ORIGINAL ARTICLE

Intraspecific variation in morphology, physiology, and ecology of wildtype relative to horticultural varieties of red maple (*Acer rubrum*)

Eleanor C. Lahr[1,2] · Kristi M. Backe[1] · Steven D. Frank[1]

Abstract

Key message **Wildtype and horticultural varieties of red maple seedlings had different morphologies and physiological responses to water stress and abundances of an insect herbivore when grown in a common garden.**

Abstract Horticultural varieties of trees (cultivars) are selected for desirable and consistent morphologies relative to wildtype trees, but the ways by which morphology influences intraspecific physiological and ecological responses are unclear. In this study, we compared morphology, gas exchange and water use efficiency, and stem herbivory for seedlings of wildtype red maple (*Acer rubrum*) and two cultivars, 'Summer Red' and 'Brandywine,' grown together in a common garden. Seedlings also received high and low water treatments. Overall, wildtype seedlings grew taller, had fewer, larger leaves, intermediate rates of photosynthesis, and lower abundances of the scale insect *Melanapsis tenebricosa*, relative to the cultivars. Summer Red seedlings had more leaves, leaves with red coloration throughout the growing season, and the highest rates of photosynthesis, although photosynthesis was higher in green relative to red leaves. Brandywine seedlings tended to be intermediate in morphology, exhibited smaller differences between the high and low water treatments, and had higher leaf water use efficiency than Summer Red seedlings in April and June but similar water use efficiency in September. Total seedling water use efficiency, measured in September, was highest for wildtype seedlings. Both cultivars had more scale insects than wildtype seedlings under well-watered conditions. These differences among types of red maple aid in understanding how intraspecific variation in tree appearance—and the *en masse* planting of a single cultivar in cities—affects physiological and ecological responses, the provision of ecosystem services, and the condition of planted trees.

Keywords *Acer rubrum* · Cultivar · Genotype · Photosynthesis · Red maple · Wildtype

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Introduction

Morphological variation plays a key role in tree physiology and ecology across environmental gradients. For example, intraspecific differences in leaf size occur across broad ranges of many tree species (Hovenden and Vander Schoor 2006; Royer et al. 2008; Uribe-Salas et al. 2008) and promote drought tolerance, resistance to herbivory, and other physiological or ecological processes that influence tree condition and longevity (Brown and Lawton 1991; Poorter et al. 2006; Rozendaal et al. 2006). In urban environments, however, such naturally occurring intraspecific variation has often been removed through the planting of genetically identical horticultural varieties (cultivars). For example, red maple (*Acer rubrum* L.), a commonly planted species in eastern and Midwestern cities (Raupp et al. 2006), has over 40 different hybrid and cultivar varieties (Adkins

et al. 2012). Red maple cultivars are valued for characteristics like beautiful autumn foliage, but selecting for this or other morphological characteristics could result in the loss of non-targeted traits and in physiological and ecological consequences for the tree (Coley and Aide 1989; Burger and Edwards 1996; Menzies et al. 2016). This is a concern in cities because the long-term benefits provided by urban trees depend not only on appearance but also on planting species and individual cultivars that maximize ecosystem services while minimizing management costs (McPherson et al. 1997; Nowak and Crane 2002; Nowak et al. 2006, Pataki et al. 2011; Roy et al. 2012; Bodnaruk et al. 2017). Understanding tradeoffs in tree morphology, physiology, and ecology is critical to improving tree breeding programs, planting and management decisions, and urban forest sustainability.

Physiological and ecological differences among red maple cultivars, and between cultivars and wildtype trees, could be magnified or diluted in demanding urban environments. Urban impervious surfaces often increase temperature and drought stress, with corresponding effects on insect pest pressure (Dale and Frank 2014, 2017; Meineke et al. 2013), photosynthesis and carbon storage (Meineke et al. 2016), and urban tree condition and longevity (Roman and Scatena 2011). Bauerle et al. (2003) found that the red maple cultivars with the highest rates of photosynthesis at the start of a drought did not have the best performance by the end of the drought. This suggests that failing to account for potential environmental stressors, particularly in urban environments, could lead to choosing a cultivar that is ultimately not suited for the site conditions it will experience once planted.

In this study, we compare physiological and ecological tradeoffs among wildtype and horticultural varieties of red maple. Red maple occurs naturally throughout the eastern and Midwestern United States (Townsend et al. 1979; Walters and Yawney 1990; Abrams 1998; Royer et al. 2008), and cultivars are being planted in cities and suburbs in increasing numbers (Raupp et al. 2006), making this a useful study species for informing management of urban forests. In Raleigh, North Carolina, USA, where this study occurred, red maple comprises over 18% of the trees in the urban street tree inventory (Dale and Frank 2017). This species is a popular choice for street plantings throughout the eastern United States due to its tolerance of a variety of environmental conditions (Santamour and McArdle 1982; Townsend and McIntosh 1993; Abrams 1998; Townsend and Douglass 1998). Here, we assess stem growth and leaf traits, gas exchange and water use efficiency, and the abundance of a common stem herbivore on seedlings grown in a common garden under high and low watering treatments. Overall, our goals were to identify potential tradeoffs in tree morphology, physiology, and ecology that could influence the performance of wildtype and cultivar trees and to investigate how these tradeoffs were moderated by environmental

stress. We asked the following questions: (1) Do cultivar and wildtype seedlings have different morphologies? (2) Does gas exchange or water use efficiency differ among cultivar and wildtype seedlings? (3) Does herbivory differ among cultivar and wildtype seedlings? and (4) Do the watering treatments influence seedling morphology, physiology, or herbivory?

Materials and methods

Study species

Red maple is a medium-sized tree with rapid growth. It has an average lifespan of 80–150 years (Walters and Yawney 1990), although the mean life expectancy of street trees is shorter (Roman and Scatena 2011). The 'Summer Red' and 'Brandywine' cultivars used in this study were bred, respectively, to have new red–purple leaves in summer that mature green and turn yellow in autumn (Shademaker Trees, Collinsville, TX, http://www.shademakertrees.com/summermaple.htm. Accessed 24 Jan 2018), and to have new green leaves in summer that mature to brilliant red–purple in autumn (United States National Arboretum, Washington, D.C., https://www.usna.usda.gov/assets/images/as_standard_image/Acer_rubrum_Brandywine.pdf. Accessed 8 Jul 2019). In Raleigh and throughout the southeastern United States, the most serious pest of red maple is gloomy scale (*Melanaspsis tenebricosa* Comstock), a piercing–sucking insect that thrives on the trunk and branches of heat- and drought-stressed urban trees (Metcalf 1912; Frank et al. 2013; Dale and Frank 2014, 2017).

Experimental design

Wildtype red maple seedlings, grown from seed, were purchased from Pinelands Nursery (Columbus, NJ, USA), and 'Summer Red' and 'Brandywine' cultivars, grown from cuttings grafted to root stock, were purchased from Rennerwood Inc. (Tennessee Colony, TX, USA). We planted seedlings in 3 gallon pots in soilless sand and bark nursery mix (Parker Bark Company, Rose Hill, NC, USA) in May, 2016, and placed them outside at the Lake Wheeler Field Complex of North Carolina State University, in Raleigh, North Carolina. Raleigh (35.772096° N 78.638614° W) has a humid subtropical climate, with an average long-term January temperature of 4.2 °C and July temperature of 25.9 °C, and average annual precipitation of 117 cm (State Climate Office of North Carolina; www.climate.ncsu.edu. Accessed 26 Oct 2016).

