

The evolution and adaptive significance of the leaf-mining habit

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Feeding inside leaves by “leaf-mining” insects has been viewed as an adaptive innovation for consuming foliage. The leaf mine presumably provides its occupant shelter from the detrimental effects of the physical environment, protection from attack by natural enemies, and potentially a means of avoiding plant defenses concentrated in particular layers of leaf tissue. We examine several hypotheses concerning the adaptive significance of leaf mining using both the published literature and experiments. We also examine the notion that leaf mining is an innovation that has led to an adaptive radiation by examining the phylogeny and species richness of leaf-mining lineages and their sister groups.

Both advantages and disadvantages accrue to insects that feed inside leaf mines in comparison to external-feeding folivores. The advantages of feeding within a leaf mine include: a lower incidence of disease infection, a microenvironment with lower evaporative demand and therefore protection from desiccation, protection from the direct and indirect effects of UV radiation by the leaf-mine epidermis, and avoidance of plant defenses resulting in higher feeding efficiencies. The disadvantages of feeding inside a leaf mine include: lower mobility resulting in higher mortality from parasites, higher mortality associated with premature leaf-abscission, and smaller average body size and lower fecundity.

Leaf mining is certainly a viable means of feeding upon foliage, and in particular instances it might be the most successful means of feeding on leaves. However, the lower species richness of leaf-mining lineages in comparison to their external-feeding sister groups indicates that the evolution of leaf mining does not represent an innovation that has led to an adaptive radiation. Perhaps the evolution of leaf-feeding per se and the evolution of the ability to feed externally on leaves rather than concealed feeding modes such as mining and galling represent innovations that have resulted in adaptive radiations in the Insecta.

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Leaf mining is a means by which some plant-eating insects consume live foliage while simultaneously dwelling inside it. Along with leaf galling, it is one of two so-called “endophagous” means of feeding upon foliage used by insects. Unlike gall-forming insects, the feeding and tunneling activities of leaf miners do not

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lead to the proliferation of undifferentiated plant tissues which are fed upon by larvae. Rather, leaf miners consume previously developed leaf tissue. While some leaf miners have the ability to slow the rate of necrosis in plant tissues surrounding the leaf mine (the cavity in the leaf created by the feeding of leaf-mining larvae) (Engelbrecht et al. 1969) there is no other evidence that leaf-mining insects can manipulate their food supply as do gall-forming insects.

In all leaf-mining taxa, only larvae feed within leaf mines. However, in many taxa adults and pupae can sometimes be found inside leaf mines because pupation occurs within the leaf mine and the adult must either break through or chew a hole through the epidermis of the leaf to emerge. The duration of leaf mining can be as short as a single instar in some taxa or as long as the entire larval and pupal period. While many species are restricted to the natal leaf mine, some can quit individual leaf mines and initiate new mines on the same or another leaf.

Numerous authors have speculated, but few provide evidence that characteristics of the leaf mine could render it an adaptive innovation when viewed in comparison to larvae feeding externally on foliage (Needham et al. 1928, Hering 1951, Feeny 1970, Southwood 1973, Mattson et al. 1988, Cornell 1989, Hespeneide 1991). While feeding within a leaf, leaf-mining larvae may be: 1) protected from attack by natural enemies (e.g., predators, parasites, and pathogens), 2) buffered from variation in the physical environment (e.g., hygrothermal balance, ultra-violet radiation, or dislodgment by rain and wind), and 3) able to avoid plant defenses occurring in tissue layers not encountered while feeding (e.g., structural or chemical). Any or all of these characteristics could result in an individual selective advantage for leaf-mining insects in comparison to external-feeding insects via higher survival rates or higher fecundity. In addition, because of the potentially higher abundance or greater persistence of species resulting from any selective advantage conferred by leaf mining, lineages possessing leaf-mining larvae may have had greater opportunity to encounter new resources, host plants, and habitats and consequently may have experienced an “adaptive radiation”. We define adaptive radiation as greater diversification in lineages possessing a new or innovative trait in comparison to closely related taxa lacking this trait (Mitter et al. 1988, Wiegmann et al. 1993).

Does the trait – possessing larvae that feed as leaf miners for all or part of the larval period – confer some ecological advantage relative to taxa whose larvae feed externally on foliage? If so, what is the adaptive significance of leaf mining, and has this advantage resulted in more rapid diversification or an “adaptive radiation” in leaf-mining taxa? We report experiments and an analysis of the published literature in an effort to determine if leaf mining confers any potential advantage relative

to feeding externally on foliage. We also examine the phylogeny and compare the species richness of leaf-mining taxa and their sister groups, using the method of multiple sister-group comparisons outlined by Mitter et al. (1988), Farrell et al. (1991) and Wiegmann et al. (1993) to determine if leaf mining could be considered an innovation that has led to diversification and adaptive radiation.

The adaptive significance of the leaf-mining habit

Escape from natural enemies

The hypothesis that the leaf mine might protect its occupant from attack is part of the broader notion that concealed feeding strategies such as leaf mining, leaf galling, leaf rolling, leaf tying, and web spinning serve as defenses against natural enemies (Needham et al. 1928, Hering 1951, Cornell 1983, Heads and Lawton 1985, Price et al. 1987, Price and Pschorn-Walcher 1988). An alternative hypothesis proposed by Cornell (1989) is that endophagous insects might be more susceptible to pathogens because the humid internal leaf environment would be conducive to persistence of pathogens. However, evidence that leaf miners suffer more or less from natural enemies in comparison to external-feeding folivores is sparse. Four kinds of evidence can be adduced to address this question: 1) data on the species richness of the parasitoid communities associated with mining versus external-feeding insects, 2) data on the success of biological control programs for leaf-mining versus external-feeding insects, 3) reports in the literature on the incidence of attack by predators, parasites, and pathogens on leaf-mining and external-feeding folivores, and 4) comparisons of life tables of leaf-mining and external-feeding folivores.

1. Parasitoid species richness

In comparisons of the species richness of insect parasitoid communities associated with insect hosts in different feeding guilds (e.g., leaf mining, gall forming, stem boring, external feeding, etc.), leaf-mining insects rank as having the most species of parasitoids per host of any feeding guild (Askew 1980, Hawkins and Lawton 1987, Hawkins 1988, Pschorn-Walcher and Altenhofer 1989, Hawkins 1990, 1994, Hawkins et al. 1992) a fact that would appear to be inconsistent with the hypothesis that the leaf mine serves as a defense against parasitism. However, Pschorn-Walcher and Altenhofer (1989) report that the taxonomic composition of the parasitoid community attacking leaf-mining sawflies (Hymenoptera: Tenthredinidae) is radically different from that attacking external-feeding sawflies. Leaf-mining sawflies have lost many species of Ichneumonidae and Braconidae parasitic on their external

feeding ancestors, but have recruited many species of Chacidoidea, particularly Eulophidae, that also parasitize leaf-mining Lepidoptera. Perhaps the initial escape from parasitism associated with the acquisition of the leaf-mining habit was sufficient impetus to selectively reinforce the tendency to feed by mining (Pschorn-Walcher and Altenhofer 1989).

2. Biological control of leaf-mining and external-feeding insects

The success rates of biological control programs based on the release of parasitoids are higher for leaf-mining than for external-feeding insects (Gross 1991, Hawkins and Gross 1992, Hawkins 1993, 1994). Hawkins and Gross (1992) interpret this pattern to indicate that leaf miners are more susceptible to parasitism and are more likely to suffer population limitation by parasitoids than external-feeding insects. However, these data represent the release of exotic parasitoids to control their associated exotic pests, so they may not accurately estimate the impact of parasitoids on native insects.

3. Incidence of attack by pathogens, predators, and parasites

We conducted a survey of the published literature to determine if the incidence of attack by natural enemies (e.g., parasites, predators, and pathogens) is similar among leaf-mining and external-feeding folivores. The survey consisted of examining and classifying every paper concerning foliage eating insects, in the orders that have leaf-mining taxa, published during the years 1981-1991 in 11 journals. We restricted our survey to orders with leaf-mining taxa to remove any potential confounding effects of phylogeny on our comparison of rates of natural enemy attack on leaf miners and external feeders. The insects examined in each paper were classified by feeding guild into leaf miners, gall formers, and external feeders. Each paper reporting evidence that the insect(s) examined were attacked by diseases (e.g., fungal, viral, bacterial, protozoan and nematode infections), parasites (e.g., insect parasitoids), and predators (e.g., insects, arachnids, or vertebrates) was counted, as were the papers that reported no evidence of natural enemy attack. Most papers reporting no evidence of attack by natural enemies simply did not address that subject. However, in order to normalize the incidence of natural enemy attack for differences in research effort among feeding guilds, we express incidence as the number of papers reporting attack by a particular group of natural enemies on a guild divided by the total number of papers published on that guild. Estimates of incidence normalized for the number of species encountered in each guild were calculated as the number of papers to be attacked by a particular group of natural enemies divided by the total number of species encountered in a guild. These data should not be interpreted as

measures of what fraction of species in each guild actually experience mortality from pathogens, predators, or parasitoids. This survey merely chronicles how commonly different classes of natural enemies are reported in the literature to affect each feeding guild. We contend that this provides an indication, albeit a very indirect one, of the impact of these categories of natural enemies on insects in each feeding guild. To some unknown extent, these data reflect the interests of researchers, but such a criticism could be lodged against the data compiled from any literature survey.

Based on the literature survey described above, it appears that the incidence of disease among leaf-mining and gall-forming insects is considerably less than that for external-feeding folivores (Table 1). Of the 303 papers on leaf-mining insects encountered in the survey, only 2.6% reported disease while for external-feeding folivores 22.0% of the 2537 papers report disease. A similarly large discrepancy is observed if the incidence of disease is reported as a percentage of species. It is unlikely that the absence of reports of disease among leaf miners is simply because no one has looked, even though such a concealed feeding habit might appear a priori to be inimical to the commonly observed per os mechanism of infection (by ingestion) for insect pathogens. Leaf miners in the genera *Liriomyza*, *Agromyza* (Diptera: Agromyzidae), and *Phyllonorycter* (Lepidoptera: Gracillariidae) are major pests of agricultural

Table 1. Incidence of reports of attack by natural enemies on leaf-mining, gall-forming, and external-feeding folivores in the literature. Based on a survey of the literature published in the following journals between 1981-1991: Ecology, Journal of Animal Ecology, Environmental Entomology, Ecological Entomology, Journal of Invertebrate Pathology, Canadian Entomologist, Journal of Economic Entomology, Annals of the Entomological Society of America, Entomophaga, Bulletin of Entomological Research, and Entomologia Experimentalis et Applicata.

	Percent of papers				p
	Leaf miners	Gall formers	External feeders	G*	
Disease	2.6	0	22.0	89.36	<1 x 10 ⁻⁷
Parasitism	40.3	50.0	18.8	32.52	<1 x 10 ⁻⁷
Predation	9.6	9.1	6.4	1.96	0.1611
Total number of papers	303	22	2537		
	Percent of species				p
	Leaf miners	Gall formers	External feeders	G*	
Disease	5.3	0	25.0	16.76	0.00004
Parasitism	58.7	46.7	36.4	6.12	0.0133
Predation	30.7	20.0	17.0	2.99	0.0837
Number of species	73	15	324		

* Results of a G-test for independence between guild and incidence of mortality based on leaf miners and external feeders only for each source of mortality. The G statistic is computed using Williams correction (Sokal and Rohlf 1995).

and orchard crops and comprise a significant portion of the literature on leaf-mining insects. If these leaf miners had pathogens, it is likely that they would have been detected and developed for biological control purposes. Several papers mention the possibility that some portion of the larval mortality that is usually attributed to "other causes", which can represent a substantial proportion for leaf-mining insects (Auerbach et al. 1995), may have been caused by disease (Faeth 1985, 1986, Faeth and Bultman 1986, Auerbach 1991). However, none of these authors have provided evidence to confirm the presence of entomopathogenic organisms in the leaf miners they study.

Since most disease organisms that infect phytophagous insects are transmitted by contact and eventual ingestion, we hypothesize that the leaf mine shields larvae from contact with pathogens reducing the probability of infection and the rate of disease transmission. Therefore, those leaf-mining taxa that are concealed within a single leaf mine for the entire larval and pupal phases would be least likely to suffer disease infections, while those taxa that move among multiple leaf mines or pupate outside the leaf mine should be more susceptible to disease.

