

Inhibition of Premature Leaf Abscission by a Leafminer and Its Adaptive Significance

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ABSTRACT We tested the possibility that a lepidopteran leafminer, *Coptotriche japoniella* Puplesis and Diškus, inhibits the host plant *Eurya japonica* Thunberg from abscising mined leaves prematurely to increase its survivorship in immature stage. We monitored abscission patterns of mined leaves with sacrificed larvae, mined leaves with living larvae, and unmined leaves from April to July 2004 and 2005 until leafminers emerged as adults. Unmined leaves rarely abscised before July. Mined leaves with sacrificed larvae fell at a constant rate after May, abscising significantly more than unmined leaves. In contrast, mined leaves with living larvae rarely fell before adult emergence; afterward they abscised rapidly. We also examined larval/pupal survivorship and mortality sources on the ground and trees after leafminers completed larval development. Leafminers on the ground suffered a higher mortality from predation than those on trees, and thus they emerged as adults on the ground less successfully. These findings suggest that the leafminer *C. japoniella* prevents the host plant from abscising mined leaves prematurely until adult emergence, thereby increasing their survivorship.

KEY WORDS *Coptotriche japoniella*, co-evolutionary process, counteradaptation, *Eurya*, plant-insect interaction

Premature leaf abscission is simply defined as early leaf fall induced by herbivore damage (Strauss and Zangerl 2002). It often causes substantial mortality in endophagous insects such as leafminers and gall-formers because of leaf desiccation and senescence on the ground. Although many authors have agreed that leafminers and gall-formers induce premature leaf abscission, they have disagreed as to the impact of abscission on the survival of those insects. Some authors suggest that premature abscission is a considerable source of mortality for leafminers and gall-formers (Naruse 1978, Faeth et al. 1981, Faeth 1985, Potter 1985, Bultman and Faeth 1986, Williams and Whitham 1986, Simberloff and Stiling 1987, Auerbach and Simberloff 1989, Stiling and Simberloff 1989, Preszler and Price 1993, Kagata and Ohgushi 2004), whereas others suggest that abscission does not increase mortality of leafminers because leafmining larvae complete feeding by the time of abscission or because leafminers in fallen leaves escape late larval and pupal parasitism (Kahn and Cornell 1983, 1989, Prichard and James 1984a, 1984b; Maier 1989, Waddell et al. 2001).

Whichever assertion is more likely, this argument raises a possibility that leafminers would have evolved a strategy to inhibit leaf abscission until they complete feeding within the leaves. This possibility would be more likely if leafminers have a long larval period in

the leaf and make a large mine for the leaf area. Larval feeding for a long time would impose a constant cost to the leaf and large mines would become a physiological stress on the leaf. Thereby, larvae would increase the risk of inducing leaf abscission. Unless such leafminers inhibit the host plant from abscising mined leaves prematurely, they would suffer a higher mortality from abscission. Furthermore, because most leafminers are specialized to their host plants, they could have often evolved inhibition of premature leaf abscission. The dipteran holly leafminer *Phytomyza ilicicola* Loew is a specialist that feeds on the American holly *Ilex opaca* Aiton and spends ≈ 10 mo in the leaves (Potter 1985, Kahn and Cornell 1989). Hence, *P. ilicicola* may be a likely candidate to inhibit leaf abscission. However, studies focusing on *P. ilicicola* have thus far ignored the possibility that *P. ilicicola* larvae inhibit leaf abscission until their completion of feeding, although it could be tested by examining leaf abscission patterns of mined leaves with living larvae and sacrificed larvae.

