

DEFENSIVE ROLE OF EXTRAFLORAL NECTARIES

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Abstract

Plants have to deal with a diverse assemblage of herbivores, which can consume significant amounts of biomass and reduce plant reproductive success. Consequently, plants have developed a diversity of structures and evolutionary strategies to provide protection against herbivory. One of these strategies is represented by extrafloral nectaries, which are nectar secretory structures involved in the indirect defense of plants. Extrafloral nectaries attract adult and predatory parasitoids, leading to a significant reduction in the number of pests that attack plants. Plant-insect interactions are old, and formed the structural basis of many of the terrestrial environments. These relationships directly involve a wide variety of consumption-resource networks, placing plants under enormous pressure of evolution caused by higher trophic levels, especially by herbivores. This paper aims to present a short review about the properties and role in defense of extrafloral nectaries.

Key words: extrafloral nectaries, defense mechanism, indirect defense.

INTRODUCTION

Nectar glands produce carbohydrate exudates and can be found on any vegetative or reproductive plant structure (Aguirre et al., 2013). These are specialized structures present on the plant components and are called floral (located on the flower organs) and extrafloral (located on vegetative organs of the plant) nectaries (Garcia de Almeida et al., 2012; Coutinho et al., 2012). It is known that floral nectar glands play a direct role in pollination providing nectar to visitors. In contrast, extrafloral nectaries are not directly involved in pollination, they play a vital role in maintaining a beneficial relationship of mutualism between plant and insects (Garcia de Almeida et al., 2012). Extrafloral nectar contains mainly sugars, so their secretion can be influenced by photosynthesis (Fang-Fang & Jin, 2015). Generally, the extrafloral nectar is known to be an indirect defense mechanism. Along with ants, other insects such as wasps and mosquitoes use this feed source, thus providing different degrees of pest protection. For example, it has been repeatedly demonstrated that the presence of ants increases the

protection of the whole plant, while other visitors can act as commensals or even plant pests (Kost & Heil, 2005).

Plants secrete nectar to attract animals that function predominantly as pollinators as in the case of floral nectar, or pest control as in the case of extrafloral nectar. Because nectars are usually aqueous solutions containing mainly sugars and aminoacids, but also other nutrients (Jamont et al., 2013), they are susceptible to infestation with microorganisms that can use nectar-like tissues as entry doors to infect the plant. Nectar secreting tissues therefore require an effective protection shield against pathogenic lesions (Escalante-Pérez et al., 2012). The aim of this study is to present a short review about the properties and role in defense of extrafloral nectaries.

GENERAL ASPECTS CONCERNING EXTRAFLORAL NECTARIES AND EXTRAFLORAL NECTAR

Knowledge of plant defense systems against pests is crucial to understanding trophic relationships in terrestrial ecosystems. Defense systems can act alone or combined during foliar

development (Calixto et al., 2015). In most food chains, herbivore insects are one of the main energy flow pipes between autotrophic plants and the rest of the food chain. Thus, it is not surprising that the pests have led to the evolution of a wide range of effective plant defense mechanisms (Agrawal, 2007). Lack of mobility restricts plants' ability to disperse pollen and signs and protect themselves from pests but is compensated by the presence of floral and extrafloral nectaries glands. Extrafloral nectar is secreted by specialized organs that can appear on all plant structures, but are not involved in pollination. These have been described for about a thousand plant species, which are part of more than 93 plant families of flowers and ferns, but are absent in gymnosperm.

Extrafloral nectar is distributed across plant structures (e.g. spikes, pedicels, buds, calyx, leaves, petioles, bractes or stems) and occurs in over 108 families and 745 genera of ferns and angiosperms (Kost & Heil, 2005; Dattilo et al., 2015). When on the leaves, extrafloral nectar secreting glands develop at the beginning of the budding period, and their activity (time and productivity) is variable among species, depending on the phenological development of the plants (Calixto et al., 2015). Extrafloral nectar glands often secrete large volumes of nectar over a much longer period than floral nectar glands (Géneau et al., 2013).

