Growth of *Lonicera caerulea* across Fertility and Moisture Conditions: Comparisons with *Lonicera villosa* and Invasive Congeners

Darren J. Hayes and Bryan J. Peterson

School of Food and Agriculture, University of Maine, 5722 Deering Hall, Orono, ME 04469

Additional index words. *Lonicera tatarica*, *Lonicera xylosteum*, controlled-release fertilizer, honeyberry, Haskap, mountain fly honeysuckle, Tatarian honeysuckle, European fly honeysuckle

Abstract. Several species of honeysuckle from Europe and Asia have proved to be invasive in North America, with substantial impacts on native ecosystems. Although shrubby honeysuckles of Eurasian origin have appeared on banned plant lists in North America and other parts of the world, cultivars of the edible blue honeysuckle (*Lonicera caerulea* L.) derived from Eurasian germplasm and marketed as honeyberry, Haskap, or sweetberry honeysuckle have recently been widely developed for agricultural use in North America, with little scrutiny of invasive potential in North America despite its documented invasion of the Scandinavian Peninsula in northern Europe. To gain insight into differences in growth strategies among congeners, we compared the growth of Eurasian *L. caerulea* with that of a closely related congener in North America [*Lonicera villosa* (Michx.) R. & S.] and two known invasive congeners from Eurasia (*Lonicera tatarica* L. and *Lonicera xylosteum* L.). In Expt. 1, *L. villosa*, *L. caerulea*, and *L. tatarica* were grown in #1 nursery containers after top-dressing with Osmocote Pro 17–5–11 4-month controlled-release fertilizer (CRF) at rates of 5, 10, 15, 20, and 25 g CRF/container. Across all fertilizer treatments, *L. caerulea* outperformed *L. villosa* by a factor of two for root and shoot dry weights, although *L. tatarica* produced more growth than either of the others and was more responsive to increasing CRF. However, *L. caerulea* more strongly resembled *L. tatarica* in form, producing leaves of greater individual size and producing significantly taller primary stems than *L. villosa*, evidence for prioritization of competitive growth. In Expt. 2, plants of the same taxa plus *L. xylosteum* were grown communally in #20 nursery containers, followed by a period in which each container was subjected to regular irrigation, withheld irrigation (dry treatment), or inundation (flooded treatment). Plant growth differed substantially among taxa, but moisture treatments did not affect growth significantly. As in Expt. 1, plants of *L. caerulea* in Expt. 2 produced greater dry biomass than plants of *L. villosa* and resembled the invasive Eurasian honeysuckles more strongly in size and form. We conclude Eurasian *L. caerulea* is distinct in growth rate and morphology from North American *L. villosa*. In light of these findings, the ecology and competitive ability of Eurasian *L. caerulea* may not be well predicted by ecological observations of its closely related North American congener.

Received for publication 24 June 2019. Accepted for publication 30 Sept. 2019.

Published online 17 January 2020.

Maine Agriculture and Forestry Experiment Station Publication Number 3711. Funding for this research was provided by U.S. Department of Agriculture National Institute of Food and Agricultural Hatch Project #ME021614 and an Agricultural Research Service Cooperative Agreement for Project 8020-21000-147-06S. We thank Bill Patterson, Nancy Sferra, The Nature Conservancy in Maine, Bradley Libby, Stephanie Burnett, Melissa Smith, and anonymous reviewers for helpful assistance.

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the National Institute of Food and Agriculture or the U.S. Department of Agriculture.

B.J.P. is the corresponding author. E-mail: bryan.j.peterson@maine.edu.

This is an open access article distributed under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

Honeysuckles (*Lonicera* spp.) introduced from Eurasia have a long history of popularity in the horticultural trade in North America. Tatarian honeysuckle (*Lonicera tatarica*) from Asia was in widespread horticultural cultivation in Europe and North America by the mid 1700s (Barnes and Cottam, 1974), whereas another popular Asian honeysuckle, *Lonicera maackii* (Rupr.) Maxim., was introduced into North America by 1896 (Luken and Thieret, 1996). In The Standard Encyclopedia of Horticulture, L.H. Bailey (1919) described honeysuckles as plants “of easy cultivation and propagation . . . quite hardy,” and among “our most popular ornamental shrubs.” The popularity of introduced shrubby honeysuckles continues into the 21st century, despite evidence as early as the 1920s suggesting they often escaped cultivation and naturalized in the wild (Luken and Thieret, 1996). Now, many widely planted shrubby and vining honey-

suckles of Eurasian origin are documented invasive pests (Batcher and Stiles, 2000), and recently have become the targets of state-level bans in parts of the United States (U.S. Department of Agriculture-Natural Resources Conservation Service, 2018). Many of the same traits that made Eurasian *Lonicera* attractive as ornamentals—such as their colorful, bird-dispersed fruits; intermediate shade tolerance; and landscape adaptability—enabled them to become serious invaders in the North American landscape (Conser et al., 2015; Koop et al., 2012). Despite broad concerns about introduced invasive species in general, and the invasive history of Eurasian *Lonicera* in particular, cultivars of honeysuckle developed from Eurasian taxa, including *Lonicera tatarica*, *Lonicera xylosteum*, *Lonicera japonica* Thunb., and others, continue to be sold in North America.

Recently, many cultivars of the edible blue honeysuckle, *Lonicera caerulea*, have been developed using germplasm collected from a wide geographic range in mainland Asia and Japan (Gerbrandt et al., 2017), and genotypes from Europe are likewise commercially available in North America. Marketed under the names honeyberry, Haskap, and sweetberry honeysuckle, these cultivars are described as an agricultural berry crop for cold climates, with fruit finding use in value-added products such as wines, jams, and confections (Celli et al., 2014). Clones are sometimes advertised as alternative crops to blueberries (Bors, 2009), and studies indicate the fruit of *L. caerulea* is high in vitamins and antioxidants (Wang et al., 2016).

