Floral Nectar Production and Nectar Sugar Composition of *Cotoneaster* Species as Determined by Structural and Environmental Features

Erika NAGY TÓTH[a] – Ágnes FARKAS[b*] – Géza KÓSA[c]

[a]Institute of Biology, Faculty of Natural Sciences, University of West Hungary, Szombathely, Hungary
[b]Department of Pharmacognosy, Medical School, University of Pécs, Pécs, Hungary
[c]Botanical Garden in Vácrátót, Institute of Ecology and Botany of the Hungarian Academy of Sciences, Vácrátót, Hungary

Abstract – The nectary structure and nectar production of several *Cotoneaster* species was investigated in the Vácrátót Botanical Garden from 2007 to 2011. Structural features were studied with light and scanning electron microscopy. Nectar volume and concentration was determined with calibrated capillaries and hand refractometers, respectively. Sugar composition was analysed with thin layer chromatography and densitometry. Significant differences were found regarding the total area and thickness of the nectary, ranging from 84,000 to 400,000 μm^2 and from 80 to 260 μm, respectively. Large nectar glands produced higher volumes of nectar compared to small nectaries (10-18 and 5 μl nectar per flower per day, respectively). Besides the genetically coded structural features, nectar production was also influenced by environmental factors. Temperatures of 23-24°C proved to be optimal for nectar production in cotoneasters, while above 26°C nectar volumes decreased significantly. The nectar of all examined species was hexose-dominant, except for *C. dammeri*, whose nectar contained sucrose, too. Apicultural ranking was based on nectar sugar value.

Keywords: **nectary / apiculture / bee / hexose / ornamental**

1. INTRODUCTION

Representatives of the *Cotoneaster* genus (Rosaceae) are popular ornamentals, widespread in the temperate regions of Europe and Asia (FRYER – HYLMÖ 2009). Despite their small size, cotoneaster flowers can secrete large volumes of nectar with sugar concentrations that are sufficiently high to attract honey bees (*Apis mellifera*) (WERYSZKO-CHMIELEWSKA et al. 2003, 2004) and bumble bees (*Bombus* sp.) (CORBET – WESTGARTH-SMITH 1992) alike. Although *Cotoneaster* sp. can be regarded as valuable bee pasture, they are among the genera being most susceptible to fire blight (VAN DER ZWET – KEIL 1979, ROBERTS et al. 1998), and bees play an important role in transmitting the causing agent *Erwinia amylovora*. Therefore, cotoneasters should be avoided in the vicinity of orchards (CORBET – WESTGARTH-SMITH 1992).

To date very little information is available regarding the structure and the nectar producing capacity of the nectary in various cotoneasters. WERYSZKO-CHMIELEWSKA et al. (2003, 2004) reported on the anatomy and nectar production of the flowers in *C. hjelmquistii*, *C. lucidus* and *C. nanshan*. Furthermore, a detailed anatomical description was provided

* Corresponding author: agnes.farkas@aok.pte.hu, 7624 Pécs, Rókus u. 2., Hungary

about the nectar glands of *C. roseus* in our previous paper (NAGY TÓTH et al. 2011). However, a larger number of *Cotoneaster* species should be investigated for at least two years to understand the underlying factors regulating the nectar secretion process of cotoneasters. The varying nectar producing capacity of different species might be explained partly by the structural differences of their nectar glands, and partly by the actual environmental circumstances that will determine the volume and sugar concentration of the nectar produced by the flowers in the given year. In order to determine which factors are decisive for nectar production in cotoneasters, we investigated the nectary structure, as well as the sugar value and composition of the nectar in several *Cotoneaster* species in three years.

2. MATERIALS AND METHODS

2.1. Location and time of studies

Field studies were carried out in the Vácrátót Botanical Garden in May and June of 2007, 2010 and 2011. Further investigations were performed in the appropriate laboratories of the University of West Hungary and the University of Pécs.

2.2. Studied species

Investigations were performed on 13, 22 and 31 *Cotoneaster* species in 2007, 2010 and 2011, respectively. For the detailed list of investigated species see *Figure 5, 6 and 7* and *Table 1*.

