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Birch Leaves as a Resource for Herbivores: Seasonal Occurrence of Increased Resistance in Foliage after Mechanical Damage of Adjacent Leaves

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Summary. Seasonal occurrence of such wound-induced reaction in birch foliage which deteriorates the quality of nearby leaves for herbivores was tested by means of bioassays. Length of the larval period was protracted in two early and mid-summer (larval period!) lepidopteran species as well as in two mid-summer hymenopteran species when larvae were reared on birch leaves whose adjacent leaves had earlier been damaged mechanically. This response was not found for two late-summer hymenopteran species. In a lepidopteran species whose larval period lasts through the whole season, retardation in growth was significant in the beginning of August but not later. Hence such response of leaves, interpreted as defensive on the part of the birch, was not efficient after leaves had gained their final size. The potential consequences of wound-induced responses of leaves for herbivores are discussed.

Introduction

An increased resistance of the plant to herbivores after herbivory-caused damage of plant tissue offers a largely underrated factor in herbivore ecology (Benz, 1974; Haukioja and Hakala, 1975; Rhoades, in press). It is of special interest because it acts in a density-dependent way and hence is a potential agent in the population regulation of herbivores. The problematics of damage-induced responses of leaves, acting against herbivores causing the damage, can be divided into more detailed questions like: 1) do such responses exist, 2) what kind of chemical and physiological reactions in plants are responsible for these reactions, 3) how variable are these responses in time and space, 4) how do they affect herbivores, 5) are they a defence on the part of the plant, etc.

In the case of the mountain birch (*Betula pubescens* ssp. *tortuosa*) and its herbivores we gave an affirmative answer to the first question in showing that larvae of a geometrid moth (*Oporinia autumnata*) expressed retarded growth when reared on leaves from trees defoliated in the previous year. Besides, a rapid damage-induced response was shown to take place in birch foliage because the same symptom was found when larvae were reared on leaves whose adjacent

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leaves had been damaged a couple of days before (Haukioja and Niemelä, 1977). The purpose of this paper is to study the seasonal duration of this response in birch foliage, i.e. to answer the first part of the third question. What kind of consequences are caused by the damaged leaf will be treated elsewhere. Problems 2 and 4 are only discussed in the present paper. As regards the fifth problem, the responses of birch foliage reported above can be interpreted as defensive on the part of the birch but also as side effects of damage and loss of photosynthetic surface. We tried to separate these alternatives by testing some predictions (Haukioja and Niemelä, 1976) and concluded that birch really has active defence responses, e.g. because their effects were stronger where the condition of the birch was better.

Materials and Methods

The experiments were carried out at the Kevo Subarctic Research Station (69°45′N, 27°E), in Finnish Lapland, in 1975–1977. The study site was situated in the birch forest at the foot of the fell Jesnalvaara. For a description of the experimental area, see Haukioja et al. (1978).

The practice for studying the existence of damage-induced reactions in birch foliage was, in 1975 and 1976, to tear birch leaves (to simulate larval feeding) and to collect intact leaves (*"induced leaves"*) from the same branches two days later for experimental animals. Control animals were reared on intact leaves from the same trees but without any known damage of neighbouring leaves (*"control leaves"*). The method is explained in more detail in an earlier paper (Haukioja and Niemelä, 1977). In the above experiments each tree was used just once because by this practice we wanted to show only the existence of such induced reactions affecting the growth of herbivores and because it was not clear that all trees responded in the same way. Using several trees, a new tree each day, increased the probability of including at least some trees able to respond to the treatment. In 1976 we were able to show that ten out of twelve birches increased leaf phenolics after mechanical damage of nearby leaves (Niemelä et al., in press). For achieving stronger induced reactions we adopted a new practice in 1977. Leaves from four trees, or from some stems of them, were torn two or three times per week to simulate larval feeding. Intact leaves (=induced leaves) were collected from these stems so that each tree was used every fourth day. Control leaves were collected each day from a new tree in the immediate vicinity of experimental trees. Thus all animals in the experiments were reared on intact birch leaves and only the adjacent leaves differed in their treatments.