Although *L. caerulea* is a promising shrub for North America, its invasive potential has not been studied experimentally. This species features prominently on the Norwegian Biodiversity Information Center’s Black List, where it ranks among Norway’s invasive organisms with the most severe ecological impact, in a category of severity above other invasive honeysuckles such as *L. tatarica* and *L. maackii* (Gederaas et al., 2012). *Lonicera caerulea* is also listed as an invasive species in Sweden, where it is described as one of the few invasive taxa that have successfully invaded natural forest communities (Tyler et al., 2015). In North America, Schimpf et al. (2011) discovered a naturalized population of honeyberry near Duluth, MN, which was subsequently visited by Peterson et al. (2018), who reported evidence of spread by seedling recruitment and probable natural layering. Application of checklist-based invasive plant screening tools by Peterson et al. (2018) indicated that blue honeysuckle cultivars developed from Eurasian germplasm pose a credible risk of becoming invasive in North America, even with conservative ratings based on limited data available for *L. caerulea*.

The exact taxonomy of blue honeysuckles is still the subject of debate, and many consider *L. caerulea* to form a single, circumboreal taxon consisting of numerous Eurasian

representatives as well as two North American relatives. However, Fernald (1925) argued that the two North American representatives differed sufficiently from their Eurasian congeners and from one another to elevate them to two distinct species, *Lonicera villosa* (mountain fly honeysuckle) in north-central and eastern North America, and *Lonicera cauriana* Fernald (blue fly honeysuckle) in western North America. A universally accepted global taxonomy based on a synthesis of genetic and morphological evaluations is elusive (Holubec et al., 2015; Hummer et al., 2012; Naudum et al., 2011, 2014). For brevity, Eurasian *L. caerulea* is henceforth referred to as *L. caerulea*, and the mountain fly honeysuckle, commonly accepted as *Lonicera caerulea* subsp. *villosa* (Michx.) A. Löve & D. Löve is referred to simply as *L. villosa*.

In contrast to several introduced members of the genus that have become widely invasive in North America, the native *L. villosa* generally forms sparse populations and is a species of conservation concern in portions of its range (Lieurance and Cipollini, 2013; U.S. Department of Agriculture-Natural Resources Conservation Service, 2018). The minor presence of *L. villosa* in the ecosystems in which it is present has been interpreted by some as evidence that introduced genotypes of *L. caerulea* are similarly unlikely to become invasive in North America (e.g., Bors et al., 2012). However, the long-standing documentation of morphological differences between Eurasian and North American blue honeysuckles (Fernald, 1925), and the absence of direct comparisons of growth between them, leave uncertain the ecological equivalence of these taxa. In fact, the degree of relatedness and known capacity for hybridization between Eurasian *L. caerulea* and *L. villosa* (Bors et al., 2012) may be cause for additional concern, as population genetic consequences of gene flow between local and introduced genotypes have been documented in various taxa (Crispo et al., 2011; Ellstrand and Shierenbeck, 2000; Saltonstall, 2002).

Nutrient and water availability are among the factors that may determine the invasive success of introduced taxa. For example, many invasive plants can rapidly assimilate nutrients and accumulate biomass when transient nutrient enrichment occurs as a result of habitat disturbances (Gioria and Osborne, 2014). *Lonicera caerulea* has rapidly invaded low-resource environments in boreal forests throughout Norway (Gederaas et al., 2012), suggesting that it may have traits that help it compete for limiting resources in an ecosystem long viewed by conservationists as resistant to invasion. In addition to nutrient availability, water stress also may affect the invasive success of an introduced taxon. *Lonicera villosa* and *L. caerulea* are both facultative wetland plants, although the latter also thrives in well-drained field soils. The comparative drought and flooding tolerances of *L. villosa*, *L. caerulea*, and honeysuckles already invasive in North America are cur-

rently unknown. Notably, *L. tatarica* is documented to invade habitats with well-drained to poorly drained soils that are acidic to alkaline and high or low in nutrient availability (Batcher and Stiles, 2000).

We conducted two experiments to understand more fully the potential similarities and differences in traits of root and shoot biomass accumulation, average leaf size, specific leaf area, and primary stem length among *L. caerulea*, *L. villosa*, and two invasive congeners under different conditions of container substrate fertility and moisture. In Expt. 1, we compared substrate fertility responses of *L. caerulea* and *L. villosa* to those of the known invader, *L. tatarica*. In Expt. 2, we assessed responses of *L. caerulea*, *L. villosa*, and the known invaders *L. tatarica* and *L. xylosteum* to late-summer moisture conditions. We included known invaders in an effort to put the magnitude of trait variation between *L. caerulea* and *L. villosa* into the broader context of honeysuckles that have invaded North America successfully. Fundamentally, our goal was to identify potential functional variation between *L. caerulea* and *L. villosa*, rather than to classify either as invasive or not invasive based on comparisons with known invaders.

Materials and Methods

Expt. 1. Twenty-five plants each of the taxa *L. caerulea*, *L. villosa*, and *L. tatarica* were produced from semihardwood stem cuttings collected from 1-year-old stock plants grown in containers at the University of Maine in Orono. Cuttings with stems 6 to 8 cm in length were collected on 16 July 2016 and rooted under intermittent mist in 510-mL vacuum pots (8.9 × 8.9 cm; Dillen-ITML, Middlefield, OH) in a substrate of 1:1 (by vol.) peat:perlite. *Lonicera tatarica* stock plants originated from a single, locally invasive genotype growing wild in the Fay Hyland Botanical Garden on the University of Maine campus (lat. 44°53'45.0"N, long. 68°40'27.8"W), whereas stock plants of *L. villosa* originated from cuttings collected from several plants in Lubec, ME (lat. 44°48'03.4"N, long. 67°07'35.4"W), and those of *L. caerulea* 'Svetlana' were purchased from a nursery in Manitoba, Canada. Rooted cuttings were overwintered in a cold-storage facility maintained at 4 °C from 16 Nov. 2016 until 14 Mar. 2017, then transplanted on 6 Apr. 2017 into #1 plastic nursery pots (2.4 L; 16.5 cm wide × 16.5 cm tall; Landmark Plastic Corp., Akron, OH) filled with two parts milled peat (Sungro, Agawam, MA), one part fine vermiculite (Whittemore Co., Inc., Lawrence, MA), and one part coarse perlite (Whittemore Co.) amended with 6 g pulverized dolomitic limestone per liter of medium.

