NOVEL ASPECTS OF HOST TREE RESISTANCE TO LEAFMINERS

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INTRODUCTION

At least 10,000 species of leafminers in four orders of insects (Lepidoptera, Diptera, Coleoptera, and Hymenoptera) are found worldwide. The common feature of all leafminers is that larvae feed within leaves for at least some larval stages. Larvae of facultative mining species feed internally but also externally as free-feeders, usually in later larval instars. Larvae of obligate mining species feed exclusively, and may also pupate, within leaves (Hering 1951, Powell 1980). Despite wide interspecific variation in life histories, leafminers are generally more closely associated with their feeding substrates than are free-feeding insects (Mattson et al. 1988). This "intimacy" (sensu Mattson et al. 1988) is the primary factor in directing their evolution and influencing population dynamics.

For leafminers, as distinct from most free-feeding insects, selection of feeding substrates is determined solely by female oviposition choice, at least for obligate leafminers. Eggs may be cemented to the surface or embedded in the leaf, so adult females determine feeding sites not only among plants but also within leaves. For facultative leafminers, female oviposition choice determines larval feeding sites for at least some larval instars, and female choice generally plays a much larger role in larval feeding sites than it does for most external feeding insects. Confinement to a single leaf means that larvae cannot behaviorally escape deteriorating abiotic or biotic conditions associated with their substrates as vagile insect larvae can, though a few species can mine more than one leaf by exiting the mine or mining through the petiole to adjacent leaves. The individual leaf is the arena where the fates of larvae are decided.

Because of this tight link between oviposition and larval feeding sites and survival, one expects strong selection on female oviposition choice. Since many free-feeding larvae are mobile, however, oviposition choice may be less critical. Furthermore, since most adult lepidopteran leafminers do not feed, selection of suitable leaves should be driven by survival and fecundity of offspring, not by feeding preferences of adults as with many free-feeding insects.

The leafminers differ from other endophagous insects, such as the stem and twig borers and gallformers, in several important ways. A leaf usually has less biomass than either a stem or a twig. Structurally, most leaves are essentially two-dimensional with little depth, while stems or twigs provide three-dimensional substrates. The two-dimensional nature of the leaf constrains the size and shape of larvae more than borers. Leafminers are the smallest of all the microlepidopterans and many are compressed dorso-ventrally. The lack of depth in leaves increases the likelihood of inter- and intraspecific interactions and may facilitate attack by natural enemies. Leaves are generally more photosynthetically active and composed of different cell types than twigs or stems. Some galling insects

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are also confined to feeding on single leaves, but by virtue of their isolating properties, galling insects are less likely to interact directly with other galling insects. Gall tissues also differ phytochemically from the leaves in which they are formed (Zucker 1982). Thus leafminers may be more intimately associated with their host leaves than are gallformers.

I contend that this close association between leafminers and the leaf substrate causes phenological and morphological traits of plants to override other factors in governing host plant resistance. The biology and ecology of a lepidopteran leafminer, *Cameraria* sp. nov. Davis (Lepidoptera: Gracillariidae, *agrifoliella* group), are presented to elucidate that argument.

HOST PLANT RESISTANCE

Strictly speaking, plant resistance consists of the intrinsic (inherited) traits of a plant which prevent herbivore damage (Williams et al. 1988). Many extrinsic factors, however, such as abiotic conditions, interactions with natural enemies and other herbivores (including microbes), and the association of the host plant with other plants, influence the susceptibility of host plants to insect herbivores. Here, resistance is considered in the broad sense, i.e. what factors generally influence abundances, and thus population dynamics, of leafminers on their host plants.

Temporal and Spatial Patterns of Abundances of *Cameraria* on Emory Oak

Cameraria sp. nov. Davis is a dominant, univoltine leafminer on *Quercus emoryi* (Fagaceae) in some localities in central Arizona. Adults emerge from puparia inside leaves as they abscise in April. Adults mate and females oviposit on newly expanded leaves in late April to early May. Eggs hatch a few days after oviposition and larvae immediately form upper-surface, blotch mines. Larvae complete three to four instars by August to September, apparently undergo a larval diapause in winter months (although larvae can be observed feeding on warmer winter days), then rapidly complete the last four to five instars in March and April, and pupate in leaves to complete the yearly cycle. Successful larvae consume most of the upper-surface leaf area of the relatively small Emory oak leaves (Bultman and Faeth 1986a).

I have monitored the temporal patterns of abundances of *Cameraria* on eight Emory oak trees at a locality in central Arizona (Oak Flat, Pinal Co.) noted for unusually high densities. Other leafmining species typically remain at very low densities ($\approx$ 1 mine/100 leaves) on Emory (Faeth 1986) and other oaks (Faeth and Simberloff 1981). Several spatio-temporal patterns emerge. Resistant trees in one season tend to be resistant in the next (Fig. 1a, 1b) and whereas overall abundances vary among trees and years, the pattern of tree resistance remains the same, as does the pattern of higher densities within trees on interior, shaded leaves (Fig. 2).

Patterns of spatial distribution at finer scales have also been observed. Within trees, *Cameraria* is highly clumped among leaves (Faeth 1990). Fewer unmined leaves and more leaves with more than two mines occur than would be expected by chance. The leafminer occupies larger leaves more frequently than small ones. Within leaves, multiple mines are clumped on the basal half of individual leaves (Faeth 1990).

Two related questions emerge: what factors determine the pattern of among-tree distribution, and how are the consequences of within-tree and within-leaf clumping of *Cameraria* related to this pattern? Both of these questions are fundamental to concepts of plant resistance. The first examines the initial colonization or habitat selection of trees and leaves, which is a function of female oviposition choice. The second question focuses on the fate of the population after colonization and thus on future colonization by leafmining survivors.

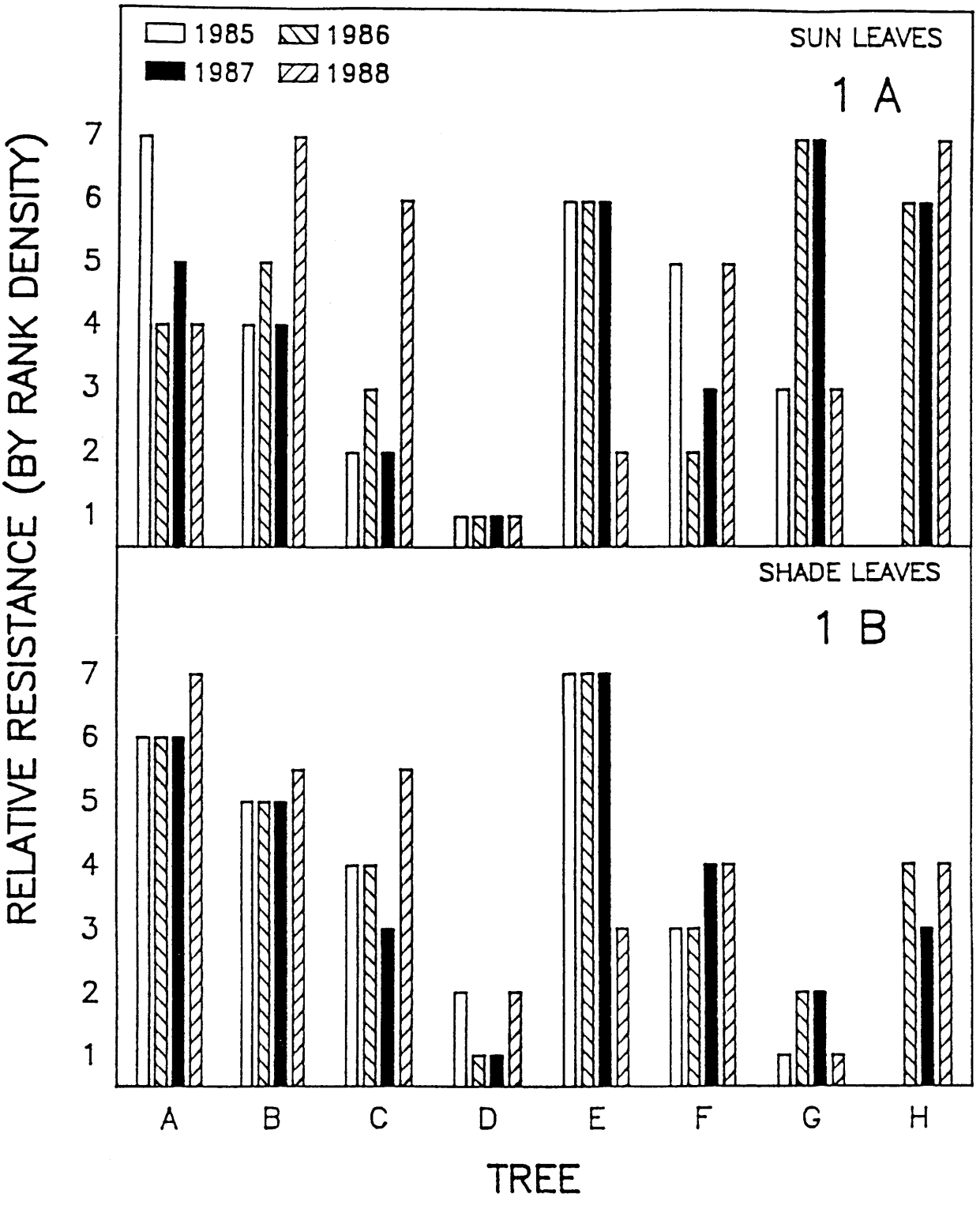

Figure 1. Relative resistance of sunny (1A) and shaded (1B) regions within eight study trees to *Cameraria* infestation over four growing seasons. Resistance is defined as rank by mean density. Ranks of sun and shade leaves in one season were tested for independence in the following season by Kendall's nonparametric test for independence and are as follows: Sun, 1985-86, $p > 0.20$; 1986-87, $p < 0.001$; 1987-88, $p > 0.40$; Shade, 1985-86, $p < 0.001$; 1986-87, $p < 0.001$; 1987-88, $p = 0.05$. Densities of Tree H were not recorded in 1985.

