Selection among leaflets of *Rosa,* according to their size and position, by a leaf-cutting bee, *Megachile sp.* (Hymenoptera, Megachilidae)

by Jean Béguinot[1, 2]

[1] Biogéosciences, Université de Bourgogne, F – 21000 Dijon
[2] Société d'Histoire naturelle du Creusot, 12 rue des Pyrénées, F – 71200 Le Creusot <jean-beguinot@orange.fr>

Abstract. – The wild Rose bush, *Rosa canina* L.*,* is a common leaf supplier for some leaf-cutting bees (*Megachile sp.*). Yet, the different leaflets of *Rosa* are far from being exploited with the same frequency and intensity by *Megachile* females, according to the position of leaflets along the leaf axis. In particular, the average number of operated cuts per leaflet strongly differs according to the position of leaflets along the leaf axis and these differences prove to be highly significant. The relative size of the leaflets, systematically correlated to their position along the leaf axis, is far from explaining the strong differences in the degree of exploitation of the leaflets by the leaf-cutting bees. Doubling of leaflet size leads to an 8-fold increase in the average number of disks cut out per leaflet, while the average size of the disks increases by a factor 1.6. Other criteria used in leaf or leaflet selection by insects are also discussed.

Résumé. – **Sélection des folioles de *Rosa*, selon leurs taille et position, par une abeille coupeuse de feuilles, *Megachile sp.* (Hymenoptera, Megachilidae)**. L'Églantier, *Rosa canina* L., figure parmi les fournisseurs de matériau préférés des abeilles coupeuses de feuilles (*Megachile sp.*). Or, il apparaît que les différentes folioles d'une même feuille d'églantier sont utilisées par les Mégachiles avec des fréquence et intensité bien différentes selon leur position d'insertion le long de l'axe de la feuille. Les écarts de fréquences d'exploitation (nombre moyen de découpes par foliole selon leur position le long de l'axe de la feuille), statistiquement hautement significatifs, sont loin de s'expliquer seulement par les écarts de taille des folioles selon leur position le long de l'axe foliaire. En effet, un doublement de taille de foliole conduit à un accroissement d'un facteur 8 du nombre moyen de disques découpés par foliole avec, en outre, une taille moyenne des disques accrue d'un facteur voisin de 1,6. Les causes complémentaires susceptibles d'orienter le choix des découpeuses sont discutées.

Keywords. – Nesting resources, leaf size, preference, selectivity, behaviour, *Rosa*.

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Leaf-cutting bees belonging to the genus *Megachile* Latreille, 1802 (Hymenoptera: Apoidea) ordinarily build a brood cell using a pre-existing tunnel of appropriate size which they line and ultimately close with a tight assemblage of small discs, cut out from the lamina of leaves or leaflets of some preferred species of plants or trees (fig. 1).

The remarkable crafts of leaf-cutting bees have generated long standing interest for these insects, tracing back, at least, to Réaumur (1742) with, later, more specific attention addressed to *Megachile* by Fabre (1915).

Leaf-cutting females cut out discs pieces of two distinctive geometries: either ellipsoids or circles. Ellipsoid pieces are designed for lining the bottom and lateral part of the future egg- and ressources-containing cell while the circular discs are essentially for serving as a cover, closing the top of the cell (Fabre, 1915). The crude geometrical distinction between ellipsoids and circles does not preclude some variability in shape and dimensions within each geometrical category. Fabre (1915) already noticed this variability and observed that lining the cell consists in displaying ellipsoidal pieces in successive layers of imbricate discs, with the inner discs being somewhat shorter than the outer ones, a point further examined in more details recently (Kim, 2007). Similarly, the circular discs that serve as a multi-layered covering for the cell, also show some slight variability in diameter, with the inner ones almost exactly tailored to the diameter of the cell and the outer ones often a little bit larger.

Fig. 1. – Specimen of *Rosa* leaf supporting several discs cut-out by bees. Leaflet 1: 2 ellipsoidal discs; leaflet 2: 2 circular discs; leaflet 3: 1 circular and ellipsoidal discs; leaflet 4: 1 ellipsoidal disc; leaflet 5: 1 circular disc.

Megachile females, when cutting out their discs, make use of their sharp mouth pieces as a minute pair of scissors and use their body as a kind of adaptable 'compass' to steer appropriately during their cutting-out operations. The insects likely select leaves or leaflets according to some specific properties that are related to the future use of the cut-out discs, probably in terms of (i) surface smoothness and homogeneity, to ensure regular cutting and (ii) fair elasticity, both strong enough for the curved ellipsoidal pieces to adhere spontaneously to the inner side of the tunnel-shaped brooding cell but not too strong in order to remain easily bent by the bee. Accordingly, the rather smooth-surface leaves of, for example, *Syringa*, *Robinia*, *Cercis*, *Betula*, *Calystegia*.... are favoured. The leaves of wild or, occasionally, cultivated species of roses (*Rosa sp*.) are also frequently selected. After identifying the proper tree or plant, within a reasonable vicinity of their brooding nest location, that satisfy their purpose, females might still have to discriminate which leaves within the tree / plant will be more appropriate and preferred, in particular in terms of size and elasticity.