We used a randomized complete block design to assign twenty wildtype, Summer Red, and Brandywine seedlings to five plots and two water treatments, with ten seedlings

of each type receiving a "high water" treatment, and ten seedlings receiving a "low water" treatment. An automatic irrigation system provided water three times per day in 2016 and two times per day in 2017. Seedlings in the high water treatment were watered seven days per week, and seedlings in the low water treatment were watered three days per week (Monday, Wednesday, and Friday). All seedlings received the same amount of natural precipitation. To verify that treatments were effective, we measured volumetric water content (Electronic Supplementary Table 1) on nine dates using a SM150 Soil Moisture Kit (Delta T Devices Ltd., Cambridge, UK).

We recorded stem height at the time of planting in May 2016. In April, June, and September 2017, we re-measured the stem height and counted the number of leaves per seedling. We qualitatively assessed leaf color on a whole-seedling basis in September 2017 by scoring seedlings for the presence of red color in 0–25% of leaves, 25–75% of leaves, or ≥ 75% of leaves. In October 2017, we collected 5–6 leaves per seedling, scanned them using a flatbed scanner (Epson Perfection V500 Photo), and calculated the area of individual leaves using the software Easy Leaf Area, according to Ealson and Bloom (2014).

We measured photosynthesis and stomatal conductance in April, June, and September 2017 using a LI-6400XT Portable Photosynthesis System with a leaf chamber fluorometer (LI-COR, Inc., Lincoln, Nebraska). Gas exchange conditions followed Lahr et al. (2018): 400 µmol mol^{-1} CO_2, 1200 µmol m^{-2} s^{-1} photosynthetically active radiation, and vapor pressure deficit between 1 and 2 kPa. Measurements were taken between the hours of 9:00 and 13:00 with a block temperature of 25 °C in April and June and 30 °C in September. After leaves had stabilized in the LI-6400XT chamber, we measured photosynthesis and stomatal conductance at 15-s intervals for 3 min. We averaged the measurements for each leaf to obtain a single value for further analyses. These measurements were performed on two fully expanded green leaves per seedling. Instantaneous water use efficiency was calculated as the ratio of photosynthesis to stomatal conductance. Total photosynthesis and total stomatal conductance per individual seedling were calculated by multiplying the mean rate of leaf photosynthesis or stomatal conductance by mean leaf size and total number of leaves in September (Electronic Supplementary Table 2). We also measured gas exchange of red leaves on a subset of Summer Red seedlings in September ($N = 8$ seedlings in the high water treatment and $N = 9$ seedlings in the low water treatment; Electronic Supplementary Table 3).

In May 2016, we used visual assessment to confirm that no gloomy scales were present on the seedlings. We then collected branches from mature red maple trees infested with gloomy scales on the campus of North Carolina State University (Raleigh, NC, USA) and placed five-centimeter-long

segments of these infested branches into floral water picks. We attached one water pick to the bottom stem of each seedling with twist-ties, so that the infested branch made contact with the seedling's stem. We repeated this process in June 2016. Gloomy scale nymphs transitioned from the infested branches to the seedlings during the summer of 2016. In December 2016, we counted gloomy scales on each seedling using an OptiVISOR binocular headband magnifier (Donegan Optical, Lenexa, Kansas) in the field. In March 2018, we harvested the seedlings and counted live gloomy scales (adults and nymphs) on the main stem using a dissecting microscope.

Statistical analyses

We performed all statistical analyses in R version 3.3.0 (R Core Team 2016). In the *lme4* package (Bates et al. 2013), we used a linear mixed model to test the efficacy of the watering treatments using treatment (high water or low water), plot (1–5), and treatment × plot as fixed effects, and tree identity as a random effect. Tree identity was included as a random effect to account for repeated measures (Baulmer et al. 2003; Jensen et al. 2018). Volumetric soil water content was the response variable. We performed Pearson correlations in the *psych* package (Revelle 2016) to examine correlations between change in stem height, number of leaves, and leaf size. We chose to use change in stem height during the experimental period as a morphological characteristic instead of actual stem height because wildtype and cultivar seedlings were obtained from different sources. We detected correlations between change in stem height and mean leaf size ($r = 0.53$, $P ≤ 0.01$) and between change in stem height and number of leaves ($r = 0.31$, $P = 0.03$), but not between number of leaves and mean leaf size ($r = -0.03$, $P = 0.8$). A principal components analysis showed that these variables did not reduce well to a single component. Therefore, we performed separate general linear mixed models for the following morphological characteristics: change in stem height between May 2016 and September 2017, number of leaves in September 2017, and mean leaf size in October 2017. We used the *lme4* package with Kenward–Roger approximation of F-statistics (Halekoh and Højsgaard 2014; Kuznetsova et al. 2016; Luke 2017; McNeish 2017) and treated seedling type (wildtype, Summer Red, or Brandywine), treatment (high water or low water), and seedling type × treatment as fixed effects. All models included initial stem height as a covariate and plot as a random effect.

To assess photosynthesis, stomatal conductance, and instantaneous water use efficiency on a per leaf basis, we used general linear mixed models with Kenward–Roger approximation of F-statistics (Halekoh and Højsgaard 2014; Kuznetsova et al. 2016; Luke 2017; McNeish 2017), with seedling type (wildtype, Summer Red, or Brandywine),

treatment (high water or low water), month of measurement (April, June, or September), and all interaction terms as fixed effects. Seedling identity was included as a random effect to account for repeated measures (Baulmer et al. 2003; Jensen et al. 2018) and plot was included as a random effect to account for blocking. Total (whole seedling) photosynthesis, stomatal conductance, and instantaneous water use efficiency were assessed using general linear mixed models with Kenward–Roger approximation of F-statistics (Halekoh and Højsgaard 2014; Kuznetsova et al. 2016; Luke 2017; McNeish 2017), with seedling type (wildtype, Summer Red, or Brandywine), treatment (high water or low water) and seedling type × treatment as fixed effects. Plot was included as a random effect to account for blocking. Total gas exchange was calculated once, in September when leaves were mature, and thus these models do not include a term to account for repeated measures. We performed an additional model for Summer Red seedlings in September, with leaf type (red or green), treatment (high water or low water) and leaf type × treatment as fixed effects and plot as a random effect. Gas exchange for red relative to green leaves was also assessed only once, in September, and this model, therefore, does not contain a term to account for repeated measures. Residuals for all models were tested for normality and met model assumptions.

We used generalized linear mixed models in the *lme4* package (Bates et al. 2013) to assess gloomy scale abundance in December 2016 and, separately, March 2018. We specified a negative binomial distribution because the count data were overdispersed when using a Poisson distribution. For 2016, seedling type (wildtype, Summer Red, or Brandywine), treatment (high water or low water), and seedling type × treatment were included as fixed effects and plot was included as a random effect. We used the same model specifications for 2018 but scaled the model by 2016 gloomy scale adult abundance to account for differences in the number of live adult scales detected the previous year. To test the significance of fixed effects and interactions, we used likelihood ratio tests to compare a model including the effect of interest with a nested model with this effect of interest removed. In 2016, there was not a significant interaction

($\chi^2 = 3.80$, $df = 2$, $P = 0.149$), and we removed the interaction term from the model. In 2018, we found a significant interaction between seedling type and watering treatment ($\chi^2 = 11.55$, $df = 2$, $P = 0.003$) and left the interaction term in the model.