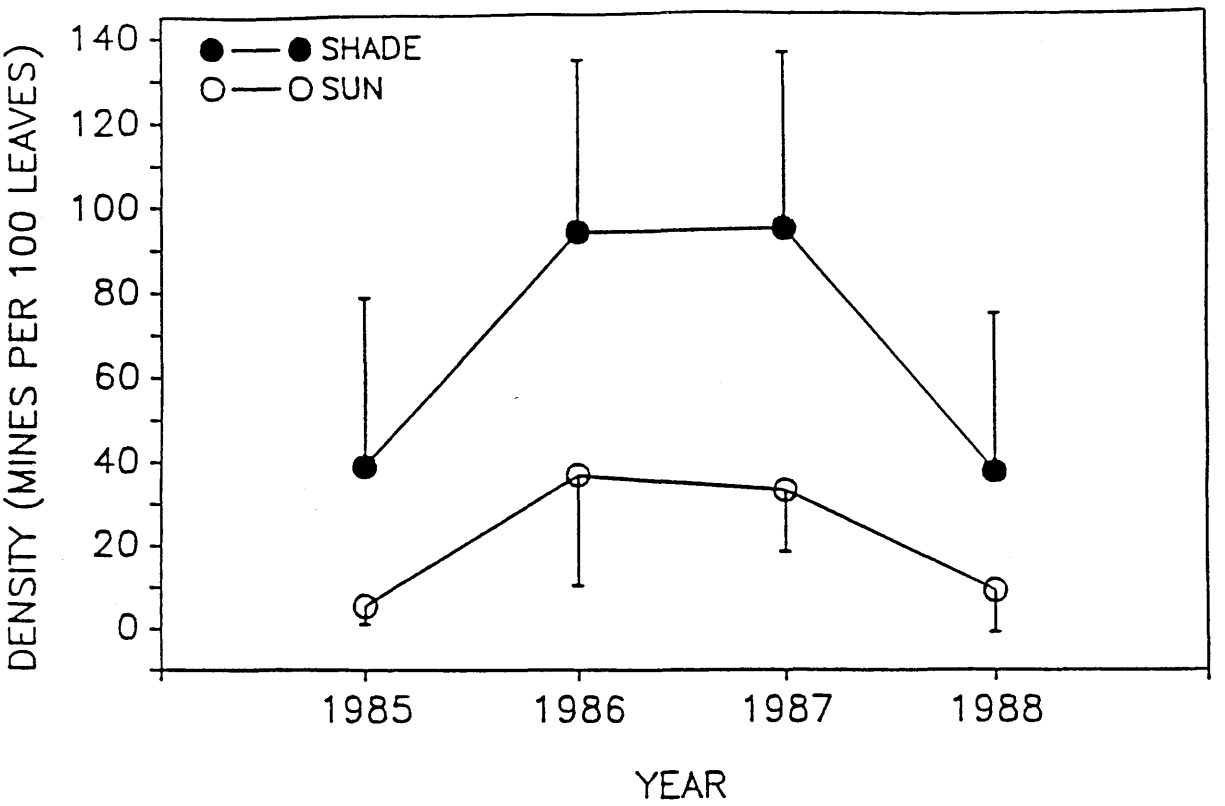

Figure 2. Mean densities of *Cameraria* (± SE) in sun and shade leaves from 1985 to 1988. Densities in shade leaves are significantly greater than sun leaves in each year, except 1985 (paired t-test, 1985, $t = 2.23$, $df = 6$, $0.10 > p > 0.05$; 1986, $t = 4.92$, $df = 7$, $p < 0.01$; 1987, $t = 4.53$, $df = 7$, $p < 0.01$; 1988, $t = 2.40$, $df = 7$, $p < 0.05$).

Oviposition Choice

The operation of selection on adult females via survival of offspring has apparently resulted in behavioral and physiological mechanisms by which they discriminate suitable leaves for oviposition, at least in some leafminers. McNeil and Quiring (1983) reported an oviposition deterring pheromone in the dipteran alfalfa blotch miner, and others have suggested that lepidopteran leafminers can detect the presence of conspecific eggs (Simberloff and Stiling 1988, Auerbach and Simberloff 1989). In all of these studies, co-occurrence with conspecifics on a leaf resulted in increased mortality. Godfray (1986) showed that observed clutch size (three per leaf) of the leafmining fly, *Pegomyza nigrotarsis*, produced greatest larval survival, suggesting that natural selection has optimized clutch size. In addition to the presence of conspecifics, adult leafminers select leaves based on phenological or morphological traits such as 1) leaf size (Bultman and Faeth 1986a, 1986d, Godfray 1986, Faeth 1990), 2) propensity of leaves to abscise (Bultman and Faeth 1986c, Stiling et al. 1987, Simberloff and Stiling 1988), 3) previous leaf damage (Faeth 1986), and 4) age of leaves (Quiring and McNeil 1987).

Budbreak phenology can also influence oviposition and, consequently, densities. Some leafmining species embed eggs only on new, supple leaves, perhaps because the ovipositor cannot penetrate mature, toughened leaves. Auerbach and Simberloff (1985) showed that atypical production of new, secondary leaves on *Quercus nigra* resulted in increased densities of two leafmining species that are restricted to oviposit and feed on new foliage. Opler (1974) suggested that late-season generations of the leafminer *Neurobathra bohartiella*, a new leaf specialist, were dependent on early-season defoliations by a dioptid moth that resulted in secondary flushing of new leaves.

It is interesting to note that all of the above features associated with habitat selection are pheromonal, phenological, or morphological, and not phytochemical. Phenological and morphological features of leaves and detection of the presence of co-occurring leafminers may be more important than phytochemistry in determining habitat selection by females. Possible reasons for the absence of correlation of habitat selection and larval survival with phytochemistry are discussed below.

Population Dynamics

Once the feeding substrate has been more or less fixed via oviposition, do factors which influence survival of larval leafminers differ from those which affect free-feeding insects?

Abiotic Factors

Feeding within leaves may ameliorate certain abiotic factors known to affect free-feeding folivorous insects. Because of insulation provided by the leaf, larvae may be less susceptible to external temperature fluctuations. Some species of leafminers continue to feed on evergreen host plants during the winter months in temperate zones despite sub-freezing external temperatures that kill externally-feeding larvae. In some cases, the translucent mine may act as a miniature greenhouse, since dome-shaped blotch mines may magnify sunlight (Hering 1951).

In warmer climates, leafminers continue to feed when external temperatures rise above lethal limits for most externally-feeding insects, perhaps due to the mine's insulation and cooling provided by evapo-transpiration from stomata. Leafmining larvae are less susceptible to desiccation since the environs of the mine provide higher relative humidity than ambient air (Hering 1951). *Cameraria*'s unusually long larval development time (11 months) in Arizona, which includes both extremely warm and sub-freezing temperatures, may be possible due to the amelioration of harsh ambient temperatures. Opler (1974) proposed that leafminers feed longer on evergreen oaks than deciduous ones because of poorer leaf quality. I suggest that these long development times are not caused by phytochemistry, but rather that internal feeding negates many of the climatic hazards of feeding externally. In addition, prolonged development in the case of *Cameraria* appears to reduce attack by predators. The larvae escape ant predation (see discussion of natural enemies below) by remaining small during the summer months when ant activity is greatest and rapidly growing in late winter to early spring when ant activity is low (Faeth, unpubl. data).

Host Plant Factors

Phytochemistry. One might hypothesize that phytochemistry is a crucial factor in survival of leafminers because of their confinement to individual leaves. Variation in phytochemistry is a prevalent explanation for non-random distribution of free-feeding insects (Denno and McClure 1983, Kareiva 1983, Spencer 1988). However, studies of leafminers have usually shown that phytochemistry is either not related to leafminer abundances or shows an inverse relation to that predicted (Feeny 1970, Faeth et al. 1981a, Faeth 1985, Stiling et al. 1982, Bultman and Faeth 1988, Rooney 1989). Similarly, in a detailed study of nutritional (protein) and allelochemical (tannin) variation among and within trees, the distribution of *Cameraria* among or within trees was not correlated with any phytochemical parameters

(Faeth 1990). Moreover, survival of *Cameraria* larvae was not related to phytochemical parameters of individual leaves (Faeth 1990).

The general failure of phytochemistry to explain host plant resistance to leafminers may be accounted for in several ways. First, leafminers, like other endophagous insects, are more specialized on host plants (narrower host ranges) than insects that feed externally (Hering 1951, Powell 1980, D. Tonkyn, pers. comm.) and therefore may be better physiologically and behaviorally adapted to the chemistry of their host plants. However, performance of specialized insects is not necessarily better on their host plants than that of generalists (Bernays and Graham 1988). Second, specialized feeding within leaves by leafminers may circumvent poor quality tissues. Some larvae feed only on selected tissue layers that may be more nutritious and contain lower amounts of fiber and secondary compounds (Needham et al. 1928, Hering 1951). However, some leafminers feed primarily on tissue layers that contain secondary compounds (e.g. palisade layers), and others mine fully through all depths of the leaf such that ingestion of some tissues with secondary compounds is certain. Third, other phenological and morphological features of leaves may override differences in chemistry. This explanation seems most likely. Although phytochemical cues may play some role in the location of adult females of appropriate host plants and leaves within host plants, phytochemical effects on offspring may be less important than phenological and morphological ones. Furthermore, effects of morphology and phenology may interact with effects of competition and natural enemies.