In this respect, the leaves of *Rosa sp*. provide an interesting opportunity for testing the preferences of *Megachile* females: each leaf of *Rosa canina* L. generally displays a set of seven leaflets with their size ordinarily increasing from the base to the tip of the composite leaf.

Hereafter, we address the question of whether a leaf-cutter bee shows any preference among the leaflets of a same leaf, in a bush of wild *Rosa canina*. Only those leaves are considered in the study that have all their leaflets with sufficient length for the extraction of at least two discs per leaflet (one disc per side). The degree of females' preference was investigated according to leaflets individual size and position along the leaf axis. The degree of preference is quantified in terms of (i) the number of discs extracted from a given leaflet, (ii) the distribution of sizes of disc(s) extracted from a given leaflet.

Methods

Eleven leaves supporting a relatively dense exploitation by *Megachile* females were sampled from a wild *Rosa canina* bush. I did not observe bees making the cuts, so it was not possible to identify which bee or bees were responsible for the leaflet cuttings. The bush was located in a pastoral environment near Le Creusot, southern Burgundy (France). Most leaves were composed of 7 leaflets but one leaf had lost two leaflets, which makes a total of 75 leaflets examined for this study. A total of 76 traces of cut-out discs were recorded from these 75 leaflets. The dimensions of each cut-out discoidal piece were measured and its larger size was considered;

that is the diameter for rounded pieces and the longer axis ('length') for the ellipsoidal discs. Leaflet size was estimated as the length of the lamina. The position of leaflets along the axis of the composite leaf is conventionally labelled using a numerical index, from 1 for the tip leaflet to 6 and 7 for the two basal leaflets, (with pair and odd numbers respectively for the left-side and right-side positions of leaflets relative to the leaf axis).

Chi-square test was applied to estimate the degree of statistical significance of the recorded difference between the numbers of cut-out discs per leaflet, according to leaflet position along the axis of the composite leaf. Student t test was implemented to estimate the degree of statistical significance of the recorded difference between leaflet's dimensions.

RESULTS

Fig. 2 shows the steadily decreasing evolution of the average leaflet length according to the leaflets position along the axis of the composite leaf, from leaf tip (distal) to leaf base (proximal).

The size distribution of the 76 discs is plotted in fig. 3. In spite of a substantial variability within each of the two types of disc geometry, circle and ellipse, the overall size distribution remains clearly bimodal, with a rather clear separation between roughly round shape (size < 14 mm) and ellipsoidal shapes (size > 14 mm).

Number of cut-out discs per leaflet according to leaflets' size and position. – The distribution of the numbers of cut-out discs per leaflet strongly depends upon the leaflet position along the axis of the composite leaf: differences according to leaflets position are highly significant: χ^2 statistical test applied to the four leaflets positions (table I): $\chi^2 = 46.3$, d.f. = 9, p << 0.001. The corresponding highlighted trend is a monotonic increase of the average number of cut-out discs from proximal to distal positions of leaflets along the leaf axis.

Also, table I shows a monotonic increase of the average number of cut-out discs with increasing length of leaflets (correlative of the preceding trend, since leaflets length increases monotonically from proximal to distal positions along the leaf axis, as shown in fig. 2). Basically, the average number 'n' of cut-out discs per leaflet varies by a factor ≈ 8 (2.0/0.23) along the range of leaflet size or leaflet position along the leaf axis. That is, the leaflets supporting 1 cut-out disc have significantly larger sizes than the leaflets supporting no cuts: Student t test on leaflet length, t = 2.70, d.f. = 48, p = 0.01. Also, the leaflets supporting 2 or 3 cut-out discs are
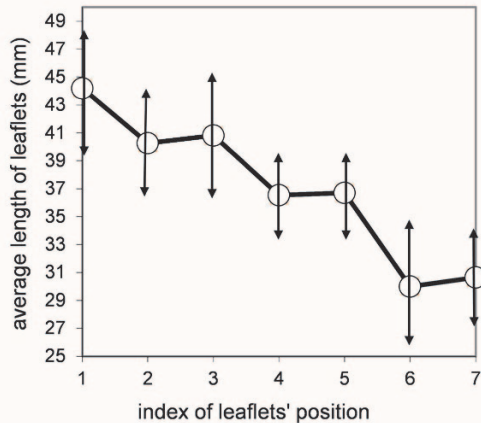

