Introduction History Influences Aboveground Biomass Allocation in Brazilian Peppertree (*Schinus terebinthifolius*)

Kelley D. Erickson, Paul D. Pratt, Min B. Rayamajhi, and Carol C. Horvitz*

Multiple introductions of an exotic species can facilitate invasion success by allowing for a wider range of expressed trait values in the adventive range. *Schinus terebinthifolius* (Brazilian peppertree) is an invasive shrub that was introduced into Florida in two separate introductions and has subsequently hybridized, resulting in three distinct lineages (eastern, western, and hybrid). To determine whether allocation of aboveground biomass differed by introduction history, we destructively sampled 257 stems from each of six populations with differing introduction histories. The proportion of aboveground biomass allocated to fruit, wood, and leaves differed among the three populations. To determine whether the relationship between stem size and several dependent variables that measure plant performance (total dry weight, wood dry weight, number of fruits, fruit dry weight, leaf dry weight, and number of leaves) differed quantitatively by introduction history, we performed analyses of covariance. Slopes of these relationships (dependent variable vs. stem size) varied by lineage. Hybrid populations had the steepest slopes for one set of dependent variables (total dry weight, wood dry weight, and leaf dry weight), while western populations had the steepest slopes for a different set of dependent variables (number of fruits, fruit dry weight, and number of leaves). The parameterized regression equations for each dependent variable and lineage were used to nondestructively estimate different kinds of production by individuals that are part of long-term longitudinal studies to understand the demographic consequences of these different biomass allocation strategies for the performance of *S. terebinthifolius* individuals across the invaded range in Florida.

Nomenclature: Brazilian peppertree, *Schinus terebinthifolius* Raddi.

Keywords: Allometry, Florida, haplotype, hybrid vigor, invasion.

The invasion history of an exotic species often includes propagule introductions from multiple source populations, which can result in a wider range of trait values exhibited by invaders in the invaded range than might be expected from a single introduction (Crawford and Whitney 2010). When an invader is introduced into a novel region via independent introductions, how does the existence of multiple founding populations affect its biology in ways that might affect the invasion process? Aspects of the biology that could affect invasion and that might well differ by source population include biomass allocation to reproduction (Lavergne et al. 2004; Moracová et al. 2015), size at maturity (Weiner et al. 2009), and population dynamics (Iles et al. 2016). Understanding the genetic history of introductions and how source population affects the life history and ecology of invaders may provide insights to improve management strategies and improve understanding of invasive characteristics of select species (Allendorf and Lundquist 2003).

Schinus terebinthifolius Raddi (Anacardiaceae) is a large dioecious woody shrub, occasionally of small tree stature, growing to 7.5 m or more in height, often with multiple branches bearing bright red fruits that appear on female plants seasonally (Tobe et al. 1998). Native to Brazil,

Paraguay, and Argentina, the shrub has become an established problematic species globally around the subtropics including Florida (Cuda et al. 2006; Ewel et al. 1982; Morton 1978). *Schinus terebinthifolius* was introduced to Florida in two separate introductions: one on the western coast near the town of Punta Gorda (Charlotte County) and one on the eastern coast near Miami (Dade County) (Williams et al. 2005). The source populations for these two introductions were from different regions of the native range of *S. terebinthifolius* (coastal southeastern Brazil and northeastern Brazil in the Bahia state, respectively) and, consequently, the genetic backgrounds of the two founding populations were distinct (Mukherjee et al. 2012; Williams et al. 2005). Today, there are three recognized genetic lineages: one originating from the western Punta Gorda introduction (the western genetic type), one originating from the eastern Miami introduction (the eastern genetic type), and a hybrid lineage resulting from intraspecific hybridization between individuals from the eastern and western introductions (the hybrid genetic type) (Mukherjee et al. 2012; Williams et al. 2005, 2007). Previous common garden experiments have suggested that individuals from hybrid populations should have an advantage over individuals from either the eastern or western genetic types (Geiger et al. 2011).

