

Circadian control of jasmonates and salicylates

The clock role in plant defense

Danielle Goodspeed, E. Wassim Chehab, Michael F. Covington[†] and Janet Braam*

Biochemistry and Cell Biology; Rice University; Houston, TX USA

[†]Current affiliation: Department of Plant Biology; College of Biological Sciences; University of California; Davis, CA USA

Keywords: *Arabidopsis thaliana*, jasmonic acid, salicylic acid, *Trichoplusia ni*, circadian rhythm, plant-insect interaction, herbivory, biotic stress, plant resistance

Plants have evolved robust mechanisms to perceive and respond to diverse environmental stimuli. The plant phytohormones jasmonates and salicylates play key roles in activating biotic stress response pathways. Recent findings demonstrate that basal levels of both jasmonates and salicylates in *Arabidopsis* are under the control of the circadian clock and that clock-controlled jasmonate accumulation may underlie clock- and jasmonate-dependent enhanced resistance of *Arabidopsis* to *Trichoplusia ni* (cabbage looper), a generalist herbivore. Here we summarize these findings and provide further evidence that a functional plant circadian clock is required for optimal herbivore defense in *Arabidopsis*. When given a choice to feed on wild-type plants or arrhythmic transgenics, *T. ni* prefer plants lacking robust circadian rhythms. Altogether these data provide strong evidence for circadian clock enabling anticipation of herbivore attack and thus contributing to overall plant fitness.

Nearly all organisms have evolved an internal clock, which influences diverse behaviors that exhibit 24-h, or circadian, rhythms.^{1,2} The circadian clock is thought to be advantageous to diverse organisms because it provides the ability to anticipate the predictable daily and seasonal changes that occur in the environment as a consequence of the rotation of the earth. A few examples of clock-dependent anticipatory daily behaviors are well known. Some plant leaves turn to expose surfaces toward the east during the night to anticipate the rising sun.³ The effect of the clock on internal physiological processes may be often underappreciated because experiments must be designed in ways to reveal the impact of the dimension of time. Given the widespread effect of the clock on plant gene expression—with approximately one-third of *Arabidopsis* transcripts showing circadian regulation of accumulation,⁴ it is predicted that the clock has broad impact on plant physiology.

Recent demonstration that over 40% of genes known to be upregulated in expression by wounding also show circadian regulation of transcript accumulation in unwounded plants⁵ led to the question of the physiological relevance of these massive daily fluctuations in gene expression. In a recent study,⁶ we sought to test the hypothesis that the circadian clock enables plants to anticipate daily fluctuations in potential herbivore behavior by mounting defenses prior to likely attack.

Remarkably, *Arabidopsis* plants, entrained in light-dark cycles and then placed in constant conditions, to remove potential behaviors influenced by changes in light and reveal only those behaviors controlled by the clock, accumulated two major defense hormones, jasmonates and salicylates, with distinct circadian patterns.⁶ In unwounded plants, jasmonate levels peaked in the middle of subjective day and salicylate levels peaked in the middle of subjective night. Thus, in unwounded, uninfected plants, hormone levels fluctuated with daily rhythmicity, perhaps in anticipation of attack.

Indeed, the circadian clock function is critical for *Arabidopsis* resistance to herbivory. The generalist herbivore, *Trichoplusia ni* larvae (commonly known as cabbage loopers) demonstrated a circadian feeding behavior, with peak feeding occurring during the middle to end of subjective day.⁶ When *Arabidopsis* plants and the cabbage loopers were entrained with in-phase light-dark cycles, the plants demonstrated substantial resistance. Cabbage looper performance was enhanced and *Arabidopsis* resistance was reduced if the plants and insects were entrained with opposite light-dark cycles. As a consequence of these opposite entrainment conditions, when the plants were experiencing subjective night (and therefore not accumulating jasmonates), the insects were experiencing subjective day and therefore were highly active in feeding. These experiments demonstrated that proper

*Correspondence to: Janet Braam; Email: braam@rice.edu.

Submitted: 12/03/12; Accepted: 12/04/12

<http://dx.doi.org/10.4161/psb.23123>

Citation: Goodspeed D, Chehab E, Covington M, Braam J. Circadian control of jasmonates and salicylates: The clock role in plant defense. *Plant Signal Behav* 2013; 8:e23123; PMID: 23299428; <http://dx.doi.org/10.4161/psb.23123>.

