored ant attendance on the plants for three censuses between five and six days after initiating treatments. We also recorded any incidence of beetle attack on control plants ($n = 5$ plants attacked). After the third census, we collected all damaged leaves and estimated the mass lost using the relationship between petiole diameter (mm) and leaf mass (mg) inferred from 100 undamaged leaves collected at that time (mass = $-0.437 + 0.861 \times \text{diameter}$; $R^2 = 0.58$; one plant was omitted that 'gained' mass in spite of evident beetle damage). The experiment was repeated with the undamaged plants using a second cohort of beetles beginning 10 July. This experiment was performed in the Kayaderosseras watershed.

Correlated distribution of Japanese knotweed and *Myrmica rubra*

We explored whether *M. rubra* was disproportionately common at sites that also included *F. japonica* by visually inspecting sites in the Hoosic watershed (the only watershed in this study with *M. rubra* populations). The Hoosic River was sampled by canoe once every kilometer of riverflow for canoe-accessible sites from Adams, MA to its confluence with the Hudson River ($n = 39$ sites). Because *M. rubra* is generally abundant where it has invaded (*personal obs*), we identified sites as 'non-invaded' if *M. rubra* was not discovered after 30 person-minutes. We also documented the presence or absence of *F. japonica* at each site at that time. These data were analyzed using a χ^2 -test (Gotelli and Graves 1996).

Statistical analysis

All statistical analyses were conducted using R (R Development Core Team). We analyzed plant responses

Table 1. Summary statistics for the effects of simulated herbivory and watershed on ant abundance at Japanese knotweed. Analyses are based on likelihood-ratio tests from generalized mixed-models with Poisson errors (Methods). Analyses were performed hierarchically and the effect of watershed was evaluated before the effect of treatment. Terms were removed from the model when not significant. T = simulated herbivory (treatment), W = watershed, C = census.

Hypothesis test	Terms removed terms remaining*	DF	χ^2	p
Does the pattern of induction vary across watersheds?	W + W × T + W × C + W × C ² + W × T × C + W × T × C ² T + C + T × C + T × C ²	6	52.2	<0.001
Is there any effect of treatment on ant attendance?	T + T × W + T × C + T × C ² + T × W × C + W × T × C ² W + C + W × C + W × C ²	6	60	<0.001
Does the temporal pattern of ant abundance vary by watershed?	W × C + W × C ² + W × T × C + W × T × C ² T + W + C + T × W + T × C + T × C ²	4	2.9	0.57
Does the temporal pattern of ant abundance vary by treatment?	T × C + T × C ² T + W + C + T × W	2	19.3	<0.001
Does the effect of treatment vary by watershed?	T × W T + W + C + T × C + T × C ²	1	6.2	0.013

*full model = terms removed + terms remaining.

to simulated herbivory using a mixed-effect linear model with plant as a random effect (Pineiro and Bates 2000). Total sugars were log + 0.5 transformed to improve normality and stabilize variances. We analyzed ant count data using a mixed-effects generalized linear model with Poisson errors and with either plant or site as a random effect to account for the grouping structure of the data (Bates and Maechler 2010). We analyzed average herbivory using either a linear model or, where data were aggregated by sites, a mixed-effects linear model with site as a random effect. For the analysis of probability of herbivory, a mixed-effects logistic regression was used with binomial errors and with plant as a random effect to account for overdispersion. All p-values reported for the analysis of herbivory are associated with a one-sided test of the hypothesis that ants reduce herbivory.

Results

Enemy release and facilitation of Japanese knotweed by ants

During the ant-exclusion experiment conducted in May–June 2007, levels of herbivory in our six sites were well below those reported in Japan (Kawano et al. 1999), averaging less than 1 percent (back-transformed 95% CI = 0.88–0.96 percent) compared to ca 36% in the native range. Neither watershed (invaded Hoosic or non-invaded Kayaderosseras) nor ant exclusion had a significant effect on total herbivory or the probability of leaf damage (Table 2, 3). Ant exclusion did cause a small but significant increase in the level of herbivory when considering damaged leaves only, and herbivory on damaged leaves was higher in the non-invaded watershed (Table 4, Fig. 3). Among the 60 ant-accessible control plants, variation in herbivory and ant attendance were not correlated (i.e. there was no induction response within this range of damage; slope \pm SE = 0.09 \pm 0.18, $z = 0.49$, $p = 0.63$).

