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Co-Evolution of Secondary Metabolites

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Co-Evolution of Secondary Metabolites

With 204 Figures and 48 Tables

Springer

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Preface

This book is like a dream project putting together metabolic aspect of complex biological processes like pollination, symbiosis, herbivory by insects, and volatiles released by plants in their atmosphere. Most of these processes are very complex and produce very small amount of metabolites, which remains a challenging task to detect and quantify. Though the chemistry and biosynthesis of secondary metabolites is increasingly well studied, less attention is paid to their evolutionary and interactive aspect. Almost all plants are attacked by insect herbivores, pests, and animals and they cannot escape from being non-movable unlike animals. Therefore, they evolve and conserve several defensive traits to combat pests by various types of chemical weapons. Improvement in tools of chemical analysis like GLC, HPLC with sensitive sensors and detectors such as mass spectrometer, and high-throughput screening along with gene expression using transcriptome analysis paved the way for analyzing, detecting, and identifying these molecules, small or large, in quantities unnoticeable with old prevailing technology.

Therefore, this book presents state of information about secondary metabolites produced in plants during interaction with parasites, pollinators, pests, and herbivores. As secondary metabolites are specialized classes of compounds biosynthesized by different pathways involving several genes, this is an interesting evolutionary mechanism to adapt to the changing host or the pests by modifying the secondary metabolites. Secondary metabolites play a crucial fundamental biological role in a plant's life, and genetic changes are required to execute the energy-expensive process.

The book *Co-evolution of Secondary Metabolites* is divided into six parts covering the entire gamut of bioactive molecules present in plants. This includes phenomena like diversity within plant, changes in secondary metabolites during adaptation of plants to life on land, and involvement of secondary metabolites in pollination, allelochemy, abiotic stress, host–parasite interaction, sensory perception, insect–plant interaction, and plant defense. These interactions are vital for survival of plants and their pests and have evolutionary consequence.

This book is planned as a reference work providing state-of-the-art knowledge composed by highly renowned scientists of the field. Well-recognized international specialists in their respective fields of research contributed the chapters. This book will be useful to all those working in the field of botany, evolutionary biology,

phytochemistry, physiology, molecular biology, biotechnology, and plant pathology. This book is arranged in 36 well-illustrated chapters.

We would like to acknowledge the cooperation, patience, and support of our contributors who have put serious efforts to ensure the high scientific quality of this book with up-to-date information. We are thankful to the staff at Springer, namely Dr. S. Blago and N. Clifford, for their professional support in this project.

February 2020

Professor Jean-Michel Mérillon
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Sugar and Polyphenolic Diversity in Floral Nectar of Cherry

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Abstract

Cherries (*Prunus avium* L. and *Prunus cerasus* L.) are economically important fruit species in the temperate region. Both are entomophilous fruit species, thus need pollinators to give high yields. Since cherry's flower is easy-to-reach, bees and other pollinators can smoothly collect nectar as a reward for doing transfer of pollen to receptive stigma. Nectar in cherry is usually attractive for insects,

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especially to honey bee (*Apis mellifera*) who is the most common pollinator. Nectar is predominantly an aqueous solution of sugars, proteins, and free amino acids among which sugars are the most dominant. Trace amounts of lipids, organic acids, iridoid glycosides, minerals, vitamins, alkaloids, plant hormones, non-protein amino, terpenoids, glucosinolates, and cardenolides can be found in nectar too. Cherry flower may secrete nectar for 2–4 days and, depending on the cultivar, produces up to 10 mg nectar with sugar concentration from 28% to 55%. Detailed chemical analysis of cherry nectar described in this chapter is focused on sugar and phenolic profile in sour cherry. The most abounded sugars in cherry nectar was fructose, glucose, and sucrose, while arabinose, rhamnose, maltose, isomaltose, trehalose, gentiobiose, turanose, panose, melezitose, maltotriose, isomaltotriose, as well as the sugar alcohols glycerol, erythritol, arabitol, galactitol, and mannitol are present as minor constituents. Regarding polyphenolics, rutin was the most abundant phenolic compound followed by naringenin and chrysin. Cherry cultivars showed different chemical composition of nectar which implies that its content is cultivar dependent.

Keywords
Prunus avium L. · *Prunus cerasus* L. · Flower · LC/MS · HPAEC · Polyphenolic profile · Sugars

1 Introduction

Cherry is the common name of several species of the genus *Prunus* originated from the common ancestor in area between the Black Sea and Caspian Sea in Asia Minor [1]. Among cherries, the sweet cherry, sour cherry, flowering ornamental cherry species, and a few other *Prunus* species used as rootstocks for cherries are considered important [2]. Cherries are members of the *Rosaceae* family, *Prunoideae* subfamily, and genus *Prunus* and are further placed within two subgenera *Cerasus* Pers. and *Padus* (Moench) Koehne [3]. The *Cerasus* Pers. subgenus and *Cerasus* Koehne section contain the diploid sweet cherry ($2n = 2x = 16$), and the tetraploid ($2n = 4x = 32$) sour cherry and ground cherry.

Cherries are one of the oldest fruit crops known to mankind. It is believed that Theophrastus has mentioned cherries roughly 300 years BC [4]. Another earlier writing suggests that Lucullus brought cherries back to Italy when he returned from the Pontu region (in present day Turkey). Archaeologists have discovered fossilized cherry pits in Stone Age caves and dwellings of western Switzerland, Bourget (France), and Parma (Italy) [5] that places cherry into the Neolithic Period (about 4000–5000 years ago).

Both sweet and sour cherry production, as the most economically important among cherries, has increased significantly during the past two decades in the traditional leading cherry-producing countries. The annual global sweet cherry production (average 2014–2016) is about 1.7 million tons and shows a slightly increasing tendency. The leading sweet cherry-producing country is Turkey,

followed by the USA, Iran, Italy, Spain, Chile, and Ukraine. Sour cherry is often called the fruit species of Eastern Europe because the most important producing countries are located in this part of the world. Global production is about 1.3 million tons (average 2014–2016). In countries where there is a keen interest in sour cherry-based products, such as the eastern European countries, production is usually machine harvested and is increasing slightly. The world's leading sour cherry-producing country is Turkey, followed by the Russian Federation, Poland, Ukraine, Iran, the USA, Serbia, and Hungary [6].

Cherries are a deciduous fruit tree, having an attractive appearance during bloom time. The cherry fruit is a nutrient dense food with relatively low caloric content and significant amounts of important nutrients and bioactive food components including fiber, polyphenols, carotenoids, vitamin C, and potassium [7]. Sweet and sour (syn. tart) cherry ripen first among stone fruits, followed by apricot, peach, and plum. Because sweet cherry is first on the fresh market, it is in high demand in the late spring and early summer. The majority of sweet cherries are consumed fresh with the remaining 20–25% processed as brined, canned, frozen, dried, or juiced. In contrast, 97% of tart cherries are processed primarily for cooking and baking and the confectionary industries [7, 8].

Pollination is a crucial part of growing quality cherries because most of the cultivated varieties of sweet cherry are self-incompatible. To set fruits, they require pollen from suitable pollinating cultivars. Thus for the commercial production of sweet cherry, a good orchard design, with enough pollinizers have to be planted [9]. Besides, pollinating insects should be present for adequate transfer of compatible pollen to the stigma. Among sour cherry cultivars, there are more and more self-compatible ones; however, foreign pollination can improve quality even at these cultivars.

According to recent research, cherry flowers are very attractive to various insects [10]. They observed activity of 31 species of insects belonging to 5 orders and 13 families of class. Honey bees (*Apis mellifera*) have been assumed to be the main pollinators in cherry [11], due to their high demand for pollen and nectar and their hairy body, which collects and disperses the pollen [12–16]. However, honey bee is not active on temperature below 12 °C or in rainy weather conditions. In that case, pollination can be also successful since other insect species belonging to the *Bombus*, *Andrena*, and *Osmia* spp. could maintain their activities on lower temperatures and during rainy days [17]. Landscapes with wild bee habitats enhance pollination, fruit set, and yield of sweet cherry, presumably due to their higher pollination efficiency [18]. Therefore, it is very important to attract honey and wild bees to proper pollination of these crops, especially commercial crop production.

