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Effects of the exotic shrub *Lonicera maackii* on the plant animal interactions in the invaded habitat

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This dissertation is dedicated to
Jenni Malie Higashiguchi,
a dear friend gone too soon

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Chapter 1

Invasive plant species alters consumer behavior by providing refuge from predation

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ABSTRACT

Understanding the effects of invasive plants on native consumers is important because consumer-mediated indirect effects have the potential to alter the dynamics of coexistence in native communities. Invasive plants may promote changes in consumer pressure due to changes in protective cover (i.e. the architectural complexity of the invaded habitat) and in food availability (i.e. subsidies of fruits and seeds). No experimental studies have evaluated the relative interplay of these two effects. In a factorial experiment, we manipulated cover and food provided by the invasive shrub Amur honeysuckle (*Lonicera maackii*) to evaluate whether this plant alters the foraging activity of native mammals. Using tracking plates to quantify mammalian foraging activity, we found that removal of honeysuckle cover, rather than changes in fruit resources it provides, reduced the activity of important seed consumers, mice in the genus *Peromyscus*. Two mesopredators, *Procyon lotor* and *Didelphis virginiana,* were affected as well. Moreover, we found rodents used *L. maackii* for cover only on cloudless nights, indicating that the effect of honeysuckle was weather-dependent. Our work provides experimental evidence that this invasive plant species changes habitat characteristics, and in so doing, alters the behavior of small- and medium-sized mammals. Changes in seed predator behavior may lead to cascading effects on the seeds that mice consume.

INTRODUCTION

Plant invasions often alter the landscape of the invaded habitat by creating a dense vegetative layer (e.g. Sheley et al. 1998; Williams et al. 2009; Forseth and Innis 2004; Levine et al. 2003, Mack et al. 2000; Mattos and Orrock 2010; Orrock et al. 2010a), thereby increasing the overall architectural complexity of the habitat (Sheley et al. 1998; Forseth and Innis 2004). In addition, this altered vegetative layer is often associated with new food sources in the form of fruits and seeds that may become available for consumers (William et al. 1992; Ingold and Craycraft 1983; Bartuszevige et al. 2006, Gosper et. al. 2006; Richardson et al. 2000; Stansbury and Vivian-Smith 2003). Although both vegetation cover and fruit availability may affect consumer behavior (Orrock et al. 2010a) experiments that manipulate both cover and food provided by an invader, to the best of our knowledge, have not been conducted.

Food and cover can interact to determine when and how long an animal will forage, as many vertebrates apparently perceive a tradeoff between food procurement and safety. This tradeoff is manifested as a dependence of the time allocated for foraging on perceived predation risk (Lima and Dill 1990; Brown et al. 1992; Kotler et al. 1997; Mohr et al. 2003; Verdolin 2006). The decision apparently can be influenced by weather conditions that might change the conspicuousness of the prey to predators (Orrock et al. 2009; Mattos and Orrock 2010). Most importantly the effects of the invasive on perceived predation risk may foster a novel effect of biological invasion, as the effects of food and shelter on consumer behavior (Matos and Orrock 2010) and abundance (Noonburg & Byers 2005; Borer et al. 2007) may result in differential predation pressure on seedlings (i.e. apparent competition: Orrock et al. 2010b).

We evaluated the relative role of shelter and food in affecting the foraging activity of a community of small mammal consumers in areas that have been invaded by Amur honeysuckle (*Lonicera maackii* (Rupr.) Maxim., Caprifoliaceae). *Lonicera maackii* is a highly successful and aggressive invasive plant in forests in much of the eastern United States, known for reducing diversity (Luken and Goessling 1995, Luken and Thieret 1996; Luken et al. 1997; Hutchinson and Vankat 1997, 1999), abundance (Gould and Gorchov 2000; Collier et al. 2002; Gorchov and Trisel 2003), species composition (Hartman and McCarthy 2008), and growth (Miller and Gorchov 2004) of native species. It also alters the behavior and abundance of native fauna (Schmidt and Whelan 1999; McCusker 2010; Mattos and Orrock 2010, Rodewald 2010). *Lonicera maackii* provides an ideal system for experimental manipulation because its branch architecture, consisting of multi-stemmed shrubs with arching branches from several trunks, produces a thick understory (Luken et al. 1997) that has been shown to serve as a refuge (here defined as cover from predation) for mammals (Meiners 2007; Mattos and Orrock 2010) and nest sites for birds (Schmidt and Whelan 1999). The plant is also known for its massive fruit production of up to 400 million berries per ha (Ingold and Craycraft 1983), or more than 20 kg of fruits in a 25 m^2 area (HPD, unpublished results), with approximately 62% escaping consumption by birds and falling to the ground (Bartuszevige et al. 2006). Small rodents are known to consume *L. maackii* fruits (Williams et al. 1992) especially underneath highly invaded areas (Meiners 2007) where seed caches are relatively common (HPD, personal observation).

We focused on the foraging activity of the most common vertebrate consumers in oak-hickory forests. Specifically, we targeted mesopredators (raccoons and opossum, respectively *Procyon lotor* and *Didelphis virginiana*) because they play a key role in regulating the abundance and diversity of both fauna and flora communities (Prugh et al. 2009) and seed predators (mice and squirrels, respectively *Peromyscus* spp. and *Sciurus* spp.). The latter are known for their negative effects on tree seedling recruitment (Ostfeld et al. 1997; Manson et al. 1998, 1999; Vander Wall 2001). *Peromyscus* spp. may also increase disease risk (Jones et al. 1998; Allan et al. 2003) and limit biological invasions (Elkinton et al. 1996; Jones et al. 1998).

We combined a factorial manipulation of *L. maackii* structure and fruit to experimentally examine how shelter and food, respectively, affect the activity of native mammals. Because consumer foraging and activity may vary with weather (e.g. Orrock et al. 2009; Mattos and Orrock 2010), we conducted our study over 14 months, which allowed us to evaluate the potential interaction of *L. maackii* with climatic factors. This factorial manipulation of *L. maackii* provides the first experimental evaluation of the relative impact of food versus cover provided by an invasive plant on consumer activity.

METHODS

Study area

This study was conducted at Busch Wildlife Conservation Area (38.70° N, 90.71° W), a 6987 ha park in Saint Charles County, Missouri. The park is open to the public for hunting and has about 1215 ha of oak/hickory forest, with a shrub layer dominated by *L. maackii* and *Rosa multiflora* (multiflora rose). Other common understory, woody plants include *Lindera benzoin* (spice bush), *Rhus aromatica* (aromatic sumac), *Cornus* spp. (dogwood) and *Symphoricarpus orbiculatus* (coral berry).

Experimental design

Three experimental blocks were established in the fall of 2006. Each block consisted of four treatment plots (30 × 30 m each). Plots were 50 m apart and blocks were at least 3 km from each other. Plots within each block were randomly assigned to one of four experimental treatments: 1) fruits and honeysuckle cover present, 2) fruits present and honeysuckle cover removed, 3) fruit removed and honeysuckle cover present, 4) both fruits and honeysuckle cover removed. Honeysuckle individuals were physically removed from treatment plots 2 and 4 by cutting the stem at the base in the fall of 2006. Pruning of resprouts continued from late fall 2006 until the end of the study. During the fall months of 2006, 2007 and 2008 we removed all fruits by hand from treatment plots 3 and 4. In 2006, fruits on treatment plots 2 were removed from the plant and left on the ground prior to plant removal. In 2007 and 2008, fruits removed from plots of treatment 3 were added to plots of treatment 2. In order to guarantee that fruit supplementation treatments mimicked the natural availability of fruit on the ground we started the removal in the end of the fruiting season when fruits are fully-grown and ripe. Fruits were gradually added to the supplemental plots throughout our harvest. Fruit addition was done by throwing fruits on the ground in a random fashion simulating an even distribution across the entire plot. Often we found fruit caches on the ground, amidst mouse feces, indicating that mice were consuming the fruits.

Tracking plates were used to quantify mammal foraging behavior. Connors et al. (2005) suggest that track plates may represent a more accurate picture of small mammals space use than trapping, as track plates do not impede animal movement. However, because one organism can visit multiple plates this technique potentially confounds activity with density. For simplicity, we refer to track plate data as foraging activity. Track plates consisted of 14 cm × 22 cm acetate sheets covered in a graphite alcohol oil mixture, fastened to aluminum flashing, and nailed to the ground surface. Plates were distributed on 20 × 20 m grid positioned at the centre of the large 30 × 30 m treatment plot. The grid consisted of 16 track plates distributed in a 4 × 4 array with approximately 5 m spacing between plates. Censuses were conducted monthly from September 2007 to December 2008. Heavy rains and snow prevented data collection for November 2007 and January 2008. For each census, track plates were left in the field for three consecutive nights and then brought to lab for track identification and quantification. We used Elbroch (2003) as reference for identifying the paw prints. One disadvantage of using track plates instead of more traditional methods such as sand boxes is that prints are marked on a flat surface, thus one may not be able to see the depth of the print, as opposed to three dimensional print left in sand. While large animals (e.g. raccoons and opossums) leave a clear print, very small mammals such as mice *Peromyscus* spp. and short-tailed shrews (*Blarina brevicauda*) do not do so (Wiewel et al. 2007). As such, we assigned all small-mammal tracks of this type to be *Peromyscus* spp. because live-trapping conducted in the study sites found that *Peromyscus* spp. comprised 588 of 600 or 98% of all captures, with *B. brevicauda* comprising the remaining 12 (HPD, unpublished data).

Cloud cover and average temperature for each night were collected from the Spirit of St. Louis airport weather station located in St. Charles, MO (quality control data, station name and ban number [SUS, 03966], http://cdo.ncdc.noaa.gov/qclcd/QCLCD SUS station, National Oceanic and Atmospheric Administration, NOAA), approximately

10 km from our field site. Hourly values for these variables were averaged over the course of each night, a 13-hour period. An hour was considered cloudy if sky cover was between 0.6 and 1 (corresponding to categories broken and overcast, according to NOAA) or if weather was classified as light rain, rainy, thunderstorm, snowy and/or foggy. These data were tallied over the course of each night to give the relative amount of time that the sky was cloudy (e.g., if 3 out of 13 h had cloudy skies then cloud cover for that night was 0.23). The fraction of the moon illuminated for each night, also used as a covariate, was obtained from published tables available from the U.S. Naval Observatory (http://www.nws.noaa.gov/climate/index.php).

To determine if invasion by *L. maackii* modified the vegetation density of the understory vegetation, we measured vegetation density along a 20 m transect by counting the number of times that any plant material touched a polyester line held 2 m above the ground for the entire extension of each transect. Vegetation density measurements were taken from transects in 18 distinct areas with different natural densities of *L. maackii.* A transect was placed in each of these areas. These areas consisted of 12 plots that were not part of the study and 6 experimental plots with cover present, On each of these areas we also recorded the number of *L. maackii* individuals within 2 m of the line. To verify if our treatment manipulations were effective in reducing the vegetation density, we also compared vegetation density between plots with honeysuckle cover present with plots from which honeysuckle had been removed. Using the same technique, we measured the vegetation density for one transect established in the middle of each 30 × 30 m plot. The ends of each transect were 5 m from the edge of the plot.

Statistical Analysis

For the 18 transects in natural vegetation, we regressed number of touches on the polyester line on the number of *L. maackii* individuals within 2 m of the line. For manipulated plots, we calculated a *t*-test for the effect of honeysuckle removal on number of touches per 20 m compared to intact plots. Regression and *t*-tests were done using R (R development core team 2010). Data are presented as means and standard errors.

Monthly proportion of track plates per plot with mammal paw prints (arc-sin square root transformed) was used as our response variable. Analyses were performed separately for mice, raccoons, opossums and squirrels. Although we found prints from canids, skunks, deer and chipmunks on our plates, we did not perform any analysis for these species due to their low incidence (less than 2% of all tracks recorded). *Peromyscus* spp. analyses consisted of mixed model using SAS Proc Glimmix (SAS Institute Inc. 2004) using a Gaussian distribution. We ran two analyses; one that used time as a factor and another one that used weather covariates (cloud cover, fraction of the moon illuminated and temperature). This approach was chosen because models did not converge when both time and covariates were incorporated in the same model due to insufficient degree of freedoms. For the first analysis we treated time, cover and fruit as fixed effects. Our model considered all three-way interactions between time (monthly sampling) and experimental manipulations (cover and fruit). We treated plot as the subject, and months as a repeated-measures factor. Blocks were used as random effects. We called this model the “temporal model”. For the second analysis, we maintained the same error structure determined by the random effects on the temporal model (repeated measures) but instead of modeling time itself we used weather covariates that are associated with this temporal variation (temperature, fraction of moon illuminated and cloud cover). We called this the “weather-model”. Analyses started with a full model including all 5-way interactions between the two treatments (fruit and cover) and 3 covariates. Model simplification was done by removing non-significant interactions unless they were marginally significant, i.e., $P < 0.15$ (Littell et al. 2006). After model simplification we used least squares means estimated for specific values of cloud cover to compare the effects of treatments at different levels of the covariates (0.25, 0.5 and 0.75 percentile).

In both models, temporal and weather, we used Kenward-Rogers method to generate the appropriate denominator degrees of freedom due to the repeated measures nature of the data (Littell et. al. 2006). Residual covariance model structure was chosen based on AICc and the treatment structure of the data following Littell et al. (2006) recommendations. Compound symmetry (CS) and autoregressive (AR[1]) yielded the lowest AICc for the temporal model and weather model, respectively.

Due to the low density of raccoons, opossum and squirrels our datasets did not meet analyses assumptions (zero inflated data), so we tallied their activity over the course of all 14 months and used mean proportion of tracks per plot as a response variable. We used Proc Mixed procedure (SAS Institute Inc. 2004) with treatments as fixed effects and block as a random factor to verify the role of food and cover in the overall foraging activity of each of these mammals.

RESULTS

Vegetation Sampling

Vegetation density was four times higher ($t = 3.27$, $P < 0.01$) on plots with honeysuckle cover present (22.7 ± 11.3 touches per 20 m) relative to plots from which honeysuckle had been removed (5.0 ± 6.9 touches). The number of honeysuckle individuals explained approximately 49% of the variation in vegetation density ($R^2 = 0.487$, $P < 0.001$; Fig. 1), supporting our hypothesis that honeysuckle increases the vegetation density of the understory.

Overall mammal activity

Our 14 censuses summed to 2688 track plate nights. Overall 48.8% (1310 plates) of these plates had signs of vertebrate activity. Mouse paw prints were found on 56.8% of the plates showing any activity, followed by raccoons, squirrels and opossums with 15.4%, 14.6% and 6.9%, respectively. Birds represented only 4.7% of the prints found. Approximately 15% of prints could not be identified because the organisms scratched and smudged the graphite suspension.

Mouse activity: temporal model

Mouse activity significantly increased throughout the duration of the study ($F_{13,26} = 5.35$, $P = 0.0001$, Fig. 2). In the beginning of the experiment mouse prints were found on approximately 20% of the track plates, increasing to approximately 35% in the summer of 2008, and going back to about 25% in the fall until a peak of 60% in December 2008 (Fig. 2). Mouse activity was always lower on plots that had honeysuckle removed, with the exception of September 2009 when mouse activity rose to more than 40% instead of the usual percentage in the mid-teens (Fig. 2). Our temporal model analysis showed that honeysuckle cover (foliage and branches) positively affected mouse foraging activity ($F_{1,6} = 15.63$, $P = 0.007$). On average 33% (± 4%) of plates on plots with honeysuckle present had mouse paw prints as opposed to 22% (± 3%) on plots that had honeysuckle removed. Although in some months of the fruiting season (September through December), there were peaks of activity in plots with fruits (Fig. 2), the effect of fruit was not statistically significant ($F_{1,6} = 0.37$, $P = 0.564$). Interactions between fruit, cover and time were not significant (Table 1a).

Mouse activity: weather model

After the removal of non-significant interactions, our final weather model was reduced to the singular effects of treatments (honeysuckle cover and fruit), covariates (temperature, cloud cover and fraction of the moon illuminated), and two two-way interactions of honeysuckle cover × cloud cover and honeysuckle cover × temperature (Table 1b). Our weather model analyses also show a significant effect of vegetation on mouse foraging activity ($F_{1,88.51} = 11.51$, $P < 0.001$). There was no effect of fruit, cloud cover, fraction of the moon illuminated or temperature (Table 1b), but there was a significant interaction of honeysuckle cover and cloud cover ($F_{1,118.8} = 8.67$, $P = 0.004$). Least square means estimates of mouse foraging activity holding constant the cloud cover covariate at 0.1, 0.26 and 0.58 (1^{st}, 2^{nd} and 3^{rd} quartile, respectively) show that differences on mouse activity between honeysuckle cover present and honeysuckle removal plots were only significant when cloud cover was low (cloud cover = 0.1, t = 4.88, D.F = 67.06, $P < 0.0001$, cloud cover = 0.26, t = 4.51, D.F. = 31.09, $P < 0.0001$), but as cloud cover intensified honeysuckle cover did not affect mouse behavior (cloud cover = 0.58, t = 0.35, D.F. = 74.28, $P = 0.724$; Fig. 3).

Raccoon, Squirrel and Opossum

Honeysuckle cover had a significant positive effect on the activity of raccoons ($F_{1,6} = 17.6$, $P = 0.006$, Fig. 4) and a marginally significant effect on opossums ($F_{1,6} =$

4.46, $P = 0.079$, Fig. 4). Mean proportion of plates in control plots with raccoon and opossum paw prints was greater (60% and 100%, respectively) than the mean for honeysuckle removal plots. Honeysuckle cover removal did not affect squirrels ($F_{1,6} = 0.40$, $P = 0.552$, Fig. 4). Fruits did not affect opossum ($F_{1,6} = 1.61$, $P = 0.252$) or squirrel ($F_{1,6} = 0.09$, $P = 0.773$, Fig. 4), but had a significant negative effect on raccoon foraging activity ($F_{1,6} = 17.60$, $P = 0.006$, Fig. 4). We did not find a significant interaction between honeysuckle cover and fruits for raccoons ($F_{1,6} = 0.03$, $P = 0.863$), opossum ($F_{1,6} = 2.63$, $P = 0.156$), or squirrels ($F_{1,6} = 0.65$, $P = 0.450$).

DISCUSSION

Traditionally, studies of the impacts of invasive plants in terrestrial ecosystems have focused on native plant species. The results of our experiment illustrate several points regarding the direct effects on native animals: invasive plants cause changes in the activity density of native consumers (Figs. 2 and 4); the primary mechanism of this effect is via the provision of a refuge, not a food source; and the impact of the refuge on activity is mitigated by abiotic conditions. As we discuss below, these findings have important implications for understanding biological invasions, for predicting the response of native consumers to invasive plants, and for interpreting field studies of plant-consumer interactions (Allan et al. 2010).

Vegetation density of after honeysuckle removal was similar to density levels found in areas not infested with *L. maackii* (Allan et al. 2010). Our honeysuckle cover removal treatment indicated that the dense vegetation density, as a result of the invasion by *L. maackii* (Fig. 1), is linked with a reduction in the foraging activity of three taxa of nocturnal mammals (mice, opossum and raccoons, Figs. 2 and 4). This result agrees with other studies that have shown reduced foraging by mammals in areas with denser vegetation (Kotler et al. 1991; Korpimäki et al. 1996; Anderson et al. 2003, 2006).

Although other studies have shown that *L. maackii* plants alters foraging activities of mice (Meiners 2007; Edalgo et al. 2009; Mattos and Orrock 2010), ours is the first to demonstrate that the dense vegetation density of this invasive is the mechanism responsible for modifying mammal behavior. This higher consumer foraging activity in areas infested with the invasive honeysuckle may lead to changes in consumer pressure, which could be critical for both plant and animal species (Orrock et al. 2010ab). One explanation for higher mouse foraging activity underneath invaded areas is that the invasive plant species increases vegetation complexity, which is then perceived as a refuge from predation (Orrock et al. 2004; Edalgo et al. 2009; Mattos and Orrock 2010). Dense horizontal vegetation reduces the chance that avian (e.g., hawks and owls) and terrestrial predators (e.g foxes and genets) will spot a mouse foraging on the ground (Lima and Dill 1990; Kotler et al. 1991; Korpimäki et al. 1996). Increased prey availability associated with invaded habitats may also be important. *Lonicera maackii* is preferred over native plants by understory nesting birds (Schmidt and Whelan 1999; Rodewald et al. 2010), increasing the availability of both eggs and nestlings, which are common prey for mice (Bradley and Marzluff 2003). These two mechanisms, shelter and indirect food sources, are not mutually exclusive: both may have contributed to higher mouse foraging activity seen on honeysuckle infested plots.

The results of our weather model are in agreement with other studies that have shown that the mouse activity may be conditioned by weather factors (Orrock and

Danielson 2004; Mattos and Orrock 2010). Mice appear take into consideration their conspicuousness to predators while foraging, since they are less likely to use areas without honeysuckle cover when cloud cover is low and visibility to avian predators is high. The interaction of cloud cover and vegetation structure corroborates our shelter hypothesis, and reveals flexibility in their behavior. This result also underscores the importance of long term studies that might reveal interactions that otherwise would be undiscovered.

In addition, we found that mouse foraging activity increased during the timespan of the study (Fig. 2). Possibly mice may have had an initial aversion to plot areas due to the human (experimental) disturbance, followed by a slow acclimation to the altered habitat. Alternatively, a severe freeze in early April 2007 had critical effects on many communities across the Midwest of the U.S. (Gu et al. 2008). The freeze could have reduced the abundance of mammals and the study period just happened to record the data when the population was recovering and overall proportion of paw prints was increasing.

The copious amount of fruit produced by honeysuckle and the evidence that mice actually consume and cache these fruits led us to believe that fruits would at least influence mouse activity if not also their abundance. Despite our expectations, we found no evidence of that *L. maackii* fruits influenced mice. However, we cannot entirely rule out this hypothesis. In the first calendar year of the study (2007), early warm temperatures in the spring sped up plant activity and were followed by a killing frost (Gu et al. 2008) that destroyed a majority of the flower buds and flowers on *L. maackii.* As a result, fruit production was severely reduced in the fall of 2007. It is important to point out that in 2006, a crew of 2-10 persons removed honeysuckle berries for approximately

8 hours daily from September to late December, while in 2007 we had only 6 field trips with the same number of people to remove all the berries. Thus the frost of 2007 may have been responsible for the lack of a fruit effect on mammal behavior in that year. One must also consider the scale of the experiment and the possibility of a spillover effect. Our plots were 900 m^2, which might be too small to affect food availability for mammals, especially considering that mice can be quite mobile. Our trapping data showed that individuals moved between plots in 24 occasions. Thus, honeysuckle fruits might not constitute an important source of food at such scale, especially considering that the surrounding vegetation matrix is full of honeysuckle shrubs and mammals could forage in these other areas and return to the experimental plots. In addition, birds are known to disperse *L. maackii* seeds and generate an extensive seed shadow (Bartuszevige and Gorchov 2006). Our treatment might not have been effective in controlling food availability for mice, as birds also feed on *L. maackii* seeds.

