

## ACTIVITY 1

# What happened to the holly leaf-miner?

### Introduction

Holly trees (*Ilex aquifolium*) are common in city squares and urban parks, and several are found in Gordon Square. In this investigation, pupils collect evidence of the food chain of the holly leaf-miner (*Phytomyza ilicis*).

Animals are usually so transitory in feeding that it is impossible to assess the ecological impact of each species on its food source. However, the leaf-miner food chain provides an opportunity to study living organisms in the field and gain experience of ecological interactions in greater detail and offers data for pupils to analyse and discuss.



Figure 5. Mines on the upper surface of a holly leaf.

### Learning outcomes

Through this activity pupils will:

- investigate energy flows through living organisms and population numbers
- consider different sampling methods
- evaluate the collection technique used to collect the primary data

### Background information for teachers

#### (a) Life cycle of the holly leaf-miner

The larvae of the Agromyzid leaf mining fly (*Phytomyza ilicis*) burrow in the mesophyll of holly leaves (*Ilex aquifolium*) and produce characteristic white patches known as mines beneath the surface (Fig. 5). The larvae (miners) are parasitized by a number of parasitic wasps and fed on by birds, such as blue tits (*Parus caeruleus*).

The tiny adult leaf-miner flies lay their eggs at the base of the midrib on the underside of the leaf in June. On hatching, the larvae enter the midrib, slowly eating their way forward until the months of September through to November when they enter the mesophyll. Further feeding produces the meandering mines which reach maximum size in March. Larvae pupate within the cuticle of the last larval stage from March to May. The cuticle is retained as a protective covering (puparium) over the pupa. Before it pupates the larva prepares a thin triangular area on the leaf cuticle against which will fit the hinged 'emergence plate' of the puparium. The adult fly escapes by pressing against the 'emergence plate' in late May – June, leaving a large emergence hole (> 1mm). The cycle continues as the adult fly lays her eggs.

## (b) The food chain of the holly tree

The basic food material of the food chain is synthesized by the holly leaf (producer), which is consumed by the larvae of the holly leaf-miner (primary consumer), which itself in turn is predated by the blue tit (secondary consumer). A v-shaped tear, with no remains of a miner inside the mine, indicates that the leaf has been opened by a beak (Fig. 6).



Figure 6. V-shaped tear on a mine indicating blue-tit predation/

Competing with the blue tits are a number of parasites. The most important of these is the parasitic wasp *Chrysocharis gemma*, which attacks leaf-miner larvae. The adult wasp has a long ovipositor which she uses to insert a single egg through the leaf cuticle into the body cavity of the larva. Attacked larvae appear flaccid and a pale, dirty yellow in colour compared with the turgid, bright, shiny, whitish-lemon appearance of healthy larvae. The parasite larva feeds within the fly larva, and eventually kills it. It then forms a shiny jet-black pupa which lies free inside the mine.

Evidence that the adult parasite has emerged is a very small, neat, round hole which may be found on either side of the mined leaf. Also leaving evidence of a small emergence hole is another parasitic wasp *Sphexigaster flavicornis* which attacks the pupa of the leaf-miner. The adult parasite bores through the leaf cuticle and the tough skin of the puparium with its ovipositor. The parasite larva bores into the leaf-miner's pupa, feeds and then pupates. The pupa of the parasite is black with a bluish tinge. Another less common parasite is *Pleurotropis amyntas*, which is unique as it may either live as a primary parasite in the pupa of the leaf miner or feed as a secondary parasite on the miner's pupal parasite *S. flavicornis*. Thus *P. amyntas* forms the fourth link in a parasite food chain.



Figure 7. Small hole indicating emergence of an adult parasite.

In practice it is difficult to distinguish easily between the different parasites that feed on the miner and to find out whether *P. amyntas* is working at the fourth trophic level. It does however provide opportunities to discuss the contrasting feeding strategies of organisms (herbivores, carnivores, parasites and hyper-parasites).

