Biological notes on the holly leaf miner, *Phytomyza ilicis* (Diptera: Agromyzidae)

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Abstract: The eggs of *Phytomyza ilicis* are deposited about the end of May in the xylem of the petiole or the base of the midrib. The larva tunnels its way up the nerve, and starts to form a blotch mine around December. It turns out that this mine in many cases is not simply an upper side mine in the middle layer of the palisade mesophyll, but that late in the larval life a lower side blotch may be added in the spongy mesophyll. Such 'doublesided' mines seem to occur in particular in relatively thick leaves. Bird predation is concentrated on large, doublesided mines, and is mostly from the leaf underside. The main cause of mortality of the larvae seems not to be parasitoids, but being squeezed to death by fast growing callus tissue in the mines. Parasitoid larvae seem to escape the danger of sharing this fate by a very early emergence. Although no more than four mines were observed in a leaf, many more eggs are often deposited. Despite this indication of competition in the first larval instar, oviposition is strongly clumped. Vacated mines play a role as acarodomatia.

Keywords: acarodomatia; bird predation; leaf mines; multiple oviposition; plant-induced mortality.

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Introduction

Although the adult fly is short-lived and quite inconspicuous, the striking and ubiquitous mines of the holly leaf miner, *Phytomyza ilicis* Curtis, are well known. In fact, both in nature and in gardens and parks it is difficult to find a holly tree without these mines.

Phytomyza ilicis stands well isolated within the large genus *Phytomyza*; it belongs to a Holarctic, mainly Nearctic, group of eight species, of which seven mine in *Ilex* (holly) species and one in *Nemopanthus*, both genera of the family Aquifoliaceae (Griffiths & Piercey-Normore, 1995). This is a rather small plant family, comprising only three genera (Richardson, 1978), and biologically forming somewhat an outsider in the Holarctic flora, in particular because of its evergreen foliage (Peterken & Lloyd, 1978). Perhaps because of this unusual life trait, possibly also because of its tough, nutrient-poor leaves, with high levels of toxic phenolic compounds and saponins (Potter & Kimmerer, 1989; Potter & Redmond, 1989), *P. ilicis* is the only leaf miner of holly in the western Palaearctic (Hering,

1957). Precisely the evergreen, leathery nature of the leaves is closely connected with the unusual biology of the fly. Most of this has been elucidated by the admirable study of Miall & Taylor (1907), later elaborated on by Cameron (1939) and Lewis & Taylor (1967). With some small additions by myself, the life history can be summarized as follows.

Oviposition takes place in the restricted window of time when the new leaves are still tender, end May – June. Eggs are deposited, one at a time, in the primary xylem of the basal part of the midrib or the distal part of the petiole. The place of the oviposition remains visible throughout the lifetime of the leaf as a distinct, somewhat swollen and suberized, scar at the underside of the petiole or midrib. The larva for the first several months tunnels its way in the midrib towards the tip of the leaf. In this stage the mine is invisible from the outside, and even in cross-section is difficult to detect, because it is only a narrow flattened cavity. After the first moult, which takes place around December, the larva leaves the midrib and starts to feed in the leaf lamina. Holly leaves have a distinct spongy mesophyll and a
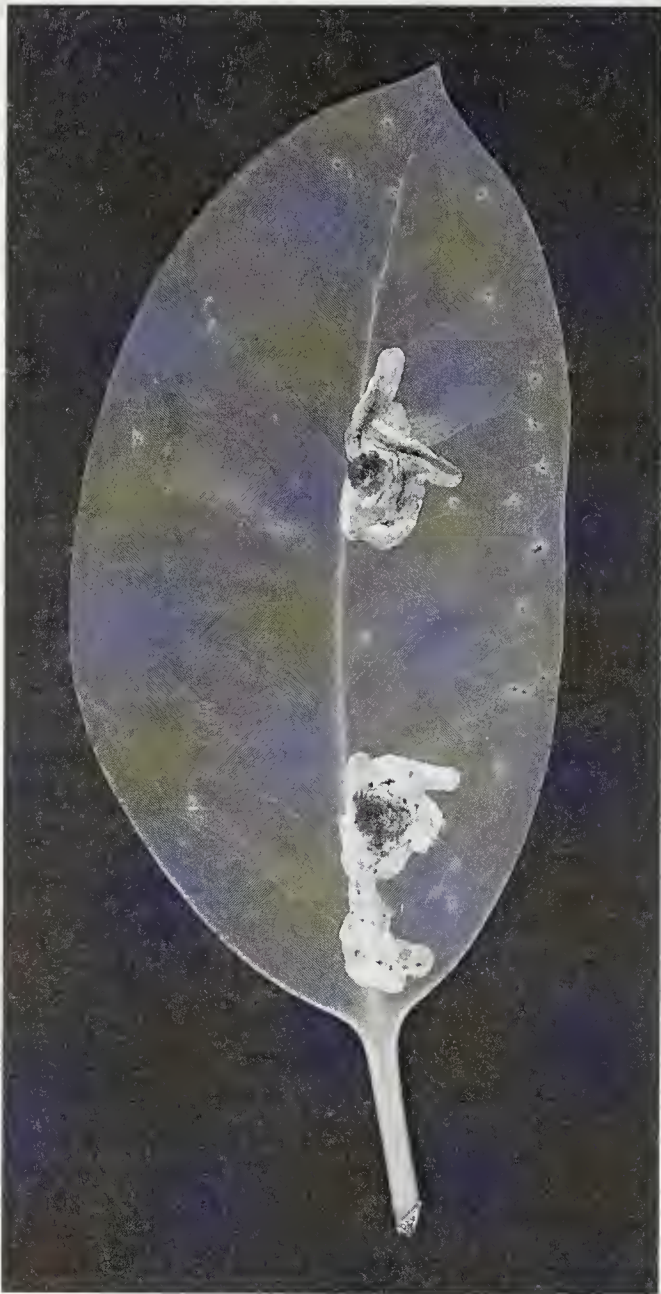
Fig. 1. Holly leaf with two mines; although not visible in the photograph, the lower one is doublesided. Dark (actually wine-red) central spots in the mines indicate the place of frass concentration. Small marks all over the leaf are scars of feeding punctures.

