

# Nectar: generation, regulation and ecological functions

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**Nectar contains water, sugars and amino acids to attract pollinators and defenders and is protected from nectar robbers and microorganisms by secondary compounds and antimicrobial proteins. Floral and extrafloral nectar secretion can be induced by jasmonic acid, it is often adjusted to consumer identity and consumption rate and depends on invertase activity. Invertases are likely to play at least three roles: the uploading of sucrose from the phloem, carbohydrate mobilization during active secretion and the postsecretory adjustment of the sucrose:hexose ratio of nectar. However, it remains to be studied how plants produce and secrete non-carbohydrate components. More research is needed to understand how plants produce nectar, the most important mediator of their interactions with mutualistic animals.**

## Nectar research – the sleeping beauty of plant science

Nectar is a sweet aqueous secretion that mediates the interactions of plants with pollinators and defenders. Many angiosperm and some gymnosperm species produce floral nectar (FN) to attract insect or vertebrate pollinators to achieve adequate fertilization and outcrossing [1,2]. Among Orchidaceae, for example, nectariferous species are more successful at setting fruits than are nectariless species [3]. Extrafloral nectar (EFN) attracts ants, parasitoids and generalist predators, and serves as an indirect defence against herbivores for more than 100 families of ferns, gymnosperms and angiosperms [4,5]. Nectar can be secreted on virtually all plant organs except roots (Figure 1). The site of nectar secretion usually coincides with its function, although nectaries that are functionally extrafloral (i.e. not involved in pollination) might be localized within inflorescences [6]. Despite its importance, nectar remains a surprisingly understudied discipline within plant science. Little is known about nectar components other than sugars and amino acids [7,8], and new classes of substances continue to be detected in nectar. Even less is known about the synthesis of nectar components and the regulation of their secretion.

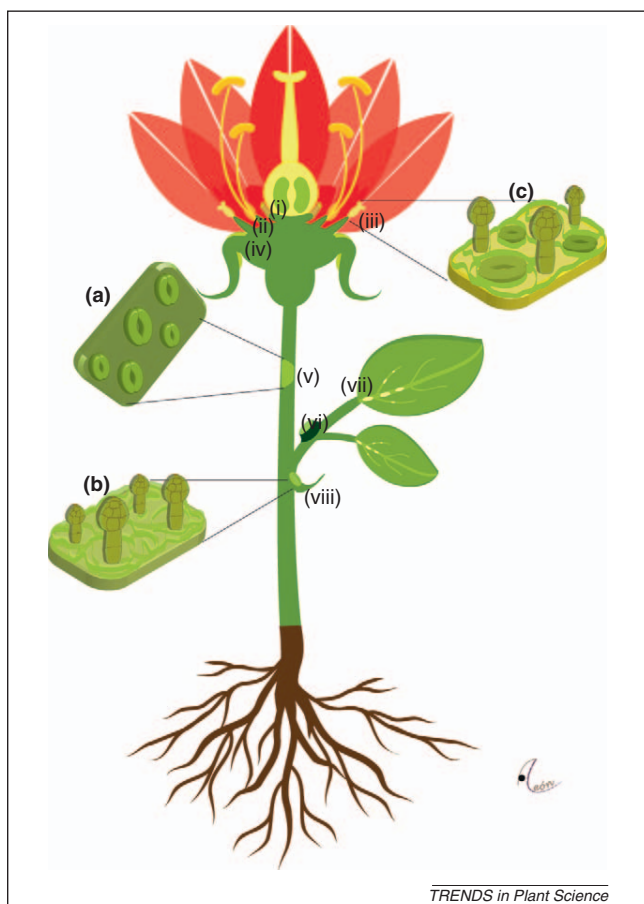
However, in the past decade several exciting discoveries have been made. Nectarins (nectar proteins) have been identified that protect tobacco (*Nicotiana* spp.) FN, *Acacia* EFN and pollination droplets of gymnosperms from microbial infection [9–13]. Enzymes have been identified that play central roles in the postsecretory tailoring of nectar chemistry [14,15], nectar odours have been found to affect pollinator behaviour [16,17], jasmonic acid (JA) has been identified as a hormone that affects the secretion of both

FN [18] and EFN [19–21], and a recently discovered gene that encodes an apoplastic invertase of *Arabidopsis* (*Arabidopsis thaliana*) represents the first gene whose function is required for FN secretion [22]. It is still not known, however, where non-carbohydrate nectar components are synthesized and how these compounds enter the nectar, how plants can adapt nectar secretion to consumption rates and/or consumer identity and how nectar that has not been consumed can be reabsorbed, although all these phenomena are well described at the phenotypic level [23–25]. Ongoing research has produced many expressed sequence tags from nectaries or used gene chips to investigate large-scale transcriptional changes during nectar secretion [26,27], and the first proteomes have been obtained from the nectars of various species [10,28–30]. The field is ready for breakthroughs with regards to the (bio)chemistry of nectar and the physiological and genetic regulation of its synthesis and secretion. In this review, I summarize the most important recent developments and suggest future research directions. I will particularly focus on the recent breakthroughs in the research on nectar proteomics and on the multiple roles of invertases in nectar secretion, because these two fields are likely to experience the most significant development in the near future.