2 Role of Nectar

Plant species that depend on insect (or other animal) pollinators for their reproduction have put lots of effort in many floral traits such as floral display, flower architecture, color, scent, and nectar [19]. To attract pollinators, plants offer different types of rewards, where floral nectar represents the main plant reward for many

pollinators [20]. Floral nectar composition, its quality, and chemical and physical features varies widely between species and type of nectary and most probably are related to different consumers and ecological factors (abiotic and biotic).

Flowers often have specialized structures that make the nectar accessible only for animals possessing appropriate morphological structures, and there are numerous examples of coevolution between nectarivores and the flowers they pollinate. The main function of nectar compounds is related to the attraction of pollinating insects. It is well-known that honey bee chemoreceptor can detect volatile substances, contained in the nectar of crop plants at distance of about 2 km [21]. In this way, pollinators are unintentionally mediating the transfer of pollen to receptive stigma, becoming a key attribute for increasing cross-pollination [22, 23]. Although floral nectar production represents a high cost for the plant, it ensures a higher possibility of fruit/seed set, higher reproductive success and gene transfer into next generation. The production of nectar (when starch granules in the parenchyma are broken down) often peaks when anthers start to shade pollen and when the stigma is the most receptive. Generally flowers secreting more nectar show more successful pollination events [24].

It is proved that secreted nectar volume correlates with flower size, which is probably due to the pleiotropic effects, where larger flowers have larger nectaries and more space for nectar [25, 26]. The amount of nectar reward is positively correlated with the number of pollinator visits, the number of flowers visited within a plant, and the duration of the visit within a flower [27]. Generally, energy received from nectar per insect (or other pollinator) must be enough to attract pollinators, but still need to encourage movement of the pollinator from flower/plant to another one. This means that nectar volume is correlating with the body size of the pollinator [28].

The attractiveness of nectar to pollinators depends on taste [29], but odor and color play an important role too [30, 31]. Characteristics such as volume, concentration, color, and taste may be related to the concentration and composition of dissolved sugar (especially glucose, fructose, and sucrose). But also other components, including minerals, phenolic compounds, and amino acids, may make a cardinal contribution to its attractiveness to honey bees [22, 32–34]. Bees prefer bright flowers, while visual and chemical associations are pushing it to navigate within the field [35].

Nectar concentration is highly influenced by geographical distribution, thus environmental factors, especially light, water, nutrients, CO_2 concentration, temperature, humidity, soil moisture, and wind [36]. Besides, nectar composition can vary between the two sexual phases of a given hermaphrodite flower [37], phenology phase, among flowers on different plants [38] and individuals, populations, cultivars, or subspecies of the same species [39]. Physiological factors such as flower age, health of plants, and damage to floral parts also affect the quality and composition of nectar.

3 Nectar Productions in Cherries

Cherry flowers are allogamous, actinomorphic, and are arranged in racemose clusters of 2–5 flowers. The sweet and sour cherry flowers are from 2.5 to 4.0 cm in

diameter, white, hermaphroditic, and are attractive for pollinators [40]. The cherry flower structure is usually characterized by stamina standing far from the pistil, thus insects can touch the stigma only during nectar collection, passing along the pistil [41]. In cherries like in most of the temperate fruit trees, sieve tubes more or less directly supply secretory parenchyma cells called the "nectariferous tissue" with pre-nectar prior to nectar secretion. The nectary is receptacular, covering the whole surface of the receptacle [42].

For the protogyn, sour cherry varieties is very important to know the periodicity of nectar production and the synchronization of the endogen rhythm with stigma receptivity and anther dehiscence. As it was stated in [43], dichogam flowers produce nectar periodically by 12th hours, the homogam ones by 6th hours, and the time of maximum production is synchronized by the stigma receptivity and anther dehiscence. In the hybrids of sweet and sour cherries, 3-h gaps can be observed in nectar production [43]. Additionally, the change of pollination strategy for the protogyn sour varieties: (i) stigma exerted – wind pollination, (ii) state of pollination chamber – beetle pollination, (iii) opening of anthers – pollination by bees and other insects was observed [44].

A sour cherry flower may secrete nectar for 2–4 days and, depending on the cultivar, produces 0.2–9.0 mg nectar. Generally, autochthonous landraces like "Cigánymeggy" or "Oblačinska sour cherry" type yield less but more concentrated nectar, with sugar values of 0.1–1.8 mg/flower/day, while cultivated varieties produce more but rather dilute nectar [45]. Among sour cherry cultivars, "Meteor korai" and "Debreceni bötermö" are one of the best nectar producers giving 10.27 μl and 7.21 μl of nectar respectively; with 13.96% and 16.6% of sugar, respectively [46].

The nectar of early blooming fruit trees such are cherries is important for honeybees in the brood rearing season, but rarely can provide unifloral honey, as well [45]. Sweet cherry blossom is more attractive for bees than sour cherry blossoms primarily because the nectar of sweet cherries is much richer in sugar (55%) than that of sour cherries (28%). But, sour cherry cultivars produce a significant amount of nectar at night [47], thus attracting night insects.

If a successful fertilization should be achieved even at self-incompatible cherry and sour cherry cultivars, all details of their pollination biology should be known, including the sugar and polyphenolic composition of nectar, as ones of the primary attractants [46, 48].

4 Nectar Compositions

The number of papers related to the examination of the chemical composition of the floral nectar is not large, although it is much more available for floral nectars than for extrafloral nectars. Mainly studies have focused on the qualitative aspect. The main components of the nectar, sugars and amino acids, were the most examined, while other solutes were not subjected to the research to that extent. This is rationale since the nectar is predominantly an aqueous solution of sugars. Also, sampling is not easy considering the duration of secretion (few hours to several days), and the amount of

nectar produced (less than 1 μL to few ml proportional to the nectary parenchyma volume).

Sugars, proteins, and free amino acids are the three major components of floral nectar among which sugars are the most dominant [49]. Nectar is highly variable at any taxonomic scale indicating that plant phylogeny can be a stronger determinant of nectar composition [50]. But, pollinator type can also shape the composition of nectar because different pollinators show preferences for solutions of different viscosity and/or sugar composition [51]. In general, insect pollinated flowers, like in cherry, produce relatively concentrated nectar.

Secondary metabolites and volatile compounds in flower nectar are appearing in low level. Compounds belonging to the secondary metabolism are either synthesized in the nectaries themselves or can also be derived directly from the phloem. They can have a range of effects on pollinator preference and performance, from fully negative to positive. More often, these compounds are usually regarded as "toxic compounds" and are involved in antimicrobial defensive functions, protection from nectar robbers, and pollinator attraction [29, 52]. On the other hand, secondary metabolites can significantly stimulate bees to feed, while indirectly, pollinators can have benefits from them by reducing gut pathogen loads [53, 54].

The phenolic compounds in nectar have several roles in attraction and/or repelling honey bees (phenolic compounds can give an astringent taste, thus inhibiting herbivores) in nutrition of pollinators, in oxidation prevention of other nectar substances, and in providing an aggregate value to honey commercialization by the certification of the botanical origin [29, 55]. In some cases, nectar constituents may also help defend the flower against invaders, which allows flower to promote out crossing and achieve its ultimate goal, and that is to set a fruit/seed [52]. Besides polyphenolic compounds, some amount of abscisic acid (ABA) can be found. The role of this plant hormone is the protection of plants in conditions of environmental stress, especially in reducing the penetration of UV-B ultraviolet radiation [56]. Also, jasmonic acid, its precursors and its derivatives, have been identified as a hormone that affects the secretion of floral nectar and defense responses [57, 58].

Volatile compounds, important cues that help insects locate flowers, mediate plant response to pathogen infection, plant-parasitoid signaling in response to herbivory, and plant-pollinator communication during flowering. Most of the floral fragrance compounds are terpenoids (most common monoterpenes), simple aromatics, amines, and hydrocarbons [59].