A lepidopteran leafminer, *Coptotriche japoniella* Puplesis and Diškus (Tischeriidae), is specialized to evergreen trees, *Eurya japonica* Thunberg and *E. emarginata* (Thunberg) (Theaceae) (Puplesis and Diškus 2003). Adults of *C. japoniella* emerge in June in Nara; females lay eggs on the lower surface of leaves aged ≥ 1 yr; larvae hatch in late August, feeding on leaf tissue within the mine through the summer and early

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winter; they overwinter as fifth-instar larvae, resume growing in early April, and pupate within the mine in mid-May to early June (unpublished data). Their fully expanded mines often occupy >50% of the leaf area (unpublished data). *C. japoniella* larvae may possibly inhibit leaf abscission of their host plant because they have a long larval period of ≈ 9 mo and because the mines are rather large for the leaf size. This study addressed three questions. First, does the host plant prematurely abscise leaves in response to herbivory? Second, if so, does the specialist leafminer *C. japoniella* inhibit the host plant from abscising mined leaves prematurely? Third, if so, is the strategy of the leafminer considered to be adaptive from the viewpoint of its survivorship? To answer these questions, we examined abscission patterns of mined leaves with sacrificed larvae, mined leaves with living larvae, and unmined leaves, and compared survivorship and mortality sources of leafminers that were placed on the ground and those which remained on trees.

Materials and Methods

Host Plant. *Eurya japonica* is an evergreen tree, occurring widely in China, Taiwan, Korea, and the middle to southern part of Japan (Kitamura and Murata 1979). New leaves break buds in early May in Nara, Japan, and mature leaves can remain on the tree for ≥ 3 yr (unpublished data).

Study Site. This study was conducted at a red pine (*Pinus densiflora* Siebold and Zuccarini) wood on Takamado Hill (34°40' N, 135°52' E, 400 m a.s.l.) near the urban area of Nara. The understorey vegetation of the forest was dominated by *E. japonica*.

Larval Development. To examine the expansion of *C. japoniella* mines in winter and spring to early summer, a leaf containing a single mine was randomly sampled from each of 10 *E. japonica* trees on 14 November 2003 and 24 March 2004 and two or three times each month from April to June 2004 and 2005. These leaves were scanned using an image scanner (GT-4000; EPSON Co., Suwa, Japan), and leaf and mine areas were measured by free software for image analysis (LIA for Windows32, ver. 0.376β1; Yamamoto 1997). Furthermore, to examine larval development after overwintering, ≈ 30 leaves containing an intact mine were randomly sampled from ≈ 10 *E. japonica* trees at intervals of ≈ 10 d from April to June 2004 and 2005. When mines had a pupal exuviae protruding from the surface or a small slit at the edge, they were classified as adult emergence. In other cases, mines were dissected to determine the larval instar and pupa. The instar was identified from the head width of the larva compared with specimens of reference (unpublished data).

Leaf Abscission Patterns. In winter and spring to early summer, leaf abscission patterns of mined and unmined leaves of *E. japonica* were examined as follows. First, on 14 November 2003, three or six shoots were randomly chosen from each of 69 trees in a quadrat of 2 by 15 m and were labeled. These shoots contained a total of 723 leaves that were ≥ 1 yr old.

Ninety-six of the 723 leaves were mined by *C. japoniella* and 627 were unmined. Digital photographs of the shoots were taken at intervals of ≈ 2 wk until 25 March 2004. At each time, we checked whether leaves remained on trees and whether mines expanded by comparing photographs with those taken before. When the expansion of a mine was found during an interval, the larva within the mine was regarded as alive.

Second, on 6 April 2004, nine trees 1.5–2 m in height were randomly chosen, and 20–30 leaves containing a *C. japoniella* mine were randomly labeled on each of the trees (a total of 200 leaves). A sharp needle was stuck into the mines of 104 leaves to kill the larvae, while it was stuck into the mines of the other 96 leaves without killing the larvae. As controls, two unmined leaves were selected for each mined leaf: one control leaf was located at the first upper node and the other at the first lower node. The needle was also stuck into these control leaves. When the node lacked a leaf or it possessed a tiny leaf, control leaf was not designated. Judging from pattern of development, each set of a mined leaf and control leaves were regarded as being of the same age. These leaves were monitored for abscission and leafminer survival at intervals of 1 wk until 29 July. When mined leaves remained on trees and mines were not torn open, mines were looked through in the light of a pocket flashlight to check whether larvae were alive or dead. At that time, stems were held between finger and thumb because handling leaves might increase abscission. It was also recorded whether mines were pleated and whether pupal exuviae protruded from the mine. Pleats indicate that the larva has completed feeding and spun for pupation. The following leaves were excluded from analyses: (1) leaves that suffered an area loss of $\geq 30\%$ by other herbivores during monitoring, (2) leaves in which mines were torn open, and (3) leaves in which leafmining larvae died before spinning for pupation. As a result, 110 unmined leaves, 92 mined leaves with sacrificed larvae, and 55 mined leaves with living larvae were used for analyses.

