The Evolutionary Ecology of Plants

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IS EXTRAFLORAL NECTAR PRODUCTION AN INDUCIBLE DEFENSE?

Suzanne Koptur

INTRODUCTION

Extrafloral nectaries are plant glands located outside the flowers; they are widespread among the angiosperms, occurring in 68 of the 337 families, in 35 of 76 orders, 5 of 6 of the subclasses of dicotyledons, and 3 of the 4 subclasses of monocots (Elías, 1983). They occur on leaves, stipules, petioles, stems, bracts, sepals, and fruits (Bentley, 1977b), and their position is often related to their ecological role (see below). Nectaries are also found in some ferns, and may have similar ecological functions in ferns as in flowering plants (Koptur et al., 1982; Page, 1982; Lawton and Heads, 1984).

Evidence for ants visiting extrafloral nectaries and providing protection against herbivores is abundant and ever-increasing (reviewed by Bentley, 1977b; Buckley, 1982; Beattie, 1984; Jolivet, 1986). Ants visiting nectaries on vegetative parts may protect foliage (Bentley, 1976; Janzen, 1966, 1967; Koptur, 1979, 1984; Tilman, 1978; Kelly, 1986) which in turn can translate into greater seed set and increased fitness for the plant with nectaries (Koptur, 1979; Stephenson, 1982; Barton, 1986). Nectaries on or near reproductive structures can provide ant protection of developing ovules and seeds (Elías and Gelband, 1975; Bentley, 1977a; Deuth, 1977; Inouye and Taylor, 1979; Schemske, 1980, 1982; Keeler, 1981; Horvitz and Schemske, 1984).

Extrafloral nectaries can also attract predators or parasitoids of the herbivores (Leius, 1967; Price et al., 1980; Hespenheide, 1985; Weis and Abrahamson, 1985). Parasitoids can serve as important biological control agents in agricultural systems (Crepps, 1975; DeBach, 1964; Hassell, 1980, 1982). This sort of plant protection can be extremely important in natural systems as well, especially in areas where ants are not abundant (Keeler, 1985; Koptur, 1985).

Some plants can respond to defoliation by producing more defensive chemicals when attacked (Fowler and Lawton, 1985; Greig-Smith, 1986). The response of individual plants to defoliated areas with increased levels of feeding deterrents or toxins has been demonstrated in a number of species (Rottger and
My goal is to test the hypothesis that plants with extrafloral nectaries will respond to damage by secreting more extrafloral nectar. Increased amounts of extrafloral nectar could lead to greater numbers of ants or parasitoids being recruited to the plant, which could lead to increased protection against herbivores. I have looked for extrafloral nectar induction in several systems, in which biotic protection by ants and/or parasitoids has been demonstrated experimentally.

METHODS

*Vicia sativa* L. (Fabaceae: Papilionoideae), the common vetch, is an annual herbaceous legume native to the Old World. The plants bear stipular nectaries, and in both exotic (Koptur 1979) and native (Koptur and Lawton, 1988) habitats, ants visiting these nectaries can provide protection against insect herbivores for foliage, flowers, and developing fruit. The flowers and fruits of most *Vicia* species are borne in the axils of the leaves, and in species with stipular nectaries (such as *V. sativa* and *V. sepium*) the flowers are sessile or on very short stalks, compared with species without nectaries (such as *V. cracca* and *V. hirsuta*) which have long peduncles. Nectar secretion in *V. sativa* begins when plants initiate anthesis, and continues through fruit maturation (Koptur and Lawton, 1988); therefore, the plants become established without the aid of biotic protection, but can benefit from ants and other nectary visitors throughout their entire reproductive life.

Fifty plants growing in a greenhouse at the University of York, England, were randomly divided into five groups of ten plants each. Initial nectar production was measured, using the technique developed by Irene and Herbert Baker for measuring small quantities: micropipettes pulled out to fine points (and therefore, uncalibrated) are used to draw up the nectar and spotted onto strips of filter paper; spot diameter can be correlated with volume (Baker, 1979). Using scissors to cut a fraction off of each leaflet, the plants were all defoliated to the level designated for the group (0%, 25%, 50%, 75% and 100% damage).

Nectar production was measured each afternoon for four days following the defoliations. Because this experiment was done in a controlled environment, it was not necessary to exclude nectary visitors or to bag plants. This experiment was initially attempted in the field, with much difficulty (rainy weather and difficulty in bagging small plants).

*Ipomoea carnea* (Convolvulaceae) is an emergent perennial morning-glory, growing in marshland in the lowland forests of...
day 2, I measured initial nectar accumulation, and then performed depletions. On day 3, I measured nectar again.

RESULTS

Vicia. Variability within groups was high (Table 1); the results are summarized (means only) in Fig. 1. The only statistically significant difference on day 1 after defoliation: analysis of variance showed that nectar production was higher with treatment (p < .05) and a posteriori tests between means showed the 25% and 50% treatments to be significantly different from the control group.

