Regulation of extrafloral nectar secretion by jasmonates in lima bean is light dependent

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To maximize fitness, plants need to perceive changes in their light environment and adjust their physiological responses accordingly. Whether and how such changes also affect the regulation of their defense responses against herbivores remains largely unclear. We addressed this issue by studying the secretion of extrafloral nectar (EFN) in lima bean (*Phaseolus lunatus*), which is known to be activated by the phytohormone jasmonic acid (JA) and functions as an indirect defense mechanism against herbivores. We found that the plant's EFN secretion in response to JA was light dependent: In the dark, JA reduced EFN secretion, whereas under light conditions, JA induced EFN secretion relative to controls. This modulation was affected by the light's spectral composition [i.e., ratio of red to far-red (R:FR) radiation], but not light intensity. These findings demonstrate a unique differential effect of JA on EFN secretion depending on the ambient light conditions. Interestingly, treatment with the isoleucine–JA conjugate (JA–Ile) enhanced EFN secretion under light conditions yet did not reduce EFN secretion in the dark. Moreover, inhibition of Ile biosynthesis in light-exposed plants significantly decreased the EFN secretion rate. This reduction could be recovered by additional application of JA–Ile, suggesting that JA–Ile is the active compound required to up-regulate EFN secretion. Finally, experiments with mechanically damaged plants revealed that light was required for the formation of JA–Ile, but not of JA. These results demonstrate that in lima bean, the light environment modulates the plant's response to jasmonates as well as JA–Ile biosynthesis, which controls the subsequent EFN secretion.

indirect plant defense | *Phaseolus lunatus* | far-red | phytohormone | jasmonic acid-isoleucine conjugate

In their natural environment, plants continuously experience diurnal (day/night) and seasonal fluctuations of their abiotic environment. Due to their sessile and obligate photoautotrophic life, selection pressures acting on plants to evolve mechanisms that allow them to anticipate predictable changes in the light environment and synchronize their physiological processes such as photosynthesis, stomatal movements, and flowering (1) accordingly, are expected to be exceedingly strong.

Light is one of the strongest and best-characterized entrainment stimuli known (2). It not only delivers the energy to fuel a plant's metabolism, but also serves as a cue to assess the current risk of herbivory, because it strongly affects feeding patterns of herbivores (3). Given that diurnal changes of the ambient light regime accurately predicted the risk of herbivory (4), being able to integrate information from the abiotic environment to predict imminent herbivore attacks and regulate its defense responses accordingly would result in a huge selective advantage.

When attacked by herbivores, plants activate defenses, which can affect the herbivore either directly (e.g., chemical defenses or physical barriers) or indirectly by attracting predatory insects [e.g., emission of volatile organic compounds (VOCs) or the secretion of extrafloral nectar (EFN)] (5). All indirect antiherbivore defenses known to date are regulated by the octadecanoid-signaling pathway, in which jasmonic acid (JA) acts as the central signaling molecule (6, 7). Besides its involvement in many plant developmental processes (8–10), jasmonates (term collectively used for JA-derived compounds) are key compounds that have been implicated in plants' responses to biotic and abiotic stresses. Recent reports on JAZ (jasmonate ZIM-domain) repressor proteins led to the discovery that in many cases, not JA itself, but its amino acid conjugate JA–Ile is the active form of the hormone (10–14). In *Arabidopsis thaliana*, it has been shown that JA–Ile formation is catalyzed by JAR1, which activates JA via adenylation and subsequently conjugates it to amino acids (14), thereby forming the active molecule that eventually triggers downstream defense responses (10). The link between jasmonate signaling and light environment has been studied extensively in the context of shade avoidance and competition (15–19): plants can sense the presence of neighbors using phytochromes, resulting in the down-regulation of antiherbivore defenses (18). Several studies have reported an interaction between JA and red to far-red (R:FR) responses (16–19). However, whether light signals also modulate indirect defense responses is completely unknown.

