

## Impact of *Ligustrum lucidum* on Leaf Morphology, Chlorophyll, and Flower Morphology of White Trout Lily (*Erythronium albidum*) in Cameron Park

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### Abstract

Invasion of non native species is reducing biodiversity worldwide. Shining privet (*Ligustrum lucidum* W.T. Aiton), a sub-canopy tree native to Asia, is diminishing native understory flora in Cameron Park in Waco, Texas. Previous data from Cameron Park indicates that privet is suppressing the vernal ephemeral, white trout lily (*Erythronium albidum* Nutt), in ravines above the Brazos River.

In February 2008, a research team from Baylor University's Environmental Studies Department sampled trout lily populations to test the hypothesis that greater privet cover increases leaf and petiole (stem) length of the ramets (individual plants) while it decreases the percentage of flowering shoots and leaf chlorophyll content. The research team categorized two sample sites along the Rio Perdido trail (Site 1) and the Hale Bopp trail (Site 2) as areas of high privet cover, moderate privet cover, or low privet cover. Average petiole lengths under low privet at Sites 1 and 2 were 1.81 cm and 1.63 cm respectively (Fig. 2). Under high privet cover average petiole lengths increased to 4.04 cm and 4.20 cm (Fig. 2). T-tests and one-way ANOVA demonstrated petiole lengths significantly increased 2.20 cm under dense privet at Site 1, and 2.50 cm at Site 2 ( $p=.05$ ) (Table 1). Site 1 had no flowering individuals under dense privet, and only 5.80% of Site 2 ramets flowered under dense privet (Fig. 1). Utilizing a CCM-200 Apogee chlorophyll meter, the research team collected chlorophyll concentration index (CCI) values. At Site 1, CCI revealed averaged values of 33.70 under low privet and 19.70 under dense privet (Fig. 4). At Site 2, under low privet CCI values averaged 34.80 and 17.50 under dense privet (Fig. 4). The research suggests that the trout lily is responding to the privet's excessive shading through etiolation, the elongation of the plant's leaf and stem to obtain sunflecks. Photosynthetic energy focuses on the extension of the plant resources, and an insufficient volume of energy is directed

towards the production of flowering ramets involved in producing new plants. Thus, the average increase in petiole and leaf lengths in these single-year data indicate the privet is suppressing populations of native spring ephemerals, such as white trout lily, and altering the morphology of the trout lily in Cameron Park.

### Introduction

From water hyacinth crowding out native lake plants to zebra mussels competing with native clams, exotic species invasion threatens the biodiversity of many different ecosystems worldwide. This well-studied phenomenon is the most serious threat to the long-term preservation of native forest in William B. Cameron Park, located in Waco, Texas. Cameron Park incorporates relic forest vernal herb communities, which are prone to species loss from disturbance. Cameron Park also provides a contiguous block of habitat for forest birds otherwise excluded from fragmented urban habitats. Seeds of ornamental trees and shrubs, some originating from lawns in or near the park and some traveling longer distances via birds or other dispersal mechanisms, have produced scattered populations of exotic plants throughout the park. Although Japanese honeysuckle (*Lonicera japonica*) is probably the most prevalent exotic on the flood plain, shining privet (*Ligustrum lucidum* W.T. Aiton) is the most widespread in the ravines and mesic slopes above the rivers (Bratton, unpublished data). Privet presents a wide threat to lower-elevation temperate forests in the Americas (Marco et al., 2002; Morris et al., 2002). The combination of a fragmented urban area on a major riparian corridor, an interface between land and streams, implies that Cameron Park will remain vulnerable to exotic shrub and vine invasion (Burton et al., 2005; Lowenstein and Lowenstein, 2005).

