

## Leaf mines: their effect on leaf longevity

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**Summary.** The effects of a number of factors, notably leaf mining insects, on the longevity of beech and holm oak leaves have been studied. The regular monitoring of individually labelled leaves was complemented by analysis of leaf fall data. Both methods confirm that these mining insects have only a slight impact on their host trees. The presence of first generation *Phyllonorycter maestingella* mines on beech leaves and winter generation *P. messaniella* mines on holm oak leaves accelerates leaf loss. Beech leaves mined by second generation *P. maestingella* and *Rhynchaenus fagi* did not show this accelerated loss. Their patterns of leaf fall can be explained by within-tree variation in both mine distribution and the timing of leaf fall. It is argued that this premature leaf fall is a damage response, and is not an attempt by the tree to regulate miner numbers.

### Introduction

In response to herbivory plants exhibit a number of responses at the individual leaf level. These range from changes in leaf chemistry to leaf loss, this latter being commonly associated with heavy herbivore consumption (Hill 1980; Hileman and Lieto 1981; Haukioja and Niemela 1977, 1979; Edwards and Wratten 1983). In this study the effect of damage by leaf mining insects, commonly found at low population densities, on leaf longevity has been considered.

A change in the balance of various chemicals within the leaf initiates senescence and abscission (Addicott and Lynch 1955; Addicott 1980). Cytokinins delay senescence and a fall in their concentration within the leaf is necessary for the initiation of these changes. The mines of *Phyllonorycter* (Lepidoptera: Gracillariidae) species are known to contain high levels of cytokinins (Engelbrecht 1971). Thus one could postulate that miners, through the action of cytokinin, could delay leaf senescence. An alternative view is that mined leaves senesce earlier than unmined leaves as a result of some form of damage response. It has also been argued that the plant could be actively trying to reduce miner numbers (Owen 1978) or it may be that mined leaves having fewer nutrients to mobilise than undamaged leaves senesce early. A third possibility is that there is no effect on leaf longevity because the effect of a single mine on a leaf is inconsequential.

Despite the leaf miner – host plant relationship providing a good study system little quantitative work on the effects of these insects on leaf longevity has been performed. Owen (1978) considered the proportion of holly (*Ilex aquifolium* L.) leaves collected by leaf fall traps that had been mined by *Phytomyza ilicis* Curtis. Faeth et al. (1981) compared the proportion of mined oak leaves collected in leaf fall traps with that of leaves remaining on the trees. As will be shown later this type of approach takes no account of within-tree variation in mine density or in the timing of leaf fall. Hileman and Lieto (1981) labelled individual *Chaemaedaphne calyculata* (L.) (Ericaceae) leaves and compared the survivorship of mined and unmined leaves.

In this study both leaf labelling and the collection of falling leaves were employed to determine the pattern of leaf fall in both holm oak and beech. This leads to a better understanding of the role of *Phyllonorycter* miners on leaf longevity and their importance in relation to other causes of leaf death. Although some experimental manipulation has been performed much of this work is based on statistical comparisons of leaf fall (or death) between damaged and undamaged leaves.

### Methods

Two beech trees (*Fagus sylvatica* L.) were sampled in Felbrigg Great Wood, near Cromer, Norfolk (TG 200 403). They were located at the boundary of an extensive area of beech trees and an area of mixed woodland (*Quercus robur* L., *Acer pseudoplatanus* L., and *Castanea sativa* Mill.). Both trees possessed substantial lower canopies, and were 20–25 m tall.

The two holm oaks (*Q. ilex* L.) were located at Taverham, Norfolk (TG 152 142), surrounded by a mixed oak/ beech/birch (*Betula pendula* Roth) woodland. Tree 1 was 15 m tall, Tree 2 10 m tall.

Data were obtained for 3 leaf miners – *Phyllonorycter maestingella* (Müller) and *Rhynchaenus fagi* L. (Coleoptera: Curculionidae) on beech and *Phyllonorycter messaniella* (Zeller) on holm oak. The monophagous *P. maestingella* is bivoltine, mining occurring during June–July and August–October. The blotch mine of the first three instars develops into the fold mine characteristic of the genus during the 4th instar. During the blotch mine stage the mine is visible only on the lower surface of the leaf, upper surface damage through feeding occurring only during the fold mine stage. After the cessation of mining the upper surface

of both types of mine may be damaged as a result of tissue death. In subsequent analysis only upper surface damage is considered. Data were obtained for the two 1982 generations. *P. messaniella* has three generations, two in summer being spent largely on deciduous oaks but the overwintering generation feeds on holm oak. Mine form is similar to that of *P. maestingella*. The data relates to the 1981/2 winter generation. *R. fagi* is univoltine. Eggs are usually laid in the midrib following bud burst, mining occurring during May/June. As the larva grows, the linear mine develops into a blotch mine which can destroy large areas of leaf tissue. Data pertain to the 1982 generation.