Light signals can vary in quantity, quality, direction, and duration. Any such variation will affect photosynthetic efficiency and hence, the production of defense-related compounds via either suppression on a regulatory level or simply the shortage of the required precursors. This should particularly hold true for carbon-based defenses such as the secretion of EFN, an aqueous solution that mainly contains sugars (20). Several studies exist on the anatomy of extrafloral nectaries, the chemical composition of EFN, as well as its defensive function in nature (21–24). However, very little information is available on the signaling cascade that governs this indirect defense and whether this regulation is affected by the ambient light conditions. To address this issue, we used lima bean (*Phaseolus lunatus* L., Fabaceae) as a model plant that secretes EFN from specialized organs called extrafloral nectaries. Previous studies have established that in this species, EFN secretion functions as an effective defense against herbivores (23), which is inducible by exogenous application of JA (22). In the current study, we investigated the functional relationship between EFN secretion, jasmonate signaling, and light availability with the aim to understand whether and how the signaling cascade that leads to the induction of this indirect defense is affected by changes in the ambient light regime.

Results

EFN Secretion in Response to Jasmonates Depends on the Availability of Light. Analyzing EFN secretion during a normal day–night cycle (12 h light–12 h darkness, 7:00 AM–7:00 PM) in lima bean, we observed that EFN secretion in control plants peaked 3 h after the onset of darkness (i.e., 10:00 PM), whereas in JA-treated plants it was maximal 3 h after the beginning of light exposure (i.e., 10:00 AM) (Fig. 1*A*). In total, untreated plants secreted significantly

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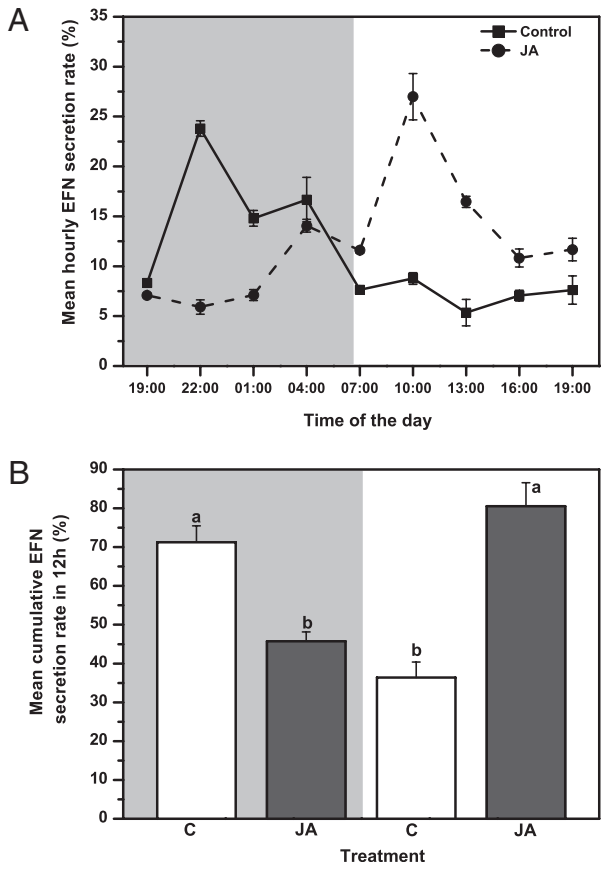

Fig. 1. EFN secretion in plants before and after JA treatment during a 12-h day/night cycle. (*A*) EFN secretion rates were monitored every 3 h for 24 h and are expressed as a percentage of total EFN secretion in plants measured in three independent experiments per treatment. Plants were treated either with JA or with tap water as control. (*B*) Mean cumulative EFN secretion rates (±95% confidence interval) of the data shown in *A*. Different letters denote significant differences (LSD post hoc test: $P < 0.04$).