Extrafloral nectaries are aqueous solutions that mainly contain sucrose, glucose and fructose, but other sugars, amino acids and other organic compounds may also be present in certain species. The secreted sugars are mainly derived from the phloem or are synthesized in the nectar region. Extrafloral nectaries secrete small amounts of nectar throughout the day. Secretion of nectar can follow circadian cycles or can be relatively constant day and night. The nectar secretion mechanisms are still poorly understood: some researchers have described secretion as a passive process, while others have described it as an elimination of excess sugars. However, there is evidence that nectar secretion is an active secretory process: requires metabolic energy consumption, and extrafloral nectaries have secretory cells that contain a large number of mitochondria and have dense protoplasts and large nucleus.

Originally discovered in *Macaranga tanarius*, it is suggested that nectar secretion is induced by leaf damage. Inhibitors that suppress the release of linolenic acid or interfere with the production of linolenic acid hydroperoxides completely suppress the EN flow induced by lesions and therefore clearly demonstrate the involvement of oxilipine signaling in EN induction. Interestingly, the attack of some *Agriotes lineatus* under the earth on cotton plants (*Gossypium herbaceum*) induces extrafloral nectar production on the aerial side of the plant. Most studies have focused on the protective effect of ants on whole plants or individual parts of plants, demonstrating a beneficial function of these insects. However, there are conflicting observations that did not detect a measurable preventive effect of the ants attracted to the EN. In these cases, the lack of plant protection can be explained by (1) differences between the degree of aggression of the attracted species of ants, (2) differences between the drilling behavior of the ant species in different habitats and (3) a variable susceptibility of the pests to the predatory ant. In addition to ants, extrafloral nectar attracts a wide spectrum of other arthropods including *Araneae*, *Diptera*, *Coleoptera* and *Hymenoptera*. Due to their predatory or parasitoid life forms, many of these non-ants, such as different species of wasps, jumping spiders (*Salticidae*), mites or flies, can also reduce the number of pests. Both ants and wasps exert beneficial effects on plants that secrete extrafloral nectar. The emission of floral aromas by plants is very important for attracting pollinators to their floral nectar. Beyond these odors, which communicate the location, abundance and quality of these nectars at higher trophic levels, other mechanisms may help guide the arthropods feeding on EN to other sources of nectar further away. First, some extrafloral nectar secretory glands are colored, providing visual indications for arthropods. In addition, increased amounts of both HIPV and EN may allow arthropods to utilize volatile organic compounds emitted as a clue to detecting nectar sources at longer distances (Arimura et al., 2005).

Understanding the role of structure, function, ecology, and evolution of plant secreting structures provides meaningful information to

understand the different types of insect-plant interactions and floral anatomy in relation to reproductive biology (Garcia de Almeida et al., 2012). Because nectar secretion is directly related to the protection of ants against pests, various factors can influence the phenotypic plasticity of a plant species to optimize the compromise between nectar secretion and defensive benefits. For example, in arid and semi-arid mediums with high day driven temperature and low humidity, many insects such as ants and pests are more active at night, mainly due to their eco-physiological limitations. Moreover, some studies have shown that pests have a greater preference for younger and tastier leaves.

It is therefore possible to expect that in these seasonal environments, plants will secrete larger amounts of nectar at night when the pest pressure is higher in more vulnerable tissues (e.g. apical branches) (Dattilo et al., 2015). Many plants interact with carnivores as an "indirect defense" against pests. For example, it is well established that plants attract, feed or host other organisms and this fact can reduce the pressure of the enemy. Plant features involved in this context may be volatile organic compounds, extrafloral nectar, feed organisms and structures used as shelters or nesting spaces (Marques Fortuna, 2013).

Nectar is an important food source for adult parasitoids and can increase longevity and fecundity and thus increase the parasitic rate. Both floral and extrafloral nectar are food sources for parasitoids. The extrafloral nectar of *Centaurea cyanus* L. (Asteraceae) is produced by sepals. The production of nectar begins two weeks before the flowering of the plants and is maintained (in parallel with the production of floral nectar) during the flowering period. It has been observed that parasitoids feed on the extrafloral nectar of *C. cyanus*, and it has been shown that this type of feeding leads to increased longevity and fertility of *Microplitis mediator*. Parasitoids must find hosts (insects) for breeding and a source of food for adult parasitoid nutrition. In the field, these two resources are often separated by space, and parasitoids have been shown to alternate the search for the host and the search for food depending on their physiological state. Parasitoids use both

olfactory and visual indicators to locate hosts and food sources (Géneau et al., 2013).