The positive effect of honeysuckle cover on mesopredators (Fig. 4) can be critical for native species. As the invasive modifies the behavior of mesopredators it has the potential to disrupt an entire ecosystem, as this guild can regulate both the diversity and abundance of plant and animal communities (Prugh et al. 2009). Higher activity of mesopredators could be related to shelter provided by the invasive plant and increased food sources in the form of nestlings. For instance, some birds suffer higher nestling predation rates in honeysuckle infested areas (Schmidt and Whelan 1999; Rodewald et al. 2010); facilitation for predators has been often suggested as the mechanism behind increased nestling predation. Studies have shown that these two factors (shelter and cover) affect mesopredator behavior (Bowman and Harris 1980; Chamberlain 2003;

Beasley et al. 2007). However, there are few predators of raccoon and opossum in the study area (HPD, personal observation). Hence, the higher abundance of birds (Schmidt and Whelan 1999) and mice (HPD, in preparation) in honeysuckle infested areas could help explain the higher foraging activity of mesopredators, as they are known to prey upon these organisms (Azevedo et al. 2006; Staller et al. 2005). The effect of fruits on raccoon activity is counter-intuitive. Areas with fruits removed had greater raccoon activity than areas with fruit present (Fig. 4). One would expect omnivores like raccoons to respond positively to fruit production, however, they were consistently more abundant in fruit removal areas in 11 out of 14 surveys. There is no evidence that raccoons consume *L. maackii* fruits, but perhaps fruits have a positive indirect effect on other raccoons' resources (i.e. birds). Resource abundance has been show to lead to more sparse distribution of raccoons (Gehrt and Fritzell 1998). Squirrels, on the other hand, did not respond to honeysuckle cover or fruits (Fig. 4), perhaps because they are mostly arboreal.

This study demonstrates an effect of an invasive plant species on the foraging behavior of native mammals. We would predict similar effects for any plant species that influences cover, including herbaceous plant species. However, densely branching shrubs and small trees should affect the greatest number of mammal species because the affected refuge habitat would be relevant for a greater range of body sizes. We were not able to demonstrate an effect of added resources in our system but we do not reject this hypothesis based on the decrease in fruit production in 2007. Experimental demonstration of such an effect will need to take into account the size of the added resource, foraging range of the target animal species, and pre-dispersal distance of fruits and seeds. The next step in our system is to uncover the indirect effects that changes in foraging behavior have on native plant species.

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Table 1. Temporal model (a) and weather model (b) for mouse foraging activity in experimental plots that had honeysuckle (HS) cover and fruits manipulated to two levels each (present or removed). Fixed effects were honeysuckle cover, honeysuckle fruit, and time for temporal model. The weather model used cloud cover, fraction of the moon illuminated and temperature as covariates (just significant interactions are shown, $P < 0.15$). Error structure for both models was a randomized block, with experimental plot identity as repeated-measures. See text for details

Effect	**DF**	**F**	**P**
A. Temporal Model			
HS Cover	1,6	15.63	0.007
HS Fruit	1,6	0.37	0.564
HS Cover X HS fruit	1,6	0.13	0.735
Time	13,26	5.35	0.0001
Time X HS cover	13,78	0.98	0.477
Time X HS fruit	13,78	0.35	0.98
HS Cover X HS fruit X time	13,78	0.79	0.66
B) Weather Model			
HS Cover	1,88.51	11.51	0.001
HS Fruit	1,27.72	0.40	0.531
Cloud	1,34.93	0.24	0.631
Moon	1,35.82	0.84	0.367
Temp	1,39.57	2.12	0.153
Moon X HS cover	1,120.1	1.72	0.192
Cloud X HS cover	1,118.8	8.67	0.004

Figure legends

Fig. 1 Regression between number of honeysuckle plants and vegetation density showing a positive relationship between these two variables (N=18 plots)

Fig. 2 Effects of honeysuckle (HS) vegetation cover and fruit production on mouse foraging behavior over time (see Table 1 and text for details). Means ± one standard error are shown (data points were slightly jittered along the x-axis to prevent overlapping of error bars)

Fig. 3 Effects of cloud cover and honeysuckle (HS) cover on mouse foraging behavior. Results are based on our weather model that used mixed model analysis of covariance (see Table 1 and text for details). Means ± one standard error are shown (data points were slightly jittered along the x-axis to prevent overlapping of error bars)

Fig. 4 Box plot showing the effects of honeysuckle (HS) vegetation cover and fruit production on raccoon, squirrel and opossum foraging behavior over time. Results are based on mixed model analysis of variance (see text for details). Circles represent the average of paw prints in each plot

Figure 1

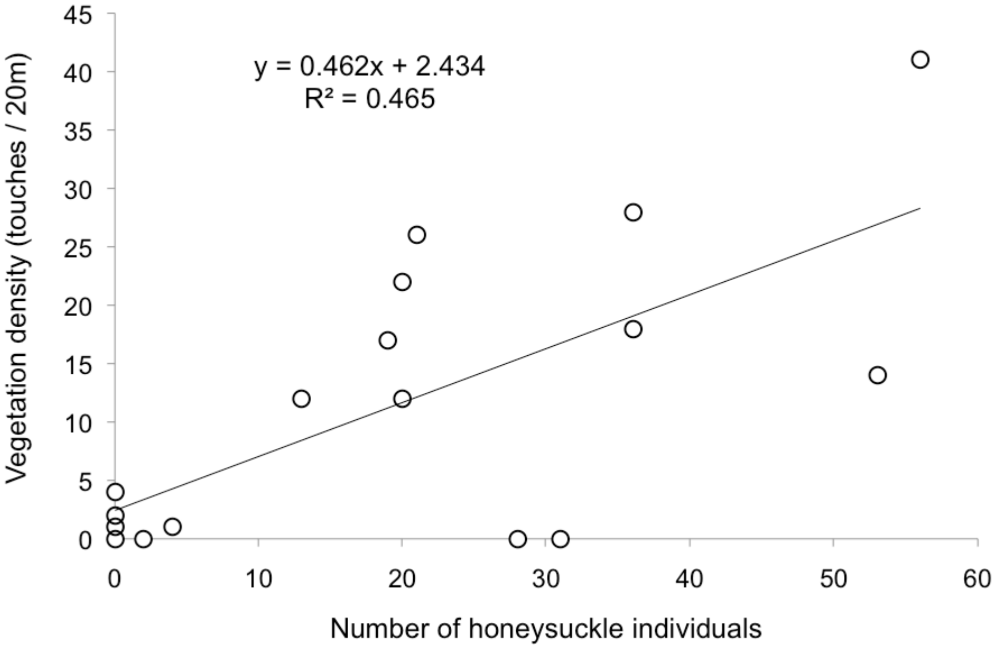

y = 0.462x + 2.434
R² = 0.465
Vegetation density (touches / 20m)
45
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
Number of honeysuckle individuals

Figure 2

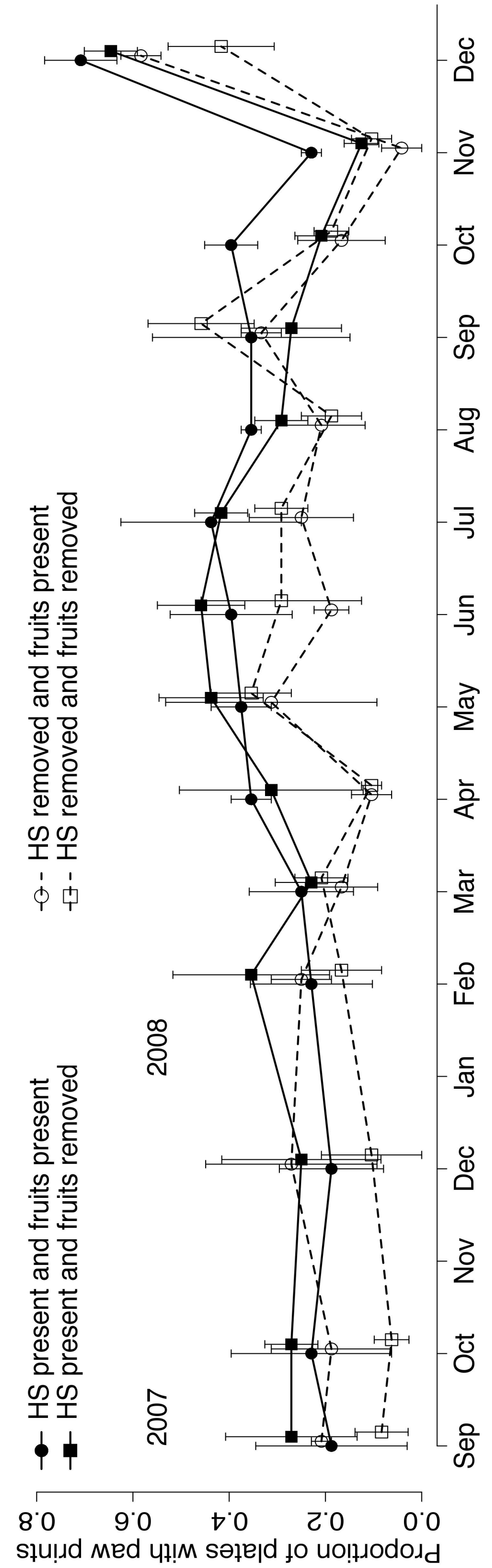

HS present and fruits present
HS present and fruits removed
HS removed and fruits present
HS removed and fruits removed
2007
2008
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Proportion of plates with paw prints
0.0
0.2
0.4
0.6
0.8

Figure 3

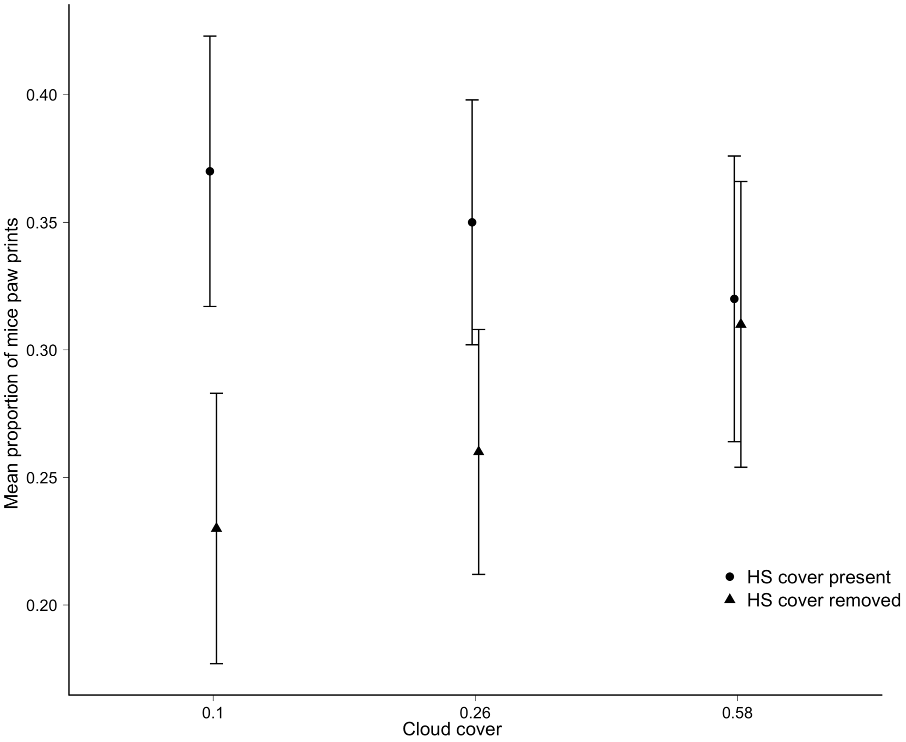
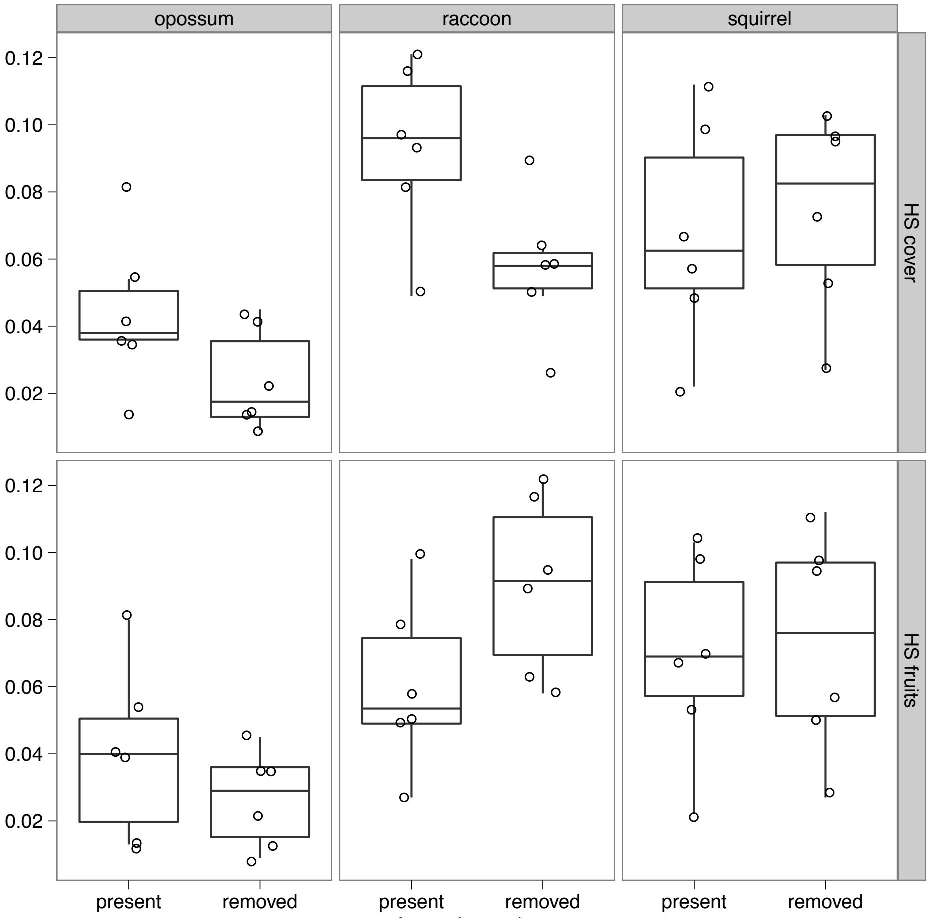

0.40
0.35
0.30
0.25
0.20
Mean proportion of mice paw prints
0.1
0.26
0.58
Cloud cover
● HS cover present
▲ HS cover removed

Figure 4


opossum
raccoon
squirrel
HS cover
HS fruits
0.12
0.10
0.08
0.06
0.04
0.02
present
removed

Chapter 2

An invasive plant species affects rodent abundance by providing food and cover

ABSTRACT

Research on invasive plants is largely focused on the direct effects of exotics on native plant communities via direct competition and as a result there is a scarcity of studies on the impacts of invasive plants on mammals. It has been hypothesized that invasive plants add structural complexity to the native vegetation cover and may also change food availability through fruit production. These two factors, food and vegetation cover, are known to interfere with mice population dynamics but to date no studies have addressed their relative impact on mice abundance. We performed a factorial experimental manipulation of cover and fruit provided by the invasive shrub Amur honeysuckle (*Lonicera maackii*) to evaluate whether this plant alters the abundance of the white-footed mouse (*Peromyscus leucopus*). Using a mark release recapture protocol to quantify mice abundance we found that both removal of honeysuckle cover and fruits had a detrimental effect on mice. Moreover, we also found that honeysuckle fruits had a marginally significant detrimental effect on the proportion of sexually active mice. We argue that honeysuckle's dense vegetation cover provide shelter for mice against predators. Additionally, our results indicate that honeysuckle fruits may constitute an abundant food source that may sustain larger populations of mice but its low nutritional value may be reproductively detrimental for individuals.

INTRODUCTION

To date most research on invasive plants has focused largely on the direct effects of exotic plants on native plant species (White et al. 2006). Numerous studies demonstrate that invasives can at times alter vegetation structure greatly (Pritekel et al. 2006; Gerber et al. 2008; Flory and Clay 2009; Johnson et al. 2009), with a predicted consequence the altering of ecosystem function (D'Antonio & Vitousek 1992,Wilcove et al. 1998, Theoharides and Dukes 2007). Plants, of course, provide the resources and habitat structure upon which all non-plant life depends. Given the ubiquity of invasive plants and their capacity to modify the vegetation which they invade, it is surprising that few studies have addressed the impact of invasive plants on the composition and abundance of consumer guilds. Furthermore, feedback loops may occur because both mammals and insects, acting as herbivores, have the potential to modify community structure themselves (Brown and Heske 1990, Lambrinos 2000, Horncastle et al. 2004, 2005, Murray et al. 2007, Marquis 2010).

The abundance of individual mammal species and overall community composition are often related to local vegetation characteristics, i.e., habitat structure. Canopy height, relative humidity, litter depth, foliage height, and plant diversity can affect rodent abundance and community composition (M'Closkey 1975, Drickamer 1990, Schmid-Holmes and Drickamer 2001). Specifically, woody biomass can be positively related to rodent abundance (Kaufman et al. 2000, Sietman et al. 1994, Swihart and Slade 1990), while the vertical complexity of woody vegetation can predict rodent abundance (Schmid-Holmes and Drimer 2001, Anderson et al. 2003, Anderson and Meikle 2006). One proximal cause for these relationships is that dense vegetation provides nesting habitat and cover from predators. By providing cover, predation risk is perceived to be lowered, resulting in increased foraging activity, higher survivorship and greater relative abundance (M'Closkey 1975, Manson and Stiles 1998, Anderson and Meikle 2006, Dutra et al. 2011).

The effects of plants on small mammals are not restricted to effects on habitat structure, but also to the resources provided. Parmenter and MacMahon (1983) hypothesized that the impact of understory structural complexity on small mammal populations is due to both food availability and vegetation cover. Complex understory vegetation is likely to provide more food in the form of edible foliage, fruit, and seeds or by hosting larger number of prey (i.e., arthropods and juvenile birds: Parmenter and MacMahon 1983, Schmidt and Whelan 1999, Anderson and Meikle 2006). The fruit and seeds of invasive plant species have been mentioned as important food sources for many native consumers (Williams et al. 1992, Ingold and Craycraft 1983, Richardson et al. 2000, Bartuszevige et al. 2006, Gosper et. al. 2005, 2006); providing fruits of native species has a positive effect on rodent abundance (Doonan and Slade 1995; Nupp and Swihart 1998, Jones et al. 1998), sexual maturation (Duquette and Millar 1995), reproductive output (Galindo-Leal and Krebs 1998), and body mass (Cittadino et al. 1994, Banks and Dickman 2000, Yunger 2002).

A clear experimental demonstration of the mechanism by which invasive plants may affect rodent abundance, however, is lacking. Both mice activity (Mattos and Orrock, 2010, Orrock and Witter 2010, Dutra et al. 2011) and abundance (Christopherson and Morrison 2004) are influenced by plant invasion. No studies have tested the relative contribution of cover versus food provided by an invasive plant species on mice abundance. In this study, we examined whether vegetation cover and fruits provided by the invasive woody shrub Amur honeysuckle (*Lonicera maackii* (Rupr.) Maxim.) modify the population dynamics of the white-footed mouse (*Peromyscus leucopus* Raf.).

Lonicera maackii was chosen for this experimental manipulation because of its dense branch architecture (Luken et al. 1997), shown to serve as a refuge for mammals (Mattos and Orrock 2010, Dutra et al. 2011), and its massive fruit production of up to 400 million berries per ha (Ingold and Craycraft 1983). *Peromyscus leucopus* feeds on *L. maackii* fruits and seeds (Williams et al. 1992, HPD personal observation). We examined the effects of *L. maackii* on *P. leucopus* abundance using an experimental approach that allowed us to disentangle the effects of the vegetation cover from fruit production to provide a better understanding of the mechanisms by which invasive plants affect rodent populations. We also evaluated if these two factors modified the proportion of sexually active individuals and pregnant females on the population, and their effects on mice body mass and body mass gain between recaptures.

METHODS

Study plant

Lonicera maackii is a highly successful and aggressive invasive plant in forests in much of the eastern United States, known for reducing diversity and recruitment of native plants (Luken and Goessling 1995, Luken and Thieret 1996, Luken et al. 1997, Hutchinson and Vankat 1997, 1999, Gould and Gorchov 2000, Gorchov and Trisel 2003). Schmidt and Whelan (1999) showed that the plant acts as an ecological trap for birds by providing nesting sites for birds that are easily accessed by predators (i.e. mice and raccoons). The plant has a copious fruit production with more than 20 kg of fruits in a 25 m^2 area (HPD, unpublished results) that are avidly consumed by birds (Bartuszevige et al. 2006, Gleditsch and Carlo 2011). Approximately 62% of these fruits fall to the ground (Bartuszevige et al. 2006) and become available for rodent consumption (Williams et al.

1992) especially underneath highly invaded areas (Meiners 2007) where seed caches are relatively common (HPD, personal observation). Recent studies also show that *L. maackii*'s vegetation cover is denser than native vegetation (Allan et al. 2010, Dutra et al. 2011). This dense cover is hypothesized to be a visual barrier that shelters small mammals from predators (Mattos and Orrock 2010, Dutra et al. 2011).

Study area

This study was conducted at Busch Wildlife Conservation Area (38.70° N, 90.71° W), a 6987 ha park in Saint Charles County, Missouri. Approximately 1215 ha of the park is oak/hickory forest with an understory dominated by *L. maackii* and *Rosa multiflora* (multiflora rose). Other common woody, understory plants include *Lindera benzoin* (spice bush), *Rhus aromatica* (aromatic sumac), *Cornus* spp. (dogwood) and *Symphoricarpus orbiculatus* (coral berry).

Experimental design

Three experimental blocks were established in the fall of 2006. Each block consisted of four treatment plots (30 × 30 m each). Plots were 50 m apart and blocks were at least 3 km from each other. Plots within each block were randomly assigned to one of four treatments: 1) fruits and honeysuckle cover present, 2) fruits present and honeysuckle cover removed, 3) fruit removed and honeysuckle cover present, 4) both fruits and honeysuckle cover removed. Honeysuckle individuals were physically removed from treatment plots 2 and 4 by cutting the stem at the base in the fall of 2006. Pruning of re-sprouts continued from late fall 2006 until the end of the study. During the fall months of 2006, 2007 and 2008, we removed all fruits by hand from treatment plots 3 and 4. In 2006, fruits on treatment plots 2 were removed from the plant and left on the ground prior to plant removal. In 2007 and 2008, fruits removed from plots of treatment 3 were added to plots of treatment 2.

A rodent trapping grid of 20 × 20 m was positioned in the center of the each treatment plot previously established to reduce edge effects. Each trapping grid consisted of 16 traps (H. B. Sherman Traps, Inc., Tallahassee, Florida - 7.5 × 9 × 23 cm) on a 4 × 4 array with approximately 6 m spacing between traps. Traps were baited with a handful of rolled oats. Cotton balls were used as bedding to reduce stress and provide thermal insulation during the fall and spring. Monthly trapping sessions took place from June 2007 to July 2009, except for winter months (November through March). Preliminary trapping sessions were highly disturbed (more than 60% of the traps) by raccoons and opossums. To prevent this kind of disruption, traps were positions inside a polyvinyl chloride squared tube (8.5 × 10 × 48 cm Lifetime Vinyl Fencing, San Jose California) with a cap on one of its ends. These tubes were staked to the ground with wood stakes and fastened with a piece of galvanized wire. Traps were left open inside vinyl tubes for four consecutive nights. The first day traps were pre-baited and left open but not activated to increase trapping efficiency (Chitty and Kempson 1949). Subsequently, rodents were captured, marked, and released for three consecutive nights. Captured individuals were marked with a uniquely numbered ear tag (National Band and Tag Company, Newport, Kentucky), and data taken on the date of capture, plot, grid location, body mass, sex, sexual activity (males: testes abdominal or descended; females: perforate or imperforate), and reproductive condition (females only: pregnant, dilated pubic symphysis, lactating nipples). Females were identified as reproductively active using any one of the three previous criteria (following Yunger 2002). All field procedures followed established guidelines (Animal Care and Use Committee 1998).

