Invasive Plant Science and Management

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Patterns in selfed seed production and germination in Amur honeysuckle (*Lonicera maackii*)

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Research Article

Cite this article: Rivera BJ, Meilan R, and Jenkins MA (2025). Patterns in selfed seed production and germination in Amur honeysuckle (*Lonicera maackii*). Invasive Plant Sci. Manag **18**(e10): 1–10. doi: 10.1017/inp.2025.5

Received: 9 July 2024
Revised: 14 October 2024
Accepted: 10 January 2025

Associate Editor:
Ryan M. Wersal, Minnesota State University

Keywords:
Fecundity; invasion biology; invasive shrub; plant reproduction; population growth; seed production

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Abstract

Amur honeysuckle [*Lonicera maackii* (Rupr.) Herder] is an aggressive invader of forests throughout the eastern United States. While self-pollination has been identified as an important trait of invasive plant species, this trait is understudied, and *L. maackii* is anecdotally described as lacking this characteristic. To examine the ability of *L. maackii* to self-pollinate, we selected 171 individual shrubs distributed across nine sites. Each site was grouped into one of three invasion types: heavy, light, and sprouting (sites on which a basal cutting treatment previously occurred, but *L. maackii* was allowed to reestablish). We compared the number of berries, seeds per berry, and seed germination rates of self- and open-pollinated flowers by pairing branches covered with pollination bags before flower emergence with uncovered branches on the same individual shrub. Out of 171 individuals, 48 produced berries from self-pollination within pollination bags (28%), with 48% of bagged branches exhibiting some degree of necrosis or chlorosis, presumably due to increased temperature and humidity. Berries from self-pollination produced 1.5 ± 1.4 (mean ± 1 SD) seeds per berry, whereas berries resulting from open pollination produced 3.3 ± 1.5 seeds per berry. In a germination trial, 47.3% of self-pollinated seeds germinated compared with 41.7% of open-pollinated seeds. This study has shown that *L. maackii* can self-pollinate and set viable seed, providing the species with an important mechanism to increase population abundance during the early stages of invasion.

Introduction

Plant invasions typically occur in three phases that mimic a logistic growth curve: establishment, expansion, and saturation (Shigesada and Kawasaki 1997). Most species' invasions fail to advance beyond the establishment phase, a lag period in population growth that often lasts for decades (Wangen and Webster 2006). A new invasion moves into the expansion phase when founding individuals have reproduced enough to become self-perpetuating and exponential growth begins (Shigesada and Kawasaki 1997). Once the expansion phase is reached, it becomes much more difficult to control the spread of the invasion (Webster et al. 2006). Thus, many have suggested that focusing on control during the establishment phase is key to preventing negative effects caused by the spread of invasive plants (Radosevich et al. 2003; Webster et al. 2006).

Many traits contribute to the innate invasiveness of a plant species (Baker 1974; Sutherland 2004). One such trait common among many invasive plants is the ability to self-pollinate (Baker 1974; Barrett et al. 2008). Despite describing different events in the process, the terms "self-pollination," "self-compatibility," "self-fertilization," "apomixis," and "selfing" are used collectively within this article to describe this ability of plants to reproduce autogamously without the need for outcross pollen. While each mechanism is distinct, further delineation between mechanisms is outside the scope of this study. In an early assessment of the potential role of self-pollination in plant invasions, Baker (1955, p. 348) stated that "with self-compatible individuals, a single propagule is sufficient to start a sexually-reproducing [*sic*] colony (after long-distance dispersal), making its establishment much more likely than if the chance of two self-incompatible yet cross-compatible individuals sufficiently close together spatially and temporally is required." Originally dubbed "Baker's law" by Stebbins (1957), the concept that invasive plants more often possess this feature has been corroborated but needs more rigorous testing (Barrett 1996; Van Kleunen and Johnson 2007).

More recent studies have examined Baker's law across a range of invasive species and have upheld selfing as playing a critical role in invasion, specifically in long-distance dispersal (Barrett et al. 2008). An analysis of 361 plant species native to Europe, but invasive in North America, found that self-compatible species had larger introduced ranges than species that are not self-compatible, leading the authors to recommend the screening of non-native species for self-pollination before allowing their import (Van Kleunen and Johnson 2007). An analysis of 11

Management Implications

Lonicera maackii (Amur honeysuckle) is a dominant invasive shrub within forests of eastern North America. Invasions of non-native species typically occur in three stages: establishment, expansion, and saturation. *Lonicera maackii*'s "lag time" for reaching the expansion phase is relatively short compared with other woody invasive species. A better understanding of the mechanisms that lead *L. maackii* to quickly invade can help managers better understand how to prevent the spread of *L. maackii*, combat the invasion of similar species, and predict which species may become invasive.

Self-pollination—or "selfing"—is the ability of plants to reproduce autogamously without the need for outcross pollen. While the association of this trait with invasiveness has been well documented, it has been relatively understudied among woody invasives. Specifically, it has recently been questioned whether *L. maackii* possesses the ability to self-pollinate, which it was previously thought unable to do. In this study, we tested *L. maackii*'s ability to self-pollinate and produce viable seed by using pollination bags to prevent outcross pollen from reaching new flowers, then subjecting the resulting seeds to a germination trial. We examined how different conditions may play a role in the rate of selfing by replicating our study across different levels of invasion types. We predict that self-pollination would result in fewer berries and seeds compared with open pollination, but that their germination rates would not differ.

Overall, we found about 1 in every 400 flowers produced a self-pollinated seed when pollinators were excluded. Self-pollinated seeds (47.3%) germinated at significantly higher rates than those pollinated in regular field conditions (41.7%). While this rate is low, *L. maackii* can produce thousands of flowers in a single year. Thus, this ability to produce viable seeds without outcross pollen could help *L. maackii* expand while outcross pollen is limited during the early stages of an invasion, when there are few individual plants.

invasive plant species in South Africa once again supported Baker's law, in that uniparental reproduction led to successful establishment and persistence (Thorne 2015). While evidence from multiple studies has supported Baker's law, not all studies are unanimously supportive. An analysis of 19,960 plant species by Sutherland (2004) revealed that self-compatibility is more common in invasive plants compared with noninvasive plants, but the author attributed this relationship to weeds being more likely to be annuals or biennials and those groups being more likely to be self-compatible. However, the datasets used in the study made an unclear distinction between non-native, invasive weeds and non-native, noninvasive weeds, and the author acknowledges this as a caveat that may have obscured relationships in the analysis (Sutherland 2004).

