

**HOST RANGE OF LICHENIVOROUS
MOTHS WITH SPECIAL REFERENCE
TO NUTRITIONAL QUALITY AND
CHEMICAL DEFENCE IN LICHENS**

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Abstract

Host use and range of herbivorous insects are determined by several factors, of which nutritional quality and secondary chemistry have been shown to play very important roles. For herbivores feeding on lichens these traits are assumed to be more critical than for species feeding on higher plants, since lichens are nutritionally poor and often contain high concentrations of secondary metabolites. I examined the role of lichens' nutritional quality and secondary chemicals on the performance of lichen-feeding Lepidopteran larvae. I also tested whether females of lichenivorous species preferably oviposit on host species of the highest nutritional quality for the growth of larvae.

Larvae of *Eilema depressum* performed best on *Melanelia exasperata*, which is of the highest nutritional quality, as indicated by the high N concentration and the absence of lichen secondary metabolites compared to the other lichens studied. Host nutritional quality did not promote the production of an additional generation. Larvae of *E. depressum* needed fewer instars and grew bigger on a high-quality diet than larvae reared on a diet of poorer quality. However, the main factor contributing to the wide variation in the number of larval instars was the question of whether or not larvae overwintered. Growth of *Cleorodes lichenaria* at the beginning of the larval period matched equally the nutritional quality of the hosts. However, the final larval period was shortest on *Ramalina* species, which was preferred by both females ovipositing their eggs and larvae searching for a host. In the field, larvae were found almost exclusively on *Ramalina* species.

Larvae of *E. depressum* were not able to survive on intact thalli of *Vulpicida pinastri* and *Hypogymnia physodes*, but after removal of lichen's secondary metabolites, larval survival remained equally high as on other lichens. Larvae also showed a clear preference towards thalli with lowered concentrations of secondary metabolites in *Parmelia sulcata*, *V. pinastri* and *H. physodes*. Parietin in *Xanthoria parietina* was the only secondary metabolite that had no impact on the survival or host selection of *E. depressum* larvae.

The present results show that the nutritional quality and some lichen secondary chemicals are important factors for the growth, survival and host selection of lichen-feeding Lepidopteran larvae. The preference-performance hypothesis is at least partly able to explain the host range of *C. lichenaria*, although it seems that there are also other factors, such as larval dispersal and host selection or top-down forces, that might contribute to host range of lichenivorous Lepidopteran larvae. Moreover, lichenivorous larvae seem to be partly responsible for their own host selection.

Keywords: foliose lichen, growth rate, host selection, Lepidoptera, lichen secondary metabolite, neonate larva, nutritional quality, preference-performance hypothesis, survival

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Abbreviations and definitions

AE	assimilation efficiency (%)
C	carbon
ECD	efficiency of conversion of digested food (%)
EFS	enemy-free space
HPLC	high-pressure liquid chromatography
N	nitrogen
P	phosphorus
RCR	relative consumption rate ($\text{mg day}^{-1} \text{mg}^{-1}$)
RGR	relative growth rate ($\text{mg day}^{-1} \text{mg}^{-1}$)
RH	relative humidity
TLC	thin-layer chromatography

List of original papers

- I Pöykkö H & Hyvärinen M (2003) Host preference and performance of lichenivorous *Eilema* spp. larvae in relation to lichen secondary chemicals. *Journal of Animal Ecology* 72: 383–390.
- II Pöykkö H, Hyvärinen M & Bačkor M (2005) Removal of lichen secondary metabolites affects food choice and survival of lichenivorous moth larvae. *Ecology* 86: 2623–2632.
- III Pöykkö H & Hyvärinen M (2005) Host quality, voltinism and variation in the number of larval instars in a lichen-feeding moth *Eilema depressum*. (Submitted)
- IV Pöykkö H (2005) Females and larvae of *Cleorodes lichenaria* prefer hosts with a low nutritional quality. (Submitted)

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1 Introduction

Insects are the most diverse group of organisms on Earth, with over one million described species and estimates of the total number of species varying between two million and one hundred million (Hawksworth & Kalin-Arroyo 1995). Intuitively, insects could have access to a wide variety of resources. However, insect herbivores, which compose approximately one fourth of all known species, mostly feed on a single or a few related plant species (Strong *et al.* 1984, Bernays & Chapmann 1994). Even the species with the widest range of food plants are unable to eat everything. The observed predominance of specialist feeding habits over generalist host use has attracted wide attention. This observation, together with the seminal paper of Ehrlich and Raven (1964), where the authors proposed that ‘the evolution of secondary plant substances and the stepwise evolutionary responses to these by phytophagous organisms have clearly been the dominant factors in the evolution of butterflies and other phytophagous groups’ and that ‘reciprocal selective responses have been greatly underrated as a factor in the origination of organic diversity’, have generated wide interest in the factors determining diet breadth, host use and host specificity of phytophagous insects.

1.1 Host nutritional quality and the performance of insect herbivores

The most important nutrients limiting the growth of herbivores are proteins, which occur in much lower concentrations in plants compared to insects (Mattson 1980, Scriber & Slansky 1981, Bernays & Chapmann 1994, Elser *et al.* 2000). Nitrogen content, which is easier to measure than proteins, is commonly used as an index of nutritional quality (Strauss & Zangerl 2002). The nutritional quality (e.g., nitrogen concentration) of a host plant often has direct effects on the survival, growth rate, size and fecundity of insects (Mattson 1980, Scriber & Slansky 1981, Leather 1988, Fischer & Fiedler 2000, Awmack & Leather 2002). For females, whose quantity and quality of eggs often depend on final body mass, larval nutrition is especially important (Haukioja & Neuvonen 1985, Honek 1993, Setamou *et al.* 1993, Tammaru *et al.* 1996, Oberhauser 1997, Fox & Czesak 2000, Wiklund *et al.* 2001). In general, females seem to suffer relatively more of poor nutritional conditions than males (Teder & Tammaru 2005). The total N concentration may not always be the best measure of the host’s nutritional quality, since N quality and

amino acid composition may also affect the performance of herbivores (Karowe & Martin 1989). The direct fitness benefits from feeding on a high-quality diet may also include an increased number of generations per year. For example, the larvae of a tortricid moth *Choristoneura rosaceana* feeding on a high-quality diet were more likely to continue development and to produce a second generation than larvae feeding on a poor-quality diet, and the nutritional quality of the larval diet had a direct effect on diapause induction (Hunter & McNeil 1997). Moreover, more larvae of *Polygonia c-album* reared on a host plant promoting rapid larval growth developed without diapause compared to larvae reared on a host resulting in a slower growth rate (Wedell *et al.* 1997). In addition to direct effects on the growth rates and hence the performance of herbivores, host nutritional quality may also have indirect effects. These include, for example, changes in sex ratio (Craig *et al.* 1992) and oviposition behaviour (Leather & Burnand 1987, Hopkins & Ekblom 1999). Host quality may also affect through male-derived nutrients in nuptial gifts during copulation (Kaitala & Wiklund 1994, Wiklund *et al.* 2001, Awmack & Leather 2002, Torres-Vila & Jennions 2005).

The nutritional requirements of herbivorous species may also be age and sex-dependent (Zalucki *et al.* 2002), as younger developmental stages tend to be more sensitive to the nutritional quality of the hosts than older ones (Zalucki *et al.* 2002). For example, Stockhoff (1993) reported that preference for a high-protein diet was highest in young larvae of *Lymantria dispar*, and that female larvae ate more protein-rich diets compared to males, possibly because of the higher protein requirements associated with egg production. Other nutritional ingredients of hosts, such as minerals, vitamins, lipids and water concentration, may affect the performance of herbivorous insects, but the host's N concentration is generally regarded as the most important determinant of the growth and fecundity of herbivorous insects (Mattson 1980, Scriber & Slansky 1981, Bernays & Chapmann 1994, Awmack & Leather 2002). Insects may have different behavioural or physiological adaptations enabling them to successfully feed and grow on diets of poor nutritional quality (Karban & Agrawal 2002). These include, for example, compensatory feeding, which means that larvae feed more on low-quality diets than on diets with higher concentrations of nutrients (e.g., Lee *et al.* 2004), feeding on several hosts in proportions that result in a better balance of nutrients than any single host alone (self-selection of optimal diets) (Waldbauer & Friedman 1991), harbouring of symbiotic bacteria that upgrade nonessential amino acids to essential ones in aphids (Douglas 1998) and host-induced resource allocation. For example, the grasshopper *Melanoplus differentialis* is able to allocate relatively more resources to its digestive system and to increase its gut size on a poor-quality diet. This increase in gut size is attributable to the ability of this species to compensate for reduced food quality (Yang & Joern 1994). The difference in the C:N ratio between plants and insects might also contribute to feeding specialization among phytophagous insects. For example, some species favour feeding on species, tissues or timeframes with high nutrient concentrations (Kraft & Denno 1982, Obermaier & Zwölfer 1999, Elser *et al.* 2000, Karban & Agrawal 2002.). According to Fagan and coauthors (2002), the role of nutrients as a driving force in the evolution of host specificity deserves more attention than it has received in the past.

