CONCHITA ALONSO

Is plant chemistry determining mortality and dispersal of young *Epirrita autumnata* larvae?

SAMANTEKT

Það er nauðsynlegt að ákvarða þá þætti sem valda mismunandi laufskaða ef skilja á þróun varna gegn jurtaætum. Úti í náttúrunni er tvennt sem orsakar það að einstök tré verða fyrir meiri laufskaða en önnur tré sömu tegundar, nefnilega: það getur staðið undir fleiri jurtaætum, eða hver einstök jurtaæta getur étið meira magn laufa mælt í lífmassa. Mismunur í atferli við fæðunám sem ekki orsakar dauða lífverunnar mun aðallega hafa áhrif það hvað lirfustigið varir lengi og endanlega stærð jurtaætunnar, á meðan breytileiki í fjölda jurtaætna mun að líkindum ákvarða mun í laufskaða plantna innan plöntustofnsins.

Val skordýra á varpstað, og dreifing og afföll á ungum lirfum ákvarða sennilega fjölda skordýra sem nærast á tiltekinni plöntu. Það er vitað fyrir haustfiðrildið, *Epirrita autumnata*, að mæður eru ekki vandfýsnar þegar þær eru að verpa. Hinsvegar er lítið vitað um dreifingu og afföll á ungum E. *autumnata* lirfum. Hér sýni ég niðurstöður úr tveimur tilraunum þar sem svifdreifing á spunaþræði, og afföll á ungum lirfum voru athuguð á einstökum birkitrjám sem vitað var að höfðu mismunandi lífefnasamsetningu. Niðurstöður úr þessum tilraunum benda til að gæði laufanna geti haft áhrif á afföll hjá ungum E. *autumnata* lirfum, en séu ekki líkleg til að hafa áhrif á dreifingu þeirra.

Traits affecting oviposition selection, dispersal and mortality of young larvae could be considered as the most efficient plant defenses against herbivorous insects since they will determine the final number of consumers sustained and, thus, the defoliation experienced by different plants. Instead, plant characteristics modifying feeding behavior without affecting mortality of individuals (i.e., *per capita* consumption), will mostly determine affect the length of larval period and the final size of individuals that will not drastically change the defoliation of the plant in the current season. Furthermore, both aspects may be determined caused by the same factors with additive or non-additive effects, and they can also modulate the responses of the natural enemies of the herbivores (Leather and Walsh 1993, Thompson 1988a, Hunter and Elkinton 2000). Furthermore, distinguishing these different steps in the interaction between plants and herbivorous insects can be also is relevant to understand the evolution of their relationships since mother selection and young larvae selection both imply active selection by the herbivore who would in turn play the role of selection pressure on plant characteristics, whereas, survival of young larvae implies differential mortality and thus plants would be in this case the selection pressure on herbivores (Thompson 1988b). Only detailed field studies of herbivore densities and defoliation can distinguish these different sources of variation under natural conditions (e.g., Hunter et al. 1997, Hunter and Elkinton 2000). In addition, indoor controlled experiments may be useful, however, to evaluate the potential relevance of these different stages and specially to discard those with low possibilities to affect the interaction between particular species.

The interaction between the autumnal moth (*Epirrita autumnata* Bkh.) and one of its main host plants the white birch (*Betula pubescens*), has been studied from many different perspectives (Ruohomäki et al. 2000 and references therein). *Epirrita autumnata* is a univoltine geometrid species. Individuals overwinter as eggs, and the new generation hatches in spring, when synchrony with leaf flush is important for larval development (Ayres and MacLean 1987). Duration of the larval stage depends on temperature and foliage quality. The pupal mass reached at the end of larval development is a good estimate of realized adult fecundity (Tammaru et al. 1996). The short-lived adults eclose in autumn. Females do not usually fly before oviposition and they are not selective while ovipositing (Tammaru et al. 1995, 1996). Although E. *autumnata* larvae are polyphagous leaf chewers, mountain birch *Betula pubescens* subsp. *czerepanovii* (Orlova) Hämet-Ahti, due to its abundance, is their main host plant in Northern Fennoscandia (Kallio and Lehtonen 1973), where the species periodically cause severe defoliations. Mountain birch leaves contain relatively high levels of different phenolic compounds (Ossipov et al. 1997) whose quantities vary among individual trees and with leaf development (Suomela et al. 1995, Nurmi et al. 1996), and can affect E. *autumnata* performance (e.g., Kause et al. 1999). Among these phenolic compounds high gallotannin concentrations are characteristic of young developing leaves (Ossipov et al. 1997, Kause et al. 1999) and thus, they are potentially suitable defensive compounds against the earliest season leaf feeders the neonate larvae of E. *autumnata*. However, little is known about the effects of birch chemistry on dispersal and mortality of young E. *autumnata* larvae.