Overall herbivory was substantially higher for the ant-exclusion experiment conducted in the presence of Japanese beetles in July–August 2010 (back-transformed 95% CI = 4.1–5.8%). In this setting, ant exclusion significantly increased herbivory by a factor of 1.8 ($F_{1,53} = 2.86$,

Table 2. ANOVA table for proportion herbivory (logit-transformed, Methods) on Japanese knotweed in the absence of Japanese beetles. Experiments were conducted at three sites in each of the invaded (Hoosic) and non-invaded (Kayaderosseras) watersheds. p-values are based on type III SS.

Parameter	DF	F-value	p-value
Intercept	1,99	3065.37	<0.001
Ant treatment	1,99	0.06	0.596
Watershed	1,4	3.28	0.144
Ant × Watershed	1,99	0.09	0.765

$p = 0.048$, Fig. 4), increased the probability of leaf damage by a factor of 1.55 (log odds \pm SE = 0.69 \pm 0.38, $z = 1.79$, $p = 0.036$, Fig. 4) but had no effect on the level of herbivory for damaged leaves only ($F_{1,42} = 0.89$, $p = 0.176$).

Correlated distribution of Japanese knotweed and *Myrmica rubra*

Within the *M. rubra*-invaded watershed, *M. rubra* and *F. japonica* were more likely to co-occur than expected by random chance ($\chi^2_{DF=1} = 12.6$, $p < 0.001$). Eighteen of the 19 sites with *M. rubra* also included *F. japonica* and 18 of the 25 sites with *F. japonica* included *M. rubra*.

Nectar production in response to leaf damage

The amount of sugar collected from EFNs on damaged plants was approximately ten-fold greater than that from undamaged control plants (leaf 2 means = 2.42 and 0.22,

Table 3. Parameter estimates and significance for the probability of leaf damage (per leaf) on Japanese knotweed in the absence of Japanese beetles. Experiments were conducted at three sites in each of the invaded (Hoosic) and non-invaded (Kayaderosseras) watersheds.

Parameter	Estimate*	z-value	p-value
Intercept (w/ants in Hoosic) [†]	-1.47	-10.82	<0.001
Δ ant exclusion [†]	-0.26	-1.62	0.947
Δ Kayaderosseras [†]	-0.15	-0.95	0.343
Δ exclusion × Kayaderosseras	-0.09	-0.14	0.782

*log-odds.

[†]with interaction-term removed.

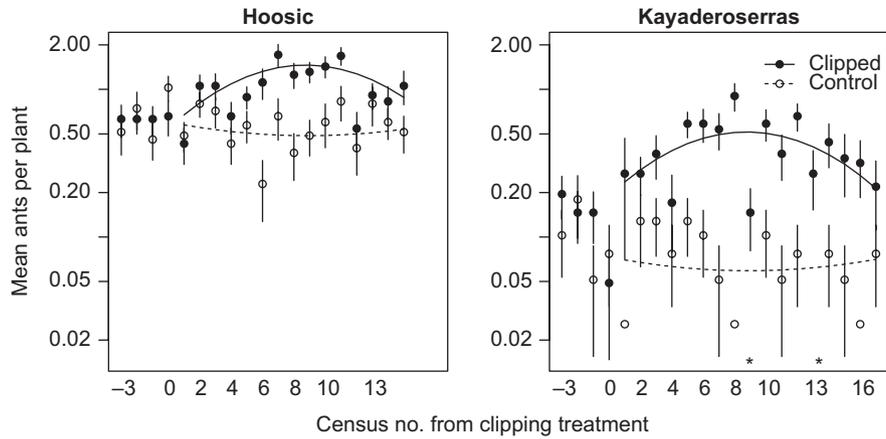


Figure 1. Temporal pattern of ant abundance at Japanese knotweed plants for control plants and plants with simulated herbivory in the Hoosic (*M. rubra* invaded) and Kayaderoserras (non-invaded) watersheds (mean \pm 1 SE). Treatments were initiated at census zero. Note the consistent temporal pattern of ant abundance between watersheds, although initial ant abundance is higher and the strength of induction is lower in the Hoosic. Asterisks indicate censuses where no ants were observed. Mean ant abundance = $\exp(-0.996 - 0.104 \times T - 2.107 \times W - 0.048 \times C + 0.003 \times C^2 + 1.064 \times T \times W + 0.027 \times T \times C - 0.016 \times T \times C^2)$, where $T = \Delta$ treatment, $W = \Delta$ watershed, $C =$ census, and the intercept defines ant abundance in the Hoosic watershed for control plants at census zero.

respectively; leaf 4 means = 2.03 and 0.21, respectively), and this among-treatment difference was significant ($F_{1,37} = 4.77$, $p = 0.035$). Mean ant attendance at control and treatment plants did not differ significantly prior to the treatment ($t = 0.87$, $DF = 37$, $p = 0.39$).

