

How Do Birch Defenses Operate?

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Abstract - Low levels of nutritive compounds and high levels of total or individual phenols describe birch trees that are unsuitable for insect pests. But analyses of hundreds of foliar compounds suggest that the mortality of insect pests on birch may be more closely related to fatty acids (many of them belonging to the octadecanoid pathway), while many phenolic compounds retard the growth of the surviving larvae. Thus birch foliar defense seems to be orchestrated by specific defense cascades, particularly the octadecanoid pathway.

I. Introduction

Plant defense is defined either in terms of its success, i.e. reduced damage to the plant, or in terms of the chemical and physical plant traits that contribute to low damage levels. These measures may correlate with the performance of pests, but correlations between insect growth and the amount of damage they cause are variable [1]. Knowledge of the chemical and physical basis of defense is essential, allowing for instance for the screening of large numbers of plants without exposing them to herbivory.

Since Feeny's classic paper on oak leaf tannins [2], tannins and other secondary compounds have been seen as the putative main chemical defenses of woody plants. Phenols have achieved their particular status because of their physiological properties, because of their often very high levels in the foliage of woody plants, and because of the availability of easy, albeit crude, analytical methods. Perhaps surprisingly, however, there is still no data-backed consensus concerning the relative role of foliar phenols among all compounds; in other words, we do not know which plant traits actually form the main defenses of woody plants [3].

In this paper I describe the attempts of our research group to elucidate the roles of numerous foliar compounds in defense of the subarctic mountain birch *Betula pubescens* ssp. *czerepanovii* against its lepidopteran and symphytan pests. The study system is described in [1].

II. Traditional Entomological Point of View: Nutritive vs. Secondary Compounds

Especially the older forest entomological literature in German [e.g. 4] emphasized the importance of low levels of nutritive factors, particularly sugars, in the control of forest pests. Later, low levels of foliage proteins and water have been seen as important problems for consumption, especially in woody plants, [1; 5-8; 9]. A major problem with the defensive role of low levels of nutritive leaf traits is that

insect pests tend to compensate for the inadequacy of nutrients by increasing consumption, which may actually lead to increased plant damage [10; 11]. Accordingly, there is not much sound evidence demonstrating that low levels of leaf nutrients actually reduce consumption on trees. This should not be interpreted as meaning that consumption is high for instance on low-protein foliage; usually it is not, but the reason may lie in covarying leaf traits other than proteins.

In birch leaves, nutrients show dramatic ontogenetic changes: proteins and leaf water go down in maturing leaves, while the originally low contents of sugars in young leaves go up, peaking in just maturing or mature leaves, depending on the compound [12; 13]. The tightly intercorrelated traits (high water, high proteins, and low toughness) which characterize young birch leaves are important for the performance of those birch-chewing insects that are adapted to young leaves [9; 14; 15]. On the other hand, the larvae of birch sawfly species, which have adapted to the exploitation of mature leaves [16], may be unable to survive on young birch leaves [17] despite of their high nutritive value.

The laboratory-measured consumption rates of birch leaf chewing insects are not well correlated with nutritive leaf traits [14]. This is not an artefact of lab studies only; the lab-measured consumption of leaves from individual trees showed a significant positive correlation with the amount of damage accumulating in the foliage of the same trees in the field [14].

Birch leaf phenols can be analyzed by shortcut methods, such as the "total phenols" revealed by the Folin-Ciocalteu method, but due to the high number of individual phenols in birch leaves [18-22], such analysis does not necessarily reveal the importance of specific phenolic compounds (see e.g. [23]). The probable compound-specific effects of phenols on insect pests are particularly noteworthy because individual phenolic groups display distinctly different seasonal dynamics [1; 12; 20; 24; 25]. The foliar contents of soluble proanthocyanidins (= condensed tannins) increase dramatically with leaf maturation. Since they also form by far the largest category within "total phenols" in birch leaves, both "total phenols" and soluble proanthocyanidins peak in August. This easily masks the fact that practically all other phenolic compounds either peak in young leaves or show no obvious seasonal trends, and that even condensed tannins are synthesized during the most active growth of birch leaves [1; 12].

Insect growth on birch leaves characteristically correlates negatively with "total phenols", or with some individual phenolic compounds, or with groups of them such as

phenolic glycosides. The problem is that the particular compounds which correlate most strongly with insect traits tend to vary from one experiment to another. One possible reason is that the absolute and relative contents of leaf phenols change in the course of leaf maturation, and the compounds showing the best correlations thus change as well. On young as well as shaded leaves, for instance, insect growth tends to correlate negatively with hydrolyzable tannins [9; 15], particularly with galloylglucoses, which peak in early season [20]. In more mature leaves, "total phenols", proanthocyanidins, flavonoids, or hydrolyzable tannins often show the most negative correlations with insect growth [9; 14; 15; 19; 23; 26]. Variable correlations between insect growth and leaf phenols may also be due to other causes, such as interactive effects between nutritive [9; 11; 14] and other leaf traits (section III), or to the species-specific adaptation of the insect to young or mature leaves [17].

