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Chinese Privet (*Ligustrum sinense)* in an elevated CO_2 Environment

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Abstract: Chinese privet (*Ligustrum sinense* Lour.) is an invasive plant currently threatening ecosystems in the southeastern United States and along its eastern seaboard. Control of this weed has generally been most effective if caught early enough to pull or dig out seedlings, meaning that effective control is accomplished with young or immature plants. The purpose of this study was to evaluate the impact of elevated atmospheric CO_2 on the growth of Chinese privet. Chinese privet, produced from seed, were grown at either 375 μmol mol^{-1} (ambient) or 575 μmol mol^{-1} (elevated) CO_2 in open top field chambers. Chinese privet seedlings grown under high CO_2 had greater numbers of branches and tended to have larger diameters and greater total root length. Increases in component part dry weights under elevated CO_2 resulted in significantly greater total plant biomass (42%). Root dry weight was significantly greater under CO_2 enriched conditions (44%); however, CO_2 did not affect root to shoot ratio nor allocation of biomass among plant organs. These finding indicate that Chinese privet will become a more troublesome weed as atmospheric CO_2 concentration continues to rise and suggests that early control may become an even more important issue.

Key words: Carbon dioxide, invasive plant, climate change, *Ligustrum sinense*

INTRODUCTION

Chinese privet (*Ligustrum sinense* Lour.) is native to China, Vietnam and Laos and was introduced to the United States in the 1850's as an ornamental and hedging plant (Miller, 2005). This species is now naturalized and a severe threat to ecosystems in the southeastern United States and along the eastern seaboard (Matlack, 2002; Langeland and Burks, 1988), as well as, Australia, New Zealand, Argentina and several Pacific Islands (Swarbrick *et al.*, 1999; Montaldo, 1993; Institute of Pacific Islands Forestry, 2000). Several mechanisms are responsible for the rapid spread of this noxious invasive plant: birds eat the berries and distribute the seeds via their droppings; roots spout new trees; seeds are spread by wild animals and deliberate plantings by humans (Miller, 2005; Batcher, 2000).

Many investigators have spent a great deal of time identifying characteristics of exotic plants that relate to that plant's invasiveness (Rejmanek, 2000); currently, several mechanisms are thought to explain the success of invasive plants. These include: escape from natural insect and disease pests (Blossey and Kamil, 1996; Klironomos, 2002; Mitchell and Power, 2003); lack of competition from native species on disturbed sites (Masters and Sheley, 2001); suppression of native vegetation through growth interactions (Callaway and Aschehoug, 2000) and rapid genetic adaptation to new environments (McDowell, 2002) resulting in increased resource use efficiency (Amarasekare, 2002; McDowell, 2002). While, these characteristics of invasive species partially explain how they became invasive, they don't predict how these plants may react to a changing global environment; however, the ability to rapidly adapt to new environments (McDowell, 2002) suggests that these weeds may have an advantage over native species.

It is well documented that the amount of carbon dioxide (CO_2) in the atmosphere is increasing (Keeling and Whorf, 2005). Several investigators have documented the stimulatory effect CO_2 has on photosynthesis, resource use efficiency and carbon allocation to belowground plant structures (Long and Drake, 1992; Rogers *et al.*, 1994; Amthor, 1995). These documented cases of stimulation in other plants species indicate that elevated CO_2 will also likely stimulate the growth and competitiveness of invasive plants (Ketner, 1990; MacDonald, 1992; Froud-Williams, 1996), perhaps to a greater degree than other plant species because of their ability to rapidly adapt to new environments (McDowell, 2002). This thought has been suggested by others as in Bright (1998), "Fast-growing, highly invasive plants may also be able to profit directly from the atmosphere's increased

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carbon content…any slower-growing natives would tend to lose out to the invaders."

Currently, very little effort has been focused on obtaining empirical data to determine the extent of increased response by invasive plant species and how this might impact the management of these species in a changing global environment. The work that has been done was recently summarized (Dukes and Mooney, 1999), nearly all invasive species showed positive growth responses to increased CO_2. In addition, Ziska (2002, 2003) reported that the stimulation of six invasive plants (Canada thistle, field bindweed, leafy spurge, perennial sowthistle, spotted knapweed and yellow star thistle) was three times greater than any species previously examined and suggested that this may have important implications for their control.