EXTRAFLOREAL NECTARIES - WANTED AND UNWANTED VISITORS

Ants are omnipresent terrestrial organisms, especially abundant in shrubs and trees of tropical forests and savannah. Many ant species use plant surfaces as feed substrates to look for both live prey and dead prey as well as for different types of plant-derived food. The ants that feed on extrafloral nectar increase individual survival, and increase their colony growth rate and reproduction. The main advantage that ants provide to plants is effective protection against natural enemies, thus improving the condition of the plant (Assunção et al., 2014).

Among plant-insect interactions, the relationship between ants and plants is a partnership where the ants are attracted to the extrafloral nectar rich in sugars, while providing protection against pests (Garcia de Almeida et al., 2012; Dattilo et al., 2015). Many resistance-related links are inducible or can be initiated for faster and stronger induction once the lesions appear. This phenotypic plasticity helps balance the costs and benefits of the defense as it ensures that costly protections are only expressed when they are effectively needed. Among the inducible traits, it appears that all plants respond to the damage caused by pests with increased emissions of volatile organic compounds (VOCs), and the plants in many taxa are also responsible for the secretion of extrafloral nectar (EN). Both VOCs and ENs attract adult and predatory parasitoids, leading to a significant reduction in the number of pests that attack plants. Plants have numerous features that offer resistance to most potential pests and pathogens. Many features act directly against these enemies through their toxic, removal or antimicrobial effects, or act as mechanical barriers (Stenberg et al., 2015).

Plant-insect interactions are old, and formed the structural basis of many of the terrestrial environments. These relationships directly involve a wide variety of consumption-resource networks, placing plants under enormous pressure of evolution caused by higher trophic

levels, especially by herbivores. In this respect, researchers are unanimous in highlighting the importance of the chemical and physical characteristics of plants that represent the first line of pest defense. Chemical defense is made up of secondary compounds, such as alkaloids and tannins. Physical defense is mainly morphological or mechanical, such as the presence of tenacity of leaves, trichomes, spinners or latex. Trichomes were considered an effective defense system against herbivorous insects. Resistance can be improved during foliage development, and fully extended leaves have a foliar hardness higher than young ones. Foliar tenacity can act as a powerful defense system that affects morphology, food behavior, and ultimately spatial and temporal patterns of pests (Calixto et al., 2015). The plants also possess other defensive strategies such as biotic defense, or association with a predator. In this situation, plants provide food and / or shelter for predators in exchange for protection. Extrafloral nectar represents the classic example for this type of defense system. Extrafloral nectaries produce a carbohydrate-rich liquid with diluted compounds of amino acids, lipids, phenols, alkaloids and volatile organic compounds, attracting a wide variety of predatory arthropods including ants. Several studies have demonstrated the effectiveness of ants in protecting plants against foliar pests. However, others have failed or have shown only a partially effective defense provided by ants at vegetative parts of plants. Indeed, Assunção et al. (2014) has shown that ants visiting extrafloral nectar secretory plants can remove pollinators and cause indirect losses. In addition to ants, spiders visiting extrafloral nectar secretory plants can also provide protection.

Interactions between plant and herbivorous insects are among the most striking challenges for ecologists around the world and have been shown to play central roles in the evolution of plant pest defenses. An important and well-known defense mechanism against pests is the indirect defense generated by a third trophic level: the enemies of the pests. An example that occurs mainly in the tropics is mediated by the supply of extrafloral nectar for ants, which protects host plants against pests. In systems involving three trophic levels, such as plants,

pests and predators, a trophic cascade describes the top-down positive effects of the third trophic level on the producing species. Associating with animal species can provide plants with protection against pests, diseases and, occasionally, competitors (do Nascimento & Del-Claro, 2010).

