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Short Communication

Herbivorous insects alter the chlorophyll metabolism of galls on host plants

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ABSTRACT

Five types of insect-induced galls derived from three host plant leaves were analyzed for their carotenoid (Car), chlorophyll (Chl), and Chl biosynthesis porphyrins such as protoporphyrinogen IX (PPIX), magnesium protoporphyrin (MGPP) and protochlorophyllide (Pchlide), and Chl degradation intermediates including chlorophyllide (Chlide), pheophytin (Phe), pheophorbide (Pho), and phytylated and dephytylated pigments, and compared to ungalled portions of the same leaf. Galls contain significantly lower levels of Chl-related compounds (CRCs) than ungalled portions of host leaves. The mole percent of porphyrin and the ratios of Chlide/Phe and phytylated/dephytylated pigments are both very different between galls and host leaves. We, therefore, conclude that leaf-derived gall is a kind of non-leaf green tissue, that herbivorous insects alter gall Chl biosynthesis and degradation pathways, that Mg-chelatase, Mg-dechelatase, and chlorophyllase may be the major non-lethal enzymes in galls, and that while ungalled host leaves take Chl → Phe → Pho and Chl → Chlide → Pho as the major and minor degradation routes, respectively, all galls are in contrast with the host leaves.

Introduction

Insect-induced galls are an atypical growth and differentiation form of plant tissue. Gall-inducers use these structures as shelters for protection and sources of nutrition. Despite our considerable knowledge of chemicals such as auxins, cytokinins, gibberellins, abscisic acids, zeatin, and their synergists, the process of gall initiation and the follow-on manipulation mechanisms are still unclear. Galls cause multiple physiological changes in host plants such as changes in pH and polarity, excess sugars and free amino acids, alterations in nutrient compositions (Stone and Schönrogge, 2003; Motta et al., 2005), deficiencies in pigment–protein complexes, lower contents of Chls, and higher contents of secondary metabolites such as phenolics, anthocyanins, and tannins, all of which impact photosynthetic capacity and plant defenses (Yang et al., 2003, 2007; Huang et al., 2011, 2012, 2014). Transcriptome analysis of gall in grape leaf confirmed the above in that galling insect increased gene expression for primary metabolism such as water, nutrient, and mineral transport, glycolysis, and fermentation, in leaf-derived gall tissues, and decreased gene expression for nonmevalonate and terpenoid synthesis, but increased that for shikimate and phenylpropanoid biosynthesis, secondary metabolite systems that alter defense status in grapes, leading to the leaf-derived galls shift from autotrophy to heterotrophy status (Nabity et al., 2013).

However, how the first cause for gall-forming insects to manipulate the morphological and physiological changes in the host leaf is an interesting myth. The gradients of reserve substances and enzyme activities associated with parasite-generated stresses vary during gall development and are fully-established in mature galls. The high metabolic activity in the inner cortex is strongly influenced when fed on by insects. The generation of reactive oxygen species (ROS) may be the primary trigger for the formation of these gradients that are important for gall metabolism and the availability of nutrients to herbivores (Oliveira et al., 2011). ROSs are important effectors in hypersensitive response (HR) signaling and possible communicators between dying cells and living plant

Abbreviations: Car, carotenoid; Chl, chlorophyll; PPIX, protoporphyrinogen IX; MGPP, magnesium protoporphyrin; Pchlide, protochlorophyllide; Chlide, chlorophyllide; Phe, pheophytin; Pho, pheophorbide; CRCs, Chl-related compounds; HR, hypersensitive response.

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tissues. The HR is a mechanism employed by plants to counteract pathogen growth and localize cell death. (Iakimova et al., 2013). Therefore, ROS production may be also a strong indication of localized HR to gall-inducing herbivores.

The activity of chlorophyllase and Mg-dechelatase, which are responsible for the first steps in the Chl degradation pathway in plants, is elicited by *Rhopalosiphum padi* and *Diuraphis noxia* (Ni et al., 2002; Wang et al., 2004). Chlorophyllase 1 of *Arabidopsis thaliana*, encoded by *AtCLH1*, is indicated to be involved in plant damage control and can modulate the balance between different plant defense pathways (Kariola et al., 2005). A previous study showed that the ungalled leaf might use Chl → Phe → Pho as the major route for chlorophyll degradation, whereas the cicedomyiid gall might use Chl → Chlide → Pho as the major route (Yang et al., 2003). Investigation on the metabolic response of larvae in bud galls induced by a Pteromalid wasp, *Trichilogaster acaciaelongifoliae*, in *Acacia longifolia* to reduced (O_2) and elevated (CO_2) indicated that larvae were tolerant of hypoxia/hypercarbia and also capable of reducing their respiratory rates to cope with hypercarbia (Haiden et al., 2012). Symbiosis between gall-inducing insects and fungi catalyzed both expansion in resource use (niche expansion) and diversification, i.e. the evolution of symbiotic interaction leads to niche expansion, which in turn catalyzes diversification (Joy, 2013).