Birch leaf phenols generally display higher negative correlations with insect growth than with consumption, casting some doubt on their defensive role. However, when the interactive effects of phenols, leaf water and leaf toughness were taken into account, phenols explained more of the variance in the consumption than in the larval growth of the geometrid *Epirrita autumnata* – though generally less than leaf water [14]. We know less about the effects of leaf traits on late season sawflies, but leaf water content and some phenols seem to be involved in their defense as well (Riipi et al., Kapari et al., unpublished data).

To sum up: leaf nutritive traits and leaf phenols are involved in birch defense against lepidopteran and sawfly chewers. The correlations between consumed amounts of foliage and leaf traits are variable, obviously for a number of reasons. However, in a strict sense even recurring negative correlations between phenols and insect growth or performance do not necessarily prove that phenols are the main defenses in birch leaves. Their true defensive role is revealed only by comparing the detrimental effects of phenols to the possibly similar effects of other foliar compounds on herbivory [3]. In the next chapter I introduce our approach to determining the relative importance of phenols and other putative leaf defenses. To my knowledge this approach has not been used earlier to the same extent in studying the defenses of woody plants.

III. Lessons from Pathogen Studies: The Importance of Specific Defense Cascades

Although the possible role of phenolic compounds has been recognized in the literature on plant defenses against pathogens, this literature – contrary to the mainstream of forest entomological studies – heavily emphasizes specific defense mechanisms [e.g. 27; 28]. During the last ten years it has become increasingly clear that the specific defense mechanisms against pathogens are governed by a few complex defense cascades [e.g. 29; 30]. The same main cascades operate in different plant species, but because they

can switch up and down large numbers of genes, these few main cascades can produce very flexible and specific outcomes. The best known defense cascades are those of the octadecanoid and salicylic acid pathways [e.g. 31], induced e.g. by hormone-like jasmonates, salicylic acid or ethylene. Jasmonic acid derivatives are nowadays widely used to trigger induced plant defenses without damaging the plants. Different defense pathways show complex interactions; they may for instance interfere in other pathways, leading to so called crosstalk between different inducers and pathways [32; 33].

Interestingly, plants seem to utilize the same defense cascades against both biotic pathogens and insects and abiotic challenges, and often in very sophisticated ways. In the entomological literature, much effort is nowadays directed to the capacity of volatile plant compounds, triggered by defense pathways, to lure parasitoids to protect the plants [34-39]. The relative role of such indirect defenses, relative to direct plant defenses (which directly reduce herbivore growth or survivorship) is actually poorly understood, and may be an underestimated major mechanism in plant defense. A particularly interesting finding is that plant volatiles may induce defenses or volatile emissions in other plant individuals [40-43].

The operation and relevance of particular defense cascades can be evaluated in different ways. The activity of the pathways can be studied by microarrays, directly measuring the switching on and off of particular genes [e.g. 44; 45]. Another alternative is to apply known inducers of defense cascades (often jasmonic acid or its methylated form) and to study changes in herbivory [e.g. 35; 36] or in other defenses, such as phenols or quinones. These last approaches have also been applied to woody plants [41; 46]. The third method is to analyze large numbers of individual compounds in plants, and to try to determine their relevance for plant defense from their correlations with insect traits.

Although the first two approaches are superior in elucidating the mechanisms of defense, the third alternative can help in resolving the relative importance of different types of defenses. An obvious problem is the necessarily large amount of analytical work that is needed, but due to recent progress in analytical chemistry in identifying huge numbers of compounds from cells (the metabolomic approach [e.g. 47; 48]) this too has become increasingly possible. These new analytical methods heavily rely on gas chromatography and mass spectrometry. By this means close to 1500 peaks have been detected for instance in birch leaves (Vladimir Ossipov, unpublished data).

Preliminary analyses by Haukioja et al. (ms), using ca 600 birch leaf traits detected by the HPLC and GC-MS methods, suggest that different classes of foliar compounds are important against different insect traits. The survivorship of *Epirrita autumnata* larvae (varying from 20 to 90 %), bagged on branches of individual mountain birch trees and therefore unaffected by predators or parasitoids, displayed the most negative correlations with several fatty acids, many of them relating to the octadecanoid pathway. The activation of defense cascades often relates to oxidative reactions [49]

(for mountain birch, Teija Ruuhola, unpublished data), and accordingly several antioxidants displayed positive correlations with larval survivorship. On the other hand, the pupal weights achieved by the surviving larvae (tree averages varying from 31 to 82 mg) displayed more numerous negative correlations with phenolic compounds than with fatty acids.

To sum up: the general defense cascades of the plant kingdom also operate in birch foliage. Our preliminary results suggest that true plant defense leading to increased larval mortality and thereby to lower damage may result from the activation of the octadecanoid pathway, while birch leaf suitability (= value of leaves for larval growth) may be more closely related to foliar phenols.

IV. Discussion

Correlations between the performance of birch herbivores and hundreds of foliar constituents suggest that levels of compounds relating to the octadecanoid pathway are good predictors of larval mortality on birch foliage, which presumably leads to true plant defense, i.e. decreasing consumption on trees. At present we do not know whether the fatty acids and other lipophilic compounds with negative correlations with larval survivorship are merely messenger compounds in the octadecanoid pathway [50], or whether they also include compounds that actually harm the herbivore.

