# Morphological plasticity: a comparative study of leaf traits between exotic honeysuckle (*Lonicera japonica*) and its native congener (*Lonicera sempervirens*)

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## Abstract

Biological invasion, one of the major processes of global change is continuously rising in its intensity in the biota. With an estimated 137 billion dollar annual deficit in the U.S. alone, there is an urgent need to understand the mechanisms of invasion. How can invasive species be so detrimental? What types of biophysical traits can enable species to function this way? In this study, we attempted to answer these questions by comparing the leaf traits of exotic invasive (*Lonicera japonica*) and native (*Lonicera sempervirens*) honeysuckle species using multivariate analysis of variance (MANOVA) method. By examining and comparing multiple traits including leaf biomass, specific leaf area, leaf thickness, stomatal length and density, main leaf vascular bundle size, proportion of photosynthetic tissues, and leaf nitrogen and carbon contents on mass basis of both species, we were able to test the hypothesis that the invasive honeysuckle outperforms its native congener owing to its possession of suites of advantageous biophysical traits associated with its leaves. Our results indicate that significant differences exist in leaf traits, which are directly related to plant photosynthetic capacity or carbon gain, between the two species. Specifically, higher carbon gain (both in biomass and carbon content), larger proportion of photosynthetic tissues, thicker leaves, larger stomatal size, higher stomatal density, and larger leaf vascular tissues were associated with the exotic species. Larger leaf area and higher nitrogen content on mass basis were found in the native species. Our results reveal that the leaves of the invasive honeysuckle are morphologically optimized for a higher $CO_2$ gas exchange and faster carbon gain. Furthermore, the lower nitrogen content found in the invasive honeysuckle leaves characterizes the invader with a relatively high nitrogen use efficiency, which enhances its growth potential. We conclude that combination of advantageous leaf traits enable the exotic honeysuckle to be more plastic and successful compared to its native congener in the invaded ecosystems.

## Introduction

Biological invasion is considered one of the major component processes of global change; it has been identified as the second greatest threat to biodiversity after habitat loss (Mack et al., 2000; Stohlgren, 2002). A rise in the impact of invasions has been recently observed in all major biomes (Baskin 2002). Typically, invasion can change the role of native species in their communities, alter the structure and function of ecosystems, and disrupt evolutionary processes (D'Antonio and Vitousek, 1992; Williamson, 1996). Consequently, invasion has caused tremendous economic losses worldwide. According to Pimentel et al. (2005), it is estimated that in the United States alone more than 137 billion dollars are spent annually on combating biological invasions. Thus, identifying the underlying mechanisms of biological invasion has emerged as a crucial area of ecological research.

How can invasive species be so detrimental? What types of traits can enable species to function this way? Studies have suggested that certain biological traits are influential; typical examples include life-history strategies, reproductive and dispersal capabilities, phenotypic plasticity, and seedling growth patterns (Usher, 1988; Rejmanek and Richardson, 1996; Pattison et al., 1998; Reichard and Hamilton, 1997; Kolar and Lodge, 2001; Richardson and Rejmanek, 2004; Hamilton et al., 2005; Rejmanek et al., 2005). However, trait-based studies have been inconclusive; in particular, consistent traits have not been identified from which the future success of an invader could be inferred.

Much of the success of invasive species is thought to be associated with their superior ability to capture resources, particularly in environments that are resource limited (Funk and Vitousek, 2007). For invasive plants, leaf carbon fixation strategy closely ties to their invasion success in the introduced habitats. A clear understanding of leaf traits associated with carbon fixation strategy is critical in the study of plant invasion. Moreover, it seems logical to compare leaf traits of invasive species with their native congeners to further our understanding of what makes a successful invader in the novel range.

In this study, we hypothesized that invasive species outperform native congeners owing to their possession of a suite of advantageous biophysical traits at the leaf level. Particularly, we thought that invasive species possess a better carbon fixation strategy than their native congeners by having a morphologically optimized leaf tissue structure for a faster carbon gain. To test the hypothesis, a comparative approach was employed to achieve our objective: the identification of leaf traits that make the invasive species superior to the native congeners. The comparison involved pairing a targeted successful invader and its native congener within the same taxonomic group in order to minimize the sources of genetic variations. If advantageous leaf traits are found in the successful invader, then these traits will help pinpoint aspects as to why the invader has been as successful in the introduced range as it has been observed. Most importantly, the identified traits can be used in assessing/predicting invasive potential in other populations still in the initial stages of introduction, or even before an introduction takes place.