2.3. Investigation of nectary structure

2.3.1. Light microscopy

Following dehydration in an ascending ethanol series, flowers were embedded in a hydroxyethyl methachrylate based resin (Technovit 7100). Medial longitudinal sections were cut with a rotary microtome (Anglia Scientific 325), stained with toluidine blue and mounted in Canada balsam. Slides were investigated with a NIKON ECLIPSE 80i microscope, and micrographs were taken with SPOT BASIC 4.0. Nectary area and thickness (at the thickest part of the gland) were measured with Image Tool 3.0 in 10 and 20 flowers in 2007 and 2010, respectively. Data were analyzed with Microsoft Excel.

2.3.2. Scanning electron microscopy

Samples were prefixed in paraformaldehyde, washed in phosphate buffer, then postfixed in osmium tetroxide. Dehydration of samples in ascending ethanol series was followed by critical point drying in amyl acetate, and gold sputter coating. Micrographs were taken in the laboratory of the University of West Hungary with a HITACHI TM3000 type microscope.

2.4. Nectar measurements

2.4.1. 24-hour nectar measurements

Nectar volume per flower was determined in the field with calibrated glass capillaries, following 24-hour isolation of the flowers by tulle nets, in order to exclude visiting insects.

Sugar concentration was measured with a hand refractometer (ATAGO N-50E). Sugar value was calculated using the formula: nectar volume (µl) * nectar concentration (%)/100.

2.4.2. Nectar sugar composition

Dried nectar samples were taken up in 70% (*v/v*) ethanol. Sugar standards (sucrose, glucose, fructose; 1 mg/ml each) and nectar samples were applied to plates (Silica gel 60 F_{254}, Merck) by microcaps. Plates were run twice in developing chambers without saturation. The mobile phase consisted of ethyl acetate : ethanol : 60% acetic acid : water coldly saturated with boric acid (50:20:10:10). Spots were visualised by dipping plates into a thymol-sulphuric acid reagent for 3 sec, then dried at room temperature, and finally at 105°C for 5 min. Densitometric evaluation was performed by a CAMAG TLC Scanner at 510 nm, using the software CATS 3.14 for quantitative measurements.

3. RESULTS

3.1. Structural features of the nectary

The floral nectary of cotoneasters is automorphic and receptacular, positioned between the ovary and the base of the stamens (*Figure 1*). The cuticle covering the nectary surface has an irregular ornamentation consisting of wrinkles and creases (*Figure 2*).

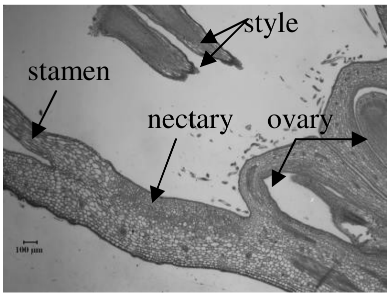

Figure 1. Nectary of Cotoneaster lancasteri

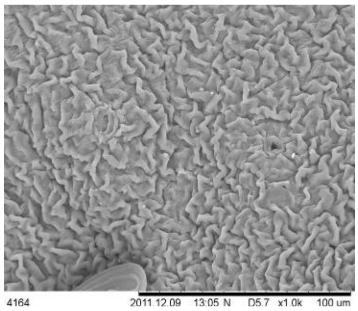

Figure 2. Nectary surface of C. kitaibelii

Nectar is secreted through stomata, whose guard cells are either in level with the epidermal cells or slightly below the level of the epidermis (*Figure 3*). Subepidermally, 3 to 4 layers of small, isodiametric cells comprise the glandular tissue; followed by the larger cells of nectary parenchyma (*Figure 4*). Calcium oxalate druses frequently occur in the parenchymatous tissues of both the gland and the receptacle. Directly beneath the nectary parenchyma vascular bundles can be observed.