Four traps were placed under each of two trees for each tree species (0.5 m<sup>2</sup> for beech and 0.25 m<sup>2</sup> for holm oak). Each trap comprised a square metal frame about 0.5 m above ground level supporting a bag of fine Terylene mesh designed to intercept falling leaves before they reached the ground. At fortnightly intervals the beech traps were emptied (monthly for holm oak) and the number of mines and leaves scored.

Because holm oak trees bear up to 4 year classes of leaves the leaves collected in the leaf fall traps were examined and sorted into age classes. Aging was based on a number of factors – leaf toughness and flexibility; mine age; leaf cleanliness (amount of algal growth) – and classified according to the year in which bud burst occurred. Because of possible aging errors of older leaves only 3 classes were used – pre-1981, 1981 and 1982 leaves.

Each leaf on individual branches were labelled for each tree species on completion of leaf expansion. On 18/5/82 629 (Tree 1) and 725 (Tree 2) beech leaves were colour-coded on their petioles with Staedtler Lumocolor pens. On 15–16/6/81 469 (Tree 1) and 432 (Tree 2A) and 523 (Tree 2B) outer canopy holm oak leaves were colour-coded on their petioles using Humbrol modelling paint. (Preliminary investigation showed no short-term leaf mortality resulting from labelling using the marking materials of the study.)

Beech leaves were examined at fortnightly intervals (monthly for holm oak) and the type and amount (% upper surface area) of damage recorded. The longevity of individual leaves, and the damage experienced by them, could thus be estimated. The length of labelled leaves was measured and their areas estimated from linear relationships established between leaf length and area for adjacent samples of leaves. Such areas were obtained from an Apple II micro-computer by delineating leaf outlines and damaged areas on to an Apple graphics tablet.

The following analysis assumes that leaves died (or were damaged) on the day of sampling, thus giving a conservative estimate of leaf damage.

## Results

For holm oak the number of labelled leaves (1981 cohort) found dead on each sample date is given in Table 1 and the number of leaves in each age class from the traps is given in Table 2. Leaf fall was continuous throughout the year but was concentrated between May and September (e.g. Tree 1 90.6% in 1981, 88.7% in 1982 of annual leaf fall.)

For beech Table 3 gives the number of labelled leaves dying between sample dates on each tree and the number of leaves collected by the traps of each tree during each

**Table 1.** The number of labelled holm oak leaves dying on each sample date

Date	Tree 1	Tree 2A	Tree 2B
1981			
Jun	36	22	76
Jul	3	6	3
Aug	2	1	0
Sep	3	1	2
Oct	0	0	0
Nov	0	0	1
Dec	8	1	0
1982			
Jan	3	0	1
Feb	19	1	7
Mar	3	4	6
Apr	3	0	1
May	1	1	3
Jun	29	49	36
Jul	18	43	50
Aug	3	10	16
Sep	7	28	7
Oct	1	3	4
Nov	0	0	0
Dec	0	1	1
1983			
Jan	0	0	3
Feb	0	2	0
Mar	0	2	0
Apr	13	11	2
May	14	15	39
Jun	154	111	172
Jul	123	111	87
Aug	16	3	6
Alive	10	6	0
Total	469	432	523

sample period. Both methods show that leaf death/loss is continuous throughout the field season although most leaves died in late October and early November. Earlier deaths were recorded in leaf fall than labelled samples, the reverse of that expected since labelling records leaf death whereas leaf fall sampling records leaf loss from the tree. As death usually precedes leaf fall an earlier distribution of deaths was expected in the labelling experiment.

## Factors affecting leaf death

### 1) Leaf size and position on the branch

Relationships between leaf area ( $X$ ) and longevity ( $Y$ ) were found for the labelled samples of each tree species (using only undamaged leaves).

For beech, although a linear relationship was significant  $r_s$  values (Spearman rank correlation) were larger than  $r$  values (Product-moment correlation) suggesting a non-linear relationship

$$\text{Tree 1 } Y = 0.502X + 158.413, n = 175, p < 0.001; \\ r = 0.2862, p < 0.001; r_s = 0.4874, p < 0.001$$

$$\text{Tree 2 } Y = 0.953X + 157.129, n = 175, p < 0.001; \\ r = 0.3908, p < 0.001; r_s = 0.5451, p < 0.001$$

Beech leaves are grouped into clusters of 2–7 (usually 4) leaves, the apical leaf typically being the largest with a progression in size down to the basal leaf. To test whether

