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Nutritional quality of specific leaf tissues and selective feeding by a specialist leafminer*

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Summary. Many folivorous insects are selective feeders which consume specific leaf tissues. For specialist herbivores feeding on plants of overall low nutritional quality, selective feeding may allow consumption of a high quality resource. Selective feeding may also allow insects to avoid structural or allelochemical defenses. We examined the structure and chemistry of leaves of American holly, *Ilex opaca* Aiton, and the feeding site of its principal insect herbivore, the native holly leafminer, *Phytomyza ilicicola* Loew (Diptera: Agromyzidae), to test the hypothesis that the leafminer consumes tissues which are of greater nutritional quality than the leaf as a whole. Holly leaves have a continuous layer of palisade mesophyll, uninterrupted by fibers or vascular bundles. The leafminer feeds entirely within this layer. The palisade mesophyll contained 196 mg/g dry wt extractable protein, more than twice as much as the leaf as a whole, and 375 mg/g dry wt saponins, more than 9 times that of the leaf as a whole. The water content of the palisade mesophyll was 66% higher than that of the leaf as a whole. The palisade mesophyll is 3–4 cell layers thick in leaves grown in full sun, but only 2 layers thick in shaded leaves. Crystals, probably of calcium oxalate, are abundant in the abaxial cell layer. These may impose mechanical constraints on larval feeding in shade leaves, which are thinner than sun leaves. Selective feeding on the middle palisade mesophyll of sun leaves allows the leafminer to consume a resource which is lacking in mechanical barriers and is rich in protein and water, but which contains large amounts of saponins.

Key words: Plant-animal-interaction – Phytomyza – Food-Selection – Ilex

Temporal and spatial variation in the nutritional and defensive characteristics of leaves can profoundly affect the suitability of plants as food for folivorous insects (Mattson 1980; Scriber and Slansky 1981; Denno and McClure 1983; and references therein). Assay methods for nutritional and allelochemical components of leaves are well-described in the literature and have been extensively applied to test for correlations between herbivory and leaf chemistry. Typically, such data are obtained by assaying whole leaf homogenates or extracts, a valid approach for studies of insects which consume entire leaves.

Many folivorous insects, however, are selective feeders, consuming certain tissues or cell types and rejecting others. Such insects include leafminers and skeletonizers, many of which consume mesophyll cells and leave behind epidermis and/or vasculature. These insects may selectively consume high-quality tissues in otherwise low-quality plants or plant organs.

There is growing evidence that the nitrogen, water, allelochemical content and toughness of leaves vary intrinsically within and among plants, and that such variation may affect the distribution and abundance of insects (Schultz 1983; Krischik and Denno 1983). However, the importance of variation in food quality among individual tissues within a leaf has received little attention. There is no reason to assume that within-leaf variability in nutritional, allelochemical and structural attributes is any less than that between leaves. For example, the chlorophyll content and chloroplast density of the palisade mesophyll is substantially higher than is that of the spongy mesophyll in many plants (Barber and Baker 1985). The protein content of the palisade mesophyll should therefore be much greater than that of the spongy mesophyll.

We hypothesized that, for specialist herbivores feeding on plants having overall low nutritional quality, selective feeding on specific tissues may allow consumption of a high-quality resource which is either rich in nutrients and water or low in allelochemical or structural defenses, or both. Feeny (1970) speculated that leafminers of oak (*Quercus robur* L.) feed selectively on spongy mesophyll, perhaps avoiding tannin-rich palisade mesophyll. However, Faeth et al. (1981) observed that most oak leafminers feed in the palisade mesophyll. *Fenusa pusila* (Lepeletier) (Hymenoptera: Tenthredinidae), a leafminer of birch, feeds in the spongy and palisade mesophyll, while another birch leafminer, *Messa nana* Klug (Hymenoptera: Tenthredinidae), feeds selectively in the palisade (DeClerck and Shorthouse 1985).

American holly, *Ilex opaca* Aiton, is a highly defended plant, with tough, spinose leaves which are low in nitrogen and water and rich in saponins and phenolics (Potter and Kimmerer 1986). The native holly leafminer, *Phytomyza ilicicola* Loew (Diptera: Agromyzidae) is one of the few insect herbivores of holly. This univoltine specialist leafminer avoids the mechanical defenses of holly, particularly the tough epidermis, by emerging in close synchrony with

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spring leaf flush and ovipositing in tender, developing leaves. The larvae then feed within leaves from early June until the following March (Potter and Kimmerer 1986). We examined the feeding site of leafminer larvae, and tested the hypothesis that the tissues on which the larvae feed are substantially different chemically and structurally from the leaf as a whole.