Amino acids are contributing to the taste of nectar and are important source of nutrients for animals, especially for those that are exclusively dependent on nectar for their nutrition, such as butterflies [60]. As it was stated in [34], phenylalanine is the most abounded one in nectar (which generally has a strong phagostimulatory effect on honeybees), followed by tyrosine, threonine, histidine, and aspartic acid. Also, it seems that some amino acids, like asparagines, are avoided by all guilds and bee families, while glycine-threonine, H-serine, serine, β-alanine, valine, leucine are bypassed by most bee families. Besides, some level of non-protein amino acids can be detected in nectar. Those compounds can be toxic and found in seeds which serve as deterrents to insect feeding. However, β-alanine, ornithine, homoserine, and γ-

aminobutyric acid (GABA) are also accumulated in nectar but are non-toxic [49]. According to the mineral analysis of nectar ion composition, concentration of K^+ is the highest, following by Na^+. Some levels of Ca^{2+} and Mg^{2+} have been also detected. According to [61], potassium and sodium chloride deter honey bees. Proteins/enzymes, or so called "nectarin" in floral nectar includes invertase, transglucosidase, transfructosidase, phosphatase, tyrosinase, alliinase, nectarin, I-superoxide dismutase, and others, playing important role in hydrolysis of sucrose, polymerization of glucose and fructose molecules, possibly defense and many more. Trace amounts of lipids, organic acids, iridoid glycosides (catalpol), vitamins, alkaloids (anabasine, gelsemine, nicotine, and caffeine), terpenoids (thymol), glucosinolates, and cardenolides can be found in nectar too.

Recently, a gene that encodes an apoplastic invertase of Arabidopsis has been discovered. This gene represents the first gene whose function is required for floral nectar secretion [62].

Chemical screening is usually done by standard chromatographic techniques hyphenated to spectral methods. New technologies and advanced techniques conquer difficulties in analyzing small fluid volumes, enabling more detail identification and quantification of nectar components.

Most of the individual studies on nectaries, nectar, and nectar consumers were included in a comprehensive book review [63]. Cherry nectar properties and chemistry were not examined to a great extent, and just a few papers discussing the composition of sour cherry floral nectar were published so far. As for the sweet cherry, no available data could be found. Therefore, presented results on cherry nectar included in this chapter rely on just a few published papers [46, 64] where nectar sugar profiles of sour cherry cultivars were reported. Most of the data on phenolics were drawn from the study carried out on "Oblačinska" sour cherry clones [64].

4.1 Nectar Carbohydrate Profile

Nectar carbohydrate profile is prevailed by three sugars, the disaccharide sucrose and its monosaccharide units, fructose, and glucose. Nectar components are believed to originate from phloem sap that is enzymatically processed and transformed within nectaries [65]. Since the phloem sap contains mostly sucrose, chemical reactions must occur to produce glucose and fructose in the nectar. The relative amounts of each are determined by hydrolyses of sucrose catalyzed by transglucosidases and transfructosidases localized in the nectaries which occur before or during nectar secretion [66].

The total sugar concentration in floral nectar can range from 5% (w/v) to 80% (w/v) [67] and may differ among individuals, populations, cultivars, or subspecies of the same species [38, 39, 68, 69]. Also, amounts and relative concentrations of the major constituents, glucose, fructose, and sucrose may vary among species from almost all sucrose to all hexose. According to [61], sucrose, maltose, glucose, fructose, trehalose, and melezitose are sweet for bees; while lactose, melibiose, raffinose, xylose,

and arabinose are tasteless; mannose and galactose are toxic to bees; where, gentiobiose and cellobiose and repellent to bees.

The nectar composition can vary greatly depending on plant species and environmental conditions [38], as well as on floral sexual phases [70], and flower position within inflorescences [71]. According to [72], between-plant variability of nectar sugar composition can be due to a casual selection of flowers of different ages, because in some cases, sucrose breakdown in nectar can be related to flower age. But this cannot be applied on the results of the recent investigation reported on sour cherry [64] were the flowers were in the same phenophase code [65], BBCH scale [73]. On the other hand, some authors considered nectar sugar composition as it is conservative taxonomic character [26, 74].

So far, sugar composition of sour cherry nectar was only explored by the two research groups. One research group was investigating sour cherry cultivars in Újfehértó, in the eastern Hungary in the period 1997–2000 [41, 46, 47]. The following sour cherry cultivars were examined: "Újfehértói fürtös," "Pándy 48," "Érdi jubileum," "Meteor USA," "Montmorency," "Debreceni bőtermő," "Nefris," "Sárándi S/Gy," "Korai pipacs," "Mej Djuk," "Kőrösi korai," "Érdi nagygyümölcsű," "Kántorjánosi 3," "Oblacsinszka," "Érdi bőtermő," "Cigány 404." Three sugar components (glucose, fructose, and sucrose) were determined by thin layer chromatography and quantitative evaluation was carried out by densitometry (CAMAG TLC Scanner II). The cultivars "Újfehértói fürtös," "Pándy 48," "Érdi jubileum," and "Érdi bőtermő" yielded nectar with high sucrose content in each season, even under varying climatic conditions, and are valued from an apicultural point of view [46].

Subsequently, in order to determine the floral insect attraction, the floral secretory product of the two cultivars, an autofertile cultivar (":Újfehértói fürtös") and an autosterile cultivar ("Pándy 48") were studied [41]. The nectar of both studied cultivars contained all three major sugar components: sucrose, glucose, and fructose. The nectar sugar composition of "Újfehértói fürtös" varied to a great extent according to the seasons. The phenomenon was explained by the great fluctuation in air temperatures, which influenced the sugar production of the cultivar to a great degree. "Pándy 48" yielded nectar with quite stable concentration and composition in the studied four seasons.

The other research group studied nectar of the most planted sour cherry cultivar in Serbian orchards, "Oblačinska" sour cherry, an autochthonous cultivar [64]. Investigation included 16 nectar samples of "Oblačinska" sour cherry clones. Both the content of sugars and sugar alcohols were studied using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC/PAD). Carbohydrates were separated on a CarboPac® PA10 pellicular anion-exchange column. Total of 14 sugars and 6 sugar alcohols were determined, showing great variability in carbohydrate profiles among studied genotypes. Nectars from several sour cherry clones stood out based on the notably different concentrations of the individual sugars and sugar alcohols. Such an unequaled nectar composition was related to the assumption that Oblačinska sour cherry is not a cultivar but mixture of many different genotypes.

As reported in [41], during 4-year study sucrose level in cultivars „Újfehértói fürtös "and „Pándy 48 "ranged from 18–59 mg/mL and from 27–59 mg/mL,

respectively. As expected, fructose, glucose, and sucrose were found to be the major constituents of all investigated "Oblačinska" sour cherry nectar samples [64]. Based on the total sugar content found in nectar, certain clones have been singled out as the most concentrated (up to 97.6 mg/mL), while the others had dilute nectars (23 mg/mL of sugars). Averagely fructose, glucose, and sucrose amounted 36.8%, 28.9%, and 30.9% of the total content of all carbohydrates, respectively, and this was in line with the other results [47]. The ranking based on fructose content takes into consideration human sensation of taste. Also, fructose in the concentration range from 15 mg/mL to 60 mg/mL in 14 sour cherry cultivars was reported [41], while in "Oblačinska" sour cherry nectars fructose content was up to 34 mg/mL [64].

On the basis of the sucrose/(glucose + fructose) quotient [20], the nectar of "Újfehértói fürtös" belonged to the sucrose-rich group each year, like the majority of sour cherry cultivars, whereas the secretory product of "Pándy 48" could be classified into the sucrose-dominant category in one of the seasons [46]. According to the proposed quotient, one "Oblačinska" sour cherry clone was hexose dominant [$S/(G + F) < 0.1$], four clones were hexose rich [$S/(G + F) = 0.1–0.49$], while other 11 were sucrose rich [$S/(G + F) = 0.5–0.99$] [64]. These results are in accordance with some previous results [46]. Proportions of sucrose over fructose and glucose have been linked with different classes of pollinators and found to be important in plant-mutualism interactions [75].

The sucrose-dominant nectar composition of 45 species belonging to tribe Sinningieae (*Gesneriaceae*) was also documented [76]. Several authors have suggested that sucrose-rich nectar is mostly found in flowers pollinated by insects with long mouth parts, whereas hexose-rich nectar has been found in flowers pollinated by short-tonged insects [20, 32, 77–79]. On contrary, an analysis in *Antirrhinum* and *Lycium* has revealed constant sugar composition despite a large variety of pollinators [78, 80].