## Nectaries

Nectaries can be extremely diverse with respect to their localizations (Figure 1), their structures and – probably – even their secretion mechanisms [6,31,32]. For example, nectaries can be ‘gestaltless’ [33] (i.e. without any externally visible structure) and in this case can only be identified as areas where nectar appears on the plant surface, or they can form anatomically distinct and sometimes highly conspicuous structures with a complex ultrastructure. Nectaries can be connected to the phloem, the xylem, both or have no direct vascular connection [31,34]. The nectar exits through modified stomata that remain permanently open or through specialized trichomes [31,34–36]. Such differences can even occur within the same plant and functional types of nectaries. For example, stipular extrafloral nectaries of cowpea (*Vigna unguiculata*) form an area of widely spaced secretory trichomes that lacks any direct connection to the vascular system, whereas extrafloral nectaries on the inflorescence stalk consist of a region with secretory, cone-shaped tissues that are connected to the phloem and release EFN through permanently open stomata [37]. Nectaries can be easily lost and gained during evolution [2,38,39], and extrafloral nectaries, and possibly

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**Figure 1.** The distribution of nectaries within a plant. Floral nectaries can be located at the base of the ovary (i), the filaments (ii) or the petals (iii), whereas extrafloral nectaries can be located on the inner or outer surfaces of the sepals (iv), on the shoot (v), leaf stalks (vi), leaf blades (vii) or stipules (viii). The most common types of nectaries secrete via constantly open stomata (a), secretory trichomes (b) or a combination of both (c).

floral nectaries, are phenotypically highly plastic [40,41]. It is probable that the ease with which nectaries are gained and lost, together with the different selective pressures on their functionality, provide explanations for this structural and mechanistic diversity.

#### Multiple functions of nectar components: attraction

The chemical composition of nectar has been reviewed repeatedly [7,8]. Therefore, I provide only a short overview here. Nectar chemistry must fulfil at least two functions: attraction of mutualists and protection from nonmutualists such as nectar robbers and nectar-infecting microorganisms (Box 1). Carbohydrates and free amino acids in the nectar are most important for the function of attraction. Because animals differ in their nutritive preferences, the composition of the nectar determines the spectrum of nectar consumers. For example, hummingbirds, butterflies, moths and long-tongued bees usually prefer sucrose-rich FNs, as do most ant species that feed on EFN, whereas short-tongued bees and flies prefer FN rich in hexoses [7,25,42,43]. However, some nectarivorous birds and ants lack the sucrose-cleaving enzyme invertase and are not able to assimilate sucrose, and therefore prefer sucrose-free nectars [14,44].

Although nectar sugars are usually approximately 100–1000 times more concentrated than amino acids, amino

acids can significantly affect the attractiveness of nectar. Birds and bats can also gain nitrogen from other sources, whereas many adult insects feed only on liquids. Thus, insect-pollinated flowers should possess more amino acids in their nectar than vertebrate-pollinated flowers do. High amino acid concentrations have indeed been reported for FNs from flowers that are adapted to butterflies [45], flies [46] or bees [47]. Similarly, ants prefer nectars rich in amino acids, and ants as well as many insect pollinators can show strong preferences for specific, usually essential amino acids [43,48,49].

Other compound classes are also involved in the nectar attraction function. Volatile organic compounds (VOCs) have been related to pollinator attraction, and scented petals have been known for centuries. By contrast, nectar odours were only recently considered a relevant signal for pollinators [50]. Butterflies and moths prefer artificial flowers containing scented nectar over those that contain pure sugar solutions [17], and parasitoid wasps localize cotton (*Gossypium hirsutum*) EFN using only its odours [51]. Flower mites use nectar odours to distinguish between host and nonhost plants [52]. The origin of FN scent has been linked to volatiles released by the petals that are absorbed and rereleased by the nectar [50]. However, a wide array of VOCs occur in the nectar of wild tobacco (*Nicotiana attenuata*), and many of these compounds have not been detected in other flower parts, suggesting that in certain species nectar emits its own scent [16]. Like other nectar compounds [7], these volatiles serve to both attract pollinators and protect from nectar robbers such as ants [16,53].

#### Multiple functions of nectar components: protection

Carbohydrates, amino acids and volatiles serve mainly in the attraction and nutrition of legitimate nectar visitors; however, nectars also contain other compounds, for example, proteins and several classes of secondary metabolites. What are their ecological functions? Nectar proteins were discovered more than 80 years ago [54,55]. One could suppose that these proteins supply nectar consumers with organic nitrogen, as described above for free amino acids. However, although a nutritive function of nectar proteins cannot be excluded at present, their main function seems to be protection.