Third, according to the same method as 2004, leaf abscission patterns were also examined from 27 April to 29 July 2005. As a result, 107 unmined leaves, 86 mined leaves with sacrificed larvae, and 64 mined leaves with living larvae were used for analyses.

Survivorship and Mortality Sources on Trees and the Ground. We examined survivorship and mortality sources of leafminers on trees and on the ground after the completion of larval feeding in two periods: 17–30 May and 7–14 June 2005. Two hundred ten and 154 leaves containing pleated mines were randomly labeled on eight trees on 17 May and 7 June, respectively; as mentioned above, pleats indicate that the larva completed feeding and spun for pupation. These leaves were left on trees until 30 May or 14 June. Furthermore, 154 and 108 leaves with pleated mines were randomly removed from ≈ 12 trees on 17 May and 7 June, respectively. These leaves were put one by one in soil-containing biodegradable cups (5 cm in diameter and 5 cm in depth), and those cups were

Table 1. Abscission frequencies of mined and unmined leaves on 69 trees in winter

Leaves	On tree	Abscised	Total	Fisher exact probability
Unmined	609	18	627	0.751
Mined	93	3	96	
Containing living larvae	50	1	51	0.598
Containing dead larvae	43	2	45	

embedded in the forest floor. On 30 May and 14 June, 174 of 210 leaves and 141 of 154 leaves were recovered from trees, respectively (the other leaves abscised by 30 May or 14 June), whereas 153 of 154 leaves and 107 of 108 leaves were recovered from the ground, respectively. The survival or mortality source of leafminers in these leaves was determined by observing external appearance, rearing in the laboratory, and/or dissecting under a microscope as follows: mines that were torn open were categorized as preyed on; mines that had small emergence holes, mines that contained dead pupae or pupal exuviae of parasitoids, and mines from which adult parasitoids emerged during rearing were categorized as parasitized; mines from which adult moths emerged during rearing were categorized as successful adult emergence; and mines that contained dead pupae of leafminers were categorized as developmental failure. Mines that were collapsed or decayed from unknown causes were excluded from analyses.

Statistical Analyses. Statistical differences among leaf fall patterns were analyzed by log-rank tests (Krebs 1997, Fox 2001). The statistical analyses including other tests were conducted using SPSS for Windows ver. 11.5J (SPSS 2002). A significance level of 0.05 was used for the tests.

Results

Larval Development and Leaf Abscission Frequencies in Winter. The mean area of *C. japoniella* mines was $27.5 \pm 7.14 \text{ mm}^2$ [SD], $n = 10$) on 14 November 2003, whereas it was $56.4 \pm 9.96 \text{ mm}^2$ ($n = 10$) on 25 March 2004. Hence, the mines expanded twice in winter. Mines occupied $12.5 \pm 5.8\%$ of the leaf area on average at the end of the winter.

Regardless of whether leaves contained mines or not, they rarely abscised during the winter (Table 1). The proportions of abscised leaves did not differ significantly between mined and unmined leaves (Fisher exact test; $P = 0.751$). Furthermore, whether mined leaves contained dead larvae or living larvae had no significant effect on leaf abscission frequency (Fisher exact test; $P = 0.598$; Table 1).

Larval Development and Leaf Fall Patterns After Overwintering. Larvae of *C. japoniella* overwintered as fifth instar (Fig. 1C and D). They gradually expanded their mine (Fig. 1A and B; data of mid- to late April 2005 were lost because of an accident) and molted into sixth instar by late April to early May (Fig. 1C and D). Mines expanded rapidly afterward. Larvae began pupation in early to mid-May and completed pupating in early June (Fig. 2A and B). Fully expanded mines had an area of $249.3 \pm 43.5 \text{ mm}^2$, occupying $61.9 \pm 20.4\%$ of the leaf area on average ($n = 38$, including supplementary data). Adult emergence started in early to mid-June and ended by late June.