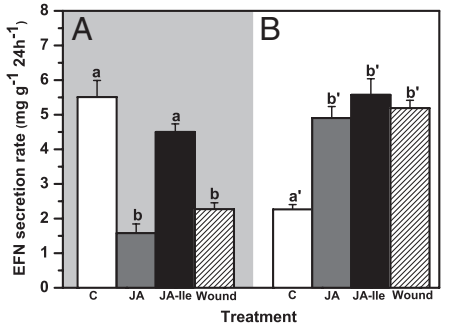

Fig. 2. Mean extrafloral nectar (EFN) secretion rates (±95% confidence interval) after JA, JA–Ile, and mechanical wounding (pattern wheel) treatments of plants exposed to prolonged dark and light conditions. Plants were exposed to (*A*) 24-h dark conditions ($n = 5$) and (*B*) 24-h light conditions (50%) ($n = 6$). EFN secretion rates are expressed as milligrams soluble solids per gram fresh weight of leaf tissue per 24 h. Different letters indicate significant differences between treatments (LSD post hoc test: $P < 0.02$).

more EFN at night than during the day, whereas JA application reversed this pattern—JA-treated plants showed higher EFN secretion rates during the day (Fig. 1*B*). To verify whether the observed inhibitory effect of JA on EFN secretion at night was indeed caused by changes of the light regime the effect of jasmonates on the EFN secretion in lima bean was investigated under prolonged light and dark conditions. For this, lima bean plants were left untreated, treated with JA, or damaged mechanically to induce the plant's de novo synthesis of JA (8–10). In addition, plants were treated with JA–Ile, to test whether this jasmonate derivative, which has been recently identified as the defense-inducing signal in other systems (11, 12), is also involved in regulating EFN secretion. After these treatments, plants were exposed for 24 h to light conditions or darkness and the EFN secretion was measured. EFN secretion was enhanced in plants that had experienced 24 h of darkness over those kept for 24 h in the light (Fig. 2*A*), which corroborated the findings of the previous experiment done under normal day/night conditions (Fig. 1). Moreover, in plants experiencing continuous darkness for 24 h, JA treatment significantly reduced EFN secretion rates relative to controls, whereas EFN production remained unchanged in JA–Ile-treated plants (Fig. 2*A*). In contrast, light exposure significantly increased EFN secretion rates in both JA- and JA–Ile-treated plants over controls (Fig. 2*B*). The composition of the EFN was unaltered before and after JA or JA–Ile treatment in light and dark conditions (Table S1). These findings suggested that in plants experiencing darkness, JA but not JA–Ile has a down-regulating effect on EFN secretion. In line with these findings, mechanical wounding of plants, which were kept in the dark, decreased EFN production relative to controls. The reducing effect on EFN secretion observed in mechanically wounded plants that were kept in the dark was statistically indistinguishable from the one observed in JA-treated plants (Fig. 2*A*). In contrast, exposing wounded plants to light, enhanced EFN secretion similarly as observed upon JA or JA–Ile treatment (Fig. 2*B*).

To verify whether the reducing effect of JA on the EFN secretion rates of darkness-exposed plants depended on the JA concentration applied (i.e., 1 mM), plants were treated with even lower concentrations of JA, exposed to prolonged darkness, and the EFN secretion was measured. The results of this experiment confirmed that JA concentrations as low as 100 μM were sufficient to inhibit EFN secretion in the so-treated plants (Fig. S1).

Taken together, these results provide strong evidence that light modulates the jasmonate-dependent regulation of EFN secretion: in the dark, JA inhibits EFN secretion relative to controls and JA–Ile treated plants, whereas under light conditions, both JA and JA–Ile show an inducing effect on EFN secretion.

Induction of EFN Secretion by Jasmonates Depends on Light Composition, but Not Intensity. After establishing that a plant's EFN secretion upon JA and JA–Ile treatment depended on the respective light conditions, we asked whether this response was affected by either the intensity or spectral composition of light to which plants are exposed. The effect of light intensity on EFN secretion was evaluated by exposing JA-treated plants to increasing light intensities, starting with complete darkness and increasing exposure in 25% steps to 100%. The results of this experiment clearly showed that already 25% of light was sufficient to significantly increase EFN secretion rates in JA-treated plants relative to both control and JA-treated plants that were kept in the dark (Fig. 3). Further increasing the light intensity to 50% and 100%, however, did not result in even higher amounts of EFN produced (Fig. 3). This observation suggested that the light-dependent inductive effect of JA shows a bimodal, switch-like behavior, rather than a continuous response to increasing light availabilities.