## Resources

### Worksheets

- 1. Holly leaf-miner photograph trail
- 2. Holly leaf-miner text key
- 3. Finding evidence in the field
- 4. What do the mines tell us?
- 5. Considering your results
- 6. Evaluation

### Accompanying CD

- PowerPoint 'Sense of Place'
- PowerPoint 'The holly leaf-miner'
- Article 'Holly leaf-miner life cycle'
- All photos from this section

### Apparatus needed

Polythene bags  
Holly leaf-miner keys (Sheets 1 and 2)

Secateurs / scissors  
Hand lenses

## Lesson Sequence

### 1. Before fieldwork

Locating a holly tree infested with mines prior to the lesson is crucial. Look around the borders on the eastern (Tavistock Square) side of Gordon Square. If you are planning to run this practical with more than one class annually, find other trees in nearby squares, and record when each tree has been used for a practical. First, this will assist you to collect worthwhile data (i.e. a tree with mines), and second it will prevent 'over plucking' of a tree.

### Introducing the investigation to the pupils

From KS2/3 the pupils will have prior knowledge of the essential characteristics of living organisms, food chains and food webs. Ask them to list three living organisms and give evidence of why they are classified as such. This allows you to remind some pupils that plants are living organisms too, and so will be affected by the environment.

Ask a few pupils to review a food web and the associated vocabulary on the board for the class. Using a photo of a mined leaf (see PowerPoint presentation accompanying CD) ask pupils what may be happening to the leaf. The life-cycle of the leaf miner fly will need to be explained at this point. The article on the CD could be used to deliver this information. Links can be made with the caterpillar and butterfly which pupils may have studied at KS2 (Nc1a).

## Key questions

- What is the holly leaf miner dependent upon for its survival to mature into a fly?
- Do all holly leaf miners become flies?  
(Discussion of energy flow and what might happen to the pupa and the fly).

This discussion should lead into the practical as evidence is now necessary to confirm possible routes the leaf miners life may take and the numbers involved at each stage.

## 2. Fieldwork

### Preliminary fieldwork

Time permitting, instead of telling the pupils what they should look for as evidence of differing deaths of the holly leaf-miner, allow the pupils to observe several mined leaves and to write down what they actually see. Leaves can be collected by the pupils or prior to the lesson. Use a hand lens to observe leaves and record any details observed. Make sure that both sides of the leaves are checked. Demonstrate how to open a mine safely and without damaging the contents by cutting around the mine and gently pulling the two epidermises apart. Again the pupils should record what they find. Sheet 3 may be used for support.

At this point the keys (Sheets 1 and 2) may be introduced. By comparing their observed information with information in the identification key, groups can tell the class how their miner died (if at all). Photographs from the CD can be projected to support this exercise.

Methods of sampling information from books and the internet can be discussed, leading to agreed methods for sampling holly trees. Sampling methods will have been taught at KS3 so this may be an ideal opportunity for pupils to devise their own method to sample a holly tree. Differing sampling methods can be presented, with advantages and disadvantages of each method discussed.

### Collecting data

- To sample 600 leaves; split class into 6 groups.
- Each group should sample 10 randomly chosen branches.
- For each branch ignore the first few young leaves and record the number of leaves with and without mines for the next 10 leaves along the branch.
- Cut off the holly leaves that have mines and place them in a plastic bag.
- Take notes of any problems or changes that had to be made during the practical.

### Safety

Caution should be exercised when collecting holly leaves. Pupils should wear gloves. Consider eye protection.

### 3. After fieldwork

#### Analysing raw data

In the classroom the pupils cut open the leaves (being careful not to cut into the mine). The top layer of the mine can then be lifted off, and the inside of the mine examined. On determining what happened to each of the mined leaves and giving it a corresponding letter (using the key) the results can be recorded in the table on Sheet 4. Class results can be collated if desired.

Pupils can use the data they have collected to draw a pyramid of numbers for the holly food chain. Sheet 5 provides further questions.



Figure 8. Careful dissection of this mine has revealed an empty pupal case of a holly leaf miner.

#### Evaluation of the method

A prompt sheet is available (Sheet 6) for pupils to evaluate the methods they used when collecting the first-hand data and to consider the validity and reliability of the data. If time is spent discussing the chosen sampling technique to be used prior to the fieldwork, evaluating the method used should be easy, and there is the option to explore different techniques that pupils feel would increase the reliability of the results.

#### Other areas for consideration

The method needs to be carefully considered by the pupils.

