On the Inside

The Function of Leaf Oil Bodies

Oil bodies are intracellular structures present in the seed and leaf cells of many land plants. The oil bodies of seeds serve as storage compartments for lipids, but the physiological function of leaf oil bodies is unknown. **Shimada et al. (pp. 105–118)** provide evidence that leaf oil bodies function as subcellular factories for the production of a stable phytoalexin in response to fungal infection and senescence. Fungal infection can dramatically affect crop production. The genus *Colletotrichum* is a common infectious and pathogenic fungus in a variety of crops, and many species of this fungus use a hemibiotrophic infectious strategy to invade host plants. Recently, the genus *Colletotrichum* genome and transcriptome have been analyzed, and an isolate of *Colletotrichum higginsianum* has been reported to infect Arabidopsis (*Arabidopsis thaliana*). The authors report that their proteomic analysis of oil bodies prepared from Arabidopsis leaves revealed caleosin (CLO3) and α-dioxygenase (α-DOX1) to be major surface components. Infection with the pathogenic fungus *C. higginsianum* promoted the formation of CLO3- and α-DOX1-positive oil bodies in perilesional areas surrounding the site of infection. The combination of α-DOX1 and CLO3 produced a stable compound, 2-hydroxyoctadecatrienoic acid (2-HOT), from α-linolenic acid. The authors report that 2-HOT has antifungal activity against members of the genus *Colletotrichum* and that infection with *C. higginsianum* induced 2-HOT production. These results lead to the conclusion that 2-HOT is an Arabidopsis phytoalexin. Thus, in this system, leaf oil bodies produce a phytoalexin under pathological conditions, revealing a new mechanism of plant defense and a new function for leaf lipid bodies.

"Mite-y" Arabidopsis

The chelicerates are a group of arthropods of major ecological and agricultural importance that include phytophagous mites that pierce plant tissue to feed on cell contents. Of these, the two-spotted spider mite (*Tetranychus urticae*; Fig. 1) is the best characterized. This species is a major agricultural pest worldwide and has been documented to feed on over 1,100 different plant species, including more than 150 crop plants. Among plant species, Arabidopsis affords exceptional opportunities for functional studies of plant-arthropod interactions. The response of Arabidopsis to herbivory by insects in several feeding guilds has already been investigated. In laboratory settings, the two-spotted spider mite is a pest of Arabidopsis and has been documented on a number of related species in the Brassicaceae family to which Arabidopsis belongs. **Zhurov et al. (pp. 384–399)** have introduced a novel platform for studies of herbivore-plant interactions that uses genomic and genetic resources developed for Arabidopsis and the two-spotted spider mite, allowing them to examine reciprocal genome-wide responses between a plant and a mite. They report that the transcriptional responses of Arabidopsis to mite herbivory resembled those observed for Lepidopteran herbivores. Mutant analysis of induced plant defense pathways revealed that only a subset of induced programs, including jasmonic acid signaling and biosynthesis of indole glucosinolates, are central to defense in Arabidopsis to mite herbivory. On the herbivore side, indole glucosinolates dramatically increased mite mortality and development times. The authors have also identified an indole glucosinolate dose-dependent increase in the number of differentially expressed mite genes belonging to pathways associated with detoxification of xenobiotics. This demonstrates that the two-spotted spider mite is sensitive to Arabidopsis defenses that are associated with deterrence of insect herbivores that are very distantly related to chelicerates.

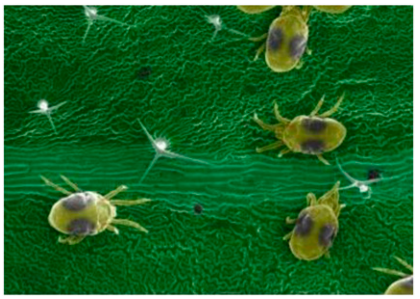

Figure 1. Two-spotted spider mites feeding on a leaf. Photo image credit: Laboratory of Zhurov et al.

Estimates of Leaf Vein Density Are Scale Dependent

Leaf vein density (LVD), defined as the total length of veins per unit area, has been linked to rates of photosynthesis, plant and leaf hydraulic conductance, leaf size and conductance, and leaf allometry. It has been suggested that an increase in vein density contributed to the phylogenetic radiation and rise to ecological dominance of the angiosperms. Despite much interest in the measurement of LVD, little consideration has been given to the effects of measurement methods on its estimate. In this issue, **Price et al. (pp. 173–180)** focus on the relationship between measurement methods and estimates of LVD. In particular, they examine the dependence of LVD on magnification, field of view (FOV; i.e. the physical size of the object studied), and image resolution. They show that estimates of LVD increase with increasing image magnification and resolution. They also demonstrate that estimates of LVD are higher and more variable at small FOV. These effects arise due to three primary factors: (1) the tradeoff between FOV and magnification, (2) geometric effects of lattices at small scales, and (3) the hierarchical nature of leaf vein networks. The first factor has the potential to influence vein density at all scales of measurement, the second factor will be most pronounced at small FOVs, and the third factor has the strongest effect as vein sample sizes get larger, both within an individual leaf and as leaves themselves get bigger. The results reported help to explain differences in previously published studies and highlight the importance of using consistent magnification and scale, when possible, when comparing LVD and other quantitative measures of venation structure across leaves.