The patterns of leaf abscission markedly differed between three leaf categories: unmined leaves, mined leaves containing a larva killed by a needle, and mined leaves containing a living larva (Fig. 2C and D). Unmined leaves rarely abscised until the end of May (2.7% in 2004 and 1.0% in 2005) and gradually fell afterward; 15.5 and 15.1% of the leaves abscised in 2004

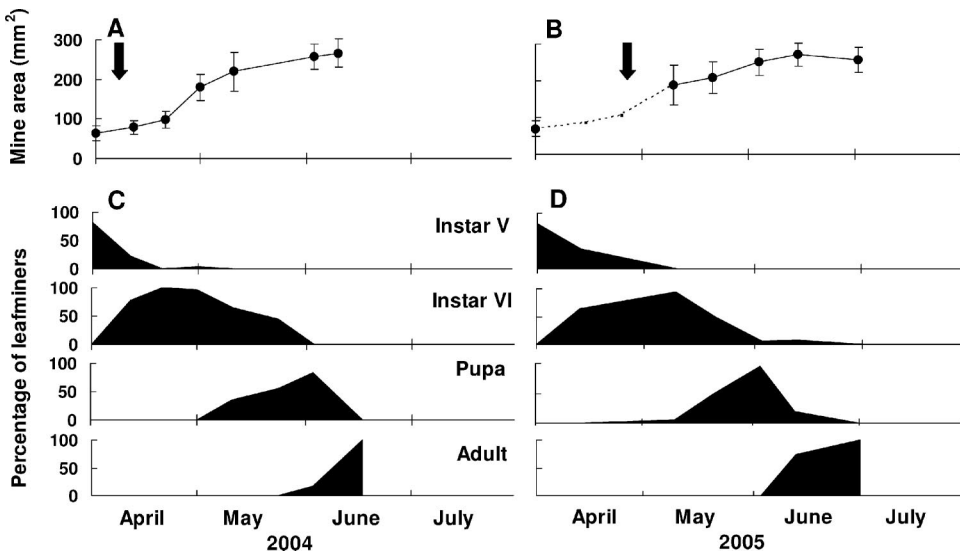


Fig. 1. Mean mine area (\pm SD) (A and B) and seasonal growth of *C. japoniella* larvae (C and D) in 2004 and 2005. Arrows indicate the dates when monitoring of leaf fall was begun (Fig. 2).