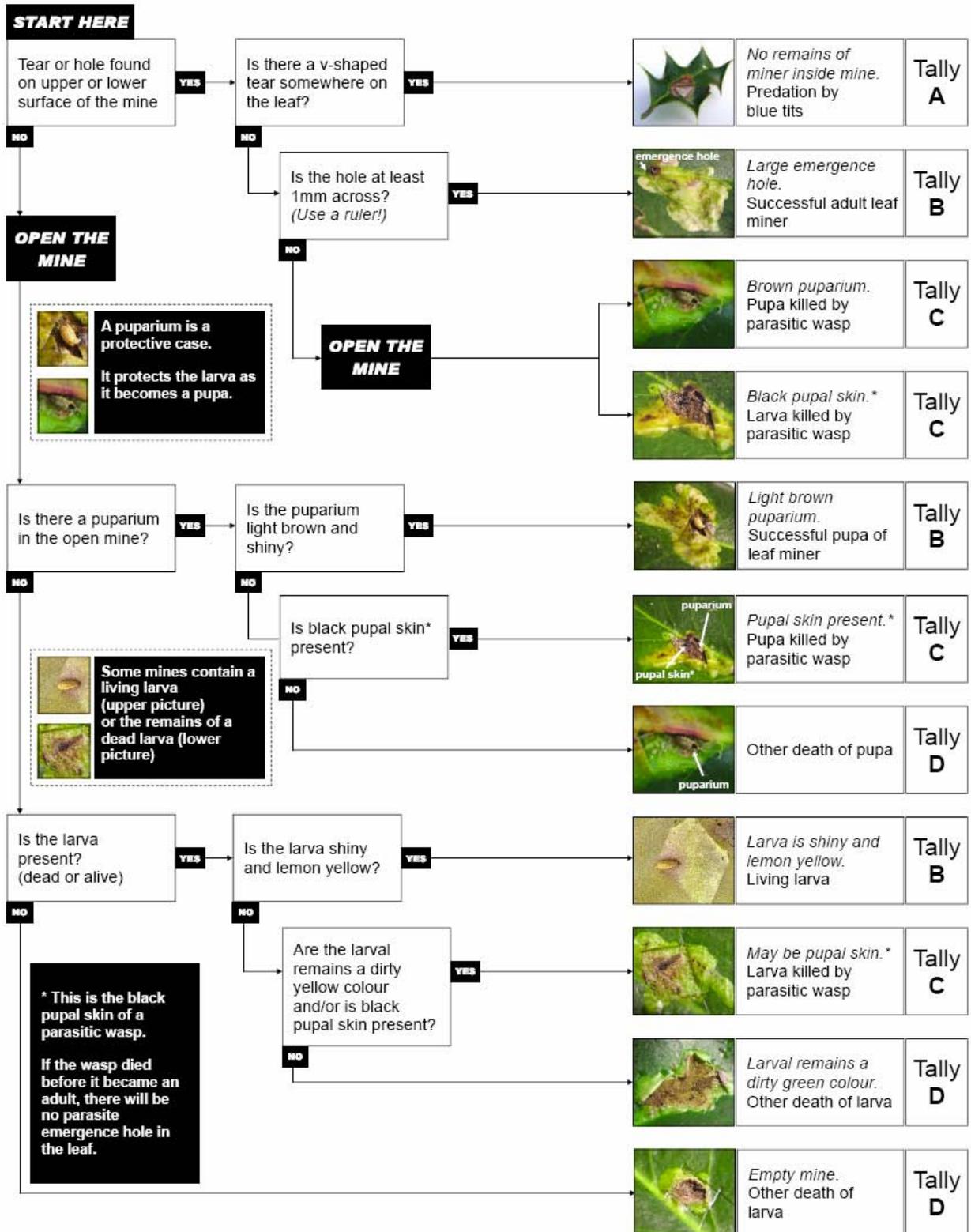
- What are the limitations of the method?
- Are there anomalies that can be explained using the site observational data?
- How would the results be different if the sampling of holly leaves was carried out at a different time of the year? (i.e. the effect of life cycles)
- If the tree had been sampled in previous years how might this affect the results?

#### Further reading

Glackin M.A., Jones M. and Norman S. (2006): What happened to the holly leaf-miner? Studying real food chains. *School Science Review*, **87** (320) pp 91-98.

