Annals of Botany **87**: 365–370, 2001
doi:10.1006/anbo.2000.1342, available online at http://www.idealibrary.com on IDEAL®

The Effect of Herbivory on Temporal and Spatial Dynamics of Foliar Nectar Production in Cotton and Castor

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Received: 4 July 2000 Returned for revision: 18 August 2000 Accepted: 20 November 2000 Published electronically: 26 January 2001

The effects of feeding *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) larvae on the quantity and distribution of extrafloral nectar production by leaves of castor (*Ricinus communis*) and cotton (*Gossypium herbaceum*) were investigated. Following larval feeding, the total volume of nectar secreted by foliar nectaries increased 2·5- and 12-fold, respectively. As HPLC-analysis showed no difference in sugar composition between extrafloral nectar from insect-damaged and control plants, it can be concluded that the plants increased the secretion of carbohydrates in response to herbivory. In damaged castor leaves, the amount of sugar excreted through extrafloral nectaries represented approx. 1‰ of the leaf's daily assimilate production. Induction of nectar production was mainly restricted to the damaged leaf, although a weaker systemic response was found in adjacent younger leaves. Spatial and temporal patterns of induced nectar production could help plants to optimize indirect defence by concentrating the recruitment of predators and parasitoids on the site, and at the time, of attack.

Key words: *Gossypium herbaceum* (L.) (Malvaceae), cotton, *Ricinus communis* (L.) (Euphorbiaceae), extrafloral nectar, indirect defence, herbivory, induction, defence distribution, spatial, temporal, constitutive defence, defence costs.

Plants possess a broad range of morphological and chemical adaptations to limit the negative impact of herbivory directly. Plants are also believed to have developed adaptations that sustain or promote the efficacy of predators and/or parasitoids, which can protect the plant indirectly. These adaptations include the emission of volatile compounds, which attract predators and parasitoids to plants with feeding herbivores (Dicke and Sabelis, 1988; Turlings *et al*., 1990), as well as structures providing shelter (domatia) and different types of nutritional supplement (food bodies, extrafloral nectaries) (Whitman, 1996).

Extrafloral nectaries have been described in approximately 1000 plant species ranging over 93 families (Koptur, 1992). They are generally believed to be catering for ants (Bentley, 1977), but they may also help sustain other predators (Bakker and Klein, 1992) or parasitoids (Lingren and Lukefahr, 1977; Koptur, 1992; Whitman, 1996; Stapel *et al*. 1997). It has been established that antagonists visiting extrafloral nectaries can reduce herbivory (O'Dowd, 1979; Wagner, 1997) and increase plant reproductive fitness (Rico-Gray and Thien, 1989; Oliveira, 1997).

Many secondary plant metabolites with a presumed role in direct plant defence have herbivore-inducible biosynthetic pathways (Baldwin, 1994), and their induction has been interpreted as optimizing defence (Edwards and Wratten, 1983; Stout *et al*., 1996; Zangerl and Rutledge, 1996). Herbivory may also result in the induction of indirect defences. For example, many plant species respond to herbivory by emitting specific volatiles (e.g. Dicke and Sabelis, 1988; Turlings *et al*., 1990). These volatiles are employed by arthropod predators (Dicke, 1994; Drukker *et al*., 1995) as well as parasitoids (Turlings *et al*., 1990; Dicke and Vet, 1998) in locating the feeding arthropods. Little information is available on the question of whether extrafloral nectar production is inducible in response to herbivory. Koptur (1989) reported that *Vicia sativa* plants responded to one of four levels of herbivory with a 2·5-fold increase in nectar volume. Three other plant species failed to show induction. Smith *et al*. (1990) found an increase in amino acid concentration following herbivory, but no effect on nectar volume. Whether plants actually increase their carbohydrate secretion in response to herbivory remains to be demonstrated. Furthermore, there is no information on the spatial dynamics of insect-induced nectar production (localized and systemic effects).