Closer examination of the papers that do report disease among leaf-mining insects supports this hypothesis. First, in a broad survey of all the literature on leaf-mining insects, we could only find reference to disease in seven species of leaf miners: the potato moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae), the cotton leafperforator, *Bucculatrix thurberiella* (Lepidoptera: Bucculatricidae), the coffee leaf miner, *Perileuoptera coffeella* (Lepidoptera: Lyonetiidae), the spruce needleminer, *Epinotia tedella* (Lepidoptera: Tortricidae), the fir needleminer, *Epinotia fraternana* (Lepidoptera: Tortricidae), the cocoon leaf miner, *Promecotheca papuana* (Coleoptera: Chrysomelidae), and the celery leafminer, *Liriomyza trifolii* (Diptera: Agromyzidae). Furthermore, only four of these seven species have indigenous associated pathogens. The pathogens reported to infect the cotton leafperforator, the coffee leaf miner, and the celery leafminer were isolated from other external feeding insect species and tested for cross-infectivity against these leaf miners. Of these species of leaf miners, *P. papuana* passes the entire larval and pupal phases in a single leaf mine, but the fungus that infects *P. papuana*, *Synnematium jonesii*, has only been reported to infect adults (Prior and Perry 1980). For the celery leaf miner, attempts have been made to apply entomopathogenic nematodes isolated and reared from other insect species to reduce infestation levels on chrysanthemum in greenhouses (Harris et al. 1990, Hara et al. 1993, Le Beck et al. 1993). However, infection only occurs when infectious stage nematodes enter the leaf mine via the oviposition scar, or via tears in the leaf mine. For the remaining species, infection occurs either prior to initiating the leaf mine

or after the larva departs the leaf mine. For the coffee leaf miner, a fungal pathogen applied to leaves can contaminate eggs so that upon hatching larvae ingest fungal spores when chewing through the egg chorion and leaf surface to initiate a mine (Villacorta 1983). For the cotton leafperforator, application of *Bacillus thuringiensis* or the nuclear polyhedrosis virus of the external feeding *Autographa californica* leads to infection, but only in 4th and 5th instar larvae and pupae. The cotton leafperforator quits the leaf mine and feeds externally beginning in the 4th instar (Bell and Romine 1982). Both the spruce and the fir needleminer are affected by indigenous pathogens, but do not develop their pathogenic infections until completing the mining phase and leaving the leaf mine to seek pupation sites (Munster-Swendsen 1989, 1991). Infection of larvae of the potato moth with its granulosis virus occurs via ingestion upon hatching from the egg which is laid in the soil, during movement between the soil and leaves and among leaves, and possibly while in the mine (Reed 1971). Larvae can move from leaf to leaf initiating new mines, and larvae also can feed on pairs of leaves webbed together and on tubers, thus encountering additional opportunities to contact virus particles in the environment. Based on experiments where leaves were sprayed with virus in water, Reed (1971) suggests that virus particles may enter the leaf mine via the stomata and infect larvae while in the mine. However, the entry of pathogens into the leaf mine via the stomata is highly unlikely since neither the moisture gradient, stomatal diameters, nor the surface tension of water would permit entry of water via the stomata by diffusion, capillarity, or droplet impact. The direction of water movement from the stomata is from the plant to the environment, so transport of pathogens into the leaf mine via the stomata is physically improbable.

It is possible that leaf miners suffer mortality or reductions in fecundity because of toxins produced by endophytic fungi (Carroll 1988, Clay 1988). Mortality caused by endophytic fungi has been reported for some gall-forming insects (Lasota et al. 1983, Taper et al. 1986, Taper and Case 1987, Sitch et al. 1988, Butin 1992, Fernandes and Price 1992, Wilson and Carroll 1994, Wilson 1995); however, reports for leaf-mining insects are limited. Since endophytic fungi invade the epidermal cells of plants directly or are transmitted from plant to plant via the seed, leaf mining or gall forming may offer no escape from the antibiotic effects of endophytic fungi. However, in studies on the effects of endophytic fungi on the leaf-mining moth, *Phyllonorycter* sp., on *Quercus grisea*, *Q. gambelii*, and their hybrids, Gaylord et al. (1996) report a diverse community of endophytic fungi, but a predominance of positive associations between *Phyllonorycter* abundance and endophyte frequency. Furthermore, Preszler et al. (1996) show that survival of *Phyllonorycter* larvae is higher in the presence of the fungal endophyte *Gnomia*

cerastis possibly because *Gnomia cerastis* is negatively associated with parasitism by insect parasitoids. Via examination of natural and experimentally manipulated populations, Faeth and Hammon (1997a, b) show that the endophytes of *Quercus emoryi* are positively associated with the abundance of a species of leaf-mining moth in the genus *Cameraria*. They also contend that the leaf mine affects the endophytes by increasing the susceptibility of the leaf to endophyte colonization, but that the endophyte does not alter foliage chemistry and has no effect on the survival of *Cameraria* (Faeth and Hammon 1997b, c). These studies show that leaf-mining insects may be less likely to be adversely affected by fungal endophytes than are gall-forming and external-feeding insects.

The incidence of reports of parasitism and predation among leaf-mining and leaf-galling insects is equal to or greater than among external-feeding folivores (Table 1). For parasitism, 40.3% of papers on leaf miners report attack while for external-feeding folivores only 18.8% report attack. For predation, 9.6% of papers on leaf miners report attack while for external-feeding folivores only 6.4% report attack. Even when expressed as a percentage of species, reports of parasitism and predation of leaf-mining and leaf-galling insects are still more common than for external-feeding insects (Table 1). The higher incidence of reports of parasitism and predation on leaf miners could reflect differences in the detectability of parasitism and predation between guilds, rather than actual differences in attack rates. Evidence of parasitism and predation in the form of emergence holes or tears is often obvious on the sedentary, persistent leaf mine, but may be harder to locate for mobile external-feeding insects. However, the identity of the parasite or predator attacking an external-feeding insect is usually reported, while for leaf-mining insects it is often unknown. This suggests more research effort has been allocated to detecting parasites and predators of external-feeding insects which might offset any differences in their detectability between guilds. In any event, estimates of the incidence of reports of attack by diseases, parasites, and predators in the literature are very indirect measures of the impact of natural enemies, the evidence presented here is inconsistent with the hypothesis that the leaf mine serves to protect its occupant from attack by parasites and predators, but consistent with the hypothesis that the leaf mine serves to protect its occupant from pathogens.

4. Life table comparisons of leaf-mining and external-feeding folivores

Data on the rates of mortality caused by natural enemies of leaf miners in comparison to external feeders or other guilds are very limited. In a comparison of life tables of leaf miners and gall formers, Cornell (1990) concluded that overall rates of mortality were equal. However, he did not report the contribution of natural

enemies. Hawkins (1993, 1994) reports the maximum observed rates of parasitism for a number of leaf-mining and external-feeding insect species, and concludes that leaf miners suffer greater rates of parasitism than external feeders. This result is inconsistent with the hypothesis that the leaf mine protects its occupant from parasitism, but the data are based on the maximum observed rates of parasitism and therefore may be sensitive to outliers. Cornell and Hawkins (1995) examined survival distributions and sources of mortality for several different feeding guilds and concluded that external-feeding insects suffer greater mortality from natural enemies than do internal-feeding insects. This pattern is consistent with the hypothesis that the leaf mine is an effective defense against natural enemies. However, Cornell and Hawkins (1995) combined all endophagous guilds including mining, galling, stem-boring, fruit-feeding, and bark- or trunk-feeding insects. Therefore, it is impossible to tell from their analysis if leaf miners suffer less mortality from natural enemies than do external-feeding folivores.

We examined published estimates of the impact of pathogens, parasites and predators on folivorous insects in different feeding guilds using the literature from the survey conducted by Cornell and Hawkins (1995). By using Cornell and Hawkins' (1995) survey, we remove any bias in the selection of studies on our part. However, the sample of studies will still reflect any potential unconscious bias on Cornell and Hawkins' (1995) part. Cornell and Hawkins (1995) report 86 studies of 84 species of insects that are either: 1) external feeders, 2) leaf tiers, leaf rollers, or web spinners, 3) mixed feeders (e.g., feeding in buds in early instars and externally on leaves in late instars), or 4) leaf miners. For 47 of these 86 studies, quantitative estimates for the effects of various mortality agents are reported in multiple decrement life tables in the literature cited by Cornell and Hawkins (1995). These 47 studies include two species of external-feeding folivores for which estimates of mortality were published by two different authors. We include these as separate estimates because their removal or inclusion has no effect on the outcome of the analyses. From these life tables we calculated the impacts of pathogens, parasitoids and predators on juvenile (larval + pupal) and on larval mortality for parasitism and predation only. Most studies included life tables for multiple generations (mean (\pm se) = 5.87 ± 0.73). For studies that did not report the average proportion of mortality caused by pathogens, parasites, and predators per generation, we calculated weighted averages, weighting by the number in each cohort.

To test the hypothesis that juvenile and larval mortality caused by pathogens, parasites, and predators is independent of guild membership, we performed an analysis of variance on the angularly transformed proportions of mortality and their sum. We detected sig-

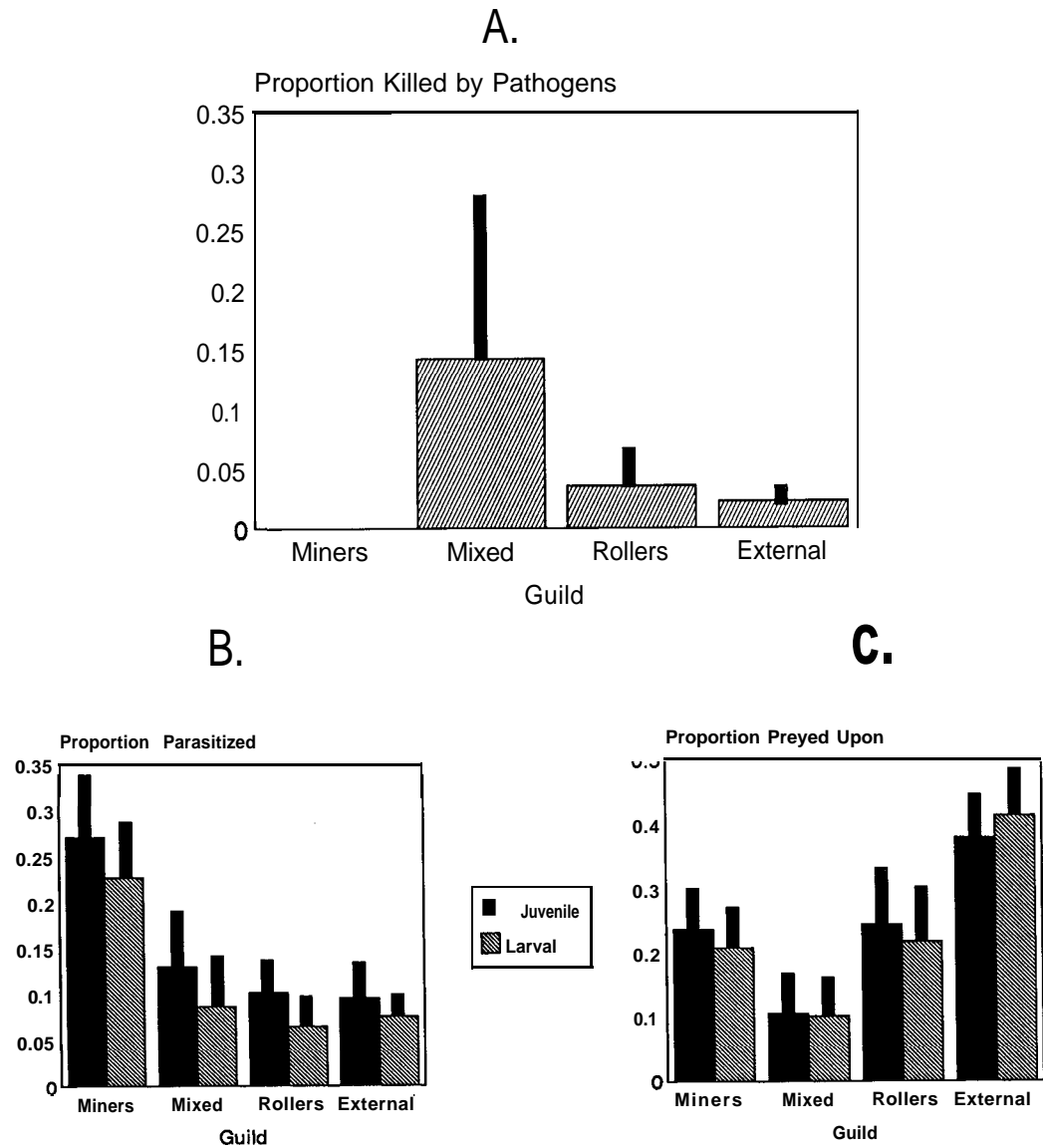


Fig. 1. Average rates of mortality caused by pathogens (A), parasites (B), and predators (C) on juvenile and larval stages of folivorous insects in four feeding guilds. Sample sizes for each guild are: leaf miners ($n = 15$), mixed feeders ($n = 5$), leaf rollers ($n = 9$), external feeders ($n = 18$). Narrow vertical bars depict one standard error.

nificant differences between guilds in juvenile mortality caused by pathogens ($F_{3,43} = 2.99$, $p = 0.0413$), in juvenile parasitism ($F_{3,41} = 2.85$, $p = 0.0489$), and in larval parasitism ($F_{3,43} = 4.4$, $p = 0.025$) with the leaf-mining guild having the lowest rate of disease induced mortality (none) and the highest rates of parasitism (Fig. 1). While there were no statistically significant differences in predation on juveniles or larvae among guilds ($F_{3,41} = 1.38$, $p = 0.262$, $F_{3,43} = 2.02$, $p = 0.126$, respectively), predation rates tended to be higher among external feeders than among leaf miners (Fig. 1).