# What happened to the holly leaf-miner?

## SHEET 1 - HOLLY LEAF-MINER PHOTOGRAPH TRAIL



# What happened to the holly leaf-miner?

## SHEET 2 - HOLLY LEAF-MINER TEXT KEY

Start in this Column	What happened to the holly leaf-miner?	Tally												
Tear or hole found on the surface of the mine, this can be on the upper or the lower surface	V-shaped tear possibly a peck mark from a beak, no remains of miner inside the mine	A												
	<table border="1"> <tr> <td>Small round exit hole found on mine</td> <td>Large exit hole (&gt;1mm)</td> <td>On opening the mine a shiny light brown puparium is found</td> <td>B</td> </tr> <tr> <td></td> <td>Small exit hole like a pin prick</td> <td>On opening the mine a brown puparium is found with a black pupal skin next to it</td> <td>C</td> </tr> <tr> <td></td> <td></td> <td>On opening the mine a black pupal skin is found but no brown puparium</td> <td>C</td> </tr> </table>	Small round exit hole found on mine	Large exit hole (>1mm)	On opening the mine a shiny light brown puparium is found	B		Small exit hole like a pin prick	On opening the mine a brown puparium is found with a black pupal skin next to it	C			On opening the mine a black pupal skin is found but no brown puparium	C	
Small round exit hole found on mine	Large exit hole (>1mm)	On opening the mine a shiny light brown puparium is found	B											
	Small exit hole like a pin prick	On opening the mine a brown puparium is found with a black pupal skin next to it	C											
		On opening the mine a black pupal skin is found but no brown puparium	C											
No hole or tear present on the upper or lower surface of the mine	On opening the mine a puparium is found	<table border="1"> <tr> <td>Puparium shiny and light brown</td> <td>Successful pupa of leaf miner</td> <td>B</td> </tr> <tr> <td>Puparium dull and dark brown</td> <td>Pupa killed by parasitic wasp</td> <td>C</td> </tr> <tr> <td></td> <td>Other death of pupa</td> <td>D</td> </tr> </table>	Puparium shiny and light brown	Successful pupa of leaf miner	B	Puparium dull and dark brown	Pupa killed by parasitic wasp	C		Other death of pupa	D			
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	Puparium dull and dark brown	Pupa killed by parasitic wasp	C											
		Other death of pupa	D											
On opening the mine a puparium is not found	<table border="1"> <tr> <td>Living larva present, shiny and lemon colour</td> <td>Successful larva of leaf miner</td> <td>B</td> </tr> <tr> <td>Remains of dead larva present</td> <td>Larva killed by parasitic wasp</td> <td>C</td> </tr> <tr> <td></td> <td>Larva killed by parasitic wasp</td> <td>C</td> </tr> <tr> <td></td> <td>Other death of larva</td> <td>D</td> </tr> </table>	Living larva present, shiny and lemon colour	Successful larva of leaf miner	B	Remains of dead larva present	Larva killed by parasitic wasp	C		Larva killed by parasitic wasp	C		Other death of larva	D	
Living larva present, shiny and lemon colour	Successful larva of leaf miner	B												
Remains of dead larva present	Larva killed by parasitic wasp	C												
	Larva killed by parasitic wasp	C												
	Other death of larva	D												
	No conspicuous larval remains	Other death of larva	D											

# What happened to the holly leaf-miner? SHEET 3 - FINDING EVIDENCE IN THE FIELD

Choose the first 10 leaves. Record the number that have mines. Remove those leaves which have mines.

		Branch number											
		1	2	3	4	5	6	7	8	9	10		
Number of leaves without mines													
Number of mines													

Looking around this habitat what other plants are found near your holly tree?

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 .....

Would they affect the holly tree you are studying? How?

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 .....  
 .....

List the difficulties you had in doing this practical.

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 .....  
 .....

# What happened to the holly leaf-miner?

## SHEET 4 - WHAT DO THE MINES TELL US? (CLASS RESULTS SHEET)

	Group Number / Tally Totals						Class Total
	1	2	3	4	5	6	
A Predation by blue tits							
B Successful larva, pupa or adult leaf mine							
C Larva or pupa killed by parasitic wasp							
D Other death of larva							
E Number of leaves without mines (from sheet 1)							
Total number of mines sampled (A + B + C + D)							
Total number of leaves and mines sampled (A + B + C + D + E)							

