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Short Communication

How Sweet is the Extra Floral Nectar Secreted by the Invasive Alien Tree of Heaven, *Ailanthus Altissima* Mill.?

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Abstract

Tree of Heaven (ToH), *Ailanthus altissima* is a fast-growing tree native to China and Taiwan, but invasive in Europe and the U.S. where it disrupts urban and natural biocenoses. This invasive brings together successful strategies or traits including defense mechanisms and extrafloral nectaries. The Extra Floral Nectar (EFN) represents a significant energy source, owing to its carbohydrate-rich content, for visiting insects. The core ecological function of EFN is an indirect defense to plants by attracting insects (generally ants) that prey on herbivores or that deter herbivores from feeding on the plants. Assemblages of natural enemies associated to ToH were shown to include a large proportion of defoliators. Despite the importance of EFN, information on the sugar composition of EFN in ToH remains sparse. We analyzed by enzymatic assays the sugar composition of EFN in ToH plants from three locations in France. The total sugar concentration of EFN was not significantly different between locations unlike the hexose ratio. The ratio of sucrose to hexose, (r) ranged from 0.501 to 0.640, ranking ToH as a sucrose rich species. As it is generally admitted that sucrose rich nectars attract generalist ants and parasitoids, this finding represents a timely and a potentially fruitful avenue for future research addressing the ecological functions of EFN in ToH including indirect defences in relation to the assemblages of natural enemies associated with ToH in both native and invasive ranges.

ABBREVIATIONS

TOH: Tree of Heaven; EFN: Extra Floral Nectar

INTRODUCTION

Extrafloral nectaries are specialized glands in vascular plants, most often located on stipules, petioles or at leaf bases [1]. Extra floral nectaries have been observed in more than 3,900 species spanning at least 108 families [2]. The nectar they produced, known as Extra Floral Nectar (EFN) represents a significant energy source, owing to its carbohydrate-rich content, for visiting insects, including ants and other plant mutualists [3-6]. EFN is involved in so-called indirect defense by attracting ants, parasitoids and other predatory arthropods such as spiders that prey on herbivores or that deter herbivores from feeding on the plants [7]. In addition, there is a mounting evidence that EFN might be a trait that facilitates colonization of new habitats and promotes invasiveness of both insects and plants [5,8,9] and that nectar-insect mutualism can be established quickly among non-coevolved (e.g. invasive) species, indicating great potential for also considering EFNs, amongst others, in a biological control management strategy [8]. *Ailanthus altissima* (Mill.) Swingle 1916, commonly known as Tree of Heaven (ToH) or the lesser used Chinese sumac common name, is a deciduous tree in the tropical Simaroubaceous family that produces EFN, is highly invasive and targeted for classical biological control. ToH first leaves have stalked nectaries with apical pores located at the base of the petioles and its completely developed pinnated leaves bear nectaries on the abaxial surface of the lamina, along the basal margins of the leaflets (Figures 1,2), [10].

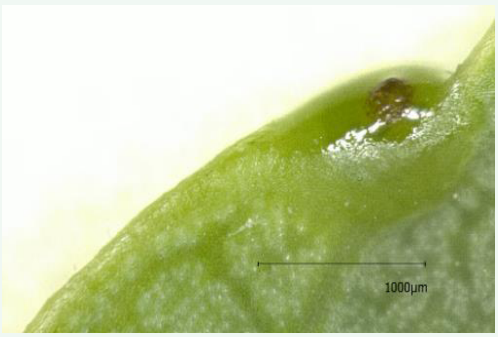

Figure 1 Active extrafloral nectary located at the margin of the ToH leaf petiole [Digital photograph taken using a Keyence digital microscope VHX-7000].

Figure 2 Observable nectar drops produced by leaflets of ToH.