Results

Our watering treatment was effective (Electronic Supplementary Table 1); volumetric soil water content was lower in pots in the low water treatment than in pots in the high water treatment ($\chi^2 = 20.19$, $df = 1$, $P < 0.001$) and was not affected by plot number ($\chi^2 = 3.44$, $df = 4$, $P = 0.488$). There was no interaction between water treatment and plot number ($\chi^2 = 0.62$, $df = 4$, $P = 0.961$). Watering treatment had a significant effect on increase in stem height, number of leaves, and mean leaf size (Table 1). Greater increases in stem height (from 2016 to 2017) occurred in the high water treatment for all seedling types (Fig. 1a), with a nearly significant seedling type × treatment interaction ($F_{2,48} = 1.77$, $P = 0.054$; Table 1) driven by a greater increase in wildtype seedlings' heights when watered compared to the increases in Summer Red or Brandywine seedlings' heights when watered (Table 1; Fig. 1a). Seedlings in the high water treatment had more leaves than seedlings in the low water treatment (Fig. 1b), and seedling type had a significant effect on number of leaves, where Summer Red seedlings had the greatest number of leaves at the end of the growing season, followed by Brandywine and then wildtype (Table 1; Fig. 1c). For mean leaf size, a significant seedling type × treatment interaction occurred (Table 1), with seedlings in the high water treatment having generally larger leaves (Fig. 1d). We also observed differences in September leaf coloration among seedling types. Of the nineteen surviving Summer Red seedlings, four had red coloration in 0–25% of their leaves, 13 seedlings had red coloration in 25–75% of their leaves, and two seedlings had red coloration in ≥ 75% of their leaves. No Brandywine seedlings had red coloration in their leaves, and three of the twenty wildtype seedlings had red coloration in 0–25% of their leaves.

Table 1 General linear mixed models test the effects of seedling type (wildtype, Summer Red, or Brandywine), treatment (high water or low water), and initial stem height on changes in stem height, number of leaves per seedling, and mean leaf size per seedling

Variable	Height Increase			Number of Leaves			Mean Leaf Size		
	F	df	P	F	df	P	F	df	P
Seedling type	2.02	2, 49	0.144	4.99	2, 49	**0.011**	1.48	2, 49	0.237
Treatment	30.13	1, 48	**≤ 0.001**	10.87	1, 48	**0.002**	7.44	1, 48	**0.009**
Initial stem height	1.56	1, 51	0.217	1.62	1, 49	0.210	0.85	1, 51	0.362
Seedling type × Treatment	3.09	2, 48	0.054	1.77	2, 48	0.182	3.270	2, 48	**0.047**

Degrees of freedom (df; numerator, denominator) are shown for the F statistic. Bold P values indicate significant effects ($\alpha = 0.05$)

Fig. 1 Selected morphological variation among seedlings by water treatment, seedling type, or their interaction. **a** Change in seedling height from spring 2016 to autumn 2017 varied by watering treatment ($F_{1,48} = 30.13$, $P < 0.001$). **b** A significant interaction ($F_{2,48} = 3.27$, $P = 0.047$) between watering treatment and seedling type explained mean leaf size per seedling in autumn 2017. **c** The number of leaves per seedling in autumn 2017 varied by watering treatment ($F_{1,48} = 10.87$, $P = 0.002$). **d** Number of leaves per seedling in autumn 2017 varied by seedling type ($F_{2,49} = 4.99$, $P = 0.011$). Full model results are shown in Table 1
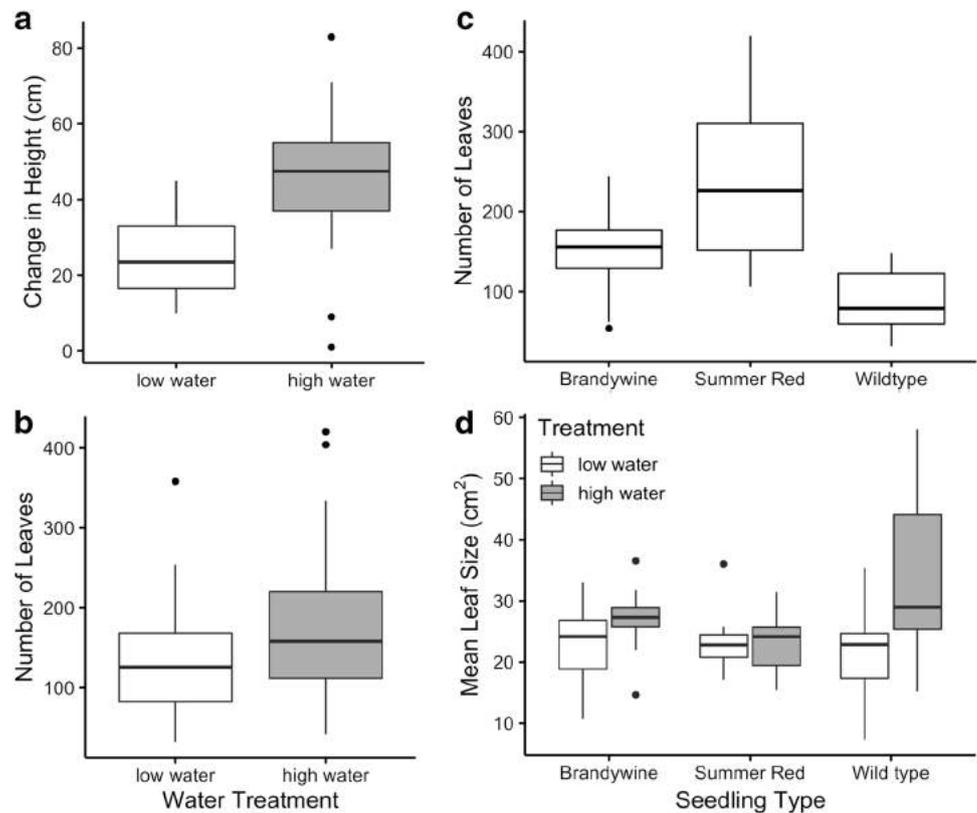
Leaf level gas exchange was significantly affected by seedling type, treatment, and month. Overall, mean rates of photosynthesis were highest in April, followed by September, and lowest in June (Fig. 2a). Photosynthesis rates were greater in the high water treatment than the low water treatment, and this difference was most apparent in April (treatment × month interaction: $F_{2,105} = 2.65$, $P = 0.075$; Table 2). We found a significant seedling type × month interaction (Table 2), where differences in photosynthetic rates between seedling types were greater in April than in June or September, with wildtype or Summer Red seedlings always having the highest mean photosynthesis rate (Fig. 2a). Brandywine seedlings showed wide variation in rates of photosynthesis, and the maximum rate of photosynthesis observed in the experiment (14.6 µ mol s^{-1} m^{-2}) occurred for a Brandywine seedling in the low water treatment in September (Electronic Supplementary Table 2). For stomatal conductance, we observed a treatment × month interaction, with the trees in the high water treatment showing higher stomatal conductance in April but not in June or September (Fig. 2b). A significant seedling type × month interaction also occurred, with Summer Red seedlings having higher stomatal conductance in April and September but not in June (Table 2; Fig. 2b). Overall, mean rates of stomatal conductance were highest in April (Fig. 2b). Instantaneous water use efficiency—the ratio of photosynthesis to stomatal conductance—was not affected by water treatment

but was significantly affected by a seedling type × month interaction (Table 2; Fig. 2c). Summer Red seedlings had the lowest water use efficiency (Fig. 2c). Similar water use efficiency occurred for Brandywine and wildtype seedlings in April, Brandywine seedlings had higher water use efficiency in June, and wildtype seedlings had higher water use efficiency in September (Fig. 2c). Total (whole seedling) photosynthesis, stomatal conductance, and water use efficiency generally followed per leaf results (Table 3), with higher photosynthesis (Table 3; Fig. 3a) and stomatal conductance (Table 3; Fig. 3b) among Summer Red seedlings and higher water use efficiency among wildtype and Brandywine seedlings (Table 3; Fig. 3c).