Herein, spatial variation in biomass allocation across these genetic types was assessed by destructively sampling a stratified random set of individuals of each type. Determining whether such differences in biomass allocation would result in differences in fitness is a question that can only be addressed by a longitudinal study of tagged individuals over time. In this context, a method for estimating aboveground biomass parameters from a set of nondestructive measurements (e.g., diameter of woody stems) is needed. Understanding biomass partitioning in

S. terebinthifolius may also be useful in assessing the impact of herbivory by potential biological control agents on growth and fitness. Therefore, the specific objectives of this study were to (1) parameterize statistical models of the dependency of several performance metrics (biomass and numbers of fruits and leaves) on stem diameter (a nondestructive metric) and (2) quantify the influence of genetic type on biomass allocation to different functions.

Materials and Methods

Study Organism and Sites. As described in Tobe et al. (1998), *S. terebinthifolius* is a large shrub that can grow to a small size tree of up to 7.5-m high with multiple stems that are underground in some cases, arise separately from the ground, and give rise to a common crown. Each stem bears multiple branches along the stem axis, resulting in a multi-branching individual that can be as tall as it is wide.

Schinus terebinthifolius plants were destructively sampled at seven sites (Figure 1, Table 1) chosen to represent the range of introduction history (eastern genetic type: Chekika, Fort Pierce, and Holiday Park; hybrid genetic type: Big Cypress and Cape Canaveral; and western genetic type: Punta Gorda and Wild Turkey). Classification of sites into genetic types was based on a growing body of literature that examines the spatial pattern of *Schinus terebinthifolius* population genetics across Florida (Mukherjee et al. 2012; Williams et al. 2005, 2007). Select stands were chosen based on landowner permission and access. We assumed that the individuals we harvested at each site are true to the genetic types as predicted by Williams et al.'s (2007) maps of genotypes.

Field Measures of Plant Size and Biomass. Field sampling took place during the winter months when seeds were present, from October 27 through February 10 over 2008 to 2011. The range of sizes was delineated at each site and we selected individuals within each portion of the size range to ensure representation of the full range of sizes present at a site. In the field, the diameter at the base of each stem (trunk) as well as the diameter at the base of each branch coming off from a main stem were measured using calipers for small individuals and DBH tapes for individuals with diameters too large to measure with calipers. For multistemmed individuals, each stem was measured separately. To determine whether nearby stems belonged to the same individual or were separate individuals, we dug down into the soil to determine whether the stems were arising from the same source below ground. Individual stems that lacked a common crown were treated as separate individuals. Individual stems were excised at the soil level and total plant fresh weight was measured while at the field site. Larger stems were subsampled by selecting approximately 50% of the branches and processing their constituent parts (leaves, fruits, and wood) at the USDA/ARS
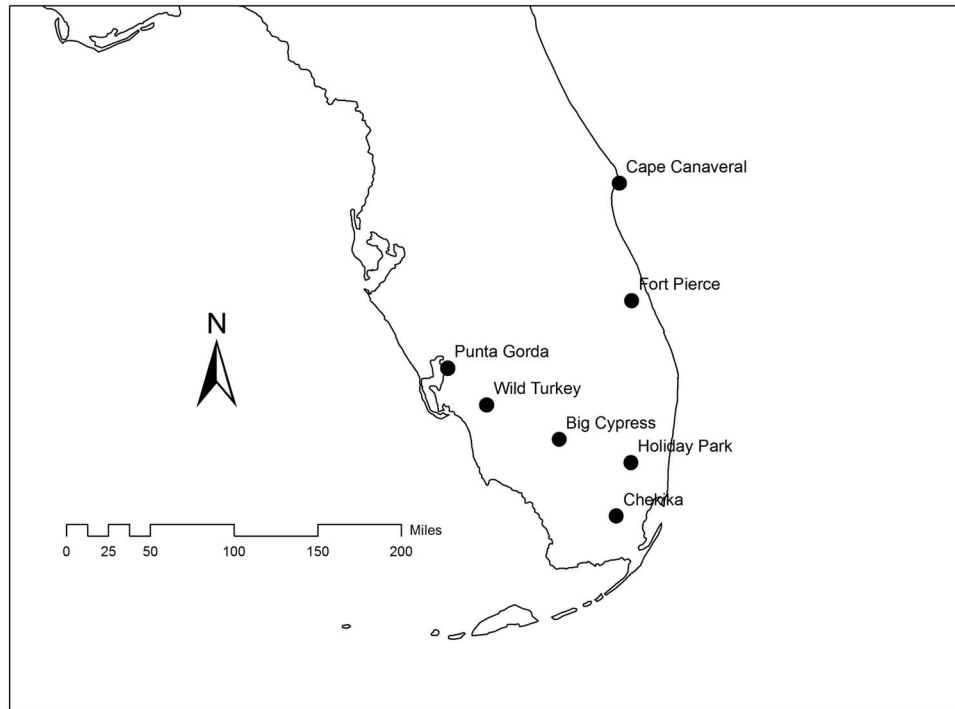
Figure 1. Location of each of the seven study sites.