**Table 2.** Monthly holm oak leaf fall

Date	Leaf age class				
	Pre-1981	1981	1982	Total	% annual leaf fall
<b>a. Tree 1</b>					
1981	Apr	35		35	1.0
	May	635		635	17.2
	Jun	1240		1322	35.9
	Jul	688	118	806	21.9
	Aug	274	53	327	8.9
	Sep	167	79	246	6.7
	Oct	25	19	44	1.2
	Nov	12	23	35	1.0
	Dec	8	14	22	0.6
1982	Jan	3	10	13	0.4
	Feb	54	56	110	3.0
	Mar	35	53	88	2.4
	Apr	59	20	79	3.2
	May	198	516	717	29.1
	Jun	269	806	1100	44.6
	Jul	56	81	142	5.8
	Aug	60	84	149	6.9
	Sep	23	38	79	3.2
	O/N	0	11	19	0.8
1983	D/F	0	18	23	0.9
	Mar	7	66	158	6.4
<b>b. Tree 2</b>					
1981	Apr	216		216	4.2
	May	1201		1201	23.4
	Jun	978	274	1252	24.3
	Jul	598	250	848	16.5
	Aug	544	54	598	11.6
	Sep	254	59	313	6.1
	Oct	19	21	40	0.8
	Nov	40	39	79	1.5
	Dec	16	15	31	0.6
1982	Jan	3	5	8	0.2
	Feb	237	118	355	6.9
	Mar	102	101	203	4.0
	Apr	39	50	89	2.0
	May	458	609	1073	24.6
	Jun	451	663	1194	27.4
	Jul	172	198	390	8.9
	Aug	121	226	475	10.9
	Sep	7	69	630	14.4
	O/N	1	40	63	1.4
1983	D/F	1	27	233	5.3
	Mar	14	86	217	5.0

(annual leaf fall = number of leaves falling off between April and March)

the size-longevity relationship was generated by sequential leaf loss according to cluster position a multiple regression was performed on data from 15 four-leaf clusters from Tree 1, the apical leaf numbered 1 and the basal leaf numbered 4:

Leaf life =  $0.535 \times \text{Leaf length} + 1.064 \times \text{Cluster position} + 130.184$ ,  $r = 0.4859$ ,  $p < 0.001$ ;

where  $t$  (leaf length) = 2.997,  $p < 0.01$   
 $t$  (cluster position) = 0.504, ns  
 $t$  (intercept) = 8.345,  $p < 0.001$

**Table 3.** The number of beech leaves recorded as dead during each fortnightly sample period

Date	Leaf fall		Labelled leaves					
	Num-ber	%	Num-ber	%	Num-ber	%		
May (1)	3	0.03	7	0.06	0	0	1	0.14
May (2)	1	0.01	14	0.12	0	0	1	0.14
Jun (1)	17	0.16	48	0.41	0	0	2	0.28
Jun (2)	12	0.11	17	0.15	0	0	1	0.14
Jul (1)	50	0.47	95	0.82	0	0	2	0.28
Jul (2)	36	0.34	91	0.78	4	0.64	1	0.14
Aug (1)	208	1.94	319	2.75	3	0.48	12	1.66
Aug (2)	338	3.16	208	1.79	12	1.91	7	0.97
Sep (1)	642	5.99	472	4.07	19	3.02	11	1.52
Sep (2)	1255	11.71	1393	12.01	35	5.56	39	5.38
Oct (1)	2459	22.95	2281	19.67	99	15.74	103	14.21
Oct (2)	5247	48.98	5273	45.46	432	68.68	271	37.38
Nov (1)	445	4.15	1328	11.45	25	3.97	262	36.14
Nov (2)	0	0	52	0.45	0	0	12	1.66
Total	10713		11598		629		725	

Where (1) relates to samples taken at the end of the first half of the month, (2) relates to samples taken at the end of the second half of the month

Thus leaf size is the important factor. This is confirmed when the leaf areas are pooled and total cluster area ( $Y$ ) related to mean leaf life ( $X$  – measured in sample periods):

$Y = 0.013X + 8.530$ ,  $n = 15$ ,  $p < 0.05$ ;

$r = 0.5875$ ,  $p < 0.05$ ;  $r_s = 0.7108$ ,  $p < 0.01$

The relationships between leaf length and longevity in holm oak explained only 3.2–6.2% of the observed variation in longevity

Tree 1  $Y = 0.081X + 17.578$ ,  $n = 170$ ,  $p < 0.05$ ,  
 $r = 0.1790$ ,  $p < 0.05$

Tree 2A  $Y = 0.085X + 17.904$ ,  $n = 105$ ,  $p < 0.05$ ,  
 $r = 0.2348$ ,  $p < 0.05$

Tree 2B  $Y = 0.107X + 17.240$ ,  $n = 264$ ,  $p < 0.001$ ,  
 $r = 0.2485$ ,  $p < 0.001$

During May and June new holm oak growth is produced from both terminal and axillary buds. Old leaves whose axillary buds develop are more likely to die during this period than leaves whose buds do not develop (Tav 1  $\chi^2 = 6.953$ ,  $p < 0.01$ ; Tav 2A  $\chi^2 = 15.709$ ,  $p < 0.001$ ; Tav 2B  $\chi^2 = 10.589$ ,  $p < 0.01$ ; all  $df = 1$ ) – using only undamaged, undamaged leaves.