However, when the mortality caused by pathogens, parasites, and predators are combined no differences were detected among guilds (juvenile mortality: $F_{3,41} = 0.343$, $p = 0.794$, larval mortality [parasitism and predation only]: $F_{3,43} = 1.78$, $p = 0.165$). These results indicate that leaf-mining insects are subjected to overall rates of mortality from natural enemies similar to those experienced by external feeders. However, leaf miners tend to suffer higher rates of parasitism and lower rates of predation and disease than external feeders (Fig. 1).

Buffering from the physical environment

The leaf mine could also serve to protect its occupant from the physical environment. Such protection could arise in several ways: 1) by providing a microenvironment where evaporative demand is low so that hygrothermal balance is readily maintained, 2) by filtering UV radiation thus protecting the larvae from its direct toxicogenic and mutagenic effects or by preventing UV stimulated photochemical reactions which alter the nutritional quality of the foliage, and 3) by preventing dislodgment caused by rain and wind and the ensuing risk of predation while attempting to relocate host plant foliage.

1. Maintenance of hygrothermal balance

The leaf mine has been suggested to provide a humid environment in which the risk of larval death due to desiccation is low in comparison to external-feeding folivores (Southwood 1973, Strong et al. 1984, Gaston et al. 1992). However, we found no evidence in the literature that the leaf mine protects its occupant from desiccation. In an examination of the distribution of gall-forming insects along environmental gradients, Fernandes and Price (1988, 1992) observe that the species richness and survival rates of gall formers is highest in arid environments. They further suggest that the protection provided by the gall against desiccation and the effects of endophytic fungi may be the primary selective advantage allowing gall formers to occupy arid environments. A similar study of the pattern of species richness and survival rates of leaf miners along an aridity gradient has not yet been conducted.

To determine if the leaf mine provides a sheltered microenvironment which prevents desiccation leading to improved larval performance, we attempted to: 1) compare internal leaf-mine to external leaf-surface temperatures, and 2) compare the growth and survival of leaf-mining larvae in intact leaf mines and in leaf mines where the protected microenvironment was breached. We used *Cameraria hamadryadella* (Lepidoptera: Gracillariidae) a leaf miner that attacks *Quercus alba* and *Q. macrocarpa* as our test species. *C. hamadryadella* is a bivoltine, outbreak species that forms blotch mines during the summer that are located just under the upper epidermis of the leaf (see Hinckley 1972, Connor 1991, Connor and Beck 1993, Connor et al. 1994 for details of its life history and population dynamics).

To determine if the thermal environment of leaf mines might reduce desiccation, we used a dual probe thermometer (YSI Model 2100) to measure internal leaf-mine and leaf-surface temperatures. Measurements were taken at thermal noon (three hours past solar noon) and at midnight. The results indicate that internal leaf-mine temperatures are more than 1.0°C lower than external leaf-surface temperatures (Fig. 2, day $t = -8.50$, $df = 19$, $p < 0.001$; night $t = -4.09$, $df = 9$,

$p < 0.003$). This result suggests that for *C. hamadryadella*, the leaf mine provides an environment buffered from the more extreme temperatures on the external leaf surface, and with reduced evaporative demand and possibly a lower chance of larval desiccation. For other species that feed during the autumn or winter, the leaf mine might also provide a warmer environment by producing a "greenhouse effect", thus permitting these species to be more active. However, no evidence is yet available to support this hypothesis.

To determine if the leaf mine microenvironment leads to higher growth and survival rates of larvae by preventing desiccation, we compared the mass gained and survival of leaf-mining larvae in intact mines and in experimentally opened mines, presumably in equilibrium with the external environment. The experimental protocol involved locating early instar first or second generation mines on several individual *Q. macrocarpa* trees and protecting them from natural enemy attack by placing mesh bags over leaves with individual mines. When mines were approximately 20 mm² in area they were assigned at random to one of four experimental treatments. The treatments consisted of either making an incision at least 1 cm in length or not, and for each of these groups applying beeswax to half of the mines to either re-seal the leaf mine or control for a "beeswax effect". Ten replicates of each treatment were established on each of three trees. The incision was made to allow the microenvironment of the leaf mine to come into equilibrium with the external environment, thus removing any differences between the internal and external environment and any benefit derived by the larva from the internal microenvironment. In the second generation experiments, the beeswax treatments were not applied, so that 40 repli-

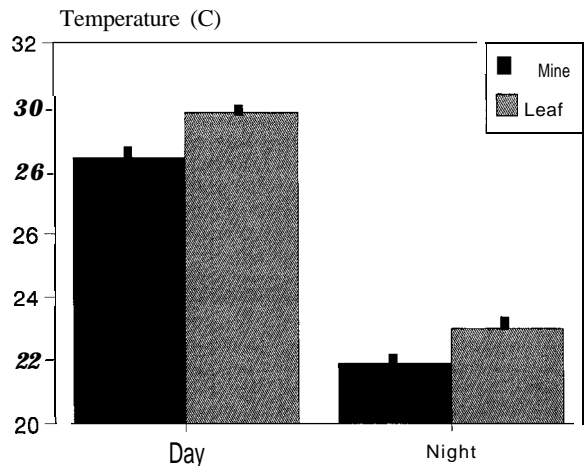
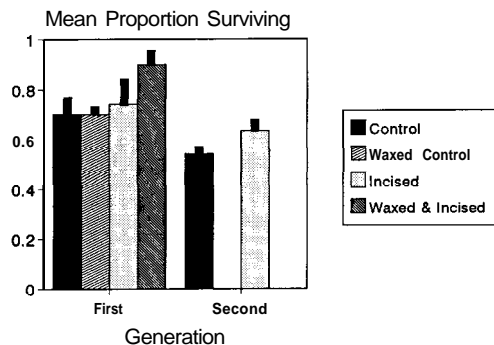


Fig. 2. Comparison of average day and night internal leaf-mine and external leaf-surface temperatures in mines of *Cameraria hamadryadella* on leaves of *Quercus alba*. Narrow vertical bars depict one standard error. Note that the y-axis begins at 20°C.

A.



B.

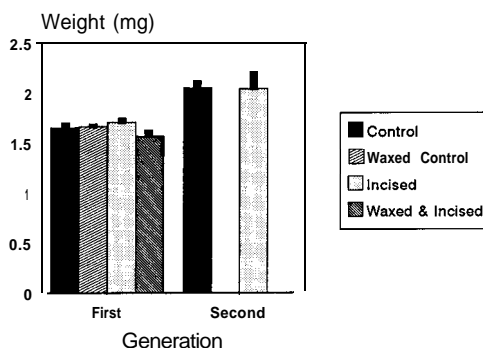


Fig. 3. Average survival rates (A) and pupal weights (B) of *Cameraria hamadryadella* on *Quercus macrocarpa* in intact leaf mines (control) and leaf mines open to the environment (incised) during 42 the first and second generation (over-wintering). The waxed treatments were applied in the first generation to control for an effect of incision per se. Narrow vertical bars depict one standard error.

cates of the un-manipulated control and 20 replicates of the incision treatment were applied on each of three trees. In the second generation experiments, leaves were allowed to abscise naturally and overwintered on the forest floor. Examination of the data on survival for each generation separately via logistic regression indicated that treatment had no effect on the probability of survival (Fig. 3; first generation, change in deviance $\chi^2 = 5.92$, $df = 3$, $p > 0.11$; second generation, change in deviance $\chi^2 = 1.68$, $df = 1$, $p > 0.19$). Examination of the data on fresh mass of pupae and dry mass of adults reveals no differences between treatments in either generation (Fig. 3, ANOVA: $F_{3,86} = 2.23$, $p > 0.05$ for first generation pupal mass, $F_{3,38} = 0.72$, $p > 0.5$ for first generation adult mass, and $F_{1,58} = 0.001$, $p > 0.9$ for second generation pupal mass). Since second generation larvae overwinter in the leaf mine, these data also argue that the leaf mine does not function to protect overwintering larvae from desiccation. These experiments sug-

gest that for *Cameraria hamadryadella*, the effect of the microenvironment of the leaf mine on maintenance of hygrothermal balance does not lead to improved growth and survival. However, the experiments we performed may not have completely removed the favorable microenvironment of the leaf mine. This is because the leaf-mine epidermis would continue to shield the larva from air movement hence decreasing the evaporative demand experienced by the larva (Willmer 1982). Perhaps experiments in which the performance of external-feeding larvae protected from wind-induced evaporation is compared to that of unprotected larvae would provide a stronger test of the role of the leaf mine in maintenance of hygrothermal balance.

2. UV radiation

The leaf mine could also offer protection to its occupant from the direct effects of UV radiation (Berenbaum 1978, Dowd 1993) or by preventing UV stimulated photochemical reactions which might alter the nutritional quality of the foliage reducing growth, survival, or reproduction (Berenbaum 1978, 1991. Caldwell et al. 1989, Larson et al. 1990, Trumble et al. 1991, Yazawa et al. 1992, Diawara et al. 1993, McCloud and Berenbaum 1994). Protection from UV radiation might be particularly important for leaf miners that feed on the upper leaf surface since, unlike other foliage eating insects, they cannot seek shelter on the lower leaf surface during the day and feed on the upper leaf surface at night.

To determine if the leaf mine might protect its occupant from UV radiation, we collected leaf mines of four different species, excised the upper leaf epidermis (which constitutes the roof of the leaf mine), and measured its ability to absorb UV radiation using a UV spectroscopy photometer. We used two species of lepidopteran (*Cameraria hamadryadella* and *Tischeria quercitella*) and one coleopteran leaf miner (*Baliosus neruosus*) that mine the leaves of *Quercus alba*, and one species of lepidopteran leaf miner (*Parectopa robinella*) that mines the leaflets of *Robinia pseudoacacia*. Each species of leaf miner produces a blotch mine under the epidermis on the upper leaf surface. Each sample was individually mounted on paper blanks (15 x 75 mm) over an opening that allowed 100% transmission of the UV light. A cuvette holder was fabricated to hold the leaf-mine samples, and the absorbance of each sample was determined for wavelengths between 280-400 nm at increments of 20 nm (the UV-B and UV-A ranges) using a Hitachi Model 100-40 UV-Visible spectrophotometer. The results indicate that at all wavelengths, greater than 95% of the UV radiation is absorbed by the leaf-mine epidermis for all species (Fig. 4). This suggests that the leaf mine may protect larvae from the direct effects of UV radiation, and to the extent that the tissue layers consumed are insulated from the photochemical effects of UV, may also protect leaf-mining larvae from the indirect effects of UV radiation.

The extent to which the leaf mine protects other leaf-mining species from UV radiation is not known. However, there is considerable variation in the opacity of the leaf mine surface to the penetration of visible light among leaf-mining species, and hence the possibility for similar variation in the penetration of UV light. For example, the leaf mines of *C. hamadryadella* are translucent and it is usually possible to see the larvae within the leaf mine even without back-lighting the leaf. On the other hand, the mines of *Baliosus nervosus* and other coleopteran leaf miners on *Quercus* are opaque and it is difficult to view the larvae in the mine even while back-lighting the leaf. A broader survey of the ability of the leaf mine to absorb UV radiation and clever manipulations of UV radiation will be necessary to determine the overall importance of the leaf mine in protecting its occupant from the harmful effects of UV radiation.