1.2 Secondary chemicals and the host use of insect herbivores

Traditionally, the evolution of host secondary chemistry and its role in the evolution of herbivores has been regarded as the most important single factor in the evolution of plant-insect interactions (Ehrlich & Raven 1964, Scheirs & De Bruyn 2002). The chemical coevolutionary concept *sensu stricto* proposed by Ehrlich and Raven (1964), which postulates that secondary chemicals serve as a driving force behind specialization on certain hosts and herbivory causes the evolution of secondary metabolites in plants, has received criticism (Jermy 1984, Bernays 1988, Thompson 1988a). However, the tendency of related phytophagous insects to feed on related host species is evident (Farrell & Mitter 1990, Farrell & Mitter 1998, Janz & Nylin 1988). There is also evidence that insect herbivores may cause the evolution secondary chemicals in plants (Mauricio & Rausher 1997). Several recent studies have proven the importance of host plant chemistry for the evolution of host use by phytophagous insects, showing that related species are more likely to be found on chemically related host species regardless of host taxonomy (Becerra 1997, Wahlberg 2001).

In addition to their role in the evolution of host range, secondary chemicals have very often a more proximate role in the host selection process of phytophagous insects. Insects may use these chemicals as cues to locate an appropriate host and as stimulants to feeding or oviposition, when a specific chemical elicits feeding or oviposition behaviour. Secondary chemicals may serve as repellents, causing movement away from a potential host, or as deterrents, inhibiting feeding or oviposition (Bernays & Chapmann 1994). In general, generalist herbivores tend to be more sensitive to defensive secondary chemicals than specialists (Bernays & Chapmann 1994). There are many examples showing that secondary chemicals have no adverse impacts on specialized herbivores (Crawley & Nachapong 1984, Macel *et al.* 2002). Several comparative and manipulative studies report that generalists suffer higher mortality or retarded growth compared to specialists when feeding on a diet containing secondary chemicals (Krischik *et al.* 1991, Landau *et al.* 1994, Johnson *et al.* 1996, Hägele & Rowell-Rahier 2000, Dyer *et al.* 2003). Secondary chemicals may also have a negative effect even on specialized herbivores (Berenbaum *et al.* 1989, Agrawal & Kurashige 2003), indicating potential ongoing evolutionary interactions between plants and herbivores (Agrawal 2004). In addition to the impact of individual secondary chemicals on the performance of herbivores, there is growing evidence of negative synergetic effects of several secondary chemicals on the growth and survival of herbivores (Berenbaum & Zangerl 1993, Calcagno *et al.* 2002, Scott *et al.* 2002, Dyer *et al.* 2003).

Herbivores have many ways to cope with secondary chemicals in their diet. They may avoid feeding on tissues or species containing harmful amounts of chemicals, circumvent the host's defences or either tolerate or detoxify them (Bernays & Chapmann 1994, Glendinning 2002). For example, the host use of a specialist silk moth appears to be dependent on its ability to degrade host toxins rapidly with midgut detoxification P450 enzymes (Johnson 1999). The ability of generalist herbivores to use several chemically different hosts may be based on the applicability of P450 enzymes to detoxify a wider array of secondary chemicals. Li and coauthors (2004) found that the cytochrome P450 protein of a generalist species effectively metabolized a wider variety of secondary

chemicals compared to that of a specialist Lepidopteran species. Specialist herbivores often sequester secondary chemicals and use them as such or as synthesized for their own defence or in sexual communication (Birch *et al.* 1990, Landolt & Phillips 1997, Nishida 2002). For example, several species in Arctiidae and Danainae sequester cardenolides and pyrrolizidine alkaloids from host plants (Cohen & Brower 1983). Larvae of *Junonia coenia* reared on a natural diet containing iridoid glycosides were predated less than larvae reared on a diet lacking iridoid glycosides (Camara 1997b). It has also been suggested that even lichenivorous Lithosiinae species in Arctiidae benefit from received unpalatability via sequestered lichen secondary chemicals (Rawlins 1984, Wink & Von Nickisch-Roseneck 1997, Weller *et al.* 1999). One example of the elaborate use of secondary chemicals in sexual behaviour is an arctiid moth, *Utetheisa ornatrix*. Males of this species have in their abdomen brush-like structures which they evert before copulation, and from which plant-derived hydroxydanaidal is aired. The female's mating decision is based on the presence and amount of hydroxydanaidal aired by the male (Conner *et al.* 1981, Iyengar *et al.* 2001). Males of *U. ornatrix* reared on a plant containing pyrrolizidine, which is used to synthesize hydroxydanaidal, grow bigger and can offer bigger spermatophores richer in hydroxydanaidal during copulation (Conner *et al.* 1990). Females, in turn, transfer the received hydroxydanaidal into their eggs, which are hence protected against predators (Eisner *et al.* 2000).

1.3 Hypotheses explaining diet breadth

In the context of host specificity, it is important to distinguish between species able or destined to complete their development on a single host plant and species whose larvae are able to move between hosts. Especially the families Noctuidae and Arctiidae include many species whose larvae are able to move between hosts and, even at an individual level, use several hosts (Dethier 1988, Gaston & Reavey 1989, Reavey & Lawton 1991, Singer & Stireman 2001). While the payoff of host specificity may emerge through faster and more accurate decisions concerning host selection, better host use efficiency or decreased predation or parasitism, the benefits of individual polyphagy may arise in different ways. Generalists have a wide selection to be included in their host list. Polyphagous individuals may also benefit from dietary mixing by either balancing their nutrition by feeding on complementary foods (Rapport 1980) or by diluting any single harmful secondary metabolite in their food (Freeland & Janzen 1974). There is a growing body of evidence to show that herbivores perform better on a mixture of food items compared to any single item (Pennings *et al.* 1993, Bernays *et al.* 1994, Hägele & Rowell-Rahier 1999, Ballabeni & Rahier 2000, Moreau *et al.* 2003). The results seem to support the hypothesis that this increase in performance is due to complementary nutrients rather than reduced impacts of single secondary chemicals (Bernays *et al.* 1994, Chambers *et al.* 1995, Hägele & Rowell-Rahier 1999). Dietary mixing increases the growth and survival of Orthoptera, but similar evidence on Hemiptera and Lepidoptera is scarce (Bernays & Minkenberg 1997).

Some recent studies have concentrated on the host use of individually polyphagous or oligophagous species that are responsible for their host selection even at the larval stage.

For example, larvae of a generalist Arctiid, *Grammia geneura*, perform better when reared on a mixed diet compared to any single-host diet. Although growth efficiency is lower on a mixed-species diet, larvae receive the benefit of an enemy-reduced space via a sequestered pyrrolizidine alkaloid deterrent against parasitoids (Singer 2001, Singer *et al.* 2004a). Larvae of a chrysomelid beetle *Oreina elongata* move between hosts to ensure better nutrition on one host and better survival on another (Ballabeni & Rahier 2000, Gotthard *et al.* 2005). These examples emphasize the role of larval behavioural adaptations that allow the use of several hosts and the combination of different selection pressures on the dietary evolution of generalist herbivores.

The key issue in studies on the host specificity of phytophagous insects is the relationship between the oviposition site preference of the female and the growth, survival and fecundity (performance) of the offspring on those hosts. One might expect that, through natural selection, the female's decisions on where to lay eggs, i.e., her preference for oviposition, should be shaped by the welfare of her progeny or, more generally, their performance (Jaenike 1978). This preference-performance hypothesis has been the cornerstone of hundreds of studies on insect-plant interactions (Thompson & Pellmyr 1991, Renwick & Chew 1994, Mayhew 1997). However, the observed relationships between adult oviposition site preference and larval performance range from good correspondence to rather poor (Thompson 1988b, Jaenike 1990, Mayhew 1997, Thompson 1998, Mayhew 2001). One reason for the poor association between preference and performance is that females may optimize their own performance. Scheirs and coauthors (2000) showed that the females of a fly *Chromatomyia nigra* oviposit on hosts which are of high quality for their own feeding but not for larval feeding. Also, the females of the common blue butterfly *Polyommatus icarus* preferred for oviposition hosts that provided a source of nectar for themselves (Niklas *et al.* 2005). These results may explain the observed patterns of preference and performance of insects whose fitness is determined partly by resources obtained during adult stages, but are probably not applicable to species whose adults do not feed. Other studies have also revealed that the whole life-cycle of the species needs to be taken into account in studies on host preference and subsequent larval performance. For example, a geometrid moth *Epirrita autumnata* shows indiscriminating oviposition site selection in relation to host quality but, instead, seems to oviposit on places ensuring some protection for the overwintering eggs against harsh environmental conditions (Tammaru *et al.* 1995)

Preference-performance hypotheses may be more applicable to monophagous species, such as butterflies, sawflies and miners, whose host selection and, subsequently, larval performance are determined by female oviposition, than to polyphagous species, whose host selection and location are often determined by larvae. Thompson (1988b) put forward four factors that may affect the preference-performance relationship and the evolution of host plant choice. First, when novel plant species are available, females may lay eggs on these plants, even though the plants are unsuitable or even lethal to larvae. Selection may take many generations to either reduce the tendency of females to oviposit on these plants or to increase the ability of larvae to survive and grow on novel plants. Second, the relative availability of host plants in space and time may affect the use of those plants by herbivores. Third, selection pressures are different for species that complete their development on a single plant and for species with grazing habits, and selection may favour the ability to use several hosts and/or mixed species diets. Fourth,

the performance of a phytophagous insect on plant species can be influenced by natural enemies: predators, parasitoids and competitors.