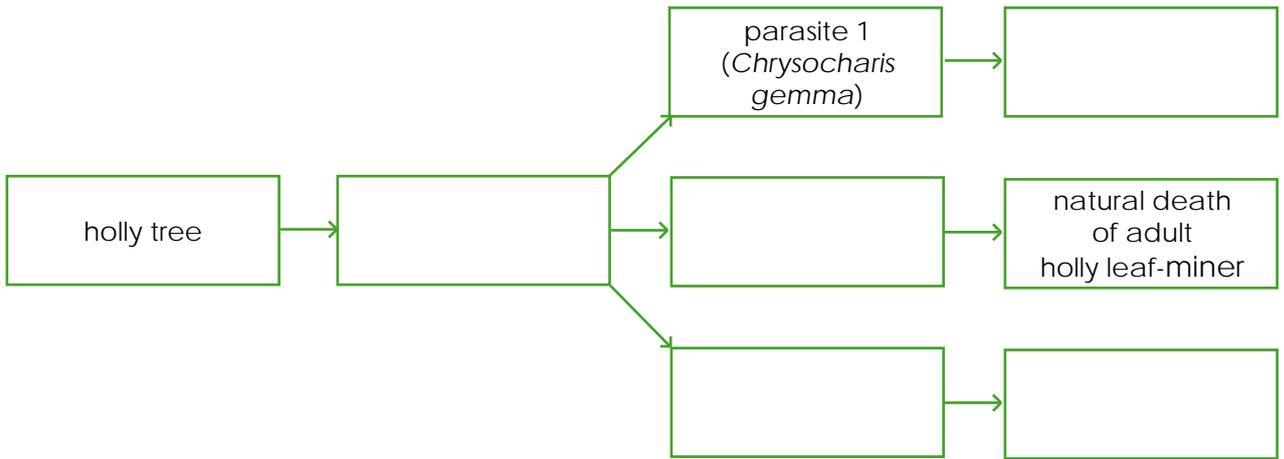
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## SHEET 5 - CONSIDERING YOUR RESULTS

1. Label the flow diagram to cover the possible pathways for energy from a holly tree. Use each one of the following labels **once** on the diagram.

Holly leaf-miner larva  
 Blue tit  
 Sparrowhawk

Adult leaf-miner fly  
 Parasite 2 (*Pleurotropis amyntas*)



2. Now use the class results to help you draw one pyramid of numbers diagram. Label each level with the species name and the type of feeder that it is (e.g. carnivore, herbivore)

3. Use your results to help **explain** your answers to the following.

(a) If the parasite (*C. gemma*) were to decrease in frequency due to a sudden change in environmental conditions, what effect would this have on (i) the number of holly leaf-miners?

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(ii) the number of blue-tits?

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(b) If the parasite (*Pleurotropis amyntas*) numbers increased rapidly, what effect would this have on

(i) the holly-leaf miner?

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.....

(ii) the other parasites?

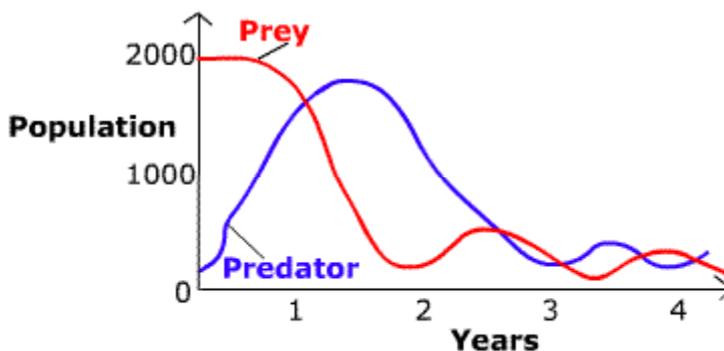
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(c) If more sparrowhawks were introduced into the Squares by humans, what effects would this have on the food chain?

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4. Extension.

The graph shows how the numbers of a prey species and the numbers of a predator species change over a period of four years.



(a) Using the graph above can you explain the changing population numbers of both predator and prey numbers over the years?

(b) Sketch a graph to show the effect on the leaf miner population if parasite numbers increased dramatically.

# What happened to the holly leaf-miner?

## SHEET 6 - EVALUATION

1. Consider your method. List the difficulties that your group found. Suggest improvements that could be made so other groups will not encounter the same problems.

Difficulties Found	Improvement
<i>To sample the bush so it took into account high and low leaves.</i>	<i>To change sample method and instead choose 3 branches from the top, 3 from the middle, three from the bottom and one randomly.</i>

2. The Royal Horticultural Society (situated near to Victoria Station), many of whose members are plant experts, is concerned that the leaf-mining fly population is going to rapidly increase in the future. They would like you to present your current findings at a meeting. How confident are you in your data? Do you feel you have enough to discuss with the Society? Explain your answer as if you are writing for the Society members.

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