Towards elucidating the differential regulation of floral and extrafloral nectar secretion

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Nectar is a rich source of sugars that serves the attraction of pollinators (floral nectar) or predatory arthropods (extrafloral nectar). We just begin to understand the similarities and differences that underlie the secretory control of these two important types of plant secretions. Jasmonates are phytohormones, which are well documented to be involved in plant developmental processes and plant defence responses against herbivores, including the secretion of extrafloral nectar. Recently, jasmonates have also been implicated in the regulation of floral nectar secretion in *Brassica napus*. Due to a trade-off between reproduction and defence, however, plants need to functionally separate the regulation of these two secretory processes. In line with this prediction, externally applying jasmonates to leaves did indeed not affect floral nectar secretion. Here we compare the current knowledge on the regulation of floral and extrafloral nectar secretion to understand similarities and dissimilarities between these two secretory processes and highlight future research directions in this context.

Jasmonic acid (JA) and other JA-derived compounds (jasmonates) control both plant developmental processes such as flowering[1,2] and anther dehiscence[3] and activate plant defence responses against herbivores.[4] For example, JA induces extrafloral nectar (EFN) secretion in various plant species from different families.[5-7] Recently, jasmonates have also been implicated in the secretion process of floral nectar (FN).[8] Floral nectar and extrafloral nectar share many chemical and functional properties[9] and apparently there is some similarity in the regulation of EFN and FN secretion. Here we compare the current knowledge on the regulation of these two processes (**Table 1**) and highlight future research directions.

Extrafloral nectar is an indirect defence trait that is used by many plant species to attract and nourish predatory arthropods, especially ants, which serve the nectar-secreting plants as 'bodyguards'[10] by effectively reducing the herbivore pressure on the EFN-secreting plant.[11,12] External application of JA induces EFN secretion in many plant species, including *Phaseolus lunatus*,[12] *Macaranga tanarius*[5] and several Acacia species[13]—an effect that is similar to the induction caused by herbivore feeding.[5] Blocking JA biosynthesis with phenidone, an inhibitor that reduces the fatty acid hydroperoxide formed by the lipoxygenase catalyzing the first step in the octadecanoid signalling pathway,[14] reduces EFN secretion.[5,13] While EFN serves defensive functions, floral nectar attracts plant pollinators and therefore significantly contributes to a plant's reproductive success.[15,16] The adaptive significance of floral nectar for mediating plant-pollinator interactions has been well studied.[17,18] Besides very few studies, however, that investigated the effect of various growth regulators on FN secretion, our understanding of the physiological processes that regulate this trait remains rather poor.[19] Recently, it was discovered that exogenous application of JA can increase FN secretion in oilseed rape (*Brassica napus*).[8] Further, blocking JA biosynthesis with

Table 1. Triggers of floral and extrafloral nectar production

	Floral nectar	Extrafloral nectar
Function	Pollinator attraction[43]	Indirect defence[12,44,45]
Consumers	Birds, insects and other pollinators[46]	Arthropods, especially ants[11,46]
Elicitor/inhibitor		
Jasmonic acid	Increases secretion[8]	Increases secretion[5]
Jasmonoyl isoleucine	Increases secretion[8]	Not known
Coronalone	Increases secretion[8]	Not known
Phenidone[1]	Reduces secretion[8]	Reduces secretion[5]
Gibberillic acid	Increases secretion[30]	Not known
Indole acetic acid	Decreases secretion[26,27]	Not known
Ethylene	Not known	Not known
Herbivory	No effect[8]/decreases secretion[47]	Increases secretion[5,11]
Florivory	Decreases secretion[38]	Not known
Plant volatiles:		
Herbivore induced	Not known	Increases secretion[34,35]
Florally emitted	Not known	Not known

[1]inhibits JA biosynthesis.

phenidone effectively reduced FN secretion, an effect that could be restored by an additional JA treatment.[8] Thus, major regulatory mechanisms appear to control the secretion of both, FN and EFN. How similar are the two mechanisms, and how can the plant physiologically separate the secretion of EFN and FN? Both types of secretion function in ecologically very different contexts and, thus, clearly need to be controlled independently.

One option would be the involvement of other jasmonates. Although JA is an important signal on its own, around 20 different JA-derived metabolites are also known to be involved in defence signalling.[20,21] Even metabolic precursors of JA may elicit different defensive phenotypes,[22,23] which opens interesting possibilities for a fine-tuning of jasmonate-dependent responses. In particular, the JA-amino acid conjugate jasmonoyl isoleucine (JA-Ile) has recently been discovered as functioning as the central signalling molecule of the jasmonate pathway.[23-25] Both JA-Ile and its structural mimic, coronalon, induced FN synthesis when applied to *Brassica napus* flowers.[8] The role of JA-Ile in EFN secretion, however, has yet to be studied.