After transplanting, rooted cuttings were grown in a glass-glazed greenhouse until they had put on an initial flush of new growth, after which plants were observed to enter ecodormancy as the minimal substrate fertility was exhausted. Shoots of plants were less

than ≈10 cm tall before the initiation of fertilizer treatments. On 27 Apr. 2017, fertilizer treatments were assigned by top-dressing Osmocote Pro 17–5–11 4-month CRF (Scotts Miracle-Gro Company, Marysville, OH) at rates of 5, 10, 15, 20, or 25 g CRF per container (0.43, 0.85, 1.28, 1.70, and 2.13 g N/L medium) for a total of five replications per combination of taxon and fertilizer treatment. A negative control was not included, because cuttings receiving no fertilizer would not continue to grow. Temperature on the bench was logged using a Watchdog 1450 micro station with radiation shield (Spectrum Technologies, Aurora, IL) positioned at plant canopy level. The temperature measured over days and nights averaged 25 °C for the study period, with a maximum reading of 47.2 °C. Photosynthetically active radiation (*PAR*) was measured once every 10 minutes using a quantum light sensor attached to the same data logger, and the daily light integral (*DLI*) was calculated from these data. The average *DLI* was 19.6 mol·m⁻²·d⁻¹, with a maximum instantaneous *PAR* reading during the experiment of 2324 μmol·m⁻²·s⁻¹. Plant placement was randomized at the start of the experiment, then rerandomized weekly for the first 5 weeks of the experiment, until the large size of some of the plants made rerandomizing too cumbersome. Plants were initially spaced on 1-ft centers, with spacing increased to 2-ft centers on the final randomization to accommodate further growth. Fertilizer release and substrate fertility were monitored during the experiment by measuring electrical conductivity (*EC*, measured in milli-Siemens per centimeter) of leachate using the PourThru extraction method (Cavins et al., 2000) with a portable pH/*EC*/TDS meter (HI991300; Hanna Instruments, Woonsocket, RI) on 18 June, 18 July, and 25 Aug. Western flower thrips (*Frankliniella occidentalis*) were observed during routine scouting in June, and plants were treated with Marathon II 1% granular pesticide on 16 June and at 3-week intervals for the remainder of the experiment.

Plants were harvested the week of 28 Aug. for data collection. We measured the length of the longest primary stem of each plant, as well as the average size of 25 fully developed leaves selected randomly from each plant. Leaves were measured by photographing them digitally and obtaining their area (measured in square centimeters) using ImageJ, version 1.51 (Schneider et al., 2012). Roots were washed to remove substrate, and roots, stems, and leaves were dried for 1 week to a constant weight in a drying room maintained at 68 °C before weighing. Stem and leaf dry weights were added to obtain total shoot dry weight, and specific leaf area (measured in square centimeters per gram) was calculated on an area-per-dry weight basis.

Statistical calculations were conducted using R, version 3.3.2 (R Core Team, 2016). Analysis of variance (ANOVA) *F* values were calculated at an alpha of 0.05 from linear models using the *car* package (Fox and Weisberg, 2011) to test for the effects

Table 1. Substrate moisture of #20 containers into which one plant each of *L. caerulea*, *L. villosa*, *L. tatarica*, and *L. xylosteum* were planted and grown for 7 weeks before initiation of moisture treatments on 1 Aug. 2017.^z

Date of measurement	Measurement location	Moisture by volume (%)		
		Dry	Regular irrigation	Flooded
16 Sept. 2017	Bottom	12.0 ± 0.6	25.9 ± 1.6	100 ± 0
	Top	9.3 ± 1.1	26.1 ± 0.8	73.0 ± 9.5
	Container average	10.7 ± 0.7	26.0 ± 1.0	86.5 ± 4.7
10 Oct. 2017	Bottom	10.5 ± 0.9	39.6 ± 5.7	100 ± 0
	Top	6.1 ± 2.1	24.2 ± 2.2	69.7 ± 9.2
	Container average	8.3 ± 1.4	31.9 ± 2.7	84.9 ± 4.6

^zThe containers in the dry treatment were allowed to dry down naturally without additional irrigation after 1 Aug., whereas the containers in the flooded treatment were fully saturated with water and not allowed to drain. The containers in the regular irrigation treatment were watered twice weekly but allowed to drain. The experiment was harvested after 10 weeks, when the substrate in the top 10 cm averaged ≈6% moisture by volume among containers in the dry treatment, as measured with a handheld theta probe. For each treatment, means ± SES were recorded from the same five containers.

of taxon, fertilizer rate, and taxon by fertilizer interactions on the response variables. ANOVA assumptions were assessed visually using residual histograms, Q-Q normal plots, and residual vs. fitted values plots. Shoot dry weights and root dry weights were square root-transformed to improve normality and homoscedasticity, whereas primary stem length and EC measurements were log₁₀-transformed. Post hoc least-square means with 95% confidence intervals were calculated for each response model using the package *lsmeans* (Lenth, 2016), omitting terms for which ANOVA F-tests failed to reject the null hypothesis. All least-square means and confidence intervals were back-transformed and reported in their original units.

Expt. 2. Fifteen plants each of *L. tatarica*, *L. caerulea* ‘Svetlana’, *L. villosa*, and *L. xylosteum* were produced from semihardwood cuttings collected on 15 July 2015. Cuttings of the first three taxa were collected from stock plants, whereas cuttings of *L. xylosteum* were collected from a plant cultivated in the Lyle E. Littlefield Ornamentals Trial Garden on the University of Maine Campus. Cuttings were rooted as described for Expt. 1, overwintered in cold storage, transplanted on 13 Apr. 2016 into 510-mL vacuum pots (8.9 × 8.9 cm, Dillen-ITML) filled with 1:1 peat:coarse perlite and top-dressed with 4 g Osmocote Pro 17–5–11 CRF, and overwintered again in cold storage from 16 Nov. 2016 until 14 Mar. 2017. On 6 Apr. 2017, cuttings were transplanted into #1 trade-gallon plastic pots containing 2:1:1 (by vol.) peat:vermiculite:perlite, and top-dressed with 10 g Osmocote Pro 17–5–11 CRF. On 13 June, the honeysuckles were transplanted communally into #20 (73.7-L) plastic nursery containers (Grip-Lip GL8000; Nursery Supplies, Inc., Chambersburg, PA) containing the same substrate, with one plant of each taxon per container, pruned to a uniform height of 10 cm, and placed under a polyfilm-glazed hoop-house with rolled-up sides and 25% mylar shade cloth. Planting location was randomized in each container, with plants spaced evenly into different quadrants. Throughout June and July, containers were fertigated to leaching twice weekly with Peters Professional 20–10–20