three-layered palisade mesophyll (a cross section is figured in Potter & Kimmerer, 1988). The larva mainly feeds on the middle layer of the palisade mesophyll; the upper layer is sometimes consumed as well, but the lower layer remains untouched. After the second (last) moult in February – March feeding activity increases. In the process, an extremely visible, pale yellow upper side blotch mine is formed (fig. 1). Usually, a wine-red central spot indicates the place where most frass is deposited. In preparation of the pupation, which takes

place in early spring, the larva (usually, but see below) eats away an oval speck of the upper palisade layer, creating a sort of semi-transparant wall, called the 'pupal blister' by Miall & Taylor. The larva then positions itself belly-up, and, upon pupation, pierces the leaf cuticle with its frontal spiracular horns. When the fly emerges a hinged semi-circular lid is pushed open, formed by the anterior part of the puparium and a part of the pupal blister. This opening is very different from the circular, generally somewhat smaller, emergence holes made by parasitoids.

It is interesting to note that the related North American *Phytomyza ilicicola* Loew, that lives on *Ilex opaca* Ait., makes a long tortuous gallery mine, without any association with the midrib (Hartzell, 1943). The phenology of the two species, however, is very similar.

In May and June, 1999, I took random samples of 100 leaves from each of twenty trees, which yielded a total of 836 leaves that had mines or at least oviposition scars. Most material (fifteen trees) came from parks in and around Amsterdam; also material was collected in a wood in the coastal dune region of The Netherlands, known as 'de Oude Hof' near Bergen, where holly forms a large part of the natural woody vegetation. Leaves from Bergen were distinctly thinner, somewhat larger in surface, and more overgrown by unicellular algae (*'Protococcus'*). All 1050 mines in the leaves were dissected under a stereo microscope. The longest (l) and shortest (s) perpendicular axis of each mine was measured to 0.1 mm, and its surface estimated as $\pi ls/4$. Representative types of mines were also hand-sectioned and studied under a light microscope.

Spongy mesophyll feeding

Although most mines remain restricted to the palisade mesophyll, this is not invariably the case. In many instances the larva, presumably near the end of its third instar, makes an extremely small opening in the lower layer of the palisade mesophyll, and from there starts feeding on the spongy mesophyll below. Usually,

Table 1. Partitioning of success over the two types of mines.

mines:	success:	
	no	yes
uppersided	694	63
doublesided	54	31

Table 2. Occurrence of uppersided and doublesided mines in the two sample sites.

mines:	locality:	
	Amsterdam	Bergen
uppersided	508	249
doublesided	68	17

there is no correspondence in part of the leaf occupied by the two compartments of the mine. Because of the loose texture of the spongy mesophyll, and because it mostly is not grazed fully down to the epidermis, this lower part of the mine remains green; only a very slight bulge and loss of distinctness of secondary leaf venation betrays its presence. Often, its presence is most obvious through the spiracular horns penetrating not the upper, but the lower epidermis. This detail of the mine is not mentioned by Hering (1957). Although many successful mines only have an upper side compartment ('uppersided mines'), a relative majority does have a lower side compartment as well ('doublesided mines'; table 1; $\chi^2 \leq$ 0.0001). Mines were scored as successful only if they had an emergence hole.

In the 31 successful doublesided mines the upper and lower surface of the mines was approximately similar (156.2 and 177.2 mm^2, respectively). In the 63 uppersided mines the average surface was 162.3 mm^2. The size of the upperside part of the mine did not differ for uppersided and doublesided mines (i.e., when only successful mines were taken into consideration).

