Adaptation of leafminers to their natural enemies:
a tritrophic interaction.

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Abstract

Leafminers face many threats in their life, ranging from plant defenses to parasitism. Plants have evolved some unique defenses against leafminers, both direct and indirect defenses. In this paper I give multiple examples of studies done on these defenses and focus more specifically at indirect defenses. Multiple studies show that plants, when damaged by herbivores, emit chemicals for attracting parasitoids of leafminers, this is known as a tritrophic interaction. Leafminers in turn have adapted to this and have evolved defense mechanisms of their own to escape from these plant defenses and prevent parasitism. Recent studies show that leafminers do discriminate between plants and actively select their host plant to escape from plants defenses, this is known as discriminate oviposition behaviour. To escape from parasitism, leafminers have developed mining patterns which are hard to track by parasitoids and thereby increase their search costs. Concluding that leafminers are in an evolutionary arms race with their host plants and parasitoids.

Introduction

Leafminers are species of insects of which the larva lives and feeds between the epidermal layers of a leaf (Borror & DeLong, 1964). They differ in the place of the mine in the leaf, the shape of the mine, and details of the excrements (Ellis, 2009). There are leafminers that belong to gall-making and deeper plant boring as well as external feeders and scavengers. Leafminers attack nearly all plant families, they can mine plants with milky juices, poisonous to higher animals, and even aquatic plants. Leafminers are widely distributed in the temperate zones but are most numerous in the tropics. Leafmining insects occur in four orders of insects: Coleoptera (beetles), Lepidoptera (moths), Diptera (flies), and Hymenoptera (sawflies). They undergo full metamorphosis, from larvae to pupae to adult. Only the larvae are able to live in the interior of leaves, the adults disperse and select the appropriate host plants for their larvae, and mate. The eggs of leafmining insects are laid on the surface of the leaf. Upon hatching the larvae must cut into the leaf, or the adult female has already cut holes and laid eggs in the leaf (Borror & White, 1970). During their stay in the mine larvae grow, forcing the mines to become wider and larger. In most cases the excrements remain in the mine, as distinctive grains or strings. Often also moulting and pupation takes place within the mine. But it also happens that larvae live only part of their life as miners: in later stages they then may live free, generally under a folded leaf margin or lobe, fixed with silk, or less often in a spinning (Ellis, 2009).

Leafminers also have an economic importance. Because some succulent leaves, such as those of parsley and delphinium, wilt as a result of their mining habit. The leaves of vegetables, fruit, and ornamental plants are often damaged the most severely by mining and as a result leafminers are often seen and treated as a pest in agriculture. That is why there is already a lot of research done on leafminers and their natural enemies. The best known example of a species that is worldwide regarded as a pest is the citrus leafminer *Phyllocnistis citrella* (Heppner, 1993; Stelinski & Czokajlo, 2010). Larvae feeding within the leaf, damage leaves which can result in yield loss (Pena *et al.*, 2000). Another negative effect is that the leaf wounds caused by *P. citrella* larvae predispose trees to infection by citrus bacterial canker. This bacteria is responsible for blemished fruit, premature fruit drop, and tree decline (Graham *et al.*, 2004). Feeding *P. citrella* larvae tear the leaf cuticle, exposing mesophyll to direct infection. These wounds do not heal readily, which in turn increases the exposure period to the bacterium. So far, leafminer repellents such as chemical sprays have had little effect on *P. citrella* due to their protection within the leaves of the plant. This is not a unique example, many more agricultural crops face similar threats of leafminer infestation and for many of these species there is still no effective repellent available.

To understand the evolution of interactions between leafmining insects and their host plants, the optimal oviposition theory is central (Thompson & Pellmyr, 1991). According to this theory, oviposition preferences of herbivorous insects should correlate with host suitability for their offspring, as they would maximize their fitness by laying eggs on high quality plants (Thompson, 1988). However, in practice such correlation was only partial or even non-existent in nearly half of the studies conducted (Mayhew, 1997). In general this was because these studies on preference-performance relationships limited their focus to ditrophic interactions between herbivore insects and their host plants (Singer *et al.*, 2004). However, leafmining insects face two substantial challenges in their life: feeding on a resource that is nutritionally poor or even toxic, and being exposed to a wide array of natural enemies and parasitoids. So the value of a plant to a leafminer depends not only on its quality as food, but also on its provision of enemy-free space (Stamp, 2001). Some ecologists have

already proposed that these challenges can be linked if herbivore success on a host plant depends on its risk of predation or parasitism on that host (Price *et al.*, 1980; Jeffries & Lawton, 1984; Berdegue *et al.*, 1996; Dicke & Van Poecke, 2002). A trade-off between food suitability and enemy avoidance may lead a leafminer to prefer plants that are lower in nutrition but on which chances of parasitism are predictably lower, over plants that are higher in nutrition but that are frequently visited by parasites and other natural enemies (Dicke, 2000; Singer *et al.*, 2004).