Invasive Plant Lab in Fort Lauderdale, FL. The fresh weights of the subsample components were recorded in the field. The subsamples and smaller individuals that were not subsampled were transported to the laboratory and dried to a constant weight at 60 C. During the drying period, each sample bag was weighed once a week, and the final dry weight of the sample was recorded when the weight of the sample reached a constant weight. The fresh weight to dry weight ratio obtained from the subsamples was used to calculate the total dry biomass of plants. Additionally, the total number of fruit and total number of leaves (which in *S. terebinthifolius* are compound with multiple leaflets) were counted per branch. Across all sites, 257 stems were destructively sampled, including 86 stems from sites with predominantly eastern genetic types, 109 stems from sites with predominantly hybrid genetic type, and 62 stems from sites with predominantly western genetic types (Table 1).

Simulation of Missing Branch-level Data for Larger Stems. Because only a subsample of branches from larger stems was sampled, the biomass fractions (branch dry weight, fruit dry weight, and leaf dry weight) and number of leaves and fruits for the remaining branches were simulated using the data from the measured branches. We first used linear regression to determine the relationship between branch basal diameter and each of the biomass allocation dependent variables. We then simulated each missing branch-level value by drawing a random value from a

Table 1. Sites used to quantify the allometry of *Schinus terebinthifolius* in natural systems of Florida. GPS data are presented in decimal degrees and genetic type refers to predominate haplotype or hybrid as characterized by Williams et al. (2007).

Site	Latitude/longitude	Genetic type	Number of individuals sampled	Number of stems sampled
Big Cypress	26.24156°N, 81.0603°W	Hybrid	44	55
Cape Canaveral	28.45195°N, 80.5419°W	Hybrid	54	54
Chekika	25.57951°N, 80.567°W	Eastern	26	37
Fort Pierce	27.438726°N, 80.434152°W	Eastern	25	32
Holiday Park	26.041°N, 80.44037°W	Eastern	6	17
Punta Gorda	26.85443°N, 82.025262°W	Western	25	28
Wild Turkey	26.54087°N, 81.6872°W	Western	27	34

normal distribution with mean and variance determined from the regression based on branch diameter.

Stem-level Biomass Variables. After missing branch-level values were filled in through simulation, all branch-level biomass variables (branch dry weight, fruit dry weight, and leaf weight) as well as the number of leaves and fruits per branch were summed by stem to arrive at the stem-level variables of interest: total dry weight, wood dry weight, fruit dry weight, leaf dry weight, number of leaves, and number of fruits). These variables all involved destructive sampling of the stems. Together, these stem-level biomass variables indicate how an individual allocates its aboveground biomass to different tissues (leaves, wood, and fruit).