The idea that the natural enemies of herbivores may contribute to the host specificity of plant feeders has received support in numerous studies (Brower 1958, Price *et al.* 1980 Jaenike 1985, Damman 1987, Bernays 1988, Bernays 1989, Bernays & Graham 1988, Denno *et al.* 1990, Berdegue *et al.* 1996). According to the concept of enemy-free space (EFS), generalist predators and parasites have a selective force to narrow down the host range of herbivores to hosts that ensure the best refuge due to either host chemistry or better concealment (Price *et al.* 1980, Bernays & Chapmann 1994, Nishida 2002). On the other hand, high mortality due to a specialist natural enemy might select for broadening of the host range of herbivores (Gratton & Welter 1999). More recent studies also emphasize the role of natural enemies as an ecologically and evolutionarily important factor determining host range in phytophagous insects. (Mira & Bernays 2002, Oppenheim & Gould 2002, Singer & Stireman 2003, Mulatu *et al.* 2004, Singer *et al.* 2004b).

One way to explain host specificity is that the genetic trade-offs in survival, development and efficient utilization between several hosts have led to the evolution of populations that specialize in different plant species. Close adaptation of a population to its primary hosts results in lower fitness on other potential hosts, and consequently selection may favour behavioural avoidance of alternative hosts. Few studies have proved the existence of such trade-offs (Fry 1990, Mackenzie 1996, Agrawal 2000). However, most studies have argued that trade-offs are not responsible for the host specialization of phytophagous insects (Futuyma & Moreno 1988, Jaenike 1990, Thompson 1995, Fry 1996, Camara 1997a, Ballabeni *et al.* 2003).

The diet breadth of herbivorous insects may be limited by their neural capacity. Consequently, constraints on the input and processing of information may lead to host specialization (Bernays & Chapmann 1994, Bernays 1998, Bernays 2001). Specialization in one host and the use of reliable cues in host selection makes host finding and selection faster. Fast and accurate decisions on host selection are assumed to be crucial for egg- and time-limited ovipositing insects (Bernays 2001). For example, Swedish polyphagous *Polygonia c-album* butterflies, which are relatively more generalists compared to English ones, which mainly use *Urtica dioica* as a host plant, oviposited more often on poor-quality hosts and used more time in decision-making concerning oviposition than English females (Janz & Nylin 1997, Janz 2003).

There are several less thoroughly tested or less fashionable hypotheses explaining the host range of herbivorous insects. For example, competition may lead to differential host use between herbivores sharing similar resources (Denno *et al.* 1995, Feder *et al.* 1995), or host selection may occur in places with good mating probabilities (Ward 1991). Experiences early in the life-cycle of herbivores in some cases set boundaries on host range in the later phases of the life-cycle (Papaj & Prokopy 1988). Furthermore, life history constraints may also limit the host range of insects (Bernays & Janzen 1988).

1.4 Lichenivory

Lichens are symbiotic organisms composed of a fungal partner, the mycobiont, and one or more photosynthetic partners, the photobionts, which may be either a green alga or a cyanobacterium. The majority of lichens develop internally stratified thalli divided into upper cortex, photobiont layer, medulla and lower cortex. Most lichen species (c. 90%) contain green algae as main photobionts (Friedl & Büdel 1996). Among the families in the order Lecanorales, which contains the majority of lichenized fungi, *Trebouxia* is the most frequent photobiont, and it has rarely been found free-living (Ahmadjian 1993, Friedl & Büdel 1996). Lichens differ in at least three principal features as hosts for phytophagous insects compared to higher plants. First, their nutritional quality, as indicated by the N concentration, is often relatively low, and they hence appear to be poor food for herbivores (Slansky & Scriber 1985, Lawrey 1987). Second, since lichens in terms of host availability can be regarded as evergreen perennials, phenological factors do not limit the host use of lichen feeders, as is often the case with herbivores feeding on higher plants. Third, the defence of lichens is mainly based on the secondary chemicals present in them since there are no physical characteristics preventing host use by lichenivorous species apart from the toughness of the cortex in a dry state. Lichens are poikilohydric organisms, whose water content is dependent on water received directly on the surface or absorbed from water vapour, and they lose most of their toughness when wet.

In lichens, secondary chemicals secreted by the fungal partner are deposited mainly on the fungal hyphae in extracellular space in either the cortical or the medullary regions of thallus (Lawrey 1984, Elix 1996). These chemicals often occur in relatively high concentrations in lichens (Fahselt 1994, Hyvärinen *et al.* 2000) and may occur only in reproductive tissues or in higher concentrations in reproductive tissues compared to somatic ones (Lawrey 1984, Hyvärinen *et al.* 2000).

There are three main biochemical pathways for lichen secondary compounds: the mevalonic acid, acetyl-polymalonyl and shikimic acid pathways. For example, usnic acid, which occurs in pendulous lichens in the genus *Usnea*, and parietin, which is attributable to the bright orange colour in *Xanthoria* species, are synthesized via the acetyl-polymanol pathway (Elix 1996). Secondary chemicals may have several roles in lichens, which can be summarized as follows: i) an antibiotic role against micro-organisms, ii) metabolites may increase the permeability of cell walls in photobionts, iii) they can absorb UV light and protect photobionts from radiation, iv) they may form a basis for defence against lichen feeders, and v) hydrophobic medullary chemicals may prevent drowning of tissue and ensure continuous gas exchange (Lawrey 1984, Fahselt 1994, Elix 1996, Huneck 1999). There is evidence for many putative roles of lichen secondary chemicals, such as inhibiting the growth of bacteria, fungi, viruses and plants (Lawrey 1984), while some others, including promoting gas exchange, have so far not received experimental support (Lange *et al.* 1997). The two most extensively studied hypotheses, the antiherbivore nature of secondary chemicals and their protective role against UV radiation, have also received considerable experimental support (Lawrey 1984, Emmerich *et al.* 1992, Emmerich *et al.* 1993, Giez *et al.* 1993, Giez *et al.* 1994, Solhaug & Gauslaa 1996, Gauslaa & Solhaug 2001, Solhaug *et al.* 2003, Nybakken *et al.* 2004).

Lichenivores are relatively common in different animal groups ranging from small invertebrates, such as mites, snails and collembolas, to big grazers, such as reindeer. Lichen-feeders occur in several insect orders, e.g., Collembola, Thysanura, Psocoptera and Lepidoptera. In Lepidoptera, lichenivory occurs in several families (e.g., Psychidae, Noctuidae, Geometridae, Arctiidae and Lycaenidae), and one of the largest radiation of lepidopteran lichenivores occurs perhaps in Arctiidae, subfamily Lithosiinae, all of whose known larvae feed on lichens, algae, liverworts or mosses (Atsatt 1981, Rawlins 1984, Lepidopterologen Arbeitsgruppe 2000). Lichen-invertebrate associations have been studied since the end of the 1800s, when Zukal (1895) proposed that secondary compounds may protect lichens from herbivory. This was contradicted by Zopf (1896), who maintained that such compounds afford lichens little protection. After that, there have been several studies of lichen-invertebrate associations, of which most have concentrated on the role of lichen secondary chemicals as antiherbivore compounds (Stahl 1904, Slansky 1979, Lawrey 1980, Lawrey 1983a, Lawrey 1983b, Reutimann & Scheidegger 1987, Blewitt & Cooper-Driver 1990, Emmerich *et al.* 1992, Emmerich *et al.* 1993, Fröberg *et al.* 1993, Giez *et al.* 1993, Giez *et al.* 1994). Apparently, lichen secondary chemicals may give a basis for chemical defence against lichenivorous species (Gauslaa 2005). However, almost all studies on the relations between lichens and invertebrates have been carried out with highly polyphagous invertebrates, which hardly use lichens in nature, and none of these studies have used specialized insect lichen feeders. It has also been suggested that the Lepidopteran species in Lithosiinae benefit from sequestration of lichen secondary chemicals as they become unpalatable or even toxic to predators (Rawlins 1984, Hesbacher *et al.* 1995, Wink & Von Nickisch-Roseneck 1997).

1.5 Aims of this study

Ecological and evolutionary factors causing and modifying host specificity in phytophagous insects are numerous and not necessarily mutually exclusive and, as outlined above, none of them alone is able to explain all observed specialization in the feeding habits of herbivorous insects. Rather, these hypotheses may be complementary, implicating, for example, coevolution constrained by neural limitation. Since lichens as hosts differ from higher plants, and studies on host specificity in specialized lichen feeders are simply lacking, the aim of this study was to explore factors in the host range of lichen-feeding moths. The first aim was to test the roles of nutritional quality and secondary chemicals of lichens on the performance of common and abundant lichenivorous moth larvae (I). As these larvae failed to survive on some lichens with high concentrations of secondary chemicals, the next step was to explore the role of lichen secondary chemicals on the survival, growth and host use of a specialized lichen feeder (II). Lichens provide a unique model to manipulate the concentration of secondary chemicals *in vivo*. During these experiments, I observed that some larvae developed to the adult stage during the same season, and that larvae of the *Eilema* species had an exceptionally high number of larval instars. Therefore, I decided to test whether the nutritional quality of the host affects the production of the second generation and the

number of larval instars (III). As a comparison to polyphagous *Eilema* species, *Cleorodes lichenaria*, a species specializing in *Ramalina* lichens, was included in the studies (IV), since the selection pressures for host use between monophagous and polyphagous species may differ. Specifically, the main objectives of this thesis can be divided into the following themes:

1. To find out the impact of the nutritional quality of lichen hosts on the performance of lichenivorous Lepidopteran species (I, III, IV)
2. To study the role of lichen secondary metabolites on the host preference and performance of lichen-feeding moth larvae (I, II)
3. To test whether the preference-performance hypothesis is applicable to explain host specificity in lichenivorous moths (IV)
4. To explore the role of larvae in the host selection of lichen feeders (I, III, IV)