As a functional group, woody species tend to be less self-compatible than non-woody species, and experience more intense inbreeding depression compared with non-woody plant species (Duminil et al. 2009). However, studies have shown that that some invasive woody species do exhibit self-compatibility. In an examination of 17 woody and non-woody invasive species in South Africa, Rambuda and Johnson (2004) found that all 13 woody species (trees, shrubs, and vines) examined displayed at least some self-compatibility. However, an in-depth study of a single invasive tree species, silver wattle (*Acacia dealbata* Link) that displayed self-compatibility found evidence of inbreeding depression in the form of fewer seeds per fruit, lower progeny survival, and decreased growth of offspring resulting from self-pollination compared with those resulting from outcrossing (Rodger and Johnson 2013). Therefore, there may be a trade-off between self-compatibility and fecundity.

Invasive woody plant species are particularly difficult and costly to control in the forests of eastern North America (Bailey et al. 2011; Rathfon and Ruble 2007; Webster et al. 2006). Amur honeysuckle [*Lonicera maackii* (Rupr.) Herder] is an archetypal example of an aggressive invasive shrub from East Asia (Luken and Thieret 1996) relying on both diffusion and long-distance dispersal invasion (Gorchov et al. 2014). While vigorous root growth, prolific sprouting, rapid maturation, high fecundity, and extended leaf phenology make *L. maackii* an aggressive competitor, the species can also alter nutrient cycling (Schuster and Dukes 2017) and may produce allelopathic chemicals (Bauer et al. 2012). These characteristics allow this species to suppress herbaceous-layer cover and species diversity (Gorchov and Trisel 2003; Hartman and McCarthy 2008; Hutchinson and Vankat 1997) and prevent the successful regeneration of overstory species (Hartman and McCarthy 2004; Shields et al. 2015).

While much is known about the pattern and rate of invasion by *L. maackii* (Deering and Vankat 1999; Shields et al. 2014), its mating system (Goodell et al. 2010), which is a critical aspect of invasion biology, remains obscure in the literature. Specifically, there has been little study of whether *L. maackii* is self-compatible. While commonly described in the literature as being self-incompatible, these descriptions, confusingly, do not cite any relevant primary literature (e.g., Deering and Vankat 1999). Examinations of selfing in *L. maackii* are limited; our review of the literature revealed just two studies. Goodell and Iler (2007) examined selfing in *L. maackii* by isolating flowers with pollination bags. They detected a low, but nonzero (<3% of bagged flowers produced a berry) rate of self-pollination in bagged flowers. They also manually supplied bagged flowers with pollen from neighboring flowers, which increased the production of berries and seeds but produced far fewer berries and seeds than open-pollinated flowers. While Goodell and Iler (2007) found clear evidence that honeysuckle possesses the ability to self-pollinate, the scale of the study was limited to just 24 individuals at just one site; thus, the statistical power to isolate the effect of each site was limited. In addition, the viability of seeds was not tested through a germination trial.

Barriball et al. (2014) used genetic parentage analysis to compare outcrossing rates and mating structure of individuals on the edge of woodlots to those in the interior. While their study was focused primarily on pollinator community behavior, their genetic tests revealed low, but varying rates of self-pollination in this population (5.4% ± 3.4% on the edge and 6.7% ± 6.6% in the interior). Once again, this study was limited by sample size (36 individuals), and it only utilized a single site. Addressing the variability in the rate of self-pollination, the authors speculated that honeysuckle might have the ability to throttle its abortion mechanism of self-pollinated flowers or seeds, depending on access to outcross pollen. Despite both of these studies having different aims, they both impressively found evidence that *L. maackii* possesses the ability to self-pollinate. However, both studies downplayed the role this trait may play in the invasiveness of the species (Barriball et al. 2014; Goodell and Iler 2007). With long-distance dispersal being an important invasion strategy for this species, more examination of its ability to self-pollinate is warranted.

Lonicera maackii invasions can remain in the establishment phase for 8 to 15 yr before progressing to the expansion and then saturation phases (Gorchov et al. 2014; Shields et al. 2014; Shigesada and Kawasaki 1997). Barriball et al. (2014) found a higher rate of selfing in the interior of the forest compared with the edge, where access to heterospecific pollen was higher. While the difference was not examined statistically, it is possible that the rate of selfing differs between invasion intensities and stages. Age and size of individuals, as well as access to heterospecific pollen, could all contribute to the rate of selfing in *L. maackii*. Self-compatibility helps advance an invasion from the establishment phase to the expansion phase (Baker 1974), so within younger, less-dense *L. maackii* invasions—which would have less access to outcross pollen, due to lower densities of reproductive-age plants—selfing may occur at a greater rate. In long-established, heavy invasions, abundant access to heterospecific pollen promotes rapid population growth and the development of a genetically diverse population. Thus, selfing rates may be lower in these heavily invaded populations. However, selfing may not only be an important mechanism in low-density invasions early in the establishment phase but may also be an important mechanism of establishment in populations that sprout back after mechanical treatment. These sprouting "reinvasions" would consist of individuals in similar life-history stages as heavy invasions but have lower densities of reproductive individuals, and thus may have less access to heterospecific pollen, similar to light invasions. All three invasion types could have similar rates of self-pollination, which would indicate that the trait is immutable within the species and does not respond to different population densities or developmental stages. Identifying the exact mechanism responsible for mediating selfing rates (hormonal response, pollinator behavior, resource allocation, etc.) is beyond the scope of this study, but establishing a pattern is a first step toward uncovering a mechanism.