Aboveground Biomass Allocation by History of Introduction. The proportion of aboveground biomass devoted to wood, leaves, and fruits was calculated for each stem by dividing the dry mass of wood, leaves, and fruits, respectively, by the total aboveground dry mass. To investigate the effect of haplotype on biomass allocation, we conducted two Kruskal-Wallis tests: one to determine whether the haplotypes differed with respect to the proportion of biomass allocated to fruit and a second to determine whether the haplotypes differed with respect to the proportion of biomass allocated to wood.

Aboveground Biomass Production by History of Introduction. To determine whether aboveground biomass production differed by history of introduction, we used Analysis of Covariance where we modeled each of the biomass and production variables (total dry weight, branch dry weight, fruit dry weight, leaf dry weight, number of fruit, and number of leaves) as a function of genetic type (eastern, hybrid, or western).

To determine the relationship between the nondestructive measure, stem size, and each of the biomass and production variables (total dry weight, branch dry weight, fruit dry weight, leaf dry weight, number of fruit, and number of leaves), we used linear regression of squared stem diameter (x^2) and each of the biomass and production variables of interest (y) by genetic type. A variety of transformations were explored, but squaring stem diameter consistently resulted in higher coefficients of determination so this transformation was used throughout. For each biomass allocation variable of interest, there is an equation of the following form:

$$y = m \cdot x^2 \qquad [1]$$

where the slope m varies depending on the genetic history of the individual stem. Lastly, a weighted average for each of the three genetic-type–specific slopes was calculated by weighting each slope by the proportion of individuals of that genetic type in our sample. In all the equations, the intercept was forced through zero.

Results and Discussion

Allocation of aboveground biomass to fruit differed statistically among the genetic types (Kruskal-Wallis test, $\chi^2 = 25.975$, df = 2, p < 0.00001), with eastern stems devoting the greatest proportion of biomass to fruit (Figure 2A). Allocation of aboveground biomass to wood also differed among the genetic types (Kruskal-Wallis test, $\chi^2 = 7.2261$, df = 2, p = 0.027): eastern and hybrid stems allocated similar proportions to wood, while western stems allocated the smallest proportion to wood (Figure 2B). The remaining biomass was allocated to leaves, with western stems having the highest allocation to leaves (Figure 2C).

For all performance variables of interest (total dry weight, branch dry weight, number of fruits, fruit dry weight, leaf dry weight, and number of leaves), the slopes for the three genetic types were significantly different from each other (Table 2). The slope indicates how quickly each of the dependent variables increases with increasing stem size. For total dry weight, wood dry weight, and leaf dry weight, hybrid populations had the steepest slopes, while for number of fruits, fruit dry weight, and number of leaves, western populations had the steepest slopes (Table 2).

Individual stems from populations with different genetic histories varied in aboveground biomass allocation. Eastern individuals devoted more of their aboveground biomass to fruits than either of the other two genetic types, while western individuals tended to devote more of their aboveground biomass to leaves than either of the other two genetic types (Figure 2). The decision about whether to allocate biomass to fruit (reproduction) or leaves (photosynthesis) is a well-studied evolutionary tradeoff in biology (Obeso 2002; Reznick 1985; Smith 1976). Previous studies have found that invaders tend to invest more biomass into tissue involved in carbon capture, allowing them to grow more quickly than in their native range (Grotkopp and Rejmanék 2007; McDowell 2002; Tho et al. 2016).