3. Secure attachment

Small, external feeding larvae may be at risk of being dislodged from their host plant by rain or wind, and if dislodged suffer high mortality while attempting to relocate a suitable host plant (Southwood 1973). Most leaf-mining larvae are restricted to a single leaf mine and are securely attached to their host plant as long as the leaf containing the leaf mine remains attached. Therefore, the leaf-mining habit could result in lower mortality rates for leaf miners because of dislodgment in comparison to similar-sized, external-feeding folivores. However, instances in which dislodgment has been reported as a source of mortality for external-feeding insects are limited, and few studies provide quantitative estimates of the mortality caused by dislodgment (Dixon 1970, Dixon and McKay 1970, Miskimen 1970, Shade et al. 1970, Dempster 1971, Helgesen and Hay-

nes 1972, Singh et al. 1974, Gross et al. 1976, Wanatabe 1976, Maelzer 1977, Hayes 1981, Raupp and Denno 1983, Feeny et al. 1985, Moran et al. 1987). This may arise in part because losses of mobile, early instar, external-feeding larvae from dislodgment are treated either as death due to other causes, dispersal, or disappearance. Therefore, it is difficult to compare the impact of dislodgment for external-feeding larvae to mortality due to leaf abscission for leaf miners. In any event, early leaf abscission usually results in the death of any leaf-mining larvae contained within the leaf, and can result in substantial rates of mortality (Faeth et al. 1981a; see Stiling and Simberloff 1989 for review of data on effects of leaf abscission on leaf-mining insects). Numerous studies on a variety of species including Diptera, Lepidoptera, and Coleoptera all show that leaf miners experience mortality from early leaf abscission (Pritchard and James 1984, Potter 1985, Stiling and Simberloff 1989, Preszler and Price 1993, Connor et al. 1994, Auerbach et al. 1995; but see Kahn and Cornell 1989). So, rather than resulting in lower mortality rates from dislodgment, leaf mining may actually lead to higher rates of mortality because of early leaf abscission.

Avoidance of plant defenses

Because leaf-mining insects tunnel inside leaves and feed on specific tissue layers, they may be able to avoid plant defenses, both structural and chemical, that occur on the exterior of the leaf or in tissue layers not encountered while feeding (Feeny 1970, Cornell 1989). On the other hand, Mattson et al. (1988) suggest that insects intimately associated with their host plants, like leaf miners and gall formers, should encounter a greater array of chemical defenses and therefore suffer higher mortality and lower growth rates than less intimately associated external-feeding insects. If either of these hypotheses is true, then the feeding efficiencies of leaf miners in comparison to external-feeding folivores should be either; 1) higher if Feeny (1970) and Cornell's (1989) hypothesis is correct or 2) lower if Mattson et al.'s (1988) hypothesis is correct. Alternatively, the leaf mine could represent a feeding strategy to create an enclosed, concealed feeding cavity, that only secondarily functions to avoid plant defenses or layers of foliage tissue with low nutritional quality.

Two kinds of evidence could be used to test the hypothesis that leaf mining is a means of avoiding plant defenses and acquiring the most nutritious tissue layers: 1) evidence that plant defenses are concentrated in tissue layers avoided by leaf miners, and 2) direct evidence that leaf-mining larvae have higher feeding efficiencies than do external feeders.

Structural plant defenses such as spines and trichomes are restricted to the plant surface (Levin 1973,

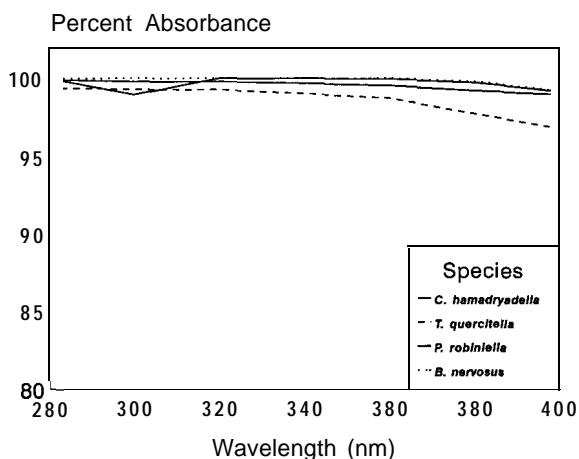


Fig. 4. Average absorbance of UV-radiation by leaf-mine epidermis in four species of leaf-mining insects. The largest standard error for any species or wavelength was 0.97%. Note that the y-axis begins at 80% absorbance.

Rathcke and Poole 1975, Stipanovic 1983, Southwood 1986), and endophagous insects such as leaf miners and leaf galls would only encounter these defenses at oviposition and emergence. Therefore, endophagy may be an effective means of avoiding most of the detrimental effects of such structural defenses. However, Ezcurra et al. (1987) report that the abundance of leaf miners was lower on *Arbutus xalapensis* (Ericaceae) with dense foliar pubescence than on individuals with little pubescence which suggests that structural defenses may be a deterrent to oviposition in some species of leaf-mining insects.

A limited literature exists on the microdistribution of chemical defenses. This literature suggests that chemical defenses are concentrated in the cuticle and epidermis, although they are by no means restricted to these tissue layers (Akai 1959, McKey 1974, 1979, Hermann 1976, Cornell 1989). Some studies of specific leaf miners demonstrate that they restrict their feeding to particular tissue layers. Mazanec (1983) has shown that the jarrah leaf miner, *Perthida glyphoba* (Lepidoptera: Incurvariidae), restricts its feeding to tissue layers with low concentrations of chemical defenses. Kimmerer and Potter (1987) have demonstrated that the holly leaf miner, *Phytomyza ilicicola* (Diptera: Agromyzidae), restricts its feeding to the palisade mesophyll where physical barriers to consumption such as fibers, vascular bundles, and calcium oxalate crystals are absent, but where the nutritional quality of the foliage is highest in terms of protein and moisture content and where defensive chemicals (saponins) are also in highest concentration. Kimmerer and Potter's (1987) study points out that selective feeding by leaf miners on specific tissue layers may involve a variety of factors including the nutritional quality of the tissue layers, as well as chemical and physical defenses. However, Faeth et al. (1981 b) report that only 1 of 18 species of leaf miners reported from oaks in north Florida avoid feeding in tannin-containing tissue layers.

To test the hypothesis that leaf mining is a strategy to avoid plant defenses or layers of foliage tissue with low nutritional quality, we attempted to obtain estimates of the feeding efficiency of leaf-mining insects and contrast these efficiencies with published estimates for external-feeding folivores. We found no published estimates of feeding efficiencies for leaf-mining insects. Therefore, we measured the feeding efficiencies for three species of leaf-mining insects. We estimated the efficiency of conversion of ingested food (ECI, Waldbauer 1968) for three species of leaf miners that feed on *Quercus alba*: *Tischeria quercitella*, *T. purinosella*, and *T. badiella* (Lepidoptera: Tischeriidae). These species were selected because of their availability and because the excrement of species in the genus *Tischeria* can be easily separated from the leaf mine. ECI is the ratio of mass gained to mass consumed and is usually expressed as a percentage. Since the eggs of

Tischeria are only 0.4 mm in length and flattened, we estimated the mass gained as the total mass of the larva using a Cahn electro-balance, not correcting for larval mass at hatching. To the extent that we do not correct for the mass of larvae at hatching, this estimate represents a slight overestimate of the mass gained. However, the estimated larval mass may not include the entire mass of shed exuviae because larvae may consume shed exuviae, but eject shed head capsules from the leaf mine. This could lead to a slight underestimate of mass gained. To estimate the mass of leaf tissue consumed, we used a technique combining gravimetric and planimetric measurements similar to Schroeder (1984). We carefully excised the leaf mine and measured its area with a LICOR LI-3000 leaf area meter and its mass on an electronic balance. We also excised a similar area of un-mined leaf tissue adjacent to each mine and measured its area and mass using the same technique. All measurements of mass were made using larvae and leaf tissue that had been dried for 24 h at 60°C. We calculated the mass consumed as follows:

$$\frac{\text{mass of un-mined leaf} - \text{mass of leaf mine}}{\text{area of un-mined leaf} - \text{area of leaf mine}} \times \text{area of leaf mine}.$$

We then calculated ECI as 100 x (mass gained/mass consumed). Most published estimates of feeding efficiencies for external-feeding folivorous Lepidoptera use late instar larvae. To obtain estimates comparable to published values, we used only leaf mines that were at least 1 cm² in area to eliminate early instar leaf miners. Although there are problems and biases involved in estimating feeding efficiencies using gravimetric procedures (Slansky 1985, Bowers et al. 1991, Barbehenn and Keddie 1992, Raubenheimer and Simpson 1992), our desire was to compare feeding efficiencies of leaf miners and external-feeding folivores estimated using similar methods.

The feeding efficiencies of the three species of *Tischeria* feeding on *Quercus alba* were twice that reported for Lepidoptera feeding externally on oak, and approximately 1.5 times that of tree feeding lepidopterous folivores in general (Fig. 5 and Table 2). Analysis of variance indicated that the feeding efficiencies of leaf miners were significantly higher than for either group of external-feeding Lepidoptera ($F_{2,14} = 5.29, p < 0.02$). Because our sample of feeding efficiencies for leaf-mining Lepidoptera is smaller and from a taxonomically more homogenous set of species than our sample for external-feeding Lepidoptera, our conclusions remain tentative. However, our result is consistent with Feeny (1970) and Cornell's (1989) hypothesis that leaf miners are able to avoid plant defenses, but could also result from leaf miners feeding selectively on the most nutritious layers of foliage tissue.

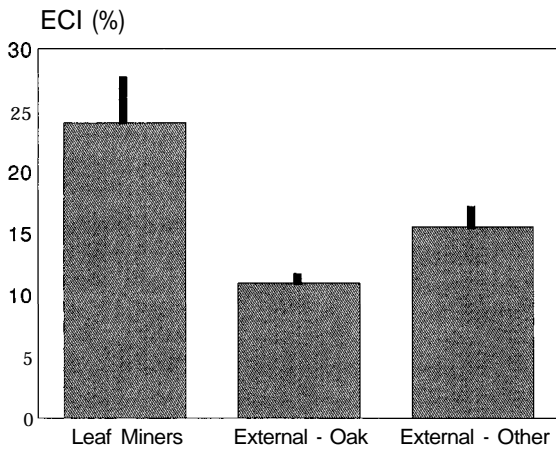


Fig. 5. Average feeding efficiencies (ECI) of leaf-mining *Tischeria* (Lepidoptera: Tischeriidae) on *Quercus alba* ($n = 3$ species) in comparison to those of external feeding Lepidoptera on oak ($n = 6$ species) and on other host tree genera ($n = 11$ species). Narrow vertical bars depict one standard error. Feeding efficiencies are significantly higher for leaf miners than for external-feeding insects.

Other differences between leaf-mining and external-feeding folivores

Leaf-mining insects could be at a selective advantage or disadvantage in comparison to external-feeding folivores because of aspects on their behavior, biology, or ecology that arise as secondary consequences of adopting the leaf-mining habit. For example, leaf miners are sedentary with larval development usually being restricted to a single leaf. As a result, leaf-mining larvae must cope with the competitive environment into which they are placed by the ovipositing female. Do leaf miners suffer more or less from inter- or intraspecific competition than do external-feeding folivores? Denno et al. (1995) suggest that external feeders experience less interspecific competition than do internal-feeding insects. However, the only study of leaf-mining insects chronicled in Denno et al.'s (1995) review indicated that among the five species of leaf-mining insects examined, none were reported to compete. Denno et al. (1995) also report that leaf-mining insects suffer more from inter-guild competition than do external feeding insects. Again, the data to support this contention are sparse. However, anecdotal evidence indicates that 3-8% of mortality among some species of leaf-mining insects is caused by leaf-mining larvae being inadvertently consumed by larger external-feeding lepidopterous larvae (Auerbach 1991, Connor and Beck 1993). On the other hand, leaf-mining insects may suffer considerable mortality as a result of intraspecific competition (Auerbach et al. 1995) but an assessment of whether leaf miners suffer more from intraspecific competition than do external-feeding folivores has not yet been attempted.

Body size is another trait that may differ between leaf-mining and external-feeding folivores. Most leaf

miners are small as larvae and adults, with body lengths usually no greater than 6-8 mm. While the eggs of leaf miners can be very small (<0.2 mm), small body size may place limits on the fecundity of leaf-mining insects in comparison to larger external-feeding folivores. However, no assessment of differences in body size or fecundity between leaf-mining and external-feeding folivores has been attempted. Such a comparison would be most informative if restricted to closely related mining and external-feeding taxa.