The southeastern United States is particularly vulnerable to invasive species due to its numerous ports of entry and mild climate. Therefore, it is important to understand how invasives already present in the Southeast will be impacted by elevated CO_2. Chinese privet is an example of an invasive plant that is prevalent in the southeastern United States and is currently threatening many ecosystems (Matlock, 2002), including the limestone cedar glade/woodland complex of the Central Basin of Tennessee, riparian forests (Morris *et al*., 2002) and others throughout the Southeast. Chinese privet establishes dense monoculture thickets where it out-competes native species for light and other resources, resulting in their decline (Batcher, 2000). The most effective control of Chinese privet is by pulling or digging out seedlings (Baker, 1998) which can only be done if the privet is discovered at a young or immature stage. Early attention to new infestations is the most effective means of control (Matlock, 2002). As the plants become larger, control moves into more labor intensive methods including herbicide control, stem cut and injection of herbicides and stump treatment with herbicides. If elevated CO_2 significantly stimulates the growth of this invasive plant species, the control applied to Chinese privet will have to be more aggressive in order to be effective. The objective of the current study was to assess the impact of elevated CO_2 on the growth of aerial and belowground parts of the Chinese privet in order to have a greater understanding of how this invasive species might respond to a changing global environment.

MATERIALS AND METHODS

Five Chinese privet seeds (*Ligustrum sinense* Lour.), a C_3 broadleaf evergreen shrub, (local source, Auburn, AL) were sown into a peat-based general purpose growing medium (PRO-MIX Bx, Premier Horticulture Inc., Quakertown, PA 18951) in 10.65 L plastic containers (TPOT4 Round Tree-pot, 22×39 cm, Stuewe and Sons Inc., Corvallis, OR 97333). When seedlings were 5-10 cm tall they were thinned to one plant per container.

Forty-eight of these plants were selected for placement in open top field chambers (OTC). The plants in these 48 containers were ranked, according to plant size and placed into four groups of 12 containers each, representing the largest 12 first in declining order down to the smallest 12; one container from each group was randomly assigned to each of the 12 OTCs (6 elevated CO_2; 6 atmospheric CO_2) used in the study (4 containers in each chamber). Initial measurements (including height, ground line diameter and number of leaves) were taken on each plant before placement in OTCs.

The study was conducted at the soil bin facilities of the USDA-ARS National Soil Dynamics Laboratory, Auburn, Alabama. The bin used for the experimental setup is 6 m wide and 76 m long and has been modified for container studies; modifications consisted of installing a geomembrane liner (20 mil) and gravel drain system to ensure a good working surface and drainage for container studies. Open top chambers, encompassing 7.3 m^2 of ground surface area, were used to continuously (24 h per day) deliver target CO_2 concentrations of 375 $\mu mol\ mol^{-1}$ (ambient) or ambient plus 200 $\mu mol\ mol^{-1}$ (elevated) using a delivery and monitoring system. Actual CO_2 concentrations over the measurement period (±SE) were as follows: ambient daytime = 396.5 (±0.3); elevated daytime = 567.5 (±0.4); ambient nighttime = 430.8 (±0.4); and elevated nighttime = 617.1 (±0.5) (daytime was taken as 7:00 AM CST to 7:00 PM CST).

The experimental design was a randomized complete block design with blocks occurring along the length of the soil bin. No significant block effect was detected. Plants were placed in the OTCs on 6 June, 2006 at which point CO_2 treatments were initiated. All plants were fertilized with Miracle-Gro®(15:30:15, N:P:K; Scotts Products Inc., Marysville, OH) on 8 June, 2006 and 21 August, 2006. Fertilization was accomplished by mixing 600 g Miracle-Gro in 130 L deionized water; each plant received 500 ml of this solution. In addition, plants received an iron chelate treatment (1:0:0, N:P:K, plus 1.25% water soluble iron, Ironite Products Co., Scottsdale, AZ) on 29 June, 2006; approximately 20 g of granular Ironite® was added to each pot.

All Chinese privet seedlings were destructively harvested on 17 November 2006, corresponding to 165 days of CO_2 exposure. Aboveground portions of all plants in each container were harvested by severing the plant(s) at the ground-line. Aboveground parameters (e.g., height, diameter, number of branches) were assessed using standard practices. Diameters were measured at ground line using high precision digital calipers. Plants were then separated into organ parts (i.e., leaves, stems) and leaf area was determined photometrically using a LI-3100 leaf area meter. Roots were separated from the growing medium using the sieve method. Root length was measured using a Comair Root Length Scanner (Hawker de Havilland, Port Melbourne, Australia). Plant organs were then dried in a forced-air oven at 55°C to a constant weight and dry weights recorded. Dry weights of each organ part were considered a measure of photosynthate partitioning; allocation among organ parts was calculated based on these weights. Data were totaled for each container and the four containers in each OTC averaged prior to analysis.