Data Analysis

To examine the effect of fruits and vegetation cover and time on mice demographics we used mixed-model analysis of variance (Littell et al. 2006) model using SAS Proc Glimmix (SAS Institute Inc. 2004) using a Gaussian distribution. We treated time, cover and fruit as fixed effects. Our model considered all three-way interactions between time and experimental manipulations (cover and fruit). We treated plot as the subject in a repeated-measures design and blocks were used as random effects. Capture data were pooled across the sampling session. To verify the effects of treatments on mice abundance we used the total number of unique individuals captured at a location during a trapping session as a response variable (Mt+1, the number of uniquely marked individuals (*sensu* Slade and Blair 2000). We used the estimator Mt+1 because it performs as well or better than closed-population estimation techniques (e.g., the Lincoln–Petersen estimator) when sample sizes are low or animals are not captured at all locations (Slade and Blair 2000). Following Anderson and Meikle (2006) we opted to not use typical abundance estimators from mark-recapture data for population size or survivorship such as Jolly-Seber or Lincoln-Petersen because our data violated many assumptions of most density estimators (Otis et al. 1978, White and Burnham 1999, Slade and Blair 2000). For instance, we had low recapture rates of just 39% of individuals. Also, we do not have standardized time intervals between trapping sessions (1 to 5 months) evidenced by the fact most recapture events are individuals that were recaptured just once. Moreover, we do not have a closed population since there was a significant increase in the population over time.

We also verified whether treatments affected mice sexual activity by using the proportion of individuals with descended testes or perforated vaginas as a response variable. Because our data had too many samples in which all individuals were sexually active, and were not normally distributed or homoscedastic, we arc-sine transformed the data and used Bartlett's correction for small samples size (Bartlett 1947). We also verified if the experimental treatments affected mice body mass. The response variable for this analysis was the average body mass of uniquely captured individuals per plot per month. We also assessed the effects of our experimental manipulation on body mass change between recaptures to see if there was a tendency for weight change for individuals that remained in the population. For this analysis we only used individuals that were recaptured at least once. We quantified body mass change as the difference in body mass between two consecutive recapture events for the entire study period. Positive values indicate that individuals gained weight between recaptures and negative values indicate weight loss. Because of low sample size we used the average body mass change between consecutive recaptures for each plot across all months as our response variable, thus removing the temporal component (time effect) for this analysis**.** This body mass change analysis is necessary because the average body mass of the population may remain constant because of recruitment of new individuals via migration but individuals that remain in the population could be going through changes in mass.

Residual covariance model structures for all analysis were chosen based on AICc and the treatment structure of the data following Littell et al. (2006) recommendations. Compound symmetry (CS) yielded the lowest AICc for all analyses. All data are presented as mean ± one standard error.

RESULTS

Our 15 trapping sessions totaled 8640 trap nights. We captured 578 *Peromyscus leucopus* individuals during our study and just 12 short-tailed shrews (*Blarina* *brevicauda*) and 2 chipmunks (*Tamias striatus*). Only 3.8% of *P. leucopus* moved between plots. Mice abundance significantly increased throughout the duration of the study ($F_{14,28}$ = 7.99, $p < 0.001$, Fig. 1, Table 1), from 3.8 ± 0.5 mice per plot in June 2007 to as many as 14.3 ± 1.1 in May 2009. Abundance usually peaked during summer (Fig. 1). Both removal of honeysuckle vegetation cover ($F_{1,6}$ =20.34, p = 0.004) and fruits ($F_{1,6}$ =6.65, p = 0.041) had a negative effect on mice abundance. A significant interaction between these two factors was also observed ($F_{1,6}$ = 7.70, p = 0.032, Fig. 2). Mice abundance was on average lower on plots that had honeysuckle vegetation removed (Fig. 1, see separation of dotted versus solid lines). Mice were more abundant in fruit removal plots early in the experiment but were consistently more abundant in fruit intact plots after July 2008 (Fig. 1, filled dots versus closed dots), indicating the positive effect of this food source on mice. We did not observe a significant interaction between time and the fruit treatment ($F_{14,84}$ = 0.95, p = 0.512), indicating that the effects of fruit removal were consistent throughout the study.

Mice sexual activity was not affected by our treatments (Table 1) with the exception of a marginally significant effect of fruits ($F_{1,6.28}$ = 4.45, p = 0.077). In fact the proportion of mice that were sexually active was slightly lower on plots with fruits present than in plots with fruits removed (Fig. 3). Sexually active mice corresponded to 85 ± 2% of the population on fruit removal plots while plots in which fruits were not manipulated had an average of 79 ± 3%. We also observed a significant effect of time on the proportion of sexually active mice ($F_{14, 26}$ = 5.37, p = 0.0001). Peaks on the proportion sexually active mice were observed in late summer (August and September 2008 and 2009) when 100% of mice had either descending testes or perforated vaginas (Fig 3). Neither honeysuckle cover nor fruits influenced the reproductive state of mice ($F_{1,6}$ = 0.12,

$p = 0.736$, $F_{1,6} = 0.52$, $p = 0.499$, Table 1). The mean percentage of pregnant females for the entire population was 64 ± 2%.

Mice body mass was on average 23.66 ± 0.28 g and although this average fluctuated with time ($F_{14,19.5} = 10.04$, $p < 0.001$) neither of our experimental manipulations, fruit or vegetation cover removal, had a significant influence on mice body mass ($F_{1,6.08} = 0.12$, $p = 0.741$, $F_{1,6.11} = 2.25$, $p = 0.183$, respectively, table 1). We also observed an average positive body mass gain between consecutive recaptures of 1.56 ± 0.16 g but again removal of neither honeysuckle vegetation cover ($F_{1,6} = 0.78$, $p = 0.410$) nor fruit ($F_{1,6} = 1.10$, $p = 0.335$) influenced body mass gain.

DISCUSSION

This study provides experimental evidence that both dense vegetation and food resources offered by the invasive plant, as well as an interaction of these two factors, determine the abundance of *Peromyscus leucopus*. This is the first experimental demonstration of the mechanisms by which invasive plants may affect rodent population dynamics. Our results are in agreement with studies that show that mice abundance responds positively to increased vegetation density (Anderson et al. 2003, Anderson and Meikle 2006), and food supplementation (Jones et al. 1998, McShea 2000, Schnurr et al. 2002, Yunger 2002). Although abundance was affected, there were no treatment effects on body mass or sexual activity.

During the fruiting season, caches of seeds and fruits were common in fruit present plots. Intact seeds and mice droppings were commonly associated with these caches indicating that mice were this fruits. Many seeds were intact in these caches indicating that the mice were acting as seed dispersers. Seed predation by rodents may have a positive effect on the spread of exotics (Ostfeld 1997) that initially may nurture larger rodent populations.

In the long run, invasive plant species often reduce plant diversity (Collier 2002, Gorchov and Trisel 2003, Miller and Gorchov 2004) and can eventually decrease mammal diversity (Horncastle et al. 2005). Perhaps, the advanced state of the invasion at our study site, evidenced by the high density of *L. maackii* individuals and reduced diversity of native seedlings explains the very low diversity at our study site, just two species of ground-dwelling rodents. Rodent abundance, on the other hand, was very high. Our peak abundance estimates (14.3 ± 1.1 per 0.09 plot or 158.89 mice per ha, see results) were high relative to other studies carried out in hardwood forest where abundance is approximately 40 mice per ha (Hansen and Batzli 1979, Anderson and Meikle 2006, McShea 2000). However, abundance estimates as high as ours (almost one mouse per trap) have been reported (Wilder and Meikle 2006).

We expected that presence of *L. maackii* fruits would have a positive effect on mice body mass, sexual activity, and pregnancy ratios. Instead we found a marginally significant detrimental effect of fruits on the proportion of sexually active mice. This result is counter-intuitive, as one would expect that the presence of additional food source (fruits) to accelerate sexual maturation, as seen in other studies (Dobson and Kjelgaard, 1985, Duquette and Millar 1995, Galindo-Leal and Krebs 1998). However, our study is different from other food supplementation studies in the sense that the supplemented fruits had a low nutritional value (sensu Stiles 1980). *Lonicera maackii* seeds have a very low fat content relativae to native species (Ingold and Craycraft 1983). Thus, even though our fruit supplementation sustained a larger mice population, the fact that the mice were slightly less sexually active indicates that honeysuckle fruit supplementation might not be beneficial for individual mice, perhaps because of a reduced nutritional value of the exotic. Alternatively, the detrimental influence of fruits on mouse reproductive status may come from the impact that the invasive fruits have on other food sources. For instance, Gerber et al. (2008) showed that insects, an important item on mice diet, had their abundances and diversity depleted due to the invasion by exotic knotweed (*Fallopia* spp.). Thus, it is possible that *L. maackii* fruits and its subsequent recruitment may have indirect effects detrimental for mice sexual activity that our experimental manipulations did not control. Future studies should examine whether reduced plant diversity due *L. maackii* invasion influences the insect community.

The response of white-footed mice to vegetation cover is well documented and several studies show small mammals respond positively to increased vertical complexity of the woody vegetation, and not simply to the presence of woody cover (M'Closkey and Lajoie 1975, Swihart and Slade 1990, Anderson et al. 2003, Anderson and Meikle 2006, Meiners 2007, Edalgo et al. 2009). Thus, it is not simply the presence or absence of woody vegetation that is important, but structure as well. In our study, the tree canopy was intact in all study plots, but removal of honeysuckle in the understory simplified the understory vegetation structure. Our hypothesis that the invasive shrub vegetation cover affects mice abundance is corroborated by an increasing number of studies that depicts rodents adjusting their foraging behavior based on these indirect cues of predation exposure (vegetation cover, cloud cover, moonlight illumination) and preferring sheltered microhabitats over exposed ones (Longland 1994, Mandelik et al. 2003, Orrock and Danielson 2004, Orrock et al. 2009, Mattos and Orrock 2010, Dutra et al. 2011). Areas with low structural complexity are less likely to be used by mice that perceive them as dangerous microhabitats because exposure to visual predators is relatively high (Orrock and Danielson 2004, Dutra et al. 2011). For instance, Kotler and collaborators (1991) showed that gerbils are more likely to be predated by owls in areas with low structural complexity. In contrast with this hypothesis that mice prefer areas with less exposure to predators, a simultaneous study done at the same location showed that mice predators (i.e. raccoon and opossum) are more active in areas with high structural complexity (Dutra et al. 2011). This suggests that mice abundance should have been low in areas with high vegetation complexity in which predators are more active. We conclude that the visual barrier created the structural complexity of the exotic *L. maackii* (Allan et al. 2010) modifies the relationship between prey and predator. The results of this study and Dutra et al. 2011 indicate that mice are more abundant and forage more often in areas infested with the invasive, that theoretically offer more shelter because the invasive forms a visual barrier but at the same time this same areas has a higher activity of predators. Perhaps, *L. maackii* infested areas in the long run act as an ecological trap for rodents. Initially these areas sustain larger populations of rodents because of the increased shelter, but over time also attract more predators. This hypothesis may also help to explain the low diversity of rodents in the study area.

Our data also show that mice abundance is determined by a significant interaction of vegetation cover and fruit. This interaction indicates that the response to our fruit manipulations is not the same for all levels of vegetation cover treatment. Fruit removal seems to reduce mice abundance when honeysuckle vegetative cover is present, but did not seem to make a difference when vegetation was removed (Fig. 2). It is been widely reported that mice foraging decisions can be the result of a tradeoff between food procurement and safety (Brown et al. 1992; Kotler et al. 1997; Mohr et al. 2003, Mattos and Orrock 2010). In our study system mice appear to be weighing these two factors simultaneously, but removal of vegetation cover seems to have a stronger detrimental effect on the mean number of mice per plot than fruits (Fig. 2). Possibly the weak effect of fruits in relation to safety (vegetation cover) also reflects the low quality of fruits. We hypothesize this relationship may change and that fruits might have a greater effect on abundance for other invasive plants that offer a more nutritious fruit.

More than merely disentangle the effects of an exotic's fruits and vegetation cover on mice abundance, our results show that an invasive plant has long term consequences for an important consumer, *P. leucopu*s. Because *P. leucopus* is the of the major seed consumer among ground-dwelling rodents in the eastern U.S. (Mason and Stiles 1998) and *Lonicera maackii* is becoming the dominant shrub in many urban forests (Hutchinson and Vankat 1997, 1999; Gould and Gorchov 2000; Gorchov and Trisel 2003), our results have critical implications for population dynamics of seed consumers and native seedling recruitment. Indeed, Meiners (2007) showed that honeysuckle removal in a small area (25 m^2) leads to reduced seed predation ratios suggesting that predators are mediating apparent competition between the invasive and natives species. Our results also indicate that *L. maackii* infested areas harbor larger mice populations, which may result in an over-consumption of seeds and seedlings in those habitats. Moreover, because *P. leucopus* is the natural host of many human diseases (Donahue et al. 1987, Morzunov 1998), *L. maackii* might also affect disease risk (Allan et al. 2010) by increasing host abundance. In summary, our results show that management strategies that reduce the infestation levels of the invasive have immediate and sustained effects on mice populations, suggesting that invasive removal may reduce the detrimental effects on seedling recruitment mediated by consumers. .

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Table 1. Effects of honeysuckle (HS) cover and fruit manipulations on mice demographics: a) population abundance; b) mice sexual activity; c) body mass; d) body mass change between recaptures, and f) proportion of pregnant female mice on the populations. Fixed effects were honeysuckle cover, fruit and time for temporal model. Error structure was a randomized block, with experimental plot identity as repeated-measures. See text for details

Effect	**DF**	**F**	**P**
A. Mice abundance			
HS Cover	1,6	20.34	0.0041
HS Fruit	1,6	6.65	0.0418
HS Cover X HS fruit	1,6	7.70	0.0322
Time	14,28	7.99	<0.0001
Time X HS cover	14,84	0.76	0.708
Time X HS fruit	14,84	0.95	0.513
HS Cover X HS fruit X time	14,84	1.17	0.315
B. Sexual activity			
HS Cover	1,6.39	0.04	0.8506
HS Fruit	1,6.28	4.45	0.077
HS Cover X HS fruit	1,6.56	2.96	0.132
Time	14,26	5.37	0.0001
Time X HS cover	1,81.6	0.94	0.5234
Time X HS fruit	14,80.1	0.72	0.7511
HS Cover X HS fruit X time	1,80.9	1.15	0.3333
C. Body mass			
HS Cover	1,6.11	2.25	0.183
HS Fruit	1,6.08	0.12	0.744
HS Cover X HS fruit	1,6.12	0.03	0.869
Time	14,19.5	10.04	<0.0001
Time X HS cover	14,70.2	0.50	0.923
Time X HS fruit	14,69.1	1.28	0.245
HS Cover X HS fruit X time	14,70.2	0.60	0.859
E. Body mass change			
HS Cover	1,6	0.78	0.410
HS Fruit	1,6	1.10	0.335
HS Cover X HS fruit	1,6	0.01	0.921
F. Proportion of pregnant females			
HS Cover	1,6	0.12	0.736
HS Fruit	1,6	0.52	0.499
HS Cover X HS fruit	1,6	0.18	0.686

Figure legends

Fig. 1 Effects of honeysuckle vegetation cover and fruit production in mouse abundance (calculated as Mt+1) over time. Mean ± one standard error is shown (data points were slightly jittered along the x-axis to prevent overlapping of error bars).

Fig. 2 Effects of honeysuckle vegetation cover and fruit production on average mouse abundance (calculated as Mt+1). Mean ± one standard error is shown.

Fig. 3 Effects of honeysuckle fruit production on the proportion of sexually active mice (individuals with descending testes or perforated vaginas. Mean ± one standard error is shown (data points were slightly jittered along the x-axis to prevent overlapping of error bars).

Figure 1

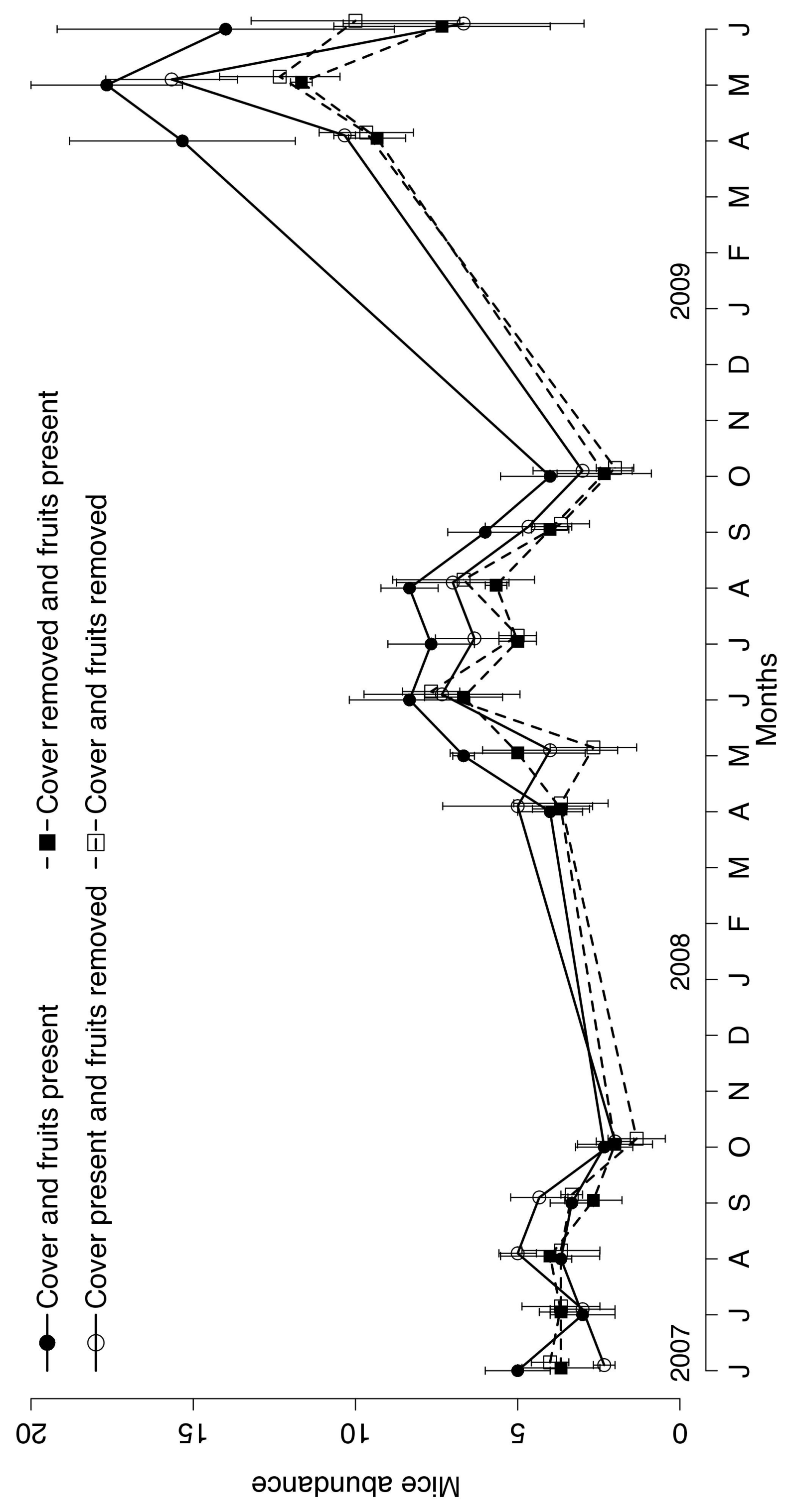
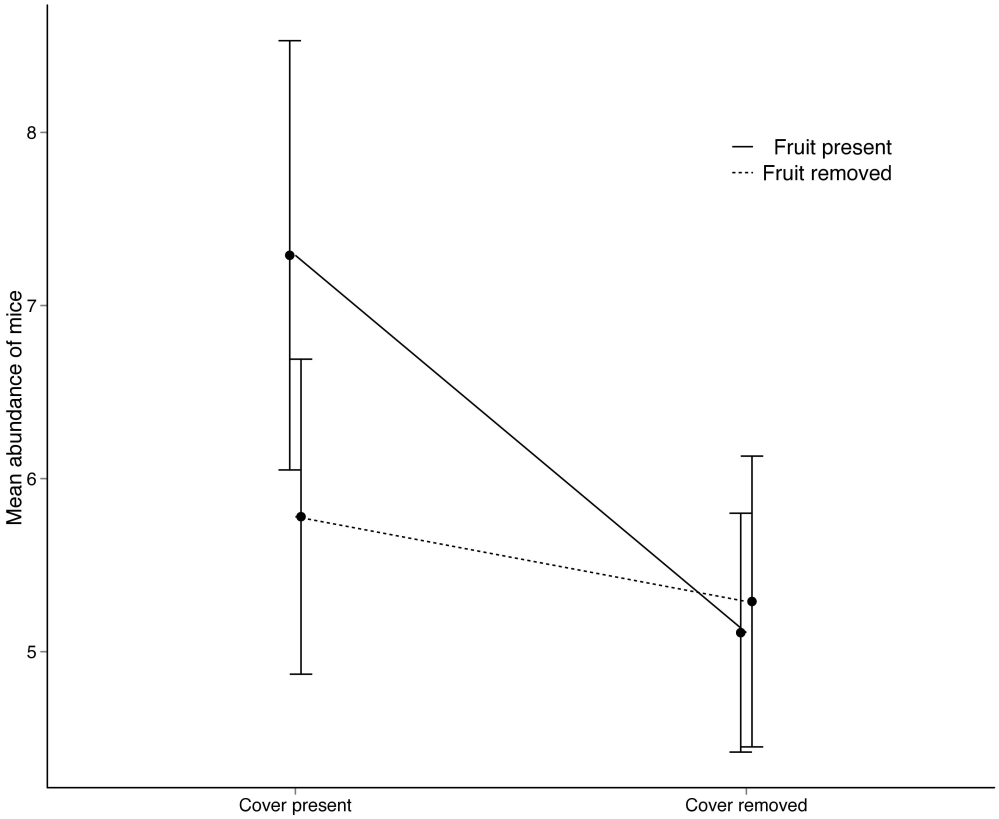
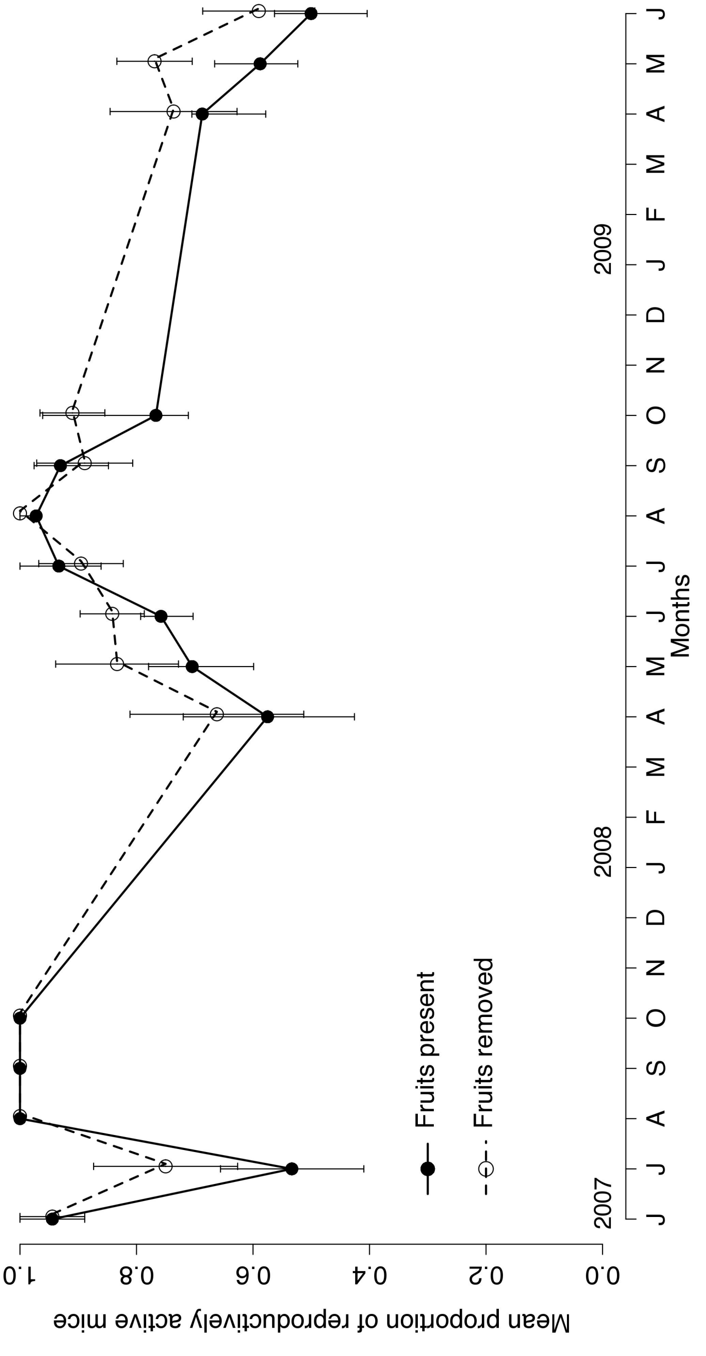

Cover and fruits present
Cover present and fruits removed
Cover removed and fruits present
Cover and fruits removed
Mice abundance
0
5
10
15
20
Months
J
J
A
S
O
N
D
J
F
M
A
M
J
J
A
S
O
N
D
J
F
M
A
M
J
2007
2008
2009

Figure 2



Mean abundance of mice
8
7
6
5
Fruit present
Fruit removed
Cover present
Cover removed

Figure 3


Mean proportion of reproductively active mice
0.0
0.2
0.4
0.6
0.8
1.0
Months
J
J
A
S
O
N
D
J
F
M
A
M
J
J
A
S
O
N
D
J
F
M
A
M
J
2007
2008
2009
Fruits present
Fruits removed

Chapter 3

Invasive honeysuckle eradication reduces tick-borne disease risk by altering host dynamics

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ABSTRACT

Despite the ubiquity of invasive organisms and their often deleterious effects on native flora and fauna, the consequences of biological invasions for human health and the ecological mechanisms by which they occur are rarely considered. Here, we demonstrate that a widespread invasive shrub in North America, Amur honeysuckle (*Lonicera maackii*), increases human risk of exposure to ehrlichiosis, an emerging infectious disease caused by bacterial pathogens transmitted by the lone star tick (*Amblyomma americanum*). Using large-scale observational surveys in natural areas across the Saint Louis, Missouri (USA) region, we found that white- tailed deer (*Odocoileus virginianus)*, a preeminent tick host and pathogen reservoir, more frequently utilized areas invaded by honeysuckle. This habitat preference translated into considerably greater numbers of ticks infected with pathogens in invaded relative to adjacent uninvaded areas. We confirm this biotic mechanism using an experimental removal of honeysuckle, which caused a decrease in deer activity and infected tick numbers, as well as a proportional shift in the blood-meals of ticks away from deer. We conclude that disease risk is likely to be reduced when honeysuckle is eradicated, and suggest that management of biological invasions may help ameliorate the burden of vector-borne diseases on human health.

