

Notes and Discussion

Damage Induced Production of Extrafloral Nectaries in Native and Invasive Seedlings of Chinese Tallow Tree (*Sapium sebiferum*)

ABSTRACT.—We conducted a pot experiment examining the effects of simulated leaf herbivory and supplemental soil nutrients on extrafloral nectary (EFN) glands of *Sapium sebiferum* seedlings derived from native Chinese and invasive Texas seed stock. We predicted that simulated herbivory and increased soil nutrients would increase EFN activity. We also predicted that Chinese genotypes would have greater EFN activity than Texas genotypes. Simulated leaf herbivory significantly stimulated effluent production on EFN glands of seedlings from both genotypes, but there were no significant differences in EFN activity between genotypes or nutrient treatments.

INTRODUCTION

Chinese Tallow Tree (*Sapium sebiferum* (L.) Roxb., Euphorbiaceae) is a major invader throughout the southeastern United States (Bruce *et al.*, 1995; Jubinsky and Anderson, 1996). Its competitive success is frequently attributed to a lack of pests and pathogens (Scheld and Cowles, 1981; Jubinsky and Anderson, 1996; Bruce *et al.*, 1997). *Sapium* loses very small amounts of leaf area to herbivory in the United States (Scheld and Cowles, 1981; Siemann and Rogers, 2001) and its leaves are presumed to contain secondary metabolites that repel herbivores (Cameron and Spencer, 1989; Jubinsky and Anderson, 1996; but *see* Siemann and Rogers, 2001). A pair of bulbous glandular stipules at the blade-petiole junction of each *Sapium* leaf have been described (Correll and Johnston, 1979), however, few details exist regarding the potential function of these glands or the conditions that stimulate their activity.

Extensive experimental evidence has shown that many plant species increase their growth and fitness by using extrafloral nectar (EFN) to attract insects (*e.g.*, predaceous ants and parasitoid wasps) that deter herbivores (Bentley, 1977; Tilman, 1978; Koptur, 1979; Stephenson, 1982; Moya-Raygoza and Larsen, 2001). Sugars and amino acids are the primary chemical constituents of EFN (Baker *et al.*, 1978; Smith *et al.*, 1990; Koptur, 1994). This chemical composition has evolved in response to nectar feeding by insects that provide protection from herbivores, but may vary greatly among and within species depending upon environmental conditions (Baker *et al.*, 1978; Bentley, 1977; Koptur, 1994; Agrawal and Rutter, 1998). Despite benefits gained by reducing detrimental activities of herbivores (Tilman, 1978; Stephenson, 1982), resource costs for defenses like EFN production can be high for plants, particularly in the absence of appreciable herbivore damage (Bentley, 1977; Baldwin, 1998). Consequently, EFN production is frequently a facultative mutualism that is induced by herbivore damage (Tilman, 1978; Smith *et al.*, 1990; Agrawal and Rutter, 1998; Heil *et al.*, 2001; Wäckers *et al.*, 2001). Active nectaries are also typically found on younger, more vulnerable plant parts (Bentley, 1977; Wäckers *et al.*, 2001). During an experiment designed to assess effects of simulated leaf herbivory and soil resources on *Sapium* seedlings derived from native Chinese and invasive Texas genotypes we observed EFN production on leaves of several seedlings. Because EFN production is often a costly response to herbivore damage, we hypothesized that recently defoliated seedlings and those receiving supplemental soil nutrients would have a greater number of active nectaries than undamaged unfertilized plants. Furthermore, based on recent findings that *Sapium* seedlings derived from invasive Texas genotypes have reduced their chemical leaf defenses in response to an absence of herbivory in its introduced range (Siemann and Rogers, 2001), we also predicted that Chinese genotypes would have greater EFN production than Texas genotypes.

MATERIALS AND METHODS

Seeds of both native Chinese and invasive Texas *Sapium* trees were germinated in a greenhouse during April–May 2001. Chinese seeds were obtained from trees at the South China Botanical Garden in Guangzhou, China (henceforth “native genotype”) while invasive seeds were collected from naturalized *Sapium* trees in Galveston County, Texas (henceforth “invasive genotype”). We germinated the seeds in single-celled, 100 ml conetainers™ (Stuewe and Sons, Inc., Corvallis, OR) filled with a homogenized mixture of topsoil, organic humus and peat. Once the seedlings had primary leaves, they were placed