The effect disappeared the next day. With greater defoliation, there was no initial increase in nectar secretion over controls.

Ipomoea. The one-day experiment gave distributions of nectar secretion shown in Fig. 2; half the plants produced no nectar at all, making parametric statistics inappropriate.

Inga. Inga brenessii nectar volumes (Table 3) were very close in the two groups before the experiment, and just as close after the experiment (Kruskal-Wallis H not significant). Most leaves secreted nectar, but the data were not normally distributed, so this non-parametric test was used.

Inga punctata nectar volumes (Table 4) showed greater differences between groups, and changes in the direction of an inducible response, but variability was great, and no differences were significant. For both species of Inga, therefore, the null hypothesis is accepted.

DISCUSSION

Unpredictable weather and the difficulties of excluding ants and bagging vetch plants in the field led to the conducting of that experiment under controlled greenhouse conditions. This was the only experiment in which any significant results were obtained. Although it was much easier to exclude ants and bag the perennial...
Table 1. *Vicia* nectar volumes from plants in greenhouse experiment, volumes are in microliters, $\bar{x}$ ± s.d.

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>25% damage</th>
<th>50%</th>
<th>75%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>.67 ± .37</td>
<td>.70 ± .52</td>
<td>.60 ± .57</td>
<td>.73 ± .56</td>
<td>.78 ± .68</td>
</tr>
<tr>
<td>1*</td>
<td>.39 ± .41</td>
<td>.96 ± .40</td>
<td>.58 ± .22</td>
<td>.52 ± .27</td>
<td>.40 ± .33</td>
</tr>
<tr>
<td>2</td>
<td>.20 ± .15</td>
<td>.37 ± .19</td>
<td>.37 ± .25</td>
<td>.33 ± .27</td>
<td>.25 ± .16</td>
</tr>
<tr>
<td>3</td>
<td>.17 ± .23</td>
<td>.35 ± .29</td>
<td>.19 ± .16</td>
<td>.15 ± .13</td>
<td>.20 ± .15</td>
</tr>
<tr>
<td>4</td>
<td>.20 ± .17</td>
<td>.24 ± .16</td>
<td>.23 ± .17</td>
<td>.16 ± .13</td>
<td>.17 ± .10</td>
</tr>
</tbody>
</table>

* Indicates only day in which there was any effect of treatment on nectar volume (by analysis of variance): "*" - $p < .025$, "**" - $p < .05$.

Table 2. *Ipomoea carnea* two day experiment. Nectar volumes produced in microliters ($\bar{x}$ ± s.d.).

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>.26 ± .27</td>
<td>.56 ± .59</td>
</tr>
<tr>
<td>Control</td>
<td>.26 ± .28</td>
<td>.47 ± .58</td>
</tr>
<tr>
<td>t-test difference</td>
<td>NS</td>
<td>t = .53, NS</td>
</tr>
</tbody>
</table>

(n = 25 each)

Figure 1. Nectar volumes from *Vicia* greenhouse experiment, to accompany Table 1. Only means were plotted. Note that each line depicts a day, with the treatment groups along the x-axis. The y-axis is mean nectar volume in microliters.

Figure 2. *Ipomoea carnea* one day experiment. Frequency distribution of nectar production for the different treatment groups.
woody plants in the field, any experiment conducted outside is subject to increased variability due to changes in ambient humidity, wind, and other factors. When small amounts of nectar were involved (the usual situation for extraloral nectaries), environmental effects on variability were accentuated. This caveat must be borne in mind before the possibility of inducible nectar production is discarded for a given species. Under greenhouse conditions, an increase in extraloral nectar production was seen only with moderate levels of defoliation. It may be that with greater leaf area loss, the reduction of photosynthate produced by a given leaf did not allow for increased nectar production. It has been suggested (Sarah Corbett, pers. comm.) that the response may simply be due to increased transpiration from cut leaf surfaces resulting in a sort of surge of liquid in the nectaries; I plan to test this by measuring floral nectar as well (since the flowers are situated in the axils of each leaf). If floral nectar increases when extraloral nectar does, then perhaps it is only a physiological response associated with increased transpiration; if, however, floral nectar is not affected as is extraloral nectar, the possible defensive role of this response will be supported.

I also plan to look for differences in nectar quality in response to defoliation. It is possible that, under stress, certain compounds change in concentration, and alter the attraction to biotic protective agents.

It is especially important to determine whether or not increased amounts of extraloral nectar result in greater protection for the plant. This will be tested in field experiments in suitable systems. We will also look for this response in more systems where biotic protection has been found to be significant, in order to determine the general circumstances under which inducible extraloral nectar production might occur.

At this point, I predict that herbaceous, annual plants will be more likely to show inducible nectar secretion than woody perennials. Annual species, which can reproduce only once, are known to expend more effort in reproduction than do perennials. They may also be able to economize on extraloral nectar production, and produce more when herbivores are present. Plants which have extraloral nectar as the sole reward for biotic protective agents will also be more likely to show this response than plants which offer a combination of rewards (e.g., food bodies and domicile in addition to nectar). Only in situations where ants are not resident in plants can more ants be recruited to plants or to plant parts with increased nectar production. Less is known about the response of parasitoids to changes in nectar rewards, and

Table 3. *Inga brennessi* nectar volumes in microliters.

<table>
<thead>
<tr>
<th>Experimental</th>
<th>Control</th>
<th>Kruskal-Wallis H</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>.16 + .09</td>
<td>14 + .57</td>
<td>&lt;.5 NS</td>
</tr>
<tr>
<td>After</td>
<td>.12 + .13</td>
<td>.10 + .25</td>
<td>&lt;.7 NS</td>
</tr>
</tbody>
</table>

Table 4. *Inga punctata* nectar volumes

<table>
<thead>
<tr>
<th>Experimental</th>
<th>Control</th>
<th>Kruskal-Wallis H</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>.04 + .08</td>
<td>.25 + .99</td>
<td>&lt;.5 NS</td>
</tr>
<tr>
<td>After</td>
<td>.24 + .39</td>
<td>.18 + .30</td>
<td>&lt;.3 NS</td>
</tr>
</tbody>
</table>
so it is tempting to say that the inducible response will be more important in plants with ant protection than parasitoid protection resulting from extrafloral nectaries; but clearly, more information is needed.

Nectaries can play a role in agricultural systems in the biological control of crop pests (de Bach, 1964; Huffaker, 1971; Simmonds, 1971; Bentley, 1976). Ants and other insects attracted to extrafloral nectaries can reduce the effects of herbivores on crop plants (Bentley, 1977b, 1983; Risch and Carroll, 1982a, b), and although these interactions have not been exploited commercially, they may hold great potential. Recruiting natural protective agents can lessen the use of pesticides, reducing pollution and producing less-contaminated crops. A number of crop plants and their wild relatives (both temperate and tropical) have extrafloral nectaries: cotton (Gossypium) (Mound, 1962; Yokoyama, 1978; Adjei-maafio and Wilson, 1983), broad bean (Vicia) (Kupicha, 1976; Koptur, 1979), passionfruit (Passiflora) (Durkee, 1982; McLain, 1983; Smiley, 1986), peaches, plums, and cherries (Prunus) (Gregory, 1915; Dorsey and Weiss, 1920; Tilman, 1978), castor bean (Ricinus) (Reed, 1923; D.A. Baker et al., 1978), sweet potato (Ipomoea) (Beckmann and Stucky, 1981; Keeler, 1977, 1980), yam (Dioscorea) (Orr, 1923; Grout and Williams, 1980), rubber (Hevea) (Parkin, 1904, and buckwheat (Polygonum) (Salisbury, 1909). These traits could be utilized (or introduced from the genome of wild relatives into cultivars) for ecologically sound crop protection.

Stress on plants caused by insect herbivores has led to the evolution of many defenses, and the ability of some plants to regulate the amounts and locations of defensive substances is ecological fine-tuning that can permit adjustment of the individual to prevailing environmental conditions.

SUMMARY AND CONCLUSIONS

A number of chemical defenses of plants have been found to increase in response to presence of, or damage by herbivores, or both. Extrafloral nectaries attract ants and other beneficial insects that can provide biotic protection against a wide variety of herbivores. My goal was to test the hypothesis that plants predisposed to this type of defense (by virtue of having extrafloral nectaries) will respond to damage by secreting more extrafloral nectar. Increased amounts of nectar could serve to attract a greater number of ants or parasitoids and thereby increase biotic protection subsequent to incidents of damage.

I tested the hypothesis in three systems: 1) the annual, herbaceous legume Vicia sativa; 2) the perennial woody morning glory, Ipomoea carnea; and 3) several Inga species, neotropical legume trees. Only in greenhouse experiments with Vicia sativa was the inducible response found: on the day following defoliation, plants defoliated to 25% and 50% levels secreted more extrafloral nectar on the average than control plants. Higher levels of defoliation (75% and 100% of the leaflets removed) did not induce any differences in nectar secretion. In field experiments with Ipomoea and two Inga species, there were no significant differences in nectar secretion between defoliated branches and controls.

More plants must be tested for this response before firm generalizations can be made. At this point, it appears that annual plants may be more likely to show inducible extrafloral nectar secretion than perennials, and herbaceous plants more than woody plants. A number of crop plants have extrafloral nectaries, and there is potential for use of this natural defense system in integrated pest management schemes. If plants are able to respond to varying levels of herbivory by adjusting their levels of extrafloral nectar secretion, this ability to economize when the defense is not needed can be an asset to increased growth and reproduction and, thereby, selective fitness.

ACKNOWLEDGMENTS

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