Another difference between leaf-mining and external-feeding folivores that emerged from the comparison of life tables described earlier was that egg mortality differed among feeding guilds ($F_{3,35} = 3.98$, $p = 0.0153$). Although few life table studies of leaf miners report data on egg mortality because eggs are often small and inconspicuous, leaf miners suffer less egg mortality than do external feeders (Fig. 6). The higher egg mortality rates of external-feeding than leaf-mining insects may arise because external-feeding species lay eggs in clusters more often than do leaf miners.

The evolution of the leaf-mining habit

Approximately 10,000 described species of leaf miners occur in at least 51 families of holometabolous insects in the orders Coleoptera, Diptera, Lepidoptera, and Hymenoptera (Needham et al. 1928, Hering 1951, Richards and Davies 1977, Bickel 1982, Brown 1982, Lawrence 1982, Evans 1987, Faeth 1991, Hespeneheide 1991). The leaf-mining habit originated at least by the early Cretaceous (Labandeira et al. 1994).

The leaf-mining habit is most widely distributed in the Lepidoptera occurring in 34 families (Needham et al. 1928, Hering 1951, Powell 1980, Munroe 1982, Stehr 1987, Powell et al. 1997; following the classification in Nielsen (1989); Table 3). Leaf mining probably evolved only once in the Lepidoptera first appearing in the primitive Heterobathmiidae (Kristensen and Nielsen 1983). The most primitive Lepidoptera, the Micropterigidae, were probably associated with moist forest habitats consuming spores and pollen of bryophytes and some angiosperms (Powell 1980, Gibbs 1983). Most non-Ditrysiac families and many of the primitive Ditrysiac families are exclusively leaf mining or at least primitively leaf mining (Powell et al. 1997). Given the pattern of distribution of leaf mining within the Lepidoptera and the tendency toward external feeding in the derived Ditrysiac and the macrolepidoptera, leaf mining should be considered a primitive trait within the Lepidoptera. The fact that the external-feeding macrolepidoptera are half again as species rich as the rest of the order, also suggests that the loss of leaf mining rather than its acquisition may represent an innovation leading to an adaptive radiation within the

Table 2. Feeding efficiencies of leaf-mining and external-feeding folivores.

Insect	Host plant	Leaf-miners ECI	SE	N	Reference
<i>Tischeria purinosella</i>	<i>Quercus alba</i>	33.0	2.5	31	this study
<i>Tischeria quercitella</i>	<i>Quercus alba</i>	16.2	2.1	10	
<i>Tischeria badiella</i>	<i>Quercus alba</i>	22.9	3.9	2	
External feeding folivores on oak					
<i>Anisota senatoria</i>	<i>Quercus alba</i>	9.9	0.2	20	Lawson et al. (1982)
	<i>Q. bicolor</i>	12.1	0.2	20	
	<i>Q. macrocarpa</i>	14.2	0.4	20	
	<i>Q. palustris</i>	11.3	0.3	20	
	<i>Q. rubra</i>	10.5	0.4	20	
<i>Alsophila pometaria</i>	<i>Q. velutina</i>	8.1	0.3	20	Lawson et al. (1984)
	<i>Quercus alba</i>	9.9	0.3	20	
	<i>Q. bicolor</i>	12.3	0.1	20	
	<i>Q. macrocarpa</i>	12.9	0.3	20	
	<i>Q. rubra</i>	10.1	0.6	20	
<i>Lymantria dispar</i>	<i>Q. velutina</i>	10.7	0.4	20	Shepard and Friedman (1990)
	<i>Q. macrocarpa</i>	12.5	0.4	10	
	<i>Q. macrocarpa</i>	8.4	0.4	9	
External feeding folivores on non-oak hosts					
<i>Malacosoma americanum</i>	<i>Prunus serotina</i>	16.6	0.4	13	Futuyma and Wasserman (1981)
<i>Malacosoma disstria</i>	<i>Prunus serotina</i>	16.6	0.5	15	Smith et al. (1986)
	<i>Nyssa aquatica</i>	5.9		175	
<i>Malacosoma neustria</i>	<i>Salix viminalis</i>	14.0			Waldbauer (1968)
<i>Spodoptera eridania</i>	<i>Sorbus americana</i>	10.7	0.6		Scriber (1982)
	<i>Betula papyrifera</i>	14.8	0.5		
	<i>Prunus serotina</i>	16.9	1.1		
<i>Hyalophora cecropia</i>	<i>Prunus serotina</i>	23.8	0.7	12	Grabstein and Scriber (1982)
	<i>Prunus serotina</i>	19.5	0.5	15	
	<i>Betula papyrifera</i>	17.3	0.5	8	
<i>Operophtera brumata</i>	<i>Acer pseudoplatanus</i>	20.4	1.7	27	Warrington (1985)
<i>Erannis defoliaria</i>		26.1	2.3	9	
<i>Agriopsis aurantaria</i>		18.9	1.7	17	
<i>Alsophila aescularia</i>		17.9	2.1	6	
<i>Bombyx mori</i>	<i>Morus alba</i>	21.0			Waldbauer (1968)
<i>Hyphantria cunea</i>	<i>Acer negundo</i>	12.0			Waldbauer (1968)

Lepidoptera. Powell et al. (1997) suggest that the limitations on size and risk of mortality from leaf abscission among leaf-mining Lepidoptera may have selected for external feeding, and resulted in several parallel transitions from primitively mining to derived external feeding lineages. Many of these transitions involved intermediate steps where larvae feed as miners in only one or two instars, and then feed externally either concealed in cases, or exposed on the leaf surface.

In the Coleoptera, leaf mining occurs in six families (Needham et al. 1928, Hering 1951, Lawrence 1982, 1991, Mitter et al. 1988; following the classification of the Coleoptera in Crowson (1981) and Kuschel (1995); Table 3), but is most extensively developed in the Chrysomelidae. Leaf mining evolved at least four times in the superfamilies Buprestoidea, Chrysomeloidea, Cucujoidea, and Curculionoidea. However, it is likely that

within these superfamilies leaf mining evolved more than once. In the Buprestoidea, Cucujoidea, and Curculionoidea, leaf mining appears to have evolved from ancestral groups that were concealed feeders in wood and under bark, or in seeds and fruits, respectively. However, in the Chrysomeloidea leaf mining arose from external leaf-feeding ancestors. In contrast to the Lepidoptera, leaf mining in the Coleoptera is a derived trait in each mining lineage. While the mining habit is most extensively developed in the chrysomelid subfamily Cassidinae within the tribe Hispini (slightly less than 3000 species), the non-mining putative sister group of the Hispini, the Cassidini are approximately equally species rich (Jolivet 1988). This argues against an hypothesis of adaptive radiation upon acquisition of the leaf-mining habit by the Hispini. The phylogenetic relationships and species richness of mining lineages and

their sister groups are not available for the remainder of the Coleoptera. However, the other mining groups are much less species rich than the Hispini.

In the Diptera, leaf mining occurs in nine families (Bickel 1982, Foote 1991; following the classification of Steyskal (1974); Table 3) but is most extensively developed in the Agromyzidae and the Ephydriidae. Leaf mining evolved at least six times in the Diptera, and also appears to be a derived trait (Spencer 1990). Phytophagous external-feeding larvae are rare within the Diptera (Southwood 1973). Leaf mining appears to have evolved from a variety of ancestral feeding habits including; internal root, stem and bark feeding (Agromyzidae), saprophagous (Tipulidae), and coprophagous (Scatophagidae) ancestors (Mitter et al. 1988). The Agromyzidae are the largest predominantly leaf-mining lineage in the Diptera with approximately 2500 species. The Clusiidae (200 species) are usually considered the sister group of the Agromyzidae and feed under the bark of trees (Steyskal 1974, Mitter et al. 1988). However, in this comparison the evolution of the trait "leaf mining" is confounded with the acquisition of the trait "leaf feeding," and if one adopts Mitter et al.'s (1988) definition of phytophagy, which restricts phytophagy to feeding on live plant tissues, leaf mining is even confounded with the acquisition of the phytophagous habit. Since Mitter et al. (1988) have demonstrated that the acquisition of the phytophagous habit by insects represents an adaptive radiation, it is impossible to infer that the higher species richness of the Agromyzidae than in the Clusiidae supports the idea that leaf mining per se has led to an adaptive radiation.

Fewer than 100 described species of leaf-mining insects occur in the Hymenoptera all in the superfamily Tenthredinoidea (D. Smith, pers. comm.). Leaf mining probably evolved six times in the Hymenoptera, and as

in the Diptera and Coleoptera is a derived trait (Pschorn-Walcher and Altenhofer 1989). Leaf mining arose from ancestral taxa that were external feeding folivores in the Hymenoptera, as they did in the Chrysomelidae (Coleoptera). The phylogeny and species richness of leaf-mining sawflies and their sister groups are better known than in any of the other orders with leaf-mining taxa (Smith 1971, 1976a, b, 1979, 1990). A comparison of the species richness of those leaf-mining sawfly lineages with their hypothesized sister groups shows that leaf-mining lineages are always less species rich than their external-feeding sister groups (Table 4). Furthermore, in the Acordulecerinae, Phylacteophaginae, and Sterictophorinae where the sister groups of the leaf-mining lineages have not been postulated, the mining lineages are comprised of no more than three species, so are unlikely to substantially exceed the species richness of their sister groups.

If the acquisition of leaf mining represents an innovation that led to an adaptive radiation relative to feeding externally on leaves, then leaf-mining lineages should contain more species than their external-feeding sister groups, since sister groups are by definition the same age (Mitter et al. 1988, Wiegmann et al. 1993). There are three lineages of leaf-mining Hymenoptera that have evolved from external-feeding ancestors for which sister groups have been hypothesized and for which we could obtain data on species richness (Smith 1971, 1976a, b, 1979, 1990) (Table 4). Either data on species richness are not available, or the sister group relationships are unknown for other leaf-mining lineages that have evolved from external-feeding ancestors. Based on the available data, leaf mining does not represent an innovation that has led to an adaptive radiation in contrast to feeding externally on leaves. This result is consistent with the evidence available for the other four lineages of leaf miners that have evolved from external feeding ancestors. Because of their low species richness, the leaf-mining sawfly subfamilies Phylacteophaginae, Acordulecerinae, and Sterictophorinae cannot be substantially more species rich than any proposed sister group. Based on approximate species richness and the tentative assignment of the Cassidini as the sister group, the leaf-mining Hispini also are no more species rich than their sister group. In combination with the repeated loss of leaf mining from primitively mining lepidopteran lineages (Powell et al. 1997) and the significantly higher species richness of external-feeding than leaf-mining Lepidoptera, then the overall tendency is for the leaf-mining lineages to be less species rich than their external-feeding sister groups.

For those leaf-mining lineages that have evolved from non-phytophagous or non-folivorous ancestors, we can offer no conclusions regarding whether leaf mining is an innovation leading to an adaptive radiation, or an evolutionary dead end. This is partly because the phylogeny and species richness of these

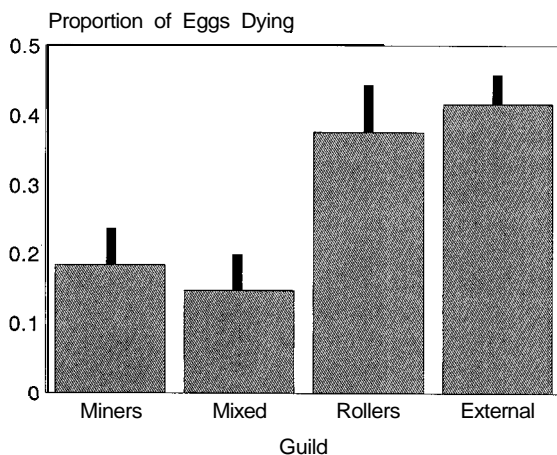


Fig. 6. Average egg mortality for folivorous insects in four feeding guilds. Sample sizes for each feeding guild are: leaf miners ($n = 9$), mixed feeders ($n = 5$), leaf rollers ($n = 9$), external feeders ($n = 16$). Narrow vertical bars depict one standard error.

Table 3. Families of insects with at least one leaf-mining member.