The objectives of our study were 2-fold: (1) determine the rate of self-pollination in *L. maackii* and how seed production and viability differ between self- and open-pollinated plants; and (2) determine whether the rates of self-pollination, seed production, and seed viability differ between invasions of different population density and developmental stage. While a full examination of the reproductive biology of *L. maackii* may be important, we are primarily focused on the scenarios where an individual is its only source of possible reproduction to better understand the early stages of invasion. To address these objectives, we established nine replicate sites evenly distributed across three types of *L. maackii* invasion, varying in density and developmental stage (heavy density, light density, and sprouting after mechanical treatment). Pollination bags covered a branch on each individual plant for closed pollination to occur, while another branch was left unbagged for open pollination. Berries were collected, and the seeds were used in a germination trial. We predicted that bagged branches (closed pollinated) would produce fewer berries and seeds compared with open-pollinated branches, but that their germination rates would not differ. We also predicted that the rate of selfing would be inversely proportional to the availability of outcross pollen; the light invasion sites would have the highest rates of selfing, followed by sites where posttreatment sprouting occurred, with the lowest rate of self-pollination occurring in the heavy invasion sites.

Materials and Methods

We established nine total replicate sites between Martell Forest (40.43232°N, 87.03882°W) and the Richard G. Lugar Forestry Farm (40.42903°N, 86.95382°W; henceforth referred to as "Lugar Farm") in north-central Indiana, near West Lafayette (these forests were a minimum of 4.8 km apart). While Lugar Farm and Martell Forest are different study areas, they are likely a part of the same forest metacommunity. The nine replicate sites were evenly distributed into one of three types of *L. maackii* invasion, based upon visual inspection: heavy invasion, light invasion, and sprouting invasion. The heavy and light classifications were assigned based on the size and abundance of *L. maackii* individuals. We classified sprouting invasions as areas that had previously been naturally invaded by *L. maackii*, where all shrubs were cut, but the site did not receive subsequent cutting treatments, and individuals were allowed to sprout. All heavy invasions were at the Lugar Farm (minimum of 0.5 km apart) and all light invasions were at the Martell Forest (minimum of 0.9 km apart). Two sprouting invasions were at the Lugar Farm (located 0.4 km from nearest heavy invasion treatment and 0.2 km apart) and one was at the Martell Forest (0.7 km from nearest light invasion treatment). At eight out of the nine sites, we haphazardly selected 20 individuals with basal diameters (measured at root collar) greater than 2 cm and with at least two branches displaying 20 or more floral buds. For each shrub, we recorded basal diameter and distance to the nearest other *L. maackii* individual displaying floral buds (Table 1). The sprouting invasion site at the Martell Forest only contained 11 individuals that met our criteria. To confirm our subjective assessments of the sites, we performed an ANOVA ($\alpha = 0.05$) on the basal diameters ($F(8, 162) = 18.54$, $P < 0.001$) and distance to nearest conspecific neighbor ($F(8, 162) = 7.78$, $P < 0.001$) between types of invasions; both variables being log transformed for the analyses. Groupings were determined using Tukey's honest significant difference (HSD) test ($\alpha = 0.05$; Table 1). Our tests generally confirmed our previous categorizations of the sites, with heavy invasion sites consisting of larger individuals growing more closely together than light invasion and sprouting sites.

In early May 2020, before anthesis (opening of the flower bud), we fastened a pollination bag (Bag Type PBS 10-1, PBS International, Salter Rd, Eastfield, Scarborough YO11 3UP, United Kingdom) to one of the preselected branches that displayed abundant floral buds (Figure 1A). Normally used in breeding trials, these bags are specifically designed to allow the transfer of air and sunlight but exclude pollen. This eliminated potential outcrossing by excluding both pollination from the wind and the primary mode of pollination: insects (McKinney and Goodell 2011). The open end of each bag was tightly wrapped with both a twist-tie and a zip-tie around a layer of nonabsorbent cotton wrapped around the branch inside the bag, thus preventing both pollen entry and damage to the stem. The goal was to mimic conditions wherein the flowers had no other outside source of reproduction—such as in the early stages of an invasion—rather than to disentangle the exact reproductive mechanisms of *L. maackii*. Thus, no hand pollinating was performed.

We removed the pollination bags in early September 2020. At this time, we counted the number of berries on both the open- and closed-pollinated (bagged) branches for each *L. maackii* individual. Additionally, we counted the number of "nodes" on each branch to standardize our measurements. A node was defined as the junction between two opposite leaves on a branch, where four flowers typically emerge. On closed-pollinated branches, we counted from the tip as far along the branch as the bag covered. For open-pollinated branches, we counted to an equivalent point on a branch. Both points were marked with a paint pen.

The bags were opened and counted before the berries reached maturity, due to a late summer heat wave that caused more bagged branches than expected to exhibit some level of chlorosis or

Table 1. Basal diameter and distance to nearest mature individual (mean ± 1 SD) for light, sprouting, and heavy invasion sites (three sites for each type) at Martell Forest and Lugar Farm, IN[a]

Invasion type	Distance m	Basal diameter cm
Heavy	0.95 (0.72) a	12.91 (7.81) a
Light	2.31 (1.45) b	7.98 (3.83) b
Resprouting	1.84 (1.89) c	6.57 (3.04) b

[a] Each site contained 20 *Lonicera maackii* individuals, except for the sprouting invasion at Martell Forest, which only contained 11 mature individuals. Lowercase letters represent groupings between invasion types within each variable and are the results of a Tukey's honest significant difference (HSD) test ($\alpha = 0.05$) on the log-transformed variables.

Figure 1. (A) A pollination bag (Bag Type PBS 10-1, PBS International) fastened to an *Lonicera maackii* branch before anthesis. (B) Germinating *L. maackii* seeds with radicle hooks extending from their seed coats.

necrosis (48%), but this did not fully prevent berry production. Berries were left on the branches to develop fully. For closed-pollinated branches that produced berries, we placed thinner, mesh bags around the branch to prevent bird frugivory and capture any berries that dropped after the removal of the thicker pollination bags used to exclude pollen, although we did not observe these mesh bags catching many berries.