INTRODUCTION

Invasive species, defined here as non-native species that spread rapidly and often become dominant members of local assemblages, constitute a significant threat to native biological diversity (Wilcove et al. 1998). Invasives often directly degrade a variety of important ecosystem-level properties, including disturbance regimes, nutrient cycling, microbial processes, and hydrology (Mack and D'Antonio 1998 Brooks et al. 2004, Ehrenfeld 2003, Bair et al. 2006, Zavaleta et al. 2001). Additional indirect effects of biological invasions frequently manifest via ecological interactions within wildlife communities (Levine et al. 2003). These indirect effects may include changes in the distribution and abundance of parasites and pathogens, which are often deeply embedded in the complex, interactive webs of wildlife communities (Ostfeld 2008). However, only recently has the field of ecology begun to address the potential consequences of biological invasions for the transmission of parasites and pathogens that cause disease in humans (e.g., Pearson & Callaway 2006).

Among the most ecologically complex disease dynamics are those involving pathogens that are transmitted among a community of hosts via arthropod vectors (Keessing et al 2006). As a result, the possible influences of invasive species on community interactions that govern host-pathogen dynamics are manifold, but may be divided into two broad mechanistic pathways. First, invasive species can alter the distribution, abundance, and/or diversity of hosts for infectious agents or their arthropod vectors (i.e., biotic pathways) (Keesing et al. 2006). Second, biological invasions can alter abiotic features of the local environent (e.g., temperature, humidity), which can potentially alter vector survival rates and ultimately their transmission rates of pathogens to hosts (Needham & Teel 1992, Civitello et al. 2008). In this study, we used surveys of communities invaded and uninvaded by an exotic shrub, Amur honeysuckle (*Lonicera maackii*), coupled with a removal experiment of the shrub, to distinguish between these pathways. In so doing, we provide a comprehensive examination of how an invasive plant affects vertebrate host communities, tick vectors, and the pathogens they carry to ultimately influence human disease risk.

Lonicera maackii (hereafter 'honeysuckle') is a woody shrub native to Asia that has become a noxious understory invader in many deciduous forests of eastern North America (Luken and Thieret 1996), with myriad biotic and abiotic consequences (Webster et al. 2006). By reducing light levels (Gorchov and Trisel 2003) and through allelopathy (Dorning and Cipollini 2006), honeysuckle has wreaked havoc on native plant diversity and abundance (Gould and Gorchov 2000, Collier et al. 2002, Miller and Gorchov 2004)

. The implications of these changes for the composition and diversity of vertebrate species that occupy these habitats are largely unknown, although some animals appear to take advantage of the thick cover provided by honeysuckle to evade predators (Mattos and Orrock 2010).

The invasion of eastern North American forests by honeysuckle has occurred throughout much of the range of the lone star tick (*Amblyomma americanum*). Once considered a nuisance but a non-vector species, the lone star tick is now known to be an important vector of infectious diseases from wildlife to humans (i.e., zoonoses) in the United States (Childs and Paddock 2003), including *Ehrlichia chaffeensis* and *E. ewingii* (agents of human ehrlichiosis). Because *E. chaffeensis* and *E. ewingii* are not transovarially transmitted (i.e., from mother to offspring), it is the acquisition of a bloodmeal from a reservoir-competent and infective host in a juvenile life stage (i.e., larvae and nymphs) that results in an infected vector life stage tick (i.e., nymphs and adults) capable of transmitting pathogens to humans (Childs and Paddock 2003). Recent insights into the ecology of lone star tick- associated zoonoses suggest that white-tailed deer (*Odocoileus virginianus)* may serve as both the primary host for the lone star tick and wildlife reservoir for multiple emerging bacterial pathogens, including *E. chaffeensis* and *E. ewingii* (Paddock and Yabsley 2007).

To quantify the impact of honeysuckle invasion on ticks and their associated pathogens, as well as on tick hosts, we conducted field surveys of paired honeysuckle-invaded and -uninvaded plots (measuring 30m x 30m) in nine natural areas throughout the Saint Louis, Missouri region (Fig. 1A). Further, to provide a strong experimental test of the underlying mechanisms by which honeysuckle invasion alters community interactions and to determine whether eradication of the invasive plant reduces tick-borne disease risk, we conducted an experimental removal of honeysuckle and measured tick survival rates in invaded and restored habitats at one of our most heavily-invaded and high disease risk study sites (Fig. 1B).

RESULTS AND DISCUSSION

Regional Survey: Ticks, deer, and disease risk in native vegetation versus honeysuckle- invaded plots. We found significantly higher abundances of both nymph (t = -4.011, P = 0.004, Fig. S1A) and adult (t = -3.117, P = 0.014) life stage ticks in honeysuckle invaded plots relative to neighboring uninvaded native vegetation in the nine surveyed natural areas. There were no significant differences in the proportion of ticks infected with pathogens across sites (range = 0.011-0.078 nymph infection prevalence). However, the density of nymphs infected with *E. chaffeensis* was ~ten times higher in honeysuckle-invaded plots relative to uninvaded plots (t = -3.766, P = 0.020, Fig. 1C), indicating that honeysuckle presence lead to a substantial increase in disease risk.

Because white-tailed deer represent the primary host for lone star ticks and several of their associated pathogens (Paddock and Yabsley 2007), we conducted field surveys to estimate their abundances based on scats found in the same plots surveyed for ticks. These surveys indicated nearly five times greater density of deer in honeysuckle-invaded areas relative to non-invaded areas (t = -3.420, P = 0.009, Fig. 1E). One possible mechanism by which deer might utilize honeysuckle- invaded areas more frequently would be if invaded areas have higher overall vegetation density, providing possible food or security to resting deer. Indeed, there was a positive relationship between the density of honeysuckle in a given area and the overall vegetation density ($R^2 = 0.74$, $P < 0.0001$), and there was an 18-fold increase in the overall density of plants in invaded relative to uninvaded areas (1 ± 1.7 contacts with vegetation per 20 m in plots of native vegetation vs. 18.1 ±13.8 contacts per 20 m in honeysuckle-invaded plots). Overall, these results suggest that increased use by deer of the densely vegetated habitat created by invasive honeysuckle may trigger a chain of ecological events that locally increases densities of ticks and their associated pathogens.

Removal Experiment: Ticks, deer, and disease risk in honeysuckle-invaded versus honeysuckle-eradicated plots. The results from our honeysuckle eradication experiment mirrored those from our surveys of naturally-invaded and -uninvaded areas. Specifically, we found significantly reduced densities of nymphs (F = 7.18, P = 0.043, Fig. S1B), but not adults

(F = 3.04, P = 0.104), in plots where honeysuckle was removed relative to when it was left intact. The density of nymphs infected with *E. ewingii* was significantly reduced in honeysuckle- removed plots (F = 5.99, P = 0.028, Fig. 1D), although the percentage of infected nymphs did not differ significantly among removal and intact plots (F = 0.24, P = 0.672). We found higher densities of deer scat in honeysuckle-intact plots (F = 11.29, P = 0.02, Fig. 1F), and vegetation density corresponded strongly with the density of honeysuckle ($R^2 = 0.82$, $P < 0.0001$; 22.7 ± 11.3 contacts per 20 m with honeysuckle intact vs. 5.0 ± 6.9 contacts per 20 m when honeysuckle was removed). Overall, these experimental results confirm the hypothesis that deer preferentially utilize areas invaded by honeysuckle, increasing the abundance of ticks in those areas, and increasing resulting disease risk.

In order to tease apart possible mechanisms which tick influenced abundances and their associated pathogen prevalence by the honeysuckle removal treatment, we performed two more detailed analyses. First, to discern whether differences in tick abundances might have been due to differences in the survivorship of ticks in honeysuckle-intact versus honeysuckle-eradicated areas as a result of abiotic differences between the habitat types (e.g., temperature, humidity), we performed a tick-survival experiment in each of the plots (Bertrand and Wilson 1997). We found no differences in survival of nymphs ($z = -0.716$, $P = 0.470$, Fig. S2A) or adults ($z = 0.728$, $P = 0.47$, Fig. S2B), suggesting that the observed differences were more likely due to honeysuckle-mediated changes in deer activity and not abiotic changes imposed by honeysuckle removal.

Second, to determine whether the distribution of tick blood-meals among hosts, and thus the potential to acquire pathogens from different hosts, changes as host abundance changes, we used molecular techniques to identify the sources of the previous instar's bloodmeal from field- collected nymphs (Allan et al. 2010, Pichon et al. 2003). We found a trend toward an increased proportion of tick blood-meals taken from deer in honeysuckle-intact, relative to honeysuckle-removed, plots ($F = 4.79$, $P = 0.079$, Fig. S3). Further, we found a significant correlation between the proportion of bloodmeals derived from deer within a plot and both the infection prevalence for *E. ewingii* ($R^2 = 0.346$, $P = 0.044$, Fig. 2A) and the density of nymphs infected with *E. ewingii* ($R^2 = 0.397$, $P = 0.028$, Fig. 2B).

CONCLUSIONS

Overall, our results show a mechanistic link between an invasive shrub and human-risk of exposure to tick-borne diseases through a cascade of ecological interactions. First and foremost, honeysuckle alters the habitat use of white-tailed deer, which in turn alters the abundance of lone star ticks and human risk of exposure to the bacterial pathogens they vector. Possible factors that cause white-tailed deer to select honeysuckle-invaded habitats are diverse, but are likely related to deer foraging on some component of honeysuckle vegetative structure (e.g., leaves or bark) and/or using dense honeysuckle stands for shelter. In the Saint Louis, Missouri region, honeysuckle invasion alters the nature of understory vegetation forming a monoculture of vegetation that is approximately 18 times denser than uninvaded areas. Further, relative to native plants, honeysuckle produces leaves earlier, and retains them longer, during the growing season (Trisel and Gorchov 1994). The combined effect of increased stem density and altered leaf phenology is to increase understory complexity and density. Wildlife may seek out such refugia for several reasons, including favorable microclimates and protection from predators (Caro 2005). Viewed in light of evidence that deer prefer to select bedsites in more densely vegetated woody habitats (Huegel et al. 1986), and that honeysuckle cover alters the behavior of other native vertebrates (Mattos and Orrock 2010), our patterns of deer habitat use are consistent with the hypothesis that honeysuckle may provide a refuge (e.g., Orrock et al. 2010) in which deer preferentially bed when not feeding.

The generality of our results as they pertain to other plant invasions and infectious agents is an area in critical need of further study. The results of our tick survival study suggest that abiotic pathways were not responsible for the increase in lone star tick abundance observed in honeysuckle-invaded plots. However, a recent study demonstrates that survival of lone star and American dog ticks is reduced by Japanese stiltgrass (*Microstegium vimineum*), an exotic annual grass invasive to eastern North America (Civitello et al. 2008). Thus abiotic effects of plant invasions on tick- borne disease risk may vary depending upon the species of tick and invasive plant. There is widespread evidence for biotic effects of environmental change on human risk of exposure to zoonotic diseases due to changes in the composition of wildlife communities (Ostfeld 2009). However, while there is phenomenological support for potential biotic effects of plant invasions on tick- borne disease risk mediated via tick hosts, mechanistic understanding has remained elusive. Studies conducted in the northeastern U.S. (Elias et al. 2006, Williams et al. 2009) demonstrate that human risk of exposure to Lyme disease, which is caused by the bacterium *Borrelia burgdorferi* and transmitted by the black-legged tick (*Ixodes scapularis*) is increased by several exotic shrubs, including honeysuckle and Japanese barberry (*Berberis thunbergii*). However, the ecological mechanisms by which these plant invasions influence Lyme disease risk remain unknown, and an enhanced understanding of the mechanisms that drive disease risk is critical to mitigation and control strategies.

Further, determining the spatial scale over which invasive honeysuckle increases tick-borne disease risk is a crucial area of future research. While our results clearly indicate an increase in disease risk at the scale of the local honeysuckle patch, increased use of invaded areas by deer could cumulatively decrease time spent in native vegetation, such that disease risk in native areas becomes reduced relative to pre-invasion conditions. Alternatively, proximity to honeysuckle-invaded sites could increase disease risk in native vegetation due to a spill-over effect of high disease risk from invaded areas. A broad-scale survey that includes large areas of uninvaded and fully invaded sites will be necessary to determine whether honeysuckle invasion increases disease risk beyond the scale of the local honeysuckle patch.

Our findings contribute to a growing body of literature that illustrates how extensively invasive species can alter interactions in native communities (Mattos and Orrock 2010, Didham et al. 2005). An accumulation of evidence indicates that the loss of biological diversity and the homogenization of wildlife communities have the potential to increase the prevalence of and risk of exposure to zoonotic diseases (Keesing et al. 2006, Pongsiri et al. 2009). Our results illustrate an underappreciated consequence of anthropogenic global change: that biological invasions may indirectly contribute to human risk of exposure to infectious diseases, mediated by how invasive species alter ecological interactions in the communities they invade. Further, our finding that removal of the invader mitigates disease risk, coupled with the benefits of invasive plant removal to wildlife communities, suggests a potential ‘win-win’ (Rosenzweig 2003) scenario for biodiversity conservation and human health.

METHODS

Regional Survey. Our survey in the Saint Louis, MO region was conducted in nine natural areas, which are naturally dominated by oak-hickory forests with an herbaceous understory (Yang et al. 2008) but are undergoing extensive invasion by honeysuckle. In each of these natural areas, we selected three plots of primarily native vegetation and three plots dominated by honeysuckle in which to conduct our surveys. Plots were intentionally selected to consist primarily of native or honeysuckle vegetation, and were therefore not selected randomly within sites. All plots were at least 30m x 30m in area, though many plots were much larger. To assess the impact of honeysuckle invasion on the complexity of understory vegetation, we measured vegetation density along one 20 m transect in one native and one invaded plot at each of the nine natural areas. Across this transect, we counted the number of times that any plant material touched a polyester line (i.e., encounters) held 2 m above the ground and the number of *L. maackii* individuals within 2 m of the line.

Human risk of exposure to tick-borne diseases is often quantified by measuring the density of vector life stages, their infection rates with pathogens, and the product of these two variables, the density of infected ticks. This latter metric is widely considered the best estimate of human risk of encountering an infected tick (Barbour et al. 1993). We sampled three native vegetation and three honeysuckle-invaded plots in each of the nine natural areas for the density of host-seeking ticks using carbon-dioxide traps baited with dry ice, a highly effective method for sampling lone star ticks (Schulze et al. 1997) Nymph and adult life stage ticks were sampled by placing two CO_2 traps, approximately 10 m apart, near the center of each of the six plots at each study site. Traps were baited with 1 kilogram of dry ice and set out for 24 hours. Sites were sampled once each in random order under constant meteorological conditions from June 12 – July 11, 2008, resulting in a total of 108 trap-nights. Sampling coincided with the peak in abundance of the nymph and adult life-stages of lone star ticks in Missouri (Kollars et al. 2000).

Dung surveys were conducted October 22 – 31, 2008, coinciding with the peak in abundance for larval life stage lone star ticks in Missouri (Kollars et al. 2000), and therefore indicative of the availability of deer for larval blood-meals at our study sites. We randomly selected one plot of native vegetation and honeysuckle at each study site, and delineated a central 20m x 20m area using stake-wire flags. The entire grid was surveyed by a single observer (BFA) who walked a transect every 2.5 m up and down each row of the survey area and scanning side-to-side for dung clusters. All dung clusters observed were marked with an additional flag to avoid recounting.

For pathogen analyses, we focused on nymph life stage ticks as other studies on tick-borne diseases have shown this is often the primary vector life stage (Barbour et al. 1993). Five of the nine natural areas yielded sufficient quantities of nymphs for pathogen analyses. We selected 90 nymphs from native vegetation plots and 90 nymphs from honeysuckle plots from each of these five areas. Briefly, we screened ticks for pathogens using a combination of polymerase chain reaction (PCR) using general primers to amplify any bacterial DNA that may be present, and reverse line blot (RLB) hybridization using a series of pathogen-specific oligonucleotide probes to identify amplified bacterial DNA from the tick samples (Pichon et al. 2003, Rijpkema et al. 1995). We used established RLB methods and probes (Allan et al. 2010) to screen for *E. chaffeensis* and *E. ewingii*.

Removal Experiment. Our honeysuckle removal experiment was conducted at one study site (Augustus A. Busch Memorial Conservation Area, Missouri Department of Conservation) that was heavily invaded by honeysuckle. We implemented a second study treatment that entailed the removal of honeysuckle fruits in order to tease apart the importance of honeysuckle vegetation versus honeysuckle fruits. We implemented this experimental removal of honeysuckle vegetation and fruits using a randomized block study design. Thus, there was one of four study treatments (honeysuckle vegetation intact or removed combined with honeysuckle fruits left intact or removed) in each of three experimental blocks.

Oak-hickory overstory was consistent between experimental blocks, and treatments were randomly assigned to each 30 x 30 m quadrant of each block. Honeysuckle individuals were physically removed by cutting the stem at the base in the fall of 2006 and continuously pruned until the end of the study. Honeysuckle berries were individually removed by hand starting in fall of 2006 and were continually removed each fall thereafter. In sites where vegetation was removed but honeysuckle fruits left intact, fruits were removed by hand and dropped on the ground prior to removing the vegetation in the first year of the study. In subsequent years, fruits from "vegetation-intact fruits-removed" plots were added to the "vegetation-removed fruits- intact" plots on a weekly basis. There were few significant effects detected from the fruit removal treatment (Table S1), suggesting the treatment had a minimally biologically relevant effect on tick-borne disease risk. Further, there were no significant interactions detected between the fruit and vegetation removal treatments in any of our analyses (Table S1), indicating that the effect of honeysuckle vegetation removal is not contingent upon the influence of fruit removal. Therefore, we focus solely on the results from the vegetation removal treatment, and conducted vegetation surveys using the same methods as described above for all 12 study plots, including removal areas.

The abundance of nymph and adult life stage ticks were sampled by CO_2 traps as described above, with two surveys in May and July of both 2007 and 2008 for a total of four surveys in each of the 12 plots. All 12 plots were sampled simultaneously with two CO_2 traps each under relatively constant meteorological conditions. To avoid any potential edge effects, white-tailed deer dung cluster surveys were performed in the inner 20 x 20 m area of each plot October 20 – 21, 2008, as described above for the regional survey.

We determined the prevalence of pathogens in nymphs for all 12 plots from the May 2007 and 2008 tick surveys. We tested at least 45 nymphs from each plot from the May 2007 survey (except for four plots that yielded less than 45 ticks – mean number tested =

41.3, SD =23.2) and exactly 45 nymphs from all 12 plots for May 2008.

For the removal experiment only, we also utilized a combination of PCR and RLB for the identification of bloodmeals derived from white-tailed deer for the above nymphs collected in May 2007 and 2008. As with our methods for pathogen detection, we used universal primers to amplify a region of vertebrate 18S rDNA that is highly conserved across vertebrate taxa. We then identified this amplified vertebrate DNA using host-specific oligonucleotide probes in a RLB panel (Allan et al. 2010).

Finally, to explore the effects of abiotic conditions in our experimental plots on tick survival, we conducted a tick survival study (Bertrhand et al. 1997) in all 12 study plots in 2008. We placed 20 nymph and 10 adult life stage ticks in each of 12 mesh bags, one of which was then partially buried in the leaf litter at the center of each study plot, protected by a cage made of chicken wire. Bags were examined weekly to determine the number of nymphs and adults surviving, until all ticks in all bags had succumbed to desiccation. The survival experiment was established on May 30, 2008, and continued for 22 weeks until November 14, 2008, when all ticks in all plots were observed to have desiccated.

Statistical Analyses. For the regional study, all samples from the three control plots of native vegetation and the three honeysuckle-invaded plots were averaged for each site allowing for paired comparisons with study sites serving as the level of replication. We used paired t-tests to determine if there was a significant difference between native vegetation versus honeysuckle for all response variables sampled (density of nymphs and adults, proportion of nymphs infected with zoonotic pathogens, density of infected nymphs, and density of deer dung clusters). All analyses were conducted in Systat.

For the experimental study, we used permutational Analysis of Variance (ANOVA) using the program ‘PERMANOVA’ (Anderson 2001, McArdle and Anderson 2001) to explore the effects of honeysuckle vegetation removal on the abundance of ticks, nymph infection rates with pathogens, density of infected nymphs, density of dung clusters, and proportion of bloodmeals from deer. We also explored for the effects of block, month (May vs. July), year (2007 vs. 2008), and all possible interactions among variables. PERMANOVA makes no particular assumptions regarding the distributions of original variables since all *P*-values are obtained by permutation. Analyses were performed with type III sums-of-squares and 9999 unrestricted permutations of the raw data using correct permutable units for the permutational ANOVA.