## 2) Damage

a) *Beech. Experimental damage:* Following leaf expansion and the initiation of *R. fagi* mining, the leaves of a branch were damaged by cutting with a pair of scissors on 18/5/82. All naturally damaged leaves, and those outside the length range 40–50 mm, were excluded from the experiment. Set amounts of damage were assigned randomly to individual leaves which were subsequently examined at weekly intervals. Each treatment consisted initially of 30 replicates but subsequent herbivore damage resulted in some leaves being

omitted from the analysis. Leaves were scored as alive or dead, for each damage category (0, 1, 5, 10, 25, 50 and 75% of leaf area removed) at approximately weekly intervals.

$\chi^2$  analyses of the cumulative number of leaves that had died by successive sample dates show no significant differences between treatments and control until 15/10/82. By this date more leaves with 75% of area removed had died than undamaged leaves ( $\chi^2=5.041$ ,  $df=1$ ,  $p<0.05$ ). Also on this date the proportion of dead leaves in each category ( $Y$ ) was linearly related to the amount (%) of damage ( $X$ ):

$$Y=0.0033X+0.1585, n=7, p<0.05; r=0.7756, p<0.05.$$

By 21/10/82 all treatments of more than 5% damage showed greater mortality than the control group (10%, 25% -  $\chi^2=10.012$ ,  $p<0.01$ ; 50% -  $\chi^2=21.849$ ,  $p<0.001$ ; 75% -  $\chi^2=25.477$ ,  $p<0.001$ ; all  $df=1$ ) and a linear relationship still held:

$$Y=0.0074X+0.4628, n=7, p<0.05; r=0.8688, p<0.05.$$

After this date leaf death was widespread, most undamaged leaves died and so significant differences with the control were lost.

Thus the removal of 10% or more of the lamina following leaf expansion leads to premature death. This was apparent towards the end of the season for about a two week period.

*Total natural damage:* Many beech leaves experience loss of photosynthetic tissue (Tree 1 - 62.2%; Tree 2 - 52.1% of labelled leaves). As the type, amount and time of occurrence of damage is known for each leaf it is possible to calculate the area lost. In the following analysis damage values have been weighted to reflect the fact that a unit area of leaf tissue lost early in the growing season is a more serious loss to the tree than the same area lost close to autumnal leaf fall.

$$\text{Cumulative damage (CD)} = \frac{\text{DH} \times \text{TH}}{\text{T}}$$

where DH = % leaf area damaged by factor H

TH = duration of damage (days)

T = "typical" leaf life (date by which over 95% of sample died

- Tree 1 170 days; Tree 2 184 days).

When the distribution of deaths in each of 4 categories (0.1-4.9%, 5-9.9%, 10-19.9% & 20+%) of cumulative damage from all natural sources is compared against that of undamaged leaves, significant differences are found at the higher damage levels. Thus for Tree 1 cumulative damage  $\geq 20\%$  ( $\chi^2=13.883$ ,  $df=1$ ,  $p<0.001$ ) and for Tree 2  $\geq 10\%$  ( $\chi^2=27.43$ ,  $df=4$ ,  $p<0.001$ ) produce earlier leaf death relative to undamaged leaves.

*P. maestingella damage:* In the following analysis all *P. maestingella* mined leaves are considered. Table 4 gives the number of first generation mined leaves captured in each sample period by the leaf fall traps.

$\chi^2$  values (based on expected values generated by multiplying the overall proportion of first generation leaves mined by the number of leaves captured during the sample

**Table 4.** The timing of first generation *P. maestingella* mined beech leaves collected in the leaf fall traps

Date	Number of mined leaves		$\frac{(O-E)^2}{E}$	Sign of O-E
	Observed	Expected		
<b>a. Tree 1</b>				
M+J	2	4.4	1.31	0
Jul (1)	20	6.7	26.40	+
Jul (2)	7	4.8	1.01	+
Aug (1)	71	27.7	67.69	+
Aug (2)	109	45.1	90.54	+
Sep (1)	95	85.6	1.03	+
Sep (2)	222	167.3	17.88	+
Oct (1)	438	327.8	37.05	+
Oct (2)	437	699.4	98.45	-
Nov (1)	27	59.3	17.59	-
Total	1428		$\chi^2=358.95$ , $df=9$ , $p<0.001$	
Proportion mined leaves = 0.1333				
<b>b. Tree 2</b>				
M+J	1	9.7	7.80	-
Jul (1)	10	10.6	0.03	0
Jul (2)	22	10.2	13.65	+
Aug (1)	89	35.6	80.1	+
Aug (2)	44	23.2	18.65	+
Sep (1)	55	52.7	0.10	+
Sep (2)	194	155.5	9.53	+
Oct (1)	320	254.6	16.8	+
Oct (2)	506	588.5	11.57	-
Nov (1)	50	148.2	65.07	-
Nov (2)	3	5.8	1.35	-
Total	1294		$\chi^2=224.65$ , $df=10$ , $p<0.001$	
Proportion mined leaves = 0.1116				