Multiple recent studies have revealed that plants attract natural enemies such as predators and parasitoids (Turlings *et al.*, 1990; Vet & Dicke, 1992; Pare *et al.*, 1999). However fewer studies have determined whether host plant species affect the risk of predation or parasitism for herbivores. This is of importance, because natural enemies can impose a significant factor in selecting a suitable host plant (Feder, 1995; Yamaga & Ohgushi, 1999), and may ultimately play an important role in the evolution of host plant choice (Price *et al.*, 1980; Bernays & Graham, 1988; Thompson, 1988; Stamp, 2001). Although natural enemies frequently play an important role in determining host plant selection, they have rarely been considered in herbivore preference-performance studies (Videla *et al.*, 2006).

The goal of this paper is to assess the research already done on the tritrophic interaction and to gain insight from an evolutionary point of view. I will give examples of different studies done on leafminers and try to illustrate the relationships with their hosts and their parasitoids. I will also look at the importance of tritrophic interactions and how each interaction between the different actors works in terms of chemistry and physics. Finally I will assess how leafminers have adapted to these interactions from an evolutionary point of view. This may help to provide insight in non-native leafminers on native plants and in native leafminers on non-native plants and may even provide useful insight into the protection of agricultural crops from leafminers.

Direct defences of host plants

Plants face a constant threat of herbivore damage and therefore have developed some unique defences. Some of these defences have been shown to directly repel leafminers while others have been shown to repel other herbivores but these most likely also imply to leafminers. Direct defenses may prevent herbivores from feeding via physical barriers, such as spines, thorns, trichomes, and waxes or chemical ones, with secondary plant metabolites, or via specialized defense proteins. Here I will focus mainly on the defenses that are of relevance to leafminers such as chemical compounds that act as direct defenses and not so much on the physical defenses such as spikes, trichomes which are of less or no relevance to leafminers.

The close association of volatile release with herbivory has suggested that these substances act in plant defence, and there is a lot of research that supports this hypothesis. According to a recent study (Unsicker *et al.*, 2009), plants produce a huge variety of chemical compounds and some of these compounds are released into the surrounding atmosphere. In addition to carbon dioxide, oxygen, water vapour, plants have been shown to emit a variety of other volatiles. The two most common constituents of these volatile blends are terpenes and green leaf volatiles.

In the last decade, evidence that vegetative volatile compounds function to directly repel herbivores has begun to accumulate (De Moraes *et al.*, 2001; Kessler & Baldwin, 2001). For example a recent study (Wang *et al.*, 2008), reported that the monoterpene volatiles of *Chrysanthemum morifolium* (florist's daisy) repel ovipositing females of the diamondback

moth (*Plutella xylostella*). This lepidopteran does not normally lay eggs on *C. morifolium*, and the repellence of the monoterpene volatiles may help explain why.

Volatiles may not only chase away adult lepidopterans but may also deter larvae from feeding. The common C5 volatile isoprene, was shown surprisingly to deter tobacco hornworm (*Manduca sexta*) caterpillars from feeding on isoprene-releasing transgenic tobacco lines and on isoprene-emitting artificial diet (Laothawornkitkul *et al.*, 2008).

Another direct defence plants use is the premature abscission of their leaves, when occupied by leafminers. Addicott was the first to look at the effect of premature leaf abscission on a species of gall aphid. He stated that leaf abscission can serve as a valuable defence mechanism used by the host plant (Addicott & Lyon, 1973). His findings are backed up by the results of Williams and Whitham, who stated that selective leaf abscission is induced by the presence of gall aphids (Williams & Whitham, 1986). Galled leaves are far more likely to be abscised than gall-free leaves. Additionally, the host response shows a rapid decline in chlorophyll content of the galled leaf only one week after colonization of the gall. This is followed a week later by the abscission of the leaf. They also found that the body mass of aphids on leaves near the abscised leaf declined in relation to those near healthy leaves. Thus the negative effects of abscission begin before leaf drop but the most significant impact arises after leaf abscission when 98% of aphids occupying abscised leaves die (Williams & Whitham, 1986).