2 Materials and methods

2.1 Study species

Several Lepidopteran species feeding on lichens during their larval period were used in this study. *Eilema* species feed as larvae on algae, lichens, mosses or dead plant material, but lichens are mentioned as their primary host resource (Rawlins 1984, Lepidopterologen Arbeitsgruppe 2000). *Eilema complanum* (L.), *E. depressum*, (Esper), *E. lurideolum* (Zincken) and *E. lutarellum* (L.) (Arctiidae, Lithosiinae) are univoltine moths flying mainly in July and August. Larvae of *Eilema* species can be regarded as polyphagous even at the individual level, since lichen secondary compounds from different species have been detected from dead specimens (Hesbacher *et al.* 1995). *E. depressum* larvae feed on lichens on tree trunks, larvae of *E. lurideolum* on lichens on trees and stones, larvae of *E. complanum* on lichens on trees and also on mosses and *E. lutarellum* larvae on lichens on the ground and on dead plant material (Lepidopterologen Arbeitsgruppe 2000). Larvae overwinter during their first instars and pupate at the following midsummer. *Cleorodes lichenaria* (Hufnagel) (Geometridae) is another univoltine geometrid moth flying mainly in July. According to the literature, larvae feed on lichens growing on deciduous trees (Mikkola *et al.* 1989). Mikkola and coauthors (1989) also mention that adult individuals have been observed flying in Åland in southern Finland around trees with high densities of *Ramalina* species. *C. lichenaria* hibernates as medium-sized larvae. In Finland, *C. lichenaria* is regarded as an endangered species due to a lack of suitable habitats or a small population size (Rassi *et al.* 2001).

2.2 Nutritional quality and secondary chemistry of host lichens

The nutritional quality of lichens was measured as N (I, IV) and P (IV) concentrations. Total N and C concentrations were analyzed using the dynamic flash combustion method with Element Analyser EA 1110 CHSN 0 (Fisons instruments) (I, II, IV). The concentration of P was determined with a spectrophotometer according to John (1970).

While manipulating lichens' chemical defence (II), the impact of the manipulation method on N, C, and P concentrations was also measured.

Secondary chemicals were analyzed using TLC and HPLC (II). *X. parietina* and *V. pinastri* were rinsed with dry acetone and *P. sulcata* and *H. physodes* with dry ethyl acetate according to the method of Solhaug and Gauslaa (1996) four times sequentially at room temperature for 5 minutes each time (II). It has been shown that acetone rinsing applied in the manner described in Solhaug and Gauslaa (1996, 2001) has no impact on lichens' viability. Solvents were allowed to evaporate completely from thalli for at least 20 hours, and control thalli were simultaneously left intact at room temperature. In first preference experiments (I), the total phenol concentration was calculated as the mean phenol concentration in the somatic and reproductive parts for a lichen species from figure 1 in Hyvärinen and coauthors (2000), where lichens from the same area were used.

Lichen specimens (10–15 mg) were placed in Eppendorf tubes and extracted at least three times in cool acetone for 60 min (Feige *et al.* 1993). A standardized TLC method for the identification of lichen products with three solvent systems (Orange *et al.* 2001) was used. Filtered acetone extracts were analyzed by gradient HPLC (Feige *et al.* 1993, Lumbsch 2002). Detection was performed at a wavelength of 245 nm. Authentic samples of lichen substances were used when available: atranorin (Sigma, the compound was mixture of atranorin and chloroatranorin), (+) usnic acid (Aldrich), vulpinic acid (isolated from the lichen *Letharia vulpina*), parietin (isolated from the lichen *Xanthoria parietina*) and pinastric acid (isolated from the lichen *Vulpicida pinastri*). Medullary secondary metabolites of *Hypogymnia physodes* and salazinic acid in *Parmelia sulcata* were determined only qualitatively against microextracts of herbarium specimens with TLC-determined chemistry.

2.3 Preference experiments

Preference experiments with *Eilema* larvae were carried out in Petri –dishes, where larvae were allowed to select their food from several alternatives. Adult females of *Eilema* species were collected using mercury lamps from different locations in southern Finland in August 2000 (I), in August 2001 (II) and in July 2003 (III), where the species are common and relatively abundant (Huldén *et al.* 2000). When the host preference of larvae among different lichen species was studied (I), larvae were allowed to select from four lichen species differing in their secondary chemistry: *Xanthoria parietina* (L.) Th. Fr. (parietin), *Vulpicida pinastri* (Scop.) J.-E. Mattsson & M. J. Lai (vulpinic and pinastric acids) and *Hypogymnia physodes* (L.) Nyl. (atranorin and physodic acid) and *Melanelia exasperata* (De Not.) Essl., which does not contain polyphenolic secondary substances (Culbertson *et al.* 1977). The role of secondary chemicals in the host preference and performance of larvae of *E. depressum* were studied in subsequent experiments (II), where larvae were allowed to select their food either from treated thalli, from which the major part of secondary chemicals had been extracted, or control thalli containing naturally occurring secondary chemicals. In another experiment, the role of secondary chemicals within lichen species was studied by providing larvae with a choice between intact control thallus and treated thallus with low concentrations of secondary

chemicals present (II). In addition to the four lichen species used earlier (I), *Parmelia sulcata* Taylor (atranorin and salazinic acid) was included in the experiments as a new species (II). The number of larvae per dish varied between six and twenty, and preference was calculated as consumed lichen biomass.

The host preference of *C. lichenaria* females was measured in the laboratory with a simultaneous choice trial, where four common lichen species occurring in the study area were provided for females to oviposit: *X. parietina*, *P. sulcata*, *Ramalina fraxinea* (L.) Ach. and *Ramalina farinacea* (L.) Ach (IV). Lichen thalli were air-dried for two days in the laboratory and for two days in a desiccator prior to weighing. One virgin female and a male were placed in a cage. When the female had oviposited her first eggs, the number of eggs was counted on each lichen every second day and detached carefully to avoid filling of any potential host. The females were allowed to lay eggs until they died. Preference was calculated as the number of oviposited eggs on each lichen species. To study the host preference of neonate *C. lichenaria* larvae, eight neonate larvae per dish were allowed to feed on the same four lichen species for twelve days in Petri dishes. During the experiment, the thalli were moistened every three days with deionized water drops. Larvae were obtained by pairing adult moths collected at larval stage from the Jomala area. Preference was calculated as the consumed lichen biomass. The distribution of larvae of *C. lichenaria* in natural conditions was checked in Jomala, Åland, where the distribution of larvae was compared to the relative abundance of macro lichen species growing on tree trunks (IV). During two nights, each sample area was checked once, and the larvae on each lichen species were counted.

2.4 Growth and survival experiments

To study whether larval growth and survival differ between host lichens (I), sixteen neonate larvae of *E. complanum* and *E. depressum* were reared individually on each of the four lichen species in a climate chamber. In subsequent experiments (II), where I studied whether lichens' secondary chemicals are responsible for the mortality and retarded growth of *E. depressum* larvae, 74 larvae/lichen species from five females were reared on control and treated thalli of three lichen species (*P. sulcata*, *H. physodes* and *X. parietina*) and 64 larvae on *V. pinastris*. Equal numbers of larvae were distributed to feed on control and treatment thalli. Twenty larvae of *C. lichenaria* on *R. fraxinea* and eighteen larvae on *R. farinacea*, *P. sulcata* and *X. parietina* (IV) were reared individually. Pupal weight (I, III, IV) or the weight of the larvae (II), the length of the larval period (I, III, IV) and survival percentage were measured or calculated. I also counted the number and duration of larval instars of *E. depressum* (III).

To test whether larval consumption and utilization of host lichens differ when larvae are reared on different lichens (I), and whether lichen secondary chemicals have an impact on host consumption and utilization (II), I reared larvae of *E. depressum* individually for four (I) or six (II) days and measured their relative growth rate (RGR), relative consumption rate (RCR), assimilation efficiency (AE) and efficiency of conversion of digested food (ECD) (Slansky & Scriber 1985). Before the experiments, all larvae had been reared on the same host lichen that was not used in the experiments.

To study whether the method of manipulation used to extract secondary chemicals *per se* has any negative effects on the nutritional quality of lichens and on the host preference and survival of *E. depressum* larvae, I reared larvae individually on *M. exasperata* treated with either acetone or ethyl acetate or on unmanipulated controls (II). I also carried out a control experiment to study whether the chemical treatments *per se* affect the host preference of *E. depressum* larvae. One thallus of *M. exasperata* was divided into three slices of similar size, which were randomly assigned to acetone treatment or ethyl acetate treatment or as control. Slices from the same thallus were placed in a dish, and six neonate larvae were allowed to feed on the thalli for two weeks. All thalli were replaced after the first week.

2.5 Statistical analyses

An ANOVA model was used to compare the host preferences of *Eilema* larvae (I). Pearson's correlation was used to compare the host preference with the N concentration and total phenol concentration (I). MANOVA was used to analyze whether the concentration of secondary chemicals and the lichen species affect the host preference of *E. depressum* larvae (II). MANOVA was also used to compare the different lichen species for larval period, pupal mass, RGR and the number of larval instars (III). Paired *t*-test was used to test the impact of lichen secondary chemicals within lichen species on the host preference of *E. depressum* larvae. The host preference of females of *C. lichenaria* was tested with repeated-measures ANOVA (IV) and the host preference of larvae with two-way ANOVA, using lichen and female sex as explanatory factors (IV).