The dispersal of neonate larvae by ballooning has been described in some other Lepidopteran species such as *Lymantria dispar* (Lymantriidae) (Hunter and Elkinton 2000), *Operophtera brumata* (Geometridae) (Tikkanen 2000), *Orgyia vetusta* (Lymantriidae) (Harrison 1995), and *Thyridopterix ephemeraeformis* (Psychidae) (Ghent 1999), in relation to host plant species, budburst phenology, natural enemies and abiotic conditions. None of these studies has tried, however, to test if larvae can use the same mechanism to discriminate conspecific plants differing in leaf characteristics other than phenology (but see Harrison 1995). Here I present results from two experiments where ballooning dispersal, and mortality of young larvae were studied in individual mountain birches known to differ in their foliage chemistry.

Materials and methods

Ballooning experiment

Ballooning studies were carried out on early June 1998 using the same 30 mountain birch trees whose leaves had been previously analyzed and tested for quality as food for E. *autumnata* larvae (Lempa et al. 2000). Two other substrates than mountain birch, a glass bar and a branch of pine, were also used to test the capability of E. *autumnata* larvae to balloon under laboratory conditions. As a standard procedure a table home ventilator (Finca©) was used to produce a continue air flow that allow larvae to escape from the host using silk filaments ('ballooning'). Both larvae found on the table and those observed while ballooning were recorded as 'ballooning' individuals. All the experiments were done at room temperature (22–23 °C). Larvae used in both experiments belonged to laboratory reared strains maintained at Kevo Subarctic Research Institute Field Station.

Fourteen neonate larvae of E. *autumnata* were placed with a fine brush at different portions of a thin glass bar in which three rubber elastic bands were placed to provided larvae with irregularities that helped them larvae to stay in the artificial branchbar. The number of larvae remaining in the bar and ballooning were recorded every five minutes for 150 min. In addition, a small portion of a pine branch was cut and placed in water. *Epirrita autumnata* larvae were transferred there and subsequently monitored recording the number of individuals ballooning. This procedure was conducted on three different dates 11, 17 and 22 of June.

On 12 June, a branch containing at least 18 short shoots was cut from every study tree (N = 30). Branches were kept in cold while collecting and immediately carried to the lab where they were placed in water to avoid desiccation of leaves. Branches were all starting to open their buds but leaves could not be observed yet. Eggs from six different broods were mixed and the hatched larvae were randomly distributed among trees. Fifteen larvae were transferred to each stembranch trying to place them around the same point, selecting some stem bifurcation if available.

Greenhouse experiment

The experiment was carried out in March 1999 at Satakunta Environmental Research Centre. I used 3 years potted mountain birch saplings obtained from seeds that were three years old at the time of the experiment. These saplings were obtained from seeds that belonged to seven identified trees whose foliage chemistry was well-known (Lempa et al. 2000). For this experiment we selected seven mother the selected trees that comprised a broad variation in concentrations of proteins and total gallotannins (Table 1). Four saplings per mother tree of similar size, phenology and appearance were used as replicates. Pots were placed on Petri dishes (12 cm diameter) containing water to avoid larval movements among plants. The system was proved to be effective for this purpose, since some of the dead larvae were found within the Petri dishes, and it was also used as watering system and its level was checked every day adding more water when necessary controlling the level of water daily.

Table 1. Mean concentrations (mg/g) of total phenolics, gallotannins, carbohydrates and proteins of the birch trees included within each class, number of trees in brackets. Values correspond to leaves collected in late June 1997. Different letters indicated significant differences between classes at P < 0.05 (Student-Newman-Keuls analyses).

Class	Total phenolics	Total gallotannins	Total carbohydrates	Total proteins
Gallotannins				
High (1)	142.17 a	27.61 a	85.57 a	178.29 a
Medium (3)	117.32 a	11.79 b	113.64 a	135.75 a
Low (3)	93.78 a	2.24 c	114.09 a	128.62 a
Proteins				
High (3)	120.96 a	15.04 a	103.17 a	169.43 a
Low (4)	103.15 a	6.15 a	114.82 a	115.78 b

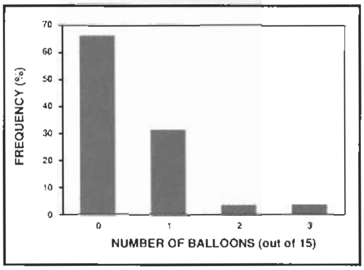

Fig 1. Percentage of birch trees from which we observed different numbers of neonate *Epirrita autumnata* larvae ballooning.