Numerous studies have documented causes and impacts of excessive growth of privet on local flora. Merriam and Feil (2002) have demonstrated that in oak-dominated forests in North Carolina, privet inhibits the reproduction of the canopy trees, reduces the diversity of understory herbs, and causes changes in the structure of the ecosystem. Lichstein et al. (2004) found that privet limited native vine recruitment in temperate forests in Argentina. Morris et al. (2000) noted that the structure of the evergreen privet (*Ligustrum lucidum*) gave it a competitive advantage over native flora. Kollmann and Grubb (1999) found that canopy environment partially controls shrub invasion. In eastern

Argentina, many of the temperate forests are overwhelmed by *Ligustrum*. Rapid growth of *Ligustrum* and effective dispersal by birds probably contributes to its successful invasion (Montaldo, 1993, 2000; Cueto et al., 2002; Aragón and Groom, 2003; Strong et al., 2005).

A number of investigators have experimented with control techniques for privet. One of the least expensive is application of herbicide, such as glyphosate. Harrington and Miller (2005) found that application of herbicide was most effective in the spring. When they applied the herbicide in April, percentage control of privet cover averaged 93% to 100%, while in June and August, control averaged 67% to 69%. Merriam and Feil (2002) found that complete clearing of an invading privet patch released the forest understory and increased herb diversity in the first year after glyphosate treatment. However, forest floor herbs are very sensitive to specific herbicides, such as glyphosate. This sensitivity must be taken into consideration when determining an effective privet control method.

Bratton (unpublished data) studied *Erythronium* populations in Cameron Park in February and March 2007 and found that trout lily patches at the edge of privet clumps not only had fewer flowers than those growing in the open, but on average these patches also had longer leaves. This suggested the trout lilies were responding to the evergreen privet shrub's intense shade by reallocating energy reserves to photosynthetic tissue, while creating fewer reproductive structures. Bratton located trout lily populations in three areas threatened by privet: the west facing slope around the Hale Bopp trail, the west facing slope above Dead River, and a knoll above a dry stream course, which was upslope from the mouth of the Bosque. Since very few herbs are present in the center of privet clumps, the privet is almost certainly suppressing herbaceous species diversity in the ravines.

As a genus, *Erythronium's* autecology is relatively well researched. The name trout lily springs from its distinct mottled green and purple leaves that resemble the body of a brook trout (Nuffer, 2007). Trout lilies grow in large colonies created by shoots from underground corms, which are thick, food-storing stems. Studies of the ecology of vernal herbs frequently incorporate *Erythronium*. The forest floor trout lily species are known to respond to sunflecks, soil temperature, and the presence of mycorrhizae, a symbiotic association of the mycelium of a fungus with the roots of plants. The alpine species are also influenced by microclimate, such as the location of snow patches. The seeds

are distributed by ants, making trout lily dispersal and reproduction vulnerable to loss of animal symbionts (Germino and Smith, 2001; Hull, 2002; Lapointe and Lerat, 2006). *Erythronium* should respond to very local increases in light and to changes in soil temperature, suggesting that minor modifications of privet cover may have a major impact on trout lily populations (Loewen et al., 2001; Swada et al., 1997).

The goal of the present study is to analyze the association between privet density and the population structure of white trout lily, *Erythronium albidum*. The primary hypothesis was that privet foliage would increase lily leaf lengths while reducing the production of flowering individuals and reducing the chlorophyll content in the leaves. This current research is a preparatory to an investigation of privet control in Cameron Park in order to increase forest floor herb diversity. Using the baselines established in this study, future researchers will determine whether the clearing of privet results in a restoration of normal white trout lily morphology.