According to [64], other carbohydrates, including the monosaccharides (arabinose and rhamnose), the disaccharides (maltose, isomaltose, trehalose, gentiobiose, turanose), the trisaccharides (panose, melezitose, maltotriose, isomaltotriose), as well as the sugar alcohols (glycerol, erythritol, arabitol, galactitol, and mannitol) were present as minor constituents in sour cherry nectars. The presence of mannitol, melezitose, panose, and maltotriose was not confirmed in some of the studied nectars. Minor sugars identified in nectars of some flowers, such as arabinose, galactose, mannose, gentiobiose, lactose, maltose, melibiose, trehalose, melezitose, raffinose, and stachyose can be toxic to potential pollinators [68, 81–84].

Among minor constituents, isomaltose, maltose, and sorbitol were the dominant in comparison to other components. For the sorbitol (a polyol with low molecular weight, highly soluble, and non-reducing compounds), results were fully expected, because this sugar alcohol is the main photosynthetic product and the primary translocated carbohydrate in *Rosaceae* [85]. Although has no influence to insects' preference, sorbitol is a frequent constituent of Mediterranean nectars [22]. Also, if it can be found in the fruit, it improves the sweet taste and texture of the mesocarp [86]. But also, its accumulation is considered as an adaptive response of plants to drought, salinity, or chilling stress [87].

Maltose is pretty rare or absent in nectars, and although it tastes sweet to honeybees, it is usually less attractive for them than sucrose [49, 88]. Earlier it was believed that maltose is synthesized in nectar itself [89], but its presence in nectar is due to the fact that maltose is a degradation product of starch (obtained from chloroplasts during starch degradation in night), while nectar secretion in flower starts with starch degradation [90]. Also, maltose is a major product of catabolism of starch in guard cells which can be found within the flower [91]. In regard to disaccharide isomaltose, it is more related to honeydew then to nectar, so that is the reason why honeydew honeys had significantly higher mean values of this sugar than the blossom honeys. In unifloral cherry honey, the concentration of the isomaltose was around 0.80% [92].

4.2 Phenolic Compounds in Cherry Nectars

Phenolic compounds are widespread natural constituents and their main function is to protect plants against various biotic and abiotic stresses. Their multiple roles in floral nectars and the relationship with pollinators were outlined in the literature [28]. Mainly, phenolics are associated with functions such as attracting pollinators or repelling nectar thieves, maintaining nectar in a microbe-free state, being important components of floral scents. Their role in cherry pollination could be the same, although yet not proved. Some phenolic compounds together with some other constituents may accumulate in floral nectar due to passive absorption by the nectar [30].

Although numerous flavonoids have been described in literature, their presence in floral nectar was not studied extensively. The same applies for the composition of cherry nectar topic. Often phenolic composition was reported only qualitatively [93], where floral nectar chemical compositions of 29 species native to Argentinian Patagonia and phenolic composition measured on qualitative scale were reported. A scarce number of papers show that nectar phenolic profile is characterized both by various phenolic aglycones and their derivatives. In rosemary nectar, kaemferol-3-sophoroside and quercetin-3-sophoroside were identified as the most abundant among 15 different flavonoids [94], while in Portuguese heather nectar (*Erica* sp.) flavonol aglycones quercetin, kaempferol, myricetin, and isorhamnetin were identified [95]. The occurrence of flavonols in higher plants was associated with lignification in cell walls and with UV absorption of flowers, as nectar guide [96]. Also, functional roles of flavonols as developmental regulators and/or signaling molecules in plants were discussed [97].

Although nectar composition of various sour cherry cultivars was examined [46], studies on floral nectar in terms of detailed phenolic characterization were not performed until the investigation of phenolic diversity in floral nectar of different "Oblačinska" sour cherry clones [64]. The phenolic complexity of sour cherry nectar was apparent and the qualitative phenolic profile was shown to be characterized mostly with flavonol glycosides. All identified glycosides were derivatives of kaempferol, quercetin, and isorhamnetin [64] (Table 1).

Table 1 Quantification of flavonol glycosides identified in floral nectars of "Oblačinska" sour cherry clones. The relative content of flavonol glycosides (in this table) was expressed as rutin equivalents (RE) per mL of nectar (μg RE/mL)

Name of identified compound	Relative content (μg RE/mL)
Kaempferol 3-*O*-(2″-*O*-hexosyl)hexoside-7-*O*-rhamnoside 1	0.002–0.197
Quercetin 3,7-di-*O*-hexoside	0.002–0.029
Quercetin 3-*O*-(2″-*O*-hexosyl)hexoside-7-*O*-rhamnoside 1	0.022–1.465
Isorhamnetin 3-*O*-(2″-*O*-hexosyl)hexoside 1	3.285–4.066
Quercetin 3-*O*-(2″-*O*-hexosyl)hexoside	0.001–0.245
Kaempferol 3-*O*-(2″-*O*-hexosyl)hexoside-7-*O*-rhamnoside 2	0.002–0.170
Isorhamnetin 3-*O*-(2″-*O*-hexosyl)hexoside 2	0.089–6.247
Quercetin 3-*O*-(2″-*O*-hexosyl)hexoside-7-*O*-rhamnoside 2	0.001–0.038
Quercetin 3-*O*-hexoside	0.002–0.225
Kaempferol 3-*O*-(6″-*O*-rhamnosyl)hexoside	0.024–6.084
Isorhamnetin 3-*O*-(6″-*O*-rhamnosyl)hexoside	0.067–6.104
Quercetin 3-*O*-pentoside 1	0.001–0.022
Kaempferol 3-*O*-hexoside	0.001–1.382
Isorhamnetin 3-*O*-hexoside	0.001–1.639
Kaempferol 3-*O*-(6″-*O*-acetyl)hexoside	0.001–0.177
Isorhamnetin 3-*O*-(6″-*O*-acetyl)hexoside	0.004–2.331
Quercetin 3-*O*-pentoside 2	0.001–0.503

Further, sour cherry nectar phenolic profile was characterized with the presence of rutin, pinocembrin, and galangin, detected in all nectar samples, while gallic acid, hesperetin, and naringin were found in some samples. In earlier work, rutin was shown to act as a feeding stimulant for some insects [98]. Also, recently was proved that rutin has the highest antimicrobial (especially antibacterial) activity in honey [99] so there is a possibility that its function in nectar is the same. Pinobanksin, naringenin, and chrysin were detected in variable amounts in sour cherry nectar. Table 2 shows the content of phenolic compounds (average values). Naringenin plays an important role in plant development and it was reported to show bactericidal and/or bacteriostatic activity [100], and antimicrobial effects against yeasts [101], but shows low activity as feeding stimulant in insect-plant interaction [102]. Moreover, naringenin influenced bee foraging behavior as deterrent [103], but no relationship could be underlined between its level and yield efficiency (yield per trunk cross sectional area), of "Oblačisnka sour cherry" clones that were studied by. As a matter of fact, group of clones that stored high content of nagingenin is showing all kind of yield effectiveness, which stands the same for the group of clones with very low level of this flavanone [64, 104].

The positive influence of abscisic acid in nectar to the immune response of worker honeybees and larvae after being parasitized with *Varroa destructor* was described previously [105]. In plants abscisic acid regulates fundamental physiological functions and accumulates in response to different environmental stresses [106, 107] and can be found in phloem and xylem sap and in nectar [95, 108]. In honey, this

Table 2 Quantification of phenolic acids and flavonoids in nectars of "Oblačinska" sour cherry clones (μg/mL)

Compound name	Content (μg/mL)
Gallic acid	0.005–0.010
Caffeic acid	0.003–0.015
Rutin	0.096–6.472
Naringin	0.026–0.092
(−)-*cis*,*trans*-abscisic acid	0.005–0.331
Naringenin	0.009–4.076
Pinobanksin	0.005–0.128
Hesperetin	0.002–0.006
Chrysin	0.030–1.597
Pinocembrin	0.010–0.764
Galangin	0.014–0.719

phytohormone comes mainly from nectar [109]. The content of this phytohormone in "Oblačinska" sour cherry clones varied from 0.005 to 0.331 μg/mL [64].