Mutualism between ants and plants is an interaction system where plants can provide habitats and/or food for ants, which in turn protects plants against pests that they hunt or remove. The most common nutritional reward that plants give to ants is extrafloral nectar. Several authors have shown that associations between ants and extrafloral nectar secretory plants can reduce foliar pests and/or increase the number of fruits. However, organisms interact not only directly but also indirectly, and indirect effects are important forces that lead ecological communities that can also cause variations in interspecific relationship outcomes. In fact, some studies have shown that the ants' positive effects on ant-plant associations are not universal (do Nascimento & Del-Claro, 2010). Plants and ants have widespread relationships that are mediated by the supply of extrafloral nectar to ants that protect plants against pests. However, these interactions between plants and ants are optional and vary in time and space, mainly depending on the characteristics of ants' species such as density and aggression. Generally, the results of these relationships are positive, but in isolated cases the presence of ants is neutral or negative for plants. Some studies suggest that aggressive attacks or simply the presence of ants could reduce the rate of sighting of pollinating insects, such as bees (Assunção et al., 2014).

Plants colonized and damaged by herbivorous insects produce a group of volatile organic compounds often called herbivore-induced vegetal volatile compounds (HIPVs), which may include chemical substances that act as repellents for herbivorous pests and as attracting agents for the antagonist organisms of these pests, such as predators and parasites. On the one hand, these signals indicate that the plant is already infested and therefore less suitable as a host, but on the other hand it may increase the search for food by predators and parasitoids. It is widely accepted that plants

respond to the attack of specific herbivore insect species through direct and indirect induced defenses. In direct defense, chemicals target the pest, resulting in delayed development or death (e.g. HIPV), while in the case of indirect defense, chemicals (e.g. HIPV) lead to increased mortality by recruiting predators and parasitoids. Studies on mechanisms leading to HIPV production have revealed the role of their specific elicitors. These elicitors can activate different signaling pathways in the plant, resulting in the accumulation or release of defensive chemicals. In addition, it has been observed that there are also intact plants that naturally produce similar VOCs without any damage from herbivores. Biological control agents (natural enemies) use a wide range of such VOCs to locate their prey (Khan et al., 2008). Activating specific responses requires recognition and adequate response to the attacking enemy, and most events that eventually lead to gene activation (the signaling pathway) occur within minutes. Among the many signaling molecules involved, reactive oxygen species (ROS) and intracellular calcium signatures belong to early events that are responsible for most chemical and molecular reaction cascades (Maffei et al., 2007).

With the coexistence of at least 100 million years, plants and insects evolved a variety of beneficial and harmful interactions. To avoid overloading, the plants have developed a chemical defense capable of producing an effective and often drastic reduction in insect feeding. These defense systems have to be orchestrated both in time and space through extremely complex regulatory networks, which are themselves further modulated by interactions with other signaling paths. Integrated responses ultimately lead to a characteristic pattern of gene expression resulting, among many other activities, in the production of phytochemicals directed against the invading or feeding organisms. This has been achieved during the evolution of constituent components (e.g. primary and secondary metabolites, but also thorns, trichomes, etc.) and induced defense (e.g. secondary metabolites, cell wall strengthening, production of ROS etc.). Direct defense is represented by plant features which by

themselves affect the performance of insects and are generally classified by their way of action. Among the secondary metabolites, many phytochemicals function as toxins by poisoning unadapted pests and by forcing adapted insects to invest scarce resources in detoxification. In addition to direct defense, plants express features that facilitate top-down control of pest populations by attracting predators and parasitoids of the herbivorous insects. Thus, indirect protection mediated by VOC release caused by insect attack has received the greatest attention, but the production of nectar-induced pests by extrafloral glands works similarly (Maffei et al., 2007).

Insects can locate their hosts, even if host plants are often hidden in a number of other plants, and volatile plant compounds play an important role in this hosting process. Furthermore, these VOC-mediated interactions of plants with organisms with higher trophic levels suggest that they communicate similarly with each other. Exposure to VOCs alone, without effective pest attack, can directly lead to increased defense. Alternatively, VOC exposure can allow nearby plants to prepare their defense for immediate use once the pest moves from the neighboring plant to attack the receiver. Moreover, volatile compounds in primary host plants can also attract other insects, such as male aphids. Interestingly, parasitoids also use herbivorous insects' responses to assess habitat returns and adapt the residence time of patches. Besides, volatile plant emissions are inducible by other biotrophic as well as abiotic agents. After their release from leaves, flowers and fruits in the atmosphere and from the roots into the soil, the phytochemical compounds of plants protect the plants against pests and pathogens or provide reproductive advantages by attracting pollinators. Furthermore, certain volatile compounds can act as air signals that amplify direct and indirect defense in the distant parts of the same plants (Maffei et al., 2007).