These are just a few examples of the many studies that address plant defenses that are induced by feeding herbivores. All the previous studies have in common that the plant response starts when the feeding damage and loss of foliage have already begun. However recent research has shown that a plant is able to respond just in time to prevent herbivores from feeding at all. Plants have evolved several mechanisms to respond to the very first step of insect attack; oviposition. These mechanisms entail both direct as well as indirect plant defenses and aim to rid the plant of eggs and kill them, and so preventing feeding damage by the larvae (Hilker & Meiners, 2002; Hilker *et al.*, 2002). Direct plant defenses induced by insect egg deposition can affect either the eggs themselves or the egg-laying female. One strategy is the formation of a neoplasm in response to egg deposition, this elevates the eggs from the leaf surface. Such elevated eggs easily drop off the leaf (Doss *et al.*, 1995). A different strategy is used by rice plants to deter planthoppers, they produce an ovicidal substance that kills the eggs in response to egg deposition (Seino *et al.*, 1996). Another strategy to get rid of the eggs is to form necrotic tissue where the eggs are laid. This hypersensitive response detaches the eggs from the plant surface and makes them fall to the ground. Larvae that hatch on the ground suffer a high mortality rate and rarely find their way back to their host plant (Shapiro & Devay, 1987). A final direct defense strategy induced by egg deposition is directed at the ovipositing female by rendering leaves deterrent that already carry eggs or are adjacent to leaves that carry eggs (Blaakmeer *et al.*, 1994).

To summarize, leafminers face many direct plant defenses such as vegetative volatile compounds designed to directly repel leafminers, leaf abscission and attacks on the larvae leafminer before it has even hatched. But still, these are not the only threats leafminers face, so which other threats do leafminers face and (how) have they adapted to all of these threats?

Indirect defences of host plants, a tritrophic interaction

As previously noted, plants use multiple chemicals to directly repel leafminers and herbivores in general, but there is also evidence that plants use chemicals as an indirect defence against leafminers. The most passive scenario of plant involvement is that leafminers and mechanical damages evoke similar semio-chemicals. This scenario has been verified in several plant families such as Cucurbitaceae, Fabaceae, Solanaceae, Ginkgoaceae, and Vitaceae (Dicke, 1999; Van Den Boom *et al.*, 2004). Using ubiquitous compounds, such as green leaf volatiles, for host localisation by general parasitoids could be an adaptation of the evolution of tritrophic interaction. In 1995 Turlings found that (Z)-3-hexenol plays an important role in the initial step of parasitoid host location. Although (Z)-3-hexenol is important, other specifically induced volatiles may be used by some parasitoids and the capability of associated learning of parasitoids are also essential in this process (Turlings *et al.*, 1995; Turlings & Wackers, 2004). Some secondary metabolites or compounds may be used to improve the precision of host location, or involve other host location cues, such as visual, contact and taste cue.

Another study done on tritrophic interactions was conducted by Dutton (Dutton *et al.*, 2000). He wondered which chemical compounds attracted the parasitoid (*Pholetesor bicolor*) of the apple leafminer (*Phyllonorycter pomonella)* and where the chemical compounds came from. Behavioural bioassays showed that the leaf epidermis damaged by the leafminer elicited ovipositional probing of parasitoid females. Whereas probing on larvae or frass was seldom observed. Hexane extracts of mines elicited the same ovipositional probing behaviour, while no response was observed with hexane extracts of larvae or frass or with methanol and diethyl ether extracts. In addition he also conducted chromatographic analyses, showing qualitatively and quantitatively different profiles of these three compounds of the host-plant complex. He found that by far the highest quantities and also the highest number of compounds was recovered from mine extracts. The compounds identified in the mine included six alkanes and squalene. A synthetic blend of the seven compounds proved to be slightly less active in biotests than the equivalent natural blend. this was shown by a time delay in female response. He concluded that this leafminer parasitoid does not rely on host-derived kairomones but instead uses plant-derived semiochemicals for host location and ovipositional probing behavior (Dutton *et al.*, 2000).

Some years later, another study was done on how parasitoids use certain vegetative chemical compounds specifically for host location (Wei *et al.*, 2007). They looked at leafminer larvae infested leaves, jasmonic acid (JA)- treated leaves and mechanically damaged leaves and looked which chemicals were emitted by the plant. They found that leafminer larvae-infested leaves and jasmonic acid (JA)- treated leaves from most plant families showed abundant productions of GLVs, monoterpenes, homoterpene, and sesquiterpenes. Interestingly, when leafminer larvae-infested leaves or JA-treated leaves were compared to mechanically damaged leaves, they found that all the investigated plant species released one or more newly produced compounds. These results indicate that several biosynthetic pathways are induced by different treatments of the plants, some of which are very common biosynthetic pathways to produce the similar compounds and some of which are less common biosynthetic pathways to generate the specific compounds.