Phenology and morphology. The link between oviposition choice and offspring survival is most evident when host plant phenology and morphology are considered. Endophagism increases risks of larval death from early leaf abscission via desiccation (Askew and Shaw 1979a, Faeth et al. 1981b, Williams and Whitham 1986, Stiling et al. 1987, but see Kahn and Cornell 1983, Pritchard and James 1984), and adults of some species may select leaves within trees based on propensity to abscise (Bultman and Faeth 1986c, Stiling et al. 1987, Simberloff and Stiling 1988). The effect of leaf abscission varies greatly depending on length and seasonality of larval development, leaf area required by larvae, normal leaf duration (i.e. deciduous versus evergreen), and external climatic conditions (early abscission in hot and dry locales may exacerbate desiccation) (Bultman and Faeth 1986c).

Variation in budbreak phenology can directly affect abundances of leafminers via availability of leaves for oviposition, but variable budbreak can also indirectly affect larval mortality. Variation in budbreak leads to differences in ages of leaves which can affect survival, often in complex ways. For example, West (1985) forced oviposition on young, spring leaves by a leafminer that normally oviposits on mature leaves and found that survival on young leaves declined due to interactions with chewing folivores that prefer new leaves. Quiring and McNeil (1987) showed that the dipteran leafminer, *Agromyza frontella*, preferentially searched and oviposited on new leaflets of alfalfa, but apparently because these leaves were less likely to be occupied by conspecifics. A late-season *Cameraria* sp. nov. (*guttifinitella* group), which normally mines mature leaves of Emory oak, will oviposit on new, secondary foliage. Survival is higher in new foliage than mature foliage because these leaves are less likely to abscise than mature leaves (Faeth 1987).

Leaf size can strongly affect larval survival and fecundity, particularly for leafminers that require relatively large areas of leaves for successful development (Bultman and Faeth 1986d). Bultman and Faeth (1986a) showed that four species of leafminers on Emory oak chose leaves for oviposition according to leaf area required for larval development (i.e. leafminers requiring small areas chose small leaves and those requiring large areas chose large leaves). However, leaf size was correlated with survival only for *Cameraria* sp., which requires large leaf areas.

Natural Enemies

Hering (1951) proposed that feeding within mines kept leafminers protected from natural enemies. Powell (1980) implied that endophagous lepidopterans have not evolved characteristics that permit exposed feeding (distastefulness, urticating hairs and glands, aposematism) because endophagism

provides protection from natural enemies. Among-guild comparisons are difficult, but apparently natural enemies of leafminers are neither less diverse nor is attack on them less intense than that on free-feeding insects. Invariably, detailed studies of leafminers reveal a wide array of parasitoid species attacking each leafminer species (Askew and Shaw 1974, 1979a, 1979b, Green 1979, Hawkins 1988, Faeth sub. man.). Moreover, natural enemies (parasitoids and predators) typically account for a large fraction, if not the majority, of the mortality of leafmining larvae and pupae (Askew and Shaw 1979a). Many parasitoid species specialize on certain leafmining species by using cues associated with the mine or frass (Hawkins 1988) or even acoustic signals of mining larvae (Sugimoto et al. 1988). At present, then, there is no evidence to suggest that leafminers are more protected from natural enemies than are other herbivorous insects.

At least in some cases, however, the opposite may be true. Gross and Price (1988) found that an obligate gelechiid leafminer on horsenettle is more vulnerable to macroparasite attack than a congeneric facultative leafminer on groundcherry that can depart the mine and move freely over the leaf surface. The horsenettle trichome morphology apparently has selected for mine architectures, which exclude free-feeding and, in turn, increase parasitism. Because of humid and perhaps warmer conditions within the mine, leafminers may also be more susceptible to infection by microbial parasites or pathogens such as fungi and bacteria (Faeth and Bultman 1986).

Natural enemies of *Cameraria* include more than 20 species of Hymenoptera, mostly in the families Encyrtidae, Eulophidae, and Braconidae. Predators are mostly adult parasitoids, which feed on larvae, and the arboreal ant *Pseudomyrmex apache*. Typically, rates of natural enemy attack are from 15 to 40 percent. Natural enemy attack varies among trees and within trees, probably as a function of leafminer densities and local distribution of natural enemies (Faeth 1980), although not necessarily in a density-dependent fashion (Heads and Lawton 1983, Stiling 1987).

Natural enemy attack may also be a complex function of interaction with other herbivores and the ensuing phytochemical and morphological changes they cause. Faeth (1986), for example, showed that leafminers feeding in leaves previously damaged either artificially or by herbivores had significantly increased rates of parasitism. Subsequent experiments indicated that both phytochemical (Faeth and Bultman 1986) and structural (Faeth, unpubl. data) changes induced by herbivory can increase the rate of natural enemy attack.

Competition

Confinement within leaves increases the probability of inter- and intra-guild interactions relative to other folivorous insects. The vagility of free-feeding insects permits behavioral avoidance of both direct (interference competition or cannibalism) and indirect competition as well as amensalistic interactions (exploitation competition, induced defenses, competition via natural enemies). Such is not the case for leafminers. Competition has been shown to occur for hymenopteran leafminers on birch trees (Tuomi et al. 1981), for dipteran leafminers on citrus (Murai 1974), alfalfa (Quiring and McNeil (1984), *Spartina* (Stiling et al. 1984), and American holly (Potter 1985), and for lepidopteran leafminers on oak (Bultman and Faeth 1986b, Auerbach and Simberloff 1989). Cannibalism or killing of co-occurring conspecifics (Condrashoff 1964, Murai 1974, Quiring and McNeil 1984, Auerbach and Simberloff 1989, Faeth 1990) is probably more common among leafminers than free-feeding folivores at comparable densities because of restriction within leaves.

The sedentary nature of leafminers also increases susceptibility to amensalistic interactions with nonleafmining insects. Punctures or tears in mines via feeding by leafchewing or sucking insects can result in desiccation and death of most larval leafminers. Insect damage to nonmined areas of the leaf can increase the propensity of the leaf to abscise (Faeth et al. 1981b, Faeth 1986), change the phytochemistry of the leaf (Faeth 1986), attract natural enemies (Faeth and Bultman 1986, Faeth unpubl.), and increase the likelihood of leaf infection by endophytic or epiphytic fungi (K. Hammon,

pers. comm.). Interactions with free-feeding insects are generally unilateral, since mining damage is usually minimal and can be avoided by mobile insects (but see Hartley and Lawton 1987).

Morphological and phenological variation may influence the intensity of intraspecific competition. Competition among leafmining larvae should be a function of relative leaf size as well as density and dispersion of the leafmining larvae. A field study is described to illustrate the complex interactions of variation in leaf size with density, dispersion, and competition among *Cameraria*.

EMORY OAK RESISTANCE TO *CAMERARIA*

Methods

To test the hypothesis that leaf size influences *Cameraria* colonization, dispersion, and subsequent survival via intraspecific interactions, 12 branches of *Q. emoryi* (two branches per six trees) were enclosed with fine mesh screening before budbreak in 1986. Twenty pupae were added to each enclosure on 24 April 1986, about a week after budbreak. Emerging adults were allowed to mate and oviposit on leaves within enclosures. Enclosures were removed at the first appearance of larval mines to allow access of natural enemies and to minimize any cage effects. All mined leaves were marked with a small dot of latex paint so that any abscised leaves could be identified (abscised leaves were collected from beneath trees bimonthly during the study period). Enclosures were again placed on the branches in late March 1987 to capture leaves during the peak of leaf abscission. Pupation and most natural enemy attack occur before this time. Leaves were collected from within enclosures on 3 and 24 April 1987; all leaves had abscised from the branches by the latter date.

Collected leaves were returned to the laboratory and each was examined for the presence and number of mines to determine densities and distribution. Distribution on each branch was tested against a random (Poisson) and, if significantly different, against a clumped (negative binomial) distribution. Densities were plotted against k of the negative binomial to determine the relationship of density and degree of clumping. Sizes of mined and unmined leaves were determined by weighing a random sample of dried leaves in each category from each branch.

Survival (presence of an emergence hole or protruding pupal case) or mortality of each leafminer was ascertained by dissecting each mine. Specific categories of mortality were not ascertained for dead leafminers. Thus I examined intraspecific competition in the broad sense, i.e. how co-occurrence affects overall mortality, be it from cannibalism, depletion of food resources, increased leaf abscission, or changes in rates of natural enemy attack. The question of interest in this study is how leaf size and densities interact to affect survival and thus population dynamics and tree resistance. Other studies in this system have examined specific categories of mortality in detail (Bultman and Faeth 1986b, 1988, Faeth and Bultman 1986, Faeth 1990).

To test for density-dependent effects at the spatial level of the branch, survival of all leafminers, of leafminers occurring alone, of those co-occurring with one conspecific, and of those co-occurring with two or more conspecifics were regressed separately against mean branch densities.