In addition to the use of direct phytochemicals, several stages are involved, starting with the detection of insect feeding to the indirect plant responses. These include leaf tissue disruption, elicitor release, signal cascades, and activation of transcription factors that ultimately lead to

cellular response of plants. Current research into the interaction between plants and insects focuses primarily on transcriptomics, genomics, proteomics and metabolomics, which are late events induced by biotic stress. In contrast, events from the first seconds to minutes that are involved in signal recognition and transduction are still poorly understood (Maffei et al., 2007).

Understanding insect-plant interaction is of interest not only from an ecological and evolutionary perspective, but also for the development of new crop protection strategies. Due to the massive damage caused by herbivores to valuable crops, the deciphering of early plant signals is one of the most interesting areas of research to defend it. ROS and calcium signaling appear to be a common event in induced pest control processes (both in chewing and perforation) and against pathogens, but the way the plant distinguishes enemies lies in the speed and intensity of the damage, as well as in the nature of the elicitor specificity delivered to the attacked plant cells. VOC production has been demonstrated for a wide range of biotrophic attacks and even in this case, the plant responds with specific but variable mixtures that can attract predators of the attacking enemies. Despite all the evidence, the link between early aggressor perception, generation of ROS and secondary messengers, and specific VOC emission is still far from clear and much work is still needed to better understand the important link between recognizing a certain biotic stress and appropriate plant responses (Maffei et al., 2007).

Pest attacks cause changes in the herbal bouquet released by plants. These volatile compounds induced by herbivorous insects (HIPVs) have been interpreted as part of the indirect defense (Turlings & Ton, 2006; Dicke & Baldwin, 2010). However, given that no study has yet investigated whether HIPV is in favor of a plant's robustness, its defensive function needs to be established. In addition, pests, pathogens, pollinators and competitors also respond to HIPV and neighboring plants from native populations also emit volatile compounds that provide a background odor. These considerations enrich the evolutionary context of HIPVs and complicate predictions

about their adaptive value (Dicke & Baldwin, 2010).

In addition to the release of volatile compounds, plants attract and maintain parasitoids providing shelter (such as cavities or tricks for ants and mites) or food (such as pollen, floral nectar, extrafloral nectar and sap) (Arimura et al., 2005; Stenberg et al., 2015).

Most species of parasitoids require more resources to complete their life cycle and to maximize their health, such as hosts, shelter and food. Adult parasitic females need to look not only for hosts to ensure the development of larvae, but also for food. Adult food is required to perform basic metabolic functions, provide large amounts of energy for flight and to acquire nutrients to be allocated to oovigens in the case of synovigene species (Jamont et al., 2013).

This food contains carbohydrates and amino acids, and is consumed by a wide range of parasitoids, most commonly during the adult stage. In particular, ingestion of nectar enhances the longevity and efficiency of parasitic predation. These types of food affect the performance, behavior and voracity of the predators, this effect can optimize the effectiveness of biological control using genotypes of plants of a certain quality. In particular, the secretion of extrafloral nectar usually reduces the number of herbivorous insects on the respective plants. In the context of horticulture, there are reports saying that plants that secrete extrafloral nectar have better protection from pests, they produce larger amounts of pollen, or provide additional shelter for ants and mites. Carbohydrate availability is a common obstacle for carnivores, while herbivorous insects are usually limited by protein supply. Therefore, carbohydrate-based rewards can shift the balance in favor of the third trophic level, even when a specific reward is also used by herbivorous insects (Stenberg et al., 2015). While the exploitation of floral nectar by parasitoids has been extensively studied, little is known about how parasitoids locate extrafloral nectar and whether the availability of extrafloral nectar increases the rate of pest parasitization in the field (Géneau et al., 2013). The presence of sugar can play a major role in the performance of parasitoids in a biological control context (Jamont et al.,