beneath a 50% shade cloth outdoors for 1 mo. To begin the 15 wk experiment, we selected 24 similar-sized *Sapium* seedlings of native and invasive genotypes and individually transplanted them into 3 gallon (7.65 liter) Stuewe Treepots™ (Stuewe and Sons, Inc., Corvallis, OR) filled with the same soil mixture. The experiment was conducted in a temperature controlled (27 C) laboratory area. The northern exposure of the laboratory was mostly windows that provided an average of 530 $\mu\text{mol}/\text{m}^2/\text{s}$ diffuse PAR during peak light as measured by an ACCUPAR Linear Ceptometer (Decagon Devices, Pullman, WA). Full spectrum lights suspended over the pots provided an additional 85 $\mu\text{mol}/\text{m}^2/\text{s}$ PAR on a 12 h light cycle. *Sapium* seedlings are able to grow efficiently in a wide range of light conditions. They are capable of rapid growth in full sunlight and sustained, moderate growth under low light levels, where their growth rates are frequently higher than many native, shade-tolerant tree species (Jones and McLeod, 1989, 1990; Rogers and Siemann, 2002). Light conditions in this study approximate those of an intact coastal prairie understory (Rogers and Siemann, 2002). Pots were watered and rotated in place daily and rotated among positions weekly.

A full-factorial experimental design was employed by randomly assigning simulated leaf herbivory and nutrient addition treatments to potted seedlings from each *Sapium* genotype. Each treatment combination was replicated six times. Supplemental nutrients were 3 g nitrogen, 1 g phosphorus, and 2 g potassium per m^2 per application added as 15-5-10 NPK fertilizer during weeks 2, 7 and 11. Thus, providing an additional total of 9 g nitrogen, 3 g phosphorus and 6 g potassium per m^2 . Herbivore defoliation was simulated by removing the front half of every full leaf blade with scissors during weeks 5 and 9. The scissors were sterilized with an alcohol wipe after defoliating each seedling to prevent the spread of disease or secondary allelochemicals. Clipped leaves that remained on seedlings until week 9 were cut in half a second time as were all newly added leaves. Both times the uppermost leaf of each seedling was excluded in order to protect the apical meristem and avoid affecting branching dynamics (Marquis, 1992). Although realistic simulation of herbivory by mechanical means is difficult because many aspects of insect chewing cannot be duplicated, simulated leaf herbivory can adequately represent decreased leaf area and mass loss experienced by herbivore damaged tree seedlings (Hendrix, 1988; Marquis, 1992; Tiffin and Inouye, 2000).

Shortly after the second simulated herbivory trial we noticed leaves on several seedlings were exuding a sticky sugary fluid from the glandular stipules at the blade-petiole junction of each *Sapium* leaf. In week 11 we counted the number of leaves on each seedling and visibly noted whether a leaf was exuding fluid from its EFN glands. Relative EFN production was calculated by dividing the number of leaves per seedling that had visibly active EFN by the total number of leaves on that seedling. We used analysis of variance (ANOVA) to compare among experimental treatments and Arcsin transformed the data in order to meet ANOVA assumptions (StatView 5.0, SAS Institute, Cary, NC). The results were back transformed for graphical presentation.

RESULTS AND DISCUSSION

Simulated leaf herbivory strongly promotes EFN activity in *Sapium* seedlings derived from both native Chinese and invasive Texas genotypes (Table 1, Fig. 1). None of the leaves on any of the seedlings were observed producing effluent prior to the second simulated leaf herbivory trial. However, in week 11 of the study, 2 wk following simulated herbivory, a significant number of damaged leaves began exuding a sugary effluent from the glands at the base of each leaf. Although often debated, this phenomenon is not likely due to a passive physiological response (Bentley, 1977; Agrawal and Rutter, 1998; Heil *et al.*, 2001). Since the nectary is located behind the damaged leaf, simple sap flow would emerge from the severed vascular tissue, but no effluent was observed along the clipped leaf edges. The vascular tissue damage caused by clipping leaves did affect EFN activity, however. Previous studies where herbivory was simulated on *Sapium* leaves with a paper hole punch, rather than severing leaves with a scissor, did not stimulate EFN production to the extent seen here (Rogers *et al.*, 2000; Rogers and Siemann, 2002; Rogers and Siemann, 2003). A possible explanation for this difference is that at least 26 insect species consume *Sapium* in its native range (Bruce *et al.*, 1997) representing a diverse array of feeding strategies, including consumption along leaf edges that damages leaf veins (W. E. Rogers, pers. obs.). In its introduced range, small chewing holes are the most common type of leaf damage observed (Rogers *et al.*, 2000), potentially explaining why *Sapium* EFN activity has not been previously attributed to herbivore damage.