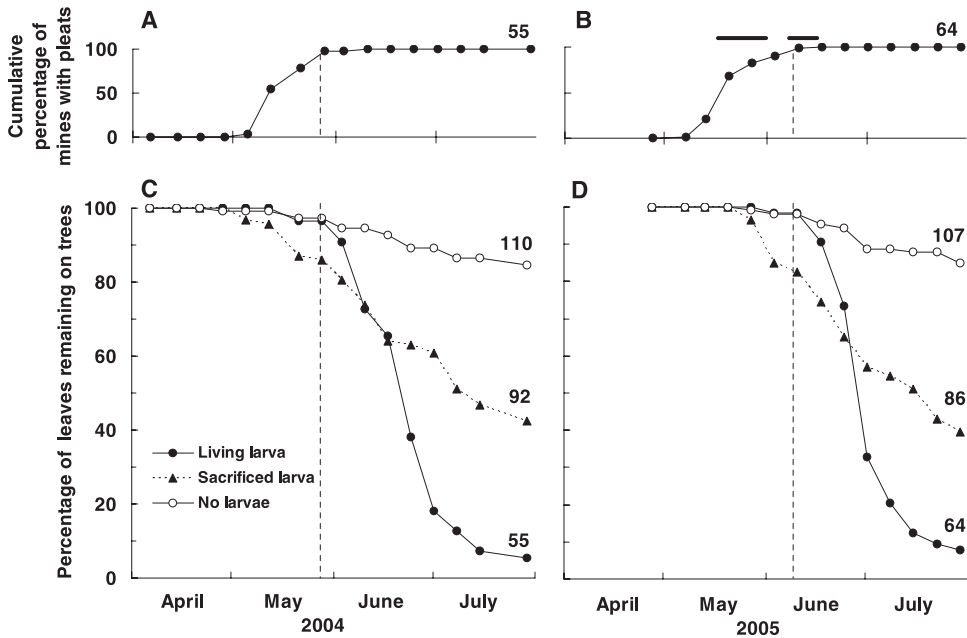


Fig. 2. Cumulative percentage of pleated mines (A and B) and leaf fall patterns of three leaf categories: mined leaves with living larvae, mined leaves with sacrificed larva, and unmined leaves (C and D). Horizontal bars in B indicate the periods when survivorship and mortality sources of leafminers were examined on trees and the ground (Tables 3 and 4); vertical broken lines indicate the border after which all larvae had pupated; numbers above lines are sample sizes.

and 2005, respectively. Mined leaves with sacrificed larvae began abscising 1 mo after larvae were killed and abscised with a constant rate until July; the final proportions of abscised leaves were 57.6% in 2004 and 60.5% in 2005. In contrast, mined leaves with living larvae rarely abscised until the end of May (3.6% in 2004 and 0% in 2005), as well as unmined leaves, but they abscised rapidly afterward; 94.5 and 92.2% of the mined leaves with living larvae abscised in 2004 and 2005, respectively.

The leaf abscission patterns were statistically compared using log-rank tests separately for the two periods of April to May and of June to July, i.e., before and after a rapid abscission of mined leaves with living larvae (Table 2; Fig. 2C and D). For the period of April to May, no significant difference in leaf abscission pattern was found between mined leaves with living

larvae and unmined leaves, whereas mined leaves with sacrificed larvae significantly differed from mined leaves with living larvae and unmined leaves (significant level was not adjusted for multiple comparisons according to Nakagawa 2004). For the period of June to July, the leaf abscission patterns of three leaf categories significantly differed from each other. These results indicated that mined leaves with living larvae remained on trees until the end of May as well as unmined leaves, and afterward, they abscised at a significantly higher rate than the other two categories of leaves.

Most of the larvae emerged as adults 3–4 wk after spinning for pupation, whereas >90% of mined leaves with living larvae abscised in and after the week of adult emergence (Fig. 3). This indicates that mined leaves with living larvae usually abscised when larvae were ready to emerge as adults or after they emerged as adults.

Survivorship and Mortality Sources of Leafminers on Trees and the Ground. Adults of a koinobiont braconid wasp, *Orgilus kumatai* Watanabe, emerged from leafminers that were left on trees or were placed on the ground. Females of *O. kumatai* seem to oviposit in early-instar larvae of *C. japoniella* between August and November and thus never attacked the host larvae in the study period of May to June (unpublished data). Accordingly, leafminers parasitized by *O. kumatai* were excluded from the analyses below (Tables 3 and 4, in which those leafminers are also given). Leafminers parasitized primarily by *O. kumatai* and secondarily by other parasitoids were, however, included in

Table 2. Log-rank tests for difference in leaf abscission patterns between mined leaves with living larvae, leaves with sacrificed larvae, and unmined leaves

	Mined leaves with living larvae versus mined leaves with sacrificed larvae		Mined leaves with living larvae versus unmined leaves		Mined leaves with sacrificed larvae versus unmined leaves	
	χ^2_{cal}	<i>P</i>	χ^2_{cal}	<i>P</i>	χ^2_{cal}	<i>P</i>
April–May 2004	4.15	0.042	0.10	0.756	8.85	0.003
June–July 2004	31.59	<0.001	125.49	<0.001	33.36	<0.001
April–May 2005	7.92	0.005	0.02	0.879	11.53	<0.001
June–July 2005	33.01	<0.001	123.51	<0.001	30.28	<0.001