Order Coleoptera	Order Diptera	Order Lepidoptera	Order Hymenoptera
Suborder Polyphaga	Suborder Cyclorrhapha	Suborder Heterobathmiina	Suborder Symphyta
Superfamily Buprestoidea Buprestidae	Superfamily Tephritoidea Tephritidae	Superfamily Heterobathmioidea Heterobathmiidae	Superfamily Tenthredinoidea Pergidae Argidae Tenthredinidae
Superfamily Cucujoidea Nitidulidae	Superfamily Drosophiloidea Ephydriidae Drosophilidae Agromyzidae	Suborder Dacnonypha Superfamily Eriocranioidea Eriocraniidae Acanthopteroctetidae	
Superfamily Chrysomeloidea Chrysomelidae	Superfamily Muscoidea Scathophagidae Anthomyiidae	Suborder Monotrysia Superfamily Nepticuloidea Nepticulidae Opotegidae	
Superfamily Curculionoidea Belidae Attelabidae Curculionidae	Suborder Nematocera Superfamily Tipuloidea Tipulidae Superfamily Culicoidea Chironomidae Superfamily Phoroidea Phoridae	Superfamily Tischerioidea Tischeriidae Superfamily Palaephatoidea Palaephatidae Superfamily Incurvarioidea Incurvariidae Prodoxidae Adelidae Heliozelidae Suborder Ditrysia Superfamily Gracillaroidea Gracillariidae Bucculatricidae Douglassiidae Roeslerstammidae Superfamily Gelechioidea Oecophoridae Elachistidae Coleophoridae Momphidae Cosmopterygidae Scythrididae Gelechiidae Superfamily Copromorpoidea Carposinidae Epermeniidae Glyphipterigidae Superfamily Yponomeutoidea Acrolepiidae Argyrestidae Yponomeutidae Heliodinidae Ochsenheimeriidae Lyonetiidae Superfamily Tortricoided Tortricidae Superfamily Pyraloidea Pyralidae Superfamily Pterophoroided Pterophoridae	

lineages and their sister groups have not yet been hypothesized and compiled. However, substantial analytical problems confront testing this hypothesis for

these lineages. This is because as outlined earlier for the comparison of the leaf-mining Agromyzidae and its sister group the Clusiidae, the acquisition of leaf mining

Table 4. Leaf-mining lineages arising from external-feeding ancestors with hypothesized sister groups and their species richness.

Taxa	Number of leaf-mining species	Total species-richness	Sister group	Sister group species-richness
Order Coleoptera				
Superfamily Chrysomeloidea				
Family Chrysomelidae				
Subfamily Cassidinae				
Tribe Hispini	< 3000	3000	Cassidini	3000
Order Hymenoptera				
Superfamily Tenthredinoidea				
Family Pergidae		> 400		
Subfamily Acordulecerinae	1	200	Phylacteophaginae*	4
Subfamily Phylacteophaginae	3	4	Acordulecerinae*	200
Family Argidae		> 650		
Subfamily Sterictophorinae	1	275	Erigeniae*	35
Family Tenthredinidae				
Subfamily Nematinae				
Tribe Pseudodineurini	20	20	Nematini	> 600
Subfamily Heterarthrinae				
Tribe Heterarthrini	15	15	Caliroini	35
Tribe Fenusini	54	54	Blennocampini	300

* These taxa represent the hypothesized sister groups of the respective subfamilies with leaf-mining members, not the specific sister groups of the leaf-mining clades.

in these lineages is confounded with a more fundamental shift in food resources from the ancestral condition of either feeding on other plant tissues, concealed or exposed, or feeding as saprophages or coprophages. Therefore, application of the methods of Mitter et al. (1988) and Wiegmann et al. (1993) could not separate that component of diversification within lineages associated with the acquisition of leaf mining from that associated with the acquisition of the folivory. Perhaps a method to separate the effects of individual traits in comparisons involving coincident acquisition of multiple traits (e.g., phytophagy, folivory, and leaf mining) could be developed based on cross lineage comparisons of species richness in instances where traits are acquired individually and in groups. For example, the magnitude of the effect of acquiring phytophagy on species richness could be estimated for all lineages, and compared to that for lineages that became folivorous, endophagous, or leaf mining. If the species richness in lineages that have acquired phytophagy, folivory, and leaf mining is consistently greater than in lineages that have only acquired phytophagy and folivory, then one might conclude that the acquisition of leaf mining from non-phytophagous ancestors represents an innovation that led to an adaptive radiation.

Synthesis

We have documented several advantages that leaf-mining insects have relative to insects that feed externally on foliage. Leaf-mining insects escape from mortality or reductions in fecundity associated with diseases more

than do external-feeding insects. Few leaf-mining insects have been reported to have diseases, and those that do become infected outside the leaf mine. Leaf-mining insects also have much higher feeding efficiencies than do external-feeding insects which suggests that they are able to either avoid plant defenses or at least consume more nutritious parts of the leaf than do external-feeding insects. The leaf mine may protect the larvae from the harmful effects of UV radiation, both the direct and indirect effects mediated by photochemical changes in plant chemistry. The leaf mine buffers temperature in the microenvironment which may reduce water loss and prevent desiccation by providing an environment with reduced evaporative demand, or may allow periods of activity to extend into times of day or seasons which would not be possible if feeding on the leaf exterior. Each of these advantages may contribute to the maintenance of leaf mining as a viable means of feeding upon foliage, and also may have played some role in the origin of the leaf-mining habit (Frumhoff and Reeve 1994). However, given the multiple evolutionary routes to leaf mining from ancestral taxa that feed as saprophages, coprophages, or concealed or exposed phytophages on leaves or other plant parts, there is likely to be no single cause for the origin of leaf mining.

On the other hand, we have also documented several disadvantages to the leaf-mining habit. The loss of mobility in the larval stage associated with leaf mining makes it more difficult to escape from parasites and has resulted in leaf-mining insects having the most species of associated parasitoids of any insect feeding guild, and to leaf miners suffering higher mortality rates from

parasites than external-feeding folivores. Mortality associated with the premature abscission of leaves also appears to be more frequent among leaf miners than is mortality associated with dislodgment among external feeding insects. Finally, leaf-mining insects also have small body sizes and hence probably lower fecundity than external-feeding folivores because of the constraints imposed by feeding within leaves.

We also document lower egg mortality rates among leaf-mining than external-feeding folivores which cannot be construed as a direct consequence of the leaf mine per se. Other differences between leaf-mining and external-feeding taxa not associated with the function of the leaf mine per se may also provide advantages or disadvantages to leaf miners. To the extent that these differences remain unknown and unaccounted, we risk misinterpreting the adaptive significance of the leaf-mining habit.

Our results parallel those reported by Price et al. (1987) in examining the adaptive nature of insect galls. Price et al. (1987) conclude that insect galls protect their occupants from desiccation and provide superior nutrition resulting in higher feeding efficiencies. Price and Pschorn-Walcher (1988) also suggest that galling nematine sawflies have lower mortality rates from parasitoids than external feeding relatives. While these authors do not report any disadvantages to the galling habit, Williams and Whitham (1986) report that galling aphids do suffer considerable mortality from early leaf abscission, and evidence collected by Lasota et al. (1983) Taper et al. (1986), Taper and Case (1987), Sitch et al. (1988) Butin (1992), Fernandes and Price (1992) Wilson and Carroll (1994) and Wilson (1995) suggest that gall-forming insects might be particularly susceptible to the effects of fungal endophytes.

The leaf-mining habit is ancient, yet continues to be acquired and lost by several lineages of phytophagous insects. Given its persistence, one could argue that leaf mining is under certain circumstances a feasible lifestyle for the consumption of foliage. However, the fact that leaf-mining lineages are less species rich than their external-feeding sister groups argues that, from an evolutionary perspective, the disadvantages of leaf mining outweigh the advantages.

Leaf mining, or more generally concealed feeding, may represent the most feasible means of surmounting the barriers to consuming foliage for lineages that are primitively concealed feeders on other plant parts, or are saprophagous or coprophagous. By using its host plant to provide both food and shelter, the leaf-mining insect overcomes the hurdles of desiccation and attachment more easily than external-feeding folivores which may require more complex morphological, physiological, and behavioral adaptations to surmount these hurdles (Southwood 1973). However, the initial advantages of leaf mining apparently can become liabilities.

Price et al. (1987) and Price and Roininen (1993) have used the terms "adaptive" and "adaptive radiation" to apply to the habit of forming galls. While there certainly exists a variety of gall morphologies and the ability of gall-forming insects to modify plant growth is uniquely characteristic of gall formers among insects, it remains unclear whether or not this is sufficient reason to conclude that such an innovation has led to an adaptive radiation as defined by Mitter et al. (1988). No analysis of the phylogeny and species richness of gall-forming lineages and their sister groups has yet been performed, although gall forming is certainly a derived trait in the Tenthredinidae (P. W. Price pers. comm.). However, a broader analysis of whether endophagy has led to an adaptive radiation which included some gall-forming lineages concluded that the evolution of endophagous feeding habits does not lead to adaptive radiations (C. Mitter pers. comm.).

Perhaps first a determination of the innovations that allow insects to consume foliage must be made rather than narrowly focusing the question upon particular concealed feeding modes such as leaf mining or gall forming. The evolutionary leap of insects to feed upon leaves per se may represent an innovation and an adaptive radiation. The status of concealed versus exposed feeding modes as further innovations leading to adaptive radiations or as liabilities and evolutionary dead ends might then be more clearly addressed.

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References

- Akai, S. 1959. Histology of defense in plants - In: Horsfall, J. and Cowling, E. B. (eds), *The diseased plant. Plant pathology: an advanced treatise*, Vol. 1. Academic Press, New York, pp. 391-434.
- Askew, R. R. 1980. The diversity of insect communities in leaf-mines and plant galls. - *J. Anim. Ecol.* 49: 817-829.
- Auerbach, M. J. 1991. Relative impact of interactions within and between trophic levels during an insect outbreak. - *Ecology* 72: 1599-1608.
- Connor, E. F. and Mopper, S. 1995. Minor miners and major miners: the population dynamics of leaf-mining insects. - In: Cappuccino, N. and Price, P. W. (eds), *Population dynamics: novel approaches and synthesis*. Academic Press, New York, pp. 83-110.
- Barbehenn, R. V. and Keddie, A. 1992. Gut contents in molting lepidopteran larvae: a source of error in nutritional studies. - *Entomol. Exp. Appl.* 62: 87-91.