## Methods

### *Study site*

This study was conducted in William B. Cameron Park, a 165.92 hectare (409.99 acres) civic park located at the confluence of the Brazos and Bosque Rivers near downtown Waco, Texas. The park, named for Waco businessman William Cameron, was dedicated on May 27, 1910. Cameron Park sits near the junction of two major vegetation areas, the Cross Timbers and Prairies to the west, and the Blackland Prairies to the east. The Park is on the White Rock Escarpment, which forms the major divide between the two vegetation areas, and sits at the edge of the Lampasas Cut Plain, a subunit of the Cross Timbers (Diggs et al., 1999; Hatch et al., 1990; Correll and Johnston, 1970). The dominant vegetation of Cameron Park is currently forest. Common trees include: bluff oak (*Quercus sinuata*), Shumard's oak (*Q. shumardii*), bur oak (*Q. macrocarpa*), hackberry (*Celtis laevigata*), black walnut (*Juglans nigra*), Texas ash (*Fraxinus texensis*), white ash (*F. americana*), American elm (*Ulmus americana*), cedar elm (*U. crassifolia*), eastern cottonwood (*Populus deltoides*), pecan (*Carya illinoensis*), and *Juniperus* hybrids, predominantly red cedar (*Juniperus virginiana*) (Blair, 1965; Jordan, 1973; Francaviglia, 1998). Taxonomy in this paper follows Diggs et al. (1999).

The soils of Cameron Park are in the Eddy-Stephen and Westwood-Yahola-Ships Formations; they are formed respectively from upland

chalks and marls—crumbly mixtures of clays, calcium, magnesium carbonates, and remnants of shells—and from floodplain alluvium (Miller, 2001). The Austin Chalk is an Upper Cretaceous limestone that underlies nearly the entire park; resulting soils are shallow, alkaline, and clayey or loamy (Bureau of Economic Geology, 1970; Miller, 2001).

Because Waco sits along the Balcones Escarpment, which acts as a natural geographical barrier, its climate alternately resembles that of hot, dry West Texas and wetter, cooler East Texas. Waco can thus undergo substantial fluctuations in temperature, rainfall, and humidity within a relatively short amount of time. Generally, however, Waco has a humid, subtropical climate and averages between 76.2 and 101.6 cm of rain each year (Spencer, 1966; Kibler and Gibbs, 2004). The warmest weather commonly occurs in June, but temperatures can exceed 37.8°C from May to September (Leach, 1978). At that temperature, the vernal herb white trout lily is likely to blossom.

The specific study locations were in steep valleys with ephemeral streams, usually running only during rainy periods in the spring. Site 1 was just above the Mouth of the Bosque parking area (at the confluence of the Brazos and the Bosque rivers), near a trail named Rio Perdido (Fig. 5). Site 2 was near the eastern boundary of the park above a trail known as Hale Bopp. Both study sites are characterized by their sheltered, west-facing slopes and their relatively moist locations. The canopy over the plots was predominantly deciduous, dominated by bur oak, bluff oak, hackberry, Shumard's oak, and cedar elm, with a few scattered red cedars.

The research team selected plots for sampling by locating areas where privet adjoined trout lily populations. In February 2007 Susan Bratton pre-sampled trout lily populations on the west facing slope on the Hale Bopp trail, on the west facing slope above the Dead River on the Rio Perdido trail, and above a dry stream above the mouth of the Bosque River. In February 2008, the research team sampled slopes at the Rio Perdido and Hale Bopp walking trails (Site 1 and Site 2, respectively) for plant propagation due to particularly high numbers of ramets. Vegetation was measured in five 10 m by 5 m areas along Site 1 and Site 2 cluster sites. The research team randomized elected points along each transect to outline foliage of the five nearest single-leafed and five nearest two-leafed ramets on 10 cm block graph paper. Transects were divided into three separate categories of privet influence. Categories included “low privet” (<10% coverage), “moderate privet” (30-40%

coverage), or “dense privet” (>70% coverage). All sampling took place between consistent blocks of time and were correlated, taking into account moisture, temperature, and weather.

In each 10 m by 5 m area, leaf quantity, dimensions and inflorescence quantity and heights were documented for the first five single-leaf and five double-leaf ramets nearest the sample point. A CCM-200 Apogee chlorophyll meter was used to collect chlorophyll concentration index (CCI) from petiole to leaf tip of each sampled plant. Degree of coverage over sample plots was calculated using a convex densitometer. Other information recorded included the composition of the canopy, slope, and aspect at the sample point.