Expected values calculated by multiplying the number of leaves per sample by the overall proportion of mined leaves

period in question) were calculated. The comparisons were highly significant for both trees (Table 4). Similarly for the labelled samples first generation mined leaves die earlier than unmined leaves on both trees (Tree 1  $\chi^2=17.929$ ,  $df=4$ ,  $p<0.001$ ; Tree 2  $\chi^2=16.439$ ,  $df=4$ ,  $p<0.001$ ). For both techniques first generation mined leaves died or fell off earlier than expected. (It should be noted that the area damaged by *P. maestingella* rarely reaches the levels shown earlier to be necessary for the premature fall of a leaf damaged by other means.)

First generation mined leaves have a significantly different temporal distribution of deaths to leaves bearing quantitatively similar levels of damage not caused by miners. On both trees the mined labelled leaves tended to die earlier (Tree 1  $\chi^2=19.602$ ,  $df=1$ ,  $p<0.001$ ; Tree 2  $\chi^2=15.314$ ,  $df=2$ ,  $p<0.001$ ).

The number of second generation mined leaves from the leaf fall traps is given in Table 5. The number of leaves in each sample recorded prior to Sept 1 has been corrected to allow for the variation in the time of mine appearance.

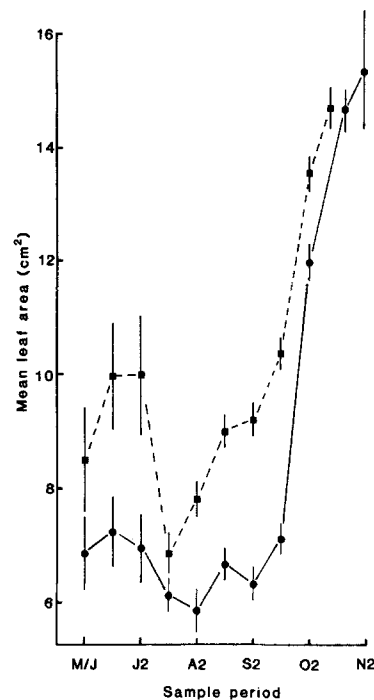
**Table 5.** The timing of second generation *P. maestingella* mined leaves collected in the leaf fall traps

Date	Number of mined leaves		Total leaves	$\frac{(O-E)^2}{E}$	Sign of O-E
	Observed	Expected			
<b>a. Tree 1</b>					
Aug (1)	0	16.4	208 (88.4)	16.4	-
Aug (2)	17	49.7	338 (268)	21.51	-
Sep (1)	93	119.1	642	5.72	-
Sep (2)	201	232.8	1255	4.34	-
Oct (1)	395	456.1	2459	8.19	-
Oct (2)	1125	973.3	5247	23.64	+
Nov (1)	99	82.5	445	3.30	+
Total	1930		10404.4	$\chi^2=83.1$ , df=6, $p<0.001$	
<b>b. Tree 2</b>					
Aug (1)	3	15.0	319 (113.6)	9.6	-
Aug (2)	9	23.3	208 (176.4)	8.78	-
Sep (1)	40	62.3	472	7.98	-
Sep (2)	115	183.7	1393	25.69	-
Oct (1)	201	300.9	2281	33.17	-
Oct (2)	925	695.5	5273	75.73	+
Nov (1)	164	175.2	1328	0.72	0
Nov (2)	6	6.9	52	0.12	0
Total	1463		11089	$\chi^2=161.79$ , df=7, $p<0.001$	

Values in parentheses are the corrected values of the number of leaves collected, based on the proportion of second generation mines visible by this time

This correction involves multiplying the number of leaves by the proportion of second generation mines discovered at the end of each sample period during the leaf labelling experiment. Comparing the observed number of mined leaves per sample with that expected on a proportionality basis, significant differences were found (Table 5). All samples prior to Oct (2) recorded fewer mined leaves than expected. For Tree 1 traps the last two sample periods produced more mined leaves than expected; for Tree 2 traps more mined leaves were recorded in Oct (2) but there was little difference between observed and expected values during November (1) and (2) (Table 5).