General-Purpose fertilizer at a concentration of 75 mg N/L. Western flower thrips (*Frankliniella occidentalis*) were observed during routine scouting in June, and plants were treated with Marathon II 1% granular pesticide on 16 June and at 3-week intervals for the duration of the experiment. Plants were also treated with insect repellent (Hot Pepper Wax Inc., Greenville, PA) in August and September to control two-spotted spider mites (*Tetranychus urticae*). A Watchdog 1450 micro station with a radiation shield and quantum sensor was used to measure temperature and PAR. Maximum and minimum daily temperatures during the experiment averaged 24.0 and 10.6 °C, respectively, with maximum and minimum temperature readings of 32.3 and 3.2 °C. Average PAR between sunrise and sunset was 228 μmol·m⁻²·s⁻¹, with a maximum instantaneous PAR of 1246 μmol·m⁻²·s⁻¹.

To evaluate the responses of plants to different late-summer moisture conditions, the #20 containers were assigned randomly to dry, regular irrigation, or flooded treatments on 1 Aug. 2017. Containers in the dry treatment were not watered after 1 Aug. to allow an ecologically realistic dry-down of the substrate over the following weeks. Containers in the regular irrigation treatment continued to receive irrigation twice weekly. Containers in the flooded treatment were prevented from draining by inserting them into 66-L plastic buckets (Large Capacity Plastic Bucket; Fortiflex, Inc., Hato Rey Norte, PR), into which they fit snugly, and irrigating until the saturated zone of the container reached the surface of the substrate, a process repeated weekly for the remainder of the study. Substrate moisture in each container was measured on two dates (16 Sept. and 10 Oct.) by inserting a handheld ML3 ThetaProbe Soil Moisture Sensor (Delta-T Devices, Ltd., Cambridge, UK) into the top of the substrate from above, and by inserting the probe into one of the drainage holes at the bottom of the container (Table 1). The probe was not used for the bottom measurement in flooded containers because the substrate was fully saturated. In an effort to provide uniform fertility among moisture treatments, no additional fertilizer was applied to any containers, and the containers

receiving the regular irrigation treatment were wetted until leaching began, but not to the point of excessive leaching.

On 10 Oct. 2017, we measured plant height (in centimeters) as well as the average size of 25 fully developed leaves selected randomly from each plant. Leaves were measured by photographing them digitally and obtaining their area (in square centimeters) using ImageJ, version 1.51. Roots were washed to remove substrate, and roots, stems, and leaves were dried to constant weight in a drying room maintained at 68 °C for 1 week before weighing. Stem and leaf weights were summed to obtain total shoot dry weights, and specific leaf area (in square centimeters per gram) was calculated on an area-per-dry weight basis. *Lonicera villosa* was excluded from leaf measurements because of its nearly complete leaf senescence during the final weeks of the experiment. Two replicates of *L. tatarica* were discarded from the regular irrigation treatment as a result of their failure to establish and grow after transplant to #20 plastic pots, before the initiation of treatments.

Statistical calculations were conducted using R, version 3.3.2 (R Core Team, 2016). ANOVA F values were calculated at an alpha of 0.05 from mixed-effect models using the *lme4* package (Bates et al., 2015) to test for the effects of taxon, moisture treatment, and taxon-by-moisture interactions on the response variables. ANOVA assumptions were assessed as for Expt. 1. Root dry weights, leaf dry weights, shoot dry weights, root system lengths, and plant heights were log₁₀-transformed; stem dry weights were square root-transformed to improve normality and homoscedasticity to meet ANOVA assumptions. Post hoc least-square means with 95% confidence intervals were calculated for each response model using the package *lsmeans* (Lenth, 2016) and Satterthwaite-adjusted degrees of freedom. Terms for which ANOVA F tests failed to reject the null hypothesis were omitted from calculations of marginal mean difference. All marginal means and confidence intervals were back-transformed and reported in their original units.

Results

Expt. 1. The main effect of taxon was significant, with the three taxa differing significantly in measures of root and shoot growth (Table 2). Compared with *L. villosa*, *L. caerulea* produced about twice the root dry weight ($P < 0.001$) and shoot dry weight ($P < 0.001$; Fig. 1). *Lonicera tatarica*, in turn, produced from twice to more than five times the root and shoot dry weights of *L. caerulea*, with a significant difference in means between the two taxa at every rate of CRF application (Fig. 1).

Rate of CRF application did not significantly alter root or shoot dry weights of *L. caerulea* or *L. villosa*, but did affect growth of *L. tatarica* (Fig. 1). Shoot dry weights of *L. tatarica* increased from 34.8 g at the lowest

Table 2. Analysis of variance F-statistics for the effects of taxon and fertilizer application rate on growth of *Lonicera caerulea*, *Lonicera villosa*, and *Lonicera tatarica*.²

Source	Root dry wt (g)	Shoot dry wt (g)	Leaf size (cm ²)	Specific leaf area (cm ² ·g ⁻¹)	Primary stem length (cm)
Taxon	207.943***	514.166***	106.894***	6.015**	70.231***
Fertilizer rate	2.892*	7.645***	0.950	0.346	1.081
Taxon × fertilizer rate	1.366	5.937***	0.597	0.924	0.760

²Fertilizer treatments were applied by top-dressing #1 containers with a controlled-release fertilizer providing 0.43, 0.85, 1.28, 1.70, and 2.13 g N/L medium.