Regardless of the similar chemical structure, only certain flavonoids are capable to absorb light in the visible region of spectra, thus rendering color. Flavone glycosides and flavonol glycosides absorb near 350 nm, but their role in the floral pigmentation is not predominant, as they are weakly colored. Usually flavonoids accompany carotenoids which are dominant in yellow pigmentation. The early work on the flower *Rudbeckia hirta* indicated flavonols as pigments responsible for ultraviolet absorption in nectar guides for bees and other insects, and it was the first interpretation of ultraviolet absorption in a nectar guide in chemical terms [96]. Due to chemical modifications at the C-8 and C-6 position on A-ring, flavonols become yellow hydroxyflavonols [110]. Also, *O*-glycosylation at the 7,4′-positions or *O*-methylation at the 3′- or 3′,5′-positions may contribute to the yellow color [110]. Also, other authors reported flavonols importance for nectar guides, such in [111] who isolated the pigment from the petals of *Brassica rapa* and identified it on the basis of MS and NMR spectroscopic data as isorhamnetin 3,7-*O*-di-beta-D-glucopyranoside.

Although some species use colored nectar as a signal for pollinators [112, 113], we assume that this could not be the case with the nectar of the "Oblačinska" sour cherry. However, based on identified phenolic compounds, certain conclusions can be made. Namely, the presence of various flavonols in nectar of "Oblačinska" sour cherry could be the reason for its pale yellow color. Several derivatives with *O*-glycosylation at 7-position were identified (Table 1). Also, isorhamnetin which is 3′-methoxylated derivative of quercetin was typical flavonol in all nectars. Of all the quantified flavonols, the largest amount of izorhamnetin 3-*O*-(2″-*O*-hexosyl) hexoside 2 was found in nectars along with rutin and therefore this specific compound could be the one that contributes to the nectar color the most.

Finally, comparison of polyphenolic profiles of "Oblačinska" sour cherry fruits [114] and nectar of the same sour cherry clones revealed some disagreements. The fruit clones stored some of the phenolics not found in the corresponding nectar, such as gallic acid, naringin, and naringenin. The opposite was found for hesperetin,

where some quantity of this flavanone was found in nectar clones but not in the fruits. Finally, rutin was one of the most abundant compounds determined in the fruits of the same "Oblačinska" sour cherry clones and its content was highest in clone II/2, in both nectar and fruit [64, 114]. As it was stressed out, no matter that floral nectar is secreted through intrafloral nectaries as a phloem solution [115] and cherry fruit is formed form ovary within the flower, it seems that those two processes are quite different and fully independent. In fact, this result is expected, because deciduous fruit trees, to which sour cherry belongs, have accumulated necessary minerals and organic compounds by the end of the previous growing season and use these reserve nutrients to support initial growth and development in the following spring. Thus, during flowering time (when leaves are just started to expand and are without photosynthetic competence), reproductive development is under total reliance on reserves stored within the tree [116]. On the contrary, during sour cherry fruit development (which occurs ≈55 days from pollination to fully ripe fruit), leaves are fully developed and are having the main role as the main source of photo assimilates [117].

5 Conclusion

Except few studies, not much was done in the analysis of cherry nectar. Sweet cherry was not an object of any study so far, so the results of this chapter are based on sour cherry nectar. According to the chemical analysis of our model plant's ("Oblačinska sour cherry") floral nectar, it can be concluded that selected clones of this cultivar showed different sugar and polyphenolic profile, where constituents showed big variation. In sugars, fructose, glucose, and sucrose were the most abounded, while arabitol, rhamnose, arabinose, turanose, gentiobiose, panose, melezitose, and matotriose, together with galactitol and mannitol, were in minor quantities. Regarding polyphenols rutin, naringenin and chrysin were found in the highest levels. Only rutin, pinocembrin, and galangin, together with ( )-cis, trans-ABA were detected in all nectar samples. Probably the cause of unequaled nectar composition (both for polyphenolics and sugars) in sour cherry is its hybrid origin (segmental allotetraploid between *Prunus cerasus* and *Prunus fruticosa*) and unstable inheritance.

In the future, nectar chemical composition, could be a breeding aim for creating a cultivar that will attract pollinators the most, and thus ensuring high yields, or have components that can protect plant from economically important bacteria/viruses/fungi. Besides, this chapter would like to support and encourage scientists to analyze nectar for all other components and connect it with the pollenizer preference.

Also in the following years, nectar of sweet cherry, and other minor cherry species like European dwarf cherry (*Prunus fruticosa* Pall.), mahaleb cherry (*Prunus mahaleb* L.), and duke cherry (*Prunus* × *gondouinii* Rehd.), and/or other agricultural plants, should be analyzed in details and connect it with honey quality.

References

1. Webster AD (1996) The taxonomic classification of sweet and sour cherries and a brief history of their cultivation. In: Webster AD, Looney NE (eds) Cherries: crop physiology, production and uses, 1st edn. CAB International, Wallingford
2. Faust N, Surányi D (2010) Origin and dissemination of cherry. In: Janick J (ed) Horticultural reviews, vol 19. Wiley, New York
3. Rehder A (1974) Manual of cultivated trees and shrubs. Hardy in North America. Macmillan, New York
4. Hedrick UP (1915) The history of cultivated cherries. In: Hedrick UP, Howe GH, Taylor OM, Tubergen CB, Wellington R (eds) The cherries of New York, 1st edn. JB Lyon Company, New York
5. De Candolle A (1886) Origin of cultivated plants. Hafner, New York
6. FAOSTAT (2018) FAO Statistical data base. http://www.fao.org/faostat/en/#data/QC. Accessed 1 Sept 2018
7. McCune LM, Kubota C, Stendell-Hollis NR, Thomson CA (2011) Cherries and health: a review. Crit Rev Food Sci Nutr 51:1–12. https://doi.org/10.1080/10408390903001719
8. Bujdosó G, Hrotkó K (2017) Cherry production. In: Quero-Garcia J, Iezzoni A, Pulawska J, Lang G (eds) Cherries: botany, production and uses, 1st edn. CAB International, Wallingford
9. Mahmoodi M, Arzani K, Bouzari N (2008) Pollination, pollen tube growth and determination of the best pollinizer for sweet cherry (*Prunus avium* L.) cv. Red rezaeieh. Acta Hortic 769:207–210. https://doi.org/10.17660/ActaHortic.2008.769.28
10. Raj H, Mattu VK (2014) Diversity and distribution of insect pollinators on various temperate fruit crops in Himachal Himalaya, India. Int J Sci Nat 5:626–631. http://scienceandnature.org/IJSN_Vol5(4)D2014/IJSN-VOL5(3)14-7.pdf. Accessed 3 Sept 2018
11. Free JB (1993) Insect pollination of crops, 2nd edn. Academic, London
12. Benedek P (1996) Insect pollination of fruit crops. In: Nyeki J, Soltesz M (eds) Floral biology of temperate zone fruit trees and small fruits, 1st edn. Akademiai Kiado, Budapest
13. Soltesz M (1996) Requirements for successful fruit set in orchards. In: Nyeki J, Soltesz M (eds) Floral biology of temperate zone fruit trees and small fruits, 1st edn. Akademiai Kiado, Budapest
14. Delaplane KS, Mayer DR (2000) Crop pollination by bees. CABI Publishing, Wallingford
15. Stern RA, Eisikowitch D, Dag A (2001) Sequential introduction of honeybee colonies and doubling their density increase cross-pollination, fruit set and yield in 'Red Delicious' apple. J Hortic Sci Biotechnol 76:17–23. https://doi.org/10.1080/14620316.2001.11511320
16. Stern RA, Goldway M, Zisovich AH, Shafir S, Dag A (2004) Sequential introduction of honeybee colonies increases cross-pollination, fruit set and yield of Spadona pear (*Pyrus communis*). J Hortic Sci Biotechnol 79:652–658. https://doi.org/10.1080/14620316.2004.11511821
17. Güler Y, Dikmen F (2013) Potential bee pollinators of sweet cherry in inclement weather conditions. J Entomol Res Soc 15:9–19. http://www.entomol.org/journal/index.php?journal=JERS&page=article&op=view&path%5B%5D=519&path%5B%5D=290. Accessed 1 Sept 2018
18. Holzschuh A, Dudenhoffer JH, Tscharntke T (2012) Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. Biol Conserv 15:101–107. https://doi.org/10.1016/j.biocon.2012.04.032
19. Nores MJ, López HA, Rudall PJ, Anton AM, Galetto L (2013) Four o'clock pollination biology: nectaries, nectar and flower visitors in *Nyctaginaceae* from southern South America. Bot J Linn Soc 171:551–567. https://doi.org/10.1111/boj.12009
20. Baker HG, Baker I (1983) Floral nectar sugar constituents in relation to pollinator type. In: Jones CE, Little RJ (eds) Handbook of experimental pollination biology, 1st edn. Van Nostrand Reinhold, New York
21. Vissche PK, Seeley TD (1982) Foraging strategy of honeybee colonies in a temperate deciduous forest. Ecology 63:1790–1801. https://doi.org/10.2307/1940121

