

COMMENTARY

Plants protect themselves from herbivores by optimizing the distribution of chemical defenses

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Plants, as a whole, are well stocked with chemical defense compounds that function in protection against herbivores and pathogens. Within individual plants, however, there is extensive variation in the amounts of chemical defenses among different organs, tissues, and

developmental stages. For example, defense compounds are typically present in greater concentrations in young compared to old leaves and in reproductive compared to vegetative organs. These patterns have been rationalized by various theories, chief among them the optimal

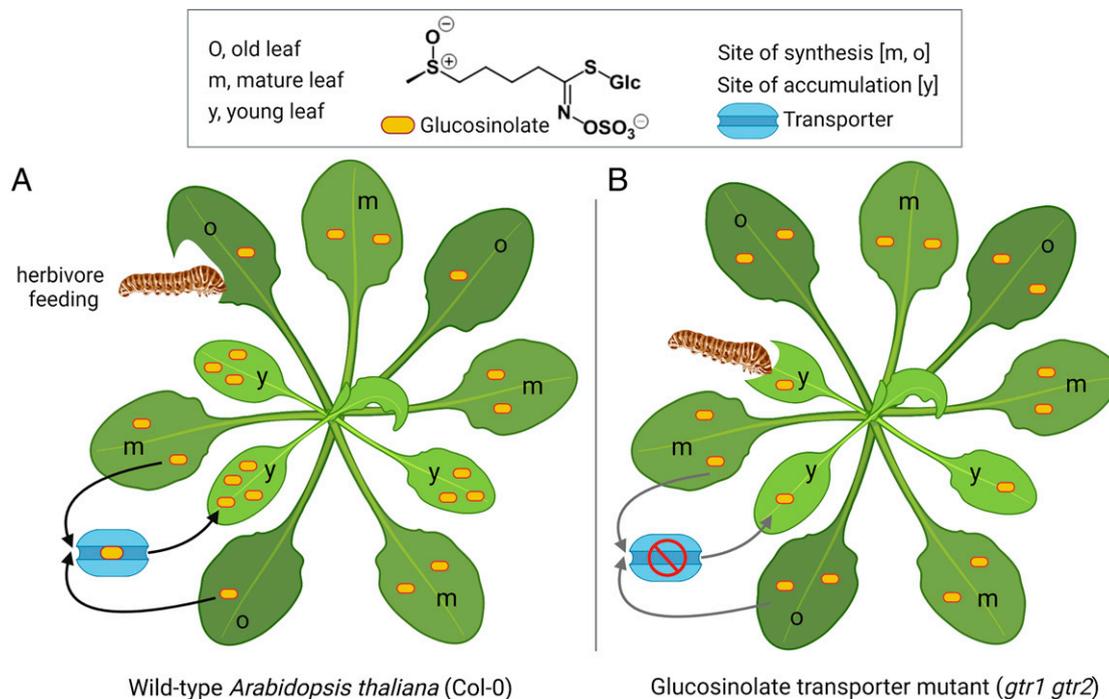


Fig. 1. Testing a classical ecological theory with a biochemical tool. The optimal defense theory posits that the distribution of chemical defenses within a plant is based on the relative fitness value of plant parts. In wild-type *A. thaliana*, glucosinolates are present in much higher concentration in young leaves (y) than in mature (m) or old (o) leaves, due to the transport of glucosinolates out of their sites of biosynthesis in the old and mature leaves to young leaves, where they accumulate (A). To test whether this pattern confers fitness benefits, Hunziker et al. (1) employ a double glucosinolate transporter mutant in which the concentration of glucosinolates is the same in all leaf age classes (B). On wild-type plants, caterpillars fed only on older leaves, with little effect on plant survival to reproduction. However, on the transporter mutant, feeding occurred only on younger leaves, leading to premature death, and showing the advantages of the wild-type distribution pattern for plant fitness. Relative glucosinolate concentration in leaves is depicted by the number of yellow ovals, and the transporter is given in blue. The chemical structure shown, 4-methylsulfinylbutyl glucosinolate, is the major glucosinolate in the leaves of *A. thaliana* (Col-0).

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Author contributions: J.G. and C.U. wrote the paper.

The authors declare no competing interest.

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See companion article, "Herbivore feeding preference corroborates optimal defense theory for specialized metabolites within plants," [10.1073/pnas.2111977118](https://doi.org/10.1073/pnas.2111977118).

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Published January 20, 2022.

defense theory, but this theory has proved very difficult to test until a recent report from Hunziker et al. (1).

Developed over many years by several authors (2–6), the optimal defense theory assumes that defenses incur costs because they redirect resources from growth and other plant processes. Hence defenses are distributed within plant tissues and organs in a way that maximizes plant fitness. The optimal intraplant distribution is hypothesized to be based on three factors: 1) value—the contribution of each tissue or organ toward evolutionary fitness; 2) risk—the chance of a tissue being attacked by herbivores; and 3) cost—the metabolic resources needed for biosynthesis and storage. The optimal defense theory has been mostly tested by correlating the distribution of defenses in various plant species with measurements of value and risk (7–9), and computational models (10) and information theory (11) have also been applied. However, the direct manipulation of intraplant defense distribution would allow a more powerful test of the theory.