For red relative to green leaves of Summer Red seedlings in September, we observed significant effects of leaf type on photosynthesis and instantaneous water use efficiency (Table 4). Green leaves had higher rates of photosynthesis (Fig. 4a), no difference in stomatal conductance (Fig. 4b) and higher water use efficiency (Fig. 4c), and this was more pronounced, although not significant, in the high water treatment (Table 4, Fig. 4). In general, higher rates of photosynthesis and lower rates of stomatal conductance for green leaves in the high water treatment led to higher water use efficiency (Electronic Supplementary Table 3).

In 2016, following initial infestation, gloomy scale abundance (Electronic Supplementary Table 4) did not differ between seedling type or water treatment ($P > 0.05$ for
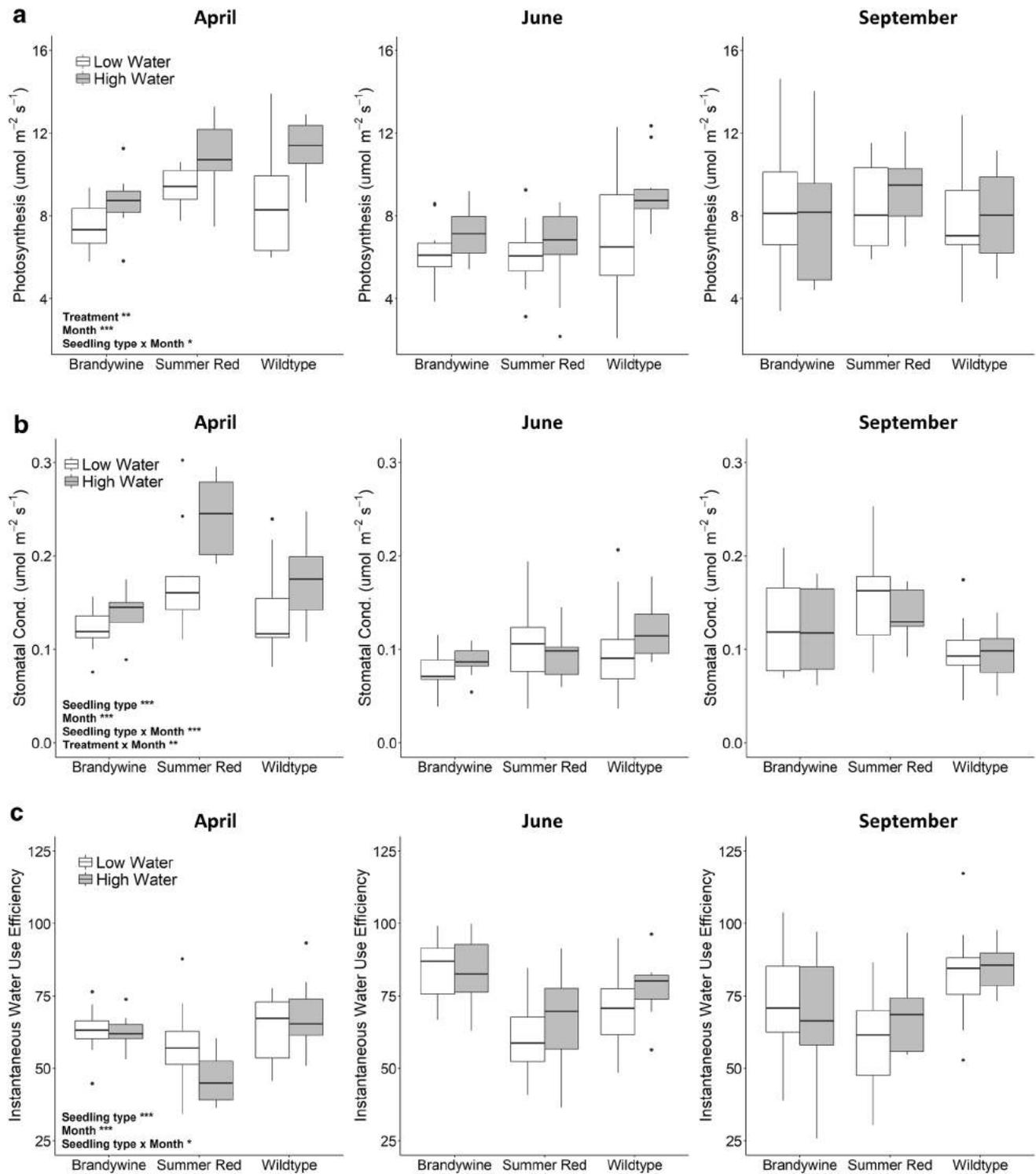
Fig. 2 Leaf gas exchange. **a** Photosynthesis in April, June, and September 2017, with significant model effects. **b** Stomatal conductance in April, June, and September 2017, with significant model effects. **c** Instantaneous water use efficiency (the ratio of photosynthesis to stomatal conductance) in April, June, and September 2017, with significant model effects. Seedling types (Brandywine, Summer Red, and wildtype) are shown on the x-axis. White boxes represent seedlings in the low water treatment and gray boxes represent seedlings in the high water treatment. Asterisks denote significance: *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$. Full model results are shown in Table 2

Table 2 General linear mixed models test the effects of seedling type (wildtype, Summer Red, or Brandywine), treatment (high water or low water), and month (April, June, or September) on leaf photosynthesis (μ mol s^{-1} m^{-2}) stomatal conductance (μ mol s^{-1} m^{-2}), and instantaneous water use efficiency (the ratio of photosynthesis to stomatal conductance)

Variable	Photosynthesis			Stomatal Cond.			Water use efficiency		
	F	df	P	F	df	P	F	df	P
Seedling type	3.14	2, 49	0.053	14.21	2, 49	**≤ 0.001**	18.5	2, 49	**≤ 0.001**
Treatment	9.59	1, 49	**0.003**	3.72	1, 49	0.060	0.45	1, 49	0.504
Month	18.62	2, 105	**≤ 0.001**	43.5	2, 105	**≤ 0.001**	17.38	2, 105	**≤ 0.001**
Seedling type×Treatment	1.26	2, 49	0.293	0.16	2, 49	0.856	0.62	2, 49	0.544
Seedling type×Month	3.34	4, 105	**0.013**	6.89	4, 105	**≤ 0.001**	3.40	4, 105	**0.012**
Treatment×Month	2.65	2, 105	0.075	5.64	2, 105	**0.005**	0.93	2, 105	0.398
Seedling type×Treatment×Month	0.64	4, 105	0.634	1.51	4, 105	0.206	1.23	4, 105	0.303

Degrees of freedom (df; numerator, denominator) are shown for the F statistic. Bold P values indicate significant effects

Table 3 General linear mixed models test the effects of seedling type (wildtype, Summer Red, or Brandywine) and treatment (high water or low water) on total seedling photosynthesis (μ mol s^{-1} m^{-2}), stomatal conductance (μ mol s^{-1} m^{-2}), and instantaneous water use efficiency (the ratio of photosynthesis to stomatal conductance) in September 2017

Variable	Photosynthesis			Stomatal Cond.			Water use efficiency		
	F	df	P	F	df	P	F	df	P
Seedling type	7.61	2, 48	**0.001**	13.19	2, 48	**≤ 0.001**	7.9	2, 48	**0.001**
Treatment	5.32	1, 48	0.228	3.54	1, 48	0.066	0.45	1, 48	0.508
Seedling type×Treatment	0.35	2, 48	0.703	0.10	2, 48	0.902	0.74	2, 48	0.487