## Materials and Methods

*Species selection*

The invader

Japanese honeysuckle (*Lonicera japonica*), a perennial woody vine, was selected as the target invasive species for the following reasons: (1) Japanese honeysuckle is a highly successful invader, ever since its introduction to the US from eastern Asia in the 1800's as an ornamental and foraging plant. It is very common in the Southeastern states and is now found in 45 States (USDA Forest Service, 2004); (2) Japanese honeysuckle has made an enormous negative impact on the native ecosystems (Dillenburg et al., 1993a, b); (3) There has been very little comparative research done on the specific traits related to its invasion success at the leaf level.

The native congener

The native congener selected was the trumpet honeysuckle (*Lonicera sempervirens*), a perennial woody vine found in dry to moist deciduous forests and thickets. This plant is now rarely found in the wild; in the near future, it could even become an endangered species in the state of Kentucky and the rest of southeastern United State. Why is the exotic honeysuckle such a successful invader and why does its native congener only occur rarely in the natural communities? What could cause such a sharp contrast between two species belonging to the same genus? The answers to the questions could further our understanding of the mechanisms of plant invasion.

*Foliage sampling*

Foliage sampling was carried out during the summer of 2007 at multiple sites close to the Hancock Biological Station on the shores of the Kentucky Lake. Leave were sampled from fifteen randomly selected plants of both honeysuckle species at the field sites. Thirty fully expanded leaves were sampled from each individual plant. Leaves were enclosed in plastic bags and maintained on ice until analysis in the laboratory on the same day.

*Leaf trait measurements*

We selected nine leaf traits in this comparative study, including leaf biomass, specific leaf area, leaf thickness, stomatal length and density, main leaf vascular bundle size, proportion of photosynthetic tissues, and leaf nitrogen and carbon contents on mass basis.

Leaf area, length, width, and perimeter were measured using a CI-202 portable leaf area meter (CID, Inc). Leaf biomass was determined after oven drying at $70^0$C for 48 hours. Specific leaf area (SLA) was calculated as the ratio of leaf area to dry weight. Dry leaves were grinded for carbon and nitrogen element analysis using complete combustion gas chromatography with Perkin Elmer 240 CHN Analyzer.

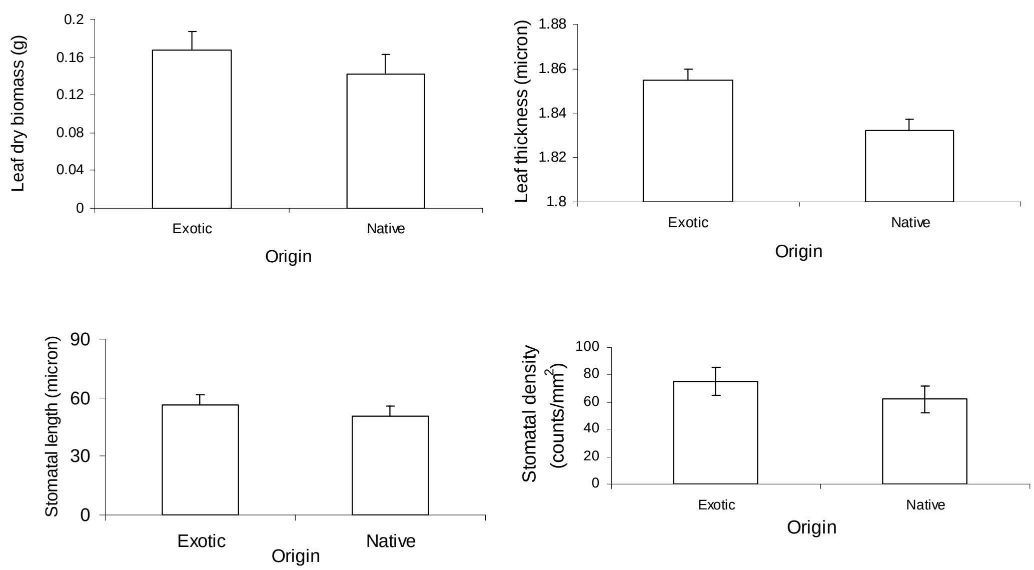
Thirty leaf transverse-section slides were prepared for both species to produce photomicrographs for examining the internal anatomical structure of leaves. The photomicrographs were made using an Olympus CX 31 light microscope under x100 and x400 respectively, and a Nikon Coolpix 8400 digital camera. Stomatal density was calculated using 30 photomicrographs for each species. Leaf thickness, stomatal size, and the proportion of photosynthetic tissue/mesophyll parenchyma were digitized using image analysis software SigmaScan Pro5 (SYSTAT Software, Inc.).

*Data analysis*

Multivariate analysis of variance (MANOVA) was performed using SYSTAT 12.0 (SYSTAT Software, Inc.) to test the differences in leaf traits between native and invasive honeysuckle species.