- Bell, M. R. and Romine, C. L. 1982. Cotton leafperforator (Lepidoptera: Lyonetiidae): effect of two microbial insecticides on field populations. - *J. Econ. Entomol.* 75: 1140-1142.
- Berenbaum, M. R. 1978. Toxicity of a furanocoumarin to armyworms: a case of biosynthetic escape from insect herbivores. - *Science* 201: 532-534.
- 1991. Coumarins - In: Rosenthal, G. A. and Berenbaum, M. R. (eds), *Herbivores, their interactions with secondary plant metabolites*, 2nd ed. Academic Press, San Diego, CA, pp. 221-249.
- Bickel, D. J. 1982. Diptera In: Parker, S. P. (ed.), *Synopsis and classification of living organisms*, Vol. 2. McGraw-Hill, New York, pp. 563-599.
- Bowers, M. D., Stamp, N. E. and Fajer, E. D. 1991. Factors affecting calculation of nutritional indices for foliage-fed insects: an experimental approach. - *Entomol. Exp. Appl.* 61: 101-116.
- Brown, W. L., Jr. 1982. Hymenoptera - In: Parker, S. P. (ed.), *Synopsis and classification of living organisms*, Vol. 2. McGraw-Hill, New York. pp. 652-680.
- Butin, H. 1992. Effect of endophytic fungi from oak (*Quercus robur* L.) on mortality of leaf inhabiting gall insects. - *Eur. J. For. Pathol.* 22: 237-246.
- Caldwell, M., Teramura, A. H. and Tevini, M. 1989. The changing solar ultraviolet climate and the ecological consequences for higher plants. - *Trends Ecol. Evol.* 4: 363-367.
- Carroll, G. 1988. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. - *Ecology* 69: 2 - 9 .
- Clay, K. 1988. Fungal endophytes of grasses: a defensive mutualism between plants and fungi. - *Ecology* 69: 10-16.
- Connor, E. F. 1991. Colonization, survival, and the causes of mortality of *Cameraria hamadryadella* (Lepidoptera: Gracillariidae) on four species of host plants. - *Ecol. Entomol.* 16: 315-322.
- and Beck, M. W. 1993. Density-related mortality in *Cameraria hamadryadella* (Lepidoptera: Gracillariidae) at epidemic and endemic densities. *Oikos* 66: 515-525.
- Adams-Manson, R. J., Carr, T. G. and Beck, M. W. 1994. The effects of host plant phenology on the demography and population dynamics of the-leaf-mining moth *Cameraria hamadryadella* (Lepidoptera: Gracillariidae). *Ecol. Entomol.* 19:111-120.
- Cornell, H. V. 1983. The secondary chemistry and complex morphology of galls formed by the Cynipinae (Hymenoptera): why and how? - *Am. Midl. Nat.* 110: 225-234.
- 1989. Endophage-ectophage ratios and plant defense. - *Evol. Ecol.* 3: 64476.
- 1990. Survivorship, life history, and concealment: a comparison of leaf miners and gall formers. - *Am. Nat.* 136: 581-597.
- and Hawkins, B. A. 1995. Survival patterns and mortality sources of herbivorous insects: some demographic trends. - *Am. Nat.* 145: 563-593.
- Crowson, R. A. 1981. *The biology of Coleoptera*. - Academic Press, New York.
- Dempster, J. P. 1971. Some observations on a population of the small copper butterfly, *Lycaena phlaeas* (Linnaeus) (Lep., Lycaenidae) *Entomol. Gaz.* 22: 199-204.
- Denno, R. F., McClure, M. S. and Ott, J. R. 1995. Interspecific interactions in phytophagous insects: competition re-examined and resurrected. *Annu. Rev. Entomol.* 40: 297-331.
- Diawara, M. M., Trumble, J. T., White, K. K., Carson, W. G. and Martinez, L. A. 1993. Toxicity of linear furanocoumarins to *Spodoptera exigua*: evidence for antagonistic interactions. *J. Chem. Ecol.* 19: 2473-2484.
- Dixon, A. F. G. 1970. Quality and availability of food for a sycamore aphid population - In: Watson, A. (ed.), *Animal populations in relation to their food resources*. Blackwell, Oxford, pp. 271-286.
- and McKay, S. 1970. Aggregation in the sycamore aphid *Drepanosiphum platanoides* (Schr.) (Hemiptera: Aphididae) and its relevance to the regulation of population growth. - *J. Anim. Ecol.* 39: 439-454.
- Dowd, P. F. 1993. Toxicity of the fungal metabolite griseofulvin to *Helicoverpa zea* and *Spodoptera frugiperda*. - *Entomol. Exp. Appl.* 69: 5-11.
- Engelbrecht, L., Orban, U. and Heese, W. 1969. Leaf-miner caterpillars and cytokinins in the "green islands" of autumn leaves. - *Nature* 223: 319-321.
- Evans, H. E. 1987. Order Hymenoptera - In: Stehr, F. W. (ed.), *Immature insects*. Kendall Hunt, Dubuque, IA, USA, pp. 597-710.
- Ezcurra, E., Gomez, J. C. and Becerra, J. 1987. Diverging patterns of host use by phytophagous insects in relation to leaf pubescence in *Arbutus xalapensis* (Ericaceae). - *Oecologia* 72: 479-480.
- Faeth, S. H. 1985. Host leaf selection by leaf miners: interactions among three trophic levels. - *Ecology* 66: 870-875.
- 1986. Indirect interactions between temporally separated herbivores mediated by the host plant. - *Ecology* 67: 479-494.
- 1991. Novel aspects of host tree resistance to leafminers In: Baranchikov, Y. N., Mattson, W. J. Hain, F. P. and Payne, T. L. (eds), *Forest insect guilds: patterns of interaction with host trees*. USDA Forest Service General Technical Report NE-153. pp. 219-239.
- and Bultman, T. L. 1986. Interacting effects of increased tannin levels on leaf-mining insects. - *Entomol. Exp. Appl.* 40: 297-300.
- and Hammon, K. E. 1997a. Fungal endophytes in oak trees. I. Long-term patterns of abundance and associations with leafminers. - *Ecology*, in press.
- and Hammon, K. E. 1997b. Fungal endophytes in oak trees. II. Experimental analyses of interactions with leafminers. - *Ecology*, in press.
- and Hammon, K. E. 1997c. Fungal endophytes and phytochemistry of oak foliage: Determinants of oviposition preference in leafminers? - *Oecologia*, in press.
- Connor, E. F. and Simberloff, D. 1981a. Early leaf abscission: a neglected source of mortality for folivores. - *Am. Nat.* 117: 409-415.
- , Mopper, S. and Simberloff, D. 1981b. Abundances and diversity of leaf-mining insects on three oak host species: effects of host-plant phenology and nitrogen content of leaves. *Oikos* 37: 238-251.
- Farrell, B., Mitter, C. and Dussourd, D. 1991. Macroevolution of plant defense: do latex/resin secretory canals spur diversification? *Am. Nat.* 138: 881-900.
- Feeny, P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. - *Ecology* 51: 565-581.
- Blau, W. S. and Kareiva, P. M. 1985. Larval growth and survivorship of the black swallowtail butterfly in central New York. - *Ecol. Monogr.* 55: 167-187.
- Fernandes, G. W. and Price, P. W. 1988. Biogeographical gradients in galling species richness. - *Oecologia* 76: 161-167.
- and Price, P. W. 1992. The adaptive significance of insect gall distribution: survivorship of species-inxeric and mesic habitats. - *Oecologia* 90: 14-20.
- Foote, B. A. 1991. Order Diptera In: Stehr, F. W. (ed.), *Immature insects*, Vol. 2. Kendall Hunt, Dubuque, IA, pp. 690-915.
- Frumhoff, P. C. and Reeve, H. K. 1994. Using phylogenies to test hypotheses of adaptation: a critique of some current proposals. - *Evolution* 48: 172-180.
- Futuyma, D. J. and Wasserman, S. S. 1981. Food plant specialization and feeding efficiency in the tent caterpillars *Malacosoma disstria* and *M. americanum*. - *Entomol. Exp. Appl.* 30: 106-110.
- Gaston, K. J., Reavey, D. and Valladares, G. R. 1992. Intimacy and fidelity: internal and external feeding by the British microlepidoptera. - *Ecol. Entomol.* 17: 86-88.

- Gaylord, E. S., Preszler, R. W. and Boecklen, W. J. 1996. Interactions between host plants, endophytic fungi, and a phytophagous insect in an oak (*Quercus grisea* x *Q. gambelii*) hybrid zone. *Oecologia* 105: 336-342.
- Gibbs, G. W. 1983. Evolution of the Micropterigidae (Lepidoptera) in the SW Pacific. - *GeoJournal* 7: 505-510.
- Grabstein, E. M. and Scriber, J. M. 1982. Host-plant utilization by *Hyalophora cercropia* as affected by prior feeding experience: - *Entomol. Exp. Appl.* 32: 262-268.
- Gross, H. R. Jr., Wiseman, B. R. and McMillian, W. W. 1976. Comparative suitability of whorl stages of sweet corn for establishment by larvae of the corn earworm. - *Environ. Entomol.* 5: 955-958.
- Gross, P. 1991. Influence of target pest feeding niche on success rates in classical biological control. *Environ. Entomol.* 20: 1217-1227.
- Hara, A. H., Kaya, H. K., Gaugler, R., LeBeck, L. M. and Mello, C. L. 1993. Entomopathogenic nematodes for biological control of the leafminer. *Liriomyza trifolii* (Diptera: Agromyzidae). *Entomophaga* 38: 359-369.
- Harris, M. A., Begley, J. W. and Warkentin, D. L. 1990. *Liriomyza trifolii* (Diptera: Agromyzidae) suppression with foliar applications of *Steinernema carpocapsae* (Rhabditia: Steinernematidae) and Abamectin. - *J. Econ. Entomol.* 83: 2380-2384.
- Hawkins, B. A. 1988. Species diversity in the third and fourth trophic levels: patterns and mechanisms. - *J. Anim. Ecol.* 57: 137-162.
- 1990. Global patterns of parasitoid assemblage size. - *J. Anim. Ecol.* 59: 57-72.
1993. Parasitoid species richness, host mortality, and biological control. - *Am. Nat.* 141: 634-641.
- 1994. Pattern and process in host-parasitoid interactions. - Cambridge Univ. Press, Cambridge.
- and Lawton, J. H. 1987. Species richness for parasitoids of British phytophagous insects. *Nature* 326:788-790.
- and Gross, P. 1992. Species richness and population limitation in insect parasitoid-host systems. *km. Nat.* 139: 417-423.
- , Shaw, M. R. and Askew, R. R. 1992. Relationships among assemblage size, host specialization and climatic variability in North American parasitoid communities. *Am. Nat.* 139: 58 -79.
- Hayes, J. L. 1981. The population ecology of a natural population of the pierid butterfly *Colias alexandra*. *Oecologia* 49: 1888200.
- Heads, P. A. and Lawton, J. H. 1985. Bracken, ants, and extrafloral nectaries. III. How insect herbivores avoid ant predation. *Ecol. Entomol.* 10: 29-42.
- Helgesen, R. G. and Haynes, D. L. 1972. Population dynamics of the cereal leaf beetle, *Oulema melanopus* (Coleoptera: Chrysomelidae): a model for age specific mortality. *Can. Entomol.* 104: 797-814.
- Hering, E. M. 1951. Biology of the leaf miners. - Junk, Gravenhage.
- Hermann, K. 1976. Flavonols and flavones in food plants: a review. *J. Food Technol.* II: 433-448.
- Hespenheide, H. A. 1991. Bionomics of leaf-mining insects. - *Annu. Rev. Entomol.* 36: 535-560.
- Hinckley, A. D. 1972. Comparative ecology of two leafminers on white oak. *Environ. Entomol.* 1: 258-261.
- Jolivet, P. 1988. Food habits and food selection of Chrysomelidae, bionomic and evolutionary perspective - In: Jolivet, P., Petitpierre, E. and Hsiao, T. H. (eds), *Biology of the Chrysomelidae*. Kluwer, Dordrecht, pp. 1-23.
- Kahn, D. M. and Cornell, H. V. 1989. Leafminers, early leaf abscission, and parasitoids: a tritrophic interaction. *Ecology* 70: 1219-1226.
- Kimmerer, T. W. and Potter, D. A. 1987. Nutritional quality of specific leaf tissues and selective feeding by a specialist leafminer. *Oecologia* 71: 548-551.
- Kristensen, N. P. and Nielsen, E. S. 1983. The Heterobthmia life history elucidated: Immature stages contradict assignment to suborder Zeugloptera (Insecta, Lepidoptera). - *Z. Zool. Syst. Evol.-forsch.* 21: 101-124.
- Kuschel, G. 1995. A phlogenetic classification of Curculionoidea to families and subfamilies. - *Mem. Entomol. Soc. Wash.* 14: 5 -33.
- Labandeira, C. C., Dilcher, D. L., Davis, D. R. and Wagner, D. L. 1994. Ninety-seven million years of angiosperm-insect association: Paleobiological insights into the meaning of coevolution. *Proc. Nat. Acad. Sci., USA* 91: 12278-12282.
- Larson, R. A., Garrison, W. J. and Carlson, R. W. 1990. Differential response of alpine and non-amine *Aquilegia* species to increased ultraviolet-B radiation.. *Plant Cell Environ.* 13: 983-987.
- Lasota, J. A., Waldvogel, M. G. and Shetlar, D. J. 1983. Fungus found in galls of *Adelges abietis* (L.) (Homoptera: Adelgidae): identification, within-tree distribution, and possible impact on insect survival. *Environ. Entomol.* 12: 2455246.
- Lawrence, J. F. 1982. Coleoptera. - In: Parker, S. P. (ed.), *Synopsis and classification of living organisms*, Vol. 2. McGraw-Hill, New York, pp.482-553.
- 1991. Order Coleoptera. In: Stehr, F. W. (ed.). *Immature insects*, Vol. 2. Kendall Hunt, Dubuque, IA, pp. 144-658.
- Lawson, D. L., Merritt, R. W., Klug, M. J. and Martin, J. S. 1982. The utilization of late season foliage by the orange striped oakworm, *Anisota senatoria*. *Entomol. Exp. Appl.* 32: 242-248.
- Merritt, R. W., Martin, M. M. and Kukor, J. J. 1984. The nutritional ecology of larvae of *Alsophila pomataria* and *Anisota senatoria* feeding on early- and late-season oak foliage. - *Entomol. Exp. Appl.* 35: 105 -114.
- LeBeck, L. M., Gaugler, R., Kaya, H. K., Hara, A. H. and Johnson, M. W. 1993. Host stage suitability of the leafminer *Liriomyza trifolii* (Diptera: Agromyzidae) to the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae). *J. Invertebr. Pathol.* 62: 58-63.
- Levin, D. A. 1973. The role of trichomes in plant defense. *Q. Rev. Biol.* 48: 3-15.
- Maelzer, D. A. 1977. The biology and main causes of changes in numbers of the rose aphid, *Macrosiphum rosae* (L.), on cultivated roses in South Australia. - *Aust. J. Zool.* 25: 2699284.
- Mattson, W. J., Lawrence, R. K., Haack, R. A., Herms, D. A. and Charles, P. 1988. Defensive strategies of woody plants against different insect-feeding guilds in relation to plant ecological strategies and intimacy of association with insects- In: Mattson, W. J., Leveux, J. and Bernard-Dagan, C. (eds), *Mechanisms of woody plant defenses against insects*. Springer-Verlag, New York, pp. 33-38.
- Mazanec, Z. 1983. The immature stages and life history of the jarrah leaf miner (*Perthida glyphoba*) Common (Lepidoptera: Incurvariidae). *J. Aust. Entomol. Soc.* 22: 101-108.
- McCloud, E. S. and Berenbaum, M. R. 1994. Stratospheric ozone depletion and plant-insect interactions: Effects of UVB radiation on foliage quality of *Citris jambhiri* for *Trichoplusia ni*. *J. Chem. Ecol.* 20: 525-539.
- McKey, D. 1974. Adaptive patterns in alkaloid physiology. *Am. Nat.* 108: 305-320.
1979. The distribution of secondary plant compounds within plants. In: Rosenthal, G. A. and Janzen, D. H. (eds), *Herbivores: their interaction with secondary plant metabolites*. Academic Press, New York, pp. 55-133.
- Miskimen, G. W. 1970. Population dynamics of the yellow sugarcane aphid, *Siphaflava*, in Puerto Rico, as affected by heavy rains. *Ann. Entomol. Soc. Am.* 63: 642-645.
- Mitter, C., Farrell, B. and Wiegmann, B. 1988. The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? - *Am. Nat.* 132: 107-128.
- Moran, V. C., Hoffman, J. H. and Basson, N. C. J. 1987. The effects of simulated rainfall on cochineal insects (Ho-