Chl accumulation is a combined effect of the Chl biosynthesis pathway and the Chl degradation pathway. *Daphnephila* induces highly diverse cecidomyiid galls on leaves of the plant genus *Machilus*. Therefore, this study used five types of galls residing on three species of host plants to determine if the alteration by herbivorous insects of Chl biosynthesis and degradation metabolism is a comprehensive phenomenon, as questioned before (Yang et al., 2003).

Materials and methods

Gall and host leaves

Ovoid, obovate, and banana-shaped galls located on the lower epidermis of *Machilus thunbergii* leaves are induced by *Daphnephila taiwanensis*, *Daphnephila sueyenae*, and *Daphnephila stenocalia* (Diptera: Cecidomyiidae), respectively. Spindle-shaped galls are induced by unidentified species 1 (Diptera: Cecidomyiidae) and grow on the epidermis of *Cyclobalanopsis glauca* (Fagaceae) leaves. Cup-shaped galls induced by unidentified species 2 (Diptera: Cecidomyiidae), reside on the epidermis of *Litsea ccuminata* (Lauraceae) leaves (Fig. 1). The former five galls were collected from Yang Ming Shan National Park in northern Taiwan and the fifth from Baoshan Dam in central Taiwan. Mature galls were detached from galled leaves and surrounding healthy tissues trimmed to avoid contamination. Data were collected from 5 to 10 galls and the ungalled portion of the same leaf from each tree, and galls from 5 to 8 trees hosting each of the five types of galls were used in the statistical analyses. The trees sampled for galls were at least 5 m tall, >10 years of age, and appeared healthy. Samples were collected from February to April 2010, at a time when the galls had reached their maximum size. All five species of gall-makers had similar life cycles. According to our field observations, larvae forming the five galls hatch from eggs in the spring, penetrate directly into leaf tissue, and remain undeveloped until autumn. Galls then begin to develop around October and mature soon thereafter. Larvae develop into second and third instars in mature galls and emerge in the early spring of the following year (Huang et al., 2011).

Pigment analysis

Galls were dissected to remove larvae. Mature leaves or galls were frozen with liquid nitrogen and then extracted with 80% acetone. The concentrations of Car and eight Chl-related compounds (i.e., PPIX, MGPP, Pchlide, Chl, Chlide, Phe, and phytylated and dephytylated pigments) were determined according to a combined procedure described by Yang et al. (1998). The mole percent of individual porphyrin is defined as [(PPIX, MGPP or Pchlide) / (PPIX + MGPP + Pchlide)] × 100%. The values of phytylated and/or dephytylated pigments in the samples are read directly at absorbances of 661 and 666 nm (i.e., A_{661} + A_{666}/g DW), respectively, with a Hitachi U3010 UV–visible spectrophotometer.

Statistical analysis

For significance levels, the means of pigment levels in galls and ungalled leaves were compared by the least significant difference (LSD) test at $p < 0.05$. All statistical analyses were conducted using JMP software, version 5.01 (SAS Institute, Cary, NC).

Results and discussion

There is a distinctive difference in the external shape and color of galls versus their corresponding host leaves, with the latter being flat and dark green whereas the galls are fruit-like and light or pale green (Fig. 1).

Changes in Chl biosynthetic intermediates

Chl and Car

Galls and ungalled portions of leaves have different Car/Chl ratios in addition to differences in Chl and Car content. While the Chl and Car contents of three ungalled portions of leaves were greater than 2000 μg/g and 530 μg/g, respectively, those of galls were reduced to 13–225 μg/g and 9–108 μg/g, respectively. Even when there was no significant change in the ratios of Car/Chl between the three galls and ungalled *M. thunbergii* leaves, those of the other two galls increased from 0.31 to 0.54 for unidentified species 1 and from 0.29 to 0.66 for unidentified species 2 (Table 1).

Porphyrins and their mole percent

Porphyrins are the Chl biosynthesis intermediates. All five of the tested mature galls accumulated much lower levels of total porphyrins (i.e., PPIX + MGPP + Pchlide) than their host leaves. Cup-shaped gall contained 2.2% and ovoid-shaped gall 72.8% of the total porphyrins found in host leaves, whereas the other three galls contained 30–40% (Table 1).

Galls not only produce significantly fewer porphyrins, but also change the mole percent of individual porphyrins. While the mole

Fig. 1. Appearance of galls analyzed in this study. Ovoid (A), obovate (B), and banana-shaped (C) galls induced by *Daphnephila taiwanensis*, *D. sueyenae*, and *D. stenocalia*, respectively; the spindle-shaped (D), and cup-shaped (E) galls induced by unidentified cecidomyiid species, respectively. All galls reside on the lower epidermis of host leaves.