In the case of labelled leaves there was no difference between second generation mined and unmined leaves in their time of death (Tree 1  $\chi^2=5.169$ , df=5, ns; Tree 2  $\chi^2=8.838$ , df=4, ns). The mean area of fallen leaves observed during the sample period increased (Fig. 1). Although positive correlations between leaf size and longevity have already been demonstrated for the labelled leaf samples these relationships are considered insufficient to generate the changes in fallen leaf area. I have shown elsewhere (Pritchard 1983) that leaf area decreased with height and that high canopy leaves tended to die earlier than mid and low canopy leaves. Thus the effects observed in the leaf fall experiment of an apparent delay in the fall of mined leaves may not be directly attributable to mining but be a consequence of the time of leaf fall varying over the trees. For a factor concentrated in the mid canopy (as were second generation *P. maestingella* mines (Pritchard 1983)) there



**Fig. 1.** Changes in leaf area in samples taken throughout the year from the beech leaf fall traps (Tree 1 -■---■-; Tree 2 -●---●-; mean  $\pm 1$  S.E.)

would be an increasing proportion of mined leaves during leaf fall, perhaps followed by a levelling off or decline as more low canopy leaves were shed. This pattern was observed for Tree 2 and to some extent for Tree 1.

*R. fagi* damage: When the number of *R. fagi* mined leaves is compared with the number expected for a constant proportion throughout the season (Table 6) both trees show significant differences. For Tree 1 during Aug (2) and Sept (2) more mined leaves were lost than expected, and during Nov (1) fewer mined leaves were shed than expected. In general however there was a close similarity between observed and expected values. In contrast during six sample periods fewer mined leaves were lost from Tree 2, whilst during Oct (1) and (2) more mined leaves were lost than expected.

The labelled leaf experiment revealed no differences between the times of death of *R. fagi* mined and unmined leaves (Tree 1  $\chi^2=1.609$ , df=4, ns; Tree 2  $\chi^2=2.50$ , df=5, ns). However using only the data for those leaves where more than 20% of the leaf area was destroyed by *R. fagi* mines showed earlier leaf death than unmined leaves (Tree 1  $\chi^2=5.227$ ,  $n=521$ , df=1,  $p<0.05$ ). There was insufficient Tree 2 data for such analysis. Thus damage by *R. fagi* miners is relatively unimportant in producing the observed leaf fall pattern.

The results of Table 6 appear contradictory. The different patterns of leaf fall indicated for the two trees may result from differences in *R. fagi* mine distribution with height. Tree 1 had an even mine distribution (at 1.5 m mines per 1,000 leaves (MPT)=314; at 10 m MPT=289) unlike Tree 2 (at 1.5 m MPT=214; at 10 m MPT=83). In Tree 2, with most mines low in the tree, the tendency for high canopy leaves to die earlier than those in the low canopy could lead to the observed pattern of initially fewer mined leaves falling followed by more mined leaves falling than

**Table 6.** Comparison of the timing of *R. fagi* mined leaf loss with a constant mined leaf loss by  $\chi^2$  analysis

Date	Number of <i>R. fagi</i> mined leaves		$\frac{(O-E)^2}{E}$	Sign of O-E
	Observed	Expected		
<b>a. Tree 1</b>				
M+J+J	27	26.4	0.014	0
Aug (1)	48	46.2	0.07	0
Aug (2)	97	75.1	6.386	+
Sep (1)	138	142.7	0.155	0
Sep (2)	304	278.9	2.259	+
Oct (1)	558	546.4	0.246	0
Oct (2)	1147	1165.9	0.306	0
Nov (1)	61	98.9	14.524	-

$\chi^2 = 23.96$ ,  $df = 7$ ,  $p < 0.01$

**b. Tree 2**

M+J	9	17.4	4.055	-
Jul (1)	10	19.2	4.408	-
Jul (2)	12	18.4	2.226	0
Aug (1)	62	64.4	0.089	0
Aug (2)	18	42.0	13.714	-
Sep (1)	96	95.2	0.007	0
Sep (2)	251	281.1	3.223	-
Oct (1)	498	460.3	3.088	+
Oct (2)	1195	1064.1	16.103	+
Nov (1)	189	268.0	23.287	-
Nov (2)	0	10.5	10.5	-

$\chi^2 = 203.82$ ,  $df = 10$ ,  $p < 0.001$

expected. With a more even distribution of *R. fagi* no difference between observed and expected values would be predicted as accords reasonably with Tree 1 data.

*b) Holm oak.* Leaf death and fall show a markedly seasonal pattern, associated with the annual leaf flush and, to a lesser extent, with severe winter weather (Pritchard 1983). In the period June-October 1982 labelled leaf mortality was 12.4-30.8%. A number of factors were identified as promoting the likelihood of leaf death.