The few phenolics among the compounds with the highest negative correlations with larval survivorship may be real, suggesting the low relative importance of phenols among all the foliar compounds for larval survivorship. The few significant correlations may also result from the way defense cascades operate. Mountain birch trees are quite variable in their phenol profiles, perhaps because the taxon was born by introgression of genes from the dwarf birch (*Betula nana*) to the European white birch (*Betula pubescens*). It is possible that in different mountain birch genets different phenols (and combinations of phenols) are critical for herbivores, and that the octadecanoid pathway activates different phenol-related genes in different trees. In such a case, the activation system of defense (such as the octadecanoid pathway) is likely to show higher correlations with insect survivorship than any individual phenol, even if the latter is detrimental for the pests. This logic indicates that an index of birch defense level could presumably be constructed from the levels of several compounds belonging to the key defense cascade, and might be used as an indicator of the general level of defense.

The fact that the octadecanoid defense cascade is implemental in birch defense, presumably more so than traditional nutritive traits or secondary compounds per se, helps to understand why correlations between insect and phenolic traits are variable. Most studies of birch defense have used poor growth of insect pests as an index of defense [9; 14; 15; 51]. If the defense cascades operate more via increased larval mortality than via the poor performance of

surviving larvae, it is not surprising to find variable correlations between insect growth and leaf traits.

Figure 1 summarizes the multiple ways in which birch defenses are known or assumed to operate. The box on the right-hand side describes plant traits (nutrients, phenols and other secondary compounds, and specific defense cascades), and emphasizes their interactions in creating "leaf quality" for herbivores. Among nutritive traits, water, proteins and some sugars (particularly glucose and fructose) regularly function as factors whose low levels may harm herbivores. However, since these compounds are also necessary for plant physiology, their low levels cannot easily function as defenses. The mechanical toughness of leaves is an important component of leaf suitability after it exceeds a threshold value [14]. Some foliar sugars (e.g. sucrose) regularly show negative or variable (galactose) correlations with insect traits [9]. The reasons are poorly understood, although galactose, the most abundant sugar in birch leaves [12], may participate in the production of compounds in the defense cascades [52]. Phenols as such do not seem to be very toxic to adapted birch herbivores, but their effects may depend on the transformation by plant oxidases of phenols into quinones [46; 53]. Oxidative reactions in general seem good candidates for the early activation of defensive cascades, and they have numerous potential connections to both direct and indirect defense.

Perhaps the most intriguing plant defenses are those relating to volatile compounds (known to be emitted from intact but especially from damaged birch leaves; Terhi Vuorinen, unpublished data). The ability of birch leaf volatiles to allure parasitoids is not known. Another potential indirect defense relates to the possible connections between host plant traits and the ability of herbivorous larvae to defend themselves against parasitoids, but to my knowledge nothing is known of this alternative.

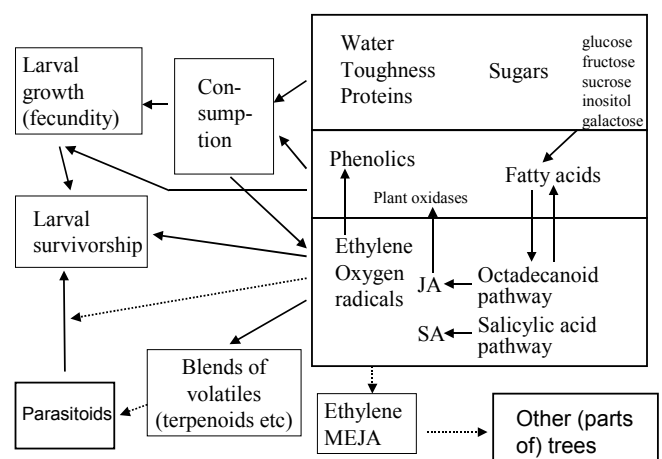


Fig. 1. Actual (black arrows) and hypothetical (dashed arrows) ecological relations between birch plant quality, herbivores and their predators.

To sum up: for several reasons, birch defense is obviously far more complex than is indicated by the ecological literature. It is presumably not organized by any single group of compounds, but is derived from different chemical and physical mechanisms, simultaneously involved in creating the outcome, "defense". Second, defense seems to result from at least partly different effects on various insect parameters, such as survival and growth or fecundity. Insect mortality may result from plant traits that prevent further consumption by directly killing the herbivore, but another obvious mechanism is recruitment of the third trophic level, i.e. parasitoids, to curtail the numbers of herbivores on the plant. Currently, we do not have a good understanding of the relative roles of direct and indirect defenses in woody or other plants. Elucidating the mechanisms that determine the importance of traditionally measured plant traits (phenols, terpenoids, nutritive traits), demands the use of more sophisticated analytical methods than to date. This is tedious, and so far no short-cut methods are available. Still numerous compounds have to be quantified if we are to understand for instance how birch defenses operate, how they have evolved, and the role of plant nutrition in defense.

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