Timely plant defenses protect against caterpillar herbivory

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As sessile, photosynthetic organisms, plants benefit greatly from a circadian clock that adjusts their overall metabolism in anticipation of the highly predictable arrival of sunrise and sunset. Thus, it is not surprising that about one-third of all plant genes show evidence of circadian regulation in their expression patterns (1, 2). However, closer examination of this diurnally regulated transcription shows that it comprises not only the predictable genes involved in photosynthesis, carbon metabolism, and water uptake but numerous genes with known functions in plant defense. Recent studies show that the success of pathogen infections is influenced by the plant circadian clock (3–5). Accumulation of salicylic acid, a key signal for the induction of antimicrobial defenses, peaks at night and may lead to elevated pathogen resistance in the early morning. Cyclical accumulation of herbivore-induced plant metabolites (6), as well as reciprocal effects of plant secondary metabolism on the circadian clock (7), suggests that resistance to insect herbivory also should show diurnal regulation. This hypothesis is confirmed by a study in PNAS (8), which provides a previously undescribed avenue for studying plant-herbivore interactions by demonstrating that plants can benefit from synchrony in the pattern of diurnally regulated defenses and insect feeding behavior.

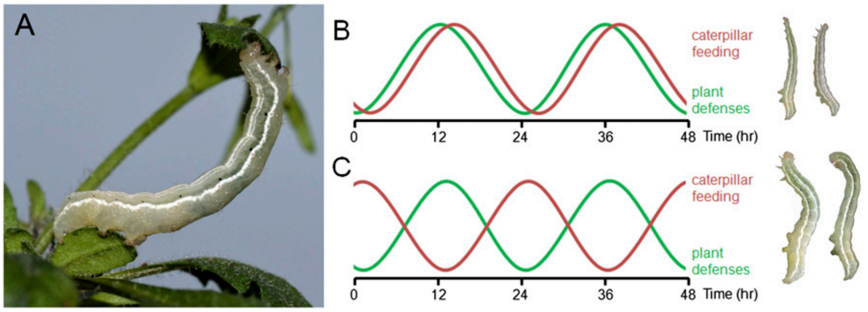

Fig. 1. (*A*) *Trichoplusia ni* (cabbage looper) caterpillar feeding on *Arabidopsis*. (*B*) Under normal growth conditions, when the circadian clocks of the caterpillars and the plants are synchronized, both caterpillar feeding activity (red line) and jasmonate-mediated plant defenses (green line) peak during the day. (*C*) If the internal clock of cabbage loopers is shifted by 12 h, maximum feeding (red line) occurs at a time of reduced *Arabidopsis* defenses (green line) and the caterpillars grow more rapidly.

The report by Goodspeed et al. (8) is focused on laboratory studies of interactions between the generalist lepidopteran herbivore *Trichoplusia ni* (cabbage looper) and the small cruciferous plant *Arabidopsis thaliana* (*Arabidopsis*) (Fig. 1*A*). Cabbage looper herbivory and accumulation of jasmonic acid, a plant hormone that triggers herbivore defenses in many plant species, follow circadian cycles that peak during the day, suggesting that *Arabidopsis* plants are allocating their resources to maximize defense when caterpillars are feeding. To test this hypothesis, the authors conducted experiments in which cabbage loopers were entrained separately from the host plants, using a light cycle that shifted their circadian clocks by 12 h. Under subsequent constant-darkness conditions, caterpillars that were feeding asynchronously with the plant defense cycle consumed more leaf material and, at the end of the experiment, were threefold as heavy as synchronously feeding control caterpillars (Fig. 1 *B* and *C*). Moreover, this effect on caterpillar growth was not observed in similar experiments with *Arabidopsis* mutants that are compromised in circadian regulation or the induction of jasmonate-mediated defense responses, showing that both of these plant functions are required for the periodicity of herbivore defenses.

The hypothesized interactions between circadian plant defense regulation and cabbage looper herbivory can be confirmed by conducting experiments with insects that have different diurnal feeding patterns. For instance, caterpillars of some species of noctuid moths, commonly called cutworms, feed almost exclusively at night and hide during the day, a behavior that may reduce predation by day-active parasitoid wasps. However, if diurnal peaks in antiherbivore defenses, which have been observed in both *Arabidopsis* and *Nicotiana attenuata* (6, 8), are a more general phenomenon in plants, nocturnal feeding may also be an adaptation to reduced nighttime plant defenses. An interesting future comparison will be to conduct experiments similar to those of Goodspeed et al. (8) but with night-feeding cutworms. In this case, one would expect that caterpillars that are clock-shifted relative to their host plants will perform less well than nonshifted control caterpillars. Other Lepidoptera, for instance, *Manduca sexta* (tobacco hornworm), are reported to show no circadian regulation of their feeding behavior (9). Thus, even though *N. attenuata,* a natural host for tobacco hornworms, shows diurnal variation in herbivore defense responses (6), one would predict few changes in caterpillar performance if it were clock-shifted relative to its host plants.

Research on the proximal causes of *Arabidopsis* resistance to insect herbivory has been focused almost exclusively on the glucosinolates, a crucifer-specific class of secondary metabolites. Tissue damage caused by herbivory brings together glucosinolates and activating enzymes called myrosinases, resulting in the formation of toxic and deterrent breakdown products. Although DNA microarray experiments clearly show diurnal regulation of glucosinolate-related genes (1, 7), there are as yet no conclusive studies that demonstrate corresponding diurnal variation in the abundance of glucosinolates or their breakdown products. Moreover, clock-shifted cabbage loopers exhibit a threefold increase in weight relative to synchronized controls (8), an effect that is greater than the growth changes observed with cabbage loopers feeding from *Arabidopsis* mutants with altered glucosinolate breakdown (10), reduced myrosinase activity (11), or even a complete absence of glucosinolates (12). Thus, although glucosinolates undoubtedly have important functions in protection against herbivory (10–12), it is likely that additional defensive metabolites, or perhaps circadian cycles in the *Arabidopsis* nutritional content, also influence the observed changes in caterpillar weight gain.

An intriguing possibility is that diurnal variation in plant metabolism, in addition to the insects' own internal clock, can have a direct effect on cabbage looper feeding patterns. For instance, behavior of *Mythimna separata* (Oriental armyworm) caterpillars, which feed at night and hide

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See companion article on page 4674.

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during the day to avoid predators, is dictated primarily by diurnal variation in volatile emissions from the host plants rather than caterpillar perception of the day-night cycle (13). If there are similar interactions between *Arabidopsis* and cabbage loopers, the clock-shift experiments conducted by Goodspeed et al. (8) could result in caterpillars that spend a greater part of the day feeding. Observations of circadian cycles in cabbage looper feeding have only been conducted with an artificial diet. Therefore, it is not yet possible to determine whether increased *Arabidopsis* tissue consumption by clock-shifted cabbage loopers is attributable to more rapid feeding during the time that is dictated by their circadian clocks or attributable to a shift in the herbivory pattern to include additional feeding times.

Although cabbage loopers are generalist herbivores that commonly feed on crucifers, there are few reports of cabbageloopers on *Arabidopsis* in the field. Thus, if the daytime peak in *Arabidopsis* jasmonate production has indeed evolved in response to herbivory, this selective pressure probably includes not only cabbage loopers but numerous other day-active species. Like most *Arabidopsis* research, the experiments conducted by Goodspeed et al. (8) involved only the well-studied Columbia-0 accession. However, there is considerable natural variation in the *Arabidopsis* circadian clock (14), production of defensive metabolites (15), and herbivore pressures that this species faces across a geographical range that extends from the equator to the Arctic Circle. Therefore, experiments with additional *Arabidopsis* accessions will likely show different circadian interactions with insect herbivores. These ideas, and likely many future studies in the area of plant–insect interactions, follow directly from a new awareness of the complex interplay between the circadian rhythms of plants and insect herbivores that has been provided by the research of Goodspeed et al. (8).

Clock-shifted cabbage loopers exhibit a threefold increase in weight relative to synchronized controls.

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