*, **, ***Significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Fig. 1. Least-square means $\pm$ 95% confidence interval (CI) for growth responses of *Lonicera* to five application rates of controlled-release fertilizer (CRF) in #1 containers. Adequate visualization of mean root and shoot dry weights did not permit showing all three taxa on one scale, so results for *L. caerulea* are compared with its two congeners in different panels. Panels show (A) shoot dry weights for *L. caerulea* vs. *L. villosa*, (B) shoot dry weights for *L. caerulea* vs. *L. tatarica*, (C) root dry weights for *L. caerulea* vs. *L. villosa*, and (D) root dry weights for *L. caerulea* vs. *L. tatarica*. Note the change in y-axis scales between panels A and B, and between panels C and D. Asterisks denote individual treatment means that were significantly different according to Fisher's protected least significant difference in the *agricolae* package for R, despite overlapping CIs; 95% CIs are much more conservative than $\alpha = 0.05$ when two independent sample means are compared.

Table 3. Least-square means $\pm$ 95% confidence interval (CI) for primary stem length, leaf size, and specific leaf area of *Lonicera villosa*, *Lonicera caerulea*, and *Lonicera tatarica* across five fertilizer application rates.²

Taxon	Measurement (95% CI)		
	Primary stem length (cm)	Leaf size (cm ²)	Specific leaf area (cm ² ·g ⁻¹)
<i>L. villosa</i>	31.4 (28.0–35.1) b	6.9 (5.5–8.3) c	121.1 (116.2–126.0) a
<i>L. caerulea</i>	68.9 (62.0–76.5) a	16.7 (15.4–18.0) b	114.8 (110.2–119.4) ab
<i>L. tatarica</i>	71.1 (64.0–79.0) a	20.9 (19.6–22.2) a	109.1 (104.5–113.6) b

²The main effect of fertilizer was not significant. Means within the same column followed by the same letter are not significantly different according to 95% CIs.

CRF application rate to nearly 80 g with the second-highest CRF application rate, a more than 2-fold increase in aboveground biomass with increasing fertility. Simple linear regression showed that both root and shoot dry weights of *L. tatarica* followed quadratic trends (P values for quadratic terms <0.01 and <0.001 , respectively) with predicted maxima at intermediate applications of CRF (Fig. 1), suggesting an inhibitory effect at the highest rates of CRF.

Average leaf size, specific leaf area, and primary stem length varied significantly by taxon (Table 2), but not by fertilizer application rate. Primary stem length was similar between *L. caerulea* and the invasive *L. tatarica*, both of which produced stems more than double the length of *L. villosa* (Table 3). *Lonicera tatarica* produced leaves of the greatest average size, whereas *L. villosa* produced the smallest. Specific leaf area of *L. caerulea* did not differ significantly from that

of the other taxa, whereas specific leaf area of *L. villosa* was significantly greater than that of *L. tatarica* (Table 3).

Across taxa, substrate EC ranged from 1.8 to 6.4 mS·cm⁻¹ on the first measurement date, indicating that CRF application rates produced substantial variation in substrate nutrient content soon after the start of the experiment (Fig. 2). Substrate EC declined for all three taxa between 18 June and 18 July, but was greatest in substrates planted with *L. tatarica*. By 25 Aug., when the experiment was ended, substrate EC had declined further, with the EC of substrates planted with *L. caerulea* or *L. villosa* (Fig. 2C). Low EC readings for *L. tatarica* at the end of the experiment suggest either that the large plants exhausted the available fertilizer or that increased substrate leaching occurred as plants grew larger and required more frequent irrigation.

Expt. 2. The main effect of taxon was significant for all variables of plant growth (Table 4). In contrast, the main effect of moisture treatment was not significant, although moisture-by-taxon interactions were detected for root dry weight, stem dry weight, and root system length (Table 4, Fig. 3), such that the main effects of taxon could not be assessed directly for these traits. Of the four taxa, the native *L. villosa* produced the least stem and root dry weights (Fig. 3A and B). *Lonicera caerulea* produced root dry weights equivalent to those of *L. tatarica* under all moisture treatments, and produced stem dry weights equivalent to *L. xylosteum* and to *L. tatarica* under some conditions (Fig. 3A and B). In terms of root system length, *L. caerulea* performed similarly to *L. xylosteum* in the dry and regular irrigation conditions and to *L. tatarica* in flooded conditions, and produced root systems that were more than twice the length of those produced by *L. villosa* (Fig. 3C). *Lonicera tatarica* produced plants with the longest root systems under dry and regular irrigation moisture conditions, more than twice the length of the other Eurasian honeysuckles, and four times the length of *L. villosa*.

Among additional traits evaluated, *L. caerulea* displayed characteristics that were intermediate between those of *L. villosa* and the invasive Eurasian honeysuckles. For instance, *L. caerulea* plants rivaled *L. tatarica* in primary stem length, reaching 70.2 cm vs. 76.2 cm, respectively (Table 5), about four times the stem length produced by the North American native, *L. villosa* (17.3 cm). The leaves of *L. caerulea* were intermediate in size compared with the other Eurasian honeysuckles, with an average leaf size 1.7 times that of *L. xylosteum* and 73% that of *L. tatarica* (Table 5). *Lonicera caerulea* also produced leaf dry weights statistically equivalent to *L. tatarica* (2.9 g vs. 4.7 g, respectively), whereas both taxa produced greater leaf biomass than *L. xylosteum* (1.0 g). The mean specific leaf area of *L. caerulea* did not differ significantly from the other non-native honeysuckles, whereas *L. xylosteum*

produced leaves of greater specific leaf area than *L. tatarica* (Table 5). As in Expt. 1, the leaves of *L. villosa* were strikingly smaller than those of *L. caerulea*, but leaf traits of *L. villosa* were not quantified during Expt. 2 because they abscised before our measurements.