Data analysis was conducted using the mixed model procedures (Proc Mixed) of the Statistical Analysis System (Littell *et al*., 1996). In all cases, differences were considered significant at the $\alpha \le 0.05$ and trends were recognized at $0.05 < \alpha \le 0.15$.

RESULTS

Over the duration of the experiment a number of plants died in the chambers (6 in the elevated chambers and 8 in the ambient chambers). These plants were not replaced and were not included in data analysis following termination of the experiment. Statistical analysis revealed no significant differences between plant mortality in ambient versus elevated CO_2 treatments (p = 0.628; data not shown) in addition statistical analysis of initial plant measurements showed no bias existed between different CO_2 treatments.

Elevated CO_2 increased number of branches (47%) for Chinese privet seedlings and also tended to increase diameter (Table 1). Plant height and leaf area were not impacted by CO_2 treatment. In addition, high CO_2 tended to increase Chinese privet seedling shoot dry weight due to changes in both leaf and stem dry weights (Table 2). While dry root biomass was significantly increased (44%) by growth in high CO_2 (Table 2), there was only a trend for increased root length (Table 1). Chinese privet total biomass was significantly greater (42%) for seedlings grown under elevated CO_2 conditions (Table 2). However, CO_2 had no effect on biomass allocation among privet component parts, resulting in no change in root to shoot ratio (Table 3).

Table 1: The response of Chinese privet seedling growth variables to ambient (375 μmol mol^{-1}) and elevated (ambient + 200 μmol mol^{-1}) CO_2. Means with associated separation statistics and percent change (ambient to elevated) are shown

Parameter	Ambient CO_2	Elevated CO_2	Change (%)	p-values
Height (cm)	21.86	22.37	2.3	0.571
Diameter (mm)	5.78	6.65	15.1	0.118
Number of Branches	41.40	60.80	46.9	0.051
Leaf Area (cm^2)	313.10	400.00	27.8	0.234
Root Length (m)	212.26	294.79	38.9	0.068

Table 2: The response of Chinese privet seedling plant component dry weights (g) to ambient (375 μmol mol^{-1}) and elevated (ambient + 200 μmol mol^{-1}) CO_2. Means with associated separation statistics and percent change (ambient to elevated) are shown

Plant part	Ambient CO_2	Elevated CO_2	Change (%)	p-values
Leaf	2.92	4.03	38.0	0.061
Stem	2.40	3.46	44.2	0.077
Total shoot	5.32	7.49	40.8	0.066
Roots	5.40	7.76	43.7	0.030
Total plant	10.73	15.25	42.1	0.044

Table 3: The response of Chinese privet seedling allocation among plant component parts (%) to ambient (375 μmol mol^{-1}) and elevated (ambient + 200 μmol mol^{-1}) CO_2. Means with associated separation statistics and percent change (ambient to elevated) are shown

Plant part	Ambient CO_2	Elevated CO_2	Change (%)	p- values
Leaves	26.19	26.28	0.3	0.938
Stems	21.68	21.77	0.4	0.940
Roots	52.12	51.94	-0.3	0.934
Root to shoot ratio	1.14	1.10	-3.5	0.688

DISCUSSION

Past research by our group and others has found that elevated CO_2 increases the growth of most plants (Kimball, 1983; Strain and Cure, 1994; Amthor, 1995; Jones and Curtis, 2000) due primarily to increased rates of photosynthesis (Long and Drake, 1992; Runion *et al*., 1999), altered carbon partitioning (Rogers *et al*., 1994) and increased water and nutrient use efficiencies (Hocking and Meyer, 1985; Patterson, 1986; Rogers *et al*., 1999). Generally, crops and weeds with the C_3 metabolic pathway have a 33-42% increase in plant biomass, while those with the C_4 pathway tend to have small responses in the range of 10-15% (Kimball, 1983; Fuhrer, 2003; Prior *et al*., 2003). We recently studied two invasive plants and found a 22% increase in sicklepod (*Arabis Canadensis,* C_3) and a 12% increase in Johnsongrass (*Sorghum halepense*, C_4) (Runion *et al*., 1999) which is in agreement with reported trends. In the present study, elevated CO_2 increased Chinese privet (a C_3 shrub) biomass by 42%; again, consistent with the general response of plants with this photosynthetic pathway.