22. Petanidou T (2005) Sugars in Mediterranean floral nectars: an ecological and evolutionary approach. J Chem Ecol 31:1065–1088. https://doi.org/10.1007/s10886-005-4248-y
23. Pacini E, Nepi M (2007) Nectar production and presentation. In: Nicolson S, Nepi M, Pacini E (eds) Nectaries and nectar, 1st edn. Springer, Dordrecht
24. Mačukanović M, Duletić S, Jocić G (2004) Nectar production in three melliferous species of *Lamiaceae* in natural and experimental conditions. Acta Vet Brno 54:475–487. https://scindeks-clanci.ceon.rs/data/pdf/0567-8315/2004/0567-83150406475M.pdf. Accessed 10 Sept 2018
25. Kaczorowski RL, Gardener MC, Holtsford TP (2005) Nectar traits in Nicotiana section *Alatae* (*Solanaceae*) in relation to floral traits, pollinators, and mating system. Am J Bot 92: 1270–1283. https://doi.org/10.3732/ajb.92.8.1270
26. Rodríguez-Riaño T, Ortega-Olivencia A, López J, Pérez-Bote JL, Navarro-Pérez ML (2014) Main sugar composition of floral nectar in three species groups of *Scrophularia* (*Scrophulariaceae*) with different principal pollinators. Plant Biol 16:1075–1086. https://doi.org/10.1111/plb.12159
27. González A, Rowe CL, Weeks PJ, Whittle D, Gilbert FS, Barnard CJ (1995) Flower choice by honey bees (*Apis mellifera* L.): sex-phase of flowers and preferences among nectar and pollen foragers. Oecologia 101:258–264. https://doi.org/10.1007/BF00317292
28. Nicolson SW (2007) Nectar consumers. In: Nicolson SW, Nepi M, Pacini E (eds) Nectaries and nectar, 1st edn. Springer, Dordrecht
29. Adler LS (2000) The ecological significance of toxic nectar. Oikos 91:409–420. https://doi.org/10.1034/j.1600-0706.2000.910301.x
30. Raguso RA (2004) Why are some floral nectars scented. Ecology 85:1486–1494. https://doi.org/10.1890/03-0410
31. Thorp RW, Briggs DL, Esters JR, Erickson EH (1975) Nectar fluorescence under ultraviolet irradiation. Science 189:476–478. https://doi.org/10.1126/science.189.4201.476
32. Baker HG, Baker I, Hodges SA (1998) Sugar composition of nectar and fruits consumed by birds and bats in the tropics and subtropics. Biotropica 30:559–586. https://doi.org/10.1111/j.1744-7429.1998.tb00097.x
33. Nicolson SW, Fleming PA (2003) Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. Plant Syst Evol 238:139–153. https://doi.org/10.1007/s00606-003-0276-7
34. Petanidou T, van Laere A, Ellis WN, Smets E (2006) What shapes amino acid and sugar composition in Mediterranean floral nectars. Oikos 115:155–169. https://doi.org/10.1111/j.2006.0030-1299.14487.x
35. Reinhard J, Srinivasan MV, Guez D, Zhang WS (2004) Floral scents induce recall of navigational and visual memories in honey bees. J Exp Biol 207:4371–4381. https://doi.org/10.1242/jeb.01306
36. Rathcke BJ (1992) Nectar distributions, pollinator behavior, and plant reproductive success. In: Hunter MD, Ohgushi T, Price PW (eds) Effects of resource distribution on animal–plant interactions, 1st edn. Academic, New York
37. Cushnie Canto A, Herrera CM, García IM, Pérez R, Vaz M (2011) Intraplant variation in nectar traits in *Helleborus foetidus* (*Ranunculaceae*) as related to floral phase, environmental conditions and pollinator exposure. Flora 206:668–675. https://doi.org/10.1016/j.flora.2011.02.003
38. Herrera CM, Pérez R, Alonso C (2006) Extreme intra-plant variation in nectar sugar composition in an insect-pollinated perennial herb. Am J Bot 93:575–581. https://doi.org/10.3732/ajb.93.4.575
39. Canto A, Pérez R, Medrano M, Castellanos MC, Herrera CM (2007) Intra-plant variation in nectar sugar composition in two *Aquilegia* species (Ranunculaceae): contrasting patterns under field and glasshouse conditions. Ann Bot 99:653–660. https://doi.org/10.1093/aob/mcl291
40. Rodrigues LC, Morales MR, Fernandes AJB, Ortiz JM (2008) Morphological characterization of sweet and sour cherry cultivars in a germplasm bank at Portugal. Genet Resour Crop Evol 55:593–601. https://doi.org/10.1007/s10722-007-9263-0
41. Bukovics P, Szabó LG, Orosz-Kovács Z, Farkas Á (2006) Nectar composition in 'Újfehértói Fürtös'and 'Pándy 48' sour cherry cultivars. Acta Bot Hung 48:271–277. https://doi.org/10.1556/ABot.48.2006.3-4.3

42. Orosz-Kovács Zs (1991) A cseresznye és a meggy nektáriumstruktúrája és nektárprodukciója. Dissertation, University of Pécs
43. Orosz-Kovács Z, Gulyás S, Halászi Z (1989) Periodicity of nectar production of sour cherry cv. Pándy Acta Bot Hungar 35:237–244. https://www.researchgate.net/profile/Agnes_Farkas/publication/228856362_Nectar_production_for_the_Hungarian_honey_industry/links/0912f50ebe8aa6ab10000000/Nectar-production-for-the-Hungarian-honey-industry.pdf. Accessed 4 Sept 2018
44. Orosz Kovács Z, Gulyás S, Kaposvári F (1992) Pollination biology of sour cherry varieties of protogyn blossoming. Acta Biol Szeged 38:47–55. http://acta.bibl.u-szeged.hu/22180/1/biologica_038_047-055.pdf. Accessed 4 Sept 2018
45. Farkas A, Zajacz E (2007) Nectar production for the Hungarian honey industry. Eur J Plant Sci Biotechnol 1:125–151. http://www.globalsciencebooks.info/Online/GSBOnline/images/0712/EJPSB_1(2)/EJPSB_1(2)125-151o.pdf. Accessed 3 Sept 2018
46. Bukovics P, Orosz-Kovács Z, Szabó LG, Farkas Á, Bubán T (2003) Composition of floral nectar and its seasonal variability in sour cherry cultivars. Acta Bot Hungar 45:259–271. https://doi.org/10.1556/ABot.45.2003.3-4.2
47. Orosz-Kovács Z, Szabó LG, Bubán T, Farkas Á, Bukovics P (2000) Sugar composition of floral nectar in sour cherry cultivars. Int J Hortic Sci 6:109–114. https://www.cabdirect.org/cabdirect/abstract/20000314329
48. Zhang FP, Yang QY, Zhang SB (2016) Dual effect of phenolic nectar on three floral visitors of *Elsholtzia rugulosa* (Lamiaceae) in SW China. PLoS One 11:e0154381. https://doi.org/10.1371/journal.pone.0154381
49. Nicolson SW, Thornburg RW (2007) Nectar chemistry. In: Nicolson SW, Nepi M, Pacini E (eds) Nectaries and nectar, 1st edn. Springer, Dordrecht
50. Nepi M, Selvi F, Pacini E (2010) Variation in nectar-sugar profile of Anchusa and allied genera (*Boraginaceae*). Bot J Linn Soc 162:616–627. https://doi.org/10.1111/j.1095-8339.2010.01036.x
51. Temeles EJ, Kress WJ (2003) Adaptation in a plant – hummingbird association. Science 300:630–633. https://doi.org/10.1126/science.1080003
52. Gonzalez-Teuber M, Heil M (2009) Nectar chemistry is tailored for both attraction of mutualists and protection from exploiters. Plant Signal Behav 4:809–813. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2802787/pdf/psb0409_0809.pdf. Accessed 20 Aug 2018
53. Liu F, Chen J, Chai J, Zhang X, Bai X, He D, Roubik DW (2007) Adaptive functions of defensive plant phenolics and a non-linear bee response to nectar components. Funct Ecol 21:96–100. https://doi.org/10.1111/j.1365-2435.2006.01200.x
54. Manson JS, Otterstatter MC, Thomson JD (2010) Consumption of a nectar alkaloid reduces pathogen load in bumble bees. Oecologia 162:81–89. https://doi.org/10.1007/s00442-009-1431-9
55. Cushnie TP, Lamb AJ (2005) Antimicrobial activity of flavonoids. Int J Antimicrob Agents 26:343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002
56. Taiz L, Zeiger E (1991) Plant physiology. The Bejamin/Cummings Publishing, Redwood City
57. Radhika V, Kost C, Boland W, Heil M (2010) The role of jasmonate signalling in floral nectar secretion. PLoS One 5:e9265. https://doi.org/10.1371/journal.pone.0009265
58. Wasternack C (2007) Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot 100:681–697. https://doi.org/10.1093/aob/mcm079
59. Knudsen JT, Tollsten L, Bergström G (1993) Floral scents, a checklist of volatile compounds isolated by headspace techniques. Phytochemistry 33:253–280. https://doi.org/10.1016/0031-9422(93)85502-I
60. Nepi M, von Aderkas P, Wagner R, Mugnaini S, Coulter A, Ettore P (2009) Nectar and pollination drops: how different are they? Ann Bot 104:205–219. https://doi.org/10.1093/aob/mcp124
61. von Frisch K (1950) Bees. Cornell University Press, Ithaca
62. Ruhlmann JM, Kram BW, Carter CJ (2010) Cell wall invertase 4 is required for nectar production in Arabidopsis. J Exp Bot 61:395–404. https://doi.org/10.1093/jxb/erp309