#### *Statistical Analysis*

An analysis of covariance (ANOVA) was performed using the statistical database SPSS version 14.0 to determine average petiole lengths, leaf lengths, leaf areas, and CCI values. Comparison of data values were performed using Student's T-tests, a statistical analysis, or when appropriate the Tukey-Kramer test, using JMP database v. 6.0. Non-parametric, one-way analyses of variances were calculated using the Kruskal-Wallis test in SPSS 14.0 (Green et al., 1997). Approximation of differences between expected versus observed distributions were calculated using the non-parametric Chi-square statistical test. A significance value of  $p=.05$  was used for all tests.

## **Results**

#### *Flowering*

In February 2007 white trout lily populations in Cameron Park had fewer flowering ramets under dense privet than those growing in open areas, while leaf lengths increased on average with privet cover. This suggested that shading of the privet causes the trout lily to focus its energy reserves on producing photosynthetic tissue and produce fewer reproductive structures. At Site 1, 50.0% of the sampled plants had no inflorescence. Areas under dense privet coverage contained no flowering individuals. Under low privet, 27.8% of the total lily shoots were flowering and 22.2% of the shoots under moderate privet coverage had flowers. At Site 2, under dense privet, only 5.8% of the present ramets were flowering. Under low privet, 29.1% of the shoots were flowering, and under moderate privet, 12.8% of the shoots had flowers

(Chi-square, Fig. 1).

#### *Leaf and Petiole Lengths*

Petiole differences among the sites were particularly pronounced for no-privet and dense-privet areas, but the petioles also differed in the area with moderate privet cover. Site 1 average petiole lengths were 1.82 cm under low privet, 4.23 cm under moderate privet, and 4.04 cm under dense privet (ANOVA, Fig. 2). The petiole lengths of the plants increased on average by 2.41 cm from low to moderate privet, decreased 0.19 cm from moderate to dense privet, and increased 2.23 cm from low to dense privet coverage ( $p=.05$ , Student's T-test) (Table 1). The minor decrease of the plant's average petiole length under moderate privet coverage was probably due to the excessive damage of the white trout lily at Site 1. Hale Bopp data displayed a similar trend of increasing petiole lengths (Fig. 2). At Site 2, average petiole lengths were 1.63 cm under low privet, 3.03 cm under moderate privet, and 4.16 cm under dense privet (Fig. 2). Average increases between different volumes of privet were 1.40 cm from low to moderate privet, 1.12 cm from moderate to dense privet, and 2.53 cm from low to dense privet (Table 1). On average, petioles at Site 1 were longer than those at Site 2 by 0.18 cm under no privet and by 1.19 cm under moderate privet. They were, however, 0.12 cm shorter under dense privet ( $p=.05$ , Tukey-Kramer).

Previous data from February 2007 showed average leaf lengths of 8.67 cm and 9.23 cm (ANOVA) at Sites 1 and 2 respectively. This apparent elongation of the leaves at the sites caused the research team to conduct further surveys to determine the reasons for the changing leaf morphology. In February 2008, Site 1 revealed average lily leaf lengths of 9.53 cm under low privet, 9.52 cm under moderate privet, and 6.98 cm under dense privet (ANOVA, Fig. 3). The mean length decreased by 2.55 cm on average from low to dense privet and 2.54 cm from moderate to heavy privet (Student's T-test, Table 1). There was an insignificant difference ( $p >.05$ ) between average leaf lengths under low and moderate privet. At Site 2, average leaf lengths were 9.27 cm under low privet, 9.88 cm under moderate privet, and 10.25 cm under dense privet (Fig. 3). Leaf length increased on average by 0.98 cm from low privet to dense privet, and 0.60 cm from low privet to moderate privet. There was an insignificant difference ( $p >.05$ ) between lengths under moderate and dense privet (Table 1). Site 1 leaf lengths under low privet were 0.25 cm longer than those at Site 2. Under moderate and dense privet,

Site 2 leaves were 0.36 cm and 3.29 cm longer (Tukey-Kramer, Table 1). Comparisons of average trout lily leaf width at Site 1 and Site 2 showed that none of the categories of privet volume had significant differences in leaf width at  $p=.05$  (Student's T-test). Strong evidence leads to the conclusion that the presence of the invasive shining privet is indeed altering the leaf morphology of the native white trout lily.