Chinese privet can be found invading homeowners yards, abandoned fields and other environments such as fence rows, ditch banks and forest edges and gaps. Data from our study are applicable to these environments and suggest that Chinese privet, given the large increase in growth, will likely become an even more troublesome invasive plant in these situations as the atmospheric CO_2 concentration continues to rise.

In addition to the environments mentioned above, Chinese privet is also prevalent in the understory of forest systems in the southeastern United States, indicating it can tolerate a high degree of shading. While many studies of CO_2 enrichment have been conducted on sun loving plants, only recently has attention been given to shade tolerant species. Seflick *et al*. (2006) found that long-term growth enhancement was 97% for plants grown in deep shade and only 47% for those grown in moderate shade relative to plants grown in ambient CO_2 under the same conditions. Perhaps, had the present study been conducted to represent a shaded environment (as opposed to growth in full sunlight), the increase in Chinese privet biomass might have exceeded the 42% that we observed; this potential certainly deserves attention.

Physiological characteristics such as leaf area may also impact the invasiveness of plants. Tremmel and Patterson (1993) studied soybean (*Glycine max*) and 5 weeds associated with that crop and found a lack of CO_2 effect in either the biomass or leaf area for the weeds. They speculated that the lack of response in both of these characteristics may make the weeds less competitive in an elevated CO_2 environment. We found that, while Chinese privet plant biomass increased, leaf surface area was not statistically impacted by CO_2 treatment. This might suggest that the increase in privet biomass will not contribute to an increased level of competitiveness for privet as suggested by Tremmel and Patterson (1993). However, we did observe a 28% increase in leaf area of Chinese privet under CO_2 enrichment. The most likely reason this large increase was not statistically significant was due to a high degree of variability among privet seedlings at termination of the study. Perhaps, had we grown Chinese privet from cuttings (rather than from seed), this variability might have been lower and the increase in leaf area significant.

In addition to the increase in biomass, our results revealed that the Chinese privet had a significantly higher number of branches when grown under elevated versus ambient CO_2 conditions. As with the discussion of leaf area, this change in branch number might also be a physiological characteristic increasing the invasiveness of Chinese privet. Further, an increased number of small branches is one of the characteristics of plant species that "fuel" forest fires (Doran *et al*., 2007). While, there are other characteristics of plants that contribute to their flammability, the prevalence of this species in the forest understory may pose an increased fire hazard in an elevated CO_2 environment. This fact would make management of this invasive species even more critical to forest health. Given that prescribed burning is a common forestry practice in southeastern pine forests, fire regimes and practices may have to be significantly altered if elevated CO_2 induced Chinese privet growth leads to higher fuel loads and/or flammability. Since, Chinese privet is common in homeowner yards, increased growth or flammability may also pose an increased fire hazard in suburban and urban communities as well.

Chinese privet is often a problem in the understory of forest communities as well as in forest gaps and edges and has the potential to displace native species. This would indicate that management of privet in a future elevated CO_2 environment will have to be done more diligently than it is now to achieve the most effective control. Chinese privet, if left unattended, will impact terrestrial ecosystems by altering the function and structure of both managed and native plant communities. Others have studied the impact of elevated CO_2 on weeds; Ziska and Bunce (1997) found that weed response to elevated CO_2 was twice that of crop plants grown under the same conditions and also concluded that C_3 weeds would have a greater negative impact on the growth of both C_3 and C_4 crop species (Ziska, 2001, 2002, 2003). The large increase in Chinese privet biomass, as a C_3 invasive plant, observed in our study suggests its impact on crops or forests will be much greater as atmospheric CO_2 levels continue to rise.

Increased root dry weight due to CO_2 enrichment is commonly observed; in fact, roots often exhibit the greatest relative dry weight increase among plant organs under high CO_2 (Rogers *et al*., 1997). Further, increased CO_2 often results in more and/or longer plant roots which could lead to greater exploration of the soil profile for water and nutrients. In our study, Chinese privet seedlings increased both root dry weight and length when exposed to elevated CO_2, indicating a greater ability to compete for belowground resources. This fact indicates it will have even greater impacts on terrestrial ecosystems and an increased need for control, in a future CO_2 enriched environment.

CONCLUSION

Our results reveal that Chinese privet increased in biomass significantly and to a greater extent than that observed by our group on any previously studied invasive species. This means that, in an elevated CO_2 environment, control of Chinese privet will have to be done more aggressively due to its faster early growth. It is unclear what this would mean in terms of terrestrial community dynamics and forest fires, if left unchecked, but these are questions worthy of consideration. Further, to evaluate the impact Chinese privet would have on understory communities, a competition study would need to be conducted in an elevated CO_2 and shaded environment.

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