For the tick survival study, we conducted Cox Proportional Hazards survival analysis for both nymphs and adults in the R programming environment using the "survival" library. The Cox Proportional Hazards approach has the benefit of being "semi-parametric", in that the baseline hazard function is left unspecified, while the covariates enter the model linearly. Again, we tested for the effects of vegetation removal, block effects, and all possible combinations of interactions on survival of nymphs and adults.

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Figure legends:

Fig. 1. Effects of honeysuckle invasion and eradication on tick-borne disease dynamics. (A) Land-use map of the Saint Louis, Missouri metropolitan area indicating the nine natural areas used in the regional survey of the effects of Amur honeysuckle invasion on tick-borne disease risk. Map produced using Geographic Resources Center, copyright 2002. (B) Amur honeysuckle (*Lonicera maackii*) in the understory of an oak-hickory forest prior to eradication. Density of *A. americanum* nymphs infected with (C) *E. chaffeensis* in native vegetation vs. Amur honeysuckle plots distributed across nine natural areas and (D) *E. ewingii* in Amur honeysuckle intact vs. removed experimental plots at Busch Conservation Area. Density of white-tailed deer dung clusters in (E) native vegetation vs. Amur honeysuckle plots distributed across nine natural areas and (F) Amur honeysuckle intact vs. removed experimental plots at Busch Conservation Area. Error bars reflect one SE.

Fig. 2. Proportion of bloodmeals from white-tailed deer vs. (A) the proportion of *A. americanum* nymphs infected with *E. ewingii* and (B) the density of *A. americanum* nymphs infected with *E. ewingii* in 12 experimental plots sampled at Busch Conservation Area in May 2008.

Table S1. Effects of fruit removal and the interaction between fruit removal and vegetation removal on the response variables measured in the honeysuckle eradication experiment.

Fig. S1. Density of *A. americanum* nymphs in (A) native vegetation vs. Amur honeysuckle plots and (B) Amur honeysuckle intact vs. removed plots. Error bars reflect one SE.

Fig. S2. Survival of *A. americanum* (A) nymphs and (B) adults in Amur honeysuckle intact vs. removed plots. Open circles indicate honeysuckle vegetation intact; closed circles indicate honeysuckle vegetation removed. Error bars reflect one SE.

Fig. S3. Proportion of bloodmeals derived from white-tailed deer detected in *A. americanum* nymphs collected from Amur honeysuckle intact vs. removed plots. Error bars reflect one SE.

Fig. 1.

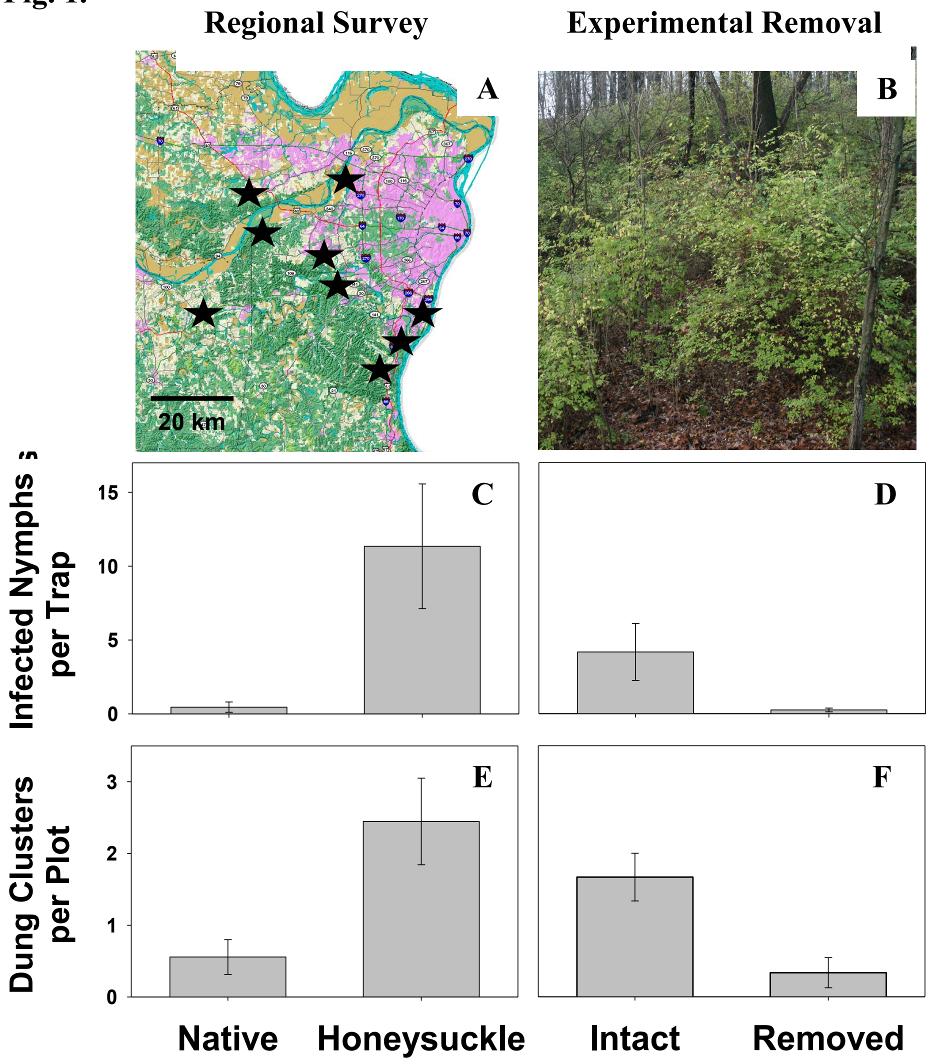

Regional Survey
Experimental Removal
A
B
20 km
C
D
E
F
Infected Nymphs per Trap
Dung Clusters per Plot
15
10
5
0
3
2
1
0
Native
Honeysuckle
Intact
Removed

Fig. 2.

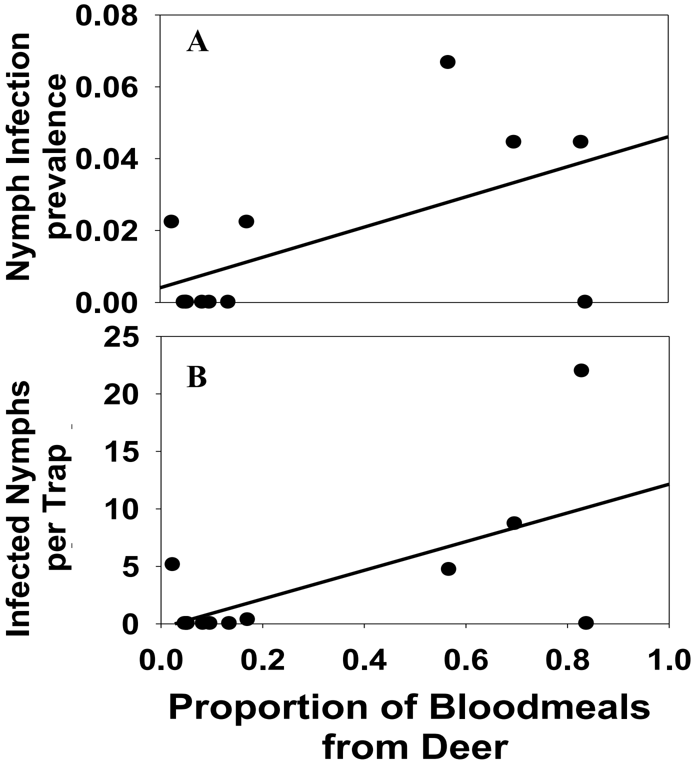

A
B
Nymph Infection prevalence
0.08
0.06
0.04
0.02
0.00
Infected Nymphs per Trap
25
20
15
10
5
0
0.0
0.2
0.4
0.6
0.8
1.0
Proportion of Bloodmeals from Deer

Table S1. Effects of fruit removal and the interaction between fruit removal and vegetation removal on the response variables measured in the honeysuckle eradication experiment at Busch Conservation Area.

Response Variable	**Treatment**	**F-value**	**P-value**
Nymph Abundance	Fruit Removal	0.73	0.454
	Fruit Removal X Vegetation Removal Interaction	1.58	0.263
Adult Abundance	Fruit Removal	1.80	0.23
	Fruit Removal X Vegetation Removal Interaction	1.09	0.368
Prevalence of *E. ewingii*	Fruit Removal	4.32	0.073
	Fruit Removal X Vegetation Removal Interaction	0.04	0.858
Density of Nymphs Infected with *E. ewingii*	Fruit Removal	0.01	0.918
	Fruit Removal X Vegetation Removal Interaction	0.10	0.817
Deer Dung Cluster Density	Fruit Removal	2.82	0.149
	Fruit Removal X Vegetation Removal Interaction	*	*
Proportion of Bloodmeals from Deer	Fruit Removal	0.43	0.564
	Fruit Removal X Vegetation Removal Interaction	0.01	0.943
		z-score	**P-value**
Nymph Survival	Fruit Removal	-3.64	0.001
	Fruit Removal X Vegetation Removal Interaction	**	**
Adult Survival	Fruit Removal	-1.69	0.09
	Fruit Removal X Vegetation Removal Interaction	**	**

* Insufficient sample size to detect interaction term via permutational ANOVA.

** Highly non-significant interaction terms were excluded from final Cox Proportional Hazards model.

Fig. S1. Density of *A. americanum* nymphs in (A) native vegetation vs. Amur honeysuckle plots and (B) Amur honeysuckle intact vs. removed plots. Error bars reflect one SE.

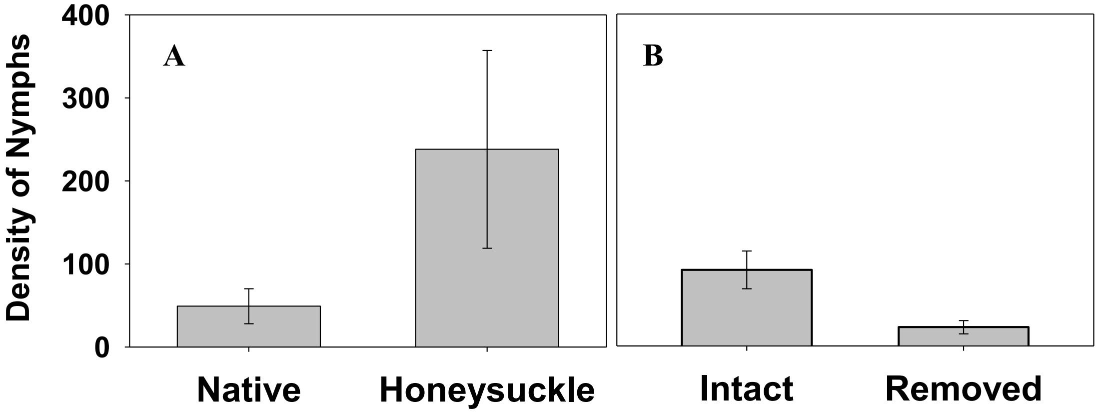


Fig. S2.

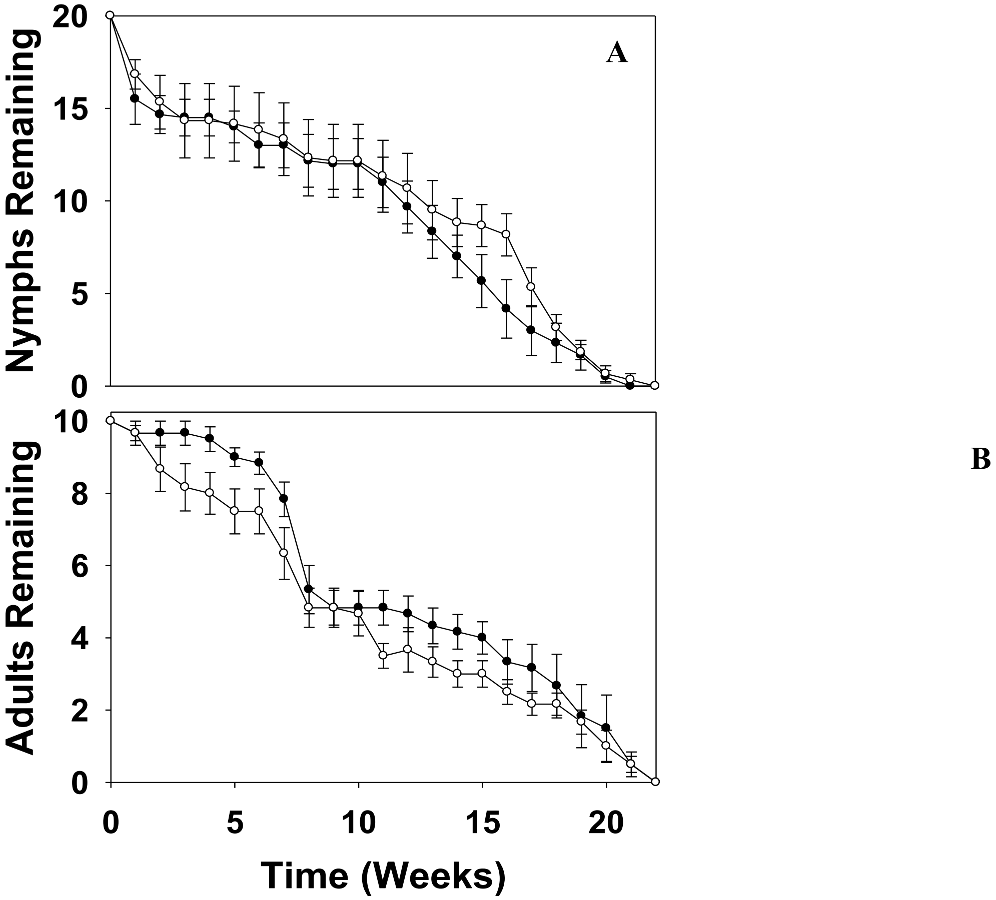
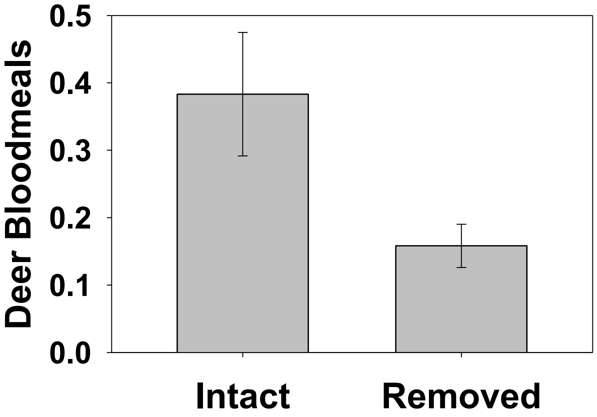

A
B
Nymphs Remaining
20
15
10
5
0
Adults Remaining
10
8
6
4
2
0
0
5
10
15
20
Time (Weeks)

Fig. S3.



Chapter 4

Invasive plant disrupts native plant community structure through apparent competition.

ABSTRACT

Understanding the effects of invasive plants on seedling recruitment is important because it shows how invasions affect forest regeneration processes. But plant invasions are also known to affect native consumers, thus consumer-mediated indirect effects (e.g. apparent competition) have the potential to alter the dynamics of coexistence in native communities. Both cover and fruit production by invasive plants may increase consumer foraging activities, which in turn may exert higher pressure on seedling recruitment. We used an experimental approach in which we manipulated invasive vegetative cover and fruit production to quantify the interplay these factors on seedling recruitment through their effect on foraging of white-footed mice. We showed that indirect effects of an exotic plant via consumer pressure on seedlings can determine species richness. However, these indirect effects do not seem to regulate seedling abundance, as the effects of consumers on overall abundance were the same across all levels of our cover and fruit manipulations. At the individual level the effects of our treatment manipulations vary from one species to another. Our findings show that both the direct and indirect effects of invasive plants via apparent competition can reduce native plant diversity and abundance. Moreover, our results show that management of this aggressive invader, *L. maackii*, can backfire and increase the invader's recruitment.

INTRODUCTION

Invasive species are the second-most significant threat to native species, having contributed to a 42% population decline in endangered and threatened species in the United States (Wilcove et al. 1998). Studies investigating the effects of invasive species provide vital information about their ecological impact, potentially leading to solutions for better management and eradication. Several lines of evidence show that invasive plant species frequently prevail over indigenous plants (Collier et al. 2002, Chornesky and Randall 2003, Daehler 2003, Miller and Gorchov 2004, Rand and Louda 2004, Lloret et al. 2005). To date, mechanistic studies of this impact of invasive plants have largely focused on their direct effects on native plant communities via competition (Lau and Strauss 2005). Nevertheless, successful plant invasions can cause detrimental indirect effects (i.e., apparent competition: Holt 1977, Holt and Lawton 1994, Holt and Kotler 1987) on native plants mediated by the local fauna (Richardson et al. 2000, Moragues and Traveset 2005, Traveset and Richardson 2006). For instance, invasive plants can intensify consumer pressure on native plants by augmenting the abundance or activity of native consumers (Orrock et al. 2010, Allan et al. 2010, Dutra et al. 2011). As a result, the consumers indirectly benefit the invader by exerting greater pressure on natives and reducing their overall abundance. This kind of indirect effect is known as apparent competition (Holt 1977, Holt and Lawton 1994, Holt and Kotler 1987).

Although rarely examined, apparent competition mediated by invasive plants can arise via two pathways, both resulting in higher seed and seedling predation for the native plant species (Orrock et al. 2010). The first pathway is referred as refuge-mediated apparent competition, a process in which the dense vegetative layer of the invasive reduces the perception of predation risk in consumers (Orrock et al. 2010, Mattos and Orrock 2010, Dangremond et al. 2010). The second is food-mediated apparent competition, occurring when the invasive plant adds new food sources for consumers (Bartuszevige and Gorchov 2006, Buckley et al. 2006, Meiners 2007, Dutra et al. 2010). These two pathways may operate simultaneously.

Recent studies on invasive plants provide supportive data for refuge-mediated apparent competition. For instance, Orrock et al. (2008) showed that prevention of re-establishment of native grasses by the invasive forb (*Brassica nigra*) resulted in higher seed consumption by mammals (squirrels, rabbits, and mice) seeking refuge in the proximity of the invasive plant. Similarly, Dangremond and collaborators (2010) showed that the endangered coastal dune plant *Lupinus tidestromii* experiences high levels of pre-dispersal seed consumption by the native rodent *Peromyscus maniculatus* when near the invasive grass, *Ammophila arenaria.*

Although many invasive plants provide food (Bartuszevige and Gorchov 2006, Buckley et al. 2006, Dutra et al. 2011) in addition to dense cover (e.g., Sheley et al. 1998, Williams et al. 2009, Allan et al. 2010), experimental studies that have manipulated food solely, or independently manipulated both of these factors have not been conducted. These types of studies are critical for determining the mechanistic basis of invader-mediated indirect effects (Orrock et al. 2010). The present study aims to accomplish this objective by studying the invasive shrub *Lonicera maackii.*

Amur honeysuckle (*Lonicera maackii* (Rupr.) Maxim., Caprifoliaceae) is an aggressive invasive plant that has become the dominant shrub in many forests in the Eastern United States (Woods 1993, Luken and Goessling 1995, Luken et al. 1997, Gould and Gorchov 2000, Collier et al. 2002, Gorchov and Trisel 2003, Miller and Gorchov 2004). *Lonicera maackii*-infested areas are ideal for the study of apparent competition because the direct negative effects of *L. maackii* on native plant diversity and recruitment, via reduced light levels and allelopathy, are already known (Woods 1993, Luken and Goessling 1995, Luken et al. 1997, Gould and Gorchov 2000, Collier et al. 2002, Gorchov and Trisel 2003, Miller and Gorchov 2004, Hartman and McCarthy 2004, Dorning and Cipollini. 2006). The thick understory created by the plant can also increase foraging and abundance of native animals (Mattos and Orrock 2010, Dutra et al. 2011). Recent evidence suggests that differential seed predation in the proximity of *L. maackii* could be a result of refuge-mediated apparent competition (Meiners 2007). Moreover, *L. maackii* also could create food-mediated apparent competition through its massive fruit production. Heavily invaded areas, fruit production reaches 400 million berries per ha (Ingold and Craycraft 1983) or more than 20 kg of fruits in a 25 m^2 area (Dutra unpublished results). Approximately 62% of the fruits produced per shrub reach the ground without being consumed by birds (Bartuszevige and Gorchov 2006). Rodents and deer commonly remove these fallen fruits (Williams et al. 1992, Vellend 2002). As a result, small mammal activity (Dutra et al. 2011) and abundance (Dutra et al. in preparation) are increased in infested areas.

We simultaneously manipulated *L. maackii* fruit abundance and vegetation cover at two levels each (present and absent) and simulated three levels of consumer pressure within each of the above four treatment combinations. We evaluated whether exclusion of seed consumers modified the net effects of *L. maackii* on native plants via apparent competition. We further documented whether the food and shelter provided by *L. maackii* affected seedling recruitment and the relative role of seed consumers on abundance of native plants, as well as the effects of consumers on plant community diversity and composition.

METHODS

This study was conducted at Busch Wildlife Conservation Area (38.70° N, 90.71° W), a 6987 ha reserve in Saint Charles County, Missouri, open to the public for hunting and recreation. The area has approximately 3000 acres of oak/hickory forest oak-hickory forests with shrub layers dominated by *L. maackii* and *Rosa multiflora* (multiflora rose). Other common plants include *Cornus* spp. (dogwood), *Symphoricarpus orbiculatus* (coral berry), *Lindera benzoin* (spice bush), and *Rhus aromatica* (aromatic sumac).

To investigate the effects of consumers on seedling recruitment and community composition we used a completely randomized factorial split-plot design. The experiment consisted of manipulating the *L. maackii* fruit and cover to two levels each (present and removed) in 12 plots (whole plot treatments). In each of these plots we sampled seedlings in areas (sub-plot) exposed to three distinct levels of consumer pressure: 1) a complete exclosure that excluded all mammalian consumers, 2) a partial exclosure that excluded large mammals (i.e., white-tailed deer) but allowed access by small mammals, such as mice, to seedlings, and 3) a control, unfenced treatment that allowed access to all consumers to seedlings (hereafter, consumers excluded, mice only, and open to consumers, respectively).

The three experimental blocks were established in the fall of 2006. Each block consisted of four treatment plots (30 × 30 m each) at a minimum distance of 50 m from each other. Experimental plots within each block were randomly assigned to one of four treatments: 1) fruits and honeysuckle cover intact, 2) fruits intact and honeysuckle cover removed, 3) fruit removed and honeysuckle cover intact, 4) both fruits and honeysuckle cover removed. Honeysuckle individuals were physically removed from treatment plots 2 and 4 by cutting the stem with a pruner or machete in the fall of 2006. Continuous pruning of sprouting honeysuckle individuals continued from late fall 2006 until the end of the study. During the fall months of 2006, 2007, and 2008 we removed all fruits by hand from treatment plots 3 and 4. In 2006, fruits on treatment plots 2 were removed from the plant and left on the ground prior to plant removal. In 2007 and 2008, fruits removed from plots of treatment 3 were added to plots of treatment 2.

Six consumer exclosure areas were established in the center of each experimental plot. Their specific location in the plot was determined randomly by throwing a small shovel over the experimenter's (HPD) shoulder. Each pair of cages was assigned to one of the three exclosure treatments. Consumer exclusion was achieved using cylindrical cages with a closed top (20 cm radius, 40 cm high made of hardware cloth). Mesh size of these cages was 0.6 cm × 0.6 cm for all consumers excluded areas, and 3 cm × 5 cm for areas that provided access to mice only. Cages were buried in the ground to a depth of 5 cm to prevent access by burrowing consumers. Exclosures were established in December 2007 and checked for seedlings in September 2008. We recorded the number and identity of each plant inside exclosures. Plants that could not be identified in the field were pressed and identified in the lab. Plants that were too small to be identified in the field or had few leaves at the time of the survey were transplanted to pots and were grown in the greenhouse until they were old enough to be identified.