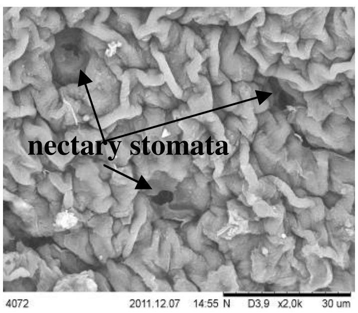

Figure 3. Nectary stomata of C. hissaricus

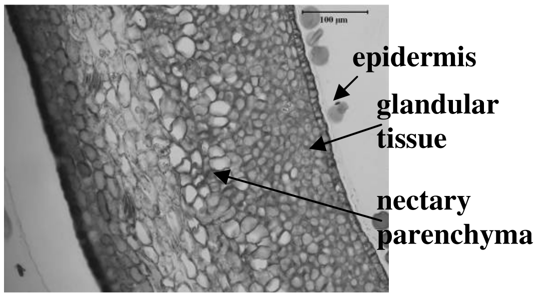

Figure 4. Nectary structure of C. glomerulata

Significant differences were found among *Cotoneaster* species, regarding both the total area (*Figure 5* and *6*) and the largest thickness of the nectary (*Table 1)*, both in 2007 and 2010.

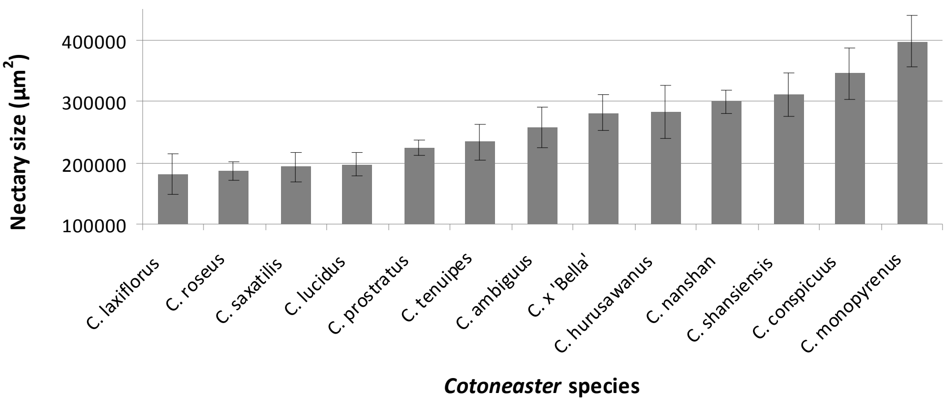

Figure 5. Nectary size of cotoneaster flowers in Vácrátót in 2007.
Data are represented as mean ± confidence interval.

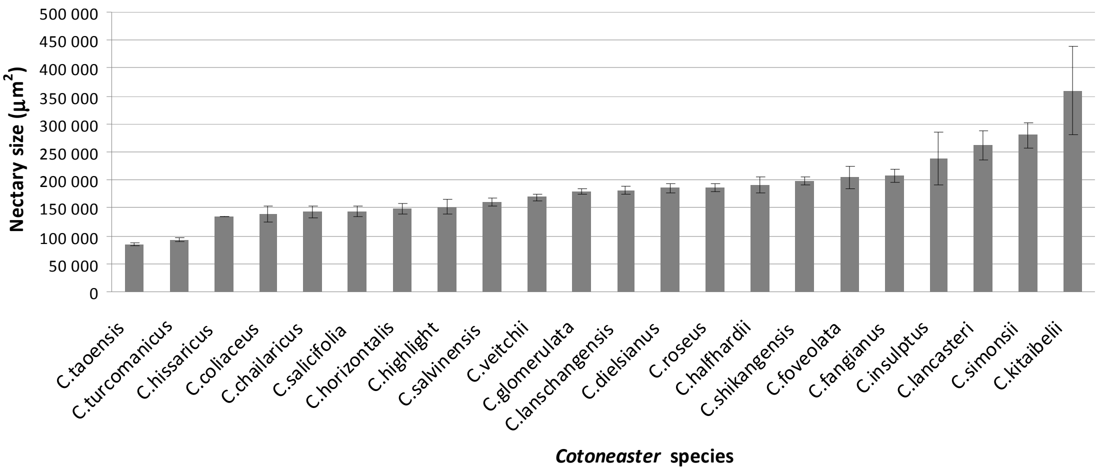

Figure 6. Nectary size of cotoneaster flowers in Vácrátót in 2010.
Data are represented as mean ± SD.

3.2. Nectar production

Cotoneaster species with large nectaries (above 250,000 μm^2) produced an average of 10-18 µl nectar per flower per day, whereas smaller nectaries (below 200,000 μm^2) were able to produce about 5 µl nectar. Sugar concentration of the nectar varied from 13 to 45%, while sugar value was in the range of 0.5-3.2 mg in 2011 (data not shown).