- moptera: Dactylopiidae): colony composition and survival on cactus cladodes. - *Ecol. Entomol.* 12: 51-60.
- Munroe, E. G. 1982. Lepidoptera. - In: Parker, S. P. (ed.), *Synopsis and classification of living organisms*, Vol. 2. McGraw-Hill, New York, pp. 612-651.
- Munster-Swendsen, M. 1989. Phenology and natural mortalities of the fir needleminer, *Epinotia fraternana* (Hw.) (Lepidoptera, Tortricidae). - *Entomol. Medd.* 57: 111-120.
- 1991. The effect of sublethal neogregarine infections in the spruce needleminer, *Epinotia tedella* (Lepidoptera: Tortricidae). - *Ecol. Entomol.* 16: 211-219.
- Needham, J. G., Frost, S. W. and Tothill, B. H. 1928. Leaf-mining insects. - Williams & Wilkins, Baltimore.
- Nielsen, E. S. 1989. Phylogeny of major lepidopteran groups. - In: Fernholm, B., Bremer, K. and Jornvall, H. (eds), *The hierarchy of life*. Elsevier, New York, pp. 281-294.
- Potter, D. A. 1985. Population regulation of the native holly leafminer, *Phytomyza ilicicola* Loew (Diptera: Agromyzidae), on American holly. - *Oecologia* 66: 499-505.
- Powell, J. A. 1980. Evolution of larval food preferences in the Lepidoptera. - *Ann. Rev. Entomol.* 25: 133-160.
- , Mitter, C. and Farrell, B. D. 1997. Evolution of larval feeding habits in Lepidoptera. - In: Kristensen, N. P. (ed.), *Handbook for zoology. Lepidoptera*, Vol. 1: Systematics and evolution. de Gruyter Publ., Berlin.
- Preszler, R. W. and Price, P. W. 1993. The influence of *Salix* leaf abscission of leaf-miner survival and life history. *Ecol. Entomol.* 18: 150-154.
- Gaylord, E. S. and Boecklen, W. J. 1996. Reduced parasitism of a leaf-mining moth on trees with high infection frequencies of an endophytic fungus. - *Oecologia* 108: 1599-166.
- Price, P. W. and Pschorn-Walcher, H. 1988. Are galling insects better protected against parasitoids than exposed feeders?: a test using tenthredinid sawflies. - *Ecol. Entomol.* 13: 195-205.
- and Roiminen, H. 1993. Adaptive radiation in gall induction. - In: Wagner, M. R. and Raffa, K. F. (eds), *Sawfly life history adaptations to woody plants*. Academic Press, San Diego, CA: pp. 229-257.
- Waring, G. L., Fernandes, G. W. 1987. Adaptive nature of insect galls. - *Environ. Entomol.* 16: 15-24.
- Prior, C. and Perry, C. H. 1980. Infection of *Promecotheca papuana* with *Synnematium jonesii*. - *J. Invertebr. Pathol.* 35: 14-19.
- Pritchard, I. M. and James, R. 1984. Leaf fall as a source of leaf-miner mortality. - *Oecologia* 64: 132-139.
- Pschorn-Walcher, H. and Altenhofer, E. 1989. The parasitoid community of leaf-mining sawflies (Fenusini and Heterarthrini): a comparative analysis. *Zool. Anz.* 222: 37-56.
- Rathcke, B. J. and Poole, R. W. 1975. Coevolutionary race continues: butterfly larval adaptation to plant trichomes. *Science* 187: 175-176.
- Raubenheimer, D. and Simpson, S. J. 1992. Analysis of covariance: an alternative to nutritional indices. *Entomol. Exp. Appl.* 62: 221-231.
- Raupp, M. J. and Denno, R. F. 1983. Leaf age as a predictor of herbivore distribution and abundance. - In: Denno, R. F. and McClure, M. S. (eds.), *Variable plants and herbivores in natural and managed systems*. Academic Press, New York, pp. 91-124.
- Reed, E. M. 1971. Factors affecting the status of a virus as a control agent for the potato moth (*Phthorimaea operculella*) (Zell.) (Lep., Gelechiidae). - *Bull. Entomol. Res.* 61: 207-222.
- Richards, O. W. and Davies, R. G. 1977. *Imms' general textbook of entomology*, Vol. 2. Classification and biology. 10th Ed. ~ Chapman and Hall, London.
- Schroeder, L. A. 1984. Comparison of gravimetry and planimetry in determining dry matter budgets for three species of phytophagous lepidopteran larvae. *Entomol. Exp. Appl.* 35: 255-261.
- Scriber, J. M. 1982. The behavior and nutritional physiology of southern armyworm as a function of plant species consumed in earlier instars. *Entomol. Exp. Appl.* 31: 359-369.
- Shade, R. E., Hansen, H. L. and Wilson, M. C. 1970. A partial life table of the cereal leaf beetle, *Oulema melanopus*, in northern Indiana. - *Ann. Entomol. Soc. Am.* 63: 52-59.
- Shepard, C. A. and Friedman, S. 1990. Influence of host plant, foliar phenology and larval dietary history on *Lymantria dispar* larval nutritional indices. - *Entomol. Exp. Appl.* 55: 247-255.
- Singh, B., Dhaliwal, J. S. and Atwal, A. S. 1974. Effect of simulated rain on the survival of first instar larvae of *Chilo partellus* (Swinhoe), Chamaebidae: Lepidoptera. - *Curr. Sci.* 43: 422-423.
- Sitch, T. A., Grewcock, D. A. and Gilbert, F. S. 1988. Factors affecting components of fitness in a gall-making wasp (*Cynips divisa* Hartig). - *Oecologia* 76: 371-375.
- Slansky, F., Jr. 1985. Food utilization by insects: interpretation of observed differences between dry weight and energy efficiencies. - *Entomol. Exp. Appl.* 39: 47-60.
- Smith, D. R. 1971. Nearctic sawflies. III. Heterarthrinae: adults and larvae (Hymenoptera: Tenthredinidae). - *Tech. Bull. No. 1420*. Agricultural Research Service, U.S. Dept of Agriculture, Washington.
- 1976a. Sawflies of the tribe Pseudodineurini in North America (Hymenoptera: Tenthredinidae). - *Proc. Entomol. Soc. Wash.* 78: 67-79.
- 1976b. World genera of the leaf-mining sawfly tribe Fenusini (Hymenoptera: Tenthredinidae). - *Entomol. Scand.* 7: 253-260.
1979. Suborder Symphyta. - In: Krombein, K. V., Hurd, P. D., Jr, Smith, D. R. and Burks, B. D. (eds.), *Catalog of Hymenoptera in America North of Mexico*. Vol. 1. Smithsonian Inst. Press, Washington. pp. 3-137.
- 1990. A synopsis of the sawflies (Hymenoptera, Symphyta) of America South of the United States: Pergidae. - *Revta Bras. Entomol.* 34: 7-200.
- Smith, J. D., Gover, R. A. and Woodring, J. P. 1986. Instar determination and growth and feeding indices of the forest tent caterpillar, *Malacosoma disstria* (Lepidoptera: Lasiocampidae), reared on Tupelo Gum, *Nyssa aquatica* L. *Ann. Entomol. Soc. Am.* 79: 304-307.
- Sokal, R. R. and Rohlf, F. J. 1995. *Biometry*. 3rd ed. Freeman, New York.
- Southwood, T. R. E. 1973. The insect/plant relationship - an evolutionary perspective. - In: van Emden, H. F. (ed.), *Insect/plant relationships*. Blackwell, Oxford, pp. 3-30.
- 1986. Plant surfaces and insects - an overview. - In: Juniper, B. and Southwood, T. R. E. (eds), *Insects and the plant surface*. Arnold, London, pp. 2-22.
- Spencer, K. A. 1990. Host specialization in the World Agromyzidae (Diptera). Kluwer, Dordrecht.
- Stehr, F. W. 1987. Order Lepidoptera. - In: Stehr, F. W. (ed.), *Immature insects*. Kendall Hunt, Dubuque, IA, pp. 288-596.
- Steyskal, G. C. 1974. Recent advances in the primary classification of the Diptera. - *Ann. Entomol. Soc. Am.* 67: 513-517.
- Stiling, P. and Simberloff, D. 1989. Leaf abscission: induced defense against pests or response to damage? - *Oikos* 55: 43-49.
- Stipanovic, R. D. 1983. Function and chemistry of plant trichomes and glands in insect resistance. - In: Hedin, P. A. (ed.), *Plant resistance to insects*. Symposium Series 208. American Chemical Society, Washington: pp. 69-100.
- Strong, D. R., Lawton, J. H. and Southwood, T. R. E. 1984. *Insects on plants*. - Harvard Univ. Press, Cambridge, MA.
- Taper, M. L. and Case, T. J. 1987. Interactions between oak tannins and parasite community structure: unexpected benefits of tannins to cynipid gall-wasps. *Oecologia* 71: 254-261.

- , Zimmerman, E. M. and Case, T. J. 1986. Sources of mortality for a cynipid gall-wasp (*Dryocosmus dubiosus* (Hymenoptera: Cynipidae)): the importance of the tannin/fungus interaction. *Oecologia* 68: 437-445.
- Trumble, J. T., Moar, W. J., Brewer, M. J. and Carlson, W. G. 1991. Impact of UV radiation on activity of linear furanocoumarins and *Bacillus thuringiensis* var. *kurstaki* against *Spodoptera exigua*: implications for tritrophic interactions. *J. Chem. Ecol.* 17: 973-987.
- Villacorta, A. 1983. Ovicidal activity of *Metarhizium anisopliae* isolate CM-14 on the coffee leaf miner, *Perileucoptera coffeella* (Lep: Lyonetiidae). - *Entomophaga* 28: 179-184.
- Waldbauer, G. P. 1968. The consumption and utilization of food by insects. - *Adv. Insect Physiol.* 5: 229-288.
- Wanatabe, M. 1976. A preliminary study on population dynamics of the swallowtail butterfly, *Papilio xuthus* L., in a deforested area. *Res. Popul. Ecol.* 17: 200-210.
- Warrington, S. 1985. Consumption rates and utilization efficiencies of four species of polyphagous Lepidoptera feeding on sycamore leaves. - *Oecologia* 67: 460-463.
- Wiegmann, B. M., Mitter, C. and Farrell, B. 1993. Diversification of carnivorous insects: extraordinary radiation or specialized dead end? - *Am. Nat.* 142: 737-754.
- Williams, A. G. and Whitham, T. G. 1986. Premature leaf abscission: an induced plant defense against gall aphids. *Ecology* 67: 1619-1627.
- Willmer, P. G. 1982. Microclimate and the environmental physiology of insects. *Adv. Insect Physiol.* 16: 1-57.
- Wilson, D. 1995. Fungal endophytes which invade insect galls: insect pathogens, benign saprophytes, or fungalinquilines? - *Oecologia* 103: 255-260.
- and Carroll, G. C. 1994. Infection studies of *Discula quercina*, an endophyte of *Quercus garryana*. *Mycologia* 86: 635-647.
- Yazawa, M., Shimizu, T. and Hirao, T. 1992. Feeding response of the silkworm, *Bombyx mori*, to UV irradiation of mulberry leaves. *J. Chem. Ecol.* 18: 561-569.