Statistical Analysis

We analyzed the effects of our experimental treatments on overall abundance, observed and rarefied species richness, and abundance of species with more than one hundred individuals and *L. maackii* seedlings with mixed model analysis using SAS Proc Glimmix (SAS Institute Inc. 2004). We used a Gaussian distribution and data were square root or log+1 transformed to improve the normality of the residual distribution and the homogeneity of variances. The decision to use one transformation over the other was based on visual inspection of a QQ-normal plot, and the residuals plotted against fitted values (Littell et al. 2006). Using the same model structure we also compared the effects of our treatments on rare and common species. Rare species were defined as any species with 52 or fewer individuals. As so defined, common species represented 65% of all individuals sampled.

We treated block as a random effect, vegetation cover, fruits and cage exclosures as fixed. Appropriate error terms were calculated using cage as a sub-plot in a split plot design. Residual covariance model structure was chosen based on AICc and the treatment structure of the data, and degrees of freedom were calculated using Kenward-Rogers (Littell et al. 2006).

Significant treatment results indicated that our experimental manipulations (cover, fruit, and consumer) affected seedling recruitment. Refuge or food mediated apparent competition would be indicated by two conditions: 1) a significant interaction between the cover or fruit treatments and exposure to mammals on native species, and 2) a greater negative effect on native species when exposed to mammals in the presence of the invasive or its fruit than when not. Because each exclosure had a variable number of individuals we calculated the estimated species richness for a standard sample size using rarefaction (Coleman curves) for each experimental treatment combination (Magurran 2004) using the software EstimateS Version 8 (Colwell, 2009). We also calculated Simpson's (1/D) diversity index for each treatment combination. Richness and diversity were calculated using the EstimateS Version 8 (Colwell, 2009), randomizing 500 times and sampling with replacement, pooling data across all unique treatment combinations in both analyses. We used the lack of overlap of the 95% confidence intervals at the end of the estimated curve to compare both species richness and diversity indexes differences among treatment groups. We opted not to use ordination techniques to assess the community dissimilarity among the 12 treatment combinations for two reasons. First, many samples had just one individual or a single species creating several outliers in the ordination. Removal of those outliers would be an arbitrary decision that inevitably would result in an unbalanced design. Second, the experiment was originally designed to assess the effects of fruit and cover on mice activity; experimentation with seedling recruitment came as an opportunity to verify the indirect effects of the exotic on natives. This resulted in a very complex structure (randomized block, two way fully crossed with a split plot). McCune and Grace (2002) suggest simplification of complex experimental designs (pooling across treatments) as an alternative for the analysis of such data. Instead, we feel that it is sufficient to show effects on community structure using the magnitude of treatment effects on observed species richness, Simpson's index, and abundance of individuals.

RESULTS

Species encountered

We recorded 2361 seedlings, representing 90 morpho species. Five species (*Carex* spp., *Geum* spp., *Ageratina altissima*, *Sanicula* sp., and *Cardamine pensylvanica*) represented 39% of all collected seedlings. The mean number of individuals per species per cage across all treatments was 11.9 ± 3.3, 6.9 ± 2.1, 6.2 ± 2.1, 4.9 ± 1.9, and 3.4 ± 2.1, respectively. The mean number of *L. maackii* seedlings per sample was 1.3 ± 0.3 per cage with just 48 individuals in all cages. There were 167 individuals representing 13 morphospecies that could not be identified because they were too young and/or did not survive the transplant to the greenhouse.

Total Abundance

Removal of *Lonicera maackii* cover increased the mean number of seedlings by 49% (from 87.4 ± 15.8 to 42.9 ± 9.9) relative to plots in which cover was left intact (Fig. 1, $F_{1,6} = 9.91$, $p = 0.019$, Table 1). In contrast, consumer exclusion had a positive effect on seedling abundance ($F_{2,16} = 14.45$, $p = 0.0003$, Fig. 1). However, there was no difference in abundance between plots from which fruits were removed or not ($F_{1,6} = 0.79$, $p = 0.41$), as well as no significant interactions among treatments (Table 1). When the analysis were performed separating common and rare species, there was a significant effect on honeysuckle cover ($F_{1,6} = 20.39$, $p = 0.004$) on rare species, but not on common species. Indeed *L. maackii* removal was responsible for approximately five fold increase in the abundance of rare species relative to plots in which cover was left intact (38.4 ± 7.3 vs. 8.0 ± 2.0). Rare species abundance was also affected by the interaction between honeysuckle cover and consumers exclusion ($F_{2,16} = 4.55$, $p = 0.027$, Table 1). Even though the abundance of rare species was low when cover was present, the effect of predator exclusion was greater in the absence of *L. maackii* cover (Fig. 2).

Individual abundances

Individual species abundances of the most common species were not affected by the fruit manipulations with the exception of *Geum* spp. that showed a marginally significant increase in abundance when *L. maackii* fruit was removed ($F_{1,6} = 8.66$, p = 0.08, Table 1, Fig. 2). On average areas in which *L. maackii* fruits were available presented 4.3 ± 1.5 seedlings per cage vs. 9.5 ± 3.9 seedlings on fruit removal areas. A marginally significant interaction between fruits and vegetation cover ($F_{1,6} = 5.34$, p = 0.06, Table 1) indicates that the effect of fruit availability on *Geum* spp. seedling recruitment was bigger when cover was removed (Fig. 2).

Vegetation cover manipulation had a marginally significant negative effect on just one of the five most common species, *Ageratina altissima* ($F_{1,6} = 4.14$, p = 0.08) (Table 1, Fig. 2). Honeysuckle cover removal plots had five times more *A. altissima* (10.6 ± 3.8) seedlings than plots in which cover was left intact (1.9 ±1.0).

Consumer manipulations significantly affected the abundance of *Geum* spp. seedlings ($F_{2,16} = 7.54$, p = 0.0049): areas open to consumers had very few *Geum* spp. seedlings (1.8 ± 0.9), a seven-fold difference relative to areas from which all consumers were excluded (12.7 ± 5.6, Fig 2). Similarly, *Carex* spp. individuals were five times more abundant ($F_{2,16} = 3.50$, p = 0.055, table 1) in areas in which consumers were excluded (Fig. 2). Consumer exclusion also had a marginally significant positive effect on the abundance of *Ageratina altissima* individuals ($F_{2,16} = 3.08$ p = 0.074, Fig. 2).

Lonicera maackii seedling recruitment was also affected by vegetation cover ($F_{1,6}$

= 29.78, $p = 0.016$). In fact, areas in which invasive cover was present had three times fewer *L. maackii* seedlings (0.7 ± 0.4) then removal areas (2 ± 0.5, Fig. 2). Fruits also had an impact on *L. maackii* recruitment ($F_{1,6} = 8.66$, $p = 0.026$), but on this case different from the impact on natives fruit removal led to a reduction in the number of seedlings (0.7 ± 0.2) relative to areas where fruits were present (2.0 ± 0.7). The fruit x cover interaction was marginally significant because the effect of fruit was increased when cover was removed. ($F_{1,6}= 4.5$, $p = 0.078$). Consumer pressure manipulations did not modify seedling abundance of *L. maackii* (Fig. 2, table 1).

Species richness

Removal of *Lonicera maackii* cover and exclusion of predators had significant effects on the observed number of species ($F_{1,6} = 21$, $p = 0.0038$, $F_{2,6} = 31.61$, $p < 0.0001$, respectively). On average cover removal plots had twice as many species (13.8 ± 1.8) relative to plots in which cover was left intact (6.5 ± 0.8, Fig. 2). Exclusion of all consumers doubled seedlings species richness relative to consumer present areas (13.75 ± 0.8 and 6.25, ± 0.88, respectively, Fig. 3), and richness was 66% higher in mice only areas relative to areas exposed to consumers (10.42 ±1.54). Our results also show a significant interaction between fruit treatment and consumer exclosure ($F_{2,16} = 3.63$, $p = 0.05$), and a marginally significant interaction of cover removal and consumer exclosure ($F_{2,16} =2.76$, $p = 0.093$). The negative effects of consumers on seedling richness were more severe in the presence of the invasive fruits than in their absence (Fig. 3).

Rarefaction analysis showed that the effects of consumers on richness varied with cover and fruit availability (Fig. 4A). For instance, only non-manipulated plots (vegetation cover and fruits present) show a significant difference (no overlap of 95% confidence interval) in expected seedling species richness between areas open to consumers and consumer excluded areas. In areas in which cover was present and fruits were removed, consumers did not affect the estimated species richness (Fig 4A). Removal of cover in the presence of fruits on the other hand changed the relationship between consumers and seedlings, with mice exerting the greatest pressure on recruitment. When both cover and fruits were removed areas exposed to all consumers had fewer species then areas with mice but were not different from areas that were exposed to all consumers.

Diversity index.

There was very little variation of species diversity among the experimental treatments. There were not any consumer effects within experimental plots (Fig. 4B). The presence of the *L. maacki* on vegetation cover however, had a small effect on the role of consumers because areas exposed to mice only in the presence of cover had a reduced diversity relative to areas with all consumers excluded and vegetation removed (Fig. 4B)

DISCUSSION

Using an experimental approach, our study illustrates several points regarding indirect effects and biological invasion. At the community level the mechanism by which the invasive had a negative effect on native seedlings was direct competition as shown by the effect of vegetation cover on species richness (Fig. 3 and 4A). There was an additional effect of apparent competition, as shown by the significant interaction of fruits and exclosure in which the presence of the invasive fruits increased consumer pressure on seedlings species richness. However, at the individual level the effects of our treatment manipulations were not consistent across species varying from a negative impact of both consumers and vegetation on seedling recruitment to neutral effects. Our results are important because they add to the growing body of literature showing that the effects of invasive plants extend beyond their direct effects through direct competition, revealing the importance of incorporating consumer pressure on biological invasion. Moreover, our results provide some information about how management of this aggressive invader, *L. maackii*, can affect the invasion.

Our findings suggest that the impact of *L. maackii* on expected and observed species richness may be modified by consumer pressure (Fig. 3 and 4A). Recent studies have shown that *L. maackii* cover is associated with increased consumer foraging activity (Dutra et al. 2011). Thus, the effects of honeysuckle on seedling species richness extend beyond direct effects only and its impact on diversity may be greater than previously thought (Gould and Gorchov 2000, Gorchov and Trisel 2003) because higher consumer activity increases seed consumption and reduces the establishment of native plant species. Indeed, accumulating evidence shows that consumers modify the impact of exotic species (Meiners 2007, Orrock et al. 2008, 2010, Dangremond et al. 2010). We agree with Meiners (2007) who suggested reduced seedling diversity as a result of indirect effects may be a common phenomenon, especially for exotics with an architecturally complex and dense vegetation cover that provides shelter for small seed predators. However, we also add that the fruits produced by the exotic plant may have the potential to augment the effects of the invasive (Figs. 2 and 3). Exotics like *L. maackii* that provide both shelter and foods for seed predators have a near perfect strategy to overcome its native competitors, as they not only shade its competitors but also recruit more consumers by offering an important food source (Dutra et al. in preparation).

Contrary to our species richness results the abundance of seedlings was not determined by an interaction of consumers and the invasive. In other words, differential seedling recruitment via apparent competition does not seem to be an important mechanism through which the invasive shrub reduces native plants abundance at our study site (Fig. 1). Some studies have concluded that *L. maackii* causes asymmetric apparent competition in *Fraxinus pennsylvanica* by observing increased seed removal by consumers under *L. maackii* cover (Meiners 2007); others are in agreement with our results and show that consumers pressure on native seeds in forests is the same regardless if the site are or are not invaded by the honeysuckle (Mattos and Orrock 2011). Orrock et al. (2010) states that apparent competition can be the initial mechanism used by the invader. Then, after its establishment the invader may maintain its control of the invaded habitat using quite distinct mechanisms that not necessarily direct competition or apparent competition. Some of these mechanisms may include long-term effects of the invader. Indeed, a long-term effect of *L. maackii* is likely considering that some native species have difficulties in re-colonizing the invaded habitat years after the exotic's removal (Luken et al. 1997, Collier et al. 2002) which is probably correlated with the exotic's allelopathic effects (Dorning and Cipollini 2006, McEwan et al. 2010), or its ability to modify soil quality and underground biota (Madritch and Lindroth 2009). Regardless of the mechanism, the historical component of the invasion cannot be ignored (Strayer et al. 2006). *Lonicera maackii* is present in the St. Louis area for more than 40 years (Yatskievych 2006), and that may have been sufficient to select for species whose abundance levels are not affected by the invader. For instance, with the exception of *Ageratina altissima* that showed a marginally significant effect for the honeysuckle cover removal, all the other common species were not affected by our *L. maackii* cover manipulations (Fig. 2). The direct effects of honeysuckle cover removal on seedling abundance are more pronounced on rare species (Table 1, Fig. 2). Interestingly, cover removal increased the effect of consumer exclusion on rare species abundance, probably because seedling densities were so low when cover was present making it difficult to access the impact of predators (Fig. 2).

Lonicera maackii's close association with frugivorous birds and its copious fruit production also seem to be a key strategy used by the plant to colonize native habitats (Bartuszevige and Gorchov 2006, McCusker et al. 2010). In fact, propagule pressure is often cited as a mechanism used by invasive plants to outcompete its native competitors (Colautti et al. 2006, Richardson and Pysek 2006). Our results report for the first time that the direct effects of *L. maackii* fruit production also have the potential to determine the abundance of certain native's seedlings (e.g. *Geum* spp. Fig. 2). Most likely *Geum* spp. seedlings compete with *L. maackii* for the same resources, with the invader having a competitive advantage over the native. Removal of *L. maackii* fruits led to a two-fold increase on *Geum* spp. recruitment (Fig. 2), but more importantly the effect of fruits on recruitment seems to be larger when the vegetation cover is removed (Fig. 2) showing that physical presence of the invasive still plays a role on inhibiting seedling recruitment.

Cover removal also had a positive effect on the invasive itself (Fig. 2). Indeed *L. maackii's* recruitment was significantly higher when cover was removed. Fruit removal areas, however, presented more seedlings than fruit present areas. Our results indicate that an interaction of both factors can indeed determine invasive's recruitment. Although our findings are in agreement with Bartuszevige and Gorchov's (2006) suggestion that seed dispersal is an important force for the invasion, our results are in disagreement with their suggestion that established *L. maackii* populations are likely to recruit more shrubs beneath their canopies. Even though we believe that recruitment in invaded areas is possible, our data suggest that intraspecific competition may be an important force limiting the invasion. Indeed other studies have suggested that intraspecific competition plays an important role in limiting the invasion (Romanek 2009). But most importantly our results show that areas with honeysuckle removed and fruits present had the greatest abundance of *L. maackii*, indicating that removal of invasive adults without curtailing the effects of seed dispersal can result in maximum recruitment.

Evidence suggests that mice and deer actively consume *L. maackii* seeds (Williams et al. 1992, Vellend 2002). In our study sites it was common to find mouse seed caches and deer droppings containing *L. maackii* fruits and seeds. However, consumers had no effect on *L. maackii* recruitment, possibly because the infestation levels at our study site were so high (Dutra et al. 2011) that the large quantities of fruits available were sufficient to satiate mice. Other studies have found that seed predation can be lower at sites with high fruit density (Manson et al. 1998).

Our study supports the hypothesis that consumer pressure can modify the impact of plant invasion on diversity, and at the same time our data show that generalization about the indirect effects of plant invasions can be as difficult as extrapolations regarding direct effects. If the overall goal of biological invasions studies is to acquire a better understanding of the invasion in order to provide tools that help mitigate their detrimental effects, then future studies on invasion should incorporate as many indirect effects as possible, as they have been proven over and again to modify the outcome of invasions.

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Figure legends

Fig. 1: Box-plot showing changes on seedling abundance due to experimental manipulations on consumer's pressure and invasive's vegetation cover and fruits availability. Circles represent the number of seedlings on each area.

Fig. 2: Box-plot showing changes on seedling abundance for the most common species and the *L. maackii* due to experimental manipulations on consumer's pressure and invasive's vegetation cover and fruits availability. Circles represent the number of seedlings on each area.

Fig. 3: Box-plots showing changes on observed seedling species richness due to experimental manipulations on consumer's pressure, invasive's vegetation cover and fruits availability. Circles represent the number of seedlings on each area.

Fig. 4. Effects of experimental manipulations (consumer's pressure, invasive vegetation cover and fruit availability) on: A) Expected seedlings' species richness at N=65; B) inverse Simpson's diversity index. Data represent mean and 95% confidence interval

Table 1. Results of mixed model analysis showing the effects of honeysuckle cover, fruits, and consumers exclusion on seedling recruitment. See text for details.

Effect	**DF**	**F**	**P**
A. Seedling abundance			
Cover	1,6	9.91	0.019
Fruit	1,6	0.79	0.41
Cover X fruit	1,6	0.10	0.76
Exclosure	2,16	14.45	0.0003
Exclosure X cover	2,16	0.14	0.87
Exclosure X fruit	2,16	0.79	0.47
Cover X fruit X exclosure	2,16	0.19	0.83
B) Observed species richness			
Cover	1,6	21	0.0038
Fruit	1,6	0.01	0.97
Cover X fruit	1,6	0.35	0.57
Exclosure	2,16	31.61	0.0001
Exclosure X cover	2,16	2.76	0.093
Exclosure X fruit	2,16	3.63	0.05
Cover X fruit X exclosure	2,16	1.02	0.38
C) Ageratina altissima			
Cover	1,6	4.14	0.088
Fruit	1,6	0.01	0.92
Cover X fruit	1,6	0.01	0.92
Exclosure	2,16	3.08	0.074
Exclosure X cover	2,16	2.05	0.16
Exclosure X fruit	2,16	0.10	0.91
Cover X fruit X exclosure	2,16	0.27	0.77

D) Carex spp.			
Cover	1,6	1.1	0.33
Fruit	1,6	1.11	0.34
Cover X fruit	1,6	0.52	0.50
Exclosure	2,16	3.5	0.055
Exclosure X cover	2,16	1.14	0.34
Exclosure X fruit	2,16	0.91	0.42
Cover X fruit X exclosure	2,16	0.01	0.99
E) Geum spp.			
Cover	1,6	0.5	0.51
Fruit	1,6	4.41	0.08
Cover X fruit	1,6	5.34	0.06
Exclosure	2,16	7.54	0.0049
Exclosure X cover	2,16	0.19	0.83
Exclosure X fruit	2,16	1.6	0.23
Cover X fruit X exclosure	2,16	0.34	0.71
F) Lonicera maackii			
Cover	1,6	29.78	0.0016
Fruit	1,6	8.66	0.026
Cover X fruit	1,6	4.50	0.078
Exclosure	2,16	0.73	0.49
Exclosure X cover	2,16	0.01	0.99
Exclosure X fruit	2,16	0.07	0.93
Cover X fruit X exclosure	2,16	1.05	0.37
G) Common species			
Cover	1,6	3.92	0.095

Fruit	1,6	1.54	0.26
Cover X fruit	1,6	1.73	0.24
Exclosure	2,16	5.38	0.016
Exclosure X cover	2,16	1.87	0.19
Exclosure X fruit	2,16	2.68	0.10
Cover X fruit X exclosure	2,16	0.78	0.47
G) Rare species			
Cover	1,6	20.39	0.004
Fruit	1,6	2.18	0.19
Cover X fruit	1,6	2.49	0.16
Exclosure	2,16	11.61	0.0008
Exclosure X cover	2,16	4.55	0.028
Exclosure X fruit	2,16	2.55	0.11
Cover X fruit X exclosure	2,16	0.93	0.42

Figure 1

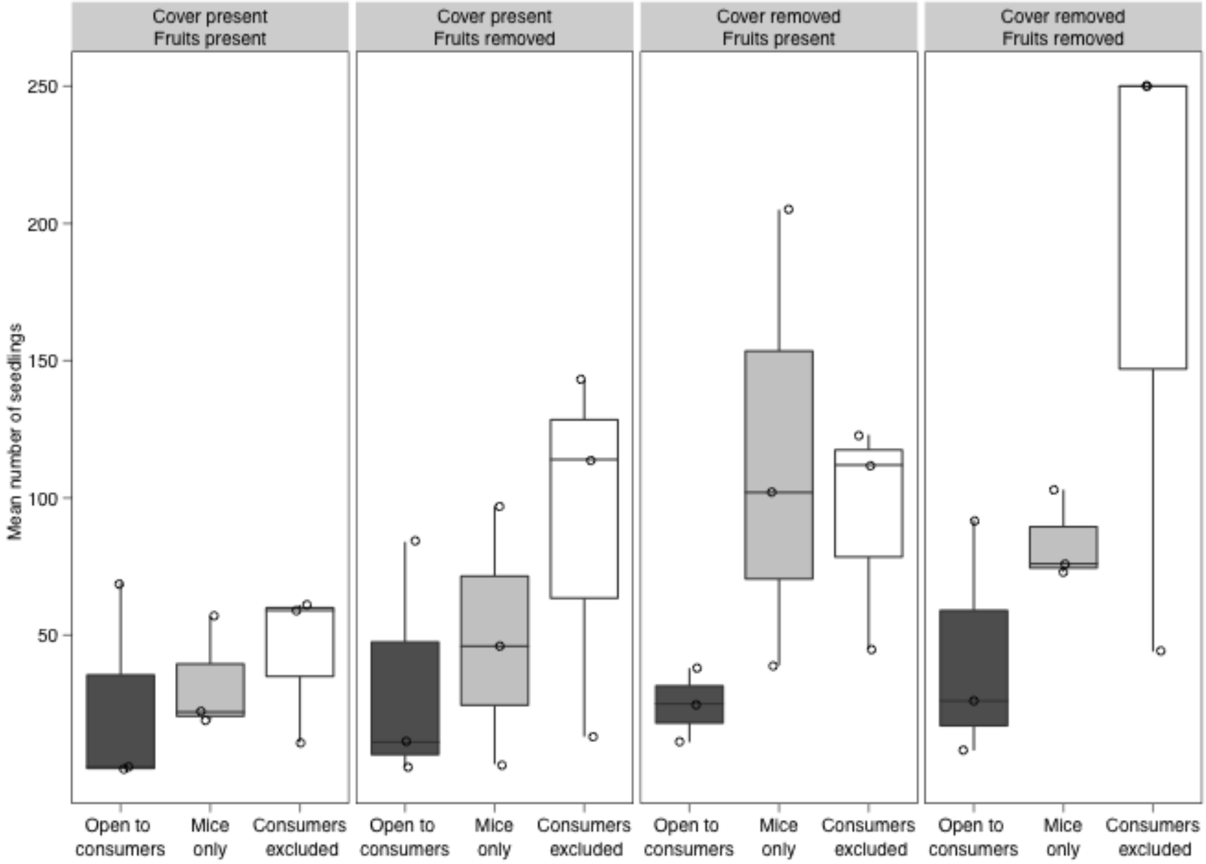
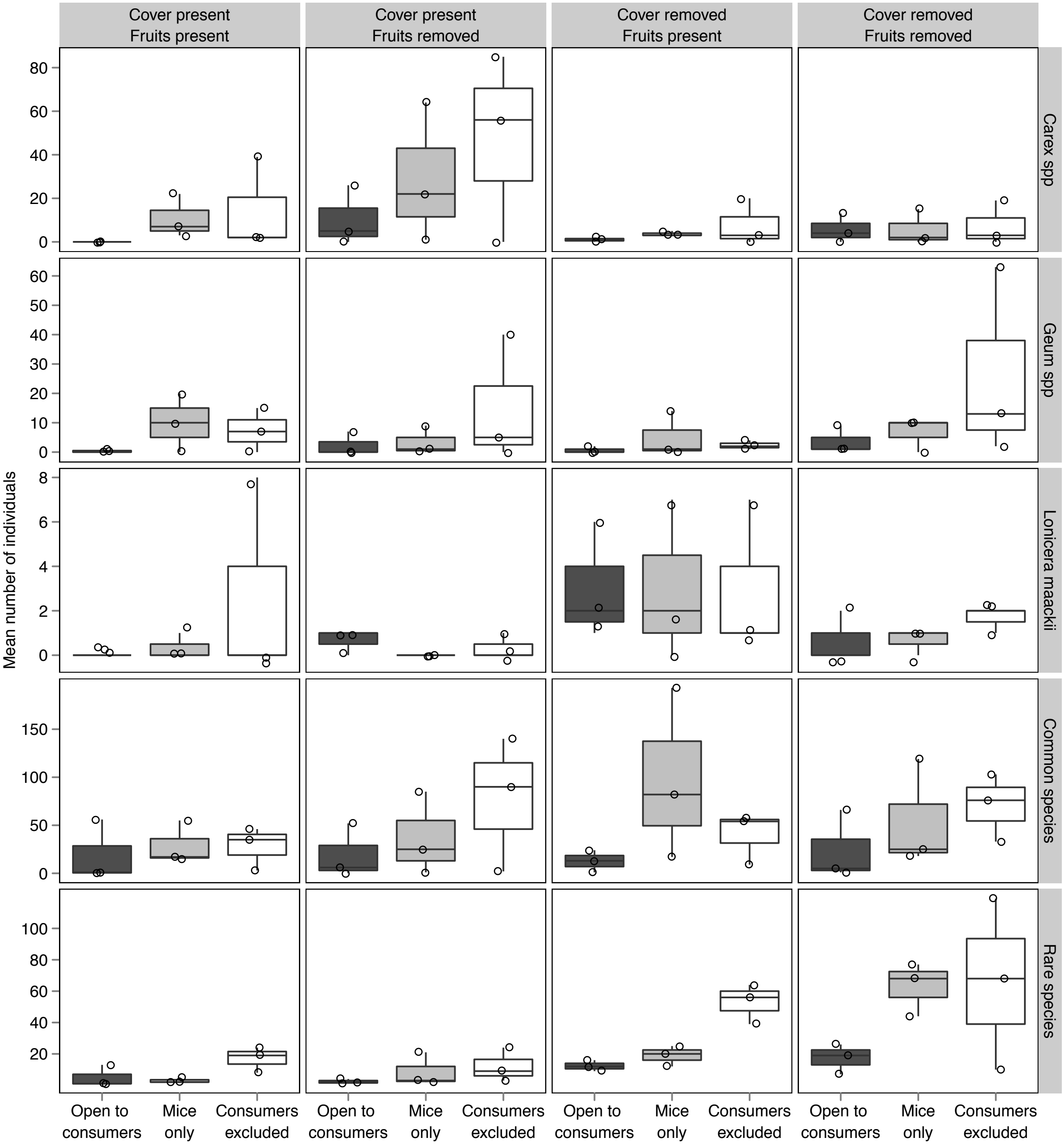