Western individuals produced the most fruits (552 fruits per cm of diameter), followed by hybrid individuals (425 fruits per cm of diameter), while eastern individuals produced the fewest fruits (400 fruits per cm of diameter; Figure 3). Overall, this means that a reproductive female with a basal stem diameter of 3.05 cm (the median stem diameter of all stems in our study) would be expected to produce anywhere between 1,220 and 1,684 fruits per year depending on its genetic type. However, a previous common-garden experiment suggests that hybrid seeds have a higher survival rate than western seeds (Geiger et al. 2011), so despite producing more fruit, recruitment of new western individuals might indeed be lower than hybrid individuals.
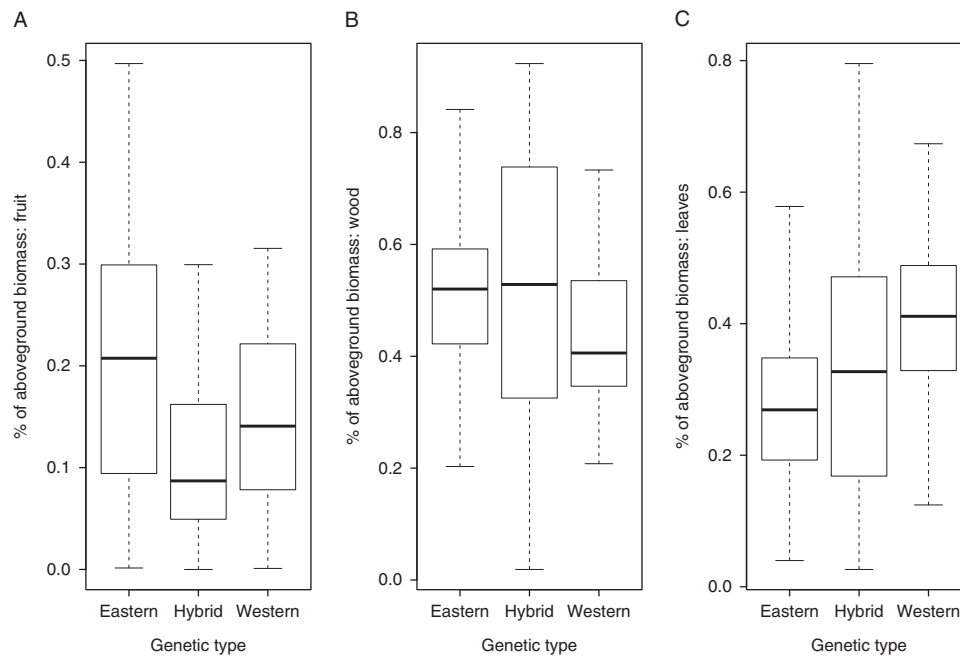
Figure 2. Biomass allocation to (A) fruit, (B) wood, and (C) leaves by genetic type.

We have shown here that populations that differ in introduction history differ in resource allocation; what is yet to be determined are the fitness consequences of such differences. In a companion study, to address the fitness consequences, we will be following populations of marked individuals from these different sources. The demographic data will be used to estimate population growth rates associated with the different allocation strategies.

Individuals from the hybrid populations exhibited the highest overall biomass per cm of stem diameter, dry weight of leaves per cm of stem diameter, dry weight of wood per cm of stem diameter, and the smallest fruit dry weight per cm of stem diameter. The rates of increase in biomass per unit size of hybrid stems were intermediate to the rates of eastern and western populations in regard to both the number of fruits and the number of leaves. An earlier common-garden experiment showed that hybrid seedlings had a higher biomass than seedlings of the western genetic type (Geiger et al. 2011). Our results suggest that hybrids maintain this advantage in total biomass throughout larger life stages, which may contribute to life-long increases in performance of the hybrid individuals, a hypothesis known as hybrid vigor.

These data indicate that there is strong evidence that genetic lineages influence biomass allocation. However, caution should be used when interpreting these results. While we assumed that all plants in each location shared the same history of introduction, it is likely that there is some overlap, with individuals of different genetic lineages living in the same population. Indeed, the proportion of eastern ancestry (in contrast to western ancestry) is predicted to fall between 0.8 to 1 for Chekika; 0.6 to 0.8 for Big Cypress, Ft. Pierce, and Holiday Park; 0.4 to 0.6 for Cape Canaveral, 0.2 to 0.4 for Wild Turkey; and between 0 and 0.2 for Punta Gorda (Figure 7 in Williams et al. 2007).