Materials and methods

Leaves for histology and chemical analysis of leaf tissues were harvested from 3 mature, 9-m-tall, 45-year-old female *I. opaca* planted in the Lexington Cemetery, Lexington, Ky. Trees had full sun exposure on the south side and were shaded on the north side. Leaves collected from these sides are henceforth referred to as sun leaves and shade leaves, respectively. Samples of several hundred sun leaves containing living *P. ilicicola* larvae were harvested in December 1985 and February 1986, when leafminers were actively feeding second and third larval instars. For histologic comparison, shade leaves were harvested from north sides of the same trees. Due to the thinness of the shade leaves, it was not possible to dissect them for chemical analysis of individual tissues.

Histology. To examine the anatomy of holly leaves and the feeding sites of leafminer larvae, ca. 20 µm thick freehand sections were cut from 100 leaves and examined unstained under a compound microscope. For detailed histologic examination and photomicrography, leaf tissue was fixed in glutaraldehyde, postfixed in OsO_4, embedded in Spurr's medium, and 1–2 m thick sections were cut and stained with Toluidine Blue O (Jarlsfors, personal communication).

Chemistry of leaf tissues. For chemical analysis of leaf tissues, leaves were frozen in liquid N_2 in the field and lyophilized. Lyophilized leaves were dissected under a microscope using microsurgical tools. For a first set of leaf samples, collected in December 1985, one half of each leaf was dissected to separate palisade mesophyll cells from the remainder of the leaf. Chemical assays were then performed on the palisade mesophyll, the remaining tissues, and samples of the intact leaf taken from the undissected half of the leaf.

In a second experiment, leaves collected in February 1986 were dissected into their component tissues. Beginning with the upper epidermis and proceeding downward, layers of cells were scraped from the leaf and collected, with separate collections for upper epidermis, palisade mesophyll, spongy mesophyll, vasculature and lower epidermis. After collection, the tissues were examined under a compound microscope, and samples which were contaminated with cells of the wrong tissue type were discarded. Cells of each tissue type were easily recognizable by their characteristic morphologies. For example, palisade mesophyll cells were dark green, elongate and unbranched, while spongy mesophyll cells were pale green and highly branched. Enough of each tissue type was collected to give at least two 2-mg-dry-wt samples per leaf. No attempt was made to collect discrete layers within the palisade mesophyll.

In a third experiment, the consumption of nutrients and allelochemicals by leafminer larvae was examined. Mined areas were dissected from leaves collected in February, using only the large blotch mines created by third instar larvae. The upper epidermis was opened to allow removal of the larva, and the mined area was then cut out, weighed and ground for chemical analysis. Larvae were saved for saponin analysis. An adjacent unmined area of the same leaf was also removed for comparison.