This study addresses the question of how castor (*Ricinus communis* L.) and cotton (*Gossypium herbaceum* L.) adjust extrafloral nectar production in response to herbivore feeding. Although mechanical damage can be sufficient to induce extrafloral nectar production (Wäckers and Wunderlin, 1999), here we studied the effect of actual insect feeding. By inflicting herbivore damage at a specified time and location, followed by repeated nectar collections at fixed time intervals, the temporal and spatial dynamics of nectar secretion in response to insect feeding were assessed.

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0305-7364/01/030365+06 $35.00/00

MATERIALS AND METHODS

Plants

Castor (*Ricinus communis* L.; Euphorbiaceae) and cotton (*Gossypium herbaceum* L.; Malvaceae) were sown individually in 5 cm diameter pots and placed in a climate chamber to germinate (14 light (L):10 dark (D); T = 26 °C (day)/10 °C (night); relative humidity (RH) 80 %). Two weeks after germination, seedlings were transferred into either 9 × 9·5 cm pots (cotton) or 16 × 18 cm pots (castor) and placed in a glasshouse (16L:8D; T = 16–30 °C; RH = 60–80 %). In the experiments, 8-week-old plants, free of any visible damage, were used.

Nectar collection experiments

Clip-on cages were constructed from a pair of Plexiglas rings of either 3·5 or 6 cm diameter. The connecting inner sides were coated with foam rubber to prevent plant damage. The outsides were covered with screen mesh.

Spodoptera littoralis was selected as the herbivore, based on the fact that both plant species serve as hosts for *S. littoralis* (Kranz *et al.*, 1977). Larvae were kindly provided by Novartis (Basle, Switzerland). Early second instars were transferred from an artificial diet to leaves of either castor or cotton. Larvae were habituated on the plant species on which they were to feed during the experiments until they moulted to the third instar. Early third instars were used in the experiment.

Experimental procedure

Castor. At the time of the experiment, the 8-week-old castor plants had produced ten–11 leaves, of which the first four or five had already abscissed. Four days before applying the cages, groups of four plants were placed in a climate chamber (16L:8D/210 µmol m^{-2} s^{-1}; T = 25 °C; RH = 99 %) and watered every other day (500 ml water per plant). The high humidity was necessary to facilitate collection of the otherwise highly viscous nectar. At the start of the experiment (day 0), a clip-on cage (6 cm diameter) was attached to the ninth leaf of all plants. Cages covered approx. 20 % of the total leaf surface. For half of the plants, a single early third instar *S. littoralis* larva was introduced into the clip-on cage (*Spodoptera* treatment), while the remaining plants had empty cages (control). After 48 h (day 2), all leaf material within the cage had been consumed and larvae and cages were removed from the plants. Nectar was collected using 5 µl micropipettes with 1 µl divisions and the collected volume was calculated based on the proportion of the pipette filled. Nectar from the three to six nectaries on each petiole was combined to permit determination of the total nectar production for each leaf. Nectar was collected at 48 h intervals, starting 2 d in advance of the treatments. On day 0, nectar was collected just before application of the clip-on cages, and the cage was removed just before nectar collection on day 2. Recordings continued until day 8, to allow time for induction effects to subside. Using this recording range it was possible to determine the onset of a potential plant response as well as its rate of decline. Each treatment (insect-damaged and control) was repeated on 20 plants.

Cotton. The 8-week-old cotton plants had produced five to six leaves, all of which were still present during the experiments. Four days before applying the cages, groups of five plants were placed into screen cages (46 × 66·5 × 70 cm) under constant climatic conditions (16L:8D/87 µmol m^{-2} s^{-1}; T = 25 °C; RH = 60 %) and subsequently watered every other day (100 ml water per plant). On day 0, clip-on cages (diameter 3·5 cm) were attached to the fourth leaf of all plants. Cages covered approx. 25 % of the total leaf area and were placed between the single nectary on the mid vein and leaf tip. The methodology and number of replicates were otherwise identical to the method described for castor.