## Results and Discussion

Our results revealed significant differences in leaf traits between the two honeysuckle species (MONOVA: Wilk's Lambda = 0.055, p<0.0001; Hotelling-Lawley Trace = 17.109, p<0.0001). These leaf traits compared in Figure 1 are directly related to plant photosynthetic capacity or carbon gain. Specifically, higher carbon gain (both in biomass and carbon content), larger proportion of photosynthetic tissues, thicker leaves, larger stomatal size, higher stomatal density, and larger leaf vascular tissues were associated with the exotic species. Larger specific leaf area and higher nitrogen content on mass basis were found in the native species.



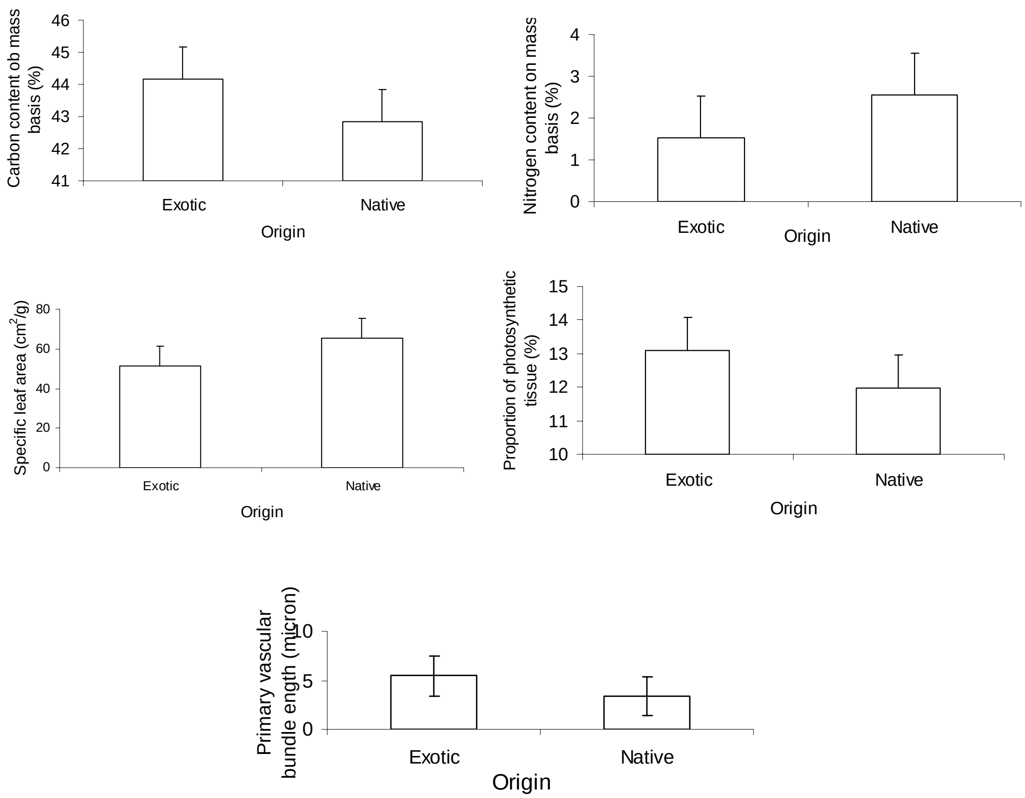



**Figure 1**. Leaf morphological and physiological traits are compared between exotic and native honeysuckle species. MANOVA test revealed significant differences in leaf biomass, thickness, stomatal length and density, carbon and nitrogen contents on mass basis, specific leaf area, proportion of photosynthetic tissues, and primary vascular bundle length.

Out results support the findings that successful invaders often possess traits that promote invasiveness as suggested by (Kolar and Lodge 2001) and Cadottee et al. (2005). We found that the leaves of the invasive honeysuckle are morphologically optimized for a higher $CO_2$ gas exchange and faster carbon gain. In particular, traits that facilitate photosynthesis are associated with exotic honeysuckle species, including higher proportion of photosynthetic tissues, large stomatal size, and stomatal density. In addition, large leaf vascular bundles, functioning in water and food conduction, are found in Japanese honeysuckle leaves. This could lead to an increased survival rate, and a faster growth rate. Thus, it might in part explain why the invader has been so successful in the field. Consequently, it might also spell out the rare occurrences of native honeysuckle in the native ecosystems as a result of interspecific competition. Furthermore, the lower nitrogen content found in the invasive honeysuckle leaves characterizes the invader with a relatively high nitrogen use efficiency, which enhances its growth potential.

We conclude that combination of advantageous leaf traits enable the exotic honeysuckle to be more plastic and successful compared to its native congener in the invaded ecosystems.

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