All berries were collected from closed-pollinated branches in late October, along with 10 berries from the paired open-pollinated branch on the same individual. If the corresponding open-pollinated branch did not contain 10 berries, then berries were collected from other open-pollinated branches on the same plant until we reached 10 berries or none were left on the individual plant. We counted the number of seeds within each berry and conducted a germination trial in accordance with a published protocol for this species (Hidayati et al. 2000). Each branch's seeds were placed in their own petri dish, which was lined with filter paper and kept moist throughout the trial. The germination trial began on November 18 and lasted 20 wk. We cold-stratified the seeds for 12 wk at ~5 to 10 C, with a 12-h photoperiod. The germination period lasted 8 wk with the same photoperiod and with daytime and nighttime temperatures of 20 and 10 C, respectively. We treated seeds with a 10 mg L^{-1} solution of gibberellic acid (GA_3) at the beginning of both the stratification period and the germination period to aid in the breaking of seed dormancy. To inhibit fungal growth within the petri dishes, we applied three treatments of a sulfur-based fungicide (Bonide®, Sulfur Plant Fungicide, 6301 Sutliff Rd. Oriskany, NY 13424) as needed throughout the 20-wk period. Each week after the seventh week, we recorded the number of germinating seeds in each petri dish and removed the germinants. Germination was determined by the presence of a "radicle hook" extending from the seed coat (Figure 1B).

Statistical Analyses

We constructed models for four dependent (response) variables using mixed-effects models in R (LME4): the number of berries resulting from open pollination ("open-pollinated berries"), the number of berries from closed-pollination ("closed-pollinated berries"), the number of seeds produced by a branch, and the proportion of seeds that germinated in the germination trial. Negative binomial distributions were chosen for models containing the following count data after checking overdispersion ($\alpha = 0.05$): open-pollinated berries ($\hat{c} = 12.32$, $P < 0.001$), closed-pollinated berries ($\hat{c} = 2.47$, $P < 0.001$), and number of seeds ($\hat{c} = 2.14$, $P < 0.001$). A zero-inflated model would not be appropriate for these data, as the large number of zeros was not generated by a separate mitigating process, which is an assumption for that type of model. The proportion of seeds that germinated in the germination trial was also analyzed in a binomial model, weighted by the number of seeds collected from the branch.

All models included the following biologically relevant explanatory independent variables: basal diameter, distance to nearest flowering conspecific neighbor, and invasion type. A relevant fixed effect for the reproductive potential of each individual (i.e., the number of flowers within a pollination bag), which varied between dependent variables, was also included (e.g., number of berries collected was included to explain the number of seeds from a branch). Site was a random effect for the models explaining number of open-pollinated and closed-pollinated berries. To predict the number of closed-pollinated berries, we also included a binary fixed effect of whether the branch displayed signs of chlorosis or necrosis in that model. Because we were only looking at individuals that resulted from selfing for the models predicting the number of seeds and number of germinants, we nested the *L. maackii* individual within site as a random effect. This allows us to avoid pseudo-replication resulting from treating the open- and closed-pollinated branches separately. Branch type (closed or open pollinated) was included as a fixed effect in these models.

For model selection, we first created a model with no interaction effects and then a model with all relevant interaction effects. Subsequent models with fewer interaction effects were included until we arrived at a model with the lowest Akaike information criterion. All final models were checked against a null model in a log-likelihood test ($\alpha = 0.05$) for overall model significance, and all were significantly better predictors.

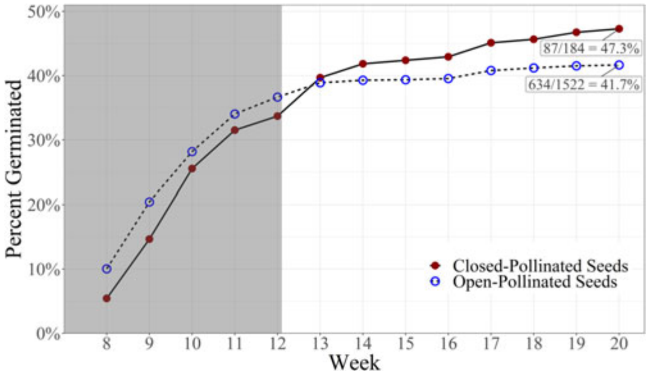

Figure 2. Percent germination of *Lonicera maackii* seeds from closed- and open-pollinated branches. The gray-shaded area represents germination during the stratification period.

We performed a type II ANOVA (CAR) on each model to determine which factors significantly predicted the response variable ($\alpha = 0.05$). Significant categorical variables were analyzed using pair-wise comparisons ($\alpha = 0.05$; EMMEANS), while significant continuous variables had their predictions mapped ($\alpha = 0.05$; EFFECTS). We displayed interaction effects using the INTERACTIONS and GGPLOT2 packages.

Results and Discussion

Berries resulting from self-pollination developed on 48 of 171 (28%) closed-pollinated branches, despite 48% of bagged branches exhibiting at least some amount of chlorosis or necrosis. Open-pollinated branches generally produced more berries per node (open mean ± SD: 0.50 ± 0.43; closed mean ± SD: 0.01 ± 0.03), which translates to one berry per 8 flowers in open-pollinated branches and one in 400 for closed-pollinated branches, as each node typically has 4 flowers. Open-pollinated branches also bore more seeds per berry (open mean ± SD: 3.25 ± 1.54; closed mean ± SD: 1.52 ± 1.40), but both open- and closed-pollinated seeds germinated at similar rates (open: 41.7%; closed: 47.3%; Figure 2).

The model that best explained the number of berries from open-pollinated branches on an *L. maackii* individual consisted of eight fixed effects, four of which were interaction effects, in a negative binomial distribution (Table 2). We found the highest-order, significant variable to be an interaction effect between an individual's basal diameter, distance to nearest conspecific individual, and invasion type (Figure 3). This means that the relationship between basal diameter and the number of open-pollinated berries not only varies across invasion types but is also mediated by how close an individual is to another conspecific.