Next we asked whether changes in the light spectral composition also affect the plant's jasmonate-controlled EFN secretion. To answer this question, we exposed plants to different ratios of R to FR radiation and measured EFN secretion 24 h after treatment with JA or JA–Ile (Fig. 4). In plants exposed to 100% FR light, application of both JA and JA–Ile significantly reduced EFN secretion (Fig. 4*A*). When the ratio of R to FR radiation was increased to 10:90, the EFN secretion was significantly increased in

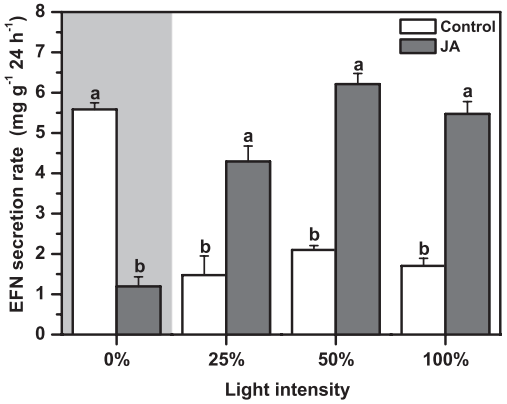

Fig. 3. Mean extrafloral nectar (EFN) secretion rates (±95% confidence interval) of plants exposed to increasing light intensities (n = 8). EFN secretion rates are expressed as milligrams soluble solids per gram fresh weight of leaf tissue per 24 h. Different letters indicate significant differences between treatments (LSD post hoc test: $P < 0.03$).

JA–Ile-treated plants over JA-treated and control plants, whereas it did not differ between JA-treated and control plants (Fig. 4*B*). Further increasing the R:FR ratio to 50:50 restored the inductive effect of both JA and JA–Ile (Fig. 4*C*). Control experiments, in which JA- or JA–Ile-treated plants were exposed to 50% light (451.7 μmol) as mentioned above (performed for each R:FR ratio experiment) confirmed that both treatments induced EFN secretion (Figs. 4*D*). In sum, our results demonstrate that the regulation of EFN secretion by jasmonates seems to be strongly affected by light composition, but—within the parameters tested—not limited by light intensity.

Biosynthesis of JA–Ile Is Light Dependent. In response to herbivory or mechanical damage, plants synthesize jasmonates de novo via the octadecanoid pathway starting from linolenic acid (10). To explore whether the biosynthesis of these phytohormones is light dependent, we analyzed JA and JA–Ile levels under 24 h light and dark conditions prior to and postmechanical damage (Fig. 5). Wounding significantly increased JA and JA–Ile levels and in both cases, maximum levels were reached about 1 h after wounding (Fig. 5). Interestingly, JA levels in wounded leaves did not differ

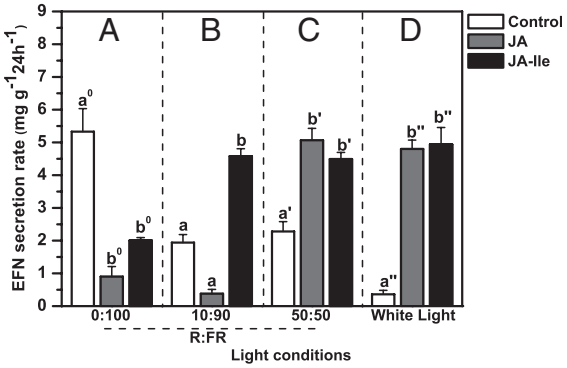

Fig. 4. Mean extrafloral nectar (EFN) secretion rates (±95% confidence interval) of JA- and JA–Ile-treated plants exposed to either different ratios of red (R), to far-red radiation, or 50% light conditions. EFN secretion rates are expressed as mg soluble solids per gram fresh weight of leaf tissue per 24 h. (*A*) FR 100% (LSD post hoc test: $P < 0.01$, n = 4), (*B*) R:FR 10:90 (LSD post hoc test: $P < 0.03$, n = 4), (*C*) R:FR 50:50 (LSD post hoc test: $P < 0.04$, n = 4), and (*D*) white light 50% (LSD post hoc test: $P < 0.04$, n = 4). Different letters indicate significant differences between treatments.