As data were not normally distributed (Normality test, StatView), the non-parametric Wilcoxon Signed-Rank test was used for statistical analysis.

Nectar analysis

Nectar for the carbohydrate analysis was collected from the fourth leaf of cotton plants treated as described above. Leaves on half of the plants had been damaged over 48 h by a single third instar *S. littoralis* larva, while the other half of the plants remained untreated (control). Nectar samples were collected from ten plants per treatment.

Composition and concentration of soluble carbohydrates in the individual nectar samples were determined by HPLC-PAD using cation-exchange chromatography. The system consisted of a Gynotek M480 high precision pump, a Gynotek Gina 50 autosampler, a BensonBC-100 Ca Column fitted with a guard column, a Jones column heater and an EG&G Model 400 electrochemical detector. The mobile phase was deionized water supplemented with Ca-EDTA (50 mg l^{-1}) at a flow rate of 0·6 ml min^{-1} at 90 °C. The carbohydrate concentrations were determined from peak area calculations related to regression curves of standards.

Data were arcsine transformed and subsequently compared using *t*-test statistics.

RESULTS

Total nectar production per plant

Castor. Undamaged castor plants produced an average of 2·3 µl nectar per day (Fig. 1). The three youngest leaves produced more than 90 % of the total extrafloral nectar per plant. Following 2 d of feeding by *S. littoralis* larvae, damaged plants increased overall nectar production 2·5-fold compared with control plants (Wilcoxon, $P < 0{\cdot}001$; Fig. 1A). An enhanced level of nectar production persisted for 4 d following removal of the larvae. This indicates that the lag time and relaxation time of induction were less than 48 and 96 h, respectively.

Cotton. Undamaged cotton plants produced an average of 0·03 µl extrafloral nectar per day. Within 48 h of the onset of feeding by *Spodoptera* larvae, damaged plants increased nectar production 12-fold compared with control

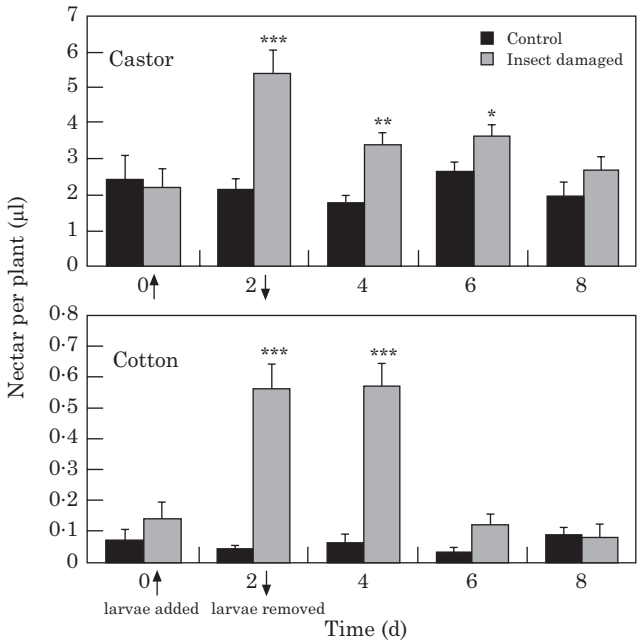

FIG. 1. Total extrafloral nectar production by insect-damaged and undamaged plants (A: castor, B: cotton) measured at 48 h intervals. *Spodoptera* larvae were applied following the nectar measurement on day 0 and removed just before the nectar measurement on day 2. Bars represent means ± s.e.m. Differences between treatments are significant at * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; and *** $P < 0{\cdot}001$ (Wilcoxon signed-rank test).

plants (Wilcoxon, $P < 0{\cdot}001$; Fig. 1C). Enhanced nectar production persisted for 2 d after removing the larvae (Wilcoxon, $P < 0{\cdot}001$), but decreased to the pre-treatment level thereafter. In this plant species both lag time and relaxation time of induction were therefore less than 48 h.