Degrees of freedom (df; numerator, denominator) are shown for the F statistic. Bold P values indicate significant effects
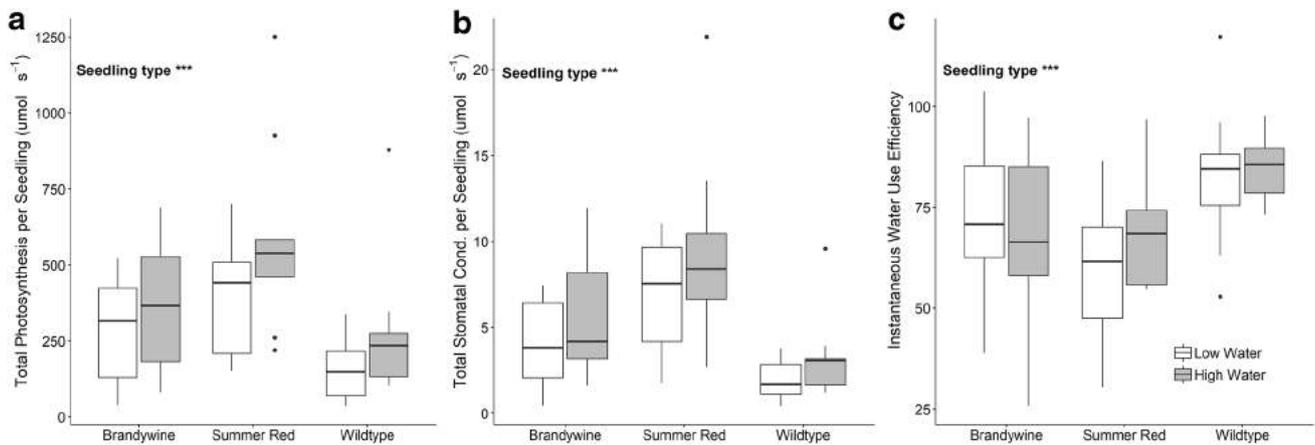

Fig. 3 Seedling gas exchange in September 2017. **a** Total photosynthesis, with significant model effects. **b** Total stomatal conductance, with significant model effects. **c** Instantaneous water use efficiency, with significant model effects. Total photosynthesis and stomatal conductance per seedling were calculated by multiplying mean photosynthesis or mean stomatal conductance by mean leaf size and the number of leaves per seedling. Instantaneous water use efficiency per seedling is the ratio of total photosynthesis to total stomatal conductance. Full calculations are shown in Electronic Supplementary Table 2. Seedling types (Brandywine, Summer Red, and wildtype) are shown on the x axis. White boxes represent seedlings in the low water treatment and gray boxes represent seedlings in the high water treatment. Asterisks denote significance: *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$. Full model results are shown in Table 3

Table 4 General linear mixed models test the effects of leaf type (red leaves or green leaves) and treatment (high water or low water) on photosynthesis (μ mol s^{-1} m^{-2}), stomatal conductance (μ mol s^{-1} m^{-2}), and instantaneous water use efficiency (the ratio of photosynthesis to stomatal conductance) for 'Summer Red' seedlings in September 2017

Variable	Photosynthesis			Stomatal Cond.			Water use efficiency		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Leaf type	7.22	1, 28	**0.012**	0.02	1, 28	0.899	6.26	1, 28	**0.019**
Treatment	0.38	1, 29	0.544	0.47	1, 29	0.500	1.58	1, 28	0.219
Leaf type×Treatment	2.45	1, 28	0.129	1.02	1, 28	0.320	0.45	1, 28	0.508

Degrees of freedom (*df*; numerator, denominator) are shown for the *F* statistic. Bold *P* values indicate significant effects
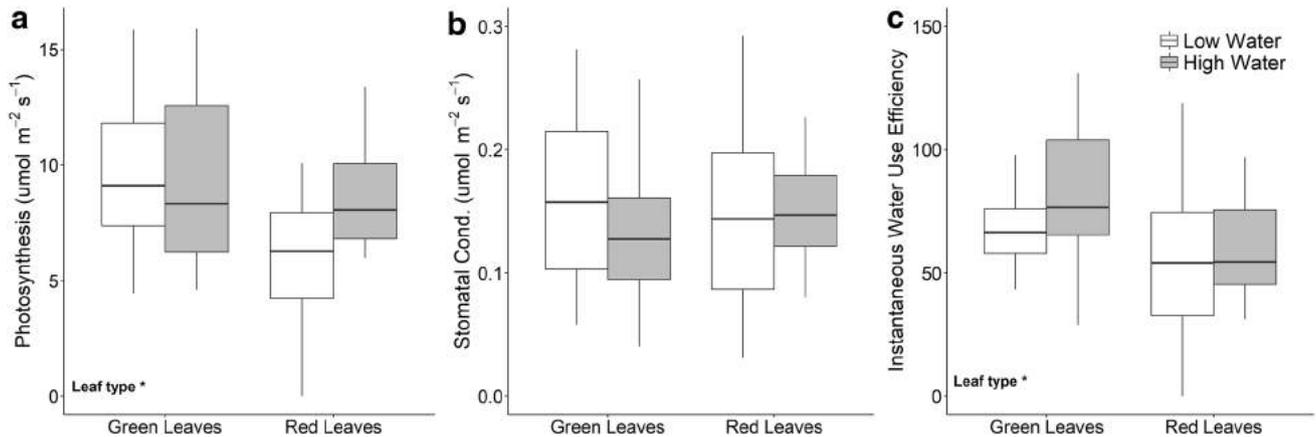

Fig. 4 Gas exchange of red and green leaves from Summer Red seedlings in September 2017. **a** Photosynthesis, with significant model effects. **b** Stomatal conductance, with significant model effects. **c** Instantaneous water use efficiency (the ratio of photosynthesis to stomatal conductance) with significant model effects. Leaf type (red or green) is shown on the *x* axis. White boxes represent seedlings in the low water treatment and gray boxes represent seedlings in the high water treatment. Asterisks denote significance: *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$. Full model results are shown in Table 4

Table 5 Generalized linear mixed model testing for differences in gloomy scale abundance in 2018

	Estimate	se	Wald *z*	*P*
Intercept (wildtype)	0.340	0.564	0.602	0.547
Seedling type (Brandywine)	− 0.766	0.838	− 0.914	0.361
Seedling type (Summer Red)	− 1.020	0.871	− 1.170	0.242
Treatment (wet)	− 2.597	1.261	− 2.058	**0.040**
Adults 2016	0.837	0.304	2.754	**0.006**
Seedling type (Brandywine)×Treatment (wet)	4.761	1.497	3.181	**0.001**
Seedling type (Summer Red)×Treatment (wet)	4.091	1.534	2.666	**0.008**