Introduced from China to Europe and the United States in the late 1700s, ToH, valued for its fast-growing ability and its resistance to insect infestation and damage, was widely planted as a shade tree for parks and public promenades in Europe and in the U.S. [11]. Over the centuries, ToH became a common invasive tree in urban, agricultural, and forested areas in temperate climates throughout the world [12-14]. ToH owes its competitive advantage to several attributes among which include its large pinnated leaves. These large compound leaves composed of 10-40 leaflets of 10-20cm in length contribute to inhibiting the growth of shade intolerant plants in the forest by blocking much of the sunlight that would reach the floor [13]. Chemical and mechanical controls of ToH are practiced, however these methods can come at an environmental and financial cost and/or are difficult to implement. Therefore, as a sustainable alternative, classical biological control is being considered for controlling ToH in North America and Europe [15,16]. As an initial stage of this biological control program, the assemblage of natural enemies, potentially biological control agents, is being monitored [17].

In order to test the hypothesis that EFN might play a mutualistic role in the interaction between ToH and its natural enemies, there remains a need for more consistent information about the sugar composition of EFN which is restricted, up to now, to the patchy data published by [18]. Therefore, this paper is aimed at investigating the sugar composition in EFNs of about one year old ToH potted plants by enzymatic assays. In this paper, we report on the use of enzymatic assays for measuring the amount of glucose, fructose and sucrose in EFNs as an alternative to High-Performance Liquid Chromatography (HPLC) [19]. Although HPLC is the most used analytical technique in determining nectar sugar composition, it is regarded as a costly strategy for a small laboratory, as it requires countless costly organics, and sophisticated equipment. As the concentrations of sugars in EFNs are relatively high, samples, in general, need to be diluted before analysis [20], sensitivity of the method is not an issue *per se* and enzymatic assays should be suitable for the present purpose. Our results were compared with those reported by [18] and discussed in terms of the possible role and function of the sugars produced by the EFNs in future interactions with the natural enemies of ToH.

MATERIALS AND METHODS

Plant Culture

ToH plants originated from three different locations i.e. near Lyon (Rhone) in Eastern France, Montferrier-sur-Lez and Saint Gély du Fesc (Hérault), in Southern France. At each location, around one year old sprouts were dug out and potted in a 4-liter volume of compost Neuhaus (Huminsubstrat N2). Potted plants were then transferred to a culture room where they were exposed to neon light for 14 hours at 22.5-22.8°C and 56% of relative humidity.

Collecting Leaf Nectar Droplets

Sampling of the nectar was conducted on a subsampling of 22 leaflets on fully expanded leaves for each locality, for which there was no significant difference between localities in the total number of nectar-producing nectaries per leaflet (one-way ANOVA $F_{2,65}$ = 0,379, P < 0,686). Each sample contained nectars pooled from all nectar-producing nectaries in one leaflet (Figure 1,2). All nectars were collected concurrently, as nectar sugar ratios may vary depending on sampling period [21]. Nectar droplets were gained using 6mm diameter paper-wicks of Whatman No. 1 of known mass [22]. We used this method for sampling nectar as an alternative to the commonly used microcapillary tubes because EFN in ToH is both produced in low volumes (< 1µL) and highly viscous. The mass of the nectar collected on each paper-wick was determined by subtracting initial mass readings from the final readings obtained with a Precisa ES225SM-DR analytical balance (Dietikon, Switzerland) and each measurement was made in triplicate. Samples were kept frozen at -24°C until further analysis. Digital photographs of the nectaries were taken using a Keyence digital microscope VHX-7000 located on site.

Analysis of Sugar Composition in Leaf Nectar

Wicks were thawed to room temperature (ca. 22°C), transferred into 2mL Lobind Eppendorf tubes and immersed in a variable volume of sterile distilled water estimated to be of 1µl per 1.2 to 1.5µg of nectar. Tubes were shaken at 350rpm for 20min, at 25°C in a Thermomix ®C (Eppendorf, Hamburg, Germany) and spun briefly to collect eluate in which sugars resuspended. The content of sucrose, free glucose and free fructose was determined using the Megazyme K-SUFRG assay kit provided by Neogen (Lansing, MI, USA) and following manufacturer's recommendations. The kit has a linear range of detection between 4 to 80µg of D glucose, fructose or sucrose per assay. Analyses of the sugar composition were repeated at least twice per sample. Data were acquired at 340nm with the Thermoscientific™ Spectrophotometer Nanodrop 2000C (Waltham, Massachusetts, USA). The sugar ratio (r) was calculated as r = sucrose/(fructose + glucose) following [23]. These authors proposed four sugar ratio categories: sucrose dominant (r > 0·999), sucrose rich (0·999-0·5), hexose rich (0·499-0·1) and hexose dominant (r < 0·1).