Fig. 2. – Length of leaflets in relation to their position on the leaf (distal position: index 1; proximal position: index 6 and 7). N = 11 leaves; vertical bars for standard deviation.
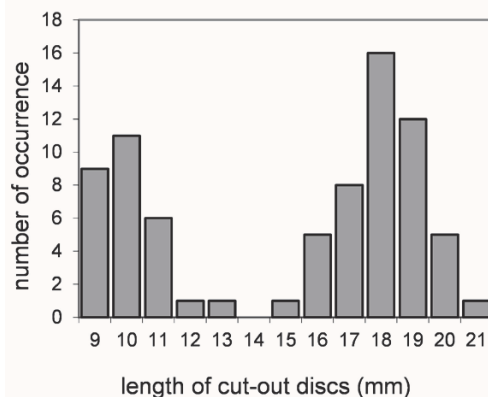

Fig. 3. – Histogram of the distribution of the size (length) of the cut-out discs. Circular discs left-hand side, ellipsoidal discs right-hand side.

Table I. – Distribution of cut-out discs among leaflets of *Rosa*. Reported are: (i) the numbers of leaflets respectively supporting n = 0, 1, 2, 3 cut-out discs according to the leaflet positions along the leaf axis and (ii) the mean (and standard deviation) of the number of cut-out discs per leaflet against the mean leaflet length.

position of leaflets along the leaf axis (index n°)	recorded numbers 'n' of discs cut-out per leaflet				total	mean 'n' per leaflet	mean leaflet length (mm)
	n = 0	n = 1	n = 2	n = 3			
distal (1)	0	1	8	1	10	2.00 ± 0.47	44.2
sub-distal (2 and **3)**	3	8	10	1	22	1.41 ± 0.80	40.5
sub-proximal (4 and **5)**	5	12	4	0	21	0.95 ± 0.67	36.6
proximal (6 and **7)**	17	5	0	0	22	0.23 ± 0.43	30.3
all positions together	25	26	22	2	75	*1.01*	

significantly larger than the leaflets supporting 1 cut-out disc: Student t test on leaflet length, t = 3.32, d.f. = 48, p = 0.002. At last, the leaflets supporting 2 or 3 cut-out discs are significantly larger than the leaflets supporting no cuts: Student t test on leaflet length, t = 5.90, d.f. = 46, p < 0.000001.

Average size of the disc(s) cut-out from a leaflet, according to the leaflet-size. – The degree of exploitation of the margins of leaflets by *Megachile* females is function not only of the number of cut-out discs but also, of course, of the length of each cut disc, which shows also a wide range of variation, from 9 to 21 mm (fig. 3). The average length of discs steadily increases with the length of the leaflets that support them (fig. 4); the trend is statistically significant: r = 0.96, n = 6, p = 0.003.

Discussion

Megachile females cut discoidal portions along the leaf or leaflets margins of a lot of plant species that have soft leaf lamina, such as, for example the leaflets of *Rosa sp*. One interesting point, in this respect, is the way bees exploit differentially the leaflets of different size and position along the leaf axis in *Rosa*. Both the size of cut-out discs and their number per leaflet vary over a large range and, accordingly, the degree of exploitation of the different leaflets of a single leaf is also highly variable.
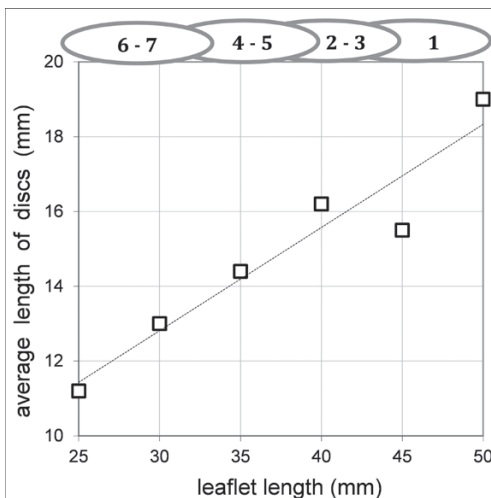

Fig. 4. – Average length of cut-out discs plotted against classes of leaflet length supporting them; positive correlation: r = 0.96, n = 6, p = 0.003.

In absolute value, the degree of exploitation of a given leaflet by cutting bees may be quantified as the product of the number 'n' of cut-out discs by their average length 'l'. As shown at table I and fig. 4, both parameters are positively correlated with the leaflet size and the position of leaflets along the leaf axis (since, on average leaflet size steadily increases from the base to the tip of leaf axis: fig. 2). These trends are both highly significant.