Virus-Based MicroRNA Silencing in Plants

MicroRNAs (miRNAs) are genome-encoded 20- to 24-nucleotide small

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RNAs that act as posttranscriptional regulators in eukaryotes. Plant miRNAs play essential roles in various biological processes, including development, signal transduction, protein degradation, response to abiotic and biotic stress, and the regulation of their own biogenesis. To date, more than 6,800 miRNAs in approximately 62 plant species have been identified. However, only a very limited number of miRNAs have been functionally characterized. **Sha et al. (pp. 36–47)** now offer a report concerning a virus-based miRNA silencing (VbMS) system that can be used for functional analysis of plant miRNAs. VbMS is performed through tobacco rattle virus (TRV)-based expression of miRNA target mimics to silence endogenous miRNAs. VbMS of either miR172 or miR165/166 caused developmental defects in *Nicotiana benthamiana*. VbMS of miR319 reduced the complexity of tomato (*Solanum lycopersicum*) compound leaves. These results demonstrate that TRV-based VbMS is a powerful tool to silence endogenous miRNAs and to dissect their functions in different plant species. The authors point out that many other available viral vectors could be used in a similar strategy as the TRV VbMS vector for functional analysis of miRNAs in a diverse range of eudicot and monocot crops.

Auxin Transporters and the Development of Compound Leaves

Leaves are derived from leaf founder cells developed at the periphery of the shoot apical meristem (SAM). The meristematic activity of the SAM is maintained by class I *KNOTTED-LIKE HOMEOBOX* (*KNOXI*) genes. Early events marking the recruitment of leaf founder cells to the incipient leaf primordia at the peripheral zone of the SAM involve the down-regulation of *KNOXI* gene expression. The MYB domain protein encoded by the *ASYMMETRIC LEAVES1/ROUGH SHEATH2/PHANTASTICA* (*ARP*) gene, together with other factors, excludes *KNOXI* gene expression from incipient leaf primordia and allows for the initiation of leaves and the specification of leaf adaxial identity. **Ge et al. (pp. 216–228)** have investigated the role of *ARP* and *KNOXI* genes in the more complex case of compound leaf development in *Medicago truncatula*. To address the role of *ARP* and *KNOXI* genes in compound leaf development in *M. truncatula*, they isolated and characterized *Tnt1* retrotransposon insertion mutants of *M. truncatula PHANTASTICA* (*PHAN*) and *BREVIPEDICELLUS* (*BP*) genes. Their results show that the *mtphan* mutant exhibits multiple defects in compound leaf development, including curling and deep serration of leaf margins, shortened petioles, increased rachises, petioles acquiring motor organ characteristics, and ectopic development of petiolules. On the other hand, the *mtbp* mutant did not exhibit visible defects in compound leaf development. They show that the altered petiole development requires ectopic expression of *ELONGATED PETIOLULE1* that encodes a lateral organ boundary domain protein and that the distal margin serration requires the auxin efflux protein *M. truncatula* PIN-FORMED10 in the *mtphan* mutant. These findings are of interest because other studies have shown that maxima in auxin activity also mark and precede leaflet and lobe initiation in compound-leafed species.

Plasticity of C_4 Biochemistry

Extreme interest in the C_4 pathway of photosynthesis has been generated by their potential for enhancing crop productivity and maintaining yield stability in the face of global warming and population pressure. Because of anatomical, metabolic, and energetic complexities, C_4 metabolism is highly sensitive to limiting light intensity and quality. Light quality has a greater influence on C_4 photosynthesis than on C_3. Leaf pigments preferentially absorb the blue and red region of the spectra and some wavelengths penetrate deeper into leaves. Earlier studies have shown in C_3 leaves that exposure to different wavelengths results in characteristic light penetrations profiles, which translated into different gradients in PSII yield, rates of ATP production, and assimilation within the leaf. In C_4 leaves, because of the concentric anatomy, light reaches mesophyll (M) cells before the deeper bundle sheath (BS) cells, and could alter the balance between light harvesting and energetic partitioning between BS and M. **Bellasio and Griffiths (pp. 466–480)** have modeled the likely profiles of light penetration for specific wavelengths associated with red, green, and blue light within a maize (*Zea mays*) M and BS leaf cross section and calculated the impact on potential ATP production for each cell type. The theoretical partitioning of ATP supply between M and BS cells was derived for these metabolic activities from simulated profiles of light penetration across a leaf. A model they have formulated suggests that transamination and the two decarboxylase systems (NADP-malic enzyme [ME] and phospho*enol*pyruvate carboxykinase) occurring in maize are critical for matching ATP and NADPH demand in BS and M when light capture was varied under contrasting light qualities. The plasticity of C_4 metabolism, in particular the possibility of shifting between malate and Asp as primary carboxylase product, may be of pivotal importance in allowing plasticity of ATP and NADPH demand. In conclusion, their study explains the extensive overlap between BS and M functions and the requirement for at least two decarboxylase systems in NADP-ME subtype plants such as maize.

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