CHARACTERISTICS OF NECTARIES AND NECTAR IN FLOWERS OF TWO *RHODODENDRON* SPECIES

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Summary

The morphology of nectaries and nectar secretion in flowers of *Rhododendron catawbiense* and *R. japonicum* were compared. The structure of the secretory epidermis was analysed using scanning electron microscopy (SEM). The weight of nectar and percentage of sugar content in nectar as well as the efficiency of different sugars from 10 flowers of the studied taxa were determined.

The nectaries in flowers of *Rhododendron* catawbiense were larger and they formed more distinctive protuberances at the base of the ovary than in *R. japonicum*. The surface of the nectaries of both species was striated at places and their upper part was covered with densely growing non-glandular trichomes. The stomata in *R. catawbiense* were distributed individually and evenly over the whole surface of the nectary, whereas in *R. japonicum* they occurred in small clusters or formed densely packed stomatal fields. The beginning of nectar secretion was observed already at the opening bud stage. Nectar was secreted by stomata which were at different growth stages throughout the whole secretion period. A positive correlation was found between nectary size and nectar production. The average weight of nectar secreted throughout the lifespan of the flower of *R. catawbiense* was 16.6 mg, whereas in R. japonicum it was 6.3mg. The sugar content in nectar reached 63% and 37%, respectively. The efficiency of the various sugars from *R. catawbiense* was 10.4 mg/flower, 4.5 times higher than in *R. japonicum* (2.4 mg/flower).

Keywords: nectary, morphology, SEM, nectar secretion, *Rhododendron catawbiense, R. japonicum*.

INTRODUCTION

The genus *Rhododendron* (Ericaceae) includes over 1200 species native mainly to the mountains of Asia (Szweykowska and Szweykowski 2003). *Rhododendron catawbiense* and *R. japonicum* are shrubs which are frequently grown in Poland (Seneta and Dolatowski 2004). The multi-coloured flowers of the shrubs of the genus *Rhododendron* provide nectar and pollen for pollinating insects (Maurizio and Grafl 1969, Sai-Chit and Corlett 2000, Weryszko-Chmielewska and Chwil 2005). Rhododendron flowers are visited by honey bees and bumblebees (Yokogawa and Hotta 1995, Escaravage and Wagner 2004).

Studies of different authors confirm the high apicultural value of *Rhododendron* (Maurizio and Grafl 1969, Szklanowska and Denisow 1994, Weryszko-Chmielewska and Chwil 2005, 2008; Chwil and Weryszko-Chmielewska 2007).

In Switzerland, Austria, Germany and Italy, monofloral honeys are obtained from Rhododendron shrubs growing in the mountains (Maurizio and Grafl 1969,

Warakomska and Wojtacki 1988, Persano-Oddo et al. 1995, Zoratti 1996). These honeys contain a high content of fructose (Maurizio and Grafl 1969).

The nectary in *Rhododendron* flowers is located at the base of a 5-ribbed ovary, forming rounded protuberances of different size and shape on particular ribs (Philipson 1985, Weryszko-Chmielewska and Chwil 2005, 2007, 2008, Chwil and Weryszko-Chmielewska 2007).

The aim of the present study was to continue earlier investigations on nectary structure and the apicultural value of Rhododendron catawbiense and *R. japonicum* flowers. The amount of nectar secreted and the sugar content in nectar as well as the morphology of floral nectaries were compared.

MATERIAL AND METHODS

Flowers of *R. catawbiense* (A. Gray) J.V. Suringar ex E.H. Wilson and *R. japonicum* Michx. were the object of the study conducted in the years 2006-2007. Shrubs grew in the Botanical Garden of the Maria Curie-Skłodowska University in Lublin, Poland. Nectar was collected throughout the lifespan of the flower at the full bloom stage of *R. japonicum* on day 4 and *R. catawbiense* shrubs on day 7. The nectar was collected at 1000 am. In addition, nectar samples were collected on day 3 from *R. japonicum* flowers and nectar was collected on day 3, 4, 6 and 8 from *R. catawbiense* flowers. Nectar secretion in the flowers was investigated using the pipette method (Jabłoński 2002, 2003). Nectar samples were collected three times per season from 10 flowers. The percentage of sugar content in the nectar of the studied taxa and the efficiency of the sugars were determined.

The results concerning the mass of nectar per 10 flowers and concentration of the sugars in the nectar were statistically analyzed. Analyses of variance for the complete randomized design (single classification) and Tukey's Test were used to determine significant differences between means ($P < 0,05$).