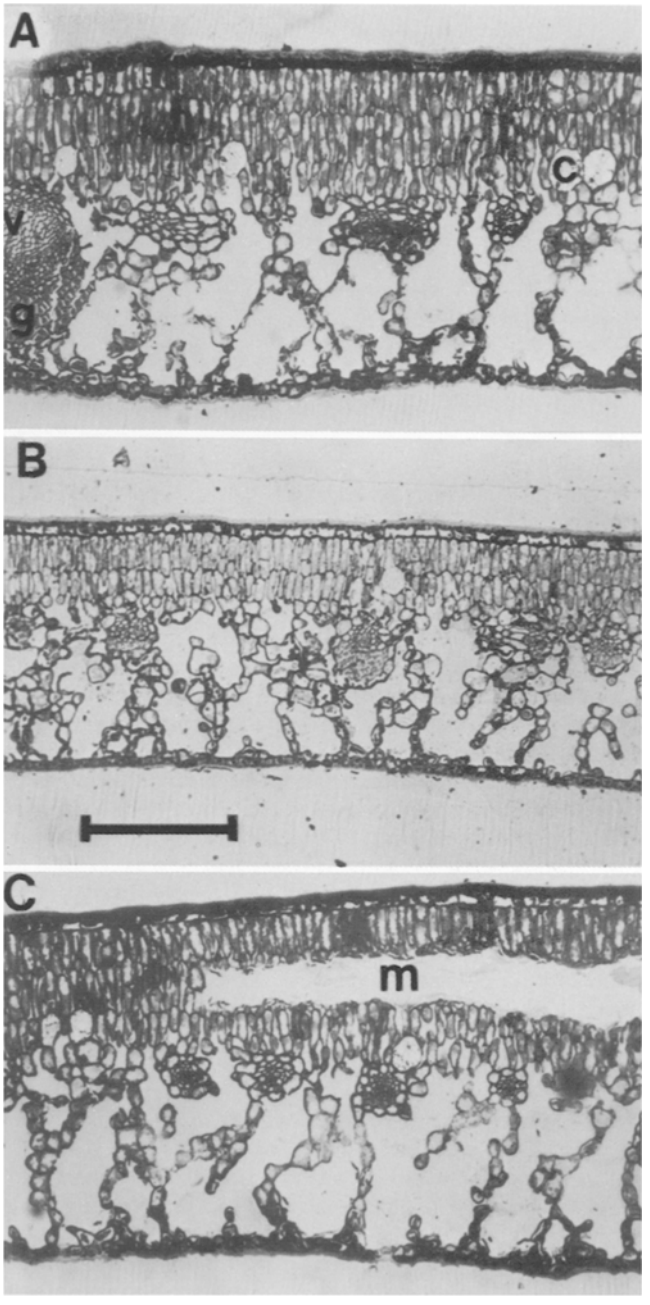

Fig. 1 A–C. Cross-sections of leaves of American holly: **A** sun leaf; **B** shade leaf; **C** sun leaf mined by *P. ilicicola* larva. *c* crystal-containing cell; *v* vascular bundle, *g* gelatinous fibers; *m* mine; Bar is 200 µm

For protein estimation, 2-mg tissue samples were extracted with 0.1 N NaOH, centrifuged at 12,000 × g for 10 min, and the supernatant was assayed for protein using Coomasie Brilliant Blue (Bradford 1976).

Saponins were assayed using a modified erythrocyte hemolysis assay (Kartnig et al. 1964), with purified holly sa-

Table 1. Histochemistry of holly leaves: protein, saponin, and water content of whole leaves, palisade mesophyll and remainder of leaf (i.e. remains of leaf after removal of palisade tissue). Samples were obtained from unmined portions of mined leaves. Data are mean ±SE of measurements on 21 leaves from 3 trees. Differences among trees were not significant

Tissue	Protein (mg/g dry wt)	Saponins (mg/g dry wt)	Water (mg/g fresh wt)
Whole leaf	89.3±2.5	40.9±1.6	463± 7
Palisade	196.3±4.6	375.1±7.0	768±12
Remainder of leaf	19.4±0.8	13.1±0.7	321±23
Significance[a]	$P<0.001$	$P<0.01$	$P<0.001$

[a] Kruskal-Wallis nonparametric analysis of variance

ponins as standard (Kimmerer and Potter, unpublished work).

The water content of fresh holly leaf tissues was estimated by dissecting tissues from fresh holly leaves in a humidified glove bag, and then obtaining fresh wt and oven-dry wt (70C) with a microbalance.

Results

Histology of the holly leaf. Holly leaves are dorsiventral, with adaxial palisade mesophyll and abaxial spongy mesophyll (Fig. 1). The palisade mesophyll is compact and deep green with abundant chloroplasts, while the sparse spongy mesophyll is nearly achlorophyllous with few chloroplasts. The palisade mesophyll was 3–4 cell layers thick in sun leaves but only 2 cell layers thick in shade leaves. The mean palisade mesophyll thickness in sun leaves was 125±6 m ($N=50$) and in shade leaves was 89±8 m ($N=20$).

The abaxial layer of palisade cells was rich in crystals, probably of calcium oxalate, but they were not observed in the middle palisade layer (Fig. 1). Similarly, in shade leaves which have only 2 palisade layers, crystals were observed only in the lower layer.

Major and minor veins were heavily invested with gelatinous fibers, which account for the toughness of holly leaves. The veins were embedded entirely within the spongy mesophyll, and no bundle sheath extensions into the palisade mesophyll were observed. The midvein, however, did extend to the upper palisade layer. Thus, each half of the holly leaf contains a solid sheet of chlorophyllous palisade mesophyll whose middle and upper layers are uninterrupted by crystal-containing cells, veins, fibers or bundle sheath extensions.

Feeding site of Phytomyza ilicicola. Mines of all instars in sun leaves occupied only the middle palisade mesophyll (Fig. 1c) and were not observed in the spongy mesophyll or in the upper or lower palisade mesophyll. In shade leaves, mines occurred primarily in the lower palisade mesophyll, with some consumption of the upper palisade layer. Mines which cross the midrib moved into the upper palisade mesophyll at the midrib.

Larvae mining in the sun leaves apparently avoid the crystal-containing cells of the lower palisade layer, the fibrous vasculature, and the spongy mesophyll by feeding in a single plane in all instars. As the larvae grow larger, the mine "roof" is pushed upward, but the larvae continue consuming the single layer of middle palisade cells. Larvae in shade leaves mine the lower palisade layer in which the crystals are abundant.