Because of leafminers' confinement within leaves, tests of density-dependent mortality of leafminers may be more appropriate at finer spatial scales (i.e. within leaves of varying sizes with varying numbers of co-occurring leafminers). Log linear analysis was therefore used to determine relationships between the response variable, survival (S), and leaf size (L), number of mines per leaf (M), and aggregation within branches (K), estimated by k of the negative binomial. Plots of k versus branch densities showed that branches fell into two categories: low and high degree of clumping (Fig. 3). Leaf sizes were divided into three categories: small (0-30 mg dry mass), medium (31-60 mg) and large (> 60 mg) leaves. Categories of mines per leaf were one, two, and > two per leaf. The

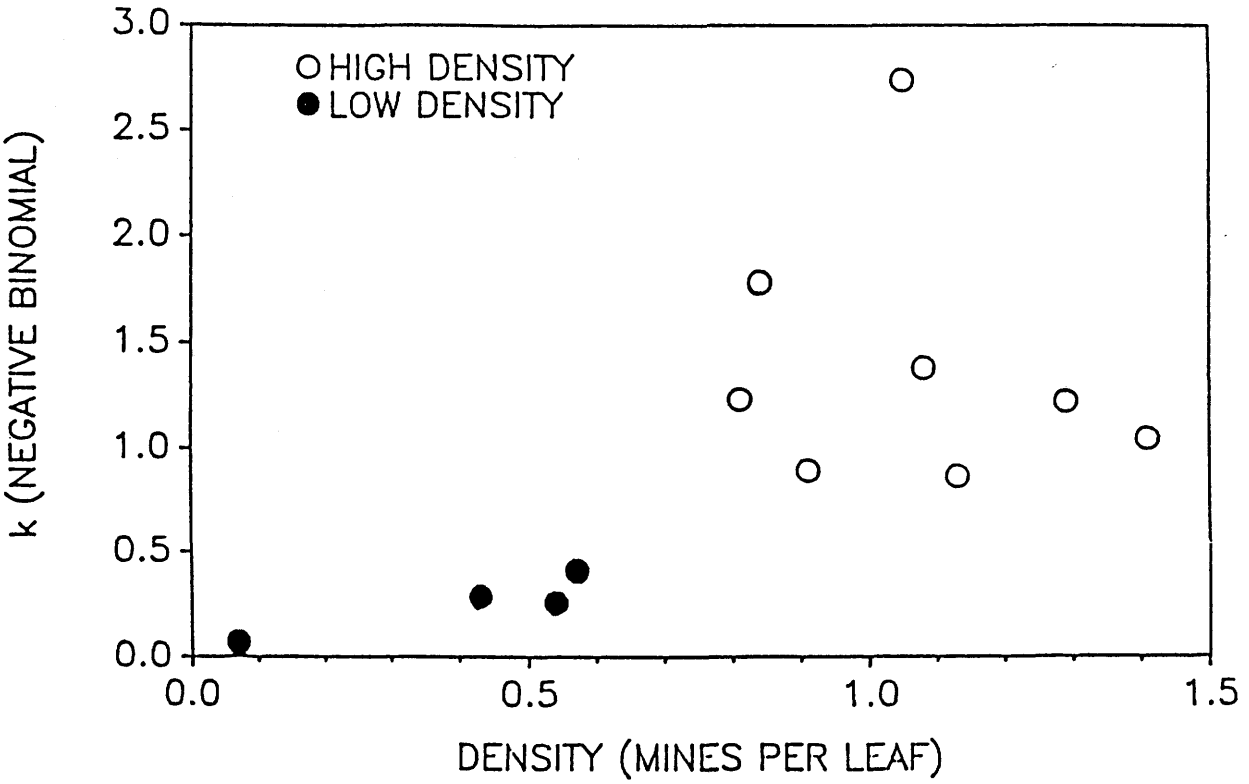

Figure 3. Relationship of density of *Cameraria* and k of the negative binomial.

response variable, survival, was simply whether each leafminer in each multi-way classification had either survived or died.

Log linear analysis is a powerful method of examining relationships among categorical variables (Jenkins 1975, Vepsäläinen et al. 1988), but it is subject to certain statistical and biological assumptions. I followed the methods of Jenkins (1975) by beginning with the most complex model (all four three-way interactions) and then testing progressively simpler models (dropping complex interaction terms) until the simplest model was found where the log-likelihood ratio was no longer significant at $p \leq .05$. This method assumes that the simplest model is the most biologically relevant one (Jenkins 1975). Data was partitioned in a biologically reasonable fashion based upon previous studies and knowledge of the system. Data was combined from all branches so that cells within the multi-way table were sufficiently large (Vepsäläinen et al. 1988).

Results

Dispersion

Dispersion of leafminers on all branches was significantly different from a random Poisson distribution (Table 1). Generally, more leaves were unmined and more leaves had multiple mines than would be expected by chance. On nine of the 12 branches, dispersions were not significantly different from that expected by a negative binomial (clumped) distribution (Table 1). Leafminers were also more aggregated (smaller k) at lower densities than higher ones (Fig. 3).

Table 1. Spatial distribution of *Cameraria* among leaves within individual branches compared to a random (Poisson) and an aggregated (negative binomial) distribution. Common letters denote branches from the same tree. Chi square tests of significance are based upon deviation of observed from expected. Observed classes with < 5 observations are lumped with previous categories. Number of classes ranged from three (0, 1, > 1 mines per leaf) to seven (0, 1, 2, 3, 4, 5, > 5 mines per leaf).

Branch	Poisson	P@	Negative binomial	P@
Chi square		Chi square		
1A	82.5	***	0.2	NS
2A	249.8	***	12.1	*
1B	161.4	***	4.7	NS
2B	258.8	***	6.0	NS
1C	65.4	***	8.8	NS
2C	183.3	***	20.8	**
1D	155.0	***	7.0	NS
2D	49.7	***	4.0	NS
1E	39.2	***	1.9	NS
2E	96.8	***	13.4	*
1F	41.1	***	1.2	NS
2F	279.8	***	7.9	NS

@ NS, not significantly different; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Leaf size

For all branches, mined leaves were significantly larger than unmined ones (Table 2).

Mortality

Mortality was shown to be inversely related to density at the spatial scale of branches whether one considers overall mortality (Fig. 4a) or mortality of leafminers occurring alone (Fig. 4b), with one conspecific (Fig. 4c), or with more than two conspecifics (Fig. 4d).

At the finer spatial scale of individual leaves, the simplest log linear relating survival (S) to clumping (K), leaf size (L), and mines per leaf (M) is: (LK)(LM)(SK)(SM). Inclusion of three-way interactions did not improve the model, nor did inclusion of the two other possible two-way interactions, (SL) and (KM). This model suggests that degree of branch clumping and number of mines per leaf are related to leaf size ((LK) and (LM) terms). Generally, more single mines and fewer multiple mines occurred on small leaves as compared to medium or large leaves (Fig. 5). Survival was a function of degree of clumping both at the level of the branch (SK term) (Fig. 6) and at the level of individual leaves (SM term) (Fig. 6). Under conditions of high branch clumping, survival was lower for leafminers occurring alone or with other conspecifics (Fig. 6). Finally, branch clumping was shown to

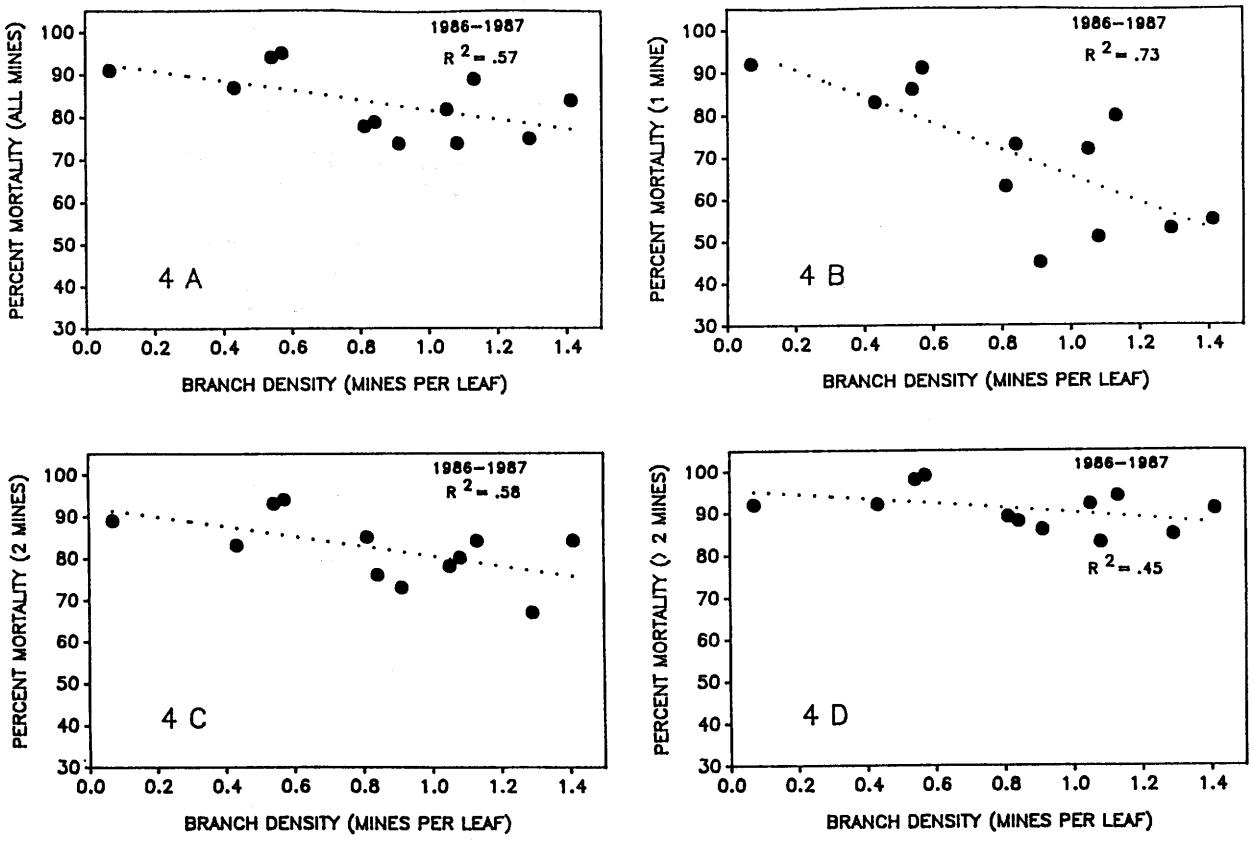

Figure 4. Regressions of mean branch density and mortality for all leafminers pooled (4A), those occupying leaves alone (4B), with one conspecific (4C), or with more than one conspecific (4D). All slopes of regressions are significantly different from zero ($p < 0.05$).