Based on the current empirical evidence we conclude that jasmonates play similar roles in the regulation of the secretion of both, FN and EFN. What about other triggers? Unfortunately, information

in this context is scattered and comparative studies that consider FN and EFN would be required to obtain a more complete picture. Exogenous application of auxin can strongly reduce floral nectar secretion in *Euphorbia pulcherrima* and *Antirrhinum majus*.[26,27] In another study, a similar reduction of FN production has been reported from snapdragon flowers upon indole acetic acid (IAA) treatment.[28] In the same study, the distribution of (^{14}C) sucrose in flowers and nectar suggested that IAA acts on the secretory process in the nectary cells, rather than on the mobilization of sugars to the nectary.[28] Recently, it was shown in *Arabidopsis thaliana* that IAA blocks FN secretion until the onset of anthesis.[29] Moreover, exogenous application of gibberellic acid (GA3), naphthalene acetic acid (NAA), indole butyric acid (IBA) and IAA to *Brassica campestris* and *Brassica oleracea* resulted in an induction of floral nectar, among which GA3 showed the strongest inducing effect in terms of nectar amount, sugar content and pollinators attracted.[30] In *A. thaliana*, an extracellular invertase has been reported to be causally involved in the mobilization of starch deposits and thus, floral nectar secretion,[31] but the hormonal control of this enzyme remains to be studied.

JA and its derivatives not only induce FN and EFN secretion but also can elicit

another indirect defence strategy: volatile organic compounds (VOCs),[32] which are released upon herbivore attack or exogenous JA treatment.[33] Besides their role in the attraction of predatory arthropods to herbivore-damaged plants, VOCs also function as a signal that is externally transmitted via the gas-phase and that systemically induces the EFN secretion of both the emitting plant[34] and of different, neighboring plant individuals.[35] Whether VOCs also affect the secretion rate of FN, however, remains to be studied.

In addition to JA, coronatine,[36] a phytotoxin isolated from the pathogenic bacterium *Pseudomonas syringae*, triggers VOC emission in many plant species.[36] Although coronatine and its structural mimic coronalon[37] induce VOC emission[22] and FN secretion,[8] it is not known to date whether these compounds also induce EFN secretion. Floral herbivory (florivory) has been reported to reduce floral nectar and the number of pollinator visits.[38] However, its effect on EFN secretion has not been studied to date. Also floral volatiles, which are attractive to pollinators, are altered qualitatively and quantitatively by florivory in *Pastinaca sativa*.[39] Similarly, those herbivore-induced volatiles that induce EFN, could likely also affect FN secretion (**Table 1**) but no empirical studies appear to exist in that context.

Although many gaps in our knowledge remain to be filled, it becomes apparent that—despite the different ecological functions of FN and EFN—there exist some similarities in their regulation (**Table 1**). Deepening our understanding on the regulatory role of jasmonates and other phytohormones for both FN and EFN secretion and elucidating how these pathways are interconnected, yet functionally separated, will provide interesting insights into the physiological basis of these processes and ultimately into the evolutionary constraints and trade-offs that shaped this regulatory separation. In particular, future work should address the following questions: (1) How do plants achieve and maintain the regulatory separation of FN and EFN secretion, although these two pathways obviously share some signalling molecules? (2) Do other phytohormones (JA-Ile, IAA, GA3, etc.,) also affect EFN

production? (3) Do herbivore-induced VOCs elicit FN secretion?

Answering these questions requires a combination of different, yet complementary methodologies: Labelling experiments, for example with ^{13}C, would allow to investigate whether or not the functional separation of FN and EFN secretion is achieved by a strictly tissue-specific production of the responsible jasmonates. This spatial separation of the sites of synthesis would, however, have to be accompanied by a barrier that limits the transport of leaf-derived jasmonates into flowers and vice-versa. Moreover, gaining a deeper understanding requires also a combination of the widely used external application of using well-characterized, specific inhibitors[40] of phytohormones with analyses of the transcriptome, proteome and metabolome of the various plant tissues and organs. Finally, using mutants that lack certain key genes, for example those that are involved in the JA signalling cascade such as *coi1* (*coronatine insensitive 1*; defective in all JA-related responses[41]) or *jar1* (*jasmonic acid resistant 1*; impaired in the biosynthesis of JA-Ile[42]) will provide mechanistic insight into the regulation of nectar secretion.

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