The growth, consumption and host use efficiency of larvae on different lichens were analyzed with one-way ANOVA (I). The effect of lichen and moth species on the survival of larvae was tested with a linear-logit model using stepwise model reduction starting from a model containing the main effects of lichen and moth species and their interactions (I). The impacts of lichen secondary chemicals within lichen species on larval mass, RGR, RCR and AE were analyzed by Student's *t*-test (II). ANOVA models were used to compare the pupal masses, larval periods, RGRs and numbers of instars of *E. depressum* on different lichens (I, III) as well as the differences in the larval weight, pupal mass and length of the larval period of *C. lichenaria* on different lichen species (IV). Two-way ANOVA with lichen and overwintering as explanatory factors was used to explore the differences in the number of larval instars before overwintering between larvae that developed directly and those that overwintered. The duration of the five first instars between larvae that developed without overwintering and those that overwintered were compared with a linear-logit model with *t*-test as a *post hoc* test within instars (III). Larval instar numbers were compared with pupal size and the length of the larval period with Spearman's correlation (III). χ^2 -test was used to compare larval survival rates on different lichens (III, IV), to test the impacts of lichens' secondary chemicals within lichen species on larval survival (II) and to compare larval distributions in nature to lichen abundance as well as to compare females' host preference and larval distribution (IV).

3 Results and discussion

3.1 Host use and performance of larvae in relation to nutritional quality of lichens

Larvae of all *Eilema* species preferred *M. exasperata*, which is of higher quality (as indicated by the N concentration) compared to the other lichens (I, II). Larvae of all species showed the same preference sequence from the most preferred lichen to the least preferred: *M. exasperata*, *X. parietina*, *H. physodes* and *V. pinastri* (Fig. 1). The host preference of the larvae correlated positively with the N concentration of the lichens (I). Larvae of *E. depressum* and *E. complanum* also reached pupal stage earlier and with a bigger mass on *M. exasperata* than on *X. parietina* and *P. sulcata* (I, III). Overwintered larvae had higher RGR and AE on *M. exasperata* than on *X. parietina* or *H. physodes* (I). One of the most important factors for the growth and performance of insect herbivores is the nutritional quality, especially the N concentration, of their hosts (Mattson 1980, Scriber & Slansky 1981, Awmack & Leather 2002). This study confirmed the crucial role of N concentration for the growth and performance of lichen-feeding insect herbivores.

Moreover, the growth of larvae of *C. lichenaria* at the beginning of their larval period corresponded to the nutritional quality of their host lichens, as larvae on *X. parietina* grew fastest and larvae on *R. farinacea* slowest (IV). Based on their N and P concentrations, lichens can be ranked in the following order, beginning from the host lichen of highest nutritional quality: *X. parietina*, *P. sulcata*, *R. fraxinea* and *R. farinacea* (IV). Surprisingly, the final larval period was shortest for the larvae reared on *R. fraxinea* and *R. farinacea* and longest for those reared on *P. sulcata* and *X. parietina*. Accordingly, growth rates were lowest on *Ramalina* species in autumn but highest after overwintering. Seasonal changes in intrinsic properties, such as the concentrations of N and secondary chemicals, of host lichens may explain the differential growth rates in the different seasons. However, there is no evidence of such changes in the lichens of the study area. Hence, there need to be either a cost to feed initially on *X. parietina* and *P. sulcata* or a benefit to feed on *Ramalina* species. Age-specific nutritional requirements of herbivorous insects (Zalucki *et al.* 2002) might explain the observed growth pattern of *C. lichenaria* larvae if *Ramalina* species were nutritionally better for older larvae.

Another possible explanation for the differential growth of larvae in autumn vs. spring might be attributable to differences in the structure of thalli. The thalli of *X. parietina* are relatively thinner than those of *P. sulcata* and *R. fraxinea*, while *R. farinacea* has the thickest thalli. Moreover, the thalli of *Ramalina* species are hard and heavy, indicating higher lichen biomass per area compared to *X. parietina* and *P. sulcata* (M. Bačkor, pers. communication). Rawlins (1984) has suggested that lichenivory is essentially nothing more than feeding on the algal partner of the thallus. If the thickness of the algal layer correlates with the thickness of the thallus, bigger larvae feeding on *X. parietina* and *P. sulcata* might have a cost for being forced to ingest more fungus compared to larvae feeding on *Ramalina* species. Bigger larvae may not be able to concentrate their feeding mainly on the algal layer due to their bigger mandibles. Observations made during the preference experiments with *E. complanum* and *E. depressum* (I, II) support the hypothesis that the algal layer is the essential part of the lichen thallus for larval nutrition, since especially young larvae concentrated their feeding on the algal layer of the thalli. These observations are in accordance with those made by Hesbacher and coauthors (1995), who reported that *E. complanum* larvae fed on the cortical and algal layers of *Cladonia pyxidata*.

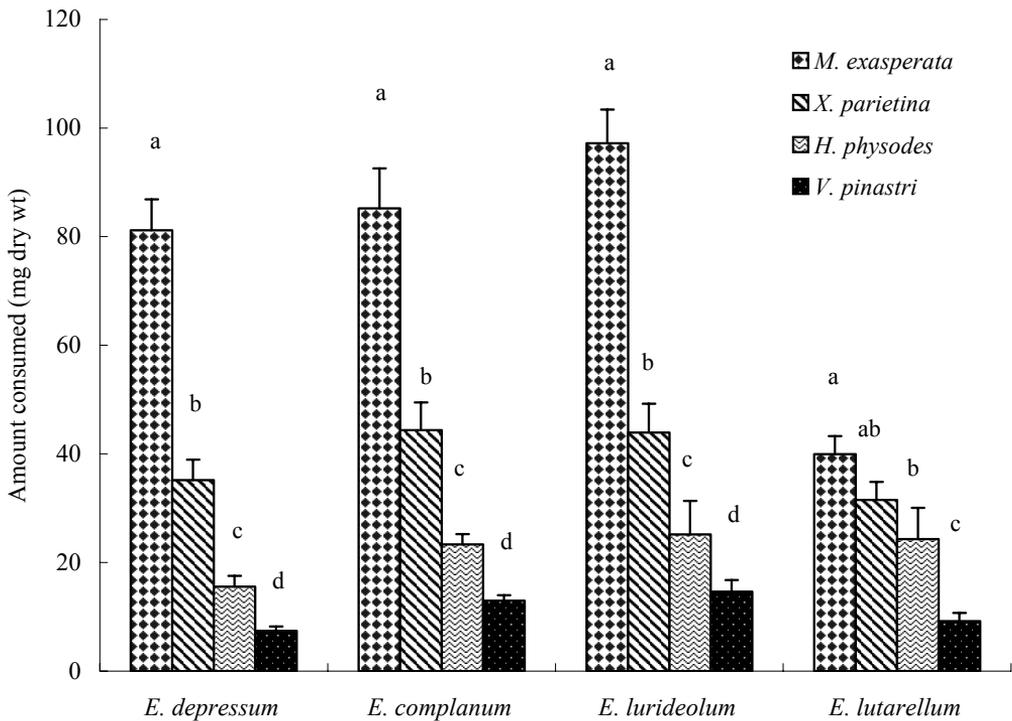


Fig. 1. Host preference of larvae of *Eilema* species measured as a consumed lichen biomass (\pm 1 SE). Letters above bars indicate significant differences between lichen species.

Although N concentration had a positive impact on the growth rates of larvae of *E. depressum*, the nutritional quality of lichens did not promote the production of an additional generation. There were no significant differences in the proportions of larvae developing to the pupal stage without overwintering on different lichens (22.2% on *M. exasperata* and *P. sulcata* and 16.7% on *X. parietina*) (III). Other factors, such as temperature, photoperiod and moisture, which are generally more important determinants of diapause induction in insects (Denlinger 2002), are probably also responsible for diapause induction in *E. depressum*. The slightly higher temperatures during the experiments in study III compared to study I might contribute to the direct development of a relatively large proportion of larvae. It may also explain why individuals of a natural second generation can be found at southern latitudes but not in the area where individuals for this study were collected (Huldén *et al.* 2000, Lepidopterologen Arbeitsgruppe 2000). It is possible that the study species originated from more southern latitudes, and that individuals of more southern origin are adapted to a longer period of growth than local specimens, or that local specimens are also able to respond to variable environmental conditions and produce a 2nd generation. Moreover, if the ability to respond flexibly to varying environmental conditions incurs no cost, it may also be preserved in northern populations.

3.2 Impact of lichen secondary chemicals on host use and performance of larvae

All larvae of *E. depressum* reared on intact *H. physodes* and *V. pinastri* died during the experiments (I, II). Larvae on *V. pinastri* died during the 2nd instar at the latest, and most larvae on *H. physodes* died during the first 50 days of growth and survival experiments, and none of them survived throughout the whole experiment (I). Overwintered larvae of *E. depressum* and *E. complanum* had very low RGR, RCR, AE and ECD on *H. physodes*. Although not statistically significant, there was a trend for a negative correlation between the secondary chemical concentration and host preference of *Eilema* spp. larvae (I). After the major part of secondary chemicals had been removed from *V. pinastri* and *H. physodes*, the survival of *E. depressum* larvae remained as high as on the other lichens, i.e., *M. exasperata*, *P. sulcata* and *X. parietina* (II, Fig. 2). Lowered concentrations of the secondary chemicals parietin and atranorin + chloroatranorin in *X. parietina* and *P. sulcata*, respectively, had no impact on the survival of larvae (II).