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be a function of available leaf sizes (LK term), and more clumping occurs when mean leaf sizes of branches are small (Fig. 7). Of interest is that the term (SL) is not in the model. Survival was not a function of leaf size (Fig. 8), although adults apparently oviposit more frequently on larger leaves (Table 2).

DISCUSSION

Contrary to expectations, mortality of *Cameraria* sp. nov. appears to be inversely density-dependent, at least at the spatial scale of branches (Fig. 4a-4d). This relationship is most pronounced for leafminers occurring alone (Fig. 4b). Given this result alone, one might conclude that intraspecific competition is not an important process in the population dynamics of this leafminer. Others (Strong et al. 1984, Lawton and MacGarvin 1986) have concluded on the basis of evidence from insect life tables that population dynamics of phytophagous insect populations are generally not driven by competition.

However, consideration of mortality only at the level of branches may obscure the complex interactions underlying the dynamics of *Cameraria*. The more appropriate scale may be that of the leaf because, after all, that is where ultimate oviposition choice and larval-larval interactions occur. When

Table 2. Mean size in mg (± SE) of all (mined and unmined leaves) and mined leaves. Sample sizes of each category for each branch are in parentheses.

Branch	Mean size of all leaves Mean ± SE (N)	Mean size of mined leaves Mean ± SE (N)
1A	26.24 ± 11.28 (50)	36.24 ± 10.18*** (41)
2A	27.46 ± 12.26 (50)	40.95 ± 13.26*** (99)
1B	32.75 ± 16.01 (50)	44.61 ± 16.47*** (99)
2B	34.00 ± 16.71 (50)	48.98 ± 18.97*** (122)
1C	38.69 ± 19.49 (50)	45.90 ± 16.10** (125)
2C	42.71 ± 19.49 (50)	51.20 ± 17.56** (172)
1D	48.63 ± 20.39 (50)	58.85 ± 19.30** (77)
2D	38.98 ± 18.57 (50)	49.72 ± 14.01*** (132)
1E	40.57 ± 19.64 (50)	52.44 ± 18.00*** (151)
2E	38.17 ± 21.71 (50)	55.29 ± 19.88*** (147)
1F	38.97 ± 19.21 (50)	48.69 ± 19.81** (126)
2F	37.68 ± 16.48 (50)	48.76 ± 15.17*** (170)

** Significantly different mean size of mined leaves compared to mean size of all leaves by Student's t test at $p < 0.01$

*** Significantly different mean size of mined leaves compared to mean size of all leaves by Student's t test at $p < 0.001$

one considers mortality at the level of leaves, a different picture emerges. Survival is a function of number of mines per leaf. Most of this increase in mortality (and decrease in pupal mass, and thus fecundity, of survivors) with mine number is attributable to cannibalism, and, secondarily, to premature leaf abscission (Faeth 1990). Translated into the spatial scale of branches, survival becomes a function of how clumped larvae are, with greater clumping leading to decreased survival (Fig. 6). Clearly, *Cameraria* mortality is density-dependent, but only when one examines the proper spatial scale.

I propose that Emory oak resistance to *Cameraria* is based in part on variation in leaf sizes, which influences the two components of tree resistance, colonization by ovipositing females and survival of larval offspring. At the level of the branch, clumping appears to be a function of available leaf sizes (LK term) (Fig. 7), with mean leaf size of the branches explaining a significant ($R^2 = 0.43$) proportion of the variation in k, an index of clumping. At the scale of individual leaves, number of mines per leaf is also related to leaf size (LM term). Rejection of small-sized leaves for oviposition (Table 2) is the likely mechanism for clumping within branches and among leaves (Faeth 1990). Rejection of small leaves may be selected through survival of offspring. A larva requires relatively large areas of Emory oak leaves successfully to complete development. Of the 1,915 mined leaves examined in this study, no leafminer survived in leaves of less than 20 mg dry mass. A critical lower size limit thus exists below which survival of even single leafminers does not occur. Thus branches or

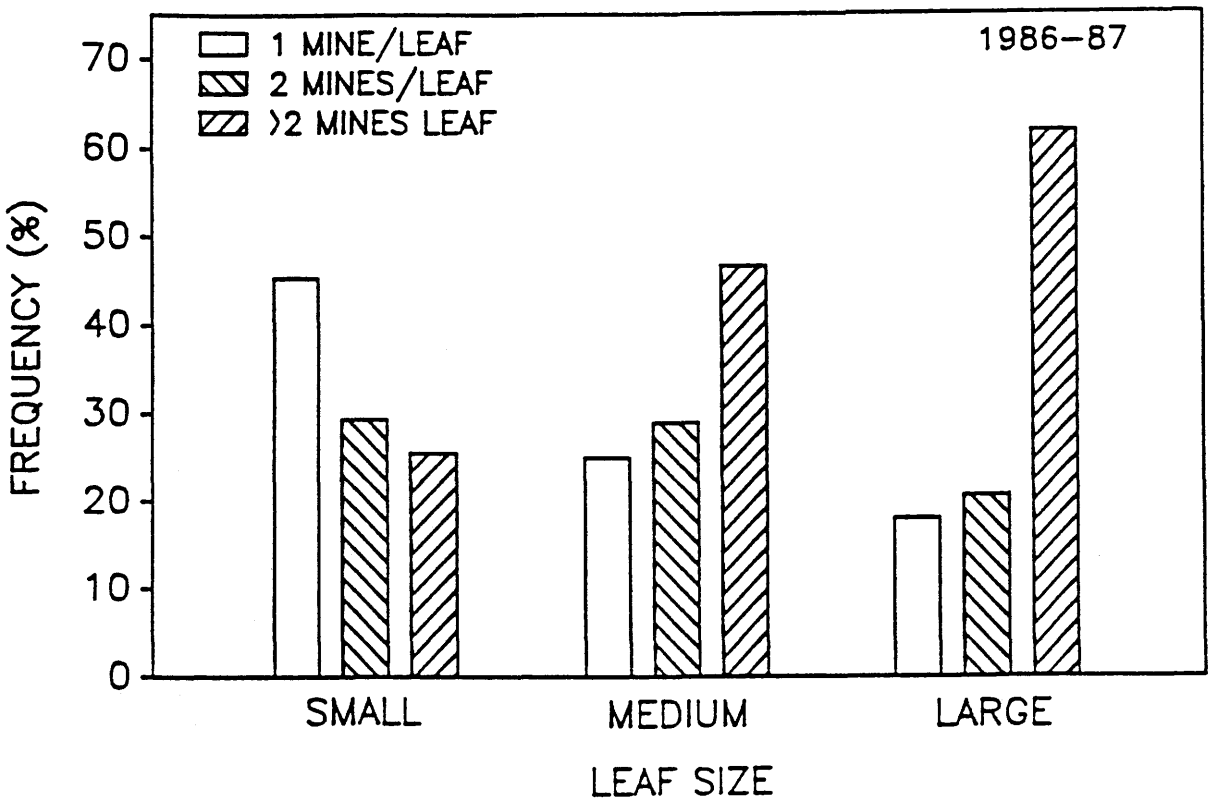

Figure 5. Percent of leafminers occurring on small-, medium- and large-sized leaves by number of mines per leaf.

trees with smaller mean leaf sizes may be more resistant at oviposition because fewer eggs are laid and those that are tend to be highly clumped on the reduced pool of suitably sized leaves. The high degree of clumping suggests that these females do not have an oviposition deterring pheromone as do some other leafminers (McNeil and Quiring 1983, Simberloff and Stiling 1988, Auerbach and Simberloff 1989), although this is yet to be determined.

Increased clumping clearly results in increased larval mortality, the second component of tree resistance. Co-occurrence exacerbates cannibalism, exploitation competition, and premature leaf abscission. Leafminers in highly clumped branches have lower survival when occupying multiply or singly-mined leaves (Fig. 4a-4d). When survival in individual leaves was examined, only 25 of the 1,617 multiply-mined leaves in this study had two larvae that survived simultaneously, and none had more than two. The consequences of confinement and co-occurrence in terms of intraspecific competition are thus severe. Colonization in the next growing season may be reduced and resistance thus reinforced if colonization occurs primarily in situ. Since pupation and emergence occur in leaves that have fallen beneath relatively isolated oak trees at the study site, this prediction may hold. Experiments are underway to test this hypothesis.

In 1987 we tested leaf size as a predictor of resistance independently by correlating leafminer densities with mean leaf size on eight Emory oak trees. Mean leaf size is a good predictor of density and explains a significant proportion of the variation in densities (R^2 = 0.41) (Fig. 9). Considering the multifarious factors that can influence host plant resistance to insects, it is noteworthy that a single parameter, mean leaf size, can predict a large part of oak tree resistance to leafminers.