Table 2. Protein and saponins in each tissue type of holly leaves. Data are mean ±SE of measurements on 12 leaves from 3 trees. Differences among trees were not significant

Tissue	Protein (mg/g dry wt)	Saponins (mg/g dry wt)
Upper epidermis	16.4±1.2	4.2± 0.3
Palisade parenchyma	180.8±9.3	353.2±11.7
Vasculature	25.4±5.2	124.6± 5.4
Spongy mesophyll	34.1±2.5	15.5± 1.0
Lower epidermis	18.5±1.4	2.1± 0.4

Table 3. Protein and saponin content of mined areas, and of unmined portions of mined leaves. Data are mean ±SE of 12 leaves from 3 plants. Differences among plants were not significant

	Protein (mg/g dry wt)	Saponins (mg/g dry wt)
Whole leaf	91.2±1.0	41.1±1.9
Mined area	81.1±2.0*	29.0±2.4*

* Significantly different at $P<0.10$, paired t-test

Chemistry of holly leaf tissues. The palisade mesophyll of holly leaves was higher in both protein and saponins than was the remainder of the leaf (Table 1), and in fact contained most of the extractable proteins and saponins. Compared with the palisade cells, all other tissues in the holly leaf were lower in protein by a factor of 5 or more, and lower in saponins by a factor of 2 or more (Table 2). The mined areas contained significantly less protein and saponins than did unmined portions of the same leaves (Table 3). However, larvae contained no detectable saponins, suggesting that they may rapidly excrete, egest or metabolize nearly all the saponins ingested.

Discussion

Holly leaves possess a seemingly formidable defensive arsenal which includes a tough epidermis, rigid spinose leaf margins, fibrous vasculature, phenolics, and saponins (Potter and Kimmerer 1986, and this study). All of the potential structural defenses are bypassed by the native holly leafminer: the female oviposits when the leaves are still soft (Potter and Kimmerer 1986) and larvae in sun leaves feed on a cell layer, the middle palisade mesophyll, which lacks

fibers and is unobstructed by any mechanical barriers except for the midrib.

The palisade mesophyll layer of holly is substantially different chemically from the leaf as a whole. While whole leaves are very low in N and water (Potter and Kimmerer 1986), the palisade mesophyll is chlorophyllous, rich in protein and contains abundant water. However, the saponin content of the palisade parenchyma is nearly 10-fold higher than that of the leaf as a whole. Thus, the leafminer larva encounters a food resource which is rich in nutrients but contains very high concentrations of allelochemicals.

Our results illustrate that the nutritional chemistry of specific plant tissues consumed by an insect may differ substantially from that of the leaf as a whole. Therefore, attempts to correlate nutritional chemistry of whole leaves with abundance or success of folivores which consume specific leaf tissues may be misleading. For example, Faeth et al. (1981) found that abundance and diversity of leafminers were negatively correlated with leaf nitrogen content, but acknowledged that whole leaf assays may obscure within-leaf variation in nitrogen and allelochemicals. The significance of tissue-specific variation in nutritional chemistry probably also applies to insects feeding on organs other than leaves, such as bark beetles which consume secondary phloem and cambium. Methods for chemical analysis of target tissues, while not easy to apply to large numbers of samples, are available and may provide more meaningful correlations with herbivory than will assays of whole leaves.

High within-plant heterogeneity in nutritional quality of leaves has been observed in several studies (e.g. Schultz 1983; Schultz et al. 1982; Whitham 1981). We suggest that some of this heterogeneity could be due to differences in the structure of leaves rather than in the nutrient content of specific tissues. For example, shade leaves of holly have only 2 layers of palisade mesophyll, compared with the 3–4 layers in sun leaves, and are lower in total nitrogen and water (Potter and Kimmerer, unpublished data). For a specialist herbivore feeding on specific tissues, heterogeneity in chemistry among leaves may be relatively unimportant.

Environmental factors may indirectly affect feeding behavior of herbivores by influencing plant or leaf structure. For example the native holly leafminer is more abundant and survivorship is greater on sun leaves than on shade leaves (authors' observations), and we suggest that this may be due to the thicker palisade mesophyll of sun leaves which allows unconstrained feeding. Leafminers are also much more common on cultivated holly than on hollies growing in the forest understory (Kulp 1965; Potter 1985). While this difference could be related to environmental factors which directly affect the insect, or to genetic differences between forest and cultivated trees, the major differences may again be structural: cultivated hollies are commonly planted in full sun or only partial shade, while holly in the forest is a tree of shaded, understory habitats. We suggest that the major constraints which limit the success of leafminers on shade leaves may be structural rather than nutritional: shade leaves do not contain a middle palisade layer free of crystal-containing cells. Crystals or rhaphides may pose considerable mechanical difficulty for phytophagous insects (Merz 1959; Ehrlich and Raven 1965). We observed little or no consumption of the upper palisade layer in our samples. Preservation of this layer may be necessary to maintain the integrity of the mine or to protect the larvae from abiotic stress, such as desiccation.

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