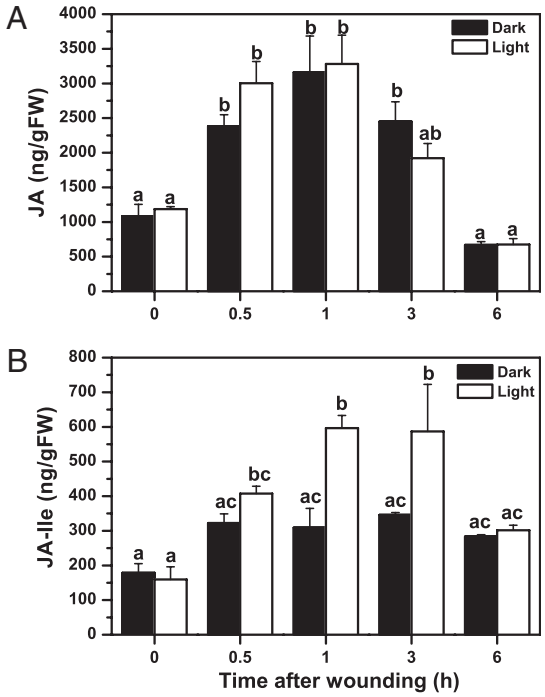

Fig. 5. Wound-induced biosynthesis of phytohormones in plants exposed for 24 h to light or dark conditions. All lima bean plants were kept in darkness for 24 h. The next day, plants were wounded mechanically (pattern wheel), exposed to either light or dark conditions, and the phytohormone levels were quantified at different time points. Mean (±95% confidence interval) levels of (*A*) JA and (*B*) JA–Ile levels expressed as nanograms per gram fresh weight of leaf tissue are shown (n = 3 for each time point and treatment). Different letters indicate significant differences (LSD post hoc: $P < 0.03$).

significantly between plants kept in the dark and those exposed to light (Fig. 5*A*), whereas JA–Ile levels were significantly higher when plants were exposed to light as compared with plants in the dark (Fig. 5*B*). Hence, these results imply a light-dependent biosynthesis of JA–Ile.

JA–Ile Formation Is Critical for EFN Secretion. Finding that light exposure of wounded plants resulted in a significant increase of their internal JA–Ile levels relative to plants that were kept in the dark while JA levels remained unchanged, suggested that probably JA–Ile rather than JA is the active signal inducing EFN secretion in lima bean.

To test this, we used coronalon (COR; 6-ethyl indanoyl isoleucine conjugate), a structural mimic of JA–Ile that shows similar biological activities (25). Application of COR to plants exposed to either light or dark conditions resulted in an EFN secretion pattern that resembled that of JA–Ile-treated plants: COR application increased EFN secretion rates in the light, whereas it showed no effect in the dark (Fig. S2*A*).

If JA–Ile is the active compound triggering EFN secretion, the conjugation between isoleucine and JA should be critical to its formation. We tested this hypothesis by applying the methyl ester of JA (MeJA) to plants that were exposed to either light or dark conditions and measured the EFN secretion rate after 24 h. In line with our expectations, MeJA neither induced EFN in plants exposed to light (Fig. S2*D*) nor inhibited EFN secretion in plants exposed to darkness as observed after JA application (Fig. S2*C*). These results imply that the presence of free JA (i.e., its nonmethylated form) is important for conjugation to Ile and, as a consequence, for the induction of EFN.

Finally, to test whether the availability of Ile is important for EFN secretion, the biosynthesis of branched chain amino acids

(including Ile) was inhibited by treating plants with the inhibitor chlorosulfuron, which blocks the acetolactate synthase (26). Indeed, inhibiting Ile biosynthesis significantly reduced the EFN secretion in light-exposed plants, whereas the reduction could be restored by the exogenous application of JA or JA–Ile (Fig. 6). Analysis of amino acid levels in plants before and after treatment with chlorosulfuron while being exposed to 24 h of light confirmed that the application of the inhibitor indeed decreased the concentration of branched chain amino acids in the so-treated plants (Fig. S3). Collectively, these results provide strong evidence that the presence of light and not only the availability of JA or Ile limits JA–Ile formation and hence the rate of EFN secreted in plants kept in the dark.

