Resource availability affects investment in carnivory in *Drosera rotundifolia*

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**Summary**

- Carnivory in plants is restricted to nutrient-poor and open habitats presumably because of high benefits and/or low costs of carnivory in these conditions. Carnivory is costly because the plants need specific adaptations to capture prey. *Drosera rotundifolia* produces sticky substances on its leaf surface to catch prey. These substances are primarily carbon-based, and their production can be expected to be lower in shade. The benefit of carnivory is in terms of the increased nutritional gain which will be low when inorganic nutrients are available in the growth medium. We expected that investment in carnivory would be lower in shade and nutrient-rich conditions.

- A factorial experiment involving shading and the addition of inorganic nutrients confirmed these predictions in the carnivorous, perennial herb *D. rotundifolia*.

- Plants growing in shade or in media with nutrients added had less sticky leaves and had reduced their investment in carnivory.

- Interestingly, the observed changes in the stickiness of the leaves were in accord with the carbon/nutrient balance theory, whereas a carbon-based secondary compound, 7-methyljuglone, in the leaves did not respond to shading or nutrient addition.

**Key words:** carnivorous plant, *Drosera* (sundew), leaf stickiness, prey capture, carbon/nutrient balance theory, shade, inorganic nutrients.


**Introduction**

Plants have several ways of taking up nutrients; one highly specialized way is through carnivory. Carnivorous plants capture and utilize nutrients in prey, which consists mostly of insects (Darwin, 1875; Juniper *et al*., 1989). They are restricted to conditions where low nutrient availability limits fitness rather than light or water (Ellison & Gotelli, 2001). They have elaborate adaptations to capture prey and use their nutrients, including specialized leaves, the secretion of sticky substances, digestive enzymes and nectar to attract prey. As these adaptations and investments in carnivory are costly, carnivorous plants have lower photosynthetic capacity, lower photosynthetic nitrogen use efficiency, weaker competitive ability and smaller root systems than noncarnivorous plants (Knight, 1992; Svensson, 1995; Karlsson *et al*., 1996; Brewer, 1998; Mendez & Karlsson, 1999). The cost of carnivory is mostly carbon-based whereas the benefits are related to the nutritional gain. If the amount of available nutrients in the growth medium increases, the benefits of carnivory will decrease while the costs remain constant and, hence, the investment in carnivory should decrease. If light becomes limiting, the relative cost of carnivory will increase as the carbon available from photosynthesis decreases while the benefits remain constant and a decrease in investment is expected.

The carnivorous plant *Drosera rotundifolia* (Droseraceae) is a small perennial carnivorous herb with a boreo-circumpolar distribution (Crowder *et al*., 1990). The plant forms basal leaf rosettes with one or several flower stalks (Slack, 1979). Each rosette overwinters as a bud of tightly rolled leaves (resting bud or hibernaculum), just below the surface, which is the only somatic part that survives the winter. The species typically grows in *Sphagnum fuscum* mats and faces competition from the *Sphagnum* moss (Svensson, 1995), but it may also grow on bare sand or peat. A substantial part of its nutrients appears to come from prey, around 50% (Millett, Jones & Waldron, 2003), and it benefits from prey capture in terms of increased growth and reproductive ability (Kraft & Handel,
In the present study we investigated the effects of different levels of light and inorganic nutrients in the growth medium on the investment in carnivory, amount of secondary metabolite, growth and reproduction in *Drosera rotundifolia*. Using a method based on work of Zamora *et al.* (1997, 1998) we quantified the investment in carnivory in terms of the stickiness of the leaves, and we expected to find low investment in carnivory under conditions of high soil nutrients and shade.

**Materials and Methods**

The experiment was conducted outdoors under ambient conditions at the experimental field of Botanical Gardens of Oulu University (Finland). Average temperature in each month from June to October was 14.3, 15.2, 11.0, 7.5 and 0.0°C, respectively. Average incoming radiation for the same period was 219, 147, 82, 70 and 16 W m⁻². The *D. rotundifolia* L. plants were grown from the middle of June to October when all the leaves had senesced and the plants were entering winter dormancy.

The plants were subjected to two treatments in a factorial design: the addition of inorganic nutrients to the growth medium (low N and high N) and light (sun and shade). We used micropropagated plants from four different genetic individuals originating from two provenances. Ten replicate plants from each genetic individual were used in each treatment group, giving a total of 160 plants. There were no interactions between genetic individuals and treatments (statistics not shown), and the genetic individuals were not further taken into account in the final statistical analyses. We measured the size of each plant as the diameter of the rosette after they had become established but before any treatment started, and this was used as the covariate in the statistical analyses.