Cover present
Fruits present
Cover present
Fruits removed
Cover removed
Fruits present
Cover removed
Fruits removed
Mean number of seedlings
250
200
150
100
50
Open to consumers
Mice only
Consumers excluded
Open to consumers
Mice only
Consumers excluded
Open to consumers
Mice only
Consumers excluded
Open to consumers
Mice only
Consumers excluded

Figure 2



Cover present
Fruits present
Cover present
Fruits removed
Cover removed
Fruits present
Cover removed
Fruits removed
Carex spp
Geum spp
Lonicera maackii
Common species
Rare species
Mean number of individuals
0
20
40
60
80
0
10
20
30
40
50
60
0
2
4
6
8
0
50
100
150
20
40
60
80
100
Open to consumers
Mice only
Consumers excluded

Figure 3

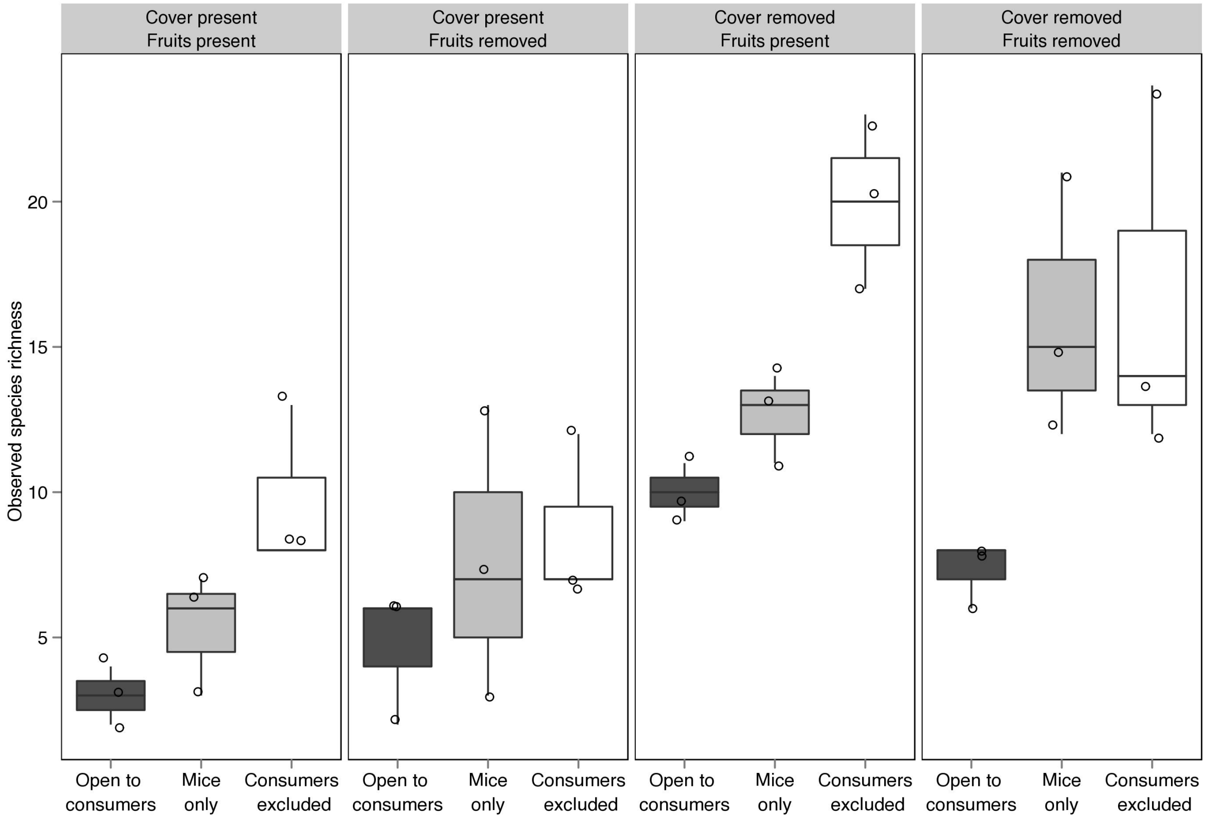
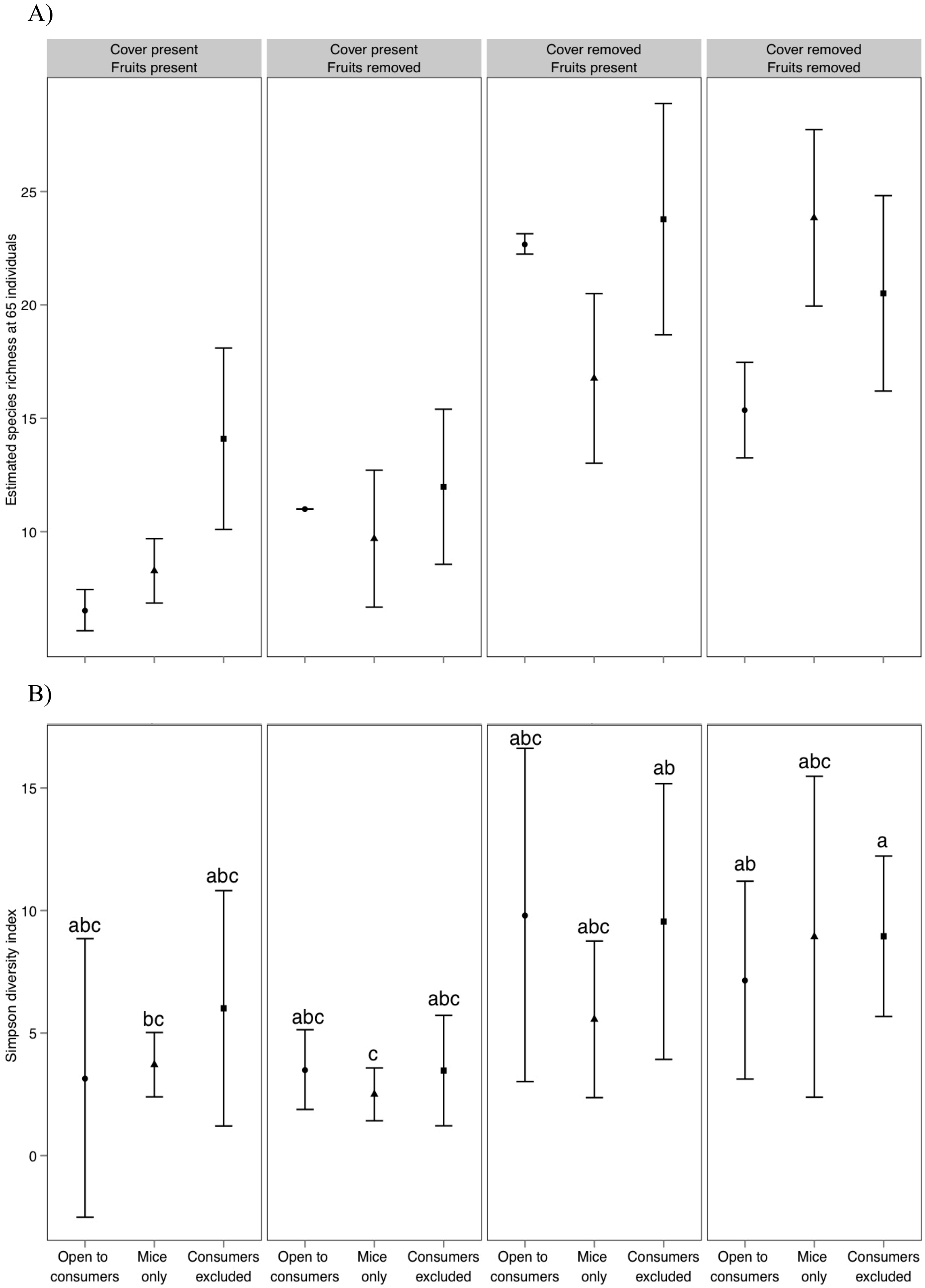

Cover present
Fruits present
Cover present
Fruits removed
Cover removed
Fruits present
Cover removed
Fruits removed
Observed species richness
20
15
10
5
Open to consumers
Mice only
Consumers excluded
Open to consumers
Mice only
Consumers excluded
Open to consumers
Mice only
Consumers excluded
Open to consumers
Mice only
Consumers excluded

Figure 4


A)
Cover present
Fruits present
Cover present
Fruits removed
Cover removed
Fruits present
Cover removed
Fruits removed
Estimated species richness at 65 individuals
25
20
15
10
B)
Simpson diversity index
15
10
5
0
abc
bc
abc
abc
c
abc
abc
abc
ab
ab
abc
a
Open to
consumers
Mice
only
Consumers
excluded
Open to
consumers
Mice
only
Consumers
excluded
Open to
consumers
Mice
only
Consumers
excluded
Open to
consumers
Mice
only
Consumers
excluded

Chapter 5

Reproductive biology of Amur Honeysuckle (*Lonicera maackii*) (Rupr.) Herder

ABSTRACT

Pollination biology can play important role in the population dynamics and success of invasive species especially when those species require pollination for seed production. And yet there are few detailed studies of the pollination biology of invasive plant species that adapted for animal pollination. In this study, we performed a detailed investigation of the breeding system of the invasive Amur honeysuckle (*Lonicera maackii*). Using a combination of 12 pollination treatments we verified if the plant's reproductive success is pollen limited and quantified the effects of autogamy, geitonogamy and xenogamy on fruit production, seed set, seed mass and germination success. Our results show that *L. maackii* is not completely self incompatible, can self on its own, and its fruit and seed production are largely dependent on pollinators. Indeed, addition of xenogamous pollen to open flowers greatly increased fruit and seed production in relation to unmanipulated flowers. Pollination treatments did not affect levels of seed germination but they did affect seed mass. Our study reports a unique situation in which inbred pollen, autogamous and geitonogamous, has a detrimental effect on fruit production, and at the same time geitonogamy yields more seed per fruits and heavier seeds then xenogamy. This suggests that geitonogamy can be an important aspect of plant invasions. The higher abundance of fruits in xenogamous treatments may have resulted in fewer resources per fruit and consequently reduced seed mass. Future studies at early stages of invasion when abundance is low and geitogamy is favored, may help clarify the role of geitonogamy in plant invasions.

INTRODUCTION

Although the ‘ideal weed’ or invasive plant species is considered to be one that is either autogamous or has very unspecialized pollination requirements (Baker, 1974), an increasing number of studies report highly invasive plant species that depend on outcrossing (Malet et al., 1992; Harrod & Taylor, 1995; Barthell 1996; Jesse et al., 2006; Stout, 2007). For invasive species that require pollinators for reproduction, the success of invasion and diverse aspects of the invasion dynamics can depend on interactions between the invasive plant and the native pollinators that it encounters. For instance, lack of proper pollination services could reduce propagule production in the invasive species. Reduced seed production may then reduce plant recruitment and diminish the spread of the invasion in absence of other limiting factors (Parker, 1997). In contrast, invasive plant species may foster their own success by recruiting pollinators away from native species, enhancing its own seed production while reducing the reproductive success of native species. Animal pollinated invasive species often have showy floral displays (White & Stiles, 1992; Vila & D’Antonio, 1998; Chitka & Schurkens, 2001) to recruit native pollinators (Harmon-Threatt et al., 2009). Despite being efficient in attracting pollinators away from native plants (Moragues & Traveset, 2005; Traveset & Richardson, 2006; Muñoz & Cavieres, 2008), reproduction in many invasive plants can be pollen- and therefore pollinator-limited (Barthell et al., 2001; Goodell & Iller, 2007; Harmon-Threatt et al., 2009).

There is still little quantitative information available on the breeding biology and pollination requirements of most invasive species. As a result, the role of pollinators in plant invasions is poor understood (Knight et al., 2005; Lloret, 2005; Harmon-Threatt et.

al., 2009). Moreover, many pollination biology studies often contrast only selfing and outcrossing with an open pollination treatment. This experimental design covers only a part of the possible types of plant breeding (Dafni, 1993). Studies with a full scheme of controls and other crosses are desirable to clarify the array of breeding system possibilities, including autogamy, apomixis, geitonogamy, and xenogamy (Dafni, 1993).

Amur honeysuckle (*Lonicera maackii* (Rupr.) Herder, Caprifoliaceae) is a highly successful and aggressive invasive, originally native to northeastern Asia. It has escaped cultivation and become the dominant shrub in many urban forests of the eastern United States (Luken & Goessling, 1995; Gould & Gorchov, 2000; Gorchov & Trisel, 2003; Miller & Gorchov, 2004; Hutchinson & Vankat, 1997). Each individual can produce thousands of large (approximately 1 cm in length), nectar-producing flowers. These flowers are visited mostly by honeybees *Apis melifera,* some native bees, and occasionally butterflies and hummingbirds (Goodell & Iller, 2007, 2010; HPD personal observation). Flowers change color from white to yellow upon being pollinated, also indicating that animal pollinators are important for reproduction, as this color change is considered to be a signal to pollinators of reduced reward in pollinated flowers (Weiss, 1991).

A recent study indicates that *Lonicera maackii* may experience pollination limitation (Goodell & Iler, 2010). The authors hypothesize that differential geitonogamous pollination ratios may explain spatial variation in fruit set. The contribution of geitonogamy in *L. maackii* reproduction is yet to be quantified.

In this study we performed a detailed investigation of the breeding system biology of *Lonicera maackii.* We used 12 pollination treatments, and associated germination experiments (see Stout, 2007), to answer the following questions:

1) What is the compatibility system of the honeysuckle? Does it produce fruits through autogamy, geitonogamy or apomixis (fruit production without fertilization)?

2) Is fruit production pollinator-limited at our study site?

3) What are the effects of inbreeding on fruit production, in terms of fruit set, number of seeds produced per fruit, seed mass, and germination success?

METHODS

This study was conducted in the St. Louis Metropolitan Area, at the Litzsinger Road Ecology Center, an urban nature reserve in Ladue, Missouri. The total area of the center is 14.5 ha, 5.6 of which are characterized as bottomland hardwood forest with a dense tree canopy system composed of *Acer negundo* (box elder), *Aesculus glabra* (Ohio buckeye), and *Platanus occidentalis* (sycamore) (Ochs, 1993). The understory is completely dominated by *L. maackii* with large patches of *Laportea canadensis* (wood nettle) and a dense ground layer of *Eunomys fortunei* (wintercreeper).

The breeding system of *L. maackii* was studied by performing a series of hand pollinations on 12 plants, each ~ 3 m tall. Plants were at least 5 m apart to ensure independence. Within each plant we selected 12 branches with 12 to 16 flowers each. Each branch was randomly assigned to one of twelve pollination treatments. The treatments were: 1) spontaneous autogamy, flower buds were bagged but no further manipulation was carried out; 2) autogamy, flowers were bagged and pollinated by bending their own dehiscent anthers to touch their stigma; 3) geitonogamy, flowers were bagged and pollinated with pollen from different flowers within same plant; 4) xenogamy, flowers were bagged and pollinated using pollen of different plants; 5) strict geitonogamy, emasculated flowers were bagged and pollinated with pollen from different flowers within the same plant; 6) strict xenogamy and emasculation, emasculated flowers were bagged and pollinated using pollen of different plants; 7) apomixis, flowers were emasculated, bagged, and no further manipulation was carried out, no pollen was added to their stigmas; 8) open flowers, pollinators were granted full access to the flowers which were not manipulated, no pollen was added; 9) autogamy supplementation, open flowers were pollinated by bending their own dehiscent anthers to touch their stigma; 10) geitonogamy supplementation, open flowers were pollinated with pollen from different flowers within same plant; 11) xenogamy supplementation, emasculated flowers were pollinated using pollen of different plants; 12) open no selfing, flowers were emasculated and no further manipulations were carried out, no pollen was added (Table 1).

Bagging was done with sewn bridal veil (1 mm mesh) bags placed on branches prior to flower opening so that all pollinators were excluded in the relevant treatments. Pollination was performed using a paintbrush, with the exception of the autogamy treatments. Brushes were cleaned with a 70% ethanol solution after each pollination. Emasculation treatments were used to control for selfing of the same flower and were performed utilizing a forceps by removing all anthers prior flower opening and anther dehiscence. Prior to the flower opening, all immature anthers were removed. Pollen for geitonogamy treatments was obtained by touching the paintbrush to five donor flowers of the focal plant. Pollen for the xenogamy treatment was obtained from at least five dehisced flowers from each of five randomly chosen plants that were not part of the experiment. Pollen was collected by clipping anthers of each flower and storing them in a plastic vial. All hand pollinations were conducted between 8:00-12:00 hrs. Honeysuckle flowers usually open at dusk. A qualitative experiment that consisted of dipping stigmas on hydrogen peroxide solution (Dafni, 1993) showed that flowers were still receptive the following morning (HPD unpublished data).

Bags were removed once all flowers were pollinated and all stigmas fell off (approximately one week after pollination) to allow development of the fruits. Fruits were counted in August but not harvested until early November 2008 when they were ripe and seeds hard. Fruits were brought to the lab and seeds were extracted and counted. Seeds were cleaned with distilled water to remove all pulp from the seeds. Seeds were then stored in paper envelopes, and allowed to dry for a week before germination experiments. We recorded the weight of 15 randomly selected seeds of each treatment (except treatments 1 and 7, that had only 7 and 6 seeds each).

An experiment in 2009 was performed to verify whether observed apomixis in the 2008 experiment was the result of contamination. Two groups of 12 to 16 flowers were selected from 12 *L. maackii* individuals and assigned to treatments 7 and 8. However, this time we used pollen proof pollination bags (Midco Enterprises, Saint Louis, MO) to prevent contamination.

A seed germination experiment was conducted to investigate how each pollination treatment affected germination success. Sixty seeds were randomly chosen from each bagged pollination treatment, except from treatments 1, 2 and 7, which produced only 7, 26 and 6 seeds, respectively, and therefore were excluded from this experiment. Seeds were stratified with gibberellic acid to break the dormancy requirements (Hydayati et al., 2000). Groups of 20 seeds were placed on two sheets of Whatman filter paper (No. 1) on Petri dishes (90 mm diameter × 15mm deep). The filter paper was moistened initially with a solution of 1000 mg/l GA_3 (potassium salt) dissolved in distilled water, and then seeds were moistened with distilled water only every 3 to 5 days. Petri dishes were double wrapped in cling wrap to prevent drying. Dishes were placed in germination chambers with a photoperiod of 12 by 12 hour cycle alternating light and dark and a thermoperiod of 25/15^{o}C. Dishes were randomly arranged in the growth chamber and randomly rotated every 3 to 5 days to spread edge effects evenly among treatments. Germination (the successful development of both cotyledons) was checked weekly for five weeks.

Statistical analysis

Response variables were proportion of fruits per number of pollinated flowers (fruit set) and mean number of seeds per fruit (seed set). Fruit set was arcsine-transformed while a cube root plus 0.5 transformations was applied to seed set to normalize the fitted residuals (Shapiro-Wilk, $w = 0.98$, $p = 0.21$, $w = 0.99$ $p = 0.81$, respectively). Breeding system data were analyzed with mixed effects models (PROC MIXED, SAS) using pollination treatment as a fixed independent variable and each plant a random block. Pre-planned contrasts were used with data subsets involving only the desired pollination treatments to test specific hypotheses and to detect differences between treatments. To verify whether the plant can self-pollinate in the absence of pollinators, we compared treatment 1 (spontaneous autogamy) with treatment 8 (open flowers). Also, to compare the effects of autogamy, geitonogamy, and xenogamy, we compared fruit and seed production between treatments 2 and 3, and between treatments 3 and 4. To verify the importance of pollinators on fruit and seed production we compared bagged treatments 2, 3, and 4 with their respective non-bagged counterparts (9, 10, and 11). Pair wise comparisons between emasculated and non-emasculated flowers were performed to evaluate the impact of selfing on apomixis (1 and 7), outcrossing (8 and 12), geitonogamy (3 and 5) and xenogamy (4 and 6). Finally pollination limitation was evaluated by comparing treatment 8 (open flowers, no pollen addition) with treatment 11 (open xenogamy) whose flowers were supplemented with outcrossed pollen. We used a one-way ANOVA (PROC GLM, SAS) to compare the effects of the pollination treatments on seed mass.