3.3. Nectar sugar composition

The nectar of all examined species was hexose-dominant, except for *C. dammeri*, whose nectar contained the disaccharide sucrose in addition to the hexoses glucose and fructose (*Figure 7*).

Table 1. Thickness of the nectary in cotoneaster flowers in Vácrátót in 2007 and 2010

***Cotoneaster* species**	**Nectary thickness (µm) 2007 (mean ± SE)**	***Cotoneaster* species**	**Nectary thickness (µm) 2010 (mean ± SE)**
C. laxiflorus	167.91 ± 20.70	*C. taoensis*	81.51 ± 2.97
C. lucidus	188.26 ±20.41	*C. turcomanicus*	89.05 ± 2.27
C. saxatilis	188.32 ±22.23	*C. hissaricus*	104.82 ± 3.81
C. prostratus	195.48 ±30.17	*C. coriaceus*	113.61 ± 8.19
C. tenuipes	199.76 ±27.59	*C. salicifolia*	122.03 ± 4.84
C. roseus	207.27 ± 16.05	*C. fangianus*	127.14 ± 6.83
C. shansiensis	212.49 ± 16.51	*C. salvinensis*	128.11 ± 8.92
C. ambiguus	222.25 ± 42.89	*C. dielsianus*	133.82 ± 5.10
C. x 'Bella'	225.01 ± 15.46	*C. halfhardii*	140.13 ± 9.9
C. nanshan	229.79 ± 29.64	*C. chailaricus*	151.75 ± 12.45
C. conspicuus	236.87 ±13.02	*C. glomerulata*	152.00 ± 12.06
C. monopyrenus	244.12 ± 26.22	*C. veitchii*	152.06 ± 7.39
C. hurusawanus	256.42 ± 49.17	*C. horizontalis*	157.00 ± 16.85
		C. highlight	157.40 ± 10.77
		C. foveolata	170.33 ± 10.08
		C. shikangensis	176.75 ± 4.26
		C. lancasteri	185.20 ± 11.93
		C. lanshangensis	191.18 ± 4.86
		C. insulptus	191.25 ± 28.22
		C. roseus	207.27 ± 5.07
		C. simmonsii	221.59 ± 20.85
		C. kitaibelii	238.00 ± 44.68

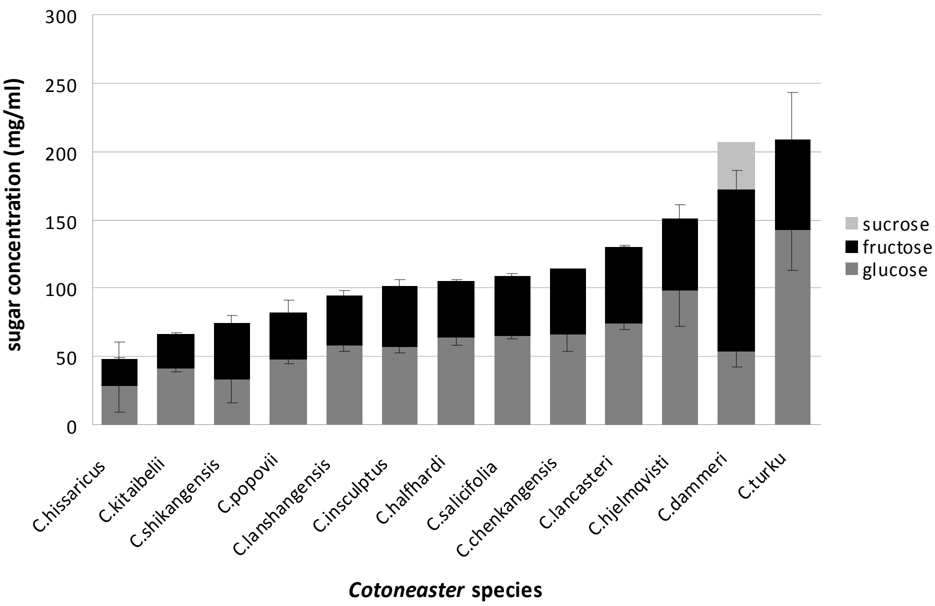

Figure 7. Nectar sugar composition of Cotoneaster species in Vácrátót in 2011. Data are represented as mean ± SD