The plants were planted individually in a very nutrient-poor peat–sand mixture in 6 × 6 × 5.5 cm pots, which were large enough for leaves and root system of *D. rotundifolia*. The pots were distributed on 16 large trays, 10 plants on each tray, with two or three plants from each genetic individual on each tray, making four different set-ups (four blocks of four trays each) which were randomized with regard to treatment. Half of the plants were placed under four screens consisting of a large enough for leaves and root system of *D. rotundifolia*, making four different set-ups (four blocks of four trays each) which were randomized with regard to treatment. Half of the plants were allocated to high nutrient treatment (1 l nutrient in water per week with nitrogen concentration of c. 0.05 mM). The nutrient was added to the plants by filling the tray to a depth of about 3 cm with nutrient solution, the plants were left standing for 24 h and then removed. This was repeated at weekly intervals. The treatment was same as that used by Karlsson *et al.* (1991). The nitrogen supplied to each pot was, if all would have been taken up, 70 mg in the high nutrients treatment and 0.7 mg in the low nutrient treatment. Total available nitrogen in the peat–sand mixture was 3 mg per pot.

Because the ability of the leaf to capture and hold insects depends on the sticky substances on the leaf surface (Zamora *et al.*, 1998), we measured the investment in carnivory in terms of the stickiness of the leaves. The stickiness depends on the amount of mucus produced and the concentration of polysaccharides in the mucus. The stickiness was measured by taking a 1 × 1 cm piece of filter paper and attaching it to a dynamometer. The piece of filter paper was pressed onto a healthy leaf, covering all the tentacles on the leaf, and the dynamometer was pulled upwards. A reading was taken when the paper separated from the leaf. This produced a rough measurement, but we are confident that it yielded relative values of the stickiness and thus the investment in carnivory. Measurements were made randomly on 10 replicate plants in each treatment group in the middle of July.

The plant produces a specific phenolic compound, 7-methyljuglone, which provides defence against microbes and herbivores (Harbone & Baxter, 1993). Extraction of 7-methyljuglone was carried out according to Bonnet *et al.* (1984), with minor modifications by Kämäräinen *et al.* (2003). When the plants were flowering, two plants from each treatment, tray and genetic individual were selected for the analysis of 7-methyljuglone (a total of 32 plants). The plants were harvested and the roots removed. The fresh weight was then measured. The plants were crushed in liquid nitrogen and extracted with toluene. Juglone (0.5 mg) was added as an internal standard. The extract was filtered and evaporated to dryness. The residue was dissolved in 1 ml acetonitrile and analysed in a gas chromatograph.

The number of flowers per plant was counted. The resting buds were harvested after the leaves had senesced. Only the weight of resting buds was measured as the roots appeared to be dead. A subsample of the resting buds was analysed for nitrogen and phosphorus concentration. Thirty-two resting buds (eight from each treatment group, two from each tray) were dissolved in HCl and analysed using the Kjeldahl method.

Factors affecting the concentration of 7-methyljuglone, number of flowers, weight of resting buds, ‘stickiness’ (relative investment in carnivory), and nitrogen and phosphorus concentrations in resting buds were determined using analysis of variance with initial size as covariate for number of flowers and bud weight. The independent variables were the light and nutrient treatments. All statistical analyses were performed with SPSS 10 (SPSS Inc., Chicago, MI, USA) and follows Sokal & Rohlf (1995).
Results

The addition of inorganic nutrients to the growth medium had a strong negative effect on the investment in carnivory: the ‘stickiness’ decreased by 45% (Fig. 1). Shading of the plants had a weaker negative effect (14%). In both cases the reduction was statistically significant (Table 1). Neither inorganic nutrients nor shade affected the production of the secondary metabolite 7-methyljuglone when measured as concentration (Tables 2 and 3) or amount produced (statistics not shown). Shaded plants produced fewer flowers (a decrease of 26%), whereas nutrient addition had no significant effect on flowering (Tables 2 and 3). The weight of the resting buds was unaffected by the treatments (Tables 2 and 3). Nitrogen concentration in the buds decreased slightly in shade (9%) but was unaffected by the addition of inorganic nutrients (Tables 1 and 3). By contrast, the phosphorus concentration in the buds increased by 13% in the nutrient addition treatment and decreased by 23% in shade (Tables 1 and 3).

Discussion

Increasing the amount of soil nutrients significantly reduced the plants’ investment in carnivory. In a recent study, Ellison & Gotelli (2002) found similar results when adding nitrogen to natural populations of the carnivorous plant *Sarracenia purpurea*. This suggests that carnivorous plants may switch their resource allocation from nutrient uptake through carnivory to nutrient uptake through roots when the soil nutrient status is improved. This switching is in accord with