Possibly the thickness of the leaves influences the relative number of doublesided mines: this number is higher in Amsterdam than in Bergen (table 2; $\chi^2 \leq 0.0001$).

Bird predation

Predation by birds, mainly tits, leaves a char-acteristic triangular flap of epidermis (Heads & Lawton, 1983; Owen, 1975); 63 leaves with this sign were encountered. Bird predation is concentrated on the largest mines; their total (upper + eventual lower) size averages 181.0, against 97.9 for the non-predated mines ($P \leq$ 0.0001). Birds also prefer doublesided mines (table 3; $\chi^2 \leq 0.0001$). This is understandable, because such mines guarantee a fully developed larva, or a puparium. Nonetheless it is remarkable that birds are capable of spotting these very inconspicuous parts of the mines. Moreover, it seems clear that the birds precisely locate the larva or puparium, perhaps by the spiracular horns, because no leaves were found with more than one stab, or other signs of poking around. Also, birds seem to 'know' quite well which side of the leaf to penetrate; an uppersided mine was never pecked from behind, and doublesided mines were attacked from the right side most of the times (table 4; $\chi^2 \leq 0.0001$).

Causes of mortality

Against 94 mines from which a fly emerged successfully, there are at least 256 in which the fly larva had succumbed to parasitoids. In 199 mines a parasitoid exit hole was present, and in 57 mines parasitoid pupae were found. Evidently, some parasitoids wait until their host has emerged, and probably oviposited, before emerging themselves. At the other hand it was striking that the mines from which parasitoids already had emerged were smaller

Table 3. Difference of the intensity of bird predation over uppersided and doublesided mines.

mines:	bird predation:	
	no	yes
uppersided	713	44
doublesided	66	19

Table 4. Distribution of the way of attack in bird predation over uppersided and doublesided mines.

mines:	bird predation:	
	upper	lower
uppersided	19	9
doublesided	0	35

Table 5. Efficiency of oviposition for different numbers of clutch sizes.

clutch	leaves	total eggs	flies emerged	efficiency %
1	243	243	20	8.23
2	190	380	14	3.68
3	124	372	9	2.42
4	91	364	16	4.4
5-18	188	1358	35	2.58

than the mines in which pupae were waiting (total surfaces 69.3 and 106.6 mm^2, respectively; $P = 0.0024$). Also, parasitized mines (vacated or not) were smaller than mines from which a fly had emerged (total surfaces 86.7 and 217.1 mm^2, respectively; $P \leq 0.0001$).

Parasitism, however, does not seem to be the main cause of mortality: 329 larvae and 75 puparia were found dead in the mines without any apparent sign of parasitism. Most of the larvae were found, totally flattened, in diminutive, often wine-red, mines that were completely filled by callus tissue. Host feeding, i.e. predation by parasitoid adults through the epidermis (Evenhuis & Soehhardjan, 1970; Talhouk & Soehardjan, 1970) does not seem to be the case, since no damage to the epidermis was obvious. It is possible that the mine became filled with callus after the death of the larvae, but it is just as conceivable that these larvae were the losers in a race between tissue removal and callus regrowth, resulting in their being squeezed to death. It is noteworthy that even in successful mines some callus appears in the central part of the mine, where there is an accumulation of frass. As noted above, this spot often has the same wine-red colouration as the aborted mines. Formation of callus in the mine has also been described by Martens & Trumble (1987) in mines caused by another agromyzid, *Liriomyza trifolii* (Burgess), and is invoked as part of the mechanism of the resistance of the Australian tree, *Eucalyptus marginata* Sm. against an incurvariid moth, *Perthida glyphopa* Common (Mazanec, 1985). Although they did not look into the mechanism involved, also Valladares & Lawton (1991) found that 'miscellaneous death', which is mortality not caused by parasitism or bird predation, is the main cause of death of *P. ilicis* larvae by far.

If plant-caused mortality of the fly larvae is so prevalent, then the parasitoid larvae run the risk to perish with their host. I guess that the sometimes surprisingly small mines from which parasitoids have emerged, mentioned above, indicate that parasitoids may escape this fate by an early, perhaps premature, emergence.

Additionally, 113 mines consisted of atypical, very narrow galleries of various length that end somewhere in the lamina, filled with callus. Hering (1957) mentions these linear mines, and attributes them to parasitism. This does not seem very plausible; the extreme narrowness of the mines seems not to agree, and no exit holes were observed. It seems that here too the larvae have been losing a battle with regrowing plant tissue, from which they have vainly tried to escape by a 'flight forward'.