Discussion

The aim of the present study was to investigate whether and how the lima bean's jasmonate-dependent EFN secretion is affected by the ambient light conditions. Our results indicate that (*i*) there is a temporal pattern of EFN secretion that peaks at night and remains relatively low during the day, (*ii*) JA inverts this pattern by inducing maximal EFN secretion rates during the day, (*iii*) JA but not JA–Ile inhibits EFN secretion in the dark, (*iv*) JA–Ile is the signal that regulates EFN secretion, and (*v*) the formation of JA–Ile is light-dependent. On the basis of these results, we conclude that in lima bean, the light environment modulates the plant's response to jasmonates as well as JA–Ile biosynthesis, which controls the subsequent EFN secretion (Fig. 7).

For plants, the ability to perceive and respond to changing environmental conditions such as light availability should be essential to maximize their fitness. Therefore it seems reasonable to expect that plants synchronize their defense responses with daily changes in their light environment. EFN secretion is reported to vary spatially (within a plant) (22) as well as temporally. For instance, in the Mexican ant acacia *Acacia hindsii*, EFN secretion peaked during the day (27). Here, EFN secretion was higher in the dark period. A similar variation of EFN production with higher secretion rates at dusk was observed in *Bixa orellana* (28) and *Macaranga tanarius* (29). From these reports it is evident that the temporal pattern of EFN secretion varies with different plant species. The underlying mechanism for this pattern, however, is unclear. The sugars, which are the main constituents of EFN, have been reported to be mainly phloem derived or synthesized at the site of the nectaries (24). For floral nectar, the required carbohydrates originate from photosynthesis or starch degradation, whereas sugars in EFN are generally believed to be mainly photosynthesis derived (24, 30). Long-term JA treatment (for 7 d) of barley seedlings reduced photosynthesis by inhibiting Rubisco biosynthesis (ribulose-1,5-bisphosphate carboxylase/oxygenase)

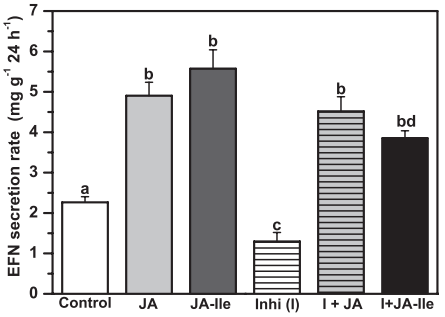

Fig. 6. Mean extrafloral nectar (EFN) secretion rate (±95% confidence interval) of plants exposed to light conditions, which have been treated with JA, JA–Ile, the inhibitor chlorsulfuron (I), inhibitor plus JA, or inhibitor plus JA–Ile (LSD post hoc test: $P < 0.01$, $n = 6$). EFN secretion rates are expressed as milligrams soluble solids per gram fresh mass of leaf tissue per 24 h.

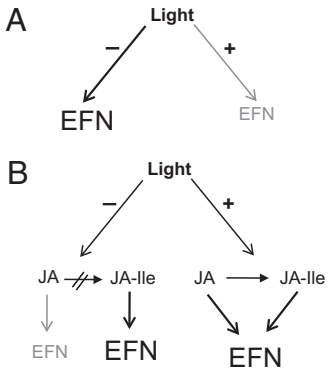

Fig. 7. A model summarizing the observed modulation of the jasmonate-dependent EFN secretion by light in lima bean. (*A*) The secretion of EFN in undamaged plants is light dependent: EFN secretion is increased in plants that experience darkness relative to plants experiencing light conditions. (*B*) The formation of JA–Ile by conjugating Ile to JA requires light. As a consequence, no JA–Ile is formed in the dark. Moreover, JA and JA–Ile show a different effect depending on the light regime to which the plants are exposed. In the dark, JA has an inhibitory effect on EFN secretion, whereas JA–Ile does not. In light, both JA and JA–Ile up-regulate EFN secretion.