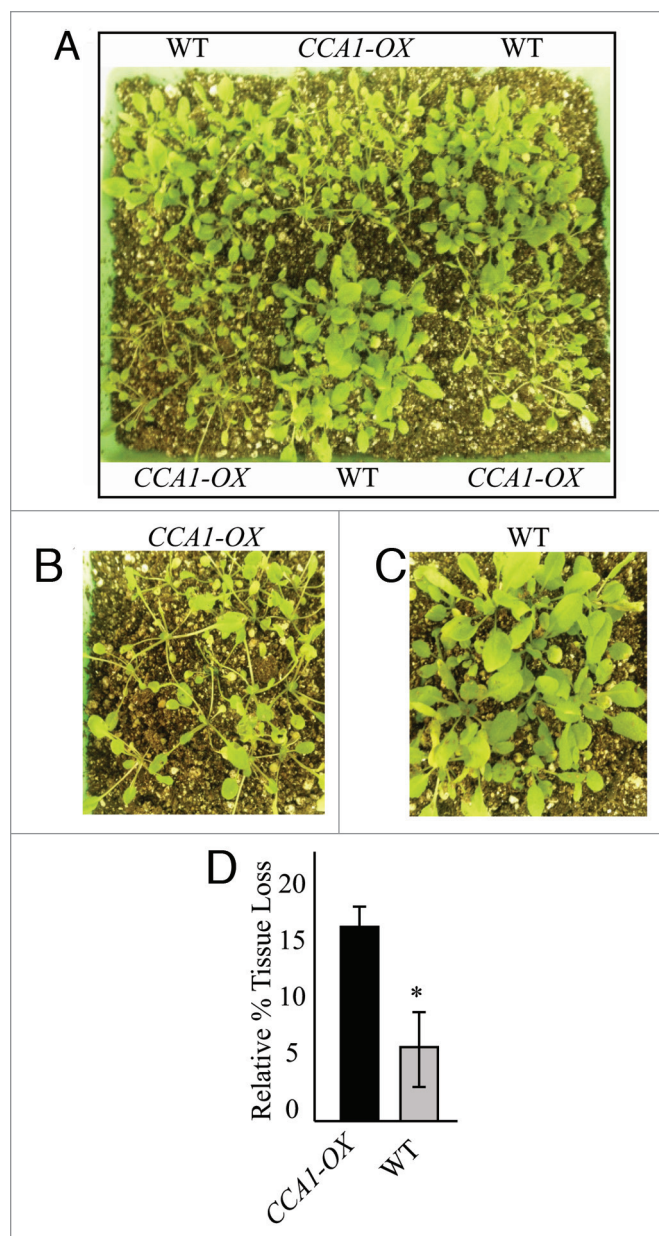


Figure 1. In a choice experiment, *T. ni* prefer to feed on arrhythmic transgenics over wild-type plants. Plants were entrained for 3 weeks and loopers were entrained for 3 d in 12-h light/dark cycles at 22C then both plants and insects were moved to constant light conditions. After 24 h of constant light, plants and loopers were co-incubated for 72 h. Loopers were evenly distributed between the two rows of plants. (A) Photograph showing the alternative arrangement of 16-plant plots of arrhythmic *CCA1-OX* plants and wild type after feeding by *T. ni* cabbage loopers. (B and C) Close up images from (A) showing representative plants and damage by *T. ni* feeding. (D) Relative percent area lost from plant tissue illustrated in (A), determined using ImageJ software (National Institutes of Health). Means \pm SE; $n = 3$; * $p < 0.05$; unpaired t-test. Experiment was repeated 3 times with similar results.

entrainment with the environment, in this case entrainment with cabbage loopers, enhances plant herbivory resistance.

We also demonstrated that both the circadian clock function and the jasmonate pathway are required for the enhanced

resistance conferred by in-phase entrainment of the plants.⁶ Plants that are arrhythmic due to clock dysfunction and mutants that cannot accumulate active jasmonates or lack the jasmonate co-receptor failed to demonstrate the in-phase enhancement of herbivore resistance. Furthermore, the data presented indicate that the clock-controlled increase in jasmonate accumulation preceded that of the clock-controlled increase in herbivore feeding behavior.⁶ The finding that plant phytohormone accumulation begins prior to the relevant insect behavior is consistent with the plant circadian clock enabling anticipation of predictable biotic stress.