Portions of the nectaries were sampled at budburst and open flower stages. The material for the scanning electron microscopy (SEM) examination was fixed in 4% glutaraldehyde and 0.1 M phosphate buffer with a pH of 7.0 at room temperature for 4 hours. After being dehydrated in acetone, the sections nectaries were dried at critical point in liquid CO_2 and coated with gold using a CS 100 Sputter Coater. Observations of the surface of the secretory epidermis were made using the scanning electron microscope BS - 340 Tesla.

RESULTS

In Lublin, *R. catawbiense* and *R. japonicum* shrubs flowered for about 3 weeks in the second half of May. *R. japonicum* flowers showed the first visible signs of corolla petal wilting on the fifth day of their life, whereas in *R. catawbiense* wilting first occurred on the eighth or ninth day. Hence, the lifespan of a single flower for the above mentioned species was, respectively, 4 - 5 and 7 - 8 days, and on the average 4 and 7 days.

R. catawbiense flowers grew in inflorescences of 5 - 20 flowers. But *R. japonicum* shrubs formed terminal corymbs with 5 – 10 flowers.

The nectaries located at the base of the ovary in flowers of the studied taxa exhibited large differences in the size and types of ribbing. The nectary glands in *R. catawbiense* flowers were larger than in *R. japonicum* (Figs 1, 2). The former reached the radial length of about 600 µm measured in longitudinal sections, the latter 350 µm. The secretory tissue situated at the base of the strongly ribbed ovary of the

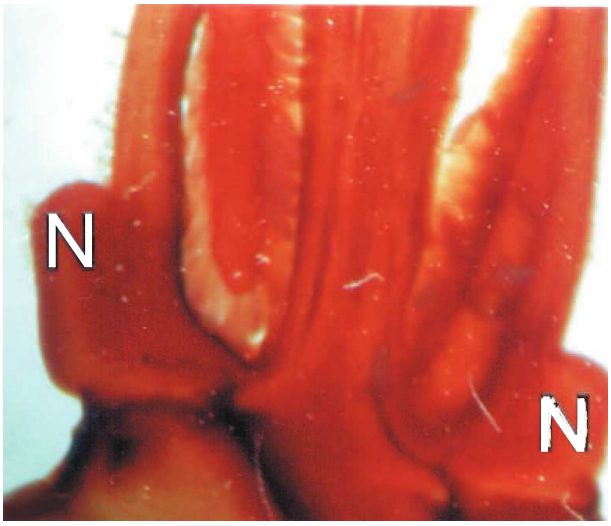

Fig. 1. Portions of the ovary with the visible nectary (N) in longitudinal section, *R. catawbiense,* (x20).

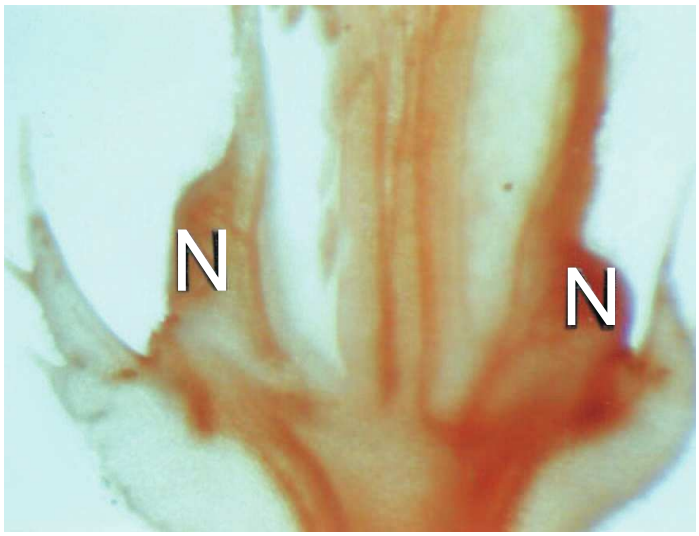

Fig. 2. Portions of the ovary with the visible nectary (N) in longitudinal section, *R. japonicum,* (x22).

R. japonicum's pistil forms a small protuberance on each of the five ribs of the ovary. It also occupies hollows between the ribs. But in *R. catawbiense*, two protuberances of the nectary were observed on each of the poorly visible five ribs of the ovary. In this species, the nectary forms a distinctive protruding collar with ten characteristic bulges, separated from the ovary by a small furrow (Figs 3, 4). The stamens were situated by deeper depressions of the nectary gland, and corolla petals were located by more shallow external depressions (Fig. 3).