Multiple oviposition and competition

Often, there is more than one oviposition scar. Female Agromyzidae use their ovipositor also to puncture leaves to feed on the cell sap. Such feeding punctures may also be made on the petiole and midrib, and it is not always possible to discriminate feeding and oviposition scars. Yet, it is beyond doubt that multiple oviposition is very common; for instance, the correlation between the estimated number of ovipositions (1 .. 18) and the number of mines (0 .. 4) in a leaf is 0.4. Valladares & Lawton

Table 6. Percentage of success in relation to the number of mines per leaf.

mines/leaf	leaves	flies emerged	% success
1	454	65	14.32
2	118	22	9.32
3	21	7	11.11
4	4	1	6.25

Table 7. Comparison, both for oviposition scars and mines, of the observed distribution of the numbers per leaf, with a random (Poisson) distribution.

number	ovipos. obs.	ovipos. exp.	mines obs.	mines exp.
0	1154	490	1336	1231
1	243	689	462	598
2	190	485	134	145
3	124	227	31	23
4	91	80	37	3
5	64	23		0
6	38	5		
7	17	1		
8	21	0		
9	12	0		
10	21	0		
11	13	0		
12	4	0		
13	5	0		
14	2	0		
18	1	0		

(1991) found in the north of England per hundred leaves approximately twice as many oviposition scars as mines, which also suggests egg overproduction. Although each oviposition act not necessarily terminates into an egg being laid, it is plausible that there is a heavy competition at the first larval stage. Probably there will be a component of space in this competition, since the only way to success is towards the leaf apex, via the narrow midrib xylem corridor.

Table 5 shows the efficiency of the oviposition for different numbers of clutch sizes, under the assumption that each oviposition scar indeed represents an egg. Clearly the efficiency is highest for single ovipositions, but in the case of multiple ovipositions, the number of eggs seems less important. Contrarily, the number of mines per leaf seems to have little or no influence on their success (table 6; linear mines, described above, are discounted in this case).

Table 7 gives, both for oviposition scars and mines, a comparison between the distribution of the observed numbers per leaf, and the distribution that is to be expected if they would be randomly distributed (Poisson distribution). In both cases the two distributions differ strongly ($\chi^2 \leq 0.0001$). Despite the competition that seems to occur in the first instar, there is a strong tendency to cluster ovipositions on a single leaf. The same, seemingly paradoxical observation, although only at the level of mines, has been reported for other leaf miners (e.g., Bultman & Faeth, 1985; Miller, 1973; Sato, 1991). The explanation that is generally given is that leaves differ in quality, and that females select superior leaves regardless of the presence of competitors.

Role of mines for the epiphyllous fauna

Through the extended lifetime of holly leaves, empty mines remain for an extended period (according to Peterken & Lloyd ,1978, five, in exceptional cases up to eight years; up to four years according to Owen, 1978). This was evidenced by the fact that often unicellular algae were growing within old vacated mines. But also it was a very regular observation that small mites were running in and out such mines. Seemingly, in plants with perennial leaves vacated mines play a role as acarodomatia (see Walter, 1996, for a review of this topic). Even though mined leaves seem to have a shorter life span than unmined ones (James & Pritchard, 1988; Owen, 1978; but see Potter, 1985), this observation may mean that, especially in wet-tropical regions where perennial leaves are the rule, plants may derive some additional protection through their leaf mines.

Acknowledgements

Samenvatting

Biologische aantekeningen over de hulstmineervlieg, Phytomyza ilicis (Diptera: Agromyzidae).

De eieren van *Phytomyza ilicis* Curtis worden omstreeks eind mei afgezet in de vaatbundel van de bladsteel of de basis van de hoofdnerf. De larve boort zich door de nerf in de richting van de bladtop, en begint omstreeks december aan de vorming van een blaasmijn in de bladschijf. Het blijkt dat deze blaasmijn in veel gevallen niet louter bovenzijdig is, maar dat de mijn ook een, laat-gevormd, onderzijdig deel heeft; dit lijkt in het bijzonder voor te komen bij relatief dikke bladeren. Predatie door vogels

treedt voornamelijk op bij grote, dubbelzijdige mijnen, en gebeurt meestal vanaf de blad-onderzijde. Meer dan door parasitoïden komen veel larven vermoedelijk om door sterke en snelle weefselwoekering in de mijn, waardoor de larve wordt doodgedrukt. Parasitoïden-larven lijken te ontkomen aan het gevaar dit lot met hun gastheer te delen door al in een vroeg stadium uit te komen. Hoewel er maar maximaal vier mijnen in een blad zijn waargenomen, worden vaak veel meer eieren per blad afgezet. Ondanks dat er daardoor een sterke concurrentie lijkt te bestaan in het eerste larvestadium, bestaat er een sterke neiging om verschillende eieren in hetzelde blad af te zetten. De oude, verlaten mijnen spelen een rol als acarodomatium, een 'thuisbasis' voor bladbewonende mijten.

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