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Table 1

<table>
<thead>
<tr>
<th></th>
<th>Stickiness</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Light (L)</strong></td>
<td>0.0048</td>
<td>28.76</td>
<td>7.66</td>
</tr>
<tr>
<td></td>
<td>7.38</td>
<td>12.28</td>
<td>81.01</td>
</tr>
<tr>
<td><strong>Light × Nutrients (N)</strong></td>
<td>0.0014</td>
<td>7.43</td>
<td>1.76</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td>0.2636</td>
<td>3.09</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th></th>
<th>7-Methyljuglone</th>
<th>Flowers</th>
<th>Resting bud</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Covariable</strong></td>
<td>0.26</td>
<td>0.26</td>
<td>1343.34</td>
</tr>
<tr>
<td><strong>Light (L)</strong></td>
<td>0.002</td>
<td>0.45</td>
<td>26.16</td>
</tr>
<tr>
<td></td>
<td>0.38</td>
<td>0.55</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Nutrients (N)</strong></td>
<td>0.002</td>
<td>0.85</td>
<td>9.47</td>
</tr>
<tr>
<td></td>
<td>0.84</td>
<td>1.68</td>
<td>1.90</td>
</tr>
<tr>
<td><strong>L × N</strong></td>
<td>0.194</td>
<td>0.16</td>
<td>6.45</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td>1.440</td>
<td>0.26</td>
<td>5791.92</td>
</tr>
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SS, type III sum of squares; F, Fischers F-value; P, probability of significance; r², error sum of squares. Mean values and SE are given in Table 3 and Fig. 1. Degrees of freedom are 1,37 for stickiness and 1,28 for nitrogen and phosphorus.

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the expected lower benefit of carnivory in nutrient richer conditions. Interestingly, Zamora et al. (1998) found an increase in the investment in carnivory of *Pinguicula vallisneriifolia* when the plants were fed by insect prey. Furthermore, a decrease in photosynthate availability because of shading resulted in a lower investment in carnivory both in Zamora et al. (1998) and in the present study. This indicates a high cost of carnivory which could explain why carnivory is restricted to certain nutrient-poor and open habitats.

Interestingly, nutrient addition to the growth medium seemed not to affect growth or reproduction significantly. The phosphorus content of the buds increased, while the nitrogen content was not affected or slightly decreased. These effects differ from previous results obtained when carnivorous plants were fed with insects. Feeding tended to increase the size of resting buds and nitrogen and phosphorus concentration in resting buds of *Drosera rotundifolia* expressed as mean values and SE

<table>
<thead>
<tr>
<th>Condition</th>
<th>7-Methyljuglone (mg g⁻¹)</th>
<th>Flowers (n)</th>
<th>Resting bud (mg)</th>
<th>Nitrogen (mg g⁻¹)</th>
<th>Phosphorus (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sun/low nutrients</td>
<td>0.70 ± 0.10</td>
<td>3.02 ± 0.34</td>
<td>13.5 ± 1.71</td>
<td>21.3 ± 0.46</td>
<td>4.02 ± 0.14</td>
</tr>
<tr>
<td>Sun/high nutrients</td>
<td>0.87 ± 0.10</td>
<td>2.95 ± 0.43</td>
<td>12.4 ± 0.95</td>
<td>20.9 ± 0.73</td>
<td>4.64 ± 0.05</td>
</tr>
<tr>
<td>Shade/low nutrients</td>
<td>0.87 ± 0.05</td>
<td>2.30 ± 0.30</td>
<td>13.8 ± 1.38</td>
<td>20.0 ± 0.54</td>
<td>3.19 ± 0.08</td>
</tr>
<tr>
<td>Shade/high nutrients</td>
<td>0.73 ± 0.06</td>
<td>2.10 ± 0.40</td>
<td>14.8 ± 1.60</td>
<td>18.4 ± 0.38</td>
<td>3.51 ± 0.14</td>
</tr>
</tbody>
</table>

Corresponding statistical analyses are shown in Tables 1 and 2.

Another aspect that deserves attention is that the observed changes in investment in carnivory are in agreement with the predictions of the carbon–nutrient balance theory of Bryant et al. (1983), which explains phenotypic plasticity in secondary metabolism by means of the relative availability of carbon and inorganic nutrients. The theory predicts that carbon-based secondary compounds tend to accumulate under conditions of low availability of inorganic nutrients and bright light. Thus, the addition of nutrients or shading should decrease the concentrations of such compounds. This was indeed reflected in the stickiness of the *Drosera* leaves. By contrast, the specific carbon-based substance, 7-methyljuglone, did not behave in this manner. There are significant genotypic differences between the plants in their 7-methyljuglone content (Kämäräinen et al., 2003; L. M. Thorén unpubl. data), but no phenotypic plasticity was observed in relation to the present manipulation of resource availability. This may indicate that either the sticky substances on the leaves and 7-methyljuglone are subject to different metabolic constraints (Koricheva et al., 1998), or that their differing responses to resource availability may be related to their differing functions, being carnivory and defence, respectively.

The present experimental study suggests that this carnivorous plant species is able to respond to an increase in the availability of inorganic nutrients in the growth medium by reducing the investment in carnivory.

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**References**


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