The model we constructed that explained the number of berries from closed-pollinated branches on an *L. maackii* individual consisted of six fixed effects, none of which were interaction effects, in a negative binomial distribution (Table 2). The number of berries from open-pollinated branches per node predicted the number of berries on closed-pollinated branches in a positive relationship (Figure 4A). Interestingly, a larger basal diameter led to fewer berries on closed-pollinated branches in our model (Figure 4B). Heavy invasions produced significantly more berries from closed-pollination than light invasions, with sprouting invasions not differing from either (Figure 5A). Additionally, the number of closed-pollinated nodes, unsurprisingly, was positively correlated with the number of berries resulting from closed pollination. As expected, branches exhibiting chlorosis or necrosis produced fewer berries than those that did not exhibit such symptoms (Figure 5B).

Table 2. Variables that best explained the number of berries from open- and closed-pollinated branches on *Lonicera maackii* individuals in a negative binomial model. Asterisked p-values denote statistical significance[a]

Variable	χ^2	df	P ($>\chi^2$)
Open-pollinated berries			
Number of open nodes	2.367	1	0.124
Basal diameter	0.018	1	0.894
Distance	1.258	1	0.262
Invasion type	3.101	2	0.212
Basal diameter : distance	9.673	1	0.002*
Basal diameter : invasion type	11.143	2	0.004*
Distance : type	4.509	2	0.105
Basal diameter : distance : invasion type	10.867	2	0.004*
Closed-pollinated berries			
Number of closed nodes	6.149	1	0.013*
Open-pollinated berries per node	7.677	1	0.006*
Bagged branch death	31.384	1	<0.001*
Basal diameter	5.711	1	0.017*
Distance	1.510	1	0.219
Invasion type	8.304	2	0.016*

[a] Individual variable significance was determined by a type II ANOVA ($\alpha = 0.05$).

To predict the number of seeds from a closed-pollinated branch, we fit a negative binomial model with six fixed effects, one of which was an interaction effect (Table 3). Branch type was a significant variable within the model, where closed-pollinated branches produced far fewer seeds than open-pollinated branches (Figure 6A). For both open- and closed-pollinated branches, our model indicated that heavy invasions produced the most seeds, followed by light invasions, and sprouting invasions produced the fewest seeds (Figure 6B). The number of berries collected was a marginally significant variable and correlated positively with the number of seeds produced.

Our best-fit model to explain the proportion of seeds that germinated from each branch was a binomial distributed regression with seven variables, two of which were interaction effects (Table 4). The number of berries from each branch has a significant, positive relationship with the proportion of seeds germinated (Figure 7A). Branch type was significant alone and in an interaction effect with type of invasion, with closed-pollinated branches having an equal or higher germination rate across invasion types (Figure 7B). Basal diameter and distance to nearest conspecific individual were not significant.

Our study demonstrated ample self-pollinated berry production by *L. maackii* and provides insight into the factors controlling berry production. Contrary to our predictions, individual plants farther away from the nearest source of outcross pollen—as measured by the distance to the nearest flowering conspecific individual—did not produce more self-pollinated berries. If distance is a good proxy of outcross pollen availability, this lack of relationship indicates that the rate of self-pollination was not influenced by this factor, at least at the range of distances measured in our study.

Based on the positive relationships we observed between the number of closed-pollinated berries and the number of berries per node on the open-pollinated branches, our results indicate that general reproductive capacity, perhaps based on carbohydrate availability, is the best predictor of the number of berries on both closed-pollinated (bagged) and open-pollinated branches. Individuals in heavy invasions were larger and likely older (Shields et al. 2014),

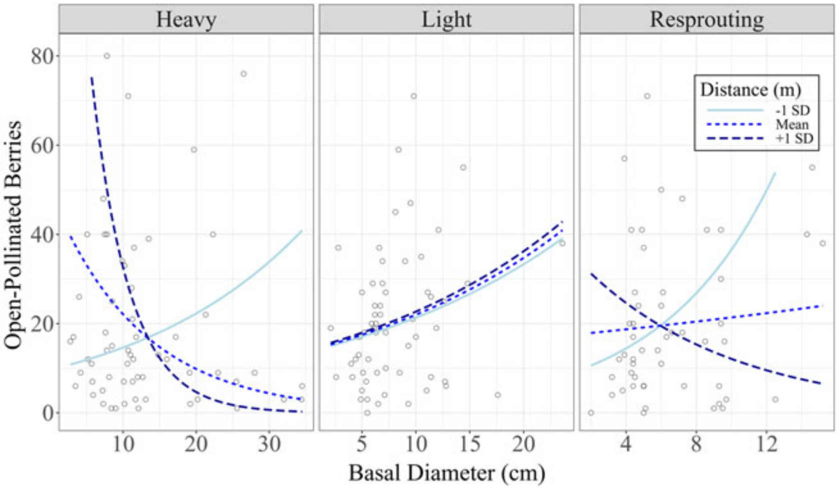

Figure 3. Relationships between basal diameter and the number of berries from open-pollinated branches across *Lonicera maackii* invasion types. Different lines represent predictions from the best-fit model and a three-way interaction effect of how the relationships change across the distribution of the variable “distance to nearest conspecific.” Because distance to nearest conspecific is a continuous variable in this interaction, we represent predictions based on the mean, 1 SD below the mean, and 1 SD below the mean distance to nearest conspecific as represented by different shades of blue and line types. Points represent raw data.