Ant responses to leaf damage

Simulated herbivory caused a significant increase in ant attendance in both sites (Table 1, Fig. 1). The temporal pattern of induction – increasing and then decreasing ant abundance – was identical between sites. Initial ant abundance was lower, however, and the relative increase in ants was higher, at the non-invaded sites that lacked *M. rubra* (Table 1, Fig. 1). Specifically, ant abundance increased by

up to a factor of 2.8 in the invaded site compared to 8.2 in the non-invaded site.

Ant responses to herbivory by Japanese beetles

The experimental addition of beetles significantly increased ant attendance over an eight-day timespan in the invaded site (Beetles \times Day|Year + Beetles + Day, $\chi^2_{DF=1} = 6.68$, $p = 0.01$; Fig. 2). In a six-day span in the non-invaded site, beetle additions to plants increased ant abundance relative to the two control groups by a factor of 7 ($\chi^2_{DF=1} = 6.95$, $p = 0.008$) and increased average herbivory by a factor of 23 (overall treatment effect, $F_{1,28} = 72.8$, $p < 0.001$; Tukey's post hoc comparisons: beetle versus beetle-control treatments, Δ beetle \pm SE = 1.3 ± 0.11 , $z = 11.8$, $p < 0.001$; beetle versus bag + beetle control treatments, Δ beetle \pm SE = 0.73 ± 0.17 , $z = 4.32$, $p < 0.01$). Notably, the increases in ant abundance were proportional to the level of beetle herbivory (slope \pm SE = 0.61 ± 0.19 , $z = 3.24$, $p = 0.001$).

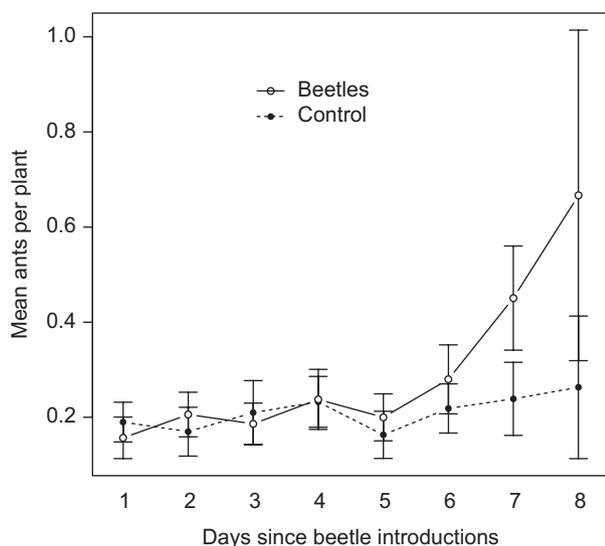


Figure 2. Temporal pattern of ant abundance (mean \pm 1 SE) at Japanese knotweed plants in the Hoosic (*M. rubra* invaded) watershed for control plants and plants with experimentally introduced Japanese beetles.

Discussion

Surprisingly few studies implicate the importance of protection mutualisms as important in the invasion of reward-producing hosts in non-native environments (Richardson

Table 4. ANOVA table for proportion herbivory (logit transformed, Methods) on damaged leaves of Japanese knotweed in the absence of Japanese beetles. Experiments were conducted at three sites in each of the invaded (Hoosic) and non-invaded (Kayaderoserras) watersheds. p-values are based on type III SS.

Parameter	DF	F-value	p-value
Intercept	1,98	3757.07	<0.001
Ant treatment	1,98	5.77	0.009
Watershed	1,4	41.35	0.003
Ant \times Watershed	1,98	0.2	0.655