Discussion

Most measured traits of *L. caerulea* exceeded those of its close relative, the native *L. villosa* (Tables 3 and 5; Figs. 1 and 3), suggesting the two taxa may not be functionally equivalent in morphology and patterns of biomass accumulation. With this insight, the minor presence of *L. villosa* in the North American landscape is poor evidence that introduced genotypes of *L. caerulea* are unlikely to become invasive in North America. Whether the differences we observed are unique only to the genotypes we assessed, or are more generalizable across these taxa, remains unknown. However, plant breeding programs generally use hybridization and focus on growth rate, disease resistance, and

yield—traits that may confer greater competitive ability in the Eurasian genotypes of *L. caerulea*. Given the recent invasive history of *L. caerulea* on the Scandinavian Peninsula (Gederaas et al., 2012), observations of a naturalized and reproducing population in Minnesota (Schimpf et al., 2011), and the results of recent evaluations by checklist-based risk assessment tools (Peterson et al., 2018), further evaluation of risk before the widespread planting of *L. caerulea* of Eurasian background seems to be prudent.

Of particular noteworthiness, differences in biomass production that we observed between the native and nonnative genotypes of blue honeysuckle were consistent between the two experiments. Although growth of the two taxa in Expt. 1 was closer in magnitude to one another than to the known invader, *L. tatarica*, differences in biomass production between the two blue honeysuckles were consistent over a range of fertility treatments (Fig. 1), with *L. caerulea* producing an average of twice the dry biomass of *L. villosa*. However, unlike the clear response of *L. tatarica* to substrate fertility, root and shoot dry weights of the blue honeysuckles were less responsive to increasing CRF application rates (Fig. 1). In the second experiment, in which plants were grown in a hoop-house providing a cooler and shadier environment that more closely simulated natural conditions, *L. caerulea* produced dramatically more growth than *L. villosa* and was strikingly similar in most measurements to its two invasive Eurasian congeners (Fig. 3). Although quantitative differences among honeysuckles in response to moisture were not detected during Expt. 2, the significant interaction terms and difference among taxa for some taxon-by-moisture treatment combinations suggest that differences may be detected in an experiment with less variation or a greater duration.

In addition to differences in general measures of biomass accumulation, our results suggest that *L. caerulea* prioritizes vertical growth, a strategy common among competitive trees and shrubs of forest ecosystems (Gaudet and Keddy, 1988) and among invasive plants (Pyšek and Richardson, 2007). Although it is difficult to pinpoint which specific traits determine invasiveness in a given study system, invaders average signif-

icantly taller than their naturalized or native congeners in similar habitats (Divíšek et al., 2018; Gallagher et al., 2015). In our experiments, stem lengths of *L. caerulea* rivaled those of *L. tatarica* in length, with the former achieving similar primary stem lengths despite only producing shoot dry weights of 20% to 25% those of *L. tatarica* in Expt. 1 and 50% those of *L. tatarica* in Expt. 2. The prioritization of vertical growth may, in part, explain the success of *L. caerulea* as an invader of established forests in Norway (Gederaas et al., 2012). Moreover, the leaves of *L. caerulea* were more than twice the size of those found on *L. villosa* (Table 3), and closer in size to those of *L. tatarica*. Such leaves could further support a strategy of intense competitive ability by shading out competitors after gaining a height advantage. Indeed, our observations (Peterson et al., 2018) of a naturalized population of *L. caerulea* in Duluth, MN revealed that plants may reach heights of several meters, often by using neighboring plants as scaffolding to support vertical growth within the partially shaded forest understory.

Our experiments did not produce differences in specific leaf area between *L. caerulea* and *L. villosa*. Low specific leaf area is often associated with increased leaf life span (Reich et al., 1991) and greater resistance to herbivory (Grime et al., 1996), traits that likely contribute to the competitive success of invasive Eurasian honeysuckles (Lieuranc and Cipollini, 2013). Here, the specific leaf area of *L. caerulea* was statistically equivalent to both *L. tatarica* and *L. villosa* in Expt. 1, and comparable to *L. tatarica* and *L. xylosteum* in Expt. 2. The specific leaf area of *L. villosa* could not be calculated in Expt. 2 because of near-complete leaf senescence of these plants before leaf senescence began in the Eurasian taxa. Given this earlier senescence, leaf retention and the functional growing season may differ under some conditions between these native and introduced congeners.

Among the aspects of invasion biology that this study did not address are the evolutionary consequences of intentional hybridization during the development of cultivars, and the population genetic consequences of introducing nonnative genotypes to native gene pools. Given the potential that *L.*

Fig. 2. Least-square means of substrate electrical conductivity (E.C.; measured in milli-Siemens per centimeter) $\pm$ 95% confidence interval (CI) measured on three dates during the culture of (A) *Lonicera caerulea*, (B) *Lonicera villosa*, and (C) *Lonicera tatarica* after application of controlled-release fertilizer (CRF) at five rates. Twenty-five plants of each species were grown in #1 containers, with five replicates per application rate of Osmocote Pro 17–5–11 4-month CRF. Ninety-five percent CIs can be compared across panels to infer differences in substrate EC among taxa.

Table 4. Analysis of variance F-statistics and degrees of freedom (df) for the effects of taxon and moisture treatment on growth of *Lonicera caerulea*, *Lonicera villosa*, *Lonicera tatarica*, and *Lonicera xylosteum* 10 weeks after treatment initiation.^z

Source	Root dry wt (g)	Stem dry wt (g)	Leaf dry wt (g)	Root system length (cm)	Plant ht (cm)	Leaf size (cm ²)	Specific leaf area (cm ² ·g ⁻¹)
Moisture-level F value	0.301NS	0.196NS	0.559NS	3.293NS	0.275NS	1.943NS	0.710NS
Moisture-level df	2	2	2	2	2	2	2
Error A df	12	12	12	12	11	11	11
Taxon F value	47.949***	31.182**	14.778***	46.545***	90.764***	46.240***	4.233*
Taxon df	3	3	2	3	3	2	2
Moisture level $\times$ taxon F value	2.929*	2.717*	1.884	2.950*	1.422	2.051	2.480
Interaction df	6	6	4	6	6	4	4
Error B df	34	34	22	34	31	20	19

^zMoisture treatments were applied as described in Table 1.

NS, *, **, ****Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Fig. 3. Least-square means $\pm$ 95% confidence interval for (A) root dry weight, (B) stem dry weight, and (C) root system length of *Lonicera villosa*, *Lonicera caerulea*, *Lonicera xylosteum*, and *Lonicera tatarica* across three moisture treatments. Although the main effect of moisture treatment was not significant, a significant interaction between taxon and moisture treatment precluded interpretation of main effects alone.