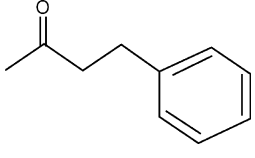

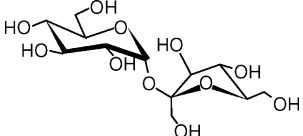

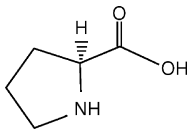

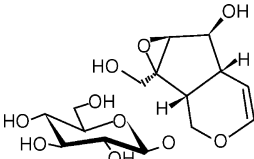

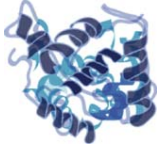

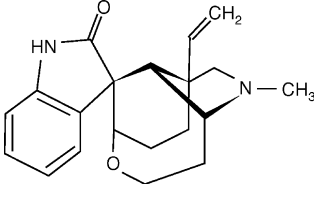
The nectarins in the FN of ornamental tobacco (*Nicotiana langsdorffii* × *Nicotiana sanderae*) have been biochemically characterized and are likely to protect FN from microbial infestation through the Nectar Redox Cycle [9,13,28,48,56]. FN proteomes seem to be small: for example, five proteins have been found in the FN of ornamental tobacco, eight proteins have been detected in the FN of *Jacaranda mimosifolia* [15] and ten in the FN of *Rhododendron irroratum* (H-G. Zha, personal communication). By contrast, we discovered more than 50 proteins in the EFN of *Acacia* myrmecophytes, which house ant colonies for their indirect defence [10]. We identified the majority of these nectarins as pathogenesis-related proteins, such as chitinases, glucanases and thaumatin-like proteins. The first two groups contributed more than 50% to the overall protein content, and similar numbers were then also found in nectars of other *Acacia* myrmecophytes [11]. Chitinases were also found in

**Box 1. The Janus-faced nature of nectar**

Nectar chemistry serves to both attract mutualists such as pollinators and defenders (e.g. ants and parasitoids) and protect from nectar robbers and nectar-infecting microorganisms (Table I). The most important attractive classes of compounds are mono- and disaccharides, amino acids and volatile components such as benzyl

acetone. Repellent effects are exhibited by secondary compounds such as gelsemine and iridoid glycosides. Interestingly, gelsemine also repels legitimate pollinators. Nectar proteins (nectarins) mainly serve to protect the nectar, and perhaps the nectary, from microbial infections.

**Table I. Interacting nectar consumers and components**

Attracted		Nectar attractants	Structure	Refs.
Pollinators		Benzyl acetone		[16]
Pollinators and defenders (ants and parasitoids)		Sugars (e.g. sucrose)		[7,8,48,49]
		Amino acids (e.g. proline)		
Repelled		Nectar repellents	Structure	
Nectar robbers		Catalpol		[67]
Microorganisms (e.g. yeasts)		Nectarin		[9–11,13,108]
Nectar robbers and pollinators		Gelsemine		[66,68]

the FN of *R. irroratum* (H-G. Zha, personal communication) and, thus, might be another common class of nectarins. The concentrations and activities of these pathogenesis-related proteins in *Acacia* EFN were quantitatively correlated, and we could demonstrate direct antifungal properties against various phytopathogens [11].

A role in antimicrobial defence has even been suggested for the GDSL lipase in the FN of blue jacaranda (*Jacaranda mimosifolia*) [15]. Most nectarins, thus, seem to be involved in protecting nectar against microorganisms.

The biological function of this effect is likely to be twofold. First, nectars are commonly infested by microorganisms, particularly yeasts, whose metabolic activities can dramatically change nectar chemistry [57,58]. The chemistry of nectar is closely linked to its function (see above). Therefore, although the presence of some nectar-infecting microorganisms or nectar robbers might have beneficial effects on the plant [59], we can assume that most plants gain a benefit from keeping the nectar as sterile as possible to maintain control over its chemical composition [58].

Second, nectaries might serve as an entrance for phytopathogens [60,61]. This hypothesis has recently gained some support, at least for fire blight caused by the bacterium *Erwinia amylovora* [62,63]. Therefore, antimicrobial nectarins should serve to protect the nectary tissue from infection. This hypothesis is in line with our observation that the EFN of myrmecophytic *Acacia* species, which is secreted constitutively, contains more antimicrobial nectarins than does the EFN of myrmecophilic *Acacia* species [11] and FN of tobacco, *J. mimosifolia* and *R. irroratum*. FNs are secreted over short time spans, and the EFN of myrmecophilic *Acacia* species is secreted transiently at low rates in response to herbivory [21]. Thus, these nectaries provide a less hostile environment for nectar-infecting microorganisms and, therefore, require less protection than do the constitutively secreting extrafloral nectaries of *Acacia* myrmecophytes. However, much more empirical evidence will be required to decide whether antimicrobial nectarins play an important role in reducing the infections of tissues other than the nectary.