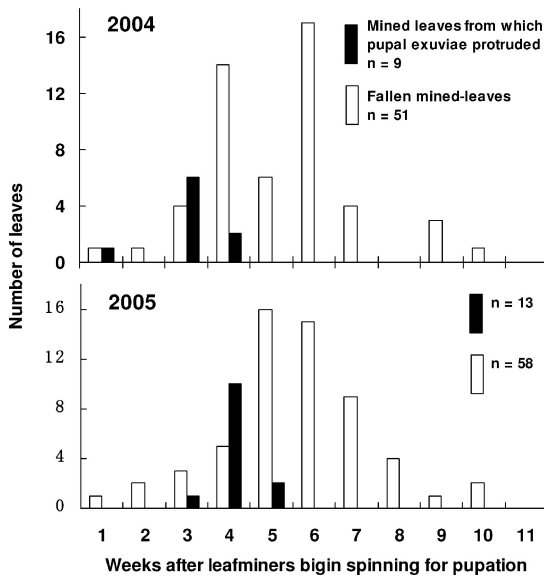


Fig. 3. Timing of adult emergence and mined-leaf fall in 2004 and 2005. Note that pupal exuviae which protruded from mines were not necessarily witnessed in mined leaves during monitoring.

the analyses. These secondary parasitoids were facultative hyperparasitoids and thus attacked host leafminers regardless of whether the hosts were already parasitized or not (unpublished data).

In the period of 17–30 May 2005, before mined leaves with living larvae began abscising rapidly (Fig. 2B), *C. japoniella* leafminers within pleated mines suffered a significantly higher mortality on the ground (53.4%) than on trees (30.0%; Fisher exact test; $P < 0.001$; Table 3). In the period of 7–14 June, when rapid leaf abscission was under way (Fig. 2D), mortalities on the two strata (trees/ground) did not significantly differ (41.2% on the ground and 39.7% on trees; Fisher exact test; $P = 0.892$). In both periods, the respective contributions of parasitism and predation as sources of mortality significantly differed between the two strata (Fisher exact test; $P < 0.001$; Table 4). In the period of 17–30 May, parasitism (46.9%) was 1.5 times as high as predation (29.4%) on trees, whereas parasitism (10.1%) was one seventh the amount of predation (75.9%) on the ground. In the period of 7–14 June, parasitism accounted for >90% of mortality on trees, whereas parasitism (50.0%) and predation (42.9%) were comparable on the ground.

Table 3. Survivorship of *C. japoniella* leafminers on trees and the ground in the periods of 17–30 May and 7–14 June 2005 after the completion of larval feeding

Period	Stratum	Successful emergence	Death	Total	Fisher exact probability
17–30 May (beginning of pupation)	On trees	119	55 (51)	174 (170)	<0.001
	On the ground	69	89 (79)	158 (148)	(<0.001)
7–14 June (end of pupation)	On trees	76	65 (50)	141 (126)	0.416
	On the ground	60	47 (42)	107 (102)	(0.892)

Figures in parentheses in the parasitism column exclude the no. of a koinobiont, *O. kumatai*.

Discussion

Abscission patterns of *E. japonica* leaves mined by *C. japoniella* and unmined leaves are summarized as follows: (1) neither leaves with active mines (those containing a living larva) nor leaves with inactive mines (those containing a dead larva) abscise prematurely in winter; (2) leaves with inactive mines abscise prematurely in spring to early summer; and (3) leaves with active mines rarely abscise before adult emergence.

Statements 1 and 2 indicate that premature leaf abscission can occur in response to herbivory by *C. japoniella* larvae in spring to early summer, but only after the larvae responsible for the leaf damage have died. Such premature leaf abscission seems to be associated with an increase in mine area and with leaf phenology of *E. japonica*. *C. japoniella* larvae greatly expand their mine area over the spring (Fig. 1A and B) and thereby they would impose a heavy physiological cost to the leaf. However, bud burst of the host plant occurs in late April, and intact leaves aged ≥ 2 yr begin falling in early May (unpublished data). *E. japonica* seems to drop not only senescent leaves but also damaged leaves after bud burst.