Because *S. terebinthifolius* is a tropical shrub lacking annual tree rings, it is difficult to reliably know the ages of individuals, so it is possible that the ages of individuals differed between the sites. It is also likely that spatial variation in environmental factors (e.g., temperature, precipitation, habitat type, soil type) across the study region may have contributed to the different patterns of biomass allocation we observed. However, the general trends we found and equations for estimating biomass components and the production of fruits and leaves should still be scalable to populations with similar genetic compositions. Also, we did not measure belowground biomass in this study; however, underground competition and alteration of the soil microbiome by *S. terebinthifolius* are likely to be important mechanisms facilitating the continued invasion and success of *S. terebinthifolius* individuals in new habitats (Dawkins and Esiobu 2016; Nickerson and Flory 2015).

In conclusion, we found that there was spatial variation in the biomass allocation of *S. terebinthifolius* stems, revealing a signature of their introduction history. Hybrid individuals tended to have a greater biomass than individuals from either of the source populations, although they tended to devote a greater proportion of their biomass to leaves and woody material than to fruit. We developed a method for estimating important plant size metrics via nondestructive

Table 2. Equation parameters for predicting how each biomass variable of interest (y) is related to basal stem diameter (x).

| Biomass variable	Genetic origin	Slope	R^2	F	df	p-value
		—m—				
Total dry weight (g)	Eastern	17.577	0.8157	374.8	3, 254	<0.0001
	Hybrid	27.6589				
	Western	22.2561				
	Weighted average	22.079				
Branch dry weight (g)	Eastern	22.234	0.4795	78.01	3, 254	<0.0001
	Hybrid	65.769				
	Western	31.146				
	Weighted average	38.9521				
Number of fruits	Eastern	400.35	0.7677	279.9	3, 254	<0.0001
	Hybrid	424.95				
	Western	551.97				
	Weighted average	445.1595				
Fruit dry weight (g)	Eastern	7.7812	0.746	248.6	3, 254	<0.0001
	Hybrid	7.2126				
	Western	9.2734				
	Weighted average	7.9509				
Leaf dry weight (g)	Eastern	9.7964	0.807	353.9	3, 254	<0.0001
	Hybrid	20.4464				
	Western	12.9826				
	Weighted average	14.1289				
Number of leaves	Eastern	24.9	0.829	410.4	3, 254	<0.0001
	Hybrid	44.8				
	Western	46.485				
	Weighted average	36.7664				
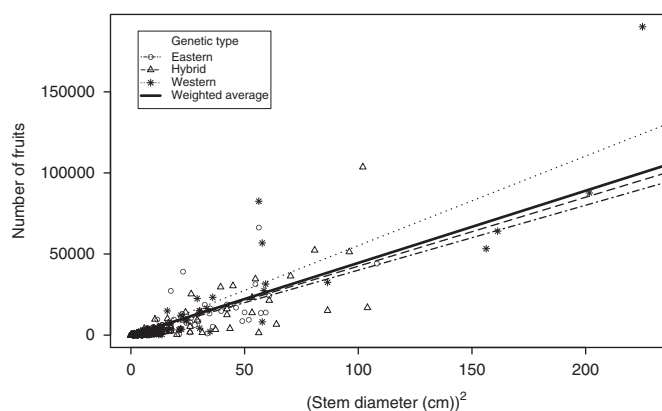
Figure 3. Scatterplot showing the relationship between squared stem diameter and number of fruits. Dash-dot lines represent the regression equations for individuals of eastern genetic type, dashed lines represent the regression equations for individuals of hybrid genetic type, dotted lines represent the regression equations for individuals of western genetic type, and solid lines represent the weighted average.

sampling that will allow us to estimate biomass production and allocation of individuals of different sizes in long-term demographic plots.

endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity employer and provider.