#### *Chlorophyll index*

Decreased chlorophyll index significantly hinders the plants' ability to reproduce and replenish populations within the ecosystem. The research team measured the chlorophyll index to discover the possible effects of privet on the trout lily's reproduction. At Site 1, measurements revealed an average CCI of 33.7 under low privet, 25.70 under moderate privet, and 19.70 under dense privet. At Site 2 CCI averaged 34.80 under low privet, 23.70 under moderate privet, and 17.50 under dense privet (ANOVA, Fig. 4). Site 2 showed that the CCI decreased an average of 17.23 between low and dense privet areas and 11.16 between low and moderate privet coverage. Site 1 CCI decreased on average by 13.72 from low to dense privet coverage and 8.23 from low to moderate privet coverage (Table 1). These figures show that privet may indeed play a role in decreasing CCI and thus inhibiting plant reproduction.

### **Discussion**

In February 2007, the research team observed that white trout lily populations under privet populations have a noticeable difference in leaf morphology. While populations growing under privet had fewer flowers than those growing in open areas, the leaves were longer on average. This study shows that the sampled ramets' leaf length has low potential for influencing whole leaf morphology at Site 2 ( $r^2 = .095$ ,  $p=.21$ ). Further regression analysis revealed that morphology is somewhat influenced by leaf area ( $r^2 = .42$ ,  $p=0.00$ ). At Site 1, correlation coefficients ( $r^2 = .25$ ,  $p= 0.00$ ) confirmed the association between the ramet's leaf length and leaf area to the overall morphology. The research concluded that leaf morphology depends on both leaf length and leaf area at the trail sites. Total leaf area is an important ecological trait in plant biomass production. To further analyze leaf morphology, the team predicted a simple mathematical relationship that nondestructively estimated the trout lily's total leaf area from corresponding petiole lengths, leaf

lengths, and widths. The variables were placed in modeling specifications in JMP with  $\hat{y}$ = leaf area,  $x_1$ =petiole length,  $x_2$ =leaf length, and  $x_3$ =leaf width. The equations  $\hat{y} = -103.2 + .52x_1 + 14.9x_2 + 56.6x_3$  for Site 2 ( $r^2=.93$ ) and  $\hat{y} = -85.31 + -2.09x_1 + 14.39x_2 + 54.19x_3$  ( $r^2=.88$ ) at Site 1 were calculated.

The results suggest that trout lily is one of the last herbs to survive as privet invades deciduous forest. Under normal forest floor conditions, the vernal herb extends its leaf length, perhaps adapting in order to locate sunflecks or light patches, or perhaps to escape fallen branches or dense patches of fallen tree leaves. Trout lily populations thus invest in longer petioles, while the chlorophyll in the leaves and number of flowers decline. These results suggest that shading most critically impacts the *Erythronium* populations. The presence of these lingering individuals suggests that trout lily can recover if the privet canopy is removed. The lack of flowering and low chlorophyll levels imply, however, that trout lily may require several years to produce new genets and to fully recover in energetic terms.