Fig. 3.

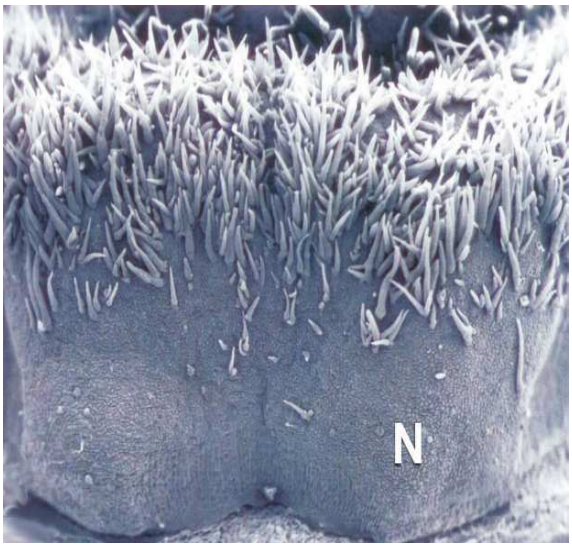

Fig. 4.

Fig. 5.

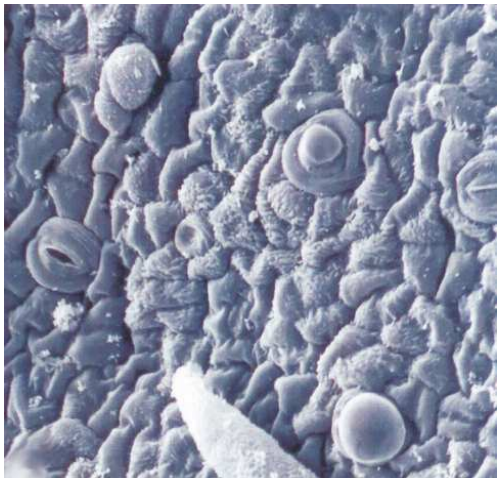

Fig. 6.

Fig. 3 - 6. Portions of the nectary of *R. catawbiense.* 3 – The nectary (N) located at the base of the pistil's ovary (O), with distinct ribbing corresponding to five ribs of the pistil's ovary; 4 – the apical part of the nectary gland with densely growing non-glandular trichomes; 5, 6 – portions of secretory epidermis in the upper (5) and middle; (6) part of the nectary, visible stomata at different growth stages.
Figs: 3 – x13; 4 – x47; 5 - x300; 6 - x450.

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 7 - 10. Portions of secretory epidermis of *R. catawbiense*. Stomata at different stages of nectar secretion: the beginning of secretion accumulation (arrow), a nectar droplet on the surface of stomatal cells surrounded by the cuticle (double arrow). Visible stomata with the closed (two arrows) and open (two arrowheads) pore and cuticular striae on the surface of epidermal cells (arrowhead).
Figs: 7 – x700; 8 – x1100; 9 – x1500; 10 – x1600.

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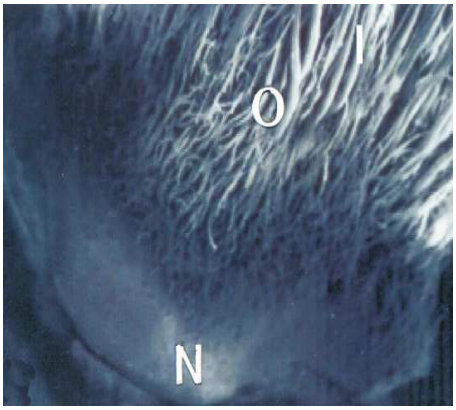
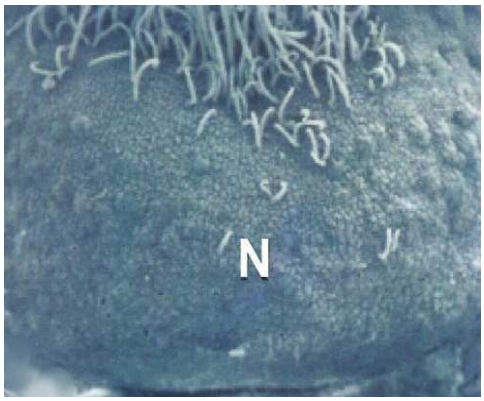


Fig. 11.