Reduction of secondary chemicals from *V. pinastri* and *H. physodes* resulted in increased RGR and RCR, and also increased AE of larvae when reared on *V. pinastri* (II). In the case of *P. sulcata*, extraction of most of secondary chemicals had a minor impact on the growth of neonate *E. depressum* larvae, but that difference disappeared within 70 days and had no impact on the host use of overwintered older larvae. Removal of secondary chemicals also had an effect on the host preference of larvae, which was most dramatic in the case of *V. pinastri*. When both control and intact thalli of *V. pinastri* were available, larvae of *E. depressum* ate almost exclusively treated thalli with lower concentrations of secondary chemicals (II). Treated thalli of *H. physodes* and *P. sulcata*

were also preferred over intact control thalli. Parietin in *X. parietina* was the only secondary chemical that had no impact on the host preference of *E. depressum* larvae (II).

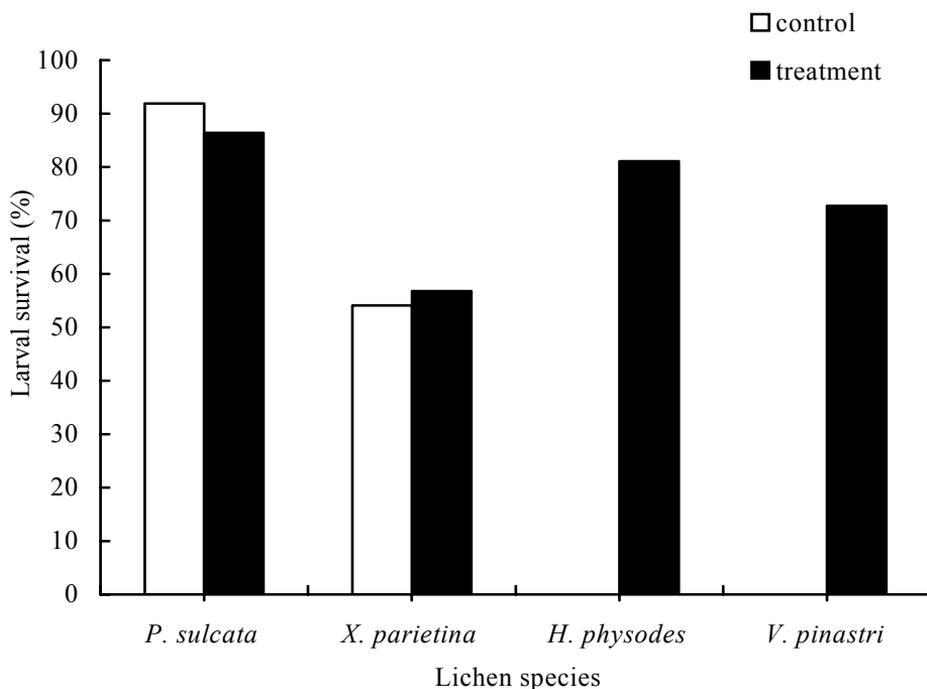


Fig. 2. Survival of *E. depressum* larvae on intact lichen thalli (open bars) with natural concentrations of lichen secondary metabolites and on treated thalli (black bars), from which major part of secondary chemicals had been removed.

There are several examples of antiherbivore impacts of vulpinic acid and usnic acid, which also occurred in *V. pinastri*, on invertebrates (Slansky 1979, Stephenson & Rundel 1979, Emmerich *et al.* 1992, Emmerich *et al.* 1993). For example, Emmerich *et al.* (1993) found that an artificial diet with added vulpinic or usnic acid resulted in reduced RGR and RCR in larvae of *Spodoptera littoralis* and also caused acute toxicity when present in high concentrations. Usnic acid was the most active compound, causing the highest mortality and retardation of growth of polyphagous larvae. In experiments with the specialized lichen feeder *E. depressum* (II), acetone treatment extracted most usnic and vulpinic acid from *V. pinastri* thalli but only about 40% of pinastric acid. Hence, the impact of secondary metabolites of *V. pinastri* on the survival, growth and host selection of *E. depressum* larvae is more probably based on usnic and vulpinic acid than on pinastric acid. However, larvae of *C. lichenaria* feeding on usnic acid containing *Ramalina* species did not suffer from excess mortality (IV) compared to other lichens. It is possible that *C. lichenaria* larvae, being specialist on *Ramalina* species, are more adapted to usnic acid than larvae of more generalist *Eilema* species. An alternative explanation is that, because usnic acid occurs in low concentrations (0.1% and 0.8% of

dry weight in *R. fraxinea* and *R. farinacea*, respectively, unpublished data), it has no impact on the growth of *C. lichenaria* larvae. The toxicity of intact *H. physodes* was probably based on physodic acids, which occur in higher concentrations than the other major secondary compound, atranorin, in *H. physodes* (Hyvärinen *et al.* 2000). Atranorin is also found in *P. sulcata*, whose secondary chemicals had no effect on larval survival (II).

There was remarkable variation in the concentration of lichen secondary chemicals between lichen species (II). The total concentration is relatively low in *H. physodes*, for example, being c. 2.5% of dry mass, higher in *X. parietina*, 3–4% of dry mass (II) (Hyvärinen *et al.* 2000), and above 5% in *V. pinastri* (II), of which half is constituted by pinastric acid (II). Secondary chemicals may have synergetic impacts on the performance of herbivores (Dyer *et al.* 2003, Macel *et al.* 2005). Dyer and coauthors (2003), for example, reported that a mixture of amids of *Piper cenocladum* increased mortality and development time and decreased pupal weight of *Spodoptera frugiperda*. Lichen secondary chemicals have also been observed to work in concert against herbivores. Slansky (1979) found out that a combination of atranorin and vulpinic acid on *Brassica oleracea* leaves resulted in the highest mortality and lowest growth rate of *Spodoptera ornithogallii* larvae compared to single chemicals during the first 12 days of the larval period, although the concentration of vulpinic acid alone was higher than the concentration of a combination of atranorin and vulpinic acid. With *E. depressum*, it is not possible to differentiate between the impact of a single secondary chemical from that of a mixture of chemicals. It may be either a single acid or a combination of several acids that caused the high mortality of *E. depressum* larvae on intact thalli of *H. physodes* and *V. pinastri*.

The location of lichen metabolites within thallus may also affect the host use and selection of lichenivorous larvae. In *V. pinastri*, usnic acid occurs in the cortical layer and vulpinic and pinastric acids in the medullary parts of thallus (Esslinger 1973). Atranorin occurs in the cortical layers of thallus both in *H. physodes* and in *P. sulcata*, whereas salazinic acid (*P. sulcata*) and physodic acids (*H. physodes*) are found mainly in the medullary parts (Culbertson 1970, Day 1978). For neonate larvae, the cortical compounds in host lichens probably play a more important role than the medullary compounds. Hence, it is possible that the location of usnic acid in the cortical layer in *V. pinastri* may be responsible for speeding up the death of *E. depressum* larvae during their 1st instar due to antifeedant activity. Atranorin, for one, seems to be a deterrent in *P. sulcata* (II). At the beginning of the larval period, larvae may be reluctant to feed on thalli containing secondary chemicals, but when no other food item is available, and atranorin has no effect on the growth and survival of larvae, they resume feeding and catch up with the larvae on control thalli.

Parietin, which occurs in *X. parietina*, had no impact on the survival, host preference or growth rate of larvae (II), and in study I, larvae consumed more lichen biomass when reared on *X. parietina* compared to other lichens. There is only some circumstantial evidence of the antiherbivore function of parietin against herbivores. Yom-Tov and Galun (1971) observed that desert snails feeding on several plant species and some lichens tended to avoid parietin-containing lichens, although those species were the most common lichen species in the habitat. The authors suggested that parietin may provide protection for these lichen species. However, more recent studies involving the

manipulation method that was used in study II yielded opposite results. Gauslaa (2005) reported that parietin did not deter grazing of a generalist herbivore snail *Cepaea hortensis*. Other recent results seem to point to a conclusion that parietin mainly provides protection against high irradiance (Solhaug & Gauslaa 1996, Solhaug *et al.* 2003).

Study II corroborates the hypothesis that a single lichen secondary metabolites or a combination of them in natural concentrations has a major defensive role against herbivores. Moreover, studies with generalist herbivores, such as snails (Gauslaa 2005) and polyphagous noctuids (Slansky 1979, Emmerich *et al.* 1992, 1993, Giez *et al.* 1993, 1994), have yielded results similar to those presented here (I, II), confirming the toxicity of some lichen metabolites, the deterrent role of some others and no herbivory-related role for yet some other metabolites. Some other studies have reported that the concentrations of some lichen secondary metabolites, e.g., parietin (Solhaug *et al.* 2003, Nybakken *et al.* 2004) and usnic acid (BeGora & Fahselt 2001, Bjerke *et al.* 2005), correlate with irradiation intensity.

The main hypotheses that explain the presence and concentration of secondary chemicals in plants include the carbon-nutrient balance hypothesis (CNB), the growth differentiation balance hypothesis, the optimal defence theory (ODT) and the specific evolutionary responses hypothesis (SER) (Rhoades 1979, Bryant *et al.* 1983, Tuomi *et al.* 1988, Herms & Mattson 1992). According to the CNB and GDB hypotheses, growth-limiting nutrients (mainly N) determine the proportion of assimilated carbon that can be allocated to growth and defence. Tuomi and coauthors (1988) suggested that the allocation of available surplus carbon to individual carbon-based secondary compounds depends on specific evolutionary responses to herbivory. In addition, SER also includes specific responses to pathogens and abiotic factors, such as UV radiation and ozone (e.g., Koricheva *et al.* 1998). There is evidence that ODT and CNB may also explain the presence and variation of secondary chemicals in lichens, although the role of herbivory in the evolution of lichen metabolites remains a challenge (Hyvärinen *et al.* 2000, 2002).