They then looked at differences in responses of parasitic wasps to some of these chemical compounds. They observed significant differences in the responses of parasitic wasps to (*Z*)-3-hexenol, TMTT and 3-methylbutanal oxime at different dosages that they tested. These

results may suggest that these three induced compound play the most important roles in host location of this parasitoid and the concentration of them is not a critical factor for attracting the parasitoids.

Further testing showed that the parasitic wasps responded preferably to (Z)-3-hexenol to either mixture of the three compounds or individual compounds. They observed higher percentages of parasitoid responses to the mixture blend than to 3-methylbutanal oxime, this could also be due to the effect of their strong attraction to one of the blend compounds, (Z)-3-hexenol. This suggests that the naive parasitoid uses (Z)-3-hexenol from mechanically- or leafminer-damaged plants as the primary damage attractant to locate the host plant because this compound with its high volatility may be the first sensed by parasitoids.

In a previous study they hypothesized that the predominant compounds in herbivore-induced volatile blends play an important role in mediating parasitoid search behavior over relatively long distances, while secondary and minor compounds improve the precision of host location over short distances (Wei & Kang, 2006). In this study the data clearly indicated that (Z)-3-hexenol is the more important general damage attractant, while TMTT (11-tridecatetraene) and 3-oxime are the important distinguishing attractants.

Evidence that this biosynthetic pathway is shared by a wide range of plants and might be used by many insect parasitoids and predators is further provided by the existence of (Z)-3-hexenol in all of the plants used for this experiment except celery. Finally, they looked at host plant preferences of parasitoids and found that leafminer host plants were preferred over two non-host plants odor. This indicates that parasitoids are able to distinguish the leafminers host plant from non-host plants based not only on (Z)-3-hexenol but also on other chemical cues involved (Wei *et al.*, 2007).

As previously discussed plants have also evolved defenses to deter insect eggs, to prevent feeding damage before it can occur. In addition to direct defenses against insect eggs they also have evolved indirect defenses against insect eggs. As is the case in indirect defenses induced by feeding or plant damage, the known indirect plant defense induced by egg deposition also act by supporting egg parasitoids to locate their hosts. For three tritrophic interactions, it has been shown that insect egg deposition induces plants to release volatiles which attracts egg parasitoids (Meiners & Hilker, 1997; Meiners & Hilker, 2000; Colazza *et al.*, 2004), whereas the study of a further system suggests that egg deposition induces the change of plant surface chemicals. By changing the plants surface chemicals, the egg parasitoid is arrested by contact cues in the vicinity of the eggs (Fatouros *et al.*, 2005).

There is a lot of evidence that plants emit volatiles to attract parasitoids, but none of these studies show if there are differences between the levels of parasitism on different host plant species. Recently, an experiment was conducted which compared the levels of parasitism on leafminers on two species of tree; box elder and black willow (Barbosa *et al.*, 2001). They found compelling evidence that the host-plant species plays an important role on the levels of parasitism. They found that parasitism of all macrolepidoptera larvae collected on box elder was significantly higher than that of larvae collected on black willow. Even more compelling evidence of the role of host species on levels of parasitism is provided by a comparison of total parasitism among larvae of species that were collected both on box elder and black willow. They found that the total level of parasitism on larvae of species on box elder was significantly higher than that on larvae of the same species when they occurred on black willow (Barbosa *et al.*, 2001). This indicates that plant species differ in their capability of attracting parasitoids and may thus play an important role in host plant selection.

The examples given above clearly illustrate that there is a lot of evidence for tritrophic interactions between leafminers, their host plants and their parasitoids. It also illustrates that the vulnerability of leafminers to parasitoids can be strongly influenced by the herbivore's host plants, this in turn can provide a strong selection pressure on leafminers. So what strategies have leafminers evolved to escape from all these potential threats?

Adaptation of leafminers to plant defences and parasitoids

Leafminers face two important threats in life, the first is direct plant defence such as chemical compounds and leaf abscission etc. and the second is indirect plant defence consisting of chemical compounds which attract natural enemies such as predators and parasitoids. In accordance with the evolution theory, leafminers have evolved different strategies to avoid these two threats.