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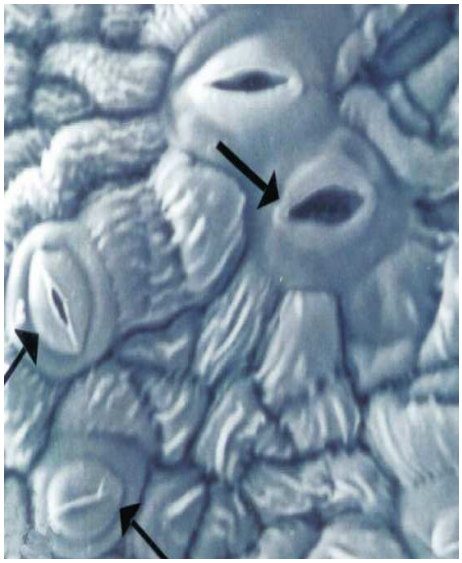

Fig. 13.

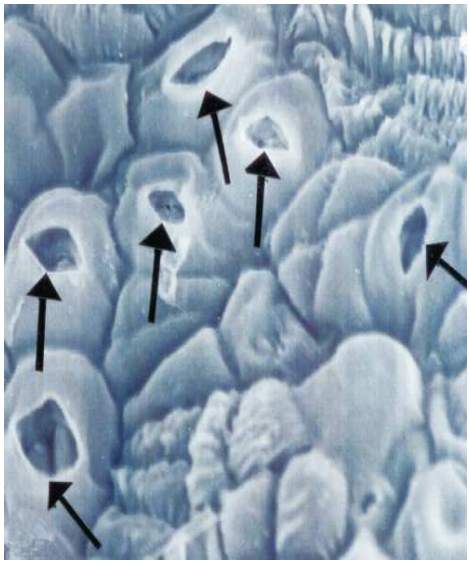

Fig. 14.

Fig. 11 - 14. Portions of the nectary of *R. japonicum* 11 - 12. The nectary (N) at the base of the pistil's ovary (O). 13 - 14 visible numerous stomata at different growth stages (arrows), forming stomatal zones at places.
Figs: 11 – x20; 12 – x55; 13, 14 – x750.

The apical portion of the nectaries in the flowers of both taxa was distinguished by the presence of densely growing non-glandular trichomes (Figs 3, 4, 11, 12). In *R. catawbiense* they were dilated at the base (Fig. 5) and covered the nectary's upper edge which was protruding outside, forming a protective shield for accumulating nectar (Figs 3, 4). Some epidermal cells were covered with a striated cuticle, others were smooth. Stomata in *R. catawbiense* were distributed individually and evenly on the whole surface of the nectary (Figs 5-10). In the nectaries of *R. japonicum*, however, clusters numbering several stomata or densely packed stomatal fields made up of even several dozen stomata were both frequently observed (Figs 13-14).

Nectar secretion took place through the stomata. Stomata were situated above the surface of other epidermal cells (the apical part) of the nectary or they were arranged at the level of other cells of this tissue (the lateral wall of the nectary). The development of the stomata on the surface of the nectaries was irregular. Even at the full bloom stage, smaller stomata with a closed aperture occurred together with fully-developed stomata (Figs 5, 6, 13).

The onset of nectar secretion was observed at the opening bud stage. Escaping secretion droplets were visible by the aperture of the stomata of *Rhododendron catawbiense* nectaries (Figs 6, 7, 9).

The weight of nectar collected from over the whole lifespan of a *R. catawbiense* flower was much higher than in *R. japonicum*, *R. catawbiense* flowers had a much longer flower lifespan and 10 of its flowers produced 165.5 mg of nectar. The same number of *R. japonicum* flowers secreted 63 mg of nectar. The sugar concentration in the nectar of *R. catawbiense* was 63%, whereas in *R. japonicum* it was 37%. Based on the obtained results, it was found that the