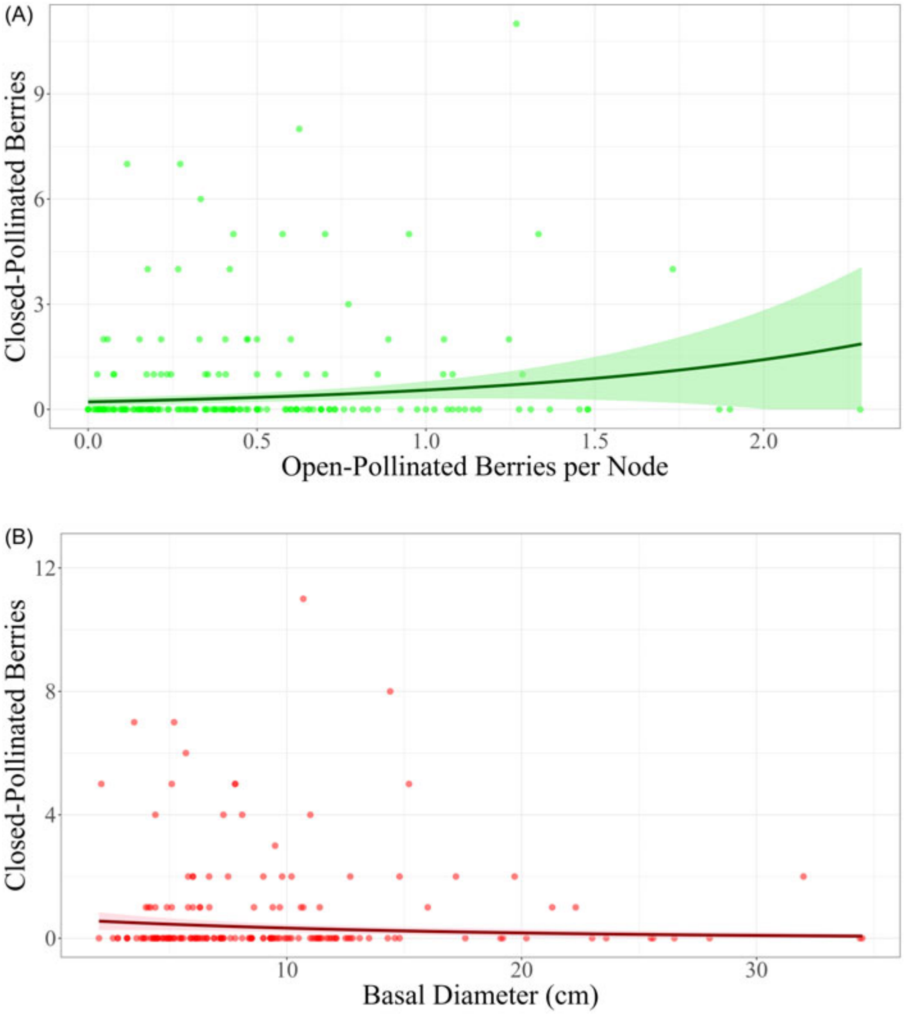

Figure 4. Predicted relationship of both the number of *Lonicera maackii* berries from open-pollinated branches per open node (A) and basal diameter (B) to the number of *L. maackii* berries from closed pollination, as determined by the best-fit negative binomial model. Points represent raw data.

Table 3. The variables that best explained the number of seeds from a given *Lonicera maackii* branch in a negative binomial model. Asterisked p-values denote statistical significance[a]

Variable	χ^2	df	P ($>\chi^2$)
Number of berries collected	2.952	1	0.086
Branch type	262.472	1	<0.001*
Basal diameter	0.146	1	0.702
Distance	0.007	1	0.935
Invasion type	5.992	2	0.050*
Number of berries collected : branch type	2.195	1	0.138

[a] Individual variable significance was determined by a type II ANOVA (α = 0.05). Branch type (open- or closed-pollinated branch) and invasion type (heavy, light, or resprouting invasion areas) were found to be significant.

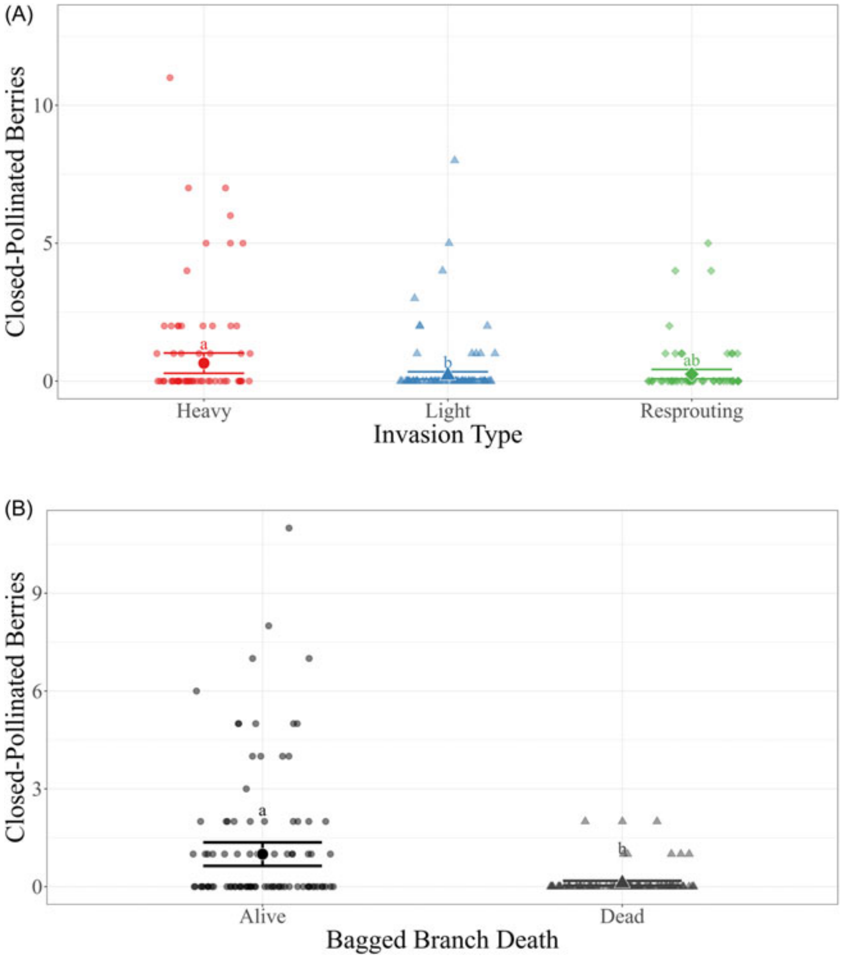

Figure 5. Predicted relationships of type of *Lonicera maackii* invasion (A) and bagged branch death (B) to the number of berries resulting from closed-pollination, as determined by the best-fit negative binomial model. Significance was determined by a post hoc pair-wise comparison (α = 0.05). Large points and error bars represent the predicted value and 95% confidence intervals. Smaller points represent raw data.

leading to greater photosynthetic capacity, which in turn contributed to overall greater production of seed. Our data show that the importance of basal diameter in relation to seed production can change, depending on other factors. Branches on plants in sprouting invasions produced the fewest seeds. Carbohydrate availability being the strongest predictor of reproductive capacity may also explain this trend, as individuals in sprouting invasions are likely to have lower energy reserves while regenerating vegetative tissues following treatment (Richburg 2005).