4. DISCUSSION

Plant taxa with larger and/or thicker nectary generally produce higher amounts of nectar than those with smaller/thinner nectariferous tissue (PETANIDOU et al. 2000, CHWIL – WERYSZKO-CHMIELEWSKA 2009). In two *Cotoneaster* species positive correlation was found between nectar volume and nectary size, as well as stoma number per unit area (WERYSZKO-CHMIELEWSKA et al. 2004). Our results confirmed in a wider range of cotoneasters that species with larger and/or thicker nectar glands can secrete higher volumes of nectar than those with smaller nectaries.

Besides the genetically coded structural features, nectar production was also influenced by environmental factors. Frost hardiness of the investigated cotoneasters ranges from -12°C to -28°C, therefore frost did not influence the development and nectar producing capacity of the plants at the study site. Temperatures of 23-24°C proved to be optimal for nectar production in cotoneasters, while above 26°C nectar volumes decreased significantly.

The apicultural value of a plant can be best characterised by the nectar sugar value, which includes both volume and concentration values of nectar. From the investigated species *C. kitaibelii* and *C. insulptus* produced nectar with sugar values above 3 mg/flower, thus being highly attractive for honey bees and valuable for apiculture. Species with sugar values below 1 mg/flower, such as *C. lanshangensis* and *C. lancasteri*, are less attractive for bees, and therefore can be recommended as ornamentals rather.

5. CONCLUSION

Nectar secretion in *Cotoneaster* species was found to be influenced both by structural features, such as the size and thickness of the nectary, and environmental factors like air temperatures. Cotoneasters with large nectar and sugar producing capacity are suitable for apicultural purposes, whereas taxa with lower nectar volumes can be recommended as ornamentals.

Acknowledgements: We wish to thank for the financial support provided by the grant TÁMOP 4.2.1.B-09/1/KONV-2010-0006.

References

CHWIL, M. – WERYSZKO-CHMIELEWSKA, E. (2009): Characteristics of nectaries and nectar in flowers of two Rhododendron species. J. Apic. Sci. 53:17-27.

CORBET, S.A. – WESTGARTH-SMITH, A. (1992): *Cotoneaster* for bumble bees and honey bees. J. Apic. Res. 31:9-14.

FRYER, J. – HYLMÖ, B. (2009): Cotoneasters: A comprehensive Guide to Shrubs for Flowers, Fruit, and Foliage. Timber Press Inc., London, 2009. 344 p.

NAGY TÓTH, E. – FILEP, R. – FARKAS, Á. (2011): Nectary Structure of *Cotoneaster roseus*. Acta Biol. Szeged. 55 (2): 243-246.

PETANIDOU, T. – GOETHALS, V. – SMETS, E. (2000): Nectary structure of Labiatae in relation to their nectar secretion and characteristics in a Mediterranean shrub community: does flowering time matter? Plant Syst. Evol. 225: 103-118.

ROBERTS, R.G. – HALE ,CN. – VAN DER ZWET, T. – MILLER, C.E. – REDLIN, S.C. (1998): The potential for spread of *Erwinia amylovora* and fire blight via commercial apple fruit; a critical review and risk assessment. Crop Protection 17 (1): 19-28.

VAN DER ZWET, T. – KEIL, H.L. (1979): Fire blight: A bacterial disease of rosaceous plants. In: Agriculture Handbook. Vol. 510. United States Department of Agriculture, Washington DC.

WERYSZKO-CHMIELEWSKA, E. – CHWIL, M. – SKRZYPEK, H. (2003): Charakterystyka kwiatów i nektarowanie irgi miseczkowatej (*Cotoneaster hjelmquistii*). Annals Univ. Mariae Curie-Sklodowska, Sect. EEE 13:137-142.

WERYSZKO-CHMIELEWSKA, E. – CHWIL, M. – KONARSKA, A. (2004): Anatomical traits of nectaries and nectar secretion by the flowers of *Cotoneaster lucidus* Schlecht. and *C. nanshan* Mottet. J. Apic. Sci. 48: 55-62.