We calculated the mean self-compatibility index (SCI), defined as average fruit set after self-pollination divided by the fruit set after cross-pollination (Lloyd and Schoen 1992). This index corrects for the success of self-pollination for poor pollinating conditions (e.g. variations in plant vigor, physiological limitations of seed production). We calculated an autogamy-SCI (mean seed set treatment 2 divided by mean seed set treatment 6) and geitonogamy-SCI (mean seed set treatment 3 divided by mean seed set treatment 6) for each plant and then calculated the mean for the population. Finally, we calculated the autofertility index (AFI), the seed set of spontaneous autogamy (treatment 1) plants divided by that of artificial cross-pollinations (treatment 6). The denominator of AFI homogenizes for the effects of variable pollinating conditions, and represents an estimate of the amount of self-fertilization (Lloyd and Schoen 1992).

Germination trials were analyzed using a repeated measures mixed effects model with time and pollination treatment as fixed effects and Petri dish identity as a random effect. Treatments 1, 2 and 7 were not included in the analysis due to small sample size.

Tropical storm Ike hit the study area in September 2008; knocking down three plants entirely and some branches of other plants. Fruit production analyses were conducted with all 12 plants except for treatment 2 that had one group of flowers knocked down in August before the storm. Seed set analysis had a variable sample size as indicated in the results (see Table 1).

RESULTS

There was a significant effect of the pollination treatments on fruit production ($F_{11,121}$ 17.24, $p < 0.0001$) and seed set ($F_{11,85} = 6.13$, $p < 0.0001$). Plant identity also affected fruit and seed production ($F_{11,121} = 2.86$, $p = 0.014$; $F_{8,85} = 3.38$, $p = 0.002$, respectively).

Autogamous self pollination and dependence on insects

The results of treatment 1, spontaneous autogamy, indicated that this plant species does little selfing, as just 15.6% ± 5.0 of flowers in this treatment became fruits and those that did mature fruits produced few seeds (0.6 ± 0.2 seeds per fruit). Flowers open to pollinators (treatment 8) were more likely to produce fruits (43.2% ± 9; $F_{1,121} = 9.86$, $p = 0.002$) and more seeds per fruit (2.1 ± 0.5; $F_{1,85} = 8.90$, $p = 0.003$) than spontaneous autogamy (treatment 1) (fig. 1). In 2009 none of 12 plants assigned to pollen proof bags produced fruits, as opposed to the open treatment in which on average, 47% ± 8 of the flowers became fruits. In addition, in 2008; emasculation did not affect fruit or seed production for the open / no pollen added treatments 8 and 12 ($F_{1,121} = 0.77$, $p = 0.38$, $F_{1,85} = 0.29$, $p = 0.59$), geitonogamous treatments 3 and 5 ($F_{1,121} = 1.20$, $p = 0.27$, $F_{1,85} = 0.1$, $p = 0.75$), and xenogamous treatments 4 and 6 ($F_{1,121} = 0.02$, $p = 0.87$, $F_{1,85}.= 0.01$, $p = 0.93$, fig. 2), indicating that the autogamous selfing contributes little to fruit and seed production. Together these results indicate that *L. maackii* fruit and seed production are largely dependent on insects.

Autogamy vs. geitonogamy vs. xenogamy

On average geitonogamous bagged flowers (treatment 3) produced a fruit 26.4% ± 8.0 of the time while 15.5% (± 4.0) of autogamous bagged flowers (treatment 2) produced fruits. This difference was not significant ($F_{1,121} = 1.19$, $p < 0.27$, fig. 1). Similarly, the number of seeds per fruit did not differ between these treatments ($F_{1,85} = 2.17$, $p = 0.14$, fig. 1). Xenogamous bagged flowers (treatment 4) produced the most fruits of all treatments ($F_{1,121} = 35.13$, $p < 0.0001$) with nearly 80% of the flowers producing a fruit, but producing slightly more seeds per fruit than bagged geitonogamous and autogamous flowers ($F_{1,85} = 3.67$, $p = 0.06$, fig. 1). A comparison between all bagged treatments vs. all non-bagged treatments (fig. 1) showed that overall, insects significantly increased fruit production by 11% ($F_{1,121} = 5.11$, $p = 0.03$). Flowers from open treatments produced 40% more seeds per fruit than bagged treatments ($F_{1,85} = 7.67$, $p = 0.006$, fig. 1).

Geitonogamous pollen supplementation to open flowers (treatment 10) did not increase fruit production relative to autogamous pollen supplementation (treatment 9) ($F_{1,121} = 0.11$, $p < 0.74$, fig. 1), but open geitonogamous treatment produced 66% more seeds per fruit than the open autogamous treatment ($F_{1,85} = 5.64$, $p = 0.02$, fig. 1). Similarly, open flowers supplemented with xenogamous pollen (treatment 11) yielded more fruits than did open geitonogamy ($F_{1,121} = 18.7$, $p < 0.001$), but xenogamous pollen supplementation did not result in more seeds per fruit than flowers pollinated with geitonogamous pollen ($F_{1,85} = 1.79$, $p = 0.18$).

Pollen limitation

Xenogamous pollen supplementation to open flowers (treatment 11) resulted in the highest fruit production of the experiment (81% ± 5), almost double that of open flowers with no pollen supplementation (treatment 8; 43% ± 9, $F_{1,121} = 15.24$, $p = 0.0002$). However, the number of seeds per fruit did not differ between treatments 8 and 11 ($F_{1,85} = 1.39$, $p = 0.24$).

Self-compatibility and auto-fertility indexes

The self compatibility index (SCI) was 0.38 ±0.09 for autogamously pollinated flower and 0.64 ±0.14 for geitonogamous flowers. The auto-fertility index (AFI) for *L. maackii* was 0.24 ±0.1.

Seed mass

Lonicera maackii seeds weighed on average 3.9 mg ± 0.0. Results of our pollination experiment showed that pollination treatments had a significant effect on seed mass ($F_{1,51} = 2.86$, $p = 0.002$). Pollinator services pollen seemed to reduce seed mass as open flowers exposed to outcrossing produced heavier seeds than bagged flowers (3.8 mg ± 1.4 and 4.4 mg ± 0.2, respectively, $F_{1,151} = 6.38$, $p = 0.01$). Similarly geitonogamous flowers (treatment 3) produced significantly heavier seeds than xenogamous flowers (treatment 4, $F_{1,151} = 4.82$, $p = 0.03$). Also, autogamous flowers (treatment 2) were heavier than xenogamous (treatment 4, $F_{1,151} = 4.35$, $p = 0.04$, fig. 1). Moreover, seeds of open flowers (treatment 8) were slightly heavier than those from open flowers supplemented with xenogamous pollen supplementation (treatment 11) ($F_{1,151}$ = 3.41, p = 0.07 figs. 1 and 2).

Seed germination

There were no significant effects of pollination treatments ($F_{8,64}$ = 1.38, p = 0.22), nor an interaction of time with treatment ($F_{24,64}$ = 1.06, p = 0.41), on seed germination. On average 92.6 ± 1.4 of seeds germinated successfully.

DISCUSSION

Our results show that pollen source (i.e., autogamy, geitonogamy, xenogamy) may influence *Lonicera maackii's* reproductive success as it affects fruit production, mean number of seeds per fruit, and seed mass. *Lonicera maackii* bagged flowers were incapable of producing fruit and seeds through apomixis (treatment 7), and the plant did little autogamy on its own (treatment 1). The plant has some degree of self-incompatibility, as indicated by the relatively low SCI levels, and it is able to produce a few fruits through autogamy. Indeed, the auto-fertility index suggests that approximately 24% of the fruit production can be a result of autogamy. Our results also suggest that *L. maackii* fruit production can be pollen limited and that geitonogamy can be an important factor in the pollination biology of this invasive. Moreover, our results add to the current body of studies (Levin, 1970; Harrod & Taylor, 1995; Barthell, 1996; Larson et al., 2002; Jesse et al., 2006; Stout, 2007) that show that highly invasive species are not completely autogamous and self-compatible as previously suggested (Baker, 1974). Indeed *L. maackii* fruit production is largely dependent on pollinators to effect cross pollination between plants.

Xenogamous pollen loads (treatments 4 and 11) yielded 3-5 times more fruits and increased seed set over that of autogamy and geitonogamy. This result indicates that self-pollen reduces fruit production in *L. mackii*, as is true for other outcrossing plant species (Barrett et al., 1996). For instance, the invasive *Rhodondendrom pontiacum* has reduced seed set both in the presence and absence of pollinators when self pollen is used to fertilize the flower (Stout, 2007). In *L. maackii* the source of self pollen (autogamy and geitonogamy) did not influence fruit production when only self pollen was used (bagged flowers). However, geitonogamy pollen supplementation to open flowers yielded 60% more seeds per fruit than autogamous open flowers, and indeed yielded the highest number of seeds per fruit of all pollination treatments.

Geitonogamy is a common strategy observed for plants with showy floral displays like *L. maackii* (Eckert, 2000; Anderson et al., 2001; Stout, 2007; Williams, 2007). Although large floral displays are effective in attracting pollinators, they can create a tradeoff between the benefits of increased pollinator visitation and the quantity of geitonogamous pollen received (Williams, 2007). High levels of geitonogamy may lead to inbreeding depression as it reduces the genetic variability of plant's offspring and may reduce fruit and seed production (Harder & Barrett, 1995). In this study, fruit production was reduced, seed production increased, and seed quality, as estimated by seed mass, was slightly increased in geitonogamous versus xenogamous crosses. *Lonicera maackii* individuals produce hundreds of thousands of flowers (Ingold & Craycraft, 1983) and often attract pollinators such as honeybees (*Apis melifera*) that are known to visit multiple flowers of the same plant, thus promoting geitonogamy (Goodell & Iler, 2010; HPD personal observation). Our study shows that geitonogamous pollen supplementation may result in a numerical advantage in terms of seeds per fruit (fig. 1) and may constitute a critical source of propagules and recruitment during the invasion

Seed mass was also affected by pollination treatments. However, contrary to expectations that self-fertilization maybe disadvantageous for the plant (Lloyd & Schoen, 1992), our results show an advantage of selfing over outcrossing as geitonogamous bagged flowers produced heavier seeds than xenogamous flowers. In fact, all bagged flowers, excluded from any xenogamous pollen, also produced heavier seeds than flowers exposed to pollinators. Moreover, both autogamous treatments (bagged and open, treatments 2 and 9) produced the fewest number of fruits and heavier seeds than their xenogamous counterparts. Because fruits compete for resources within the plant (Galen et al., 1985, Broyles & Wyatt, 1993; Martin & Lee, 1993) it is possible that the higher number of fruits in xenogamous treatments resulted in fewer resources per fruit and consequently reduced seed mass relative to geitonogamy and autogamy, each which produced fewer fruits per flower. Indeed, posterior analysis of the data set shows a negative correlation between mean number of seeds and seed weight ($r = -0.67$, $z = 0.81$, $p = 0.05$), indicating that an interaction between the pollen source and number of fruits produced may determine seed mass.

Although several studies link seed size to germination success (e.g., Kalisz, 1989; Simons & Johnston, 2000; Halpern, 2005), our germination trials show that all treatments have similar germination levels regardless of treatment effects on seed mass. Thus, although pollination treatments affected fruit set, seed production, and seed mass, they did not affect seed quality, at least as measure in the laboratory. It is important to keep in mind that we used a chemical to break *L. maackii* dormancy requirements (Hydayati et al., 2000), and that under natural field conditions seed mass could be critical for seedling recruitment. Future studies should evaluate not only the effects of seed mass on cold stratification success but also on the relationship between seed mass and longevity in the seed bank, as this trait is also known to affect seedling recruitment and invasion success (Vivian-Smith & Panetta, 2009).

Pollinator exclusion severely reduced fruit and seed set, indicating that plant invasion success can be determined by the ability of *L. maackii* to attract the native pollinator community. In fact, just 16% of the fruits produced by the plant are a result of autogamy (Treatment 1) and open flowers without pollen supplementation can produce almost triple the number of fruits. *Lonicera maackii* is known for its copious fruit production (Ingold & Craycraft, 1983; Bartuszevige & Gorchov 2006), but surprisingly the plant is pollinator-limited and could be more productive depending on the pollinator services offered. Goodell & Iler (2010) also report pollination limitation in *L. maackii*. Because pollen limitation can be a function of population size (Ågren 1996; Spigler & Chang, 2009), it would be interesting to evaluate how the plant's breeding system varies with plant densities.

In conclusion, our study reports a unique situation in which geitonogamous pollen supplementation in an outcrossing pollinator limited invasive plant yields fewer fruits per flower but more seeds per fruit then xenogamy. In addition seeds produced by selfing (either autogamy or geitonogamy) can be heavier than seeds from outcrossing treatments (fig. 1). Therefore, selfing could potentiatlly result in a quantitative advantage in terms of seeds per fruit, and may also be qualitative superior because of the larger mass. However, this is counterbalanced by the massive fruit production resulted from xenogamy. The question that remains is to what extent mean seed number and seed mass influence the outcome of the invasion. Future studies should evaluate not only the interplay of xenogamy and selfing on plant recruitment but also verify the importance of these processes at different stages of the invasion. Eckert (2000) suggests that at early stages of the colonization, when plant densities are low, geitonogamy might be more frequent than xenogamy. In the case of *L. maackii* it is difficult to predict what is going to happen because we do not know what is more important for plant recruitment, fruit production, number of seeds per fruit, or seed mass. Possibly the significance of these factors and the different breeding systems used by the plant will vary with plant densities, and some forms pollination (i.e. autogamy, geitonogamy, xenogamy) might be favored over the other. Understanding the importance of each of these reproductive strategies seems to be critical for a better comprehension of plant invasions, and in the case of *L. maackii*, these strategies seem to affect propagule production and quality. Our study adds to the current body of literature reporting invasive plants matching the entire range of breeding systems (see Harmon-Threatt et. al., 2009 for review) and shows a successful invader that does not rely exclusively on selfing to produce seeds. It is becoming increasingly difficult to find a set of traits, including those that characterize the breeding system (Barrett et al., 2008), that are common to most invasive plants species.

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Table 1. Treatments used in the pollination experiment. Treatments are classified according to the manipulation performed: pollen source, whether flowers were bagged to exclude pollinators, and whether flowers were emasculated. Pollen sources are indicated as no pollen added by hand (None), autogamous pollen (A), geitonogamous pollen (G), and xenogamous pollen (X). Sample size refers to the number of plants used for estimating seed set (see text for details)

Treatment	**Pollen source**	**Bag**	**Emasculation**	**Sample size**
1. Spontaneous autogamy	None	Yes	No	9
2. Autogamy	A	Yes	No	8
3. Geitonogamy	G	Yes	No	9
4. Xenogamy	X	Yes	No	9
5. Strict Geitonogamy	G	Yes	Yes	9
6. Strict Xenogamy	X	Yes	Yes	9
7. Apomixis	None	Yes	Yes	9
8. Open flowers	None	No	No	9
9. Autogamy supplementation	A	No	No	9
10. Geitogamy supplementation	G	No	No	8
11. Xenogamy supplementation	X	No	No	9
12. Open, no selfing	None	No	Yes	9

Figure legends

Fig. 1. Effect of pollinator exclosure on proportion of fruit set, seed set, and seed mass of after being hand-pollinated with autogamous, geitonogamous, and xenogamous pollen. Numbers on the x-axis indicate treatment number (see Table 1 for details). Data are presented as means ± standard errors.

Fig. 2. Effect of emasculation on proportion of fruit set, seed set, and seed mass, after being hand-pollinated with autogamous, geitonogamous, and xenogamous or not receiving a pollen load. Numbers on the x-axis indicate treatment number (see Table 1 for details). Data are presented as means ± standard errors.

Figure 1

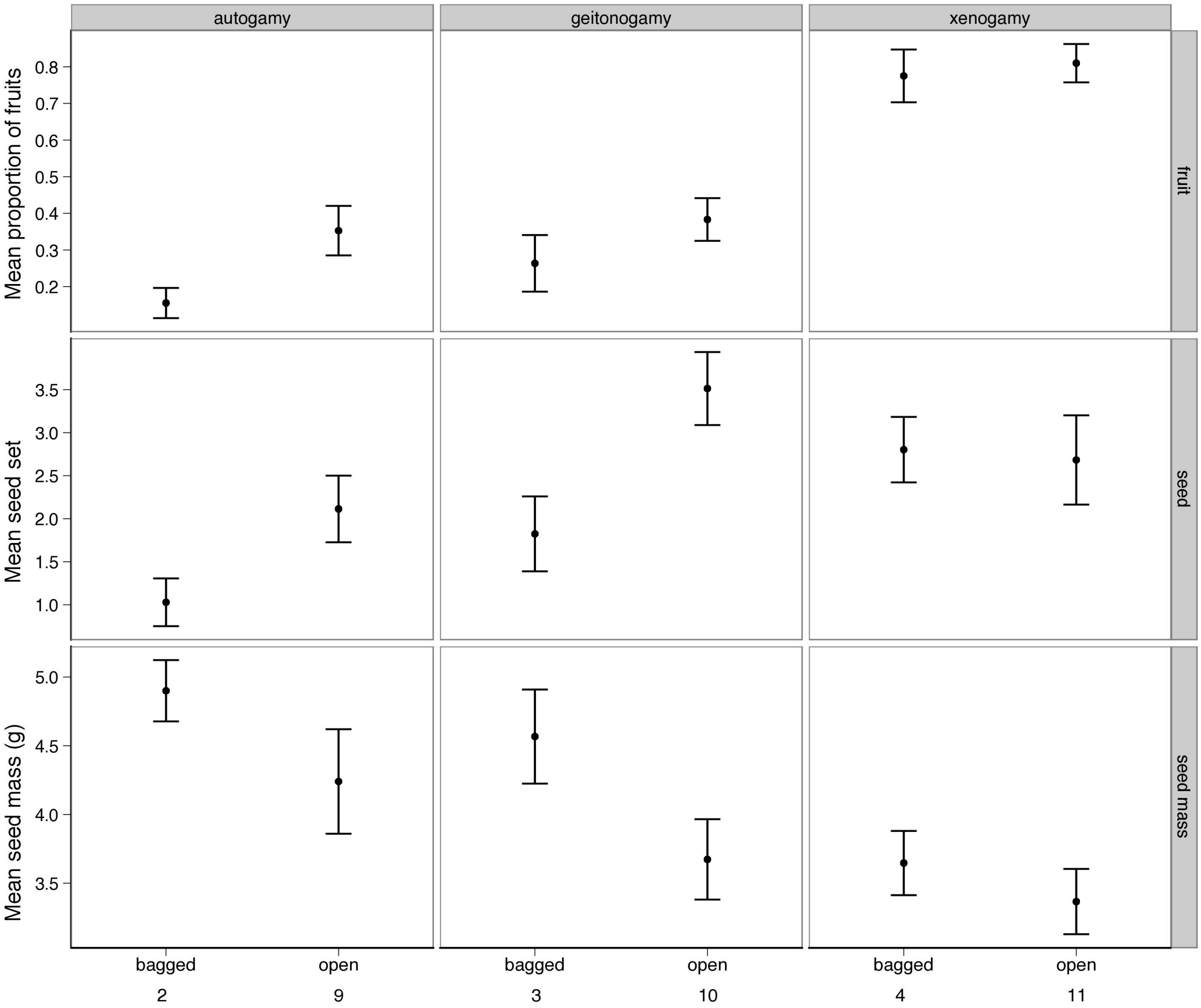
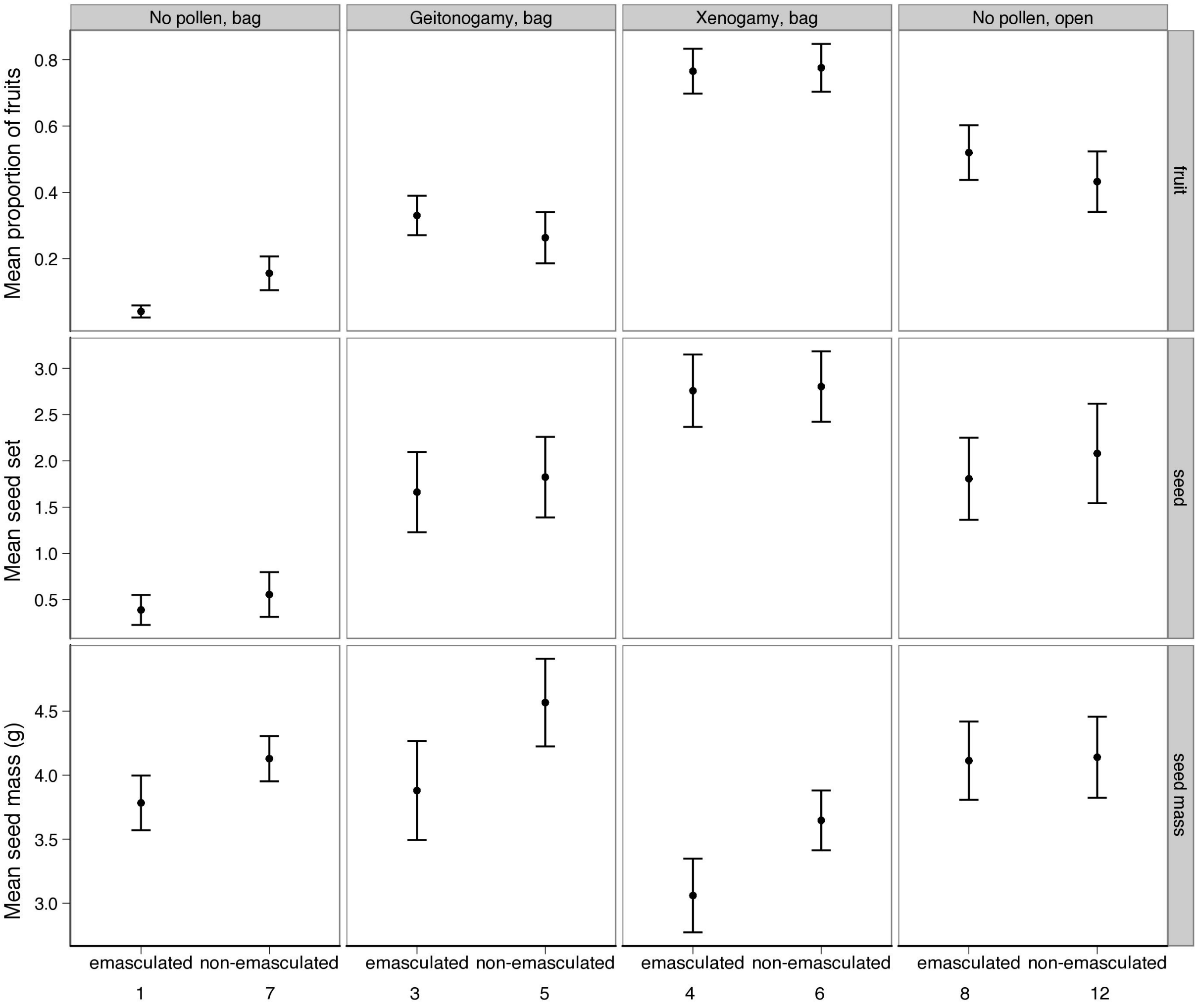

autogamy
geitonogamy
xenogamy
fruit
seed
seed mass
Mean proportion of fruits
0.8
0.7
0.6
0.5
0.4
0.3
0.2
Mean seed set
3.5
3.0
2.5
2.0
1.5
1.0
Mean seed mass (g)
5.0
4.5
4.0
3.5
bagged
open
bagged
open
bagged
open
2
9
3
10
4
11

Figure 2


No pollen, bag
Geitonogamy, bag
Xenogamy, bag
No pollen, open
Mean proportion of fruits
0.8
0.6
0.4
0.2
fruit
Mean seed set
3.0
2.5
2.0
1.5
1.0
0.5
seed
Mean seed mass (g)
4.5
4.0
3.5
3.0
seed mass
emasculated
non-emasculated
1
7
3
5
4
6
8
12