The protection of the nectar itself is often assumed to be the function of secondary metabolites (Box 1). Although nectar robbers do not necessarily reduce plant fitness [59,64], it is generally assumed that nectar consumption by nonmutualists represents a loss of a valuable resource. Thus, the FNs of numerous plant families are 'toxic' owing to their content of non-protein amino acids, phenolics and alkaloids [65,66]. For example, the FN of *Catalpa speciosa* contains iridoid glycosides that fend off nectar robbers but not legitimate pollinators [67]. However, toxic nectar components seem to function as a double-edged sword. For example, Adler and Irwin [68] experimentally manipulated the concentration of gelsemine, which represents the principal FN alkaloid of *Gelsemium sempervirens*. Supplementing the FN with gelsemine also deterred effective plant pollinators, thereby decreasing the number of flowers probed and the time spent per flower by both pollinators and nectar robbers. Why should plants produce FN that deters pollinators? Several scenarios are possible [66,68]. First, short visitation times by pollinators do not necessarily represent a fitness disadvantage for plants [69]. Second, preventing the loss of nectar to robbers might even be worth some reduction in pollinator visits. Third, gelsemine lowers the rate of selfing by reducing the proportion of pollen from the same individual that is transferred in *G. sempervirens* [70]. Finally, secondary metabolites in nectar could inhibit microbial growth [66] and, thus, complement the function of nectarins. However, no clear fitness benefits for plants with toxic nectars have been demonstrated to date [68]. An alternative explanation might be that secondary compounds, which are transported through the vascular system in a functional context of systemically induced defence, passively 'leak' into the nectar [66,71]. Identifying the sites of synthesis of toxic nectar components and elucidating their secretion mechanisms would be the first steps towards an explanation of their functions.

### Nectar quantity and function

Plants and nectar consumers have their own, often conflicting, interests. It is in the best interests of the plant to obtain maximum service with minimum investment,

whereas the selection pressure on the animal should be to obtain the maximum amount of reward for the minimum investment of energy and time. The mere presence of nectar clearly benefits plants by increasing pollination rates and the efficiency of indirect defence [3,5]. However, does more nectar always mean more service, and what can plants do to minimize the resulting costs?

The presence of benzyl acetone in the FN of wild tobacco plants increased the number of pollinator visits whereas nicotine reduced the nectar uptake during single visits. The combination of these compounds increased the number of floral visits, particularly by hummingbirds [16,69]. By contrast, *Petunia* flowers that were bred to produce reduced amounts of nectar paid for this attempt to 'cheat' their pollinators with reduced visitation frequency by *Manduca sexta* moths and a concomitant reduction in seed production (A. Brandenburg, personal communication) [72]. Do plants gain or lose fitness when reducing their FN secretion? Generalizations are impossible based on these two studies. However, it seems probable that the *Petunia* system represents the more usual scenario because *N. attenuata*, owing to its special ecology, does not usually compete for pollinators with other species.

Increased EFN secretion rates have been shown to increase the number of ant workers foraging on plants [19,73], have been related to higher survival rates of ant workers [74] and other predators [75] and can increase ant activity and aggressiveness [24,76,77]. Indeed, indirect defence via ants represents one of the few antiherbivore defence strategies for which a clear effect on net herbivory rates and plant fitness has been shown for different species [78]. For lima bean (*Phaseolus lunatus*), we linked an increased defence against herbivores unambiguously to an augmented EFN supply [73]. Thus, a positive correlation of investment with benefit for the plant has been shown for EFN, but the generality of this assumption has yet to be proven for FN.

### Phenotypic regulation of nectar quantity

Although nectar sugars in some species represent less than 2% of the current net photosynthates [32], nectar production by *Asclepias syriaca* can consume up to 37% of daily assimilated carbon during blossoming [79]. Nectar secretion undoubtedly can be costly, although many more studies on different plant groups will be required to obtain a realistic estimate of the 'usual' costs of nectar production. Therefore, it does not seem surprising that plants adjust nectar production rates to the intensity of consumption and reabsorb nectar that has not been consumed [80]. Unfortunately, many of the studies that have tried to measure nectar secretion have used inadequate methods and have, for example, quantified either nectar volume or its concentration, but not both. Because these parameters change with air temperature and humidity, both must be considered together to calculate the realistic amounts of secreted soluble solids [81–84].