Table 7 shows that leaves bearing one fold mine (from the 1982-2 generation) have a higher mortality than unmined leaves for each sample at this time. This increased mortality was not shown for leaves bearing single blotch mines, but when all blotch mined leaves were considered both Tree 2 samples showed significantly higher leaf mortality - there were few leaves bearing more than one blotch mine on Tree 1. Similarly, increased numbers of fold mines per leaf lead to higher leaf mortality. Confirmation of this pattern comes from consideration of the leaf fall data. Table 8 gives the number of mined 1981 leaves per sampling period and compares this with that expected as a constant proportion throughout the year. For each tree greater mined leaf mortality was noted during May and June 1982 than expected on a proportionality basis.

The number of caterpillar damaged leaves was low in most samples. However Tree 2A leaves that had experienced caterpillar damage  $\geq 10\%$  of leaf area in June 1981 showed a significantly higher June-October 1982 mortality than undamaged leaves ( $\chi^2 = 4.354$ ,  $df = 1$ ,  $p < 0.05$ ).

**Table 7.** Comparison of numbers of *P. messaniella* mined and unmined holm oak leaves dying between June and October 1982

No. mines	Type	$\chi^2$	<i>p</i>
<b>a. Tree 1</b>			
1	Blotch	0.037	ns
1 or more	Blotch	0.126	ns
1	Fold	20.892	<0.001
1 or more	Fold	28.132	<0.001
<b>b. Tree 2A</b>			
1	Blotch	1.786	ns
1 or more	Blotch	6.526	<0.01
1	Fold	28.516	<0.001
1 or more	Fold	41.255	<0.001
<b>c. Tree 2B</b>			
1	Blotch	2.927	ns
1 or more	Blotch	8.189	<0.01
1	Fold	8.682	<0.01
1 or more	Fold	12.456	<0.001

(all  $df = 1$ )

Using only undamaged leaves without new shoot growth

**Table 8.** The number of *P. messaniella* mined leaves from the leaf fall traps compared against numbers expected on a constant proportion basis

Date	Mined leaves (Observed)	Mined leaves (Expected)	$\frac{(O-E)^2}{E}$	Sign of O-E
<b>a. Tree 1</b>				
N/J	5	19.8	11.063	-
F	10	31.2	14.405	-
M	10	24.9	8.916	-
A	14	22.4	3.15	-
M	279	202.2	29.170	+
J	333	309.0	1.864	+
J	31	43.3	3.494	-
A	30	38.5	1.877	-
S/F	10	19.0	4.263	-
M	7	18.7	7.320	-
Total	729		$\chi^2 = 85.522$ , $df = 9$ , $p < 0.001$	
<b>b. Tree 2</b>				
N/J	5	23.7	14.755	-
F	42	71.3	12.041	-
M	29	40.8	3.413	-
A	14	17.9	0.850	0
M	308	211.2	44.367	+
J	258	223.7	5.259	+
J	49	74.3	8.615	-
A	51	69.5	4.924	-
S	5	13.9	5.699	-
O/F	4	13.7	6.868	-
M	12	17.3	1.624	0
Total	777		$\chi^2 = 108.415$ , $df = 10$ , $p < 0.001$	

## Discussion

In this study evidence of mined and caterpillar-grazed leaves of beech and holm oak dying earlier than undamaged leaves is given.

The presence of first generation *P. maestingella* mines on beech and winter generation *P. messaniella* mines on holm oak appears to promote leaf senescence. There was no clear evidence of *R. fagi* and *P. maestingella* second generation mines leading to premature leaf senescence, except when *R. fagi* removed a large portion ( $\geq 20\%$ ) of the leaf.

Leaf longevity in beech is to some extent related to its height on the tree. It was observed in the field that leaf senescence and fall were initiated in the upper canopy, gradually spreading down the trees. Thus when mines are patchily distributed over a tree it can be expected that the temporal pattern of mortality of mined leaves will differ from that of unmined leaves.

The effect of within-tree variation in mine density on leaf loss has received little attention. For example Faeth et al. (1981) state that their data support the hypothesis that mined leaves are shed earlier than unmined leaves. They compare the percentage of mined leaves on the tree (from an unspecified location) with that of fallen leaves in leaf fall traps on each sample date. However the effects of height and mine distribution on leaf fall could explain the observed trends. A similar criticism could be applied to Owen's (1978) methodology, where variations in the percentage of mined leaves collected by leaf fall traps were explained as premature leaf loss in response to miners.

During leaf senescence *Phyllonorycter* mines are easily observed as the leaf tissue above the mine remains green whilst the rest of the leaf turns brown ("green islands"). This phenomenon is attributed to concentrations of cytokinins in the mine vicinity (Engelbrecht 1971). Since these chemicals are involved in the inhibition of leaf senescence (Addicott 1980) it could be hypothesized that their presence in increased concentrations alters the life expectancy of individual leaves. However the contrasting patterns of mined leaf loss for the two *P. maestingella* generations and the lack of a difference in mortality between second generation mined and unmined labelled leaves militates against this view.