One of these evolved strategies against direct and indirect plant defences was shown by a recent study (Videla *et al.*, 2006). They did a study on host plant preference of the leafminer *Liriomyza huidobrensis*. They found that adult *L. huidobrensis* females and males varied in size depending on the host plant on which they had developed. Such variation was highly correlated with the apparent preferences of the leafminer in the field. This result suggests a link between preference and performance, because female size is frequently associated with reproductive potential (Valladares & Lawton, 1991; Honek, 1993; Harris *et al.*, 2001). Analyses of other performance indicators in the laboratory also supported a positive relationship between female host preference and offspring performance. Plant species affected development time, particularly for larvae, which are actively feeding and thus are more exposed to the nutritional quality and defensive compounds of plant tissues than other life stages. Shorter development times may increase herbivore fitness by decreasing susceptibility to parasitoids (Williams, 1999). Vulnerability of leafminers to parasitoids is highest during the larval stage (Hendrickson *et al.*, 1983), particularly for serpentine mines, where larvae are easily detected by the visual cues offered by the mine (Salvo & Valladares, 2004). Thus they concluded, *L. huidobrensis* larvae should benefit from reduced exposure to parasitoids by actively choosing their host plant for ovipositing, which is also backed up by data they got from the field on parasitism rates (Videla *et al.*, 2006).

Another example of discriminate oviposition behaviour was presented by Uesugi (Uesugi, 2008). He hypothesized that because herbivores are often less adapted to using a novel host than their native hosts, herbivores are expected to either become physiologically adapted to the novel host or to evolve oviposition-avoidance behaviour. He looked at the host plant selection of the leafminer *Amauromyza flavifrons* on the native host plant *Saponaria officinalis* (bouncing bet) and the non-native host plant *Beta vulgaris* (sugar beet). The results from his study support the hypothesis that oviposition on *B. vulgaris* is selected against in populations near sugar beet farms. In a population in the state of Michigan (MI) that was surrounded by sugar beet farms, fewer females accepted *B. vulgaris*, and when they did, they laid fewer eggs on it than New York (NY) females. As the intrinsic rate of oviposition on its normal host, *S. officinalis*, did not differ between the populations, the observed pattern was solely in response to the novel host, *B. vulgaris*. In agreement with Scheffer's (Scheffer, 1999) observation that larvae hatch on *B. vulgaris* but die soon afterwards, larval development on *B. vulgaris* was not observed in either population, suggesting that physiological adaptation has not occurred. This lack of larval viability on *B. vulgaris* implies that indiscriminate oviposition behavior would be strongly deleterious, as a female's fitness decreases every time

she makes an oviposition mistake. Opportunities for selection provided by the presence of sugar beet farms seem to have led to the evolution of *B. vulgaris* avoidance in a subpopulation of *A. flavifrons* (Uesugi, 2008). This provides a clear example of discriminate ovipositing behaviour, a strategy to avoid direct plant defences.

Discriminate ovipositing behaviour is not the only defence of leafminers against host plant defences, another direct plant defence illustrated above is the ability of plants to abscise their leaves when occupied by a leafminer. However, a recent study suggests that leafminers have evolved a way to prevent this. They found that mined leaves with sacrificed larvae fell at a constant rate, abscising significantly more than unmined leaves. In contrast, mined leaves with living larvae rarely fell before the adults emerged from the leaves, while afterwards they abscised rapidly. These findings combined with the observation that leafminers on the ground suffered a higher mortality rate from predation than those on trees, suggest that the leafminer prevents the host plant from abscising mined leaves prematurely until adult emergence, thereby increasing their survival (Oishi & Sato, 2007). Although these findings suggest compelling evidence for the prevention of premature leaf abscission, the mechanisms behind this process are still not known.

Thus far we have seen adaptations of leafminers against different plant defences. However, even if discriminate oviposition behaviour is a useful protection against direct plant defences and to some extent against indirect plant defences as well, it is not a direct protection against parasitoids and predators. So what if a parasitoid or predator manages to locate their mine? Compelling evidence suggests that leafminers have evolved their mining patterns in such a way as a defence against parasites and predators.
Kato was the first to look at the adaptive significance of leaf-mining pattern as an anti-parasitoid strategy (Kato, 1985). Because leafmining patterns frequently branch and cross they cannot be explained by the theory of the effective resource use. Kato came up with a theoretical model that suggested that the complex mining patterns had developed as an advantageous strategy of parasitoid avoidance. He considered three mining patterns, linear mine, branching mine and crossing mine. Locating a leafminer at an end of the mine is more time-consuming for a parasitoid than one lying midway in the mine because, when the leafminer lies midway of the mine, a parasitoid searches two or more smaller divided parts and this makes the searching distance short. He