(31). Also, a rapid photosynthate export to roots accompanied by a decrease in leaf starch content after treatment with JA has been reported in *Populus tremuloides*, indicating that JA reduces starch accumulation (32). However, it is unknown whether EFN can also be be derived from starch hydrolysis and whether this process is also affected by JA. Experiments to measure starch levels in lima bean plants exposed to different light regime and JA treatment are currently being performed in our laboratory. Although it is not clear whether a low photosynthetic activity also translates into decreased EFN secretion rates, this has been studied for other plant defense responses. For instance, in *Nicotiana attenuata*, *RCA* (Rubisco activase)-silenced plants were not only impaired in their photosynthetic capacity, but also showed reduced defense metabolite (trypsin proteinase inhibitors and diterpene glycosides) and JA–Ile levels, which were attributed to the reduced carbon availability (33). Also, the emission of VOCs, another indirect defense mechanism, is known to vary diurnally (34–36). In contrast to EFN secretion, the mechanism of temporal variation in the emission of VOCs has been elucidated for lima bean. It was demonstrated that continuous mechanical damage during day or night resulted in increased JA levels, but the emission of the VOC (*E,Z*)-β-ocimene started only during the day, because the formation of its precursors via the 2-C-methyl-D-erythritol 4-P pathway was limited by photosynthesis (34). Future studies in both lima bean and other EFN-secreting plant species should identify the source of EFN carbohydrates as well as unravel how the allocation of carbohydrates to EFN is affected by different induction and environmental conditions.

Light signals can modulate plant responses by interacting with the biosynthetic pathways, a process that involves both perception and signaling mechanisms (37). Whether or not light signals can interfere with the response to defense elicitor hormones like JA was the major focus of this study. Here, we report that JA can have different effects on EFN secretion depending on the ambient light regime induction in light and reduction in dark. Our results indicate that the formation of JA–Ile is essential for EFN secretion in lima bean and that this step is light dependent. This interpretation is in line with published evidence from *N. attenuata* showing that silencing threonine deaminase (TD), an enzyme that catalyzes the first step of Ile biosynthesis, leads to plants that are susceptible to herbivore attack due to reduced defense levels (38). Furthermore, Thines and coworkers established in their pioneering work that JA–Ile is highly active in promoting the inter-

actions between COI1 and JAZ1 proteins, which are essential to initiate defense responses (11). Therefore, although JA has been shown to play a crucial role in herbivore resistance, recent evidence suggests that the amino acid conjugate JA–Ile is the active jasmonate derivative (10–13). In our study, we report that JA and JA–Ile induce different responses in terms of EFN secretion in the lima bean depending on the ambient light conditions: in dark, JA reduces EFN secretion whereas JA–Ile does not, indicating that JA and JA–Ile can play distinct roles depending on the available light (Fig. 7).

Plant–herbivore interactions in the context of different light environments have largely focused on changes in the light availability caused by plant canopies, which have a major effect on the light's spectral composition, namely the ratio of R:FR radiation (39). For example, in *N. attenuata*, it has been shown that exposure of plants to FR radiation resulted in reduced chemical defense levels (e.g., the herbivore-induced accumulation of phenolics) (18), whereas in *A. thaliana*, FR radiation improved the tissue quality and reduced the plants' sensitivity to JA (16). These studies aimed at understanding how FR signals are used by plants to increase their competitive ability, as well as how plants tradeoff competition versus herbivore defense. In our investigation, however, we studied the effect of different ratios of R:FR radiation, asking how the light spectral composition modulates jasmonate-mediated EFN secretion. The corresponding experiments indicated that the light composition but not intensity crucially modulates the jasmonate-dependent EFN secretion.

Recently it has been reported in *A. thaliana* that phytochrome A and jasmonate signaling are integrated via the stability of the JAZ1 protein (40). In this study, *phyA* mutants showed reduced JA-regulated growth inhibition and altered oxylipin content relative to the wild type. Further, the COI1-mediated degradation of JAZ1 in response to JA treatment or wounding required phyA, indicating that FR and defense pathways are integrative, not mutually exclusive (40). Whether JAR1, the enzyme involved in conjugation of JA to Ile (14), is also involved in phyA signaling is yet to be studied. Unfortunately, confirmation of this hypothesis using molecular methods, such as the generation of *TD* or *JAR1* knockout mutants, is not possible in lima bean due to a lack of the required methodology. Also, using other well-developed model systems such as *A. thaliana*, tomato, or *Nicotiana tabacum* is not an option, because none of these plant species feature extrafloral nectaries. Nevertheless, our classical biochemical approach provides unambiguous evidence that JA–Ile rather than JA is the active compound that controls EFN secretion in lima bean. Future studies should verify whether this also holds true for other plant species in which JA application has been shown to induce EFN secretion during the day [e.g., different *Acacia* species (5), *M. tanarius* (6), and *Ricinus communis* (21, 22)]. Field studies at the lima bean's natural growing site should focus on the diurnal changes in ant activity patterns and unravel whether these correlate with EFN secretion and/or the feeding rhythms of the plant's natural herbivores. Such studies will help to shed light on the evolutionary and ecological significance of the observed EFN secretion patterns.