Although reports must be interpreted carefully, plants undoubtedly control nectar production. An adjustment of FN net production to consumption rates has been demonstrated in various species [85–87]. *Macaranga tanarius* has been shown to reduce EFN secretion in the absence of

consumers and increase it immediately after consumers were allowed to feed for one day on the nectaries [23]. We also discovered that obligate myrmecophytes among Central American *Acacia* species can adjust EFN secretion to the identity of the inhabiting ants and provide high levels of the resource only to defending mutualists but much lower amounts to nondefending parasites [24]. Unfortunately, the cues that enable the defending partner to be identified are not yet known. However, quantitative adjustments of apparent net production could result from either an inhibited *de novo* secretion or the reabsorption of accumulated nectar. Although it is not known whether plants really can adjust the *de novo* secretion, a reabsorption of FN has been shown unambiguously with different methods [25], including labelling studies that demonstrated the uptake and allocation to other plant organs of externally applied  $^{14}\text{C}$ -labelled sucrose or glutamine [88,89]. The reabsorption of nonconsumed FN seems common, although this phenomenon has yet to be demonstrated for EFN [25]. The reabsorption of nonsecreted nectar by the postsecretory floral nectary has been related to programmed cell death in the nectary tissue in combination with a phloem that remains active and the resulting changes in source–sink relationships [35,37]. Under this scenario, extrafloral nectaries are likely to lack the capacity to reabsorb nectar because the regulation of EFN secretion is not dependent on ontogenetically programmed patterns.

Many plants produce their FNs or EFNs in diurnal rhythms and these seem, at least in part, adapted to consumer activity [23,37,85,90]. Besides these probable endogenous rhythms, the secretion of EFN with few exceptions is induced by mechanical damage or herbivory. This induction is mediated via the octadecanoid signalling pathway and can be elicited by the application of JA [19,21,39,91]. We recently found that FN secretion by oilseed rape (*Brassica napus*) is under the control of the same hormone [18]. The probable general role of JA in controlling nectar secretion is further underlined by the observation that the expression of several genes related to JA biosynthesis or transcriptional responses to JA are upregulated in actively secreting *A. thaliana* Columbia-0 nectaries [27]. Further hormones such as auxins and gibberellins are differentially produced in nectaries and likely to play a role in the control of nectar flow as well [27], but current empirical evidence is too scattered to allow any general conclusions on their detailed roles.

### Secretion mechanisms

As demonstrated above, nectar secretion rates can be affected by endogenous as well as exogenous factors. Floral and EFN secretion is, at least in part, under the control of JA, although EFN secretion is subject to a stronger phenotypic plasticity than FN secretion is and, therefore, requires additional regulatory mechanisms. How do plants control nectar flow and where are the components of nectar synthesized?

In most nectaries, the phloem and/or xylem vascular traces do not reach the epidermis. For example, Wist and Davis [34] reported that 50% of Asteraceae lack direct vascular connections in their floral nectaries. A

taxonomically broader review also found that nectaries of more than one-third of all plant species lack any direct vascularization [31]. Rather, the vascular system more or less directly supplies secretory parenchyma cells called the 'nectariferous tissue' [31]. However, most authors agree that nectar represents 'secreted phloem sap' for most species [2,31,55,92–94]. A detailed pathway has not yet been elucidated to describe how carbohydrates and other nectar components are uploaded from the phloem to the nectariferous tissue, metabolized and secreted to the outside. Some authors favour a symplastic pathway, whereas others support transport through the apoplasm. For the symplastic pathway, transport via endocytosis and exocytosis, molecular transport across the plasmalemmae and transport via plasmodesmata have all been considered. All points of view have been controversially discussed before [27,31,35,36,95]; however, we are now starting to identify the first general phenomena that seem to be regularly associated with an active secretion (Box 2). Most likely, prenectar is transported in vesicles that move through the symplast via plasmodesmata and are secreted via exocytosis [95]. Nectar carbohydrates can be prestored in the nectariferous tissue, a step that at least seems to be involved in the secretion of nectars that are more concentrated than the phloem. However, any true breakthrough in this field will require a much more detailed knowledge of the genetic basis of nectar secretion and the localization of the enzymes involved and/or channel proteins and ion pumps.

### Origins of nectar components

Concerning the origins of carbohydrates, which form the quantitatively dominant class of compounds, two alternative, nonexclusive scenarios have been suggested: (i) direct transport from the phloem and (ii) accumulation of starch in the developing nectary and its hydrolysis during active secretion (see [95] for a recent review on secretion mechanisms). Some carbohydrates might even stem from *in situ* photosynthesis. Empirical evidence exists for both mechanisms, and owing to the repeated evolution of nectaries it seems probable that both contribute to secretion, although with varying degrees of importance. The direct secretion of the products of the current assimilation process has been shown repeatedly for FN, using the girdling of flower shoots as well as darkening and defoliation experiments [35,96]. We used experiments with  $^{13}\text{C}$ -labelled  $\text{CO}_2$  to demonstrate that EFN also contains sugars that have been assimilated during the last hours before secretion [97]. The second scenario is supported by studies showing that nectaries of ornamental tobacco and *Arabidopsis* accumulate large quantities of starch and that the degradation of this starch into mono- and disaccharides coincides with the onset of nectar secretion during anthesis [98,99]. A breakdown of accumulated starch as well as programmed cell death during and after secretion have also been described for further, taxonomically unrelated species such as soy bean (*Glycine max*) and common foxglove (*Digitalis purpurea*) [35,80,100]. Many species possess amyloplasts in their nectary tissue [80] that can become directly connected to the vacuole and consecutively emptied during the phase of most active FN secretion [35].