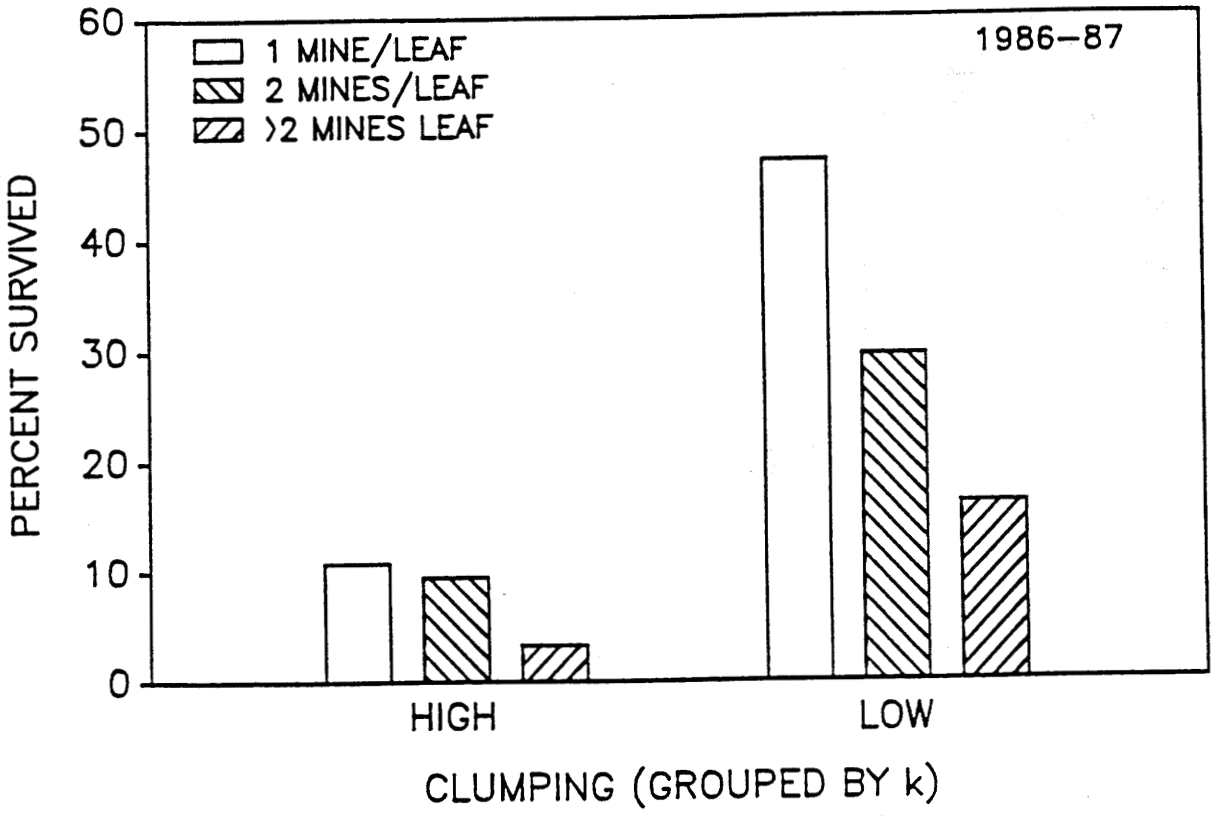

Figure 6. Survival of leafminers occurring alone, and with one or more conspecifics on branches grouped by high (n = 4) or low (n = 12) degree of branch clumping as indicated by k of the negative binomial.

Certainly, leaf size does not explain all the variation in tree resistance to leafminers. Two other phenological factors, variation in premature leaf abscission and timing of budbreak, can also influence colonization and survival. For example, Bultman and Faeth (1986c) showed that leaf abscission rates are greater in sun leaves compared than leaves and can explain some variation in both distribution and mortality. Others (Askew and Shaw 1979a, Faeth et al. 1981b, Potter 1985, Stiling et al. 1987, Simberloff and Stiling 1988, Auerbach and Simberloff 1989) have shown that premature leaf abscission is an important factor in leafminer survival. Higher rates of premature leaf abscission for sun leaves (Bultman and Faeth 1986c) may explain why leaf size accounts for less variation in *Cameraria* densities on sunny than shaded leaves (Fig. 9) (sun leaves R^2 = 0.27, shade leaves R^2 = 0.51).

Variable budbreak probably influences initial colonization also, since colonization is closely synchronized with budbreak. For example, unusually warm weather in the spring of 1988 caused early budbreak accompanied by emergence of leafmining adults. A late frost then occurred, many new leaves were destroyed, and further budbreak ceased on some trees until after adults had disappeared. These events may explain some reversals in tree resistance in 1988. Tree C, for example, exhibited delayed budbreak, and resistance greatly increased in 1988 as compared to previous years (Fig. 1). Obviously, both variable budbreak and abscission may act to intensify intraspecific interactions if the pool of suitable leaves for oviposition is enlarged and increased clumping ensues.

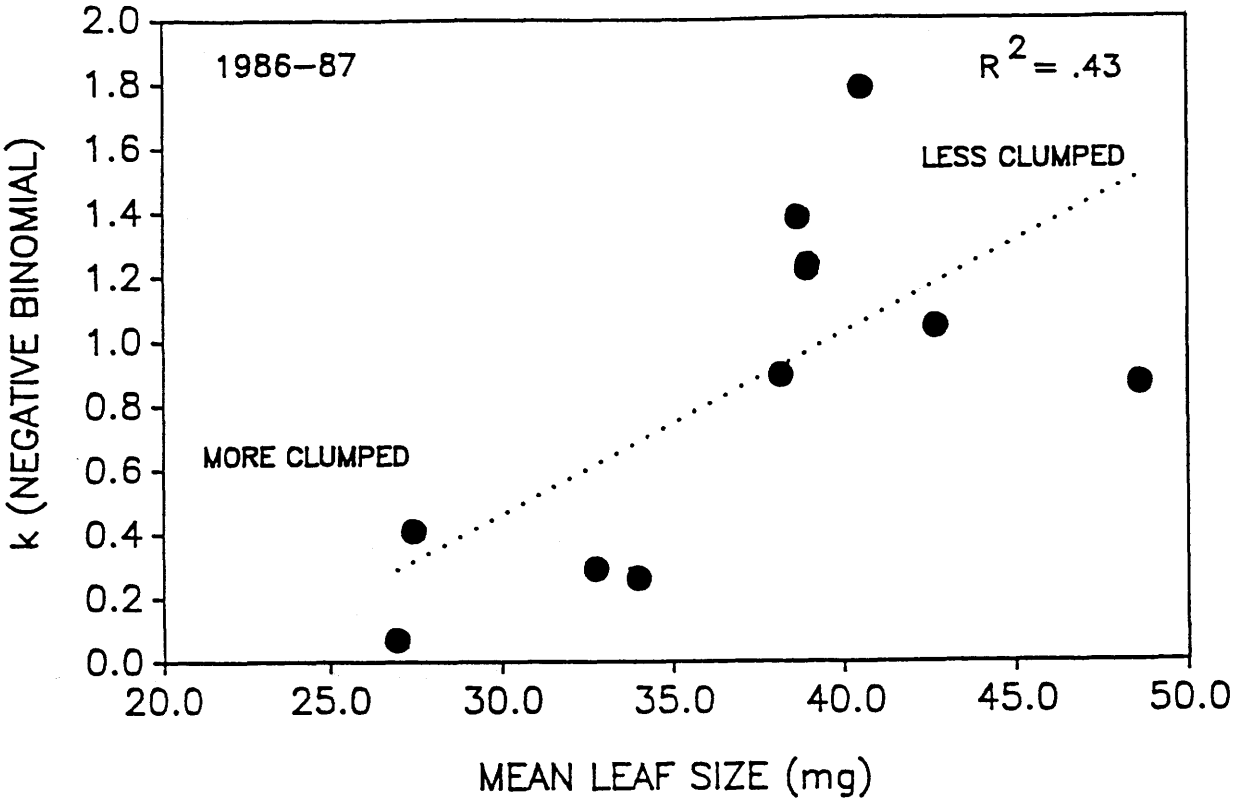

Figure 7. Relationship of clumping (k) and mean leaf size of individual branches. Slope of the regression is significantly different from zero ($p < 0.05$).

It is important to note that neither foliar nutrition nor allelochemical variation, long the bastion for explaining resistance of host plants to phytophagous insects, appears to determine Emory oak resistance to *Cameraria* (Faeth 1990). This is not to say that phytochemistry is not important in adult selection of, or larval survival in, leaves. Leaf size, for example, probably co-varies with some phytochemical parameter used by ovipositing females. It does seem, however, that variable phenology overrides variable phytochemistry in the dynamics of this leafminer.