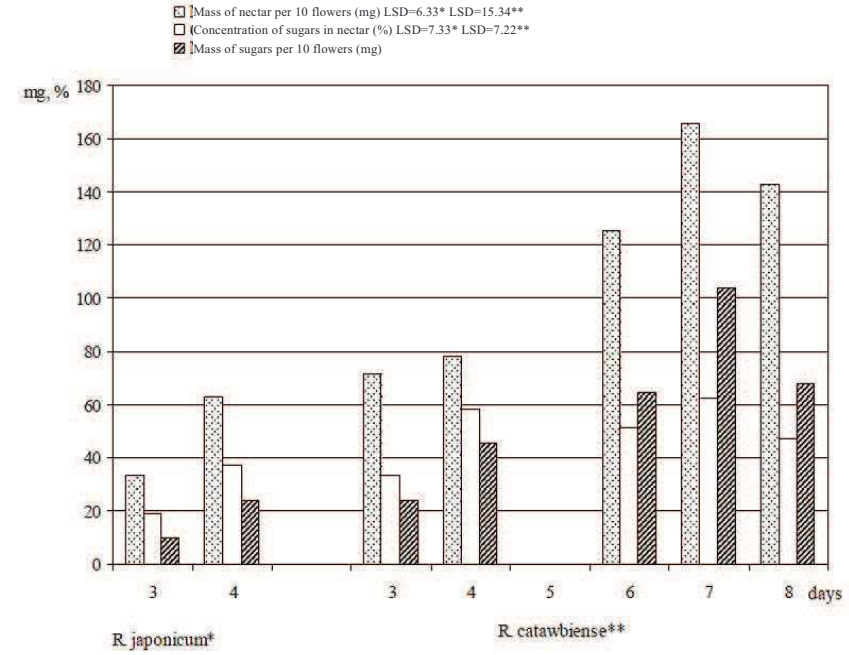

Fig 15. Characteristics of nectar production on particular days of flower life of two *Rhododendron* species.

efficiency of the various sugars of *R. catawbiense* (104 mg/10 flowers) was much higher than in *R. japonicum* (23 mg/10 flowers) (Fig. 15).

The statistically proven differences in *R. japonicum*'s mass of nectar per 10 flowers and the concentration of the different sugars in nectar between the 3rd and 4th day of the flowers' lifespan were recorded. There were no statistical differences in *R. catawbiense*'s mass of nectar between the 3rd and 4th as well as 6th and 8th day of its flowers' lifespan, whereas statistically not significant changes in the concentration of the various sugars in the nectar of this species where found between the 4th and 6th, 4th and 7th as well as 6th and 7th day of the flowers' lifespan. Other differences of both parameters were statistically significant.

When analysing nectar secretion in two *Rhododendron* species on the third and fourth day of a flower's lifespan, it was also found that on these days *R. catawbiense* flowers secreted more nectar than flowers of the other taxon (Fig 15). Also, the concentration of the various sugars in the nectar of *R. catawbiense* and their weight was greater than in *R. japonicum* (Fig. 15).

It was demonstrated that the weight of nectar in R. catawbiense flowers increased on the successive days of nectar secretion (day 3, 4, 6, 7). It was only on the eight day that a reduction in the amount of this secretion was found (Fig. 15).

DISCUSSION

In the present study involving the investigation of floral nectaries of *Rhododendron* catawbiense and *R. japonicum* as well as of the earlier described *R. luteum* (Weryszko-Chmielewska and Chwil 2005), a positive correlation was found between nectary size and nectar production.

Earlier observations also demonstrate that the nectaries in *R. catawbiense* flowers formed higher protuberances (1.3 mm) on the surface of the ribs of the ovary compared to the nectaries of *R. japonicum* (0.7 mm) (Chwil and Weryszko-Chmielewska 2007, Weryszko-Chmielewska and Chwil 2007, Weryszko-Chmielewska et al. 2007).

The onset of nectar secretion in the studied flowers was found already at the opening bud stage. Secretion droplets by the aperture of the stomata (at different development stages) were observed. In *R. catawbiense* a reduction in the weight of nectar was found on the last day of a flower's lifespan, which may result from its resorption.

Stomatal cells in the three investigated species of *Rhododendron* were at different development stages at anthesis. They were located on the same level or above other epidermal cells (Weryszko-Chmielewska et al. 2003, Weryszko-Chmielewska and Chwil 2005, 2008).

Flowers of the *Rhododendron* shrubs investigated in the study produced nectar abundantly. The amount of nectar secreted throughout the lifespan of 10 flowers ranged between 63 and 166 mg. The weight of nectar and the efficiency of the various sugars of *R. catawbiense* were, respectively, 2.5 and 4.5 higher than these parameters in R. japonicum. The maximum sugar concentration in the nectar for these species was 62.7% and 37%, respectively.

A study conducted by Sai-Chit and Corlett (2000) confirmed intensive nectar secretion in the flowers of six Rhododendron species growing in Hong Kong. Daily nectar production ranged between 0.9 and 38.7 micro L/flower. The dominant sugar in their nectar was sucrose.