Wildtype seedlings in the low water treatment are the baseline. Adults 2016 is a covariate accounting for the number of adult scale insects counted on a seedling in 2016

all likelihood ratio tests). In 2018, there was a significant interaction between seedling type and watering treatment ($\chi^2 = 11.55$, $df = 2$, $P = 0.003$) (Table 5; Fig. 5). Gloomy scale abundance did not significantly differ for seedlings in the low water treatment. Compared to the low water treatment, the high water treatment reduced scale abundance on wildtype seedlings but increased scale abundance on Brandywine and Summer Red seedlings (Table 5; Fig. 5). The difference in scale abundance between wildtype seedlings and Brandywine seedlings was greater in the high water treatment than in the low water treatment, which was also true for the difference in scale abundance between wildtype seedlings and Summer Red seedlings (Table 5; Fig. 5).
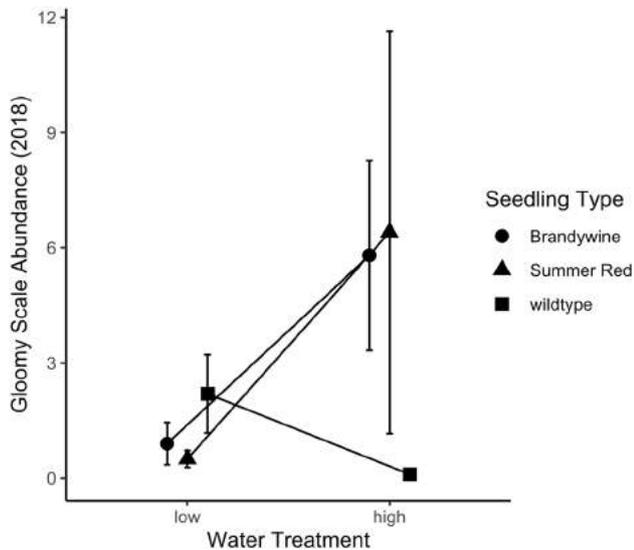
Fig. 5 Interaction plot showing mean (± standard error of the mean) gloomy scale abundance in 2018 for each seedling type in the high and low water treatments. There is a significant interaction between seedling type and water treatment ($\chi^2 = 11.55$, $df = 2$, $P = 0.003$) (Table 5), where the differences between wildtype seedlings and Brandywine seedlings and the differences between wildtype seedlings and Summer Red seedlings are greater in the high water treatment than in the low water treatment

Discussion

In this study, we found intraspecific variation in morphology, gas exchange, and stem herbivore abundance among different types of red maple seedlings. Furthermore, we observed within-cultivar differences in gas exchange in red leaves relative to green leaves on a cultivar selected or bred to produce new red leaves during the summer. While we did not find that one seedling type—wildtype, Brandywine, or Summer Red—consistently outperformed the others, nearly every variable we considered was affected in some way by our watering treatment, demonstrating the potential for tradeoffs that are moderated by local environmental stress. Month of measurement (April, June, or September) had a significant effect on seedling physiology, which was not surprising in itself, but seedling type × month interactions demonstrated patterns that were not consistent throughout the growing season; rather than a uniform response to seasonal change, seedling type also contributed to physiological responses over time. Thus, red maple cultivars should not be expected to perform similarly or equally well across the entire species range. This is important to consider given the popularity of red maple for use in urban environments (Raupp et al. 2006), because, currently, a cultivar could be selected in the northeastern United States, propagated clonally, grown in a Pacific Northwest nursery, and then planted in a city in the southeastern United States. Our findings highlight the

importance of understanding how intraspecific variation and selection for cultivars affect physiological and ecological tradeoffs, and choice of cultivar thus affects the ability of urban trees to contend with regional climates and insect pests, and to provide urban ecosystem services.

Red maple is one of many tree species that exhibit intraspecific variation in leaf morphology, with subsequent physiological and ecological effects (Hovenden and Vander Schoor 2006; Royer et al. 2008; Uribe-Salas et al. 2008). In urban environments, morphological differences in leaf size or leaf number among different cultivar varieties or wildtype red maples could affect several types of ecosystem services: rainwater throughfall and infiltration into the soil (Park and Cameron 2008; Kirnbauer et al. 2013), particulate filtration and air quality (Nowak et al. 2006; Selmi et al. 2016), and transpiration and air temperature in cities (Kjelgren and Montague 1998; Royer et al. 2009; Pataki et al. 2011). These leaf morphological variables also indicate differences in the amount of leaf area available for gas exchange. Wildtype seedlings had the fewest leaves (Table 1; Fig. 1c), but because they had larger leaves than the two cultivars in the high water treatment (Fig. 1d), there was a smaller difference in leaf area than would be expected based on leaf number alone.

Tree height plays an important role in providing shade or blocking wind, but height also affects the overall aesthetic appearance of urban trees. The ungainly appearance of wildtype red maples, due in part to their fast growth as seedlings, is one reason for the development of numerous horticultural varieties with consistent growth forms (Santamour and McArdle 1982; Townsend and McIntosh 1993; Abrams 1998; Townsend and Douglass 1998; Adkins 2012). In the low water treatment, we did not see differences in stem height from the beginning to the end of the experiment, but in the high water treatment, wildtype seedlings grew more than both cultivars (Table 1; Fig. 1a). A nearly significant treatment × seedling type interaction further suggests that the high water treatment had a greater effect on the growth of wildtype seedlings than on Summer Red or Brandywine seedlings' heights when watered (Table 1; Fig. 1a). From a management perspective, these results suggest there are benefits to planting wildtype trees in sites where privacy is valued over individual tree appearance, such as buffer areas or park borders, because of the ability of wildtype red maples to respond to natural precipitation and grow more quickly.

Intraspecific differences in gas exchange and water use efficiency are known to affect the long-term growth of trees and their ability to grow under challenging environmental conditions such as drought (Percival et al. 2006; Swoczyna et al. 2015; Tesky et al. 2015). Photosynthesis, stomatal conductance, and water use efficiency had seedling × month interactions indicating that patterns were not consistent throughout the growing season. For example, Summer Red

leaves had higher rates of photosynthesis than Brandywine leaves in April but not in June (Fig. 2a), and Brandywine leaves had highest water use efficiency in June (driven by lower rates of stomatal conductance) while wildtype leaves had the highest water use efficiency in September (Fig. 2c). This seasonal variation is not uncommon (Fang et al. 1996; Swoczyna et al. 2015) and speaks to the importance of understanding how intraspecific variation and cultivar selection affect physiology and ecosystem services over a tree's lifetime (Tesky et al. 2015). Overall, we did find that photosynthesis was higher in the high water treatment across all months, and despite monthly variation in photosynthesis and stomatal conductance, Summer Red leaves had the lowest water use efficiency during all months, suggesting they may be least suitable for hot, drought-prone urban sites.

As an estimate of the combination of morphological and physiological variables on red maple gas exchange, we calculated whole plant photosynthesis, stomatal conductance, and water use efficiency based on total leaf number and mean leaf size per seedling. These results indicate important tradeoffs between photosynthesis and water use efficiency for both wildtype and Summer Red seedlings. Wildtype seedlings had lower overall photosynthesis rates (Fig. 3a) but higher water use efficiency in both high and low water treatments (Table 3; Fig. 3c), and Summer Red seedlings had high photosynthesis rates (Fig. 3a) but the lowest water use efficiency (Table 3; Fig. 3c). Furthermore, red leaves on Summer Red seedlings had lower rates of photosynthesis than green leaves (Table 4; Fig. 4a). Selection for this trait could reduce the total photosynthetic potential of Summer Red or other cultivars that have red rather than green leaves during the growing season, as demonstrated in other species with variable leaf color (Burger and Edwards 1996; Menzies et al. 2016). Physiological differences in gas exchange and water use efficiency have long-term effects on mature trees, particularly under conditions of lower water availability or warmer temperatures which are common occurrences in urban environments (Meineke et al. 2016, 2018; Dale and Frank 2017). Potential tradeoffs between leaf traits and water use efficiency are, thus, important considerations in selecting the best cultivars for urban environments and should be considered in the context of both planting location and regional climate. Even if tradeoffs are reduced in mature trees, negative effects on younger trees will occur during a critical establishment window in challenging urban environments (Nowak et al. 1990; Roman et al. 2016).