It is premature to suggest that host plant resistance to other species of leafminers is driven mainly by phenological factors and their effects via competition. Leaf size, for example, may have little effect on leafmining species that require relatively small leaf areas for development or remain at low densities such that co-occurrence on leaves is rare. The latter situation is typical for most leafmining species, as it is for other guilds of phytophagous insects (Schultz 1983, Barbosa and Schultz 1987). A multitude of biotic and abiotic factors may be responsible for keeping populations of most phytophagous insects at low levels. I suggest, however, that the "ceiling" (sensu Strong 1986) on population densities of *Cameraria* and possibly other species in the leafmining guild is set by the interaction of phenological and morphological factors and competition. For example, in 1987, 50 pupae were introduced into the same branch enclosures (2.5x the number introduced in 1986) in an effort to boost densities and thus interactions. Yet leafminer abundances were not different from those in 1986 (1986 x = 0.844 (± 0.37) mines/leaf, 1987 x = 0.986 (± 0.73) mines/leaf, paired t = 1.04, df = 11, and $p > 0.20$). Branches and trees may thus be saturated in this system, although many leaves were not mined because they were either 1) of unsuitable size, 2) likely to abscise, or

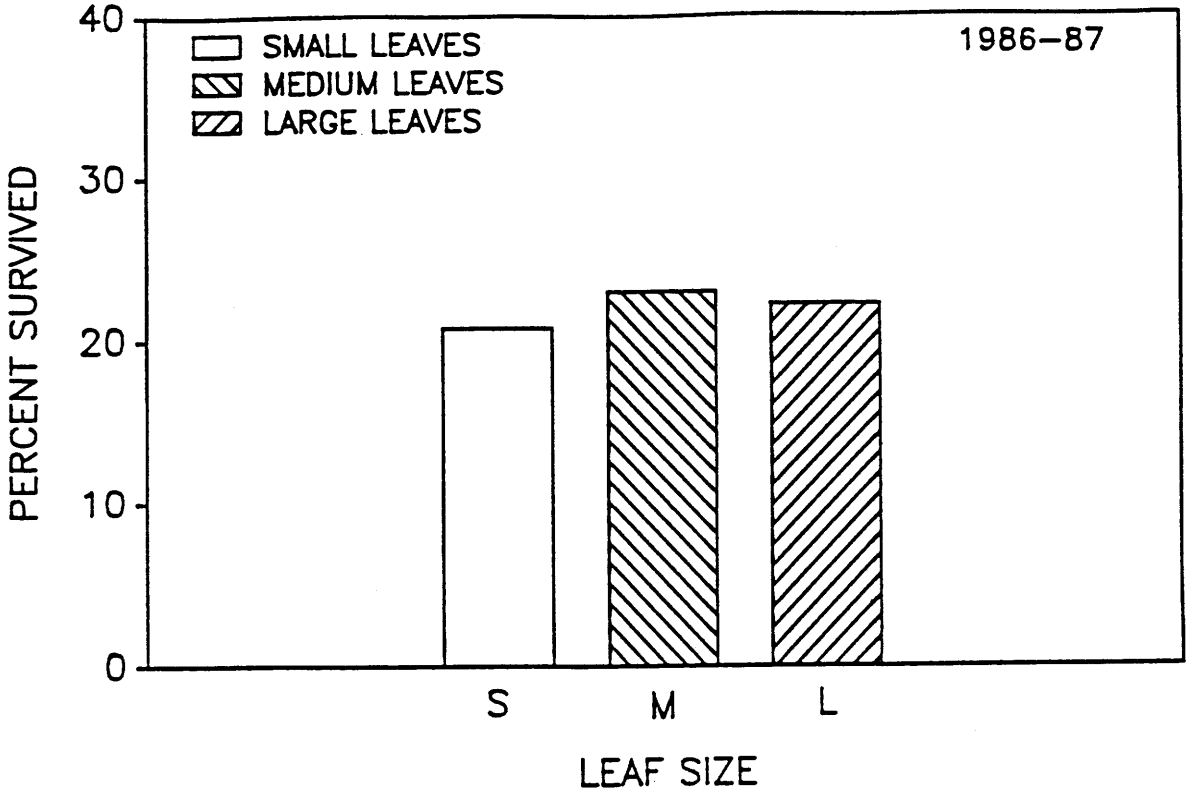

Figure 8. Percent of leafminers surviving on small-, medium-, and large-sized leaves. Data from leaves with varying numbers of mines per leaf were pooled. The interaction of leaf size and survival is not a significant term in the log linear model.

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3) unavailable for oviposition due to variable budbreak or time and energy constraints on female search. Saturation is further indicated in that leaf size per se, is not related to survival (Fig. 8). The risks of co-occurring with other conspecifics on larger leaves may be balanced by risks of mining alone on smaller leaves when densities are saturated.

The "ceiling" on *Cameraria* populations may be much lower than expectations based upon simple density estimates, especially if one considers the relationship between density and mortality at the wrong spatial scale. Variation in phenology and morphology and search constraints on females may restrict the pool of high quality leaves such that competition occurs at relatively low densities.

SUMMARY

Leafminers are endophagous insects that spend all or part of their larval feeding stages confined within leaves. Oviposition thus determines larval feeding sites among and within host trees and leaves. Host tree resistance to leafminers may be a function of initial colonization via female ovipositional preferences and the way in which these preferences translate into survival of offspring. For leafminers, ovipositional choice and offspring survival may be more closely linked to variation in tree and leaf phenology and morphology than they are for free-feeding insects.

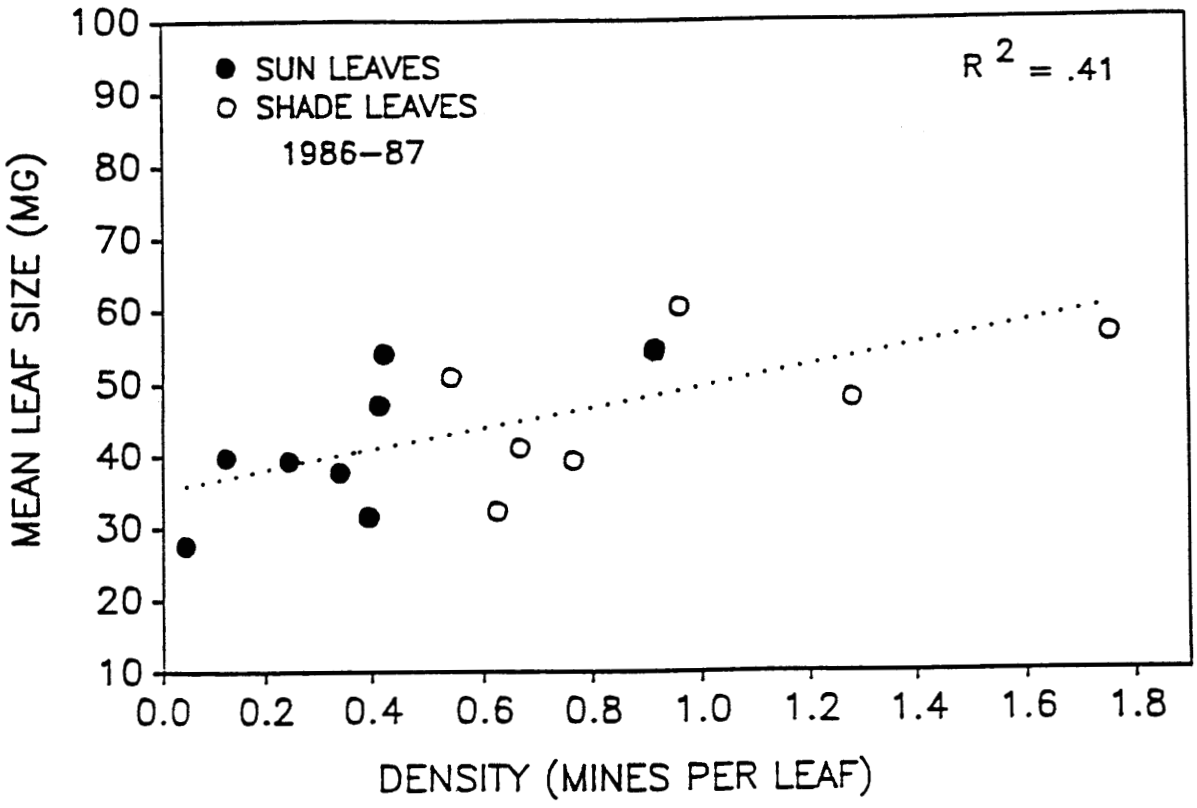

Figure 9. Regression of mean size against density of *Cameraria* on sun and shade leaves from eight Emory oak trees. Slope of the overall regression (sun and shade data combined) is significantly different from zero ($p < 0.05$).

To illustrate these arguments, patterns of abundances and distribution of a dominant leafminer, *Cameraria* sp. nov., on *Quercus emoryi* are described. These patterns appear to be determined mostly by variation in leaf sizes among and within trees, which in turn causes variation in intensity of intraspecific competition among ovipositing females and their larval offspring.

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LITERATURE CITED

ASKEW, R.R. and SHAW, M.R. 1974. An account of the Chalcidoidea (Hymenoptera) parasitising leaf-mining insects of deciduous trees in Britain. Biol. J. Linn. Soc. 6: 289-335.

ASKEW, R.R. and SHAW, M.R. 1979a. Mortality factors affecting the leaf-mining stages of *Phyllonorycter* (Lepidoptera: Gracillariidae) on oak and birch. I. Analysis of the mortality factors. Zool. J. Linn. Soc. 67: 31-49.

ASKEW, R.R. and SHAW, M.R. 1979b. Mortality factors affecting the leaf-mining stages of *Phyllonorycter* (Lepidoptera: Gracillariidae) on oak and birch. II. Biology of the parasite species. Zool. J. Linn. Soc. 67: 51-64.

AUERBACH, M.J. and SIMBERLOFF, D. 1985. Responses of leaf miners to atypical leaf production patterns. Ecol. Entomol. 9: 361-367.

AUERBACH, M.J. and SIMBERLOFF, D. 1989. Oviposition site preference and larval mortality in a leaf-mining moth. Ecol. Entomol. 14: 131-140.

BARBOSA, P. and SCHULTZ, J.C., eds. 1987. Insect Outbreaks. Academic Press, San Diego, CA. 578 p.

BERNAYS, E. and GRAHAM, M. 1988. On the evolution of host specificity in phytophagous arthropods. Ecology 69: 886-892.

BULTMAN, T.L. and FAETH, S.H. 1986a. Leaf size selection by leaf-mining insects on *Quercus emoryi* (Fagaceae). Oikos 46: 311-316.

BULTMAN, T.L. and FAETH, S.H. 1986b. Experimental evidence for intraspecific competition in a leaf-mining insect, *Cameraria* sp. nov. (Lepidoptera: Gracillariidae). Ecology 67: 442-448.

BULTMAN, T.L. and FAETH, S.H. 1986c. Selective oviposition by a leaf miner in response to temporal variation in abscission. Oecologia 69: 117-120.