Statistics

Statistical analyses were performed using XLSTATS-Basic+version 2022 (Addinsoft Inc., New York, NY). For establishing the means difference between groups data related to sugar concentration, fructose/glucose ratio, the sugar ratio *r*, a general ANOVA analysis was conducted. Comparisons between mean values of each of the analyzed criteria i.e. sugar content, fructose/glucose ratio, the sugar ratio *r*) for all studied plants were undertaken using non-parametric Kruskal-Wallis tests, and multiple Wilcoxon signed ranks tests were then used to determine which plants differed from one another. The level of statistical significance required to measure differences between the means for all analyses was $P = 0.05$. Data are presented as mean values ± SD (standard deviation).

RESULTS AND DISCUSSION

The total sugar content produced by a nectary which ranged from 0,276 grs (Saint- Gély du-Fesc) to 0,340 grs (Lyon) was not significantly different between locations (estimate ± SD: 0,324 ± 0,191, $F_{2,65} = 2,924$, $P = 0,232$). However, significant differences were observed between locations for the fructose/glucose ratio ($F_{2,65} = 13,550$, $P < 0,0001$) which ranged from 1,002 (Lyon) to 1,114 (Saint Gely du Fesc), (Figure 3; Table 1). The present study revealed that the fructose/glucose ratio was twice less than the fructose/glucose ratio (2,02) presented by Bory and Clair-Maczulajtys (1986), but with similar ratio of glucose and fructose in the leaf nectars. No significant differences were observed between locations for the sugar *r* ratio ($F_{2,65} = 0,023$, $P > 0,977$) which ranged from 0.501 (Montferrier-sur-Lez) to 0.640 (Saint Gély du Fesc), (Table 1). The present study revealed that the sugar *r* ratio can be more than two times higher than the *r* ratio (0,27) presented by Bory and Clair-Maczulajtys (1986). Following [23], ToH should be categorized as a sucrose rich species. It is generally admitted from the literature that nectars with high sucrose content attract insect parasitoids [24] and generalist ants, whereas nectars with higher hexose content are preferred by specialist ants [4]. However, the relevance of the EFN to the nutrition of its consumers and hence, to the structuring of the arthropod communities associated with ToH aspect has been poorly explored so far. The strong fetid odor emitted by the ToH flowers of both genders [25], is thought to attract a large assemblage of floral visitors among which some are pollinators, most notably flies and bees [25].

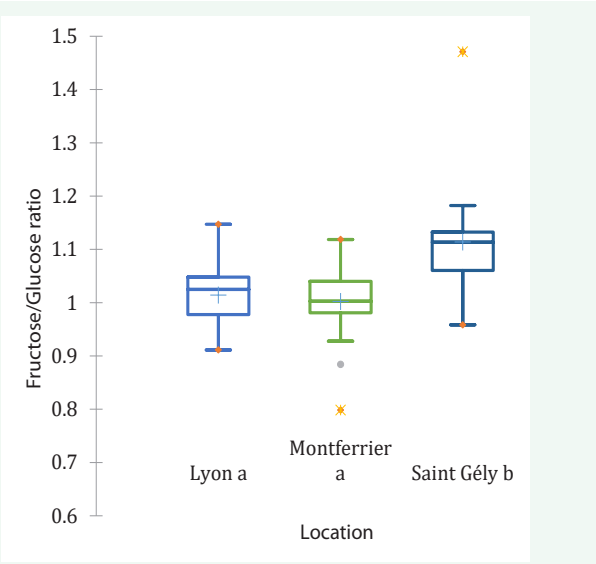

Figure 3 Box plots representing the Fructose/Glucose ratio evidenced in Extra Floral Nectars collected from ToH plants at different locations in France (Lyon, Montferrier-sur-Lez and Saint-Gély-du-Fesc). Different letters indicate meaningful differences (n = 66).