When analysing the present results relating to nectar production in these two

Rhododendron species and data on other taxa of the genus *Rhododendron* published in literature (*R. arboretum*, *R. barbatum* and in Poland *R. luteum*), it can be stated that the sugar content in nectar of these taxa falls within a wide range of from 17% to 63% (Maurizio and Grafl 1969, Martini et al. 1990, Weryszko-Chmielewska and Chwil 2005). This probably depends not only on the respective species, but also on the geographic location of plants.

We observed that the sugar concentration in the nectar of the investigated *Rhododendron* species was dependent on temperature. In Lublin in 2006, the daily mean temperature in May was 14.4°C, whereas in 2007 it was 16.0°C. The higher temperature in 2007 was accompanied by higher sugar content in the nectar, which applied to both *Rhododendron* species. For example, in the case of *R. japonicum* these values were 39.5-42% (2007) and 26-29.5% (2006) on the fourth day of the flower's lifespan. Daily mean relative humidity in the above mentioned years was similar and it was 62.8% and 65.0%, respectively.

Thise days *Rhododendron* shrubs have become very popular in Poland as additions to the gardens. Their long (3 weeks) and abundant flowering can supplement spring rewards for insects (Weryszko-Chmielewska and Chwil 2005, 2008; Stout et al. 2006). Literature reports show that flowers of different *Rhododendron* taxa are frequently visited by honey bees and bumblebees (Sai-Chit and Corlett 2000, Zhang et al. 2007).

Honeys from *Rhododendron* nectar are characterised by a variable content of pollen from flowers of this genus (Wille et al. 1990, Ferrazzi and Gerlero 1995, Stout et. al. 2006, Ünal and Küplülü 2006).

CONCLUSIONS

1. Single *R. catawbiense* flowers lived twice as long (7-8 days) as *R. japonicum* flowers (4-5 days).
2. In comparable species, the glandular tissue formed a distinctive protuberance on the surface of the ovary and it differed in size and shape.
3. Throughout their lifetime, *R. catawbiense* flowers secreted 62% more nectar than *R. japonicum* flowers.
4. During the study period, the average sugar concentration in the nectar of the two species of *Rhododendron* was within the range of 19% - 63%. The sugar yield from the whole lifespan of 10 flowers of *R. catawbiense* was 77.5% higher than in *R. japonicum* (23 mg).

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CHARAKTERYSTYKA NEKTARNIKÓW I OBFITOŚĆ NEKTAROWANIA DWÓCH GATUNKÓW *RHODODENDRON*

Chwil M., Weryszko-Chmielewska E.

S t r e s z c z e n i e

Porównano morfologię nektarników i nektarowanie kwiatów *Rhododendron catawbiense* i *R. japonicum.* Strukturę epidermy sekrecyjnej analizowano w skaningowym mikroskopie elektronowym (SEM). Określono masę nektaru i procentową zawartość cukrów w nektarze oraz wydajność cukrową 10 kwiatów badanych taksonów.

Nektarniki w kwiatach *Rhododendron* catawbiense były większe i tworzyły wyraźniejsze uwypuklenie u nasady zalążni niż u *R. japonicum*. Powierzchnia nektarników obu gatunków była miejscami prążkowana, a górna ich część była pokryta gęsto wyrastającymi włoskami mechanicznymi. Aparaty szparkowe u *R. catawbiense* były rozmieszczone pojedynczo i równomiernie na całej powierzchni nektarnika, natomiast u *R. japonicum* występowały w małych skupieniach lub tworzyły zwarte pola szparkowe. Początek sekrecji nektaru obserwowano już w fazie otwierającego się pąka. Nektar był wydzielany przez aparaty szparkowe, które przez cały okres sekrecji znajdowały się w różnych stadiach rozwojowych. Stwierdzono dodatnią zależność pomiędzy wielkością nektarnika i obfitością nektarowania. Średnia masa nektaru wydzielanego w ciągu całego życia kwiatu u *R. catawbiense* wynosiła 16,6 mg, zaś u *R. japonicum* 6,3 mg. Zawartość cukrów w nektarze osiągnęła wartość odpowiednio 63% i 37%. Obfitość nektarowania *R. catawbiense* wynosiła 10,4 mg/kwiat i była 4,5 razy większa niż u *R. japonicum* (2,4 mg/kwiat).

Słowa kluczowe: sekrecja nektaru, nektarniki, morfologia, SEM, *Rhododendron catawbiense*, *R. japonicum*