Larvae of *Oporinia autumnata* (Lep., Geometridae), *Brephos parthenias* (Lep., Geometridae), *Eriogaster lanestris* (Lep., Lasiocampidae), *Pristiphora* sp. (Hym. Tenthredinidae), *Pteronidea* sp. (Hym., Tenthredinidae), *Dineura viridiдorsata* (Hym., Tenthredinidae) and *Trichiosoma lucorum* (Hym., Cimbicidae) served as test-animals in the bioassays. The species were chosen so that the larval periods covered the whole season when birch has leaves (Fig. 1).

Larvae were kept singly in plastic tubes (for details, see Haukioja et al., 1978) and they were fed daily or, in late summer when leaves do not dry easily, every second day by excised leaves. All leaves were picked in the morning to eliminate diurnal variation in the usability of leaves (see Haukioja et al., 1978a). Rearing tubes were kept out of doors, except in the case of *Dineura* in 1975, which – due to a very cold summer – were kept in a room with an open window. Larvae of *Eriogaster lanestris*, a gregarious species, were reared in plastic pails in groups of about 80 animals and were fed on twigs with leaves, not with detached leaves.

Young larvae of *Brephos* and *Dineura* in 1975 and 1976, as well as *Trichiosoma* in 1976, were collected from trees. They were ordered according to their weight and every second individual was included in the experimental batch and every second in the control batch. In other cases larvae originated from eggs hatching in the laboratory, and the experimental and control groups consisted of newly hatched larvae, usually of siblings. In 1977 larvae of *Pristiphora, Pteronidea, Dineura* and *Trichiosoma* originated from non-mated females and therefore – due to the haplo-diploid determination of sex – were males.

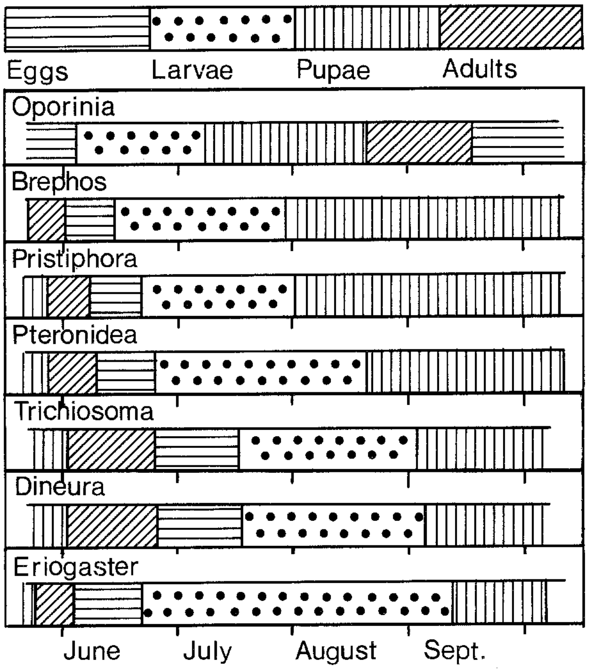

Fig. 1. A schematic representation of life cycles in the test species

Retardation of growth was evaluated from the length of the larval period (for criteria of pupation in some species, see Haukioja et al., 1978) and from weight of larvae or pupae.

Results

Growth of larvae of *Oporinia autumnata* was retarded when they were reared on induced leaves after two days induction period in 1975 and 1976 (Haukioja and Niemelä, 1977; Niemelä et al., in press). The same was true in 1977 when more continuous damage was applied (Fig. 2). Because the larval stage of *Oporinia* lasts from the first half of June to July (Fig. 1), at least during some part of this period birch leaves respond to mechanical damage in a way that is arduous for the herbivore. The same result was found when larvae of *Brephos* and of two species of saw-flies, *Pristiphora* and *Pteronidea*, were tested (Fig. 3). These tests indicated that the damage-induced response in birch leaves took place in July, and perhaps also in August. They also showed that not just lepidopteran but also hymenopteran larvae were sensitive to the damage-induced deterioration of birch foliage. Figure 4 gives results for two more hymenopteran species with seasonally late larval periods (Fig. 1). No retardation in larval growth could be detected in animals reared on induced leaves. On the contrary, the reverse might be true in *Dineura*.