63. Nicolson SW, Nepi M, Pacini E (2007) Nectaries and nectar. Springer, New York
64. Guffa B, Nedić N, Dabić Zagorac DČ, Tosti TB, Gašić UM, Natić MM, Fotirić Akšić MM (2017) Characterization of sugar and polyphenolic diversity in floral nectar of different 'Oblačinska' sour cherry clones. Chem Biodivers 14:e1700061. https://doi.org/10.1002/cbdv.201700061
65. De La Barrera E, Nobel PS (2004) Nectar: properties, floral aspects, and speculations on origin. Trends Plant Sci 9:65–69. https://doi.org/10.1016/j.tplants.2003.12.003
66. Heil M (2011) Nectar: generation, regulation and ecological functions. Trends Plant Sci 16:191–200. https://doi.org/10.1016/j.tplants.2011.01.003
67. Bertazzini M, Forlani G (2016) Intraspecific variability of floral nectar volume and composition in rapeseed (*Brassica napus* L. var. *oleifera*). Front Plant Sci 7:art. 288. https://doi.org/10.3389/fpls.2016.00288
68. Frey-Wyssling A (1955) The phloem supply to the nectaries. Acta Bot Neerl 4:358–369. https://doi.org/10.1111/j.1438-8677.1955.tb00337.x
69. Galetto L, Bernardello G (2005) Nectar. In: Dafni A, Kevan P, Husband BC (eds) Practical pollination biology, 1st edn. Enviroquest, Cambridge
70. Antoñ S, Denisow B (2014) Nectar production and carbohydrate composition across floral sexual phases: contrasting patterns in two protandrous *Aconitum* species (Delphinieae, Ranunculaceae). Flora 209:464–470. https://doi.org/10.1016/j.flora.2014.07.001
71. Lu NN, Li XH, Li L, Zhao ZG (2015) Variation of nectar production in relation to plant characteristics in protandrous *Aconitum gymnandrum*. J Plant Ecol 8:122–129. https://doi.org/10.1093/jpe/rtv020
72. Petanidou T, van Laere AJ, Smets E (1996) Change in floral nectar components from fresh to senescent flowers of *Capparis spinosa* (Capparidaceae), a nocturnally flowering Mediterranean shrub. Plant Syst Evol 199:79–92. https://doi.org/10.1007/BF00985919
73. Meier U, Graf H, Hack H, Heß M, Kennel W, Klose R, Mappes D, Seipp D, Stauß R, Streif J, van den Boom T (1994) Phänologische Entwick-lungsstadien des Kernobstes (Malus domestica Borkh. und Pyrus communis L.), des Steinobstes (Prunus-Arten), der Johannisbeere (Ribes-Arten) und der Erdbeere (*Fragaria x ananassa* Duch.). Nachrichtenbl Dtsch Pflanzenschutzdienstes (Braunschweig, Ger.) 46:141–153. https://ojs.openagrar.de/index.php/NachrichtenblattDPD/issue/view/1354. Accessed 25 Aug 2018
74. Chalcoff VR, Aizen MA, Galetto L (2006) Nectar concentration and composition of 26 species from the temperate forest of South America. Ann Bot 97:413–421. https://doi.org/10.1093/aob/mcj043
75. Heil M, Rattke J, Boland W (2005) Postsecretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. Science 308:560–563. https://doi.org/10.1126/science.1107536
76. Perret M, Chautems A, Spichiger R, Peixoto M, Savolainen V (2001) Nectar sugar composition in relation to pollination syndromes in Sinningieae (Gesneriaceae). Ann Bot 87:267–273. https://doi.org/10.1006/anbo.2000.1331
77. Lammers TG, Freeman CE (1986) Ornithophily among the Hawaiian Lobelioideae (Campanulaceae): evidence from floral nectar sugar composition. Am J Bot 73:1613–1619. https://doi.org/10.2307/2443929
78. Elisens WJ, Freeman CE (1988) Floral nectar sugar composition and pollinator type among New World genera in tribe Antirrhineae (Scrophulariaceae). Am J Bot 75:971–978. https://doi.org/10.2307/2443763
79. Stiles FG, Freeman CE (1993) Patterns in floral nectar characteristics of some bird-visited plant species from Costa Rica. Biotropica 25:191–205. https://doi.org/10.2307/2389183
80. Galetto L, Bernardello G, Sosa CA (1998) The relationship between floral nectar composition and visitors in Lycium (Solanaceae) from Argentina and Chile: what does it reflect. Flora 193:303–314. https://doi.org/10.1016/S0367-2530(17)30851-4
81. Roy R, Schmitt AJ, Thomas JB, Carter CJ (2017) Review: nectar biology: from molecules to ecosystems. Plant Sci 262:148–164. https://doi.org/10.1016/j.plantsci.2017.04.012
82. Baker H, Baker I (1983) A brief historical review of chemistry of floral nectar. In: Bentley BL (ed) The biology of nectaries, 1st edn. Columbia University Press, New York