All pots were placed in 4 rows (= blocks) in the same greenhouse bench with a randomized block design and saplings from each mother-tree were randomly located within rows, separated by 10 cm, with double distance between rows.

Overwintering E. *autumnata* eggs were taken from seven different broods and placed at room temperature until they hatched. One larva per family was placed on each plant up to a total of seven larvae per sapling. Larvae were allowed to freely feed within the assigned plant. After molting to the second instar every larva was individually weighed, marked with fast drying paint, and reweighed after marking. Weight at the end of the instar was also recorded, and growth during the instar was thus estimated as the difference between final weight and weight after marking.

Data analyses

All statistical analyses were conducted with SAS-package (SAS Institute 1996).

Differences between mother trees (trees hereafter) in the proportion of larvae that ballooned or survived to the end of second instar was analyzed by fitting a Generalized Linear Model (GENMOD Procedure, distribution = binomial, link function = probit; SAS Institute 1996). Overdispersion problems associated with the binomial distribution models were controlled by estimating the dispersion parameter as Pearson's chi-square (SAS Institute 1996).

In the case of survival in the greenhouse experiment pre-planned contrasts between trees differing in either concentration of gallotannins, concentration of proteins, or both were done to test whether these factors were affecting affected larval survivorship. Variation among trees on growth during second instar was also studied. Since growth of larvae was normally distributed, differences between trees were analyzed by fitting a General Linear Model (GLM Procedure). Again pre-planned contrasts between trees differing in either concentration of gallotannins, concentration of proteins, or both were conducted to test for the effects of these factors on larval growth. Power of the design to detect differences between trees was calculated with GPOWER (Buchner et al. 1997), note that the power of pre-planned contrasts is always higher (SAS Institute 1996). Larvae that lose their mark or were weighed after molting to 3rd third instar were excluded from growth analyses.

Results

Ballooning experiment

The experimental procedure was found to be effective since most of larvae placed on both the glass bar and the pine branch moved away in less than one hour. However, only 14 out of 459 neonate larvae did balloon from the stems of mountain birch branches of the 30 experimental birch trees. Although these balloons were not uniformly distributed among trees (Wald's $\chi^2 = 131.6$, df = 28, $P \ll 0.0001$) in no case we observed more than 3 larvae ballooning (Fig. 1) suggesting that variation is very low and presumably biologically irrelevant.

Greenhouse experiment

Only two larvae out of the 196 initially placed on plants did not survive to the second instar. Mean (± SD) larvae body mass at the beginning of the second instar was on average (± SD) 0.73 ± 0.12 mg, and differences in the mean body mass of larvae feeding on different trees were not statistically significant (P > 0.5). Power of the design at P = 0.05 was higher than 0.90 from an effect size of 0.31, that represented a difference of 0.037 mg in larval weight.

Eighteen percent of the larvae

died during the second instar. Larvae that survived were significantly heavier at the beginning of the instar (0.75 ± 0.11 mg) than those that died (0.68 ± 0.13; $F_{1,177} = 11.5$, $P = < 0.0009001$). Survival rates varied between trees in such a way that differences were statistically significant for the interaction between proteins and gallotannins levels (Table 2) with survival being higher in trees with low levels of both (96 %) than in those with high levels of both (70 %). An increment of protein concentration increased mortality (Fig. 2a), particularly when gallotannin levels were low (Table 2). The increment of gallotannins also increased mortality (Fig. 2b), although differences were only marginally significant when protein concentration was low (Table 2), and non significant when concentration of proteins was high (Table 2).

As regards growth, the mean increment of body mass during the second instar was 1.79 mg (± 0.37), and the mean larval mass at the end of the instar was 2.54 mg (± 0.41). I did not find significant differences between growth of larvae feeding on different trees, and none of the pre-planned contrasts was statistically significant ($P > 0.5$).

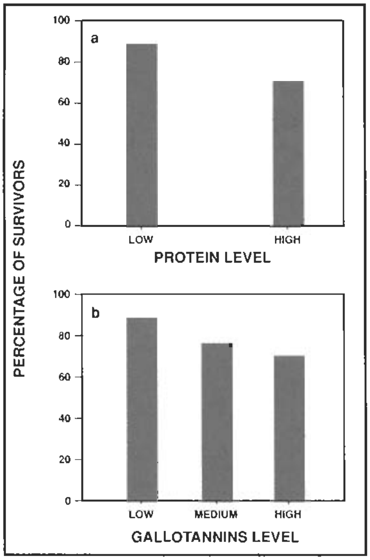

Fig. 2. *Epirrita autumnata* survivorship observed in the greenhouse experiment depending on a) proteins concentration, b) gallotannins concentration.