Table 1 summarizes the mean final weight of larvae, or the weight of pupae. It is essential that the protracted larval periods were accompanied with lower weight gain especially in the early season species, *Oporinia*. Mortality figures in Table 1 indicate that – in species whose growth was retarded on induced

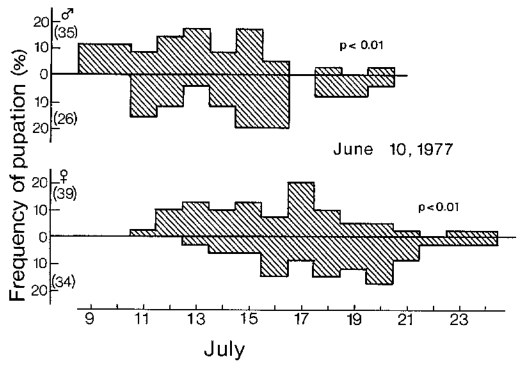

Fig. 2. Dates of pupation in larvae of *Oporinia autumnata* reared on control (*above line*) and induced (*below line*) leaves. Starting date of the experiment is given as well as the sample size (*in parentheses*). Differences in the time of pupation were tested by the Mann-Whitney U-test (two-tailed)

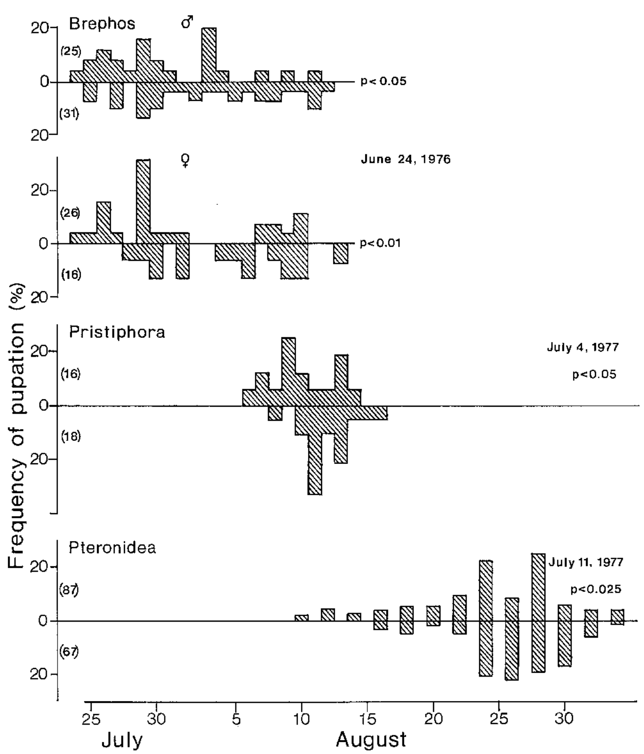

Fig. 3. Dates of pupation in larvae of *Brephos parthenias*, *Pristiphora* sp. and *Pteronidea* sp. on control (*above line*) and induced (*below line*) leaves. For other explanations, see Fig. 2

leaves – larvae also suffered a higher mortality rate on induced diet but the difference was statistically significant in one case only.

Mechanical damage of birch leaves thus led to poor growth in the herbivore species tested on induced leaves up to the first half of August. This may depend on the reaction ceasing to take place after leaves had completed their growth in late July (Haukioja and Iso-Iivari, 1976), or because late season species