TABLE 1.—Analysis of variance table for relative extrafloral nectary production (Arcsin transformed) by *Sapium* seedlings derived from Chinese and Texas genotypes (G) and subjected to factorial combinations of supplemental nutrient additions (N) and simulated leaf herbivory (H)

Variable	df	MS	F value	P value
Genotype (G)	1	0.073	2.852	0.0990
Nutrient (N)	1	0.037	1.459	0.2342
Herbivory (H)	1	1.508	59.28	<0.0001
G × N	1	0.0001	0.004	0.9500
G × H	1	0.068	2.687	0.1090
N × H	1	0.083	3.267	0.0782
G × N × H	1	0.024	0.947	0.3364
error	40	0.025		

EFN production has been observed on artificially damaged *Sapium* leaves in field conditions of the introduced range, but the nectar is ephemeral and its production is not sustained in the absence of additional leaf damage (W. E. Rogers, pers. obs.). This dynamic EFN activity has been studied in many other plant species and appears to frequently be associated with the foraging activities of predaceous ants (Bentley, 1977; Tilman, 1978; Koptur, 1979; Stephenson, 1982; Agrawal and Rutter, 1998; Moya-Raygoza and Larsen, 2001). Ants have been observed at *Sapium* EFN in its introduced range (E. Siemann, pers. obs.), but the protection provided is uncertain since *Sapium* loses less than 3% leaf area to herbivores in its introduced range.

Assuming that EFN activity imposes resource costs (Bentley, 1977; Tilman, 1978; Baldwin, 1998; Heil *et al.*, 2001) it is reasonable to expect production of effluent to be infrequent on *Sapium* in the absence of herbivore damage. However, we did not observe any statistically significant effects of NPK nutrient additions on EFN production (Table 1) suggesting that resource costs are either small or based on different biological currency, such as carbon (Smith *et al.*, 1990; Koptur, 1994; Baldwin, 1998; Wäckers *et al.*, 2001). We do not know whether *Sapium* can minimize potential costs by reabsorbing unused nectar (Bentley, 1977).

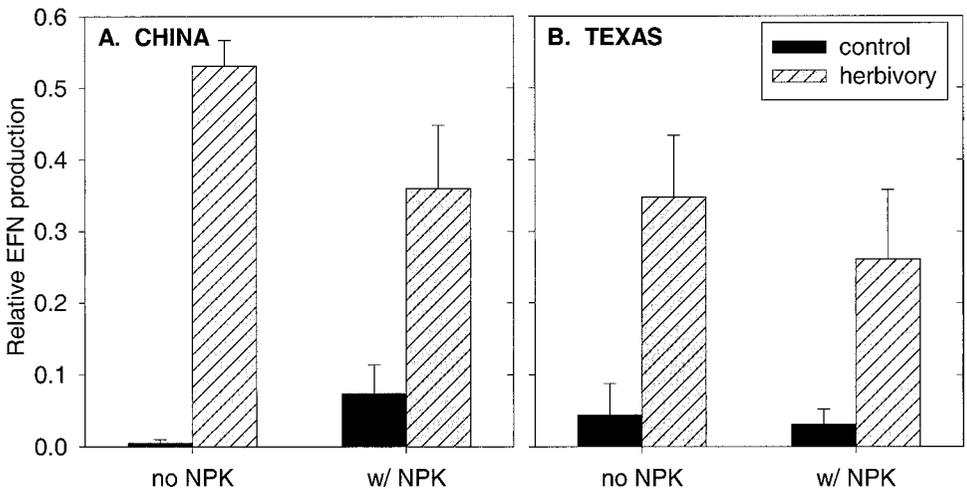


FIG. 1.—Relative extrafloral nectary (EFN) production (means +1 SE) in *Sapium* seedlings derived from (A) Chinese and (B) Texas genotypes and subjected to factorial combinations of supplemental nutrient additions (NPK) and simulated leaf herbivory

Because invasive Texas genotypes have high growth rates but lower quality, poorly defended leaves compared to native Chinese genotypes (Siemann and Rogers, 2001) we predicted that *Sapium* seedlings derived from Texas seeds would exhibit less EFN activity than seedlings derived from Chinese seeds. However, there were no statistically significant differences in EFN activity between genotypes (Table 1). Both Texas and Chinese genotypes increased EFN activity following simulated herbivory damage. These results suggest that although invasive *Sapium* genotypes have reduced their chemical leaf defenses due to a lack of herbivory in its introduced range (Siemann and Rogers, 2001), inducible EFN activity has been retained as a possible anti-herbivore defense. Further studies are necessary to determine whether differences exist in the quality (e.g., amino acid and sugar composition) and quantity of EFN production by invasive Texas and native Chinese genotypes.

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