BULTMAN, T.L. and FAETH, S.H. 1986d. Effect of within-leaf density and leaf size on pupal weight of the leaf-mining insect, *Cameraria* sp. nov. (Lepidoptera: Gracillariidae). Southwest. Nat. 31: 210-206.

BULTMAN, T.L. and FAETH, S.H. 1988. Abundance and mortality of leaf miners on artificially shaded Emory oak. Ecol. Entomol. 13: 131-142.

CONDRASHOFF, S.F. 1964. Bionomics of the aspen leaf miner, *Phyllocnistis populiella* Cham. (Lepidoptera: Gracillariidae). Can. Entomol. 96: 857-874.

DENNO, R.F. and McCLURE, M.S., eds. 1983. Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, New York. 717 p.

FAETH, S.H. 1980. Invertebrate predation of leaf-miners at low densities. Ecol. Entomol. 5: 111-114.

FAETH, S.H. 1985. Quantitative defense theory and patterns of feeding by oak insects. Oecologia 68: 34-40.

FAETH, S.H. 1986. Indirect interactions between temporally-separated herbivores mediated by the host plant. Ecology 67: 479-494.

FAETH, S.H. 1987. Community structure and folivorous insect outbreaks: the roles of vertical and horizontal interactions, p. 135-171. In Barbosa, P. and Schultz, J.C., eds. Insect Outbreaks. Academic Press, New York.

FAETH, S.H. 1990. Aggregation of a leafminer, *Cameraria* sp. nov. (Davis): consequences and causes. J. Anim. Ecol. 59: 569-586.

FAETH, S.H. and BULTMAN, T.L. 1986. Interacting effects of increased tannin levels on leaf-mining insects. Entomol. Exp. Appl. 40: 297-300.

FAETH, S.H. and SIMBERLOFF, D. 1981. Population regulation of a leaf-mining insect, *Cameraria* sp. nov., at increased field densities. Ecology 62: 620-624.

FAETH, S.H., MOPPER, S., and SIMBERLOFF, D. 1981a. Abundance and diversity of leaf-mining insects on three oak species: effects of host-plant phenology and nitrogen content of leaves. Oikos 37: 238-251.

FAETH, S.H., CONNOR, E.F., and SIMBERLOFF, D. 1981b. Early leaf abscission: a neglected source of mortality for folivores. Am. Nat. 117: 409-415.

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.

GODFRAY, H.C.J. 1986. Clutch size in a leaf-mining fly (*Pegomyza nigrotarsis*: Anthomyiidae). Ecol. Entomol. 11: 75-81.

GREEN, D.S. 1979. Ecology and host-specificity of parasitoids of leaf-mining Lepidoptera on *Quercus agrifolia* (Fagaceae) in California. Ph.D. diss. Univ. of California, Berkeley, CA.

GROSS, P. and PRICE, P.W. 1988. Plant influences on parasitism of two leafminers: a test of enemy-free space. Ecology 69: 1506-1516.

HARTLEY, S.E. and LAWTON, J.H. 1987. Effects of different types of damage on the chemistry of birch foliage, and the responses of birch feeding insects. Oecologia 74: 432-437.

HAWKINS, B.A. 1988. Foliar damage, parasitoids and indirect competition: a test using herbivores of birch. Ecol. Entomol. 13: 301-308.

HEADS, P.A. and LAWTON, J.H. 1983. Studies on the natural enemy complex of the holly leaf-miner: the effects of scale on the detection of aggregative responses and the implications for biological control. Oikos 40: 267-276.

HERING, E.M. 1951. The biology of the leaf miners. Uitgeveverij Dr W. Junk, Gravenhage, Netherlands. 420 p.

JENKINS, S.H. 1975. Food selection by beavers: a multidimensional contingency table analysis. Oecologia 21: 157-173.

KAHN, D.M. and CORNELL, H.V. 1983. Early leaf abscission and folivores: comments and considerations. Am. Nat. 122: 428-432.

KAREIVA, P. 1983. Influence of vegetation texture on herbivore populations: resource concentration and herbivore movement, p. 259-512. In Denno, R.F. and McClure, M.S., eds. Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, New York.

LAWTON, J.H. and MACGARVIN, M. 1986. The organization of herbivore communities, p. 163-186. In Kikkawa, J. and Anderson, D.J., eds. Community Ecology: Pattern and process. Blackwell, Oxford.

MATTSON, W.J., LAWRENCE, R.K., HAACK, R.A., HERMS, D.A., and CHARLES, P.-J. 1988. Defensive strategies of woody plants against different insect-feeding guilds in relation to plant ecological strategies and intimacy of association with insects, p. 3-38. In Mattson, W.J, Levieux, J., and Bernard-Dagan, C., eds. Mechanisms of Woody Plant Defenses against Insects: Search for Pattern. Springer-Verlag, New York.

McNEIL, J.N. and QUIRING, D.T. 1983. Evidence of an oviposition-deterring pheromone in the alfalfa blotch leafminer, *Agromyza frontella* (Rondani) (Diptera: Agromyzidae). Environ. Entomol. 12: 990-992.

MURAI, M. 1974. Studies on the interference among larvae of the citrus leaf miner, *Phyllocnisti citrella* Stainton (Lepidoptera: Phyllocnistidae). Res. Popul. Ecol. (Kyoto) 16: 80-111.

NEEDHAM, J.G., FROST, S.W., and TOTHILL, B.H. 1928. Leaf-mining insects. Williams & Wilkin Co., Baltimore, MD. 351 p.

OPLER, P.A. 1974. Biology, ecology, and host specificity of microlepidoptera associated with *Quercus agrifolia* (Fagaceae). Univ. of California Press, Berkeley, CA.

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 microlepidoptera. Annu. Rev. Entomol. 25: 133-159.

PRITCHARD, I.M. and JAMES, R. 1984. Leaf fall as a source of miner mortality. Oecologia 64: 140-141.

QUIRING, D.T. and McNEIL, J.N. 1984. Exploitation and interference intraspecific larval competition in the dipteran leaf miner, *Agromyza frontella* (Rondani). Can. J. Zool. 62: 421-427.

QUIRING, D.T. and McNEIL, J.N. 1987. Foraging behavior of a dipteran leaf miner on exploited and unexploited hosts. Oecologia 73: 7-15.

ROONEY, R.F., III. 1989. The effects of altered phenology on folivory in Gambel oak (*Quercus gambelii*). M.S. thesis. Arizona State Univ., Tempe, AZ.

SCHULTZ, J.C. 1983. Habitat selection and foraging tactics of caterpillars in heterogenous trees. p. 61-90. In Denno, R.F. and McClure, M.S., eds. Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, New York.

SIMBERLOFF, D. and STILING, P. 1988. Larval dispersion and survivorship in a leaf-mining moth. Ecology 69: 1647-1657.

SPENCER, K.C., ed. 1988. Chemical Mediation of Coevolution. Academic Press, San Diego, CA. 609 p.

STILING, P.D. 1987. The frequency of density dependence in insect host-parasitoid systems. Ecology 68: 844-856.

STILING, P.D., BRODBECK, B., and STRONG, D.R. 1982. Foliar nitrogen and larval parasitism as determinants of leafminer distribution patterns on *Spartina alterniflora*. Ecol. Entomol. 7: 447-452.

STILING, P.D., BRODBECK, B., and STRONG, D.R. 1984. Intraspecific competition on *Hydrellia valida* (Diptera: Ephydridae), a leaf miner of *Spartina alterniflora*. Ecology 65: 660-662.

STILING, P.D., SIMBERLOFF, D., and ANDERSON, L.C. 1987. Non-random distribution patterns of leaf miners on oak trees. Oecologia 73: 116-119.

STRONG, D.R. 1986. Density vagueness: abiding the variance in the demography of real populations, p. 257-268. In Diamond, J. and Case, T.J., eds. Community Ecology. Harper & Row, New York.

STRONG, D.R., Jr., LAWTON, J.H., and SOUTHWOOD, T.R.E. 1984. Insects on plants. Community Patterns and Mechanisms. Blackwell Scientific, London. 313 p.

SUGIMOTO, T., SHIMONO, Y., HATA, Y., NAKAI, A., and YAHARA, M. 1988. Foraging for patchily-distributed leaf-miners by the parasitoid, *Dapsilarthra rufiventris* (Hymenoptera: Braconidae). III. Visual and acoustic cues to close range patch-location. Appl. Entomol. Zool. 23: 113-121.

TUOMI, J., NIEMELÄ, P., and MANNILA, R. 1981. Leaves as islands: interactions of *Scolioneura betuleti* (Hymenoptera) miners in birch leaves. Oikos 37: 146-152.

VEPSÄLÄINEN, K., SAVOLAINEN, R., and PENTTINEN, A. 1988. Causal reasoning in modelling multiway contingency tables. Oikos 53: 281-285.

WEST, C. 1985. Factors underlying the late seasonal appearance of the lepidopterous leaf-mining guild on oak. Ecol. Entomol. 10: 111-120.

WILLIAMS, A.G. and WHITHAM, T.G. 1986. Premature leaf abscission: an induced defense against gall aphids. Ecology 67: 1619-1627.

WILLIAMS, H.J., ELZEN, G.W., and VINSON, S.B. 1988. Parasitoid-host-plant interactions, emphasizing cotton (*Gossypium*), p. 171-200. In Barbosa, P. and Letourneau, D.K., eds. Novel Aspects of Insect-Plant Interactions. John Wiley & Sons, New York.

ZUCKER, W.V. 1982. How aphids choose leaves: the roles of phenolics in host selection by a galling aphid. Ecology 63: 972-981.