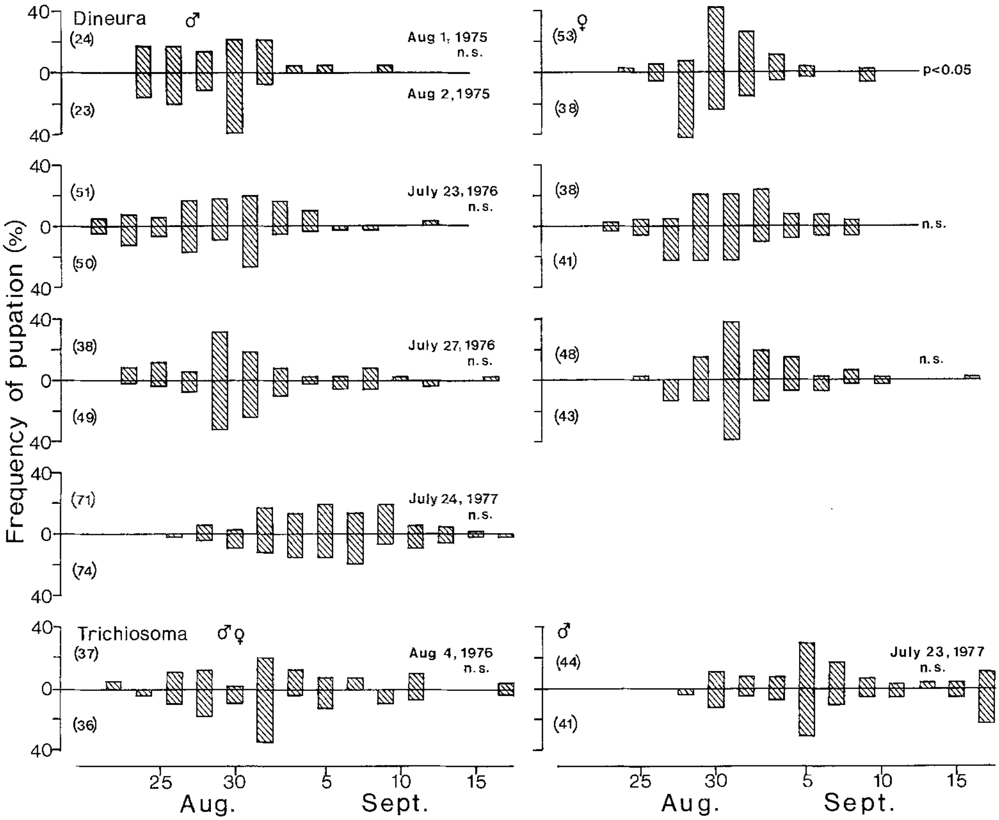

Fig. 4. Dates of pupation in larvae of *Trichiosoma lucorum* and *Dineura viridorsata* reared on control (*above line*) and induced (*below line*) leaves. For other explanations, see Fig. 2

were able to resist the response of leaves. The latter might be true in the case of *Dineura*. The larva of *Dineura* eats a small portion of the leaf surface and moves to a neighbouring leaf to do the same. The animal must be adapted to the consequences of this characteristic feeding habit and the perhaps better growth of larvae on induced leaves is in accordance with the feeding behaviour of *Dineura*. No effect, on the other hand, was observed in the other late season species, *Trichiosoma* (Fig. 4). Two other results also indicate that the response of the plant was lacking after the beginning of August. Weight of larvae of *Eriogaster* on induced leaves was significantly lower than the weight of control animals in the beginning of August, but not later (Table 2). This shows that even a gregarious species, which must cause a lot of leaf damage, was not able to cope completely successfully with induced reactions in the foliage. In *Pteronidea* the weight of larvae on induced leaves was also lower than on control leaves when weighed on August 4th:

induced	10.9 mg (s.d. 2.91, $n=69$)
control	12.2 mg (s.d. 3.59, $n=88$)

Table 1. Final larval or pupal weight and mortality of test species after rearing larvae on control leaves and on induced leaves. Differences in weights were tested by Student's t-test and in mortality by χ^2-test