While we observed similar rates of germination between closed- and open-pollinated seeds, we did not grow the germinants beyond this early stage to assess fitness. If plants resulting from closed-pollination are less fit, there may be fewer carbohydrates available for self-pollinated berries for shrubs in light invasions. However, unlike other studies examining self-compatibility in woody plant species (Duminil et al. 2009; Rodger and Johnson 2013), we found no evidence of inbreeding depression in the germination rates of *L. maackii* seeds. Overall, seeds resulting from self-pollination germinated at higher rates than open-pollinated seeds, with the highest rates occurring in light and sprouting invasions (Figure 7A). However, effects of inbreeding depression may be exhibited at a later life stage. For example, Rodger and

Table 4. The variables that best explained the proportion of germinating seeds from a given *Lonicera maackii* branch in a binomial model. Asterisked p-values denote statistical significance[a]

Variable	χ^2	df	P (>χ^2)
Number of berries	3.860	1	0.049*
Branch type	5.112	1	0.024*
Basal diameter	1.536	1	0.215
Distance	2.025	1	0.155
Invasion type	2.729	2	0.255
Branch type : invasion type	44.758	2	<0.001*
Basal diameter : distance	3.268	1	0.071

[a] Individual variable significance was determined by a type II ANOVA (α = 0.05). The number of berries collected from that branch, the branch type (open- or closed-pollinated branch), and the interaction between branch type and invasion type (heavy, light, or resprouting invasion areas) were found to be significant.

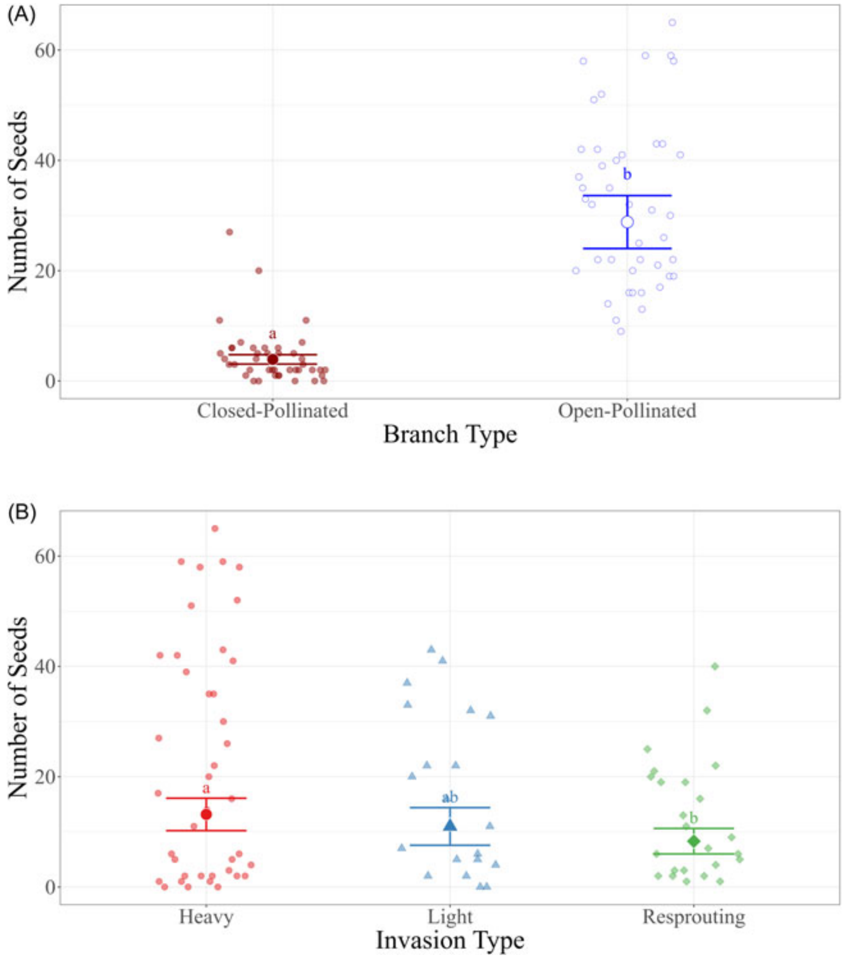

Figure 6. Predicted relationships of branch type (A) and invasion type (B) to the number of *Lonicera maackii* seeds of both closed- and open-pollinated branches, as determined by the best-fit negative binomial model. Significance was determined by a post hoc pair-wise comparison (α = 0.05). Large points and error bars represent the predicted value and 95% confidence intervals. Smaller points represent raw data.

Johnson (2013) observed slower growth in progeny of *A. dealbata* resulting from self-fertilization compared with progeny resulting from outcrossing 3 mo after sowing, even though germination rates between the two breeding groups were similar (Rodger and Johnson 2013).

When outcross pollen was excluded, *L. maackii* individuals in our study only produced self-pollinated seed on 1 out of every 400 flowers, which is lower than values reported in other, similar studies (~3 in 100 flowers in Goodell and Iler [2007]). Our study revealing a lower rate of selfing compared with the previous studies is likely due to a higher than expected necrosis rate on bagged branches (48%). We left the pollination bags on branches well past the end of flowering, and future studies should take care to remove bags earlier. However, given that a single *L. maackii* individual is capable of producing thousands of flowers in a single year (McNeish and McEwan 2016), and those seeds resulting from self-pollination germinate at similar rates to seeds from open-pollinated plants, we can conclude that *L. maackii*'s ability to