Statements 2 and 3 indicate that leafminers prevent their host plant from abscising mined leaves before adult emergence. Although previous studies have revealed that mined leaves abscise earlier than unmined leaves, none of them has shown prevention of early leaf abscission by leafminers (Narusse 1978, Faeth et al. 1981, Faeth 1985, Potter 1985, Bultman and Faeth 1986, Simberloff and Stiling 1987, Auerbach and Simberloff 1989, Stiling and Simberloff 1989, Preszler and Price 1993, Waddell et al. 2001, Kagata and Ohgushi 2004). This is mainly because these studies examined leaf abscission patterns without separating leaves with active mines and leaves with inactive mines. Additionally, they dealt with leafminers that made a small mine for the leaf area.

This study predicted that *C. japoniella* larvae would have evolved a strategy to inhibit leaf abscission because they spend a long period of ≈ 9 mo within a leaf and make a large mine for the leaf area (see Introduction). These results prove that this prediction of inhibition is true but that part of the basis is wrong. *E. japonica* does not abscise leaves regardless of whether they are mined or not in winter when mines are relatively small in size. It abscises mined leaves in spring to early summer when mines enlarge. Thus, *C. japo-*

Table 4. Mortality sources of *C. japonella* leafminers in leaves on trees and the ground in the periods of 17–30 May and 7–14 June 2005 after the completion of larval feeding

Period	Stratum	Developmental failure	Parasitism	Predation	Total	Fisher exact probability
17–30 May (beginning of pupation)	On trees	7	33 (29)	15	55 (51)	<0.001
	On the ground	11	18 (8)	60	89 (79)	(<0.001)
7–14 June (end of pupation)	On trees	2	63 (48)	0	65 (50)	<0.001
	On the ground	3	26 (21)	18	47 (42)	(<0.001)

Figures in parenthesis in the parasitism column exclude the no. of a koinobiont, *O. kumatai*.

niella evolved the strategy not because of its prolonged larval period but because of its large mine.

A co-evolutionary process in interacting species consists of reciprocal adaptations to each other (Futuyma 2005). For instance, species A attacks species B, and species B evolves a defense against species A; in turn, species A overcomes the defense of species B. Certainly, premature leaf abscission has a latent capability to starve leafminers to death through leaf desiccation and senescence. However, premature leaf abscission should be considered not to be a damage-induced defense of the host plant against leafminers but to be a simple wound response on the part of the tree (Stiling and Simberloff 1989, Stiling, 1996). Even if it is a response to damage and not the herbivore, leafminers need to overcome premature leaf abscission to increase their survivorship. Indeed, this study revealed that *C. japonella* inhibits the host plant from abscising mined leaves prematurely. Thus, this behavior of *C. japonella* can be regarded as a counteradaptation to the premature leaf abscission of the host plant.

If leafminers reduce the mortality from starvation caused by leaf abscission, inhibition until the completion of larval feeding should be sufficient for leafminers. Larvae of *C. japonella*, however, continue inhibiting leaf abscission until adult emergence. Before discussing its adaptive significance, we consider growth stages of leafminers that were placed on the ground and were left on trees to examine their survivorship and mortality sources (Tables 3 and 4). 17 May was ≈ 1 wk after spinning larvae or pupae were found at the first time in the season. Hence, most of the leafminers that were examined in the period of 17–30 May are judged to have only just pupated (Figs. 1D and 2B). However, adult emergence had already begun before 7 June (Fig. 1D). Hence, most of the leafminers that were examined in the period of 7–14 June were judged to have been ready to emerge as adults. This difference in the growth stage of leafminers in the two periods should be taken into consideration in the discussion below.

Why do *C. japonella* leafminers inhibit their host plant from abscising mined leaves prematurely even after they complete larval feeding? Its adaptive significance depends on the risk of mortality on trees. As Table 3 shows, in the period of 17–30 May, before mined leaves with living larvae began abscising rapidly, leafminers suffer a higher mortality on the ground than on trees. The cause of this difference can be attributed to more intensive predation on the ground

(Table 4). Most leafminers in this period have only just pupated (see above), and thus leafminers within abscised leaves would be exposed to the high risk of being preyed on until they emerge as adults. However, in the period of 7–14 June, when rapid leaf abscission was under way, leafminers suffered similar mortalities on the two strata (ground/trees). Most leafminers in this period are ready to emerge as adults (see above), and thus leafminers within abscised leaves could escape predation because of the short time to adult emergence (Table 4). As a consequence, *C. japonella* leafminers reduce the risk of mortality by inhibiting premature leaf abscission until adult emergence.