**Box 2. A putative mechanism of nectar secretion**

Current evidence suggests that prenectar is mainly transported in vesicles, in which its composition can be changed, and then is secreted by exocytosis [95] (Figure 1). The prestorage of carbohydrates might be involved, and invertases play a dual role in the uploading of prenectar from the phloem and in the mobilization of carbohydrates during active secretion [22,95]. Several aspects support this model. First, nectar secretion via trichomes excludes an apoplastic transport for the respective species owing to apoplastic barriers in the external cells walls in the stalk and intermediate cells of the trichomes [31,37]. Second, vesicles are common in nectariferous tissues [31,37,109]. The number of dictyosomes in secreting and

nonsecreting nectary cells of *Billbergia nutans* (Bromeliaceae) differed in coincidence with secretory activity [109]. Third, secreted nectars are characterized by a wide range of concentrations and sucrose:hexose ratios [45,81] and, therefore, cannot be produced only by a passive flow or transport mechanisms exclusively driven by sucrose-cleaving enzymes. Fourth, non-carbohydrates such as lipids and proteins [9,15] are likely to be synthesized in the nectariferous tissue and then must enter the prenectar before its secretion (Figure 2). Finally, nectar secretion depends on several fast-acting control mechanisms, which cannot result solely from passive source-sink relationships.



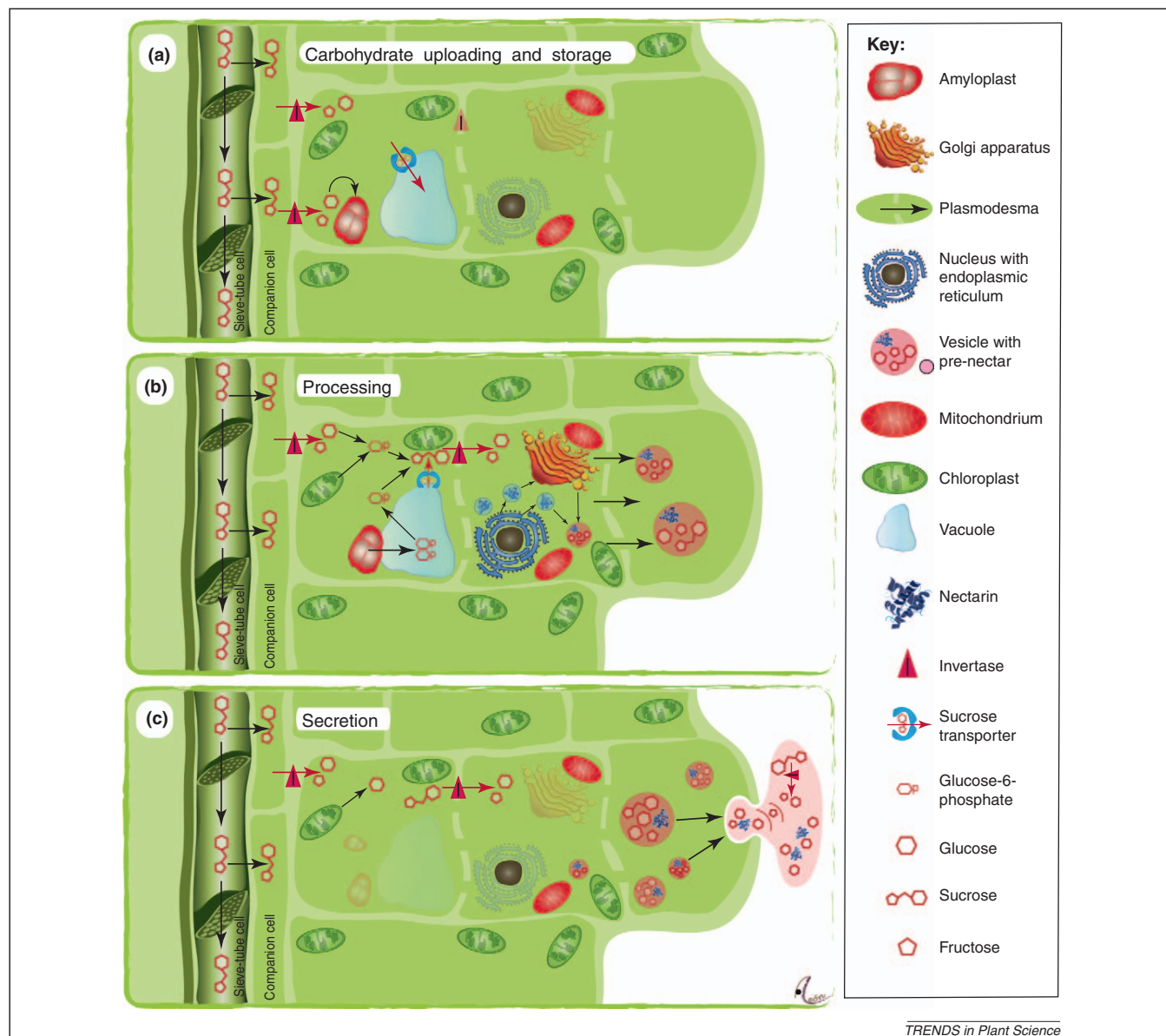
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**Figure 1.** The ultrastructure of nectar secretion. The hypothesis that prenectar is formed, metabolized and transported in vesicles within the nectariferous tissue is supported by the ultrastructural properties of this tissue [95]. The high density of plasmodesmata, which is common in the nectariferous tissue [37,110], allows vesicles to move rapidly among cells. In the case of floral nectaries, the prestorage of carbohydrates in amyloplasts can be involved (upper region), enabling floral nectaries to reach high peak sugar secretion rates owing to a combination of starch breakdown and the current uploading of sucrose from the phloem [99,103]. These peak rates are achieved when starch-containing plastids come into direct contact with the vacuole [35]. However, the prestorage of starch is not an essential prerequisite for nectar secretion (lower region) because it is not likely to occur in extrafloral nectaries.