Species	Sex	Median date of population	Weight (mg; $\bar{x}$, s.e., n) on						P	Larval mortality (%) on		P
			control leaves			induced leaves				control leaves	induced leaves	
Oporinia autumnata	♂	July 14, 1977	72.9	1.39	35	68.1	1.59	25	<0.05	23	32	n.s.
	♀	July 16, 1977	82.1	1.65	37	75.8	1.31	33	<0.01			
Brephos parthenias	♂	July 29, 1976	99.1	1.50	21	94.4	1.90	31	n.s.	8	19	n.s.
	♀	July 29, 1976	111.8	2.45	26	110.1	3.11	15	n.s.			
Pristiphora sp.	♂	Aug. 9, 1977	29.4	0.60	16	29.3	0.41	18	n.s.	24	40	n.s.
Pteronidea sp.	♂	Aug. 24, 1977	32.6	0.36	83	32.0	0.35	67	n.s.	17	33	<0.01
Dineura viridiorsata	♂	Aug. 30, 1975	17.7	1.52	24	15.7	1.15	23	n.s.	20	32	n.s.
	♀	Aug. 30, 1975	31.0	0.98	53	29.8	1.08	38	n.s.			
	♂	Aug. 29, 1976	12.4	0.33	51	13.1	0.29	50	n.s.	7	5	n.s.
	♀	Aug. 31, 1976	24.6	0.42	38	24.7	0.43	41	n.s.			
	♂	Aug. 29, 1976	12.7	0.33	38	13.6	0.26	49	<0.05	13	7	n.s.
	♀	Aug. 31, 1976	23.6	0.31	48	23.8	0.33	43	n.s.			
	♂	Sept. 7, 1977	14.3	0.15	71	14.2	0.17	74	n.s.	28	24	n.s.
Trichiosoma lucorum	♂♀	Sept. 1, 1976	270[a]	23.0	37	255[a]	22.6	36	n.s.	0	3	n.s.
	♂	Sept. 5, 1977	344	7.7	46	351	9.2	41	n.s.	8	18	n.s.

[a] Weighed on August 16

Table 2. Mean weight of larvae of *Eriogaster lanestris* reared on control and induced leaves. Differences were tested by Student's t-test. The experiment began on June 16, 1977

Date	Weight (mg; $\bar{x}$, s.e., n) on						P
	control leaves			induced leaves			
August 5	423.6	7.58	64	377.6	5.64	73	<0.001
August 17	954.4	20.95	63	969.2	22.16	72	n.s.
August 28	1,232.8	25.92	63	1,177.1	24.59	72	n.s.
September 5	1,278.7	27.15	62	1,240.9	24.05	72	n.s.

This difference half-way through the larval period was statistically significant ($p < 0.025$), Student's t-test). However, larvae were able to achieve almost the same mean weight in both groups up to the time of pupation. This just took a longer time in the induced group (Fig. 3).

Discussion

The results given above showed that mechanical damage of leaves changed the quality of adjacent leaves enough to be monitored by a rather simple experimental design. This response of leaves most probably ceased before the middle of August, i.e. at the time when leaves had completed their growth. An obvious explanation for this is that late in the season leaves are not so much worth protecting because they are falling in any case in September. A possible reason at another level is that leaf-phenolics (which were shown to concentrate after damage of nearby leaves, Niemelä et al., in press) tend to increase in the course of the season (Haukioja et al., 1978). An extra increase in the concentration of phenolics is perhaps no longer adaptive when the basic level is high. This opinion is supported by observations showing that at study sites on the middle slope of Jesnalvaara, where the usability of birch leaves to herbivores is lowest, damaging adjacent leaves did not lead to retarded growth of larvae even in early summer (unpublished). Hence damage-induced increase in the resistance of neighbouring leaves seems to be a tactic applied by birch foliage where the basic level of resistance of the leaves is low either in space or time.

Results concerning the late summer may look like somewhat contradictory. The feeding habit of *Dineura* makes it understandable why it is not susceptible to, and may even benefit from, damage-induced reactions of neighbouring leaves. But if *Dineura* really benefits from the damage-induced responses of the plant, changes in leaf quality must occur after the wounding of nearby leaves even in late summer. Whether such possible changes are identical with responses of leaves in early summer, is not known. Irrespective of which reactions take place in birch foliage, results concerning the latest herbivore species indicate that damage of nearby leaves is not problematic for larvae consuming the foliage of the mountain birch in late summer.

As regards the nature of the plant's response, phenolics play at least a partial role. In a preliminary paper (Niemelä et al., in press) it was shown that phenolic compounds, in early summer, were responsible for the ability of leaf extracts to inhibit trypsin and that extracts from induced leaves were more efficient than extracts from control leaves. Further it was shown that the moisture content of induced and control leaves was similar. Thus drying of leaves after mechanical damage of adjacent leaves – the most simple potential explanation – was not the reason of retarded growth in larvae. The question of how information from the site of wounding is transmitted to nearby leaves was not studied. Obviously it demands functioning of some hormone-like substances which have been shown to operate in analogical functions in other plants (e.g. Ryan and Green, 1974; Peng and Black, 1976).