The discovery of transporter proteins with high affinities for plant defense compounds provides a way to alter the distribution of chemical defenses in intact plants. Hunziker et al. (1) exploit membrane transporters to test the influence of glucosinolate distribution on caterpillar herbivory in the model plant *Arabidopsis thaliana*. Glucosinolates are the characteristic mustard oil glucoside defenses of *A. thaliana* and other Brassicaceae species, such as cabbages, broccoli, and rapeseed (12). Upon plant damage, glucosinolates are activated by glucose cleavage, leading to the production of toxic hydrolysis products. The distribution of glucosinolates in *A. thaliana* was altered by knocking out the genes encoding three glucosinolate transporters (13). These H⁺/symporters mobilize glucosinolates from older to younger leaves, leading to higher concentrations in young (2 $\mu\text{mol}\cdot\text{g}^{-1}$ to 4 $\mu\text{mol}\cdot\text{g}^{-1}$ fresh weight) compared to mature (1 $\mu\text{mol}\cdot\text{g}^{-1}$) and older (<0.5 $\mu\text{mol}\cdot\text{g}^{-1}$) leaves. However, in transporter mutants, there was a uniform concentration of glucosinolates in leaves of all age classes (averaging about 2 $\mu\text{mol}\cdot\text{g}^{-1}$) (Fig. 1).

Such dramatic differences in glucosinolate distribution provide an ideal chance to test the predictions of the optimal defense hypothesis. When newly hatched Egyptian cotton leaf-worm (*Spodoptera littoralis*) caterpillars, a supergeneralist feeder, were allowed to feed on wild-type *A. thaliana*, they fed exclusively on old leaves because of their significantly reduced glucosinolate concentrations, as low as one-tenth of the concentrations found in young leaves (1). However, on the double glucosinolate transporter mutant *gtr1gtr2*, caterpillars switched completely to feeding on young leaves. Since young leaves are less tough, more digestible, and more nutritious than old leaves, they should be a far superior food source in the absence of any difference in defenses.

An important control was the use of a quadruple mutant in glucosinolate biosynthetic genes that produces no residual glucosinolates (1, 14). With this *A. thaliana* line, caterpillars also fed predominantly on young leaves, suggesting that the distribution of glucosinolates was the key factor mediating leaf preference in the transporter mutants, not differential distributions of other metabolites. Caterpillar feeding choice in these experiments was tightly correlated with plant fitness. When only young leaves were damaged, due to their low glucosinolate content, none of the plants survived to reproduction. In contrast, when only old leaves were damaged, all of the plants survived to reproduce.

Hunziker et al. (1) test another prediction of the optimal defense theory: that defenses are allocated in direct proportion

to the risk of herbivore attack. Risk to plant tissue would be highest when other tissues of the same plant are also under attack, and, indeed, after herbivory, the accumulation of several types of glucosinolates was demonstrated to increase in the remaining unconsumed leaves. Glucosinolates and other defense metabolites have been shown to increase after insect herbivory in many plant species (15).

The research of Hunziker et al. is an outstanding example of how the discoveries of plant biochemistry can provide tools to test a classic hypothesis in plant ecology.

Concerning the distribution of defenses within the plant, the results of this investigation (1) can be generalized to other species and other classes of chemical defenses. Defense compounds are often present in greater concentrations in young than in old leaves (16), and this pattern can now be assumed to have significant fitness benefits for plants in general. In *A. thaliana*, this pattern is created by the transport of glucosinolates from old to young leaves. Similar transport processes can be inferred to occur in other species, although the site of biosynthesis may differ. In this study, glucosinolate biosynthesis was deduced to occur mainly in old and mature leaves of *A. thaliana*, based on greater expression of biosynthetic genes than in young leaves. However, in other species, a skewed distribution of defenses in different leaf age classes could arise from synthesis elsewhere, for example, in young leaves or in another organ such as roots (17), followed by selective transfer to leaves of different ages. Unfortunately, the sites of synthesis of most plant defenses and the basis of selective transport are not known.

The transfer of plant defenses among organs and tissues is not a general feature of all classes of defense compounds but is likely restricted to water-soluble substances such as alkaloids, nonprotein amino acids, glucosinolates, cyanogenic glycosides, iridoid glycosides, and other glycosides. Lipophilic defenses, which include many terpenes, phenolic compounds, and other components of resins and essential oils, are usually synthesized in the cells of glandular trichomes, resin ducts, or other secretory structures, and then stored there without further mobility (18). Nevertheless, the distribution of lipophilic defenses in plants may adopt the same patterns predicted by the optimal defense theory, although the resulting gradient of defense concentration would then arise from selective biosynthesis, not selective transport.

The optimal defense theory has generally been applied to plant defenses against herbivores and not pathogens, but the distribution of both constitutive and inducible defenses against pathogens could also be governed by the same basic principles, resulting in varying levels of protection in different organs, although much more investigation is needed. Defenses against pathogens are also mobile in plants, with initial infection in one organ sometimes inducing a systemic resistance throughout the plant (19). Although this so-called systemic acquired resistance is usually ascribed to mobile signaling molecules, the actual antipathogen agents could also be mobile, as seen in the present study for glucosinolates (1).

The research of Hunziker et al. (1) is an outstanding example of how the discoveries of plant biochemistry can provide tools to test a classic hypothesis in plant ecology. Our knowledge of plant defense transporters has increased rapidly in recent years

(20, 21), and these proteins can be employed in a range of biochemical, physiological, and ecological studies. In the realm of plant protection, transporters give plants the ability to mobilize chemical defenses rapidly to where they are needed and to

reclaim them from an organ when no longer needed. These proteins may thus be key innovations in allowing plants to persist under herbivore and pathogen attack without breaking their metabolic budgets.

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