2013). Carbohydrate sources provide the parasitoids with the essential energy and nutrients needed to meet their nutritional needs and thus can play a major role in the success of parasitoid reproduction. The availability of adequate carbohydrate sources for increasing survival rate and fertility of adult parasitoids has been demonstrated. If the crop does not provide sources of nectar, sugars can be obtained from vegetation that is not part of the crop or from honey produced by insects in *Sternorrhyncha* that feed on the plant. Floral nectar may be abundant, but is limited to the, often short, flowering period and may not be accessible to parasitoids in deep corolla flowers due to their short mouths. Parasitoids can also have strong competition for floral nectar with other nectar-feeding insects such as lepidopterans, bees and *Syrphidae* family (Géneau et al., 2013).

Many aphids are major agricultural pests due to their unparalleled reproductive ability and ability to manipulate the physiology of host plants. The growth of aphid population and its impact on plant health are strongly influenced by interactions with other organisms, including plant pathogens, endophytes, aphids endosymbionts, predators, parasites, ants and other herbivorous insects. Numerous molecular and genomic resources have recently been developed to identify the sources of aphids resistance in plants, as well as potential innovative targets for aphids control. Furthermore, the same model systems that are used to explore direct molecular interactions between plants and aphids can be used to study the ecological context in which they occur. The Aphididae family comprises more than 4300 species, all of them specialized in feeding on the sap of the plants. Aphids can have a negative impact on the host plants largely due to their ability to quickly populate the surrounding space. Unlike most insects, they can reproduce clonally and give birth to young life, and the aphid's embryonic development begins before the birth of its mother. These features allow for short-term generations; the nymphs of certain species of aphids can reach maturity in just five days. Depending on the densities of the population, the aphid colonies invest in the production of morphs without wings with high fertility or less prolific

offspring with wings that can be dispersed in new host plants. This wing dimorphism allows aphids to utilize ephemeral herbaceous hosts in summer and migrate to perennial super-terrestrial hosts in the fall. Such host alternation is associated with cyclic parthenogenesis, in which aphids reproduce cloned on summer hosts and produce an egg-over-egg stage by sexual reproduction in autumn (Goggin, 2007). Floral nectar and pollen or extrafloral nectar can also enhance predation by attracting natural enemies, supporting the life stages of non-carnivorous parasites and providing alternative food sources for predators when prey abundance is low. Although these alternative food sources are preferred, they may interfere with biological control for plant protection. In some cases, the direct defense of plants against pests can be counterintuitive to indirect defense. Aphid population growth is also influenced by mutual or antagonistic relationships with other insects (Goggin, 2007).

CONCLUSIONS

Nectariferous glands are glands that produce carbohydrate exudates and can be located on any vegetative or reproductive structure of the plant. These are specialized structures, present on the plant components and are called floral (located on the flower organs) and extrafloral (located on the vegetative organs of the plant). The position and type of secreted nectar are often correlated with the efficiency of reproduction.

Extrafloral nectar contains mainly sugars, so their secretion can be influenced by photosynthesis. Extrafloral nectar is used as a food source by several groups of insects. EN is produced for a longer period of time compared to floral nectar, appearing earlier and continuing to be produced after flowering. The composition of EN differs depending on the species. The production of EN depends on the photosynthetic activity, the nutrients available in the soil, the health of the plant, the air temperature and its humidity.

Plant defense mechanisms against pests are not limited to physical and chemical barriers that directly aim to affect attackers, thus becoming increasingly obvious that plants also use indirect defense strategies. One form of indirect

defense of plants is to attract predators and parasitoids by signaling the presence of a potential prey or host with the help of the extrafloral nectaries. This attraction of the third trophic level is one of the supposed functions of the plant volatile compounds induced by herbivorous insects (HIPV), which are released more or less specifically in response to the attack of pests.

The survival strategies of plants are associated with their secretory tissues in different environments, which probably results from the two evolutionary tendencies: one aimed at protecting against pests and the other related to attracting pollinators.

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