### Conclusion

Based on collected statistical data, the research team concluded that the presence of the invasive privet caused the white trout lily population to adapt through an ecological transition known as etiolation, a process which occurs when a plant grows in partial or no light. Etiolation results in longer, weaker plant stems and fewer leaves. The plant's growing tip will stretch towards any source of light, causing the leaf lengths to increase. As a response to decreasing light, the petioles of the trout lily leaves grow longer in order to seek light, and they transition from producing a singular inflorescent, the flowering part of the plant, accompanied by two ramets to a single ramet with no inflorescent present. This change in morphology indicates a secondary state for the lily population. The lily is focusing energy reserves in order to produce photosynthetic tissue and reduce the number of inflorescences for future seeding.

Data collected consistently revealed evidence of an overall changing plant structure exhibited in the white trout lily under privet cover at the research sites. ANOVA comparisons revealed that the average petiole length under dense privet was significantly longer than those under moderate or no foliage (Fig. 2). Inflorescence also decreased among the populations under privet (Fig. 1). Research showed that CCI

values declined in populations under dense privet (Fig. 4). The research team did not expect the changing leaf geometry dimensions among the samples at Site 1 that occurred in this study. Quantitative leaf dimensions plotted graphically revealed a steady transition of the leaf morphology at Site 2; an increasing presence of privet caused leaves to become longer and not wider ( $r^2 = 0.93$ ). In contrast, the leaf morphology at Site 1 showed that an increasing presence of privet caused the leaves to become shorter and not wider ( $r^2 = 0.88$ ). This trend may be due to excessive leaf damage among the sampled populations at Site 1. The research did show that the white trout lily was indeed responding to the dense coverage of shining privet by etiolation. Etiolation of the leaves and petioles was determined to be a secondary state for the trout lily due to the lack of inflorescent ramets and low chlorophyll levels among the populations in dense privet covered sites.

This research was a prologue to future studies that the team hopes to conduct on the white trout lily in Cameron Park. Knowing that etiolation of white trout lily leaves is a secondary state for the plant will influence the team's choice of methods of population restoration. By clearing the shining privet through applications of herbicide and other methods, the team may discover that the plants are able to regenerate sustainable populations.

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APPENDIX OF TABLES AND FIGURES

| Outcome variables          | Low to moderate privet | Moderate to dense Privet | Low to dense privet |
|----------------------------|------------------------|--------------------------|---------------------|
| Petiole length(cm) Site 1  | +2.41                  | +0.19                    | +2.23               |
| Petiole length (cm) Site 2 | +1.40                  | +1.12                    | +2.53               |
| Inflorescence Site 1       | -11.2%                 | -44.4%                   | -100.0%             |
| Inflorescence Site 2       | -22.0%                 | -27.0%                   | -49.0%              |
| CCI Site 1                 | -8.23                  | -5.49                    | -13.72              |
| CCI Site 2                 | -11.16                 | -6.07                    | -17.23              |
| Leaf Length (cm) Site 1    | Insignificant          | +2.54                    | +2.55               |
| Leaf Length (cm) Site 2    | +0.60                  | +0.38                    | +0.98               |
| Leaf Area Site 1           | -25.22                 | -31.56                   | -56.78              |
| Leaf Area Site 2           | +7.38                  | +11.13                   | +18.51              |