3.3 Variation in the number of larval instars

Larvae of *E. depressum* showed large variation in the number of larval instars, which was mainly attributable to overwintering (III). Larvae that did not overwinter needed c. 7 instars to complete their larval period, whereas overwintered larvae reached pupal stage after c. 11 instars (Fig. 3). Moreover, the host lichen had a minor impact on the mean number of instars, since overwintered larvae reared on *M. exasperata* had 10.6 instars and larvae on *P. sulcata* and *X. parietina* 11.6 and 11.8 instars, respectively (III).

Instar number and variation in instar number have been largely ignored as important life-history traits of Lepidopteran species, although there may occur relatively large variation in the number of larval instars. If variation occurs, it most often depends on sex, morph or population or is controlled by environmental factors such as temperature, day length and nutritional quality of diet (Daly 1985, Sehnal 1985, Slansky & Scriber 1985, Clare & Singh 1990). Lichen nutritional quality had a significant impact on the number of instars of *E. depressum* (III). Larvae of *E. depressum* that overwintered on nutritionally better *M. exasperata* reached pupal stage after c. 10.6 instars, whereas larvae

overwintering on poor-quality diets needed one extra instar to mature and still remained smaller compared to individuals reared on *M. exasperata* (III). Adult size is often the main determinant of female fecundity in Lepidopteran species (Oberhauser 1997, Wedell & Cook 1998, Fox & Czesak 2000, Awmack & Leather 2002), and biomass accumulation in insect larvae mostly occurs during the last few instars. Tammaru (1998) estimated that growing for one day longer should result in increased fitness of females of *E. autumnata*. Hence, for *E. depressum*, the benefits of reaching bigger size by allocating resources to an extra instar on a poor-quality diet may override the costs of spending more time at the larval stage if there still exist mating opportunities after a longer period of development.

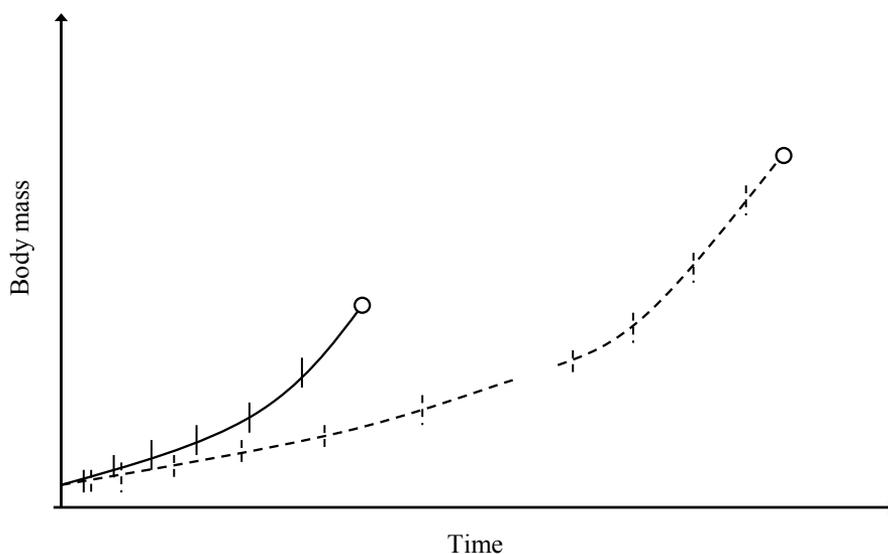


Fig. 3. Growth curves of overwintering (dotted line) and directly developing (solid line) *E. depressum* larvae. Vertical lines on curves indicate moultings and circles pupations. A gap in the growth curve indicates overwintering.

Larvae of *E. depressum* that did not overwinter reached the pupal stage after c. 7 instars at high growth rates, whereas overwintering larvae needed almost 12 instars at slower growth rates to mature. However, in this case, longer development led to larger body size upon maturation (Fig. 3, III). This is unusual, since according to the theory of life-history evolution (Stearns & Koella 1986), the reaction norms for age and size at maturation should follow an L-shaped curve. According to life-history theories, a short developmental period and large adult mass, which in the case of herbivorous insects often depend on the nutritional quality of their hosts, are thought to incur fitness benefits (Roff 1992, Stearns 1992). However, there may also exist trade-offs between size and developmental period (Danks 1994). It may be beneficial to grow slowly, although that leads to longer development or smaller adult size (Abrams *et al.* 1996). On the other

hand, increasing growth rate at the expense of final size, when growth is time-limited, probably incurs a fitness benefit (Abrams *et al.* 1996).

Insects in temperate regions, where individuals face development-limiting time horizons, have been shown to possess plastic growth patterns in relation to environmental cues (Leimar 1996, Margraf *et al.* 2003). A shorter juvenile period (at the expense of size) probably incurs a fitness benefit if there is enough time to produce a whole new generation that can reach the overwintering stage before the onset of the winter (Hunter & McNeil 1997, Gotthard 2001). During moulting, larvae are especially vulnerable to attack by predators, and a high number of instars in itself may therefore include a severe fitness cost. On the other hand, fast growing larvae of *E. depressum* maturing during the same season may minimize this cost with the lower number of instars.

Variation in instar numbers seems to have an important role in the life-history of *E. depressum*, affecting adult size and larval period. Individuals able to respond to environmental cues and take advantage of favourable conditions may increase their fitness if they are able to mate during the same season, and their progeny have enough time to prepare for diapause (Gotthard 2001). If larvae do not reach the pupal stage or grow to a size big enough, the possibility to overwinter several times may also increase the fitness of an individual. On the other hand, in favourable environmental conditions, a partial extra generation within a season – as is the case with *E. depressum* at more southern latitudes (Lepidopterologen Arbeitsgruppe 2000) – may also incur fitness benefits. Similar results have been obtained with a burnet moth, *Zygaena trifolii*, which is perhaps the best studied species in relation to flexibility in instar numbers and has 5–11 instars during the larval period (Wipking 1988, Wipking & Mengelkoch 1994, Wipking 1995).

3.4 Host selection by lichenivorous moths

The larvae of all the studied species showed clear host preference patterns. The larvae of both *Eilema* spp. and *C. lichenaria* preferred host lichens that ensured the shortest larval period and, in the case of the *Eilema* species, also the maximum pupal mass (I, IV). Moreover, the larvae of *E. depressum* avoided lichens with secondary chemicals present in natural concentrations, except *X. parietina* (II). These lichen secondary chemicals clearly affect the host selection of larvae of *E. depressum*.

For lichenivorous larvae feeding on relatively low-quality diet potentially containing high concentrations of toxic and deterrent secondary chemicals (Lawrey 1984, Hyvärinen *et al.* 2000, II), host selection is crucial for subsequent growth. The larvae of both studied species are able to locate the best host for their growth (I, IV) and are hence at least partly responsible for their host selection. Moreover, the larvae of *E. depressum* prefer host lichens with low concentrations of lichen secondary metabolites (II). Since oviposition site selection does not always correspond to the performance of larvae (Thompson 1988a, Mayhew 2001), selection may favour larval adaptations that increase larval survival and performance. These could include, for example, the use of several different hosts or increased longevity of neonate larvae. Interestingly, when studying the survival of neonate larvae, Reavey (1992) found that lichenivorous *Eilema lurideolum* larvae were

able to survive for a mean of approximately 20 days without food. Among all studied species, this was the longest observed mean time. Whether this pattern also occurs with other lichenivorous species and has something to do with host use and host selection remains unanswered. Furthermore, selection in the case of *C. lichenaria* could favour individuals that are able to locate the host lichen that minimizes the length of the larval period both in adult and larval stages. Oviposition behaviour could co-evolve with offspring performance on that lichen (Wolf & Brodie 1998). However, only a few studies so far have concentrated on the host preferences of both females and larvae with subsequent larval performance, and the results from these studies have been controversial (Nylín & Janz 1996, Berdegue *et al.* 1998).

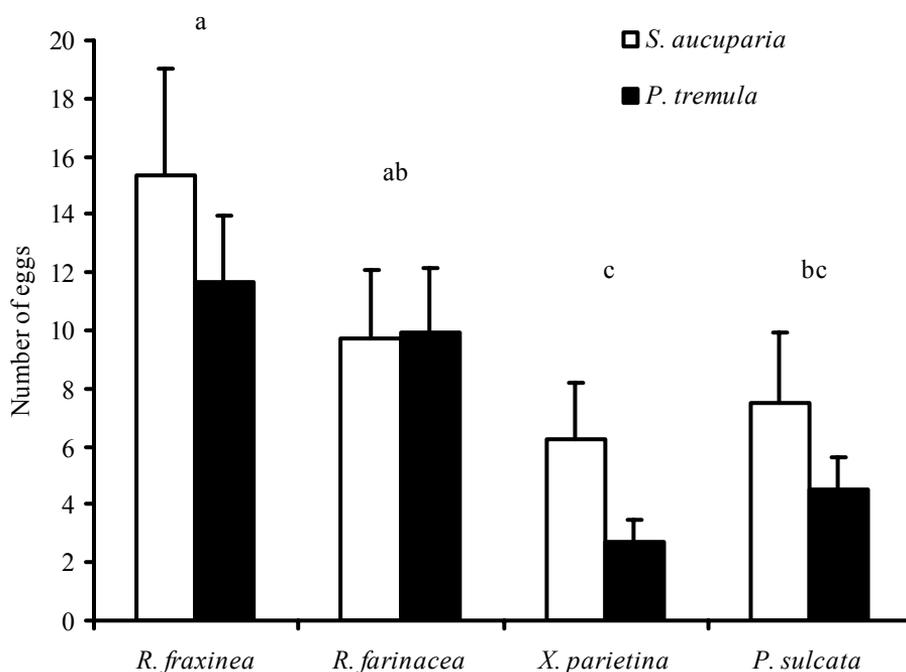


Fig. 4. Host preference of *E. depressum* females measured as a number of eggs oviposited on different hosts. Letters above bars indicate significant differences between lichens by Tukey's *post hoc* test.