All these processes seem linked to the mobilization of nectar carbohydrates (Figure 2).

In summary, carbohydrates are uploaded as sucrose from the phloem to the secretory tissue where they are stored and/or processed [95,101]. During active secretion, sucrose is metabolized by cell wall invertases, which serve to produce hexose-rich nectars and create the required source-sink relationships [92,94,102] (Figure 2). In fact, genes coding for complete sucrose biosynthesis are up-regulated in *A. thaliana* nectaries [27], and the expression patterns of genes involved in starch metabolism allow a

clear separation of an anabolic phase before anthesis and a catabolic phase during secretion in nectaries of ornamental tobacco [103]. Sucrose can also be eliminated from nectar by postsecretory hydrolysis, which is mediated by invertases that are secreted into the nectar itself [14,102]. An apoplastic invertase has recently been discovered in *A. thaliana* that is required to create the sink status for active nectar secretion [22]. A mutant line, which lacked this activity, showed reduced levels of starch accumulation within the nectary, demonstrating that apoplastic invertases might also play a central role in the uploading of



**Figure 2.** Nectar formation and secretion. Nectar formation and secretion probably require three metabolically different phases (see online supplementary movie): (a) optional carbohydrate uploading and storage, (b) nectar processing and the synthesis of non-carbohydrate components and (c) secretion. Invertases seem to be involved in several steps: (i) the uploading of sucrose from the phloem into the nectariferous tissue [22]; (ii) the formation of the sink required for the secretion of sugars into the extracellular space [22,92,94,95,102]; and (iii) the formation of hexose-rich nectars. The last step can be catalyzed by invertases that are localized in the nectariferous tissue or in the secreted nectar itself [14,102]. *In situ* photosynthesis by chloroplasts in the nectariferous tissue might provide additional carbohydrates. Nectariferous tissues are usually characterized by a dense, rough endoplasmic reticulum, the presence of many dictyosomes and an active Golgi apparatus [109,110]. The presence of protein-rich inclusions [37] points towards the synthesis and storage of nectarins in the nectariferous tissue itself, and the generally high abundance of mitochondria [34,109,110] indicates high metabolic activity. Therefore, many of the non-carbohydrate components of the nectar are likely to be synthesized in the nectariferous tissue. It remains to be elucidated whether the vesicles stem from the Golgi apparatus or directly from the endoplasmic reticulum, and where carbohydrates and the other nectar components are combined to form the final nectar.

sucrose from the phloem and its subsequent storage in the nectariferous tissue [22,95].

However, quantitative considerations demonstrate that starch accumulation can only account for a part of the sugar that is secreted during the peak activity of floral nectaries [35,99]. Moreover, extrafloral nectaries to date have not been reported to store starch [80]. In these cases, all carbohydrates are likely to stem directly from the phloem, and nectar formation and secretion depend on vesicle-based mechanisms. Because floral nectaries are phylogenetically derived from extrafloral nectaries [104,105], direct transport from the phloem seems to

represent the original mechanism, whereas starch accumulation might be a derived strategy for the secretion of large amounts of sugar during the peak activities of floral nectaries [2].