Discussion

Life-history traits have been suggested to modulate the selective behavior of Lepidopterans (Tammaru and Haukioja 1996). Simple non-selective oviposition behavior is usually associated to polyphagous species with non-feeding adults and a low flight capability of the females. Polyphagy decreases the risks of non-selectivity but still there might be a conflict between mother selection and offspring performance (e.g., Nylin and Janz 1996). Larval dispersal may contribute to alleviate this conflict, and in fact ballooning has been linked to flightless (Roff 1990) and hence to the same group of Lepidopteran species described above. *Epirrita autumnata* has been classified among capital breeders even when adult females can eat and fly because they do not apparently do it (Tammaru and Haukioja 1996, Ruohomäki et al. 2000), ovipositing females do not select between host and non-host species nor between birch trees differing in leaf quality (Tammaru et al. 1995). However, under laboratory conditions larval performance is affected by the individual host-tree in which they feed (e.g., Kause et al. 1999, Lempa et al. 2000) suggesting that individual trees differ in their quality as a host. It rested to know whether larvae were more prone to disperse from trees where their performance was worse and here I checked it by using the same trees than Lempa et al. (2000). Results from the ballooning experiment suggested that neonate larvae have the capability to move from the plant in which they hatch but this behavior is only used when there is no food available (e.g., when they hatch in a non-host plant), but not for selecting host quality at intraspecific level. Similar results have been found by Harrison (1995) in *Orgyia vetusta*, larvae only dispersed from dead bushes but did not moved away from live respond bushes differing in their to the level of defoliation level of alive bushes. Thus, risks associated to this type of uncontrolled dispersal may preclude larvae to escape from any suitable food plant and in natural conditions rates of dispersal from individual plants would be mostly determined by wind and microhabitat location (e.g., Ghent 1999,

Table 2. Results of the Generalized Linear Model fitted to test for differences in survival of second instar E. *autumnata* larvae between trees differing in concentration of either proteins, gallotannins, or both.

Contrast	Compared trees	Wald's χ^2	*P*
Between **gallotannin** levels when concentration of proteins is high	7 *vs.* 9	0.11	0.74
Between **gallotannin** levels when concentration of proteins is low	21,27 *vs.* 4,11	5.36	0.02
Between **protein** concentrations when gallotannins are medium	11 *vs.* 29	4.15	0.042
Between **protein** concentrations when gallotannins are low	7 *vs.* 21,27	9.90	**0.0017**
Low proteins **low** gallotannins *vs.* **high** proteins **high** gallotannins	21,27 *vs.* 9	12.37	**0.0004**

but see Hunter and Elkinton 1999).

As regards mortality of young larvae, in a greenhouse experiment where natural enemies and abiotic conditions were under control, the biggest difference in survival to the end of second instar was found between trees with low contents of both proteins and hydrolizable tannins (96 % of survivors) and trees with high levels of both proteins and hydrolizable tannins (70 %).

Although the causes of this pattern are uncertain, likely, bounding of proteins was more effective when concentration of both proteins and tannins were in was high concentration (see Zucker 1983 for further discussion), this would decrease larval growth and subsequently the probabilities of dying increased. Interestingly, individuals who died were those recording lightest weight at the beginning of the instar. This last finding is particularly relevant when we try to extrapolate the results to natural conditions. Mortality in the greenhouse was unusually low in first instar compared to natural patterns (personal observation) and causes of death during the second instar were presumably not the same as in the field. However, results suggest that the main effect of a poor food quality food is to weaken individuals feeding on it and this will probably interact with other abiotic factors such as temperature and natural enemies (Virtanen and Neuvonen 1999).

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References

AYRES, M. P. and MACLEAN, S. F. 1987. Development of birch leaves and the growth energetics of *Epirrita autumnata* (Geometridae). Ecology 68:558-568.

BUCHNER, A., ERDFELDER, E. and FAUL, F. 1997. How to Use G*Power. Available at: http://www. psychologie.uni-trier.de: 8000/projects/gpower/how_to_use_gpower.html.

GHENT, A. W. 1999. Studies of ballooning and resulting patterns of locally contagious distribution of the bagworm *Tryridopteryx ephemeraeformis* (Haworth) (Lepidoptera: Psychidae). Am. Mid. Nat. 142: 291-313.

HARRISON, S. 1995. Lack of strong induced and maternal effects in tussock moths (*Orgyia vetusta*) on bush lupine (*Lupinus arboreus*). Oecologia 103: 343-348.