Table 1: Average differences of variables

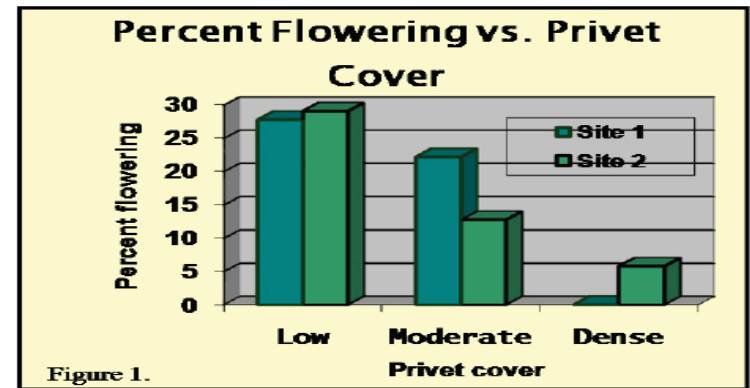


Fig. 1 Average percentage flowering individuals vs. intensity of privet cover

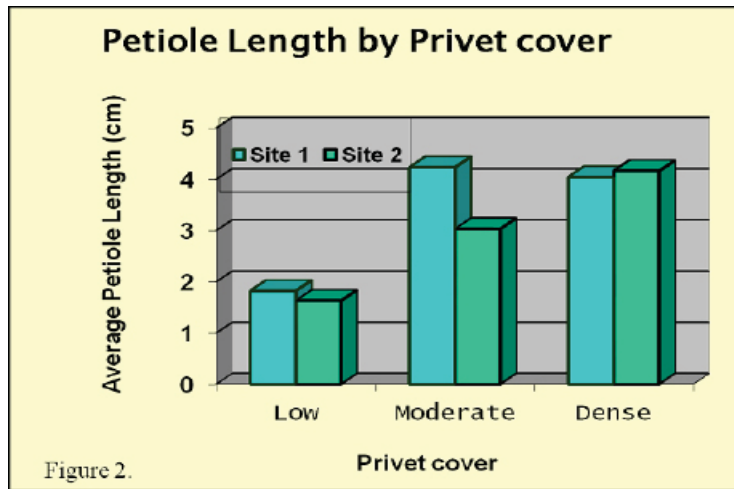


Figure 2.

Fig. 2 Average petiole lengths vs intensity of privet cover

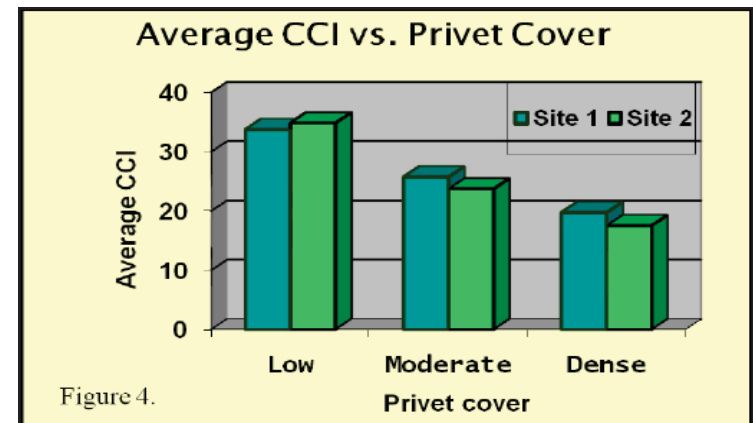


Figure 4.

Average chlorophyll concentration index (CCI) vs intensity of privet cover

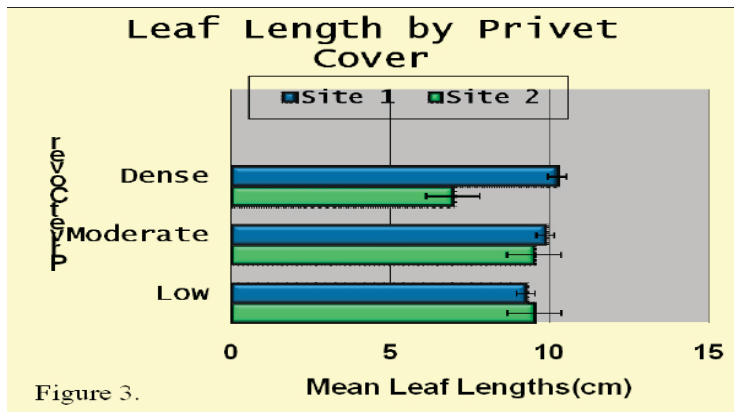


Figure 3.

Fig. 3 Average lengths of leaf vs intensity of privet cover



*Erythium albidum* under low privet





*Erythronium albidum* under dense privet



*Erythronium albidum* (Nutt)



Shinning privet (*Ligustrum lucidum* W. T. Aiton)