Unfortunately, all previous hypotheses about the mechanisms of nectar production and secretion have focused exclusively on FN carbohydrates [2,35,36,95,101]. Where non-carbohydrate nectar constituents are produced, where and how they are added to the prenectar and how they are secreted remain open questions. Considering the abundance and chemical diversity of nectary proteins and the lack of reports of many of these nectarins

from other tissues, synthesis in the nectary tissue seems probable. In fact, the secretory cells of *V. unguiculata* extrafloral nectaries contain protein-rich inclusions [37] and all *NECTARIN* genes that correspond to nectar proteins in the FN of ornamental tobacco are expressed in the nectary tissue [9,106], where some of them are under the control of a MYB305 transcription factor [107]. Furthermore, tobacco nectarins contain signal peptides for secretion and can, therefore, only be secreted by the fusion of vesicles with the plasma membrane. Similar evidence for other systems is lacking but urgently needed to understand how plants produce nectar compounds and secrete them into nectar.

### Future steps

Every general model concerning nectar production and its control will have to: (i) consider the differences and similarities between FN and EFN secretion and the JA responsiveness of both types of secretion [18,19]; (ii) explain why net flow can occur in both directions (secretion or reabsorption); (iii) integrate the two models of the direct allocation of phloem content versus the usage of prestored macromolecules; and (iv) include the synthesis and secretion mechanisms of non-carbohydrates.

The physiological differences between FN and EFN provide us with a starting point. Flowers secrete nectar in an ontogenetically programmed pattern and can reabsorb nonconsumed FN, whereas EFN secretion can be induced by herbivory and is subject to a reversible inhibition when not consumed [23]. Thus, programmed cell death and other irreversible mechanisms can play a role in controlling the secretion of FN [35], but apparently not of EFN. Although floral nectaries are likely to have evolved from extrafloral nectaries [104,105], both nectary types have been subject to different selective pressures to which they have responded with the evolution of secretion mechanisms that are in parts different. Focusing on these differences will help find those processes that are inevitably required for functioning nectar secretion.

### Concluding remarks

Nectar was an important topic in plant research during the late 19th and the first half of the 20th century (Box 3). Although central questions remained unresolved at that time, physiological and molecular research into nectar secretion mechanisms has not made much progress until recently. Which factors have inhibited nectar research since then? The field has probably suffered because of the focus of the plant sciences on model plants that either do not produce nectar (i.e. wind-pollinated crops such as maize, rice and wheat) or do not rely on nectar because they are highly selfing (*Arabidopsis*). However, 'omics' techniques have enabled nonmodel plants to be studied, and the first nectar-producing species (in particular, cotton, poplar, tobacco and *Petunia* species) have now reached the status of genetically tractable model systems. Future research should include the biochemical analyses of both nectar and phloem sap to identify nectar compounds that stem exclusively from the nectary itself. The use of large-scale untargeted attempts such as transcriptomics, proteomics and metabolomics could help in this context and would be most

### Box 3. Nectar research: a renaissance

The word 'nectar' has its origin in the old Greek term 'nektar' (νεκταρ), which is assumed to be derived from the ancient Egyptian term 'ntry' (divine) [111] or composed from 'nek' (death, see necro-) and '-tar' (overcoming). Its current meaning 'sweet plant secretion' was first recorded in 1609, and referred to FN only (<http://www.etymonline.com/index.php?search=nectar&searchmode=none>). Owing to the highly desirable effect that is expressed in the ancient Greek word, nectar was regarded as the alimentation of gods and Phylarchos considered nectar as having a strong 'sympathy' to the sun (Athenaios 693 e-f, cited in [112]). Finding that nectar secretion is dependent on light [113] represents a contemporary confirmation of mythological ideas. Do further recent reports also represent confirmatory work rather than truly novel discoveries? The answer is 'yes'. Matile [104] suggested in 1965 that floral nectaries are evolutionarily derived from extrafloral ones. Nectar proteins were discovered in 1927 [54] and their invertase activity in the 1950s [102]. Nectar reabsorption was reported by Bonnier in 1878 [114]. The defensive function of EFN was independently suggested by Delpino and Belt in 1874 [115–117], questioned by Darwin two years later [118] and regarded as 'finally rejected' by Nieuwenhuis von Uexküll-Güldenband in 1906 [102,119]. Another controversial discussion that also dates back to the 19th century concerns the mechanisms of nectar secretion. Behrens related starch degradation to nectar secretion in 1879 (see [35]), whereas the direct involvement of the phloem was suggested by von Wettstein in 1889 [120]. In summary, the current discoveries in nectar research represent a renaissance of a previously fashionable field rather than a truly novel development.

fruitful when following comparative strategies, using functionally similar nectaries of taxonomically unrelated species and functionally different nectaries within the same species. In this context, the functional differences between inducible EFN secretion and ontogenetically programmed FN secretion should provide an attractive tool. This strategy should enable an understanding of the general principles that underlie the synthesis of nectar components and the regulation of nectar secretion.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.tplants.2011.01.003](https://doi.org/10.1016/j.tplants.2011.01.003).

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