HUNTER, A. F. and ELKINTON, J. S. 2000. Effects of synchrony with host plant on populations of a spring feeding Lepidopteran. Ecology 81: 1248-1261.

HUNTER, M. D., VARLEY, G. C. and GRADWELL, G. R. 1997. Estimating the relative roles of top-down and bottom-up forces on insect herbivore populations: a classic study revisited. Proc. Natl. Acad. Sci. USA 94: 9176-9181.

KALLIO, P. and LEHTONEN, J. 1973. Birch forest damage caused by *Oporinia autumnata* (Bhk.) in Utsjoki, N. Finland. Rep. Kevo Subarctic Res. Stat. 10: 55-69.

KAUSE, A., OSSIPOV, V., HAUKIOJA, E., LEMPA, K. and HANHIMÄKI, S. 1999. Multiplicity of biochemical factors of insect resistance in mountain birch. Oecologia 120:102-112.

LEATHER, S. R . and WALSH, P. J. 1993. Sublethal plant defences the paradox remains. Oecologia 93: 153-155.

LEMPA, K., MARTEL, J., KORICHEVA, J., HAUKIOJA, E., OSSIPOV, V., OSSIPOVA, S. and PIHLAJA, K. 2000. Covariation of fluctuating asymmetry, herbivory and chemistry during birch leaf expansion. Oecologia 122: 354-360.

NURMI, K., OSSIPOV, V., HAUKIOJA, E., PIHLAJA, K. 1996. Variation of total phenolic content and individual low-molecular-weight phenolics in foliage of mountain birch trees (*Betula pubescens* ssp. *tortuosssa*). J. Chem. Ecol. 22: 2023-2040.

NYLIN, S. and JANZ, N. 1996. Host plant preferences in the comma butterfly (*Polygonia c-album*): do parents and offspring agree? Ecoscience 3: 285-289.

OSSIPOV, V., LOPONEN, J., OSSIPOVA, S., HAUKIOJA, E. and PIHLAJA, K. 1997. Gallotannins of birch *Betula pubescens* leaves: HPLC separation and quantification. Biochem. Syst. Ecol. 25:493-504.

ROFF, D. A. 1990. The evolution of flightlessness in insects. Ecol. Monogr. 60: 389-421.

RUOHOMÄKI, K., TANHUANPÄÄ, M., AYRES, M. P., KAITANIEMI, P., TAMMARU, T. and HAUKIOJA, E. 2000 [*in press*]: Causes of cyclicity of *Epirrita autumnata* (Lepidoptera, Geometridae) - grandiose theory and tedious practice. Pop. Ecol. 00: 0000-0000.

SAS INSTITUTE. 1996. SAS/STAT software: changes and enhancements through Release 6.11. SAS Institute, Cary, North Carolina, USA.

SUOMELA, J., OSSIPOV, V. and HAUKIOJA, E. 1995. Variation among and within mountain birch trees in foliage phenols, carbohydrates and amino acids, and in growth of *Epirrita autumnata* larvae. J. Chem. Ecol. 21:1421-1446.

TAMMARU, T. and HAUKIOJA, E. 1996. Capital breeders and income breeders among Lepidoptera: consequences to population dynamics. Oikos 77: 561-564.

TAMMARU, T., KAITANIEMI, P. and RUOHOMÄKI, K. 1995. Oviposition choices of *Epirrita autumnata* (Lepidoptera, Geometridae) in relation to its eruptive population dynamics. Oikos 74: 296-304.

TAMMARU, T., KAITANIEMI, P. and RUOHOMÄKI, K. 1996. Realized fecundity in *Epirrita autumnata* (Lepidoptera: Geometridae): relation to body size and consequences to population dynamics. Oikos 77:407-416.

THOMPSON, J. N. 1988a. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomol. Exp. Appl. 47: 3-14.

THOMPSON, J. N. 1988b. Coevolution and alternative hypothesis on insect/ plant interactions. Ecology 69: 893-895.

TIKKANEN, O.-P. 2000. Adaptation of a generalist moth, *Operophtera brumata*, to host plants. Ph.D. Dissertations in Biology 1. University of Joensuu, Finland. Abstract available at http://bio.joensuu.fi/PhD/tikkanen.pdf.

VIRTANEN, T. and NEUVONEN, S. 1999. Performance of moth larvae on birch in relation to altitude, climate, host quality and parasitoids. Oecologia 120: 92-101.

ZUCKER, W. V. 1983. Tannins: does structure determine function? An ecological perspective. Am. Nat. 121: 335-365.