The effects of wound-induced responses of leaves on herbivores may seem slight in the light of the above results. However, at least for two reasons our experimental procedure yields qualitative rather than quantitative results. First, in the experiments of the present paper we have used only intact leaves in rearing larvae. This practice was adopted as the first step to discover whether there are defensive responses in birch foliage, and to study whether leaf damage yields more than quite local consequences. For demonstrating just the existence – e.g. seasonal duration – of such defensive reactions, the practice is applicable. But to simulate the consequences of natural damage, caused under high larval densities, also leaves damaged previously must be included in the diet of larvae. In the case of *Oporinia* our unpublished results showed that larvae fed on leaves which were damaged earlier produced pupae more than a quarter lighter than those larvae reared on control leaves. The second reason why results reported here were milder than in the field, derives from the fact that excised leaves support the growth of *Oporinia* better than leaves growing in trees (Haukioja and Niemelä, 1976). Further, mortality of *Oporinia* larvae was much lower on excised leaves than on growing leaves (unpublished). Therefore, in our best studied species, *Oporinia autumnata,* damage-induced changes in the usability of birch leaves have such large effects on survival and – via the size of adult females – on fecundity that they form a remarkable component among factors governing the vastly fluctuating density of this species.

In saw-flies the results are not unambiguous. Although the mid-season species had a protracted larval period on induced leaves, it did not affect their final weight and hence probably not their fecundity either. But even here the higher mortality rate of larvae on induced leaves may make the behaviour of the host plant a potentially appreciable factor in the population dynamics of the herbivore.

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References

Benz, G.: Negative Rückkoppelung durch Raum- und Nahrungskonkurrenz sowie zyklische Veränderung der Nahrungsgrundlage als Regelprinzip in der Populationsdynamik des Grauen Lärchenwicklers, Zeiraphera diniana (Guenée) (Lep., Tortricidae). Z. Ang. Ent. **76**, 196–228 (1974)

Haukioja, E., Hakala, T.: Herbivore cycles and periodic outbreaks. Formulation of a general hypothesis. Rep. Kevo Subarctic Res. Stat. **12**, 1–9 (1975)

Haukioja, E., Iso-Iivari, L.: Local and annual variation in secondary production by Dineura viridi-dorsata (Hym., Tenthredinidae). Rep. Kevo Subarctic Res. Stat. **13**, 26–32 (1976)

Haukioja, E., Niemelä, P.: Does birch defend itself actively against herbivores? Rep. Kevo Subarctic Res. Stat. **13**, 44–47 (1976)

Haukioja, E., Niemelä, P.: Retarded growth of a geometrid larva after mechanical damage to leaves of its host tree. Ann. Zool. Fennici **14**, 48–52 (1977)

Haukioja, E., Niemelä, P., Iso-Iivari, L.: Birch leaves as a resource for herbivores. II. Diurnal variation in the usability of leaves for Oporinia autumnata and Dineura viridiorsata. Rep. Kevo Subarctic Res. Stat. **14**, 21–24 (1978a)

Haukioja, E., Niemelä, P., Iso-Iivari, L., Ojala, H., Aro, E.-M.: Birch leaves as a resource for herbivores. I. Variation in the suitability of leaves. Rep. Kevo Subarctic Res. Stat. **14**, 5–12 (1978)

Niemelä, P., Aro, E.-M., Haukioja, E.: Birch leaves as a resource for herbivores. Damage-induced increase in leaf phenolics with trypsin-inhibiting effects. Rep. Kevo Subarctic Res. Stat.

Peng, J.H., Black, L.L.: Increased proteinase inhibitor activity in response to infection of resistant tomato plants by Phytophora infestans. Phytopathology **66**, 958–963 (1976)

Rhoades, D.F.: Evolution of plant chemical defense against herbivores. In: Interrelationships of secondary plant constituents with herbivores (P.H. Janzen, G.A. Rosenthal, eds.). Academic Press. (in press)

Ryan, C.A., Green, T.R.: Proteinase inhibitors in natural plant protection. In: Recent advances in phytochemistry, Vol. 8, Metabolism and regulation of secondary plant products (V.C. Runeckles, E.E. Conn, eds.), pp. 123–140. New York-London: Academic Press 1974

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