Gloomy scale is a significant pest of urban red maples in the southeastern United States and reduces tree condition (Metcalf 1912; Dale and Frank 2014, 2017). Infestations are difficult to treat, and prevention via planting choices is the most reasonable management approach in the long term (Dale et al. 2016, Backe and Frank 2019). Previous work

has focused on placement of red maples in the landscape (Frank et al. 2013; Dale et al. 2016; Dale and Frank 2017; Just et al. 2018; Backe and Frank 2019), and in this study, we considered how red maple seedling type may play a role. After introducing gloomy scales to previously scale-free seedlings, we found that wildtype seedlings in the high water treatment had fewer scale insects than Brandywine or Summer Red seedlings (Fig. 5). Previously, high gloomy scale abundance was observed on urban cultivars (Youngsteadt et al. 2014, Dale and Frank 2014, Just et al. 2018) and a reduction in scale insect fecundity occurred with watering (Dale and Frank 2017). Our study adds the first results from a controlled experimental setting. Our results suggest that selection of traits in cultivars may result in an important tradeoff—higher susceptibility to a key pest—under certain environmental conditions.

Intraspecific variation in red maple enables this species to grow naturally throughout the eastern half of the United States (Royer et al. 2008, 2009) and provides baseline genetic variation for the development of new horticultural varieties with diverse characteristics. To date, however, although horticultural varieties are primarily planted in suburban and urban areas, traits under selection have been those related to appearance (Santamour and McArdle 1982; Sibley et al. 1995) or susceptibility to a leafhopper insect pest (Townsend and McIntosh 1993; Townsend and Douglass 1998), and not yet traits such as water use efficiency that could improve urban tree condition. Genetic diversity is also important for ecological resilience (Schaberg et al. 2008; Tooker and Frank 2012) and is generally lacking in urban tree canopies (Raupp et al. 2006). For red maple, planting a mix of horticultural varieties rather than a single cultivar will improve urban forest resilience, while still maintaining a more uniform appearance along streets, and planting locally sourced, wildtype red maple in areas where tree appearance is less critical would further improve the sustainability of urban forests in the face of abiotic and biotic stressors. Horticultural varieties will remain desirable due to their consistent morphologies relative to wildtype trees, but urban foresters can facilitate the evaluation of ecosystem services and management costs by including wildtype or specific cultivar identity in their lists of approved urban trees and in their urban tree inventories. This will allow for long-term evaluation of the susceptibility, condition, and performance of different varieties in diverse urban locations, and in cities in multiple climate zones. Such data may then be used to improve both horticultural breeding and urban forest health and sustainability.

References

Abrams MD (1998) The red maple paradox. Bioscience 48:355–364. https://doi.org/10.2307/1313374

Adkins CR, Braman SK, Chappell MR, Chong J-H, Derr JF, Dunwell WC, Frank SD, Fulcher AF, Hale FA, Klingeman WE et al. (2012) IPM for select deciduous trees in southeastern US nursery production. Fulcher AF, White SA, Eds. Knoxville, TN: Southern Nursery IPM Working Group

Backe KM, Frank SD (2019) Chronology of gloomy scale (Hemiptera: Diaspididae) infestations on urban trees. Environ Entomol. https://doi.org/10.1093/ee/nvz094

Bates D, Maechler M, Bolker B, Walker (2013) lme4: linear mixed-effects models using Eigen and S4. R package version 1.0-5. http://CRAN.R-project.org/package=lme4

Bauerle WL, Dudley JB, Grimes LW (2003) Genotypic variability in photosynthesis, water use, and light absorption among red and Freeman maple cultivars in response to drought stress. J Am Soc Hort Sci 128(3):337–342

Baulmer ER, Harrist RB, Carvajal S (2003) Analysis of repeated measures data. In: Reise SP, Duan N (eds) Multilevel modeling: methodological advances, issues, and applications. Lawrence Erlbaum Associates, Publishers, Mahwah

Bodnaruk EW, Kroll CN, Yang Y, Hirabayashi S, Nowak DJ, Endreny TA (2017) Where to plant urban trees? A spatially explicit methodology to explore ecosystem service tradeoffs. Landsc Urban Plan 157:457–467. https://doi.org/10.1016/j.landurbplan.2016.08.016

Brown VK, Lawton JH (1991) Herbivory and the evolution of leaf size and shape. Proc R Soc B 333:265–272. https://doi.org/10.1098/rstb.1991.0076

Burger J, Edwards GE (1996) Photosynthetic efficiency, and photodamage by UV and visible radiation, in red versus green leaf Coleus varieties. Plant Cell Physiol 37:395–399. https://doi.org/10.1093/oxfordjournals.pcp.a028959

Coley PD, Aide MT (1989) Red coloration of tropical young leaves: a possible antifungal defence? J Trop Ecol 5:293–300. https://doi.org/10.1017/s0266467400003667

Dale AG, Frank SD (2014) The effects of urban warming on herbivore abundance and street tree condition. PLoS One 9(7):e102996. https://doi.org/10.1371/journal.pone.0102996

Dale AG, Frank SD (2017) Warming and drought combine to increase pest insect fitness on urban trees. PLoS One 12(3):e0173844. https://doi.org/10.1371/journal.pone.0173844

Dale AG, Youngsteadt EK, Frank SD (2016) Forecasting the effects of heat and pests on urban trees: impervious surface thresholds and the 'Pace-to-Plant' technique. Arboric Urb For 42:181–191

Ealson HM, Bloom AJ (2014) Easy leaf area: automated digital image analysis for rapid and accurate measurement of leaf area. Appl Plant Sci 2(7):1400033. https://doi.org/10.3732/apps.1400033

Fang C, Monson RK, Cowling EB (1996) Isoprene emission, photosynthesis, and growth in sweetgum (*Liquidambar styraciflua*) seedlings exposed to short- and long-term drying cycles. Tree Physiol 16:441–446

Frank SD, Klingeman WE, White SA, Fulcher A (2013) Biology, injury, and management of maple tree pests in nurseries and urban landscapes. J Integr Pest Manag. https://doi.org/10.1630/ipm12007

Halekoh U, Højsgaard S (2014) A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models: the R package pbkrtest. J Stat Soft 59:1–30. https://doi.org/10.18637/jss.v059.i09

Hovenden MJ, Vander Schoor JK (2006) The response of leaf morphology to irradiance depends on altitude of origin in *Nothofagus cunninghamii*. New Phytol 169:291–297. https://doi.org/10.1111/j.1469-8137.2005.01585.x

Jensen SM, Schaarschmidt F, Onofri A, Ritz C (2018) Experimental design matters for statistical analysis: how to handle blocking. Pest Manag Sci 74:523–534

Just MG, Frank SD, Dale AG (2018) Impervious surface thresholds for urban tree site selection. Urb For Urb Green 34:141–146. https://doi.org/10.1016/j.ufug.2018.06.008

Kirnbauer MC, Baetz BW, Kenney WA (2013) Estimating the stormwater attenuation benefits derived from planting four monoculture species of deciduous trees on vacant and underutilized urban land parcels. Urb For Urb Green 12:401–407. https://doi.org/10.1016/j.ufug.2013.03.003

Kjelgren R, Montague T (1998) Urban tree transpiration over turf and asphalt surfaces. Atmos Environ 32:35–41

Kuznetsova A, Brockhoff PB, Christensen RHB (2016) lmerTest: tests in linear mixed effects models. R package version 2.0–30. http://CRAN.R-project.org/package=lmerTest

Lahr EC, Dunn RR, Frank SD (2018) Variation in photosynthesis and stomatal conductance among red maple (*Acer rubrum*) urban planted cultivars and wildtype trees in the southeastern United States. PLoS One 13(5):e197866

Luke SG (2017) Evaluating significance in linear mixed-effects models in R. Behav Res Methods 49:1494–1502

McNeish D (2017) Small sample methods for multilevel modeling: a colloquial elucidation of REML and the Kenward-Roger correction. Multivar Behav Res 52:661–670. https://doi.org/10.1080/00273171.2017.1344538