Literature Cited

Allendorf FW, Lundquist LL (2003) Introduction: population biology, evolution, and control of invasive species. Conserv Biol 17:24–30

Crawford KM, Whitney KD (2010) Population genetic diversity influences colonization success. Mol Ecol 19:1253–1263

Cuda JP, Ferriter AP, Manrique V, Medal JC (2006) Florida's Brazilian Peppertree Management Plan: Recommendations from the Brazilian Peppertree Task Force. West Palm Beach, FL: Florida Exotic Pest Plant Council. 82 p

Dawkins K, Esiobu N (2016) Emerging insights on Brazilian pepper tree (*Schinus terebinthifolius*) invasion: the potential role of soil microorganisms. Front Plant Sci 7, 10.3389/fpls.2016.00712

Ewel JJ, Ojima DS, Karl DA, DeBusk WF (1982) Schinus in Successional Ecosystems of Everglades National Park. Technical Report T-676. Homestead, FL: Everglades National Park, South Florida Research Center. 141 p

Geiger JH, Pratt PD, Wheeler GS, Williams DA (2011) Hybrid vigor for the invasive exotic Brazilian peppertree (*Schinus terebinthifolius* Raddi., Anacardiaceae) in Florida. Int J Plant Sci 172:655–663

Grotkopp E, Rejmánek M (2007) High seedling relative growth rate and specific leaf area are traits of invasive species: phylogenetically independent contrasts of woody angiosperms. Am J Bot 94:525–532

Iles DT, Salguero-Gomez R, Adler PB, Koons DN (2016) Linking transient dynamics and life history to biological invasion success. J Ecol 104:399–408

Lavergne S, Thompson JD, Garnier E, Debussche M (2004) The biology and ecology of narrow endemic and widespread plants: a comparative study of trait variation in 20 congeneric pairs. Oikos 107:505–518

McDowell SCL (2002) Photosynthetic characteristics of invasive and non-invasive species of *Rubus* (Rosaceae). Am J Bot 89:1431–1438

Moracová L, Pyšek P, Jarošík V, Pergl J (2015) Getting the right traits: reproductive and dispersal characteristics predict the invasiveness of herbaceous plant species. PLoS ONE 10, 10.1371/journal.pone.0123634

Morton J (1978) Brazilian pepper—its impact on people, animals and the environment. Econ Bot 32:353–359

Mukherjee A, Williams DA, Wheeler GS, Cuda JP, Pal S, Overholt WA (2012) Brazilian peppertree (*Schinus terebintifolius*) in Florida and South America: evidence of a possible niche shift driven by hybridization. Biol Invasions 14:1415–1430

Nickerson K, Flory SL (2015) Competitive and allelopathic effects of the invasive shrub *Schinus terebinthifolius* (Brazilian peppertree). Biol Invasions 17:555–564

Obeso JR (2002) The costs of reproduction in plants. New Phytol 155:321–348

Reznick D (1985) Cost of reproduction: an evaluation of the empirical evidence. Oikos 44:257–267

Smith CC (1976) When and how much to reproduce: the trade-off between power and efficiency. Am Zool 16:763–774

Tho BT, Sorrell BK, Lambertini C, Eller F, Brix H (2016) *Phragmites australis*: How do genotypes of different phylogeographic origins differ from their invasive genotypes in growth nitrogen allocation and gas exchange? Biol Invasions 18:2563–2576

Tobe JD, Craddock Burks K., Cantrell RW, Garland MA, Sweeley ME, Hall DW, Wallace P, Anglin G, Nelson G, Cooper JR, Bickner D, Gilbert K, Aymond N, Greenwood K, Raymond N (1998) Florida Wetland Plants: An Identification Manual. Tallahassee, FL: Florida Department of Environmental Protection. 588 p

Weiner J, Campbell LG, Pino J, Echarte L (2009) The allometry of reproduction within plant populations. J Ecol 97:1220–1233

Williams DA, Overholt WA, Cuda JP, Hughes CR (2005) Chloroplast and microsatellite DNA diversities reveal the introduction history of Brazilian peppertree (*Schinus terebinthifolius*) in Florida. Mol Ecol 14:3643–3656

Williams DA, Muchugu E, Overholt WA, Cuda JP (2007) Colonization patterns of the invasive Brazilian peppertree, *Schinus terebinthifolius*, in Florida. Heredity 98:284–293