*Phyllonorycter* larvae remove all green tissue from the mine only if pupation occurs (and even then a green area may remain). Premature senescence of this region was noted in both tree species. In the second *P. maestingella* generation autumnal leaf fall usually precedes this localized senescence. Damage caused by first generation *P. maestingella* mines was shown to have a qualitatively different influence on senescence to similar amounts of damage caused by other agents. *Phyllonorycter* mines and their contents, being in contact with physiologically active leaf tissue from mining until leaf or tissue death, may thus be more disruptive to leaf functioning than damage from external feeders which can be isolated from active tissue by wound responses. *R. fagi* consumes all the green tissue encompassed by the mine, leaving only a network of veins and the cuticle intact. Thus its effect is somewhat similar to caterpillar feeding (*R. fagi* mined leaves on Tree 1 with  $\geq 20\%$  leaf area damaged died earlier than undamaged leaves. This is a similar effect to that of experimental damage to beech leaves).

In holm oak heavy leaf mortality occurs during the production of new growth. Summer leaf fall during leaf flush

**Table 9.** The amount (%) of leaf tissue (as calculated using cumulative damage) removed from the labelled leaf samples

### 1. Holm oak

Sample	Causal agents of herbivore damage			
	<i>P. mes-saniella</i>	Other leaf miners	Caterpillars	Total
Tree 1	1.54 (44.3%)	0	1.94	3.48
Tree 2A	1.25 (34.0%)	0	2.43	3.68
Tree 2B	0.68 (55.3%)	0.02	0.53	1.23

### 2. Beech

Sample	Causal agents of herbivore damage			
	<i>P. maestingella</i>	Other leaf miners	Caterpillars and weevils	Total
Tree 1	0.35 (8.9%)	1.94	1.65	3.94
Tree 2	0.37 (12.0%)	1.29	1.43	3.09

Where figures in parentheses are the proportions of total herbivore damage caused by the *Phyllonorycter* species

is a common phenomenon of evergreens (Owen 1978; Faeth et al. 1981; Shaver 1981; Hileman and Lieto 1981). Leaves whose axillary buds developed showed a higher mortality than leaves with dormant buds. Competition between organs within the tree for resources can lead to the senescence of individual leaves as a direct consequence of their location (Thomas and Stoddart 1980). Nutrient translocation from old leaves to new shoots is thought to be the cause of this mortality (Shaver 1981). The high mortality of damaged (both mined and caterpillar-grazed) leaves may be a result of nutrient transfer from less efficient leaves to the new growth. Many plants have been shown to shed diseased or damaged leaves (Jacobs 1962). The mechanism of this response is not fully understood, owing to the complex interactions of many plant compounds (Thomas and Stoddart 1980). Whatever the cause, it may prove beneficial for the plant to shed inefficient leaves. This may be especially true of evergreens such as holm oak where new growth supplants the previous leaf cohort in much of its photosynthetic work (Hileman and Lieto 1981). Small leaves may be less efficient than large leaves leading to their earlier mortality seen in both tree species.

As a defence strategy leaf fall could be effective against insects such as miners where larval survival is dependent on that of the leaf. However *Phyllonorycter* miners caused less than 1.6% cumulative damage to the labelled leaf cohorts (although in some samples they accounted for a major portion of total herbivore damage (Table 9)). These values are the proportion of the total amount of leaf tissue damaged by the various herbivores, which is more than the amount of herbivore consumption since holes in leaf tissue expand during leaf growth (Nielsen 1978). (It should be noted that the analysis used here assumes that the value to the plant of a unit area of leaf declines linearly with

time. Such an assumption is almost certainly incorrect (Janzen 1979.) However mobile herbivores such as caterpillars can move off damaged leaves and, as miners often form only a minor component of herbivore damage due to their low levels of abundance other defence responses are likely to be of greater importance. In this study the majority of mined leaf loss occurred weeks, or even months, after mining activity has ceased. The fanciful notion (Owen 1978) that leaves containing active miners are shed by the trees in order to regulate miner numbers and thus reduce herbivore damage is not supported by the data presented here.

Although *Phyllonorycter* miners usually remove a small proportion of the photosynthetic tissue available they may invoke senescent responses causing the loss of the remainder of the leaf. The loss of some leaves, especially damaged ones, may have a negligible effect on tree growth because the trees may not be photosynthesizing at their maximum rate (Maggs 1964; Sweet and Wareing 1966) or because they can compensate for low levels of herbivory (McNaughton 1983).

In an evolutionary context the impact of herbivores on plants is evaluated in terms of their effect on plant fitness (Janzen 1979). Commonly plant fitness is measured in terms of seed production (McNaughton 1983). The low mine densities observed in the field, the small amount of leaf area lost to them, and the long reproductive life-span of the tree species considered here implies that these mining species have only a trivial impact on their hosts.

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