Now, it makes more sense to consider the absolute degree of exploitation of a given leaflet by comparison with the available resource, i.e. to the size 'L' of the leaflet. Thus, a more relevant criterion quantifying the intensity of exploitation of a leaflet by cutting bees would be the relative, rather than absolute value. Thus defined, the relative exploitation ratio is: E = n × l/L.

Along the range of variation of leaflets length 'L', from L = 25 to L = 50 mm, the average length 'l' of cut-out discs varies by a factor ≈ 1.6 (fig. 4; l-11 mm versus l-18 mm) and the average number 'n' of discs per leaflet varies by a factor ≈ 8 (table I; 0.23 cuts / leaflet versus 2.00 cuts / leaflet). Accordingly, the absolute exploitation intensity E increases by a factor ≈ 13 when the leaflet size increases by double. The relative exploitation ratio E thus varies by a factor ≈ 6.

This, indeed, is very different of what is expected from the null hypothesis, according to which the degree of exploitation should be proportional to the resource, i.e. to the leaflet length. This expected null hypothesis would thus require E remaining independent of leaflet size. To the contrary, the reported 6-fold range of variation of the exploitation ratio E, according to leaflets size and position, strongly differs from expectation.

This strong, unexpected choosiness of *Megachile* females, in favour of largest leaflets then calls for explanations.

One reason for *Megachile* females being so selective in favour of larger leaves could be that they systematically avoid having to cut the middle rib of leaflet, either because they are unable to do so or because the mechanical properties of the resulting disc would be too much heterogeneous. This would then compel females to limit the discs' width to no more than half the width of leaflets, which, in turn, would possibly cause rejection of shorter leaflets for their correlated insufficient width. Yet, careful examination reveals that this would only partially explain the weak exploitation of the shortest leaflets in proximal position 6-7 and, in any case, would leave entirely unexplained the decrease of exploitation of leaflets of intermediate sizes, located in intermediate positions 4-5 and 2-3, as compared to the distal leaflet.

Thus, the preference of *Megachile* females for longer (and, correlatively, more distal) leaflets far oversteps the available resource, proportionate to the leaflets size. Accordingly, there seems to be something more (in addition to the larger width and longer contour of the leaflets), which strongly attracts *Megachile* females towards either longer or more distal leaflets. For example, besides their larger average size, the leaflets in more distal positions may also be more readily approached by arriving bees and, accordingly, more frequently probed and thus more frequently adopted by females, in addition to the attractive dimensions of the lamina. Alternatively, the physical properties of the lamina may be involved in bees' preference; for example, the elasticity of leaflets lamina might seem to bees, on average, a little bit insufficient in *Rosa*; it might then be conceived that bees tend to prefer larger leaflets not because of their size, but instead possibly due to their greater elasticity. This is, yet, rather unlikely since leaves substantially softer than those or *Rosa* —for example those of *Calystegia*— are also commonly selected by *Megachile*.

Interestingly enough, similar trends of preference, in favour of a strongly enhanced exploitation of the larger and more distal leaflets of *Rosa canina*, are also observed in a series of gall-inducing insects upon wild roses leaflets: the sawfly *Blennocampa phyllocolpa* Viitasaari & Vikberg, 1985, the cynipoid wasp *Diplolepis* gr. *eglanteriae* (Hartig, 1840) and the midge *Wachtliella rosarum* (Hardy, 1850) (Béguinot, unpublished results).

Whatever the true reason, the general aptitude of many insects to discriminate and select among leaves or leaflets of a single plant, so as to satisfy their needs, has been extensively documented, especially for gall-inducing or mine-forming species: SUOMELA & AYRES (1994); WALLIN & RAFFA (1998); GRIPENBERG (2007); GRIPENBERG & ROSLIN (2005, 2007); ROSLIN *et al.* (2006); GRIPENBERG *et al.* (2007a, b); CORNELISSEN *et al.* (2008); BÉGUINOT (2009a, b, 2011, 2012). In particular, mothers' selection according to *leaf size* within a single tree is often reported: WHITHAM (1978); KIMBERLING *et al.* (1990); WHITHAM (1992); KAGATA & OHGUSHI (2001); CORNELISSEN *et al.* (2008).

The reasons for such preference in many gall-inducers and other herbivore insects have also been analysed and discussed extensively, and the "host-plant vigour hypothesis" has often been referred to in this respect (KIMBERLING *et al.*, 1990; PRICE, 1991). This hypothesis might seem

more likely for gall-makers since the galled tissues are intended to perform at best regarding both their ability to insure proper gall development and their capacity as a resource supplier for the enclosed larva. For discs' extractors, such as *Megachile*, the argument seems less convincing.

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