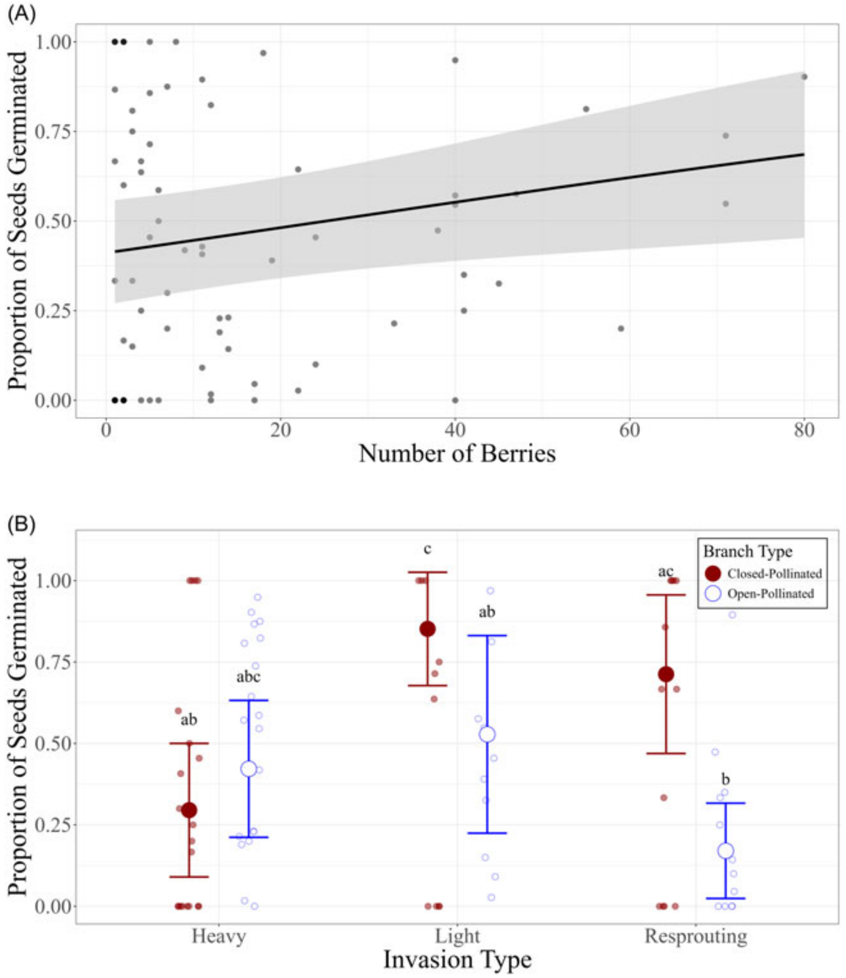

Figure 7. (A) Predicted interaction effect between branch type and total number of *Lonicera maackii* berries originally found on each branch, which significantly predicted the number of germinating seeds in the best-fit binomial model. Points represent raw data, with darker points representing overlapping data points. (B) Predicted interaction effect between branch type and invasion type, which significantly predicted the number of germinating seeds in the best-fit binomial model. Significance was determined by a post hoc pair-wise comparison (α = 0.05). Large points and error bars represent the predicted value and 95% confidence intervals. Smaller points represent raw data.

self-pollinate likely contributes to the species' ability to invade new, isolated forest patches.

Birds are a major vector of *L. maackii* seeds (Bartuszevige and Gorchov 2006; Ingold and Craycraft 1983), and this method of dispersal may result in a limited number of seeds reaching a distal site (Gosper et al. 2005). While a bird or deer (Guiden et al. 2015) may deposit more than one seed in a long-distance dispersal event, there are several factors that could keep the ability to self-pollinate advantageous to establishing a new population. For example, not all dispersed seeds are guaranteed to reach sexual maturity, which would limit outcrossing potential. It is possible all dispersed seeds come from the same or a closely related parent, and thus a tolerance to inbreeding depression or increased capability to reproduce with similar pollen would help foster berry production, and overall density could still be low enough as to not provide sufficient outcross pollen to avoid selfing. While not clear yet, it is possible that propagules resulting from self-pollination can reduce the lag time in the establishment phase of a species' invasion (Shigesada and Kawasaki 1997). Information on the establishment phase of woody invasions is limited, but *L. maackii* is known to have a much shorter lag period (10 to 15 yr in Shields et al. [2014]; 8 to 9 yr in Gorchov et al. [2014]) in the establishment phase than the 34 yr needed for the invasive tree Norway maple (*Acer L. plantanoides*; Wangen and Webster 2006). While it is difficult to compare directly between trees to shrubs due to differences in how quickly the two growth forms may reach sexual maturity, species in the genus *Acer* rarely have the ability to self-pollinate (Sullivan 1983), so self-compatibility contribute to the shorter lag period in L. *maackii*. Moreover, if self-pollinated plants become established, when more distantly related individuals do eventually disperse to the area, there will be more flowers ready to receive and provide outcross pollen. Thus, the species would be able to more rapidly reproduce, spread, and establish in comparison to scenarios where viable self-pollination is not possible.

Additionally, this study does not distinguish between the various mechanisms by which a plant could reproduce when outcross pollen is excluded. Whether *L. maackii* is able to produce seed sexually through selfing with its own pollen or through apomixis, which requires no pollen at all, could affect how this species invades and establishes in cases of long-distance dispersal. Further research into this area could provide insights in how to manage and prevent these invasions.

Self-pollination has been consistently downplayed in the invasion biology of *L. maackii* (Barriball et al. 2014; Goodell and Iler 2007). However, our results suggest that self-pollination is an important trait that facilitates the early establishment of

L. maackii and may reduce the duration of the lag period during early population growth. Future studies should examine the exact reproductive biology of *L. maackii* (selective abortion of fruits, exact mechanism by which berries form without outcross pollen, etc.); explore how this trait affects invasion speed through simulation or examination of seed dispersal; and determine how plants produced from self-pollinated propagules perform over time in their physiology, growth, and survival. Self-pollination should also be more closely studied in other problematic invasive plants.

Acknowledgments. We thank Jim McKenna for the pollination bags, the advice, and always checking in on our progress. We would also like to thank Brian Beheler for help with finding field sites and the access to the Wright Center. Rucha Karve provided GA_3 formulations which was very helpful. Finally, we thank Sarah Rademacher for her friendship, as well as her help with fieldwork.

Funding statement. This research received no specific grant from any funding agency or the commercial or not-for-profit sectors.

Competing interests. The authors declare no conflicts of interest.

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