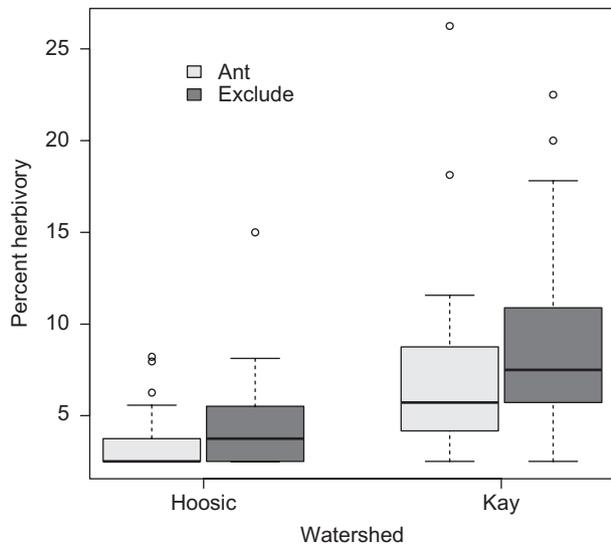


Figure 3. Boxplots showing the effect of ants on the percent leaf herbivory (leaf area removed per plant) for leaves with some damage in the Kayaderoseras (non-invaded) and Hoosic (*M. rubra* invaded) watersheds in the absence of Japanese beetles. Boxes show the interquartile range (IQR), the horizontal line within boxes shows the median. Whiskers show the most extreme value no greater than 1.5 times the IQR, and points show 'outside' values greater than 1.5 times the IQR (Chambers et al. 1983). Herbivory increased significantly with ant-exclusion and was significantly higher in the Hoosic watershed, but with no interaction between these variables (Table 4).

et al. 2000, but see Koptur 1979, Bach 1991, Abbott and Green 2007, Lach et al. 2010). This dearth of studies is consistent with a role for enemy release in successful invasions. However, reviews of both the facilitation and enemy release hypotheses have emphasized the need for evidence drawn from new systems (Colautti et al. 2004, Liu and Stiling 2006, Simberloff 2006). The tri-partite interactions among natural enemies, reward-producing hosts and prospective protectors in protection mutualisms provide a novel approach for evaluating the importance of positive and negative interactions for biological invasions, and for exploring how invasions can alter the consequence of existing interactions.

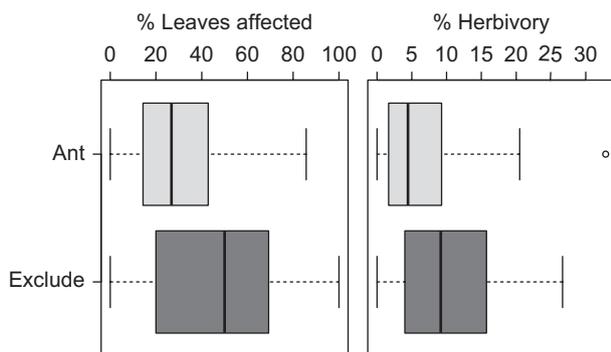


Figure 4. Boxplots showing the effect of ants on the percent leaf herbivory (leaf area removed per plant) and probability of leaf herbivory in the presence of Japanese beetles in Hoosic (*M. rubra* invaded) watershed. Box and whisker plots as in Fig. 3.

Our results suggest that enemy release can moderate the effect of prospective protection mutualisms. In particular, we found extremely low levels of herbivory for Japanese knotweed in the absence of the introduced herbivore, Japanese beetles, especially in contrast to levels reported in its native range (ca 1% vs 36%, respectively; Kawano et al. 1999). These low levels of herbivory are consistent with the hypothesis that enemy release has contributed to the success of knotweed in its introduced range. Moreover, the low levels of herbivory that we observed precluded a strong positive effect of protection by both native and introduced ants in the absence of introduced beetles. Although the significant reduction in herbivory for damaged leaves with ants indicates that ants can effectively deter herbivores, the low levels of herbivory that we found was associated with an insignificant effect of ants on overall plant herbivory. Thus, our results are consistent with the hypothesis that enemy release for Japanese knotweed can limit the protective benefits provided by ants, including both native and introduced species.

If the food resources provided to introduced ants by prospective mutualists are important for their invasions (Hoffman et al. 1999, Tillberg et al. 2007, Savage et al. 2009, Helms et al. 2011), Japanese knotweed may facilitate invasions by *M. rubra* even in the absence of substantial reciprocal benefit. However, in our study site the benefit provided by knotweed-derived extrafloral nectar to ant populations appears to be limited by the typically low levels of herbivory. In particular, leaf damage induces sugar production at knotweed EFNs, as observed in other systems (Agrawal 1998a, Ness 2003a, Rogers et al. 2003, Lach et al. 2009). In our sites, significant levels of herbivory were contingent upon the presence of introduced Japanese beetles, and the damage that plants typically experience in the absence of the beetles (i.e. in May–June) appears to be insufficient to induce meaningful responses by the plants or ants. These findings, and the pronounced spatiotemporal variation in beetle density irrespective of interactions with plant-protecting ants (Dalthorp et al. 2000), suggest that facilitation between knotweed and *M. rubra* is rare (or at least inconsistent).