Predators of leafminers on trees and on the ground were not identified in this study. Several studies have reported predation of lepidopteran and dipteran leafminers by ants, adult parasitoids, and vespid wasps on trees and by carabid beetles in fallen leaves (Hespenheide 1991). Ants were not frequently observed on *E. japonica* trees during survey. Vespid wasps were not so active in foraging until early summer. Many carabid beetles (*Carabus* sp., *Leptocarabus* sp.) were, however, collected using pit-fall traps in the study period (unpublished data). Hence, adult parasitoids and carabid beetles are likely to be the main predators of *C. japonella* leafminers on trees and on the ground, respectively.

Faeth et al. (1981) have proposed a hypothesis that premature leaf abscission is a significant source of mortality for leafminers. This hypothesis has been supported by many studies (Naruse 1978, Faeth 1985, Potter 1985, Bultman and Faeth 1986, Simberloff and Stiling 1987, Auerbach and Simberloff 1989, Stiling and Simberloff 1989, Preszler and Price 1993, Kagata and Ohgushi 2004). However, as mentioned before, these studies did not separate active mines and inactive mines. This study has shown that *E. japonica* prematurely abscises not leaves with active mines but those with inactive mines. Consequently, premature leaf abscission makes little contribution to an increase in leafminer mortality.

In contrast to Faeth et al. (1981), Kahn and Cornell (1983) have argued that premature leaf abscission can benefit leafminers because it provides them with parasitoid-free space. They suggested that leafminers within abscised leaves could avoid the risk of being parasitized because adult parasitoids usually search hosts on trees according to their stereotypic host-search behavior. In this study, the parasitism rate on the ground was significantly lower than that on trees (Table 4). Clearly, part of leafminers placed on the

ground had been already attacked when they were on trees. Hence, the parasitism rate obtained on the ground must be higher than an actual parasitism rate at which leafminers suffered on the ground. This supports the view of Kahn and Cornell (1983) that premature leaf abscission drops leafminers into parasitoid-free space. However, predators such as carabid beetles frequently attack leafminers on the ground, leading to a higher mortality (Tables 3 and 4). Consequently, even if premature leaf abscission provides *C. japoniella* with parasitoid-free space, it would scarcely benefit the leafminers because of a high risk of being preyed on on the ground.

Larvae of some leafmining species, unlike *C. japoniella*, leave mines to drop onto the ground at the end of feeding. Most larvae of the family Nepticulidae (Lepidoptera) descend through a silken thread on the ground and spine tough flat, oval cocoons for pupation (Johansson et al. 1990). Larvae of *Tischeria decidua* Wocke (Tischeriidae, Lepidoptera) cut a disk-like case out of the leaf, pupating within it on the ground (Sato 1993). The inside of the case is strongly reinforced with silken threads. Tough cocoons or pupal cases must enable these larvae to protect themselves against predation by carabid beetles on the ground. In contrast to *C. japoniella*, these leafminers seem to drop onto the ground as parasitoid-free space, increasing survivorship during pupal stage.

Some leafminers are well known for keeping their mines green in discolored autumn leaves (Hering 1951). These mines are called "green islands." They preserve chlorophyll and may make it possible for leafminers to continue feeding on leaf tissue even after the leaves fall (Prichard and James 1984a). They contain high levels of cytokinins, which are perhaps secreted by the leafminers (Engelbrecht et al. 1969). Cytokinins are effective retardants of not only leaf senescence but also abscission (Addicott 1982). Recent studies have shown that concentrations of indoleacetic acid and ethylene directly regulate leaf abscission, whereas cytokinins influence the production of these hormones and change the sensitivity of abscission zone to them (Sexton 2001). Hence, one can hypothesize that larvae of *C. japoniella* secrete cytokinins and/or other hormones to inhibit leaf abscission. We are still studying how *C. japoniella* leafminers control leaf abscission.

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