Table 1: Sugar content produced by a nectary (grs), Fructose/Glucose ratio and ratio (*r*) Sucrose/Fructose + Glucose in the Extra Floral Nectars collected from ToH plants from three different locations in France.

Location	Lyon	Montferrier-sur-Lez	Saint-Gély-du-Fesc
Sugar content/nectary (grs)	0,340 ± 0,119	0,356 ± 0,275	0,276 ± 0,138
Ratio Fructose/Glucose	1,014 ± 0,061	1,002 ± 0,074	1,114 ± 0,096
Ratio (*r*) Sucrose/Fructose + Glucose	0,600 ± 0,306	0,501 ± 0,265	0,640 ± 0,354

Much less studied are the mutualists i.e. non pollinators such as the ants. Ants commonly patrol the leaves of ToH, removing nectar from the flowers and from extra-floral nectaries [Aldrich, personal observation]. These resources may serve as a reward to the ants for protection from herbivores, augmenting Ailanthus' defenses, although ants might also deter potential pollinators as well. Although there is mounting evidence that Extrafloral nectaries on leaves increased the production of EFN (volume and calories) after simulated herbivory, thus contributing to plant defense [26], this was not yet demonstrated for ToH. In addition, the orientation of natural enemies may be facilitated by specific scents released by extrafloral nectaries [27]. In their literature search of the natural enemies associated with ToH in Asia, [17] identified 46 phytophagous arthropod species from five orders, most of them being defoliators. One of them is *Lycorma delicatula* White (Hemiptera: Fulgoridae), a phloem feeding planthopper native to China, Japan and Vietnam and invasive in the United States [28].

This pest of many host-plants, is also a "natural enemy" of ToH. Interestingly, *Lycorma delicatula* has been shown to prefer feeding on hosts with particular sugar content ratios and survived longer when fed sugar solutions similar to those produced by ToH, of which branches were shown to be rich in sucrose, followed by fructose and glucose [28]. Tree of heaven is also the preferred host of vagrant eriophyid mites including *Aculus taihangensis* Hong & Xue (Eriophyidae) (Acari; Prostigmata) a promising biocontrol agent of ToH, that lives on the underside of leaflets but occasionally on the upper side of leaflets and can build up large populations on *Ailanthus altissima* causing leaf deformation, yellowing, necrosis, premature leaf drop, and death in young seedlings [16,29,30]. On the other hand, in laboratory conditions, A. *taihangensis* is also a suitable prey for the phytoseiid mite *Euseius stipulatus* Athias-Henriot (Acari; Mesostigmata) also occurring on ToH [31]. *Euseius stipulatus* is a generalist phytoseiid species, capable of developing when feeding

on pollen as alternative food and for which the provisioning of sucrose enhances the conservation of populations [32]. To date, the possibility that the EFN may potentially sustains reproduction and development for this predatory mite on ToH has certainly been neglected in biological studies of the mite. Thus, providing information about extra floral nectar production might be useful to start addressing the possible role and function of the sugars of the EFN in the interaction with the mites.

CONCLUSION

From data shown in the present report, it is clear that EFN in ToH is sucrose rich. Our findings represent a timely and a potentially fruitful avenue for future research addressing the ecological functions of EFN in ToH including indirect defense in relation to the assemblages of natural enemies associated with ToH in both native and invasive ranges. This study may also lead to an interesting implementation of a biological control management by understanding the role of EFN in repelling or attracting selected natural enemies. It will be particularly important to understand if the presence of EFN can disrupt biological control using eriophyid mites by promoting the presence of predatory mites.

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