Table 5. Least-square means $\pm$ 95% confidence interval (CI) for primary stem length, leaf dry weight, average leaf size, and specific leaf area of *Lonicera caerulea*, *Lonicera villosa*, *Lonicera tatarica*, and *Lonicera xylosteum* across three moisture treatments.²

Taxon	Measurement (95% CI)			
	Primary stem length (cm)	Leaf dry wt (g)	Avg leaf size (cm ²)	Specific leaf area (cm ² ·g ⁻¹)
<i>L. villosa</i>	17.3 (14.7–20.4) c	—	—	—
<i>L. caerulea</i>	70.2 (59.6–82.7) a	2.9 (1.9–4.4) a	11.3 (9.6–13.0) b	185.1 (173.1–197.1) a
<i>L. tatarica</i>	76.2 (63.8–91.0) a	4.7 (3.0–7.5) a	7.6 (5.9–9.3) a	196.3 (183.8–208.8) a
<i>L. xylosteum</i>	47.7 (40.5–56.2) b	1.0 (0.6–1.5) b	17.7 (15.8–19.6) c	177.5 (164.7–190.3) a

²The main effect of moisture treatment was not significant. *Lonicera villosa* was excluded from leaf measurements because of early leaf senescence. Means within the same column followed by the same letter are not significantly different according to 95% CIs.

caerulea might be able to invade habitats in North America, a close relationship between invasive genotypes and indigenous genotypes could lead to changes in genetic structure, genetic identity, and the decline or replacement of recipient populations (Ellstrand and Rieseberg, 2016). Introgression of nonnative genes into native populations has been observed for many agricultural crops that occur near related wild populations (Ellstrand et al., 2013). Introduced alleles can spread rapidly through native populations as hybrid progeny backcross with parental lineages, causing local extinctions of native populations within a few generations (Wolf et al., 2001). The specific risks, or benefits, of natural hybridization and introgression among multiple subspecies of *L. caerulea* (including *L. villosa*) from disparate provenances around the globe are largely unknown and should be studied further.

In conclusion, although *L. caerulea* did not exhibit growth or responses to fertility to the extent of the confirmed high-impact invader *L. tatarica* in the context of Expt. 1, it produced more dry biomass than *L. villosa* throughout the course of both experiments and was more similar in growth form and size to *L. tatarica* and *L. xylosteum*. Although it is difficult to predict invasiveness based on trait studies, the agricultural blue honeysuckles developed from Eurasian germplasm should not be assumed functionally or ecologically equivalent to their native North American congeners. When considering our results in conjunction with the invasive history of *L. caerulea* in the Scandinavian Peninsula, the long history of North American invasion by Eurasian *Lonicera*, the high risk of invasion indicated by checklist-based screening tools, and the uncertain potential for population-genetic consequences, this study illustrates the benefits of further trait studies before the

widespread planting of *L. caerulea* cultivars across North America.

Literature Cited

- Bailey, L.H. 1919. The standard cyclopedia of horticulture. Vol. 2. Macmillan, New York, NY.
- Barnes, W.J. and G. Cottam. 1974. Some autecological studies of the *Lonicera* $\times$ *bella* complex. Ecology 55:40–50.
- Batcher, M.S. and S.A. Stiles. 2000. Element stewardship abstract for *Lonicera maackii* (Amur honeysuckle), *Lonicera morrowii* (Morrow's honeysuckle), *Lonicera tatarica* (Tatarian honeysuckle), and *Lonicera* $\times$ *bella* (Bell's honeysuckle): The bush honeysuckles. The Nature Conservancy, Arlington, VA. <https://www.invasive.org/gist/esadocs/docmnts/loni_sp.pdf>.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1–48.
- Bors, B. 2009. Breeding of *Lonicera caerulea* L. for Saskatchewan and Canada. 20 June 2019. <<http://lonicera-conference.narod.ru/articles/Bors.pdf>>.
- Bors, B., J. Thomson, E. Sawchuk, P. Reimer, R. Sawatzky, and T. Sander. 2012. Haskap breeding and production (final report). Saskatchewan Agriculture, Regina, SK. ADF2008-0042. 20 June 2019. <<http://www.agriculture.gov.sk.ca/apps/adf/ADFAdminReport/20080042.pdf>>.
- Cavins, T.J., B.E. Whipker, W.C. Fonteno, B. Harden, I. McCall, and J.L. Gibson. 2000. Monitoring and managing pH and EC using the PourThru extraction method. North Carolina State University Hort. Info. Lft. 590.
- Celli, G.B., A. Ghanem, and M.S.L. Brooks. 2014. Haskap berries (*Lonicera caerulea* L.): A critical review of antioxidant capacity and health-related studies for potential value-added products. Food Bioprocess Technol. 7:1541–1554.
- Conser, C., L. Seebacher, D.W. Funjino, S. Reichard, and J.M. DiTomaso. 2015. The development of a plant risk evaluation (PRE) tool for assessing the invasive potential of ornamental plants. PLoS One 10:e0121053.
- Crispo, E., J.S. Moore, J.A. Lee-Yaw, S.M. Gray, and B.C. Haller. 2011. Broken barriers: Human-induced changes to gene flow and introgression in animals. BioEssays 33:508–518.
- Divišek, J., M. Chytrý, B. Beckage, N.J. Gotelli, Z. Lososová, P. Pyšek, D.M. Richardson, and J. Molofsky. 2018. Similarity of introduced plant species to native ones facilitates naturalization, but differences enhance invasion success. Nat. Commun. 9:4631.
- Ellstrand, N.C., P. Meirmans, J. Rong, D. Bartsch, A. Ghosh, T.J. de Jong, P. Haccou, B.-R. Lu, A.A. Snow, C.N. Stewart, J.L. Strasburg, P.H. van Tienderen, K. Vrieling, and D. Hoofman. 2013. Introgression of crop alleles into wild or weedy populations. Annu. Rev. Ecol. Evol. Syst. 44:325–345.
- Ellstrand, N.C. and L.H. Rieseberg. 2016. When gene flow really matters: Gene flow in applied evolutionary biology. Evol. Appl. 9:833–836.
- Ellstrand, N.C. and K.A. Shierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proc. Natl. Acad. Sci. USA 97:7043–7050.
- Fernald, M.L. 1925. The American representatives of *Lonicera caerulea*. Rhodora 27:1–11.
- Fox, J. and S. Weisberg. 2011. An R companion to applied regression. 2nd ed. Sage Publishing, Thousand Oaks, CA.