To summarize, we have demonstrated that the light environment is crucially involved in regulating the lima bean's response to jasmonates and hence, subsequent EFN secretion (Fig. 7). In untreated plants, EFN secretion rates were highest in the dark phase, which was reversed by JA, but not by JA–Ile treatment. The light-dependent inductive effect of JA varies with light spectral composition, and shows a bimodal, switch-like behavior, rather than a continuous response to increasing light intensities. Finally, our results strongly suggest that this light-controlled EFN regulation proceeds via the biosynthesis of JA–Ile, the active elicitor molecule for this indirect defense response.

Materials and Methods

Plant Growth and Light Conditions. Lima bean plants (*P. lunatus*) were cultivated from seeds derived from a native population growing near Puerto Escondido, Mexico (29). Plants were grown in climate chambers (Snijders Microclima MC1000E, Snijders Scientific) at 27 °C, 65% humidity, in a 16-h photoperiod. Experiments were performed with 4-wk-old plants (i.e., five to six leaves per plant). For artificial night experiments, plants were kept in complete darkness for 24 h. Diurnal changes of EFN secretion were monitored continuously for a period of 24 h under the 16-h photoperiod (457.1 μmol) at 27 °C and 65% humidity. For experiments regarding EFN secretion at increasing light intensities, plants were exposed to 0% (0.02 μmol), 25% (241.7 μmol), 50% (451.7 μmol), and 100% (712.8 μmol) (measured using a LI-COR 250A light meter, LI-COR Biosciences) at 27 °C and 65% humidity in a climate chamber. For experiments with different R:FR ratios of light, plants were kept in growth chambers (Percival Scientific) at 27 °C that contain LED lampbanks (CLF floralLED series, CLF Plant Climatics, with overall light intensity up to 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$) as the light source, where each light wavelength (red LED λ_{max} = 673 nm; far red LED λ_{max} = 745 nm) can be programmed to desirable intensities ranging from 1 to 100%. The fluence rates were rechecked using a GER1500 spectroradiometer (Geophysical and Environmental Research) (Fig. S4). The red LED showed peak emission between 635 and 690 nm and the far-red LED between 700 and 780 nm (Fig. S4).

Quantification of EFN Secretion. At the beginning of each experiment, extrafloral nectaries were thoroughly washed with tap water to remove residual EFN and plants were allowed to dry. Then EFN secretion was induced by spraying an aqueous solution of the focal inducer (1 mM unless stated otherwise) on the leaves until runoff. Plants were treated twice with the desired compound at an interval of 30 min. After that, leaves were allowed to dry for 1 h before plants were placed back into the climate chambers. The EFN secreted 24 h posttreatment was quantified as the amount of soluble solids (i.e., sugars and amino acids). The concentration of EFN was measured immediately upon removal from the nectary using a temperature-compensating refractometer (ATAGO N-10E refractometer; Leo Kübler) and the nectar volume was quantified using 5-μL microcapillaries as described (6, 29). EFN measurements from all nectaries of an individual leaf were pooled. The EFN was quantified as the amount of soluble solids per gram dry weight of leaf material secreted in 24 h. JA was synthesized from commercially available MeJA (Sigma) by saponification. JA–Ile and coronalon were synthesized according to published procedures (25, 41).

Phytohormone and Amino Acid Analysis. Analysis and quantification of phytohormones was performed using standard liquid chromatography/mass spectrometry (LC/MS) protocols (42). Amino acid analysis was carried out after derivatization with mercaptoethanol and O-phthaldialdehyde (43).

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