McPherson EG, Nowack D, Heisler G, Grimmond S, Sough C, Grant R, Rowntree R (1997) Quantifying urban forest structure, function, and value: the Chicago Urban Forest Climate Project. Urban Ecosyst 1:49–61

Meineke EK, Frank SD (2018) Water availability drives tree growth responses to herbivory and warming. J Appl Ecol. https://doi.org/10.1111/1365-2664.13130

Meineke EK, Dunn RR, Sexton JO, Frank SD (2013) Urban warming drives insect pest abundance on street trees. PLoS One 8(3):e59687

Meineke EK, Youngsteadt E, Dunn RR, Frank SD (2016) Urban warming reduces aboveground carbon storage. Pro R Soc B 283:20161574. https://doi.org/10.1098/rspb.2016.1574

Menzies IJ, Youard LW, Lord JM, Carpenter KL, van Klink JW, Perry NB, Schaefer HM, Gould KS (2016) Leaf color polymorphisms: a balance between plant defence and photosynthesis. J Ecol 104:104–113. https://doi.org/10.1111/1365-2745.12494

Metcalf ZP (1912) The gloomy scale, an important enemy of shade trees in North Carolina. J Elisha Mitchell Sci Soc 28:88–91

Nowak DJ, Crane DE (2002) Carbon sequestration by urban trees in the USA. Environ Pollut 116:381–389. https://doi.org/10.1016/s0269-7491(01)00214-7

Nowak DJ, McBride JR, Beatty RA (1990) Newly planted street tree growth and mortality. J Arboric 16:124–129

Nowak DJ, Crane DE, Stevens JC (2006) Air pollution removal by urban trees and shrubs in the United States. Urb For Urb Green 4:115–123. https://doi.org/10.1016/j.ufug.2006.01.007

Onyiah LC (2009) Design and analysis of experiments: classical and regression approaches with SAS. CRC Press, Boca Raton

Park A, Cameron JL (2008) The influence of canopy traits on through-fall and stemflow in five tropical trees growing in a Panamanian plantation. Forest Ecol Manag 255:1915–1925. https://doi.org/10.1016/j.foreco.2007.12.025

Pataki DE, Carreiro MM, Cherrier J, Grulke NE, Jennings V, Pincetl S, Pouyat RV, Whitlow TH, Zipperer WC (2011) Coupling biogeochemical cycles in urban environments: ecosystem services, green solutions, and misconceptions. Front Ecol Environ 9:27–36. https://doi.org/10.1890/090220

Percival GC, Keary IP, Al-Habsi S (2006) An assessment of the drought tolerance of Fraxinus genotypes for urban landscape plantings. Urb For Urb Green 5:17–27. https://doi.org/10.1016/j.ufug.2006.03.002

Poorter L, Bongers L, Bongers F (2006) Architecture of 54 moist-forest tree species: traits, trade-offs, and functional groups. Ecology 87:1289–1301. https://doi.org/10.1890/0012-9658(2006)87%5b1289:aomtst%5d2.0.co;2

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Raupp MJ, Cumming AB, Raupp EC (2006) Street tree diversity in eastern North America and its potential for tree loss to exotic borers. Arboric Urban For 32:297–304

Revelle W (2016) psych: procedures for Personality and Psychological Research, Northwestern University, Evanston, Illinois, USA. http://CRAN.R-project.org/package=psych Version = 1.6.4

Roman LA, Scatena FN (2011) Street tree survival rates: meta-analysis of previous studies and application to a field survey in Philadelphia, PA, USA. Urb For Urb Green 10:269–274. https://doi.org/10.1016/j.ufug.2011.05.008

Roman LA, Battles JJ, McBride JR (2016) Urban tree mortality: a primer on demographic approaches USDA Forest Service General Technical Report NRS-158

Roy S, Byrne J, Pickering C (2012) A systematic quantitative review of urban tree benefits, costs, and assessment methods across cities in different climatic zones. Urb For Urb Green 11:351–363

Royer DL, McElwain JC, Adams JM, Wilf P (2008) Sensitivity of leaf size and shape to climate within *Acer rubrum* and *Quercus kelloggi*. New Phytol 179:808–817. https://doi.org/10.1111/j.1469-8137.2008.02496.x

Royer DL, Meyerson LA, Robertson KM, Adams JM (2009) Phenotypic plasticity of leaf shape along a temperature gradient in *Acer rubrum*. PLoS One 4(10):e7653. https://doi.org/10.1371/journal.pone.0007653

Rozendaal DMA, Hurtado VH, Poorter L (2006) Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. Funct Ecol 20:207–216. https://doi.org/10.1111/j.1365-2435.2006.01105.x

Santamour FS Jr, McArdle AJ (1982) Checklist of cultivated maples: I. *Acer rubrum* L. J Arboric 8:110–112

Schaberg PG, DeHayes DH, Hawley GJ, Nijensohn SE (2008) Anthropogenic alterations of genetic diversity within tree populations: implications for forest ecosystem resilience. Forest Ecol Manag 256:855–862. https://doi.org/10.1016/j.foreco.2008.06.038

Selmi W, Weber C, Rivière Blond N, Mehdi L, Nowak D (2016) Air pollution removal by trees in public green spaces in Strasbourg city, France. Urb For Urb Green 17:192–201. https://doi.org/10.1016/j.ufug.2016.04.010

Sibley JL, Eakes DJ, Gilliam CH, Keever GJ, Dozier WA Jr, Peterson CM (1995) Anatomy and morphology of select red maple cultivars. J Arboric 21:136–144

Swoczyna T, Kalaji HM, Pietkiewicz S, Borowski J (2015) Ability of various tree species to acclimation in urban environments probed with the JIP-test. Urb For Urb Green 14:544–553. https://doi.org/10.1016/j.ufug.2015.05.005

Tesky R, Wertin T, Bauweraerts I, Ameye M, McGure MA, Steppe K (2015) Responses of tree species to heat waves and extreme heat events. PCE 38:1699–1712. https://doi.org/10.1111/pce.12417

Tooker JF, Frank SD (2012) Genotypically diverse cultivar mixtures for insect pest management and increased crop yields. J Appl Ecol 49:974–985. https://doi.org/10.1111/j.1365-2664.2012.02173.x

Townsend AM, Douglass LW (1998) Evaluation of various traits of 40 selections and cultivars of Red Maple and Freeman Maple growing in Maryland. J Environ Hortic 16:189–194

Townsend AM, McIntosh MS (1993) Variation among full-sib progenies of Red Maple in growth, autumn leaf color, and leafhopper injury. J Environ Hortic 11:72–75

Townsend AM, Wright JW, Kwolek WF, Beineke WF, Lester DT, Mohn CA, Dodge AF (1979) Geographic variation in young red maple grown in north central United States. Silvae Genet 28:33–36

Uribe-Salas D, Sáenz-Romero C, González-Rodríguez A, Téllez-Valdéz O, Oyama K (2008) Foliar morphological variation in the white oak *Quercus rugose* Née (Fagaceae) along a latitudinal gradient in Mexico: potential implications for management and conservation. Forest Ecol Manag 256:2121–2126. https://doi.org/10.1016/j.foreco.2008.08.002

Walters RS, Yawney HW (1990). Red maple. In: Burns RM, Honkala BH (eds) Silvics of North America Volume 2: Hardwoods. USDA Forest Service Agricultural Handbook 654, pp 60–69

Youngsteadt EK, Dale AG, Terando AJ, Dunn RR, Frank SD (2014) Do cities simulate climate change? A comparison of herbivore response to urban and global warming. Global Change Biol 21(1):97–105. https://doi.org/10.1111/gcb.12692