- Gallagher, R.V., R.P. Randall, and M.R. Leishman. 2015. Trait differences between naturalized and invasive plant species independent of residence time and phylogeny. *Soc. Conserv. Biol.* 29:360–369.
- Gaudet, C.L. and P.A. Keddy. 1988. A comparative approach to predicting competitive ability from plant traits. *Nature* 334:242–243.
- Gederaas, L., T.L. Moen, S. Skjelseth, and L.K. Larsen. 2012. Alien species in Norway: With the Norwegian black list 2012. The Norwegian Biodiversity Information Centre, Norway.
- Gerbrandt, E.M., R.H. Bors, R.N. Chibbar, and T.E. Baumann. 2017. Spring phenological adaptation of improved blue honeysuckle (*Lonicera caerulea* L.) germplasm to a temperate climate. *Euphytica* 213:172.
- Gioria, M. and B.A. Osborne. 2014. Resource competition in plant invasions: Emerging patterns and research needs. *Front. Plant Sci.* 5:501.
- Grime, J.P., J.H.C. Cornelissen, K. Thompson, and J.G. Hodgson. 1996. Evidence of a causal connection between anti-herbivore defence and the decomposition rate of leaves. *Oikos* 77:489–494.
- Holubec, V., T. Smekalova, F. Paprštejn, L. Štocková, and V. Řezníček. 2015. Potential of minor fruit crop wild relatives (CWR) as new crops in breeding for market diversification. In: R. Redden, S.S. Yadav, N. Maxted, M.E. Dulloo, L. Guarino, and P. Smith (eds.). *Crop wild relatives and climate change*. 2nd ed. Wiley, Hoboken, NJ.
- Hummer, K.E., K.W. Pomper, J. Postman, C.J. Graham, E. Stover, E.W. Mercure, M. Aradhya, C.H. Crisosto, L. Ferguson, M.M. Thompson, P. Byers, and F. Zee. 2012. Emerging fruit crops. In: M.L. Badenes and D.H. Byrne (eds.). *Fruit breeding: Handbook of plant breeding*. Vol. 8. Springer Science+Business Media, New York, NY.
- Koop, A.L., L. Fowler, L.P. Newton, and B.P. Caton. 2012. Development and validation of a weed screening tool for the United States. *Biol. Invasions* 14:273–294.
- Lenth, R.V. 2016. Least-squares means: The R package lsmmeans. *J. Stat. Softw.* 69:1–33.
- Lieurance, D. and D. Cipollini. 2013. Exotic *Lonicera* species both escape and resist specialist and generalist herbivores in the introduced range in North America. *Biol. Invasions* 15:1713–1724.
- Luken, J.O. and J.W. Thieret. 1996. Amur honeysuckle, its fall from grace. *Bioscience* 46:18–24.
- Naugžemys, D., S. Žilinskaitė, V. Kleizaitė, A. Skridaila, and D. Žvingila. 2011. Assessment of genetic variation among elite and wild germplasm of blue honeysuckle (*Lonicera caerulea* L.). *Balt. For.* 17:8–16.
- Naugžemys, D., S. Žilinskaitė, A. Skridaila, and D. Žvingila. 2014. Phylogenetic analysis of the polymorphic 4x species complex *Lonicera caerulea* (Caprifoliaceae) using RAPD markers and noncoding chloroplast DNA sequences. *Biologia* 69:585–593.
- Peterson, B.J., L. Stack, and D.J. Hayes. 2018. What do we know about the invasive potential of *Lonicera caerulea* cultivars in North America? Third International Symposium on Woody Ornamentals of the Temperate Zone. *Acta Hort.* 1191:129–137.
- Pyšek, P. and D.M. Richardson. 2007. Traits associated with invasiveness in alien plants: Where do we stand? In: W. Nentwig (ed.). *Biological invasions*. Ecological Studies, Vol. 193. Springer-Verlag, Berlin, Germany.
- R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 14 Nov. 2019. <<https://www.R-project.org/>>.
- Reich, P.B., C. Uhl, M.B. Walters, and D.S. Ellsworth. 1991. Leaf lifespan as a determinant of leaf structure and function among 23 Amazonian tree species. *Oecologia* 86:16–24.
- Saltonstall, K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proc. Natl. Acad. Sci. USA* 99:2445–2449.
- Schimpf, D.J., D.L. Pomroy, and S.C. Garske. 2011. Noteworthy collections. *Mich. Bot.* 50:26–38.
- Schneider, C.A., W.S. Rasband, and K.W. Eliceiri. 2012. NIH Image to ImageJ: 25 Years of image analysis. *Nat. Methods* 9:671–675.
- Tyler, T., T. Karlsson, P. Milberg, U. Sahlin, and S. Sundberg. 2015. Invasive plant species in the Swedish flora: Developing criteria and definitions, and assessing the invasiveness of individual taxa. *Nord. J. Bot.* 33:300–317.
- U.S. Department of Agriculture-Natural Resources Conservation Service. 2018. The PLANTS Database. National Plant Data Team, Greensboro, NC. 14 Feb. 2018. <<http://plants.usda.gov>>.
- Wang, Y., J. Zhu, X. Meng, S. Liu, J. Mu, and C. Ning. 2016. Comparison of polyphenol, anthocyanin and antioxidant capacity in four varieties of *Lonicera caerulea* berry extracts. *Food Chem.* 197:522–529.
- Wolf, D.E., N. Takebayashi, and L.H. Rieseberg. 2001. Predicting the risk of extinction through hybridization. *Conserv. Biol.* 15:1039–1053.