In contrast to the results highlighted above, the strong spatial association of *M. rubra* with knotweed that we observed in this study is consistent with the hypothesis of reciprocal facilitation between these species. Although further studies are needed to evaluate the benefits of Japanese knotweed for ant populations, we expect some combination of four factors to contribute to the observed pattern of spatial correlation. First, the strong spatial association may indicate that access to pulses of plant-produced resources can be meaningful to the ants (see Byk and Del-Claro 2011 regarding the positive effects of EFN on growth rate and size of associated ant colonies). Second, some resource provision occurs irrespective of natural enemy attack – ant foraging is particularly pronounced when young knotweed shoots are growing (Kawano et al. 1999, Ness and Morales unpubl.). Third, the value of even modest rewards may be heightened by the rarity of nectar rewards in these riparian habitats, where they are limited to a few plants such as *Catalpa speciosa*, and ant-tended hemipterans such as *Publilia concava*. Lastly, shared habitat requirements and

dispersal dynamics also likely contribute to the positive spatial association between knotweed and *M. rubra* seen in this study (Ness and Morales unpubl.). As a result, a combination of abiotic and biotic factors (see also Grosholz 2005) may generate invasional hotspots characterized by a high diversity of introduced species.

While we find equivocal evidence for strong reciprocal facilitation between the introduced ant *M. rubra* and knotweed, our most striking result may be that novel introductions can qualitatively change the magnitude of reciprocally beneficial interactions between previously weakly interacting introduced species. The effect of Japanese beetles on levels of herbivory, ant abundance, and plant protection demonstrate that introduction of a third introduced species in the food web can dramatically change the ant–plant interaction. On-plant foraging by the ants, a currency for both plant protection and resource collection by the ants (Ness 2003b), increases by an order of magnitude when plants are damaged by introduced beetles or experimentally damaged in a fashion that quantitatively mimics the levels of damage typical in the plant's native habitat. Here, the introduced beetle is not only the 'ant antagonist' but also the trigger for the provision of greater reward production by the introduced plant.

Strong versions of the facilitation hypothesis (i.e. invasional meltdown) may require that the services provided to knotweed by *M. rubra* exceed those performed by native ants. Our comparisons of results from the invaded and non-invaded watersheds suggest that native ants and *M. rubra* did not differ in efficacy of protection (Table 1–3, Fig. 1, 3). Although the non-invaded ant community showed a stronger induction response, both initial and post-induction ant densities of *M. rubra* were higher. This result is consistent with the observation that *M. rubra* reaches very high nest densities in invaded sites (Grodén et al. 2005, personal obs.) and with comparisons of the effectiveness of *M. rubra* and native ants as mutualists in the protection mutualism with the treehopper *Publilia concava* (Morales unpubl.). Further, our observation that the distribution of knotweed is independent of that of *M. rubra* within mutually invaded watersheds is consistent with the interpretation that *M. rubra* and native ants affect the plants similarly. Of course, comparisons between native ants and *M. rubra* in the current study are also confounded with watershed differences – a landscape artifact necessitated by the strong competitive effects of *M. rubra*. Future studies will be needed to quantify differences in the pattern of ant abundance and protection provided by these species.

Protection mutualisms provide a unique lens through which to explore the relative importance of positive and negative interactions for species invasions and the manner in which the importance of these interactions can change as communities are enriched. Overall, our results suggest that enemy release can moderate the potential effect of protection mutualism for both partners in these interactions. Our results also suggest that species introductions can cause qualitative shifts in the dynamics of interactions between existing introduced species – a finding with important implications in the context of biological control. For example, the introduction of folivorous insects as biological control agents for Japanese knotweed (Shaw and

Seiger 2002) in New England could unexpectedly (and literally) fuel the invasion of *M. rubra* in the region. Biological control is typically evaluated in the context of host specificity, asking whether an intentionally introduced consumer is likely to attack other plants. The prospect that these consumers could influence the mutualists of the target species, let alone to the benefit of those mutualists to a degree that could influence other species of the larger community, is largely unexplored. Our results argue that biological control needs to be evaluated within a community context, particularly as our biota becomes increasingly homogenized (McKinney and Lockwood 1999). Finally, because enemy release and facilitation are predicated on the rarity versus presence of particular combinations of introduced species, respectively, we predict that interactions of the latter type will become increasingly common as the duration of time in the new environs increases. This is particularly likely to be the case with protection mutualisms – facilitation fueled by these interactions are predicated on a robust natural enemy community that may develop over centuries in the introduced environs (Hawkes 2007, Mitchell et al. 2010).

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