83. Barker RJ (1977) Some carbohydrates found in pollen and pollen substitutes are toxic to honey bees. J Nutr 107:1859–1862. https://doi.org/10.1093/jn/107.10.1859
84. Sols A, Cadenas E, Alvarado F (1960) Enzymatic basis of mannose toxicity in honey bees. Science 131:297–298. https://doi.org/10.1126/science.131.3396.297
85. Liu D, Ni J, Wu R, Teng Y (2013) High temperature alters sorbitol metabolism in *Pyrus pyrifolia* leaves and fruit flesh during late stages of fruit enlargement. J Am Soc Hortic Sci 138:443–451. http://journal.ashspublications.org/content/138/6/443.full.pdf+html. Accessed 27 Aug 2018
86. Skrzyński J, Leja M, Gonkiewicz A, Banach P (2016) Cultivar effect on the sweet cherry antioxidant and some chemical attributes. Folia Hortic 28:95–102. https://doi.org/10.1515/fhort-2016-0011
87. Escobar Gutierrez AJ, Gaudillere JP (1996) Distribution, metabolisme et roˆle du sorbitol chez les plantes superieures. Synth Agronomie 16:281–298. https://doi.org/10.1051/agro:19960502
88. Tinti JM, Nofre C (2001) Responses of the ant *Lasiusniger* to various compounds perceived as sweet in humans: a structure-activity relationship study. Chem Senses 26:231–237. https://doi.org/10.1093/chemse/26.3.231
89. Rowley FA (1976) The sugars of some common Philippine nectars. J Apic Res 15:19–22. https://doi.org/10.1080/00218839.1976.11099828
90. Lu Y, Sharkey TD (2006) The importance of maltose in transitory starch breakdown. Plant Cell Environ 29:353–366. https://doi.org/10.1111/j.1365-3040.2005.01480.x
91. Ritte G, Raschke K (2003) Metabolite export of isolated guard cell chloroplasts of *Vicia faba*. New Phytol 159:195–202. https://doi.org/10.1046/j.1469-8137.2003.00789.x
92. Nayik GA, Dar BN, Nanda V (2016) Physico-chemical, rheological and sugar profile of different unifloral honeys from Kashmir valley of India. Arab J Chem (in press). https://doi.org/10.1016/j.arabjc.2015.08.017. https://ac.els-cdn.com/S1878535215002579/1-s2.0-S1878$32#535215002579-main.pdf?
93. Forcone A, Galetto L, Bernardello L (1997) Floral nectar chemical composition of some species from Patagonia. Biochem Syst Ecol 25(5):95–402. https://doi.org/10.1016/S0305-1978(97)00030-6
94. Gil MI, Ferreres F, Ortiz A, Subra E, Tomás-Barberán FA (1995) Plant phenolic metabolites and floral origin of rosemary honey. J Agric Food Chem 43:2833–2838. https://doi.org/10.1021/jf00059a012
95. Ferreres F, Andrade P, Gil MI, Tomás-Barberán FA (1996) Floral nectar phenolics as biochemical markers for the botanical origin of heather honey. Z Lebensmittelunters Forsch 202:40–44. https://doi.org/10.1007/BF01229682
96. Thompson WR, Meinwald J, Aneshansley D, Eisner T (1972) Flavonols: pigments responsible for ultraviolet absorption in nectar guide of flower. Science 177:528–530. https://doi.org/10.1126/science.177.4048.528
97. Pollastri S, Tattini M (2011) Flavonols: old compounds for old roles. Ann Bot 108:1225–1233. https://doi.org/10.1093/aob/mcr234
98. De Boer G, Hanson FE (1987) Feeding responses to solanaceous allelochemicals by larvae of the tobacco hornworm *Manduca sexta*. Entomol Exp Appl 45:123–131. https://doi.org/10.1111/j.1570-7458.1987.tb01071.x
99. Pimentel RB, da Costa CA, Alburquerque PM, Junior SD (2013) Antimicrobial activity and rutin identification of honey produced by the stingless bee *Melipona compressipes manaosensis* and commercial honey. BMC Complement Altern Med 13:151. https://doi.org/10.1186/1472-6882-13-151
100. Vandeputte OM, Kiendrebeogo M, Rasamiravaka T, Stévigny C, Duez P, Rajaonson S, Diallo B, Mol A, Baucher M, El Jazir M (2011) The flavanone naringenin reduces the production of quorum sensing-controlled virulence factors in *Pseudomonas aeruginosa* PAO1. Microbiology 157:2120–2132. https://doi.org/10.1099/mic.0.049338-0
101. Ataç U, Kadriye S, Özant Ö, Dilşah Ç, Ömür G, Bekir S (2005) Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. Microbiol Res 160:189–195. https://doi.org/10.1016/j.micres.2005.01.002

102. Levy EC, Ishaaya I, Gurevitz E, Cooper R, Lavie D (1974) Isolation and identification of host compounds eliciting attraction and bite stimuli in the fruit tree bark beetle, *Scolytus mediterraneus*. J Agric Food Chem 22:376–379. https://doi.org/10.1021/jf60193a042
103. Verónica CS, de los Ángeles FM, Claudio RG, Fernanda SM (2014) Analysis of phenolic compounds in onion nectar by miniaturized off-line solid phase extraction-capillary zone electrophoresis. Anal Methods 6:4878–4884. https://doi.org/10.1039/c4ay00240g
104. Rakonjac V, Fotirić Akšić M, Nikolić D, Milatović D, Čolić S (2010) Morphological characterization of 'Oblačinska' sour cherry by multivariate analysis. Sci Hortic 125:679–684. https://doi.org/10.1016/j.scienta.2010.05.029
105. Negri P, Maggi MD, Ramirez L, De Feudis L, Szwarski N, Quintana S, Eguaras MJ, Lamattina L (2015) Abscisic acid enhances the immune response in *Apis mellifera* and contributes to the colony fitness. Apidologie 46:542–557. https://doi.org/10.1007/s13592-014-0345-7
106. Ruggiero B, Koiwa H, Manabe Y, Quist TM, Inan G, Saccardo F, Joly RJ, Hasegawa PM, Bressan RA, Maggio A (2004) Uncoupling the effects of abscisic acid on plant growth and water relations. Analysis of sto1/nced3, an abscisic acid-deficient but salt stress-tolerant mutant in Arabidopsis. Plant Physiol 136:3134–3147. https://doi.org/10.1104/pp.104.046169
107. Ramirez L, Negri P, Sturla L, Guida L, Vigliarolo T, Maggi M, Eguaras M, Zocchi E, Lamattina L (2017) Abscisic acid enhances cold tolerance in honeybee larvae. Proc Biol Sci 284:pii: 20162140. https://doi.org/10.1098/rspb.2016.2140
108. Deiana V, Tuberoso C, Satta A, Pinna C, Camarda I, Spano N, Ciulu M, Floris I (2015) Relationship between markers of botanical origin in nectar and honey of the strawberry tree (*Arbutus unedo*) throughout flowering periods in different years and in different geographical areas. J Apic Res 54:342–349. https://doi.org/10.1080/00218839.2016.1164540
109. Lipp J (1990) Detection and origin of abscisic acid and proline in honey. Apidologie 21:249–259. https://eurekamag.com/research/002/067/002067209.php. Accessed 20 Aug 2018
110. Harborne JB (1976) Functions of flavonoids in plants. In: Goodwin TW (ed) Chemistry and biochemistry of plant pigments, vol 1, 1st edn. Academic, New York
111. Sasaki K, Takahashi T (2002) A flavonoid from *Brassica rapa* flower as the UV-absorbing nectar guide. Phytochemistry 61:339–343. https://ezproxy.nb.rs:2129/S0031942202002376/1-s2.0-S0031942202002376-main.pdf?_tid=c123f00f-7101-4a7d-ab86-917b930f9b73&acdnat=1538599302_398f9345813081e30f8b77d3f21a1d86. Accessed 25 Aug 2018
112. Hansen DM, Olesen JM, Mione T, Johnson SD, Müller CB (2007) Coloured nectar: distribution, ecology, and evolution of an enigmatic floral trait. Biol Rev Camb Philos Soc 82:83–111. https://doi.org/10.1111/j.1469-185X.2006.00005.x
113. Zhang FP, Larson-Rabin Z, Li DZ, Wang H (2012) Colored nectar as an honest signal in plant-animal interactions. Plant Signal Behav 7:811–812. https://doi.org/10.4161/psb.20645
114. Alrgei HOV, Dabić D, Natić M, Rakonjac V, Milojković-Opsenica D, Tešić Ž, Fotirić Akšić M (2016) Chemical profile of major taste- and health-related compounds of Oblačinska sour cherry. J Sci Food Agric 96:1241–1251. https://doi.org/10.1002/jsfa.7212
115. Brandenburg A, dell'Olivo A, Bshary R, Kuhlemeier C (2009) The sweetest thing: advances in nectar research. Curr Opin Plant Biol 12:486–490. https://doi.org/10.1016/j.pbi.2009.04.002
116. Fotirić Akšić M, Tosti T, Nedić N, Marković M, Ličina V, Milojković-Opsenica D, Tešić Ž (2015) Influence of frost damage on the sugars and sugar alcohol composition in quince (*Cydonia oblonga* Mill.) floral nectar. Acta Physiol Plant 37:1701. https://doi.org/10.1007/s11738-014-1701-y
117. Santos A, Ribeiro RS, Cavalheiro J, Coreiro V, Lousada JL (2006) Initial growth and fruiting of 'Summit' sweet cherry (*Prunus avium*) on five rootstocks. N Z J Crop Hortic Sci 34:269–277. https://doi.org/10.1080/01140671.2006.9514416