Lichen species and brood had significant impacts on the host preference of females of *C. lichenaria* in an oviposition site selection experiment (IV). Females showed a clear host preference towards *Ramalina* species (Fig. 4). *Ramalina fraxinea* was the most preferred host, and females laid significantly more eggs on *R. fraxinea* than on *X. parietina* or *P. sulcata* (IV). There may be several reasons for females and larvae to prefer *Ramalina* lichens over others. For example, for *C. lichenaria* feeding on relatively poor fodder at the northern border of its geographical range (Huldén *et al.* 2000), timing may be crucial

for adult eclosion and larval growth before winter. Feeding on hosts resulting in a long larval period may lead to a loss of mating opportunities, and small larvae may be unprepared for hibernation. On the other hand, the physical and chemical properties of *Ramalina* lichens may also contribute to the performance of larvae. *Ramalina* species have fruticous appearance, whereas *X. parietina* and *P. sulcata* are foliose lichens with their lobes tightly on the tree trunk. Larvae may be more exposed to predators on foliose lichens than on lichens with branching and erect thallus, which may provide physical protection against predators. Hence, host preference may be due to differential predation and parasitism pressures on different lichen species. *Ramalina* species contain usnic acid, which has an antiherbivore function against polyphagous herbivores and is at least partly responsible for the high mortality of *E. depressum* larvae (II). Thus, one can speculate that larvae of *C. lichenaria* may become unpalatable to predators by sequestering usnic acid from host lichens. The branching and bush-like thallus of *Ramalina* species may also provide a physical refuge for larvae, preventing them from falling down from thallus or, since larvae overwinter in trees (pers. observation), allowing them to find a sheltered place for overwintering.

One might also hypothesize, on the basis of the optimal oviposition theory, that females would prefer *X. parietina* for ovipositing if the selection of the oviposition site is based exclusively on the nutritional quality of the host. In that case, larvae could benefit from high growth rates both at the beginning of the larval period on *X. parietina* and after changing the host lichen later during the larval period to *Ramalina* species. However, this scenario may include severe costs. First, high growth rates may be expensive because of increased predation pressure. Gotthard (2000), for example, showed that the faster growing larvae of *Pararge aegeria* were more likely to be killed by generalist predators compared to slower growing larvae. Second, since moving may increase the predation pressure on larvae (Bernays 1997), the costs of changing host lichen may override the benefits of higher growth rates at the beginning of the larval period. However, Bernays (1997) made their experiments with larvae feeding in the daytime, and the situation may be different with nocturnally active larvae, such as *C. lichenaria*.

There was a significant positive connection between host selection by *C. lichenaria* females and subsequent larval performance (IV), since females preferred *Ramalina* species and larvae performed best on them. Furthermore, larvae were found even more often on *Ramalina* species than on other lichens than expected on the basis of the females' oviposition pattern, indicating that other factors besides oviposition may contribute to the host use of *C. lichenaria* larvae. For example, since *C. lichenaria* larvae can be regarded more as grazers than as parasitic feeders according to Thompson (1988b), the ability of larvae to find and select *Ramalina* species may contribute to the observed larval distribution. As the diversity of epiphytic lichens may be high within a few square decimetres (Hyvärinen *et al.* 1992), and larvae prefer *Ramalina* species to other lichens (IV), larvae are able to find *Ramalina* species and compensate for the possible oviposition "mistakes" made by females.

3.5 Evolution of host range of lichenivorous moths

Most Lepidopteran species are phytophagous, i.e., feed on higher plants, and lichenivorous species occur within these families. For example, there are many species in Noctuidae, Geometridae and Psychidae that feed on lichens and dozens of species in Arctiidae and Lycaenidae that feed on lichens and fungi (Atsatt 1981, Skou 1986, Lepidopterologen Arbeitsgruppe 2000). *Eilema* species belong to the subfamily of Lithosiinae in Arctiidae, and *C. lichenaria* belongs to the subfamily Ennomini in Geometridae. Therefore, lichenivorous feeding habits must have evolved several times in Lepidoptera, and there may be several factors that have led to lichen feeding. However, evolutionary changes in the morphology, physiology and ecology of insect herbivores as a prerequisite for or a consequence of adaptation to lichen feeding are almost entirely unexplored.

Ehrlich and Raven (1964) proposed that the evolution of secondary metabolites and the responses to these by phytophagous insects have been the primary factors behind the evolution and diversification of many phytophagous groups. This hypothesis may explain the evolution of host use in lichenivorous moths within families (e.g., in *Eilema* species) but probably not at higher levels (e.g., between families), as lichens and higher plants are evolutionarily separate entities. There were no differences in host preference and survival among *Eilema* species (I), indicating that none of the studied species are adapted to secondary chemicals better than the others. However, the lichen species used in this study represent only a minority of all lichen species, and further studies may show differences in host use ability and survival in relation to lichen secondary metabolites among Lithosiinae species. The subfamily of Ennominae also includes another lichenivorous geometrid moth, *Alcis jubatus* (Thunberg), which feeds on hanging lichens in the genus *Usnea* and *Alectoria*. Interestingly, these lichens contain usnic acid, which is also found in the host lichens of *C. lichenaria*. Usnic acid itself is probably not the driving force in the evolution of the host use of lichenivorous geometrid moths, as all the other species in Ennominae feed on higher plants. However, it is tempting to speculate that the host shift from higher plants has proceeded to usnic acid containing lichens if usnic acid is easy to metabolize or has some function increasing the fitness of lichenivorous moths. For instance, usnic acid might provide protection for larvae against predators and parasites

Inter-specific interactions, such as competition and natural enemies, are potential factors causing a narrow host range for phytophagous insects (Bernays & Chapmann 1994). *C. lichenaria* is a monophagous species feeding on *Ramalina* species in the study area (IV), but it is able to efficiently use other host lichens. As larvae are relatively exposed on tree trunks and, hence, potential prey to predators, predation pressure on other lichens may underlie the evolution of the narrow host range of *C. lichenaria*. Furthermore, an enemy-free space may be obtained, at least partly, by ingesting usnic acid from host lichens.

4 Conclusions

Lichen nutritional quality has a significant role in the performance of lichen-feeding Lepidopteran larvae. Larvae of *E. depressum* reared on *M. exasperata* with the highest N concentration grew faster, reached higher pupal mass and even needed fewer instars during their larval period to reach pupal stage compared to larvae reared on *X. parietina* and *P. sulcata*. Although nutritional quality affected the growth rates of the larvae of *E. depressum*, it did not promote the production of an additional generation during the same season. Also, young larvae of *C. lichenaria* grew better on lichens with high nutritional quality. However, the final larval period was shortest on lichens with lower nutritional quality, and the host lichen had no impact on pupal weight.

Some lichen secondary metabolites appeared to be highly toxic to young Lepidopteran larvae in natural concentrations. All larvae reared on intact *V. pinastri* and *H. physodes* thalli died during the experiments. When most secondary metabolites were chemically removed from these lichens, the survival of larvae was between 60 and 90%. In *V. pinastri*, toxicity is probably based on vulpinic or usnic acid or a combination of these acids, whereas in *H. physodes* it is based on physodic acids. Furthermore, the secondary chemicals in *P. sulcata*, *V. pinastri* and *H. physodes* had a remarkable impact on the host preference of larvae since thalli with lowered concentrations of secondary chemicals were preferred to intact ones. Atranorin in *P. sulcata* was found to have a deterrent role for larvae and to slightly slow down larval growth at the beginning of the larval period. Parietin was not found to affect the performance or host preference of larvae.

While ovipositing, females of *C. lichenaria* clearly preferred *Ramalina* species, on which larvae needed less time to reach the pupal stage. Hence, the results seem to support the preference-performance hypothesis. However, there also seem to be other factors that determine the host range of *C. lichenaria* larvae in the field, since more larvae than expected based on females' oviposition pattern were found on *Ramalina* species. These factors could include predators and parasites or host selection by larvae.

The larvae of all studied species showed a clear pattern in host preference, preferring host lichens that resulted in the biggest pupal mass and/or the shortest larval period. Thus, larvae of these lichenivorous species are able to locate appropriate hosts themselves and seem to be at least partly responsible for their host selection.

Many questions were answered during this project, but new interesting ones also arose. Nitrogen concentration had a significant direct impact on many measured life-

history traits of lichenivorous species. However, in the future, the potential indirect effects of host quality will merit more investigation. These effects include the impact of nutrition on male mating success and on the viability of eggs. The role of a single lichen metabolite vs. a synergetic impact of several chemicals on the performance of larvae is one question that was not addressed in this study. As lichen secondary metabolites probably do not work as a selective agent towards host specialization in lichenivorous species, the role of top-down forces in the evolution of the host range and the host use of lichen-feeding larvae remains a challenging field to be explored. Also, the marked plasticity in the number of larval instars and the observed positive correlation between developmental time and body mass in maturation deserve further attention.

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