Within plant effects

Figure 2 shows the distribution of nectar production within cotton plants over 6 d. Before application of the clip-on cages (day 0), control and insect-damaged plants did not differ with respect to nectar production or distribution. Following 2 d of herbivory (day 2), the larval-damaged leaf showed a ten-fold increase in nectar production (Wilcoxon, $P < 0{\cdot}001$), while an additional (systemic) increase in nectar production was found in the younger leaves (Wilcoxon, $P = 0{\cdot}01$ and $P = 0{\cdot}005$ for leaf 5 and 6, respectively). Two days after removing the larvae (day 4), the leaves had a similar pattern of induced nectar production as recorded for day 2 (Wilcoxon, $P < 0{\cdot}001$, $P = 0.001$ and $P = 0{\cdot}001$ for leaf 4, 5 and 6, respectively). Nectar was produced primarily by the damaged leaf, indicating that induction was localized. Both localized and systemic induction decreased to pre-treatment levels within 4 d of removing larvae (day 6). Similar spatial induction patterns were found in castor (Wäckers, unpubl. res.).

Nectar analysis

Larval feeding did not affect the carbohydrate composition of the extrafloral nectar. The nectar collected from

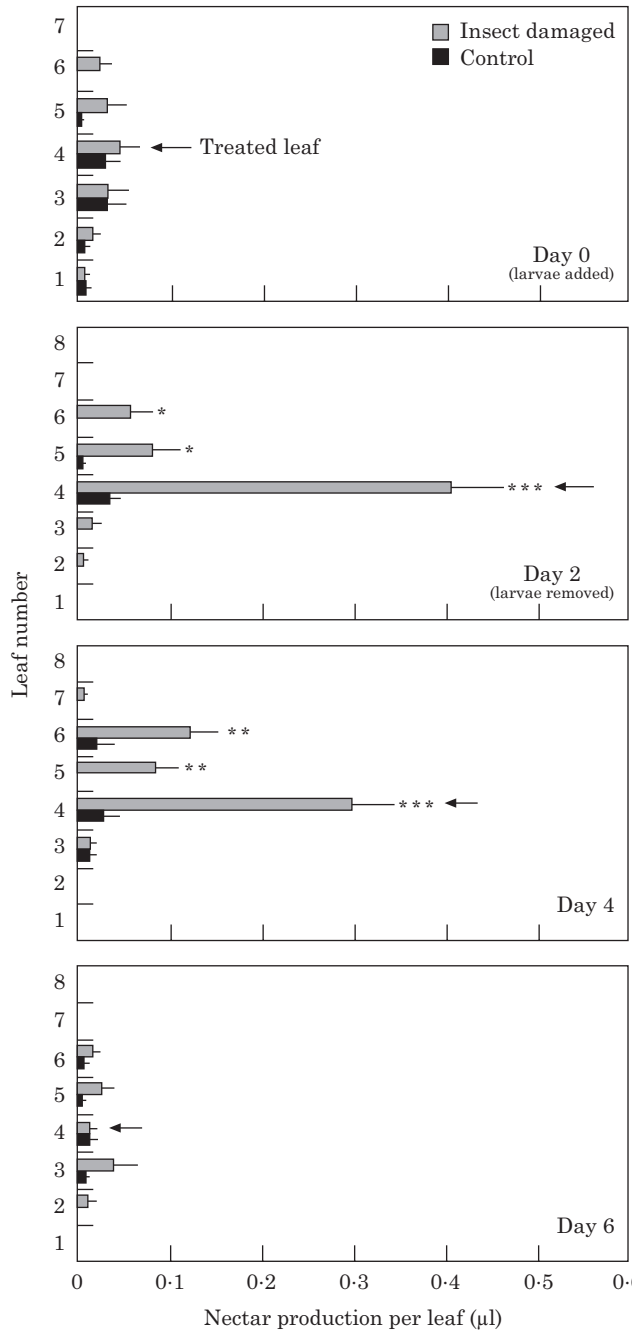

FIG. 2. Distribution of nectar production within insect-damaged and undamaged cotton plants. Data represent nectar production by individual cotton leaves over a 48 h period. *Spodoptera* larvae were applied to leaf number four following the nectar measurement on day 0 and removed just before the nectar measurement on day 2. Bars represent means ± s.e.m. Differences between treatments are significant at * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; and *** $P < 0{\cdot}001$ (Wilcoxon signed-rank test).

undamaged and insect-damaged leaves contained sucrose, fructose and glucose at comparable concentrations (Table 1). The fact that the total concentration of carbohydrates in the nectar did not differ between undamaged and *S. littoralis*-damaged plants shows that the induced

TABLE 1. *Sugar composition (% by weight) of extrafloral nectar from undamaged and insect-damaged cotton plants. Values are means of ten plants ± s.e.m*

	Sugar composition (% by weight)			
	Sucrose	Glucose	Fructose	Total
Cotton				
undamaged (control)	12·8 ± 3·1	19·2 ± 3·6	29·7 ± 6·6	61·7 ± 11·7
insect-damaged	14·5 ± 4·5	18·1 ± 4·6	26·5 ± 5·6	59·1 ± 12·3
P-value (*t*-test)				0·67
Castor				
undamaged (control)	26·7 ± 7·3	18·4 ± 6·1	28·9 ± 6·4	74·0 ± 18·4
insect-damaged	28·2 ± 8·8	21·1 ± 7·6	26·3 ± 7·8	75·6 ± 12·3
P-value (*t*-test)				0·79

increase in nectar volume was associated with a corresponding increase in carbohydrate secretion.

DISCUSSION

Both plant species produced extrafloral nectar in the absence of insect damage, but they differed considerably with regard to the level of this baseline production. Such constitutive nectar production may provide a degree of prophylactic protection, as it allows plants to accommodate some natural enemies before herbivores arrive. Prophylactic protection by natural enemies may include, for example, the prevention of herbivore oviposition, or removal of herbivore eggs (Whitman, 1996).

In each plant species, sucrose and its components, glucose and fructose, were the only nectar sugars recorded. These sugars and their relative concentrations in *G. herbaceum* correspond to the sugar composition reported for foliar nectaries in *G. hirsutum* and *G. barbadense* (Butler *et al.*, 1972). The overall sugar concentration in both *G. herbaceum* and *R. communis* was remarkably high, and exceeded concentrations reported for most floral nectars (Baker and Baker, 1983). This is probably because the extrafloral nectaries are more exposed than most floral nectaries, subjecting extrafloral nectar to additional evaporation. The high sugar concentrations might also serve to improve indirect defence, as high sugar concentrations reduce intake by visiting ants and increase durations of ant visits (Josens *et al.*, 1998). A further benefit of concentrated extrafloral nectar is that viscous extrafloral nectar prevents nectar use by a range of non-intended visitors. This applies especially to Lepidoptera, whose mouthpart morphologies restrict them to feeding on nectar with relatively low sugar concentrations.

Following herbivory, both cotton and castor increased extrafloral nectar production above the constitutive level. The fact that extrafloral nectar production was inducible in two unrelated plant species suggests that this active plant response might be a more widespread phenomenon. As herbivory did not affect the sugar composition of the extrafloral nectar, it can be concluded that the increase in nectar volume was not simply a dilution effect. This finding appears to constitute the first conclusive evidence that plants can actually raise their carbohydrate secretion in response to herbivory.

It has been widely assumed that the primary benefit of inducible defence is economic, as it restricts investment in defence to periods during which plants are actually under attack (McKey, 1974; Rhoades, 1979; Zangerl and Rutledge, 1996). With respect to the costs of nectar production, Pyke (1991) demonstrated a trade-off between floral nectar secretion and seed production in hand-pollinated *Brandfordia nobilis*. Comparable studies on the consequences for fitness of extrafloral nectar production have yet to be conducted, but strong indirect evidence for the costliness of extrafloral nectar production is provided by the finding that some plant species have lost extrafloral nectaries in ecosystems without mutualist ant species (e.g. Bentley, 1977; Rickson, 1977).

Of the potential costs of extrafloral nectar production, the direct energetic investment is probably the most obvious and the easiest to measure. In the present experiments, damaged castor leaves produced up to 4·7 mg of nectar per day. Given the 75 % (by weight) sugar concentration, this is equivalent to a daily sugar excretion of 3·6 mg per leaf. Castor has a maximum rate of photosynthesis of 61 μmol CO_2 m^{-2} s^{-1} (Dai *et al.*, 1992), which in the present plants (leaf surface approx. 350 cm^2) would translate to 5·4 g CO_2 per leaf d^{-1} (16L : 8D). Since 1 g of fixed CO_2 yields 648 mg sucrose (Penning de Vries *et al.*, 1974), this level of nectar production corresponds to a daily photosynthetic production of approx. 3·5 g sucrose per leaf, or 1‰ of its daily assimilates. This may be an underestimate since it is unlikely that the plants achieved the maximum rate of photosynthesis. Even though this cost may not seem very substantial, its cumulative nature could lead to a rapid compounding of costs over the total period of plant growth.

Besides carbohydrates, the extrafloral nectar also contained various amino acids. Despite their relatively low concentration in nectar, amino acids may represent a considerable cost factor, as plants are commonly N-limited rather than C-limited (Bazzaz, 1997). Finally, nectar secretion also constitutes a loss of water, which may be especially important for plants growing under water-limited conditions (Pimentel, 1988).

In addition to the direct costs of diverting primary metabolites and water to nectar production, the production

of extrafloral nectar is also likely to entail substantial indirect (ecological) costs, as extrafloral nectaries are frequently exploited by herbivores (McEwen and Liber, 1995). When herbivores are attracted or retained by extrafloral nectaries, herbivory levels on nectary-bearing plants may be increased (Adjei-Maafo and Wilson, 1983; Rogers, 1985; McEwen and Liber, 1995). Further ecological costs may arise when extrafloral nectaries distract pollinators away from flowers (Koptur, 1992) or when recruited ants attack flower visitors (Buys, 1990).

Induction of extrafloral nectar production allows plants simultaneously to minimize all of the above cost factors. In the absence of herbivory, costs of nectar production may be all but eliminated, while the full costs are assumed only during periods of herbivory. The cost-saving benefit of defence induction is countered by the loss of prophylactic protection (Zangerl and Bazzaz, 1992). Any damage inflicted during the time required for the plant to respond (lag time of induction) should be included in the costs of induced defence. In comparison with direct defences, indirect defences usually entail a longer lag time, arising from the delay in natural enemy response. For extrafloral nectaries this delay can include nectar encounter time, as well as the time for potential nestmate recruitment. Maintaining some baseline nectar production in undamaged plants could be a way of minimizing the lag time in nectar-mediated plant defence. By accommodating at least a few natural enemies, indirect defence can begin to operate quickly once the plant is attacked.

In addition to the economic benefits, induction of indirect defences may also enhance the effectiveness of natural enemy recruitment as it results in an accumulation of natural enemies on the leaf under attack (Wäckers and Frei, unpubl. res.). Such enhanced recruitment allows the plant actively to increase the probability of ants detecting and removing the herbivore. Positioning of the nectaries can further improve recruitment in localized induction. In castor, for instance, several nectaries are arranged along the petiole, from the stem node to the base of the lamina, such that localized induction provides natural enemies, walking up the stem, with a trail to potential folivores. In situations in which a plant is visited by several ant species, a localized increase in nectar secretion can further improve ant-mediated defence because the most aggressive species monopolize the most productive nectar source within a plant (Del-Claro and Oliveira, 1993).

ACKNOWLEDGEMENTS

We thank Bradley Binder, P.J. Edwards, J.H. Tumlinson, A.R. Zangerl, and an anonymous reviewer for helpful comments on a previous version of this manuscript. F. Keller performed the nectar analysis.

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