To further test the importance of circadian clock function in herbivore resistance, we conducted herbivore choice experiments, in which cabbage loopers were given the choice to feed upon either clock-defective *CCA1-OX* transgenic plants⁷ or wild type (Col-0). Two identical trays of six sets of plants (16 plants per plot) were arranged such that the two genotypes were interspersed, with wild type at top left, top right, and bottom center, and *CCA1-OX* at top center, bottom left, and bottom right (Fig. 1A). Both plant genotypes and the cabbage loopers were entrained in coincident 12-h light-dark cycles before co-incubation of insects with plants at constant light. One tray of plants was used as a control in that the plants were not exposed to the cabbage loopers. With the second tray of plants, 15 *T. ni* cabbage loopers were evenly distributed at the center interface between the top and bottom rows of plants and allowed to feed for 72 h. We compared the amount of tissue remaining among plants exposed to the cabbage loopers to that of plants not exposed to the insect herbivore to calculate percent tissue lost. Figure 1 reveals that a significantly greater percentage of tissue is lost from the *CCA1-OX* transgenic plants than wild type due to herbivory. Greater tissue damage among the *CCA1-OX* plants than the wild type is apparent in photographs of the plants (Fig. 1B and C). Quantitation of tissue loss verifies that the herbivore damage was significantly greater on the arrhythmic plants (*CCA1-OX*) than on the wild type controls (Fig. 1D). Therefore, when cabbage loopers are given a choice between wild-type plants and plants with dysfunctional circadian clocks, the arrhythmic plants are preferred over the wild-type control. Thus, a functional *Arabidopsis* circadian clock is clearly advantageous for plant cabbage looper resistance.

In conclusion, the plant circadian clock can significantly enhance and is required for robust herbivore resistance. The demonstration that two major plant defense hormones, jasmonates and salicylates, fluctuate with daily rhythms and finding that the essential role the jasmonate pathway in clock-dependent looper resistance in *Arabidopsis* are strong evidence that plants use the circadian clock to anticipate attack and prepare in advance to best fight off attackers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This material is based upon work supported by the National Science Foundation under Grant No. MCB 0817976 Grant to J.B.

References

1. Bell-Pedersen D, Cassone VM, Earnest DJ, Golden SS, Hardin PE, Thomas TL, et al. Circadian rhythms from multiple oscillators: lessons from diverse organisms. *Nat Rev Genet* 2005; 6:544-56; PMID:15951747; <http://dx.doi.org/10.1038/nrg1633>.
2. Harmer SL. The circadian system in higher plants. *Annu Rev Plant Biol* 2009; 60:357-77; PMID:19575587; <http://dx.doi.org/10.1146/annurev.arplant.043008.092054>.
3. Schwartz A, Koller D. Diurnal phototropism in solar tracking leaves of *Lavatera cretica*. *Plant Physiol* 1986; 80:778-81; PMID:16664701; <http://dx.doi.org/10.1104/pp.80.3.778>.
4. Covington MF, Maloof JN, Staume M, Kay SA, Harmer SL. Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development. *Genome Biol* 9, R130.1 (2008).
5. Walley JW, Coughlan S, Hudson ME, Covington MF, Kaspi R, Banu G, et al. Mechanical stress induces biotic and abiotic stress responses via a novel cis-element. *PLoS Genet* 2007; 3:1800-12; PMID:17953483; <http://dx.doi.org/10.1371/journal.pgen.0030172>.
6. Goodspeed D, Chehab EW, Min-Venditti A, Braam J, Covington MF. Arabidopsis synchronizes jasmonate-mediated defense with insect circadian behavior. *Proc Natl Acad Sci U S A* 2012; 109:4674-7; PMID:22331878; <http://dx.doi.org/10.1073/pnas.1116368109>.
7. Wang Z-Y, Tobin EM. Constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) gene disrupts circadian rhythms and suppresses its own expression. *Cell* 1998; 93:1207-17; PMID:9657153; [http://dx.doi.org/10.1016/S0092-8674\(00\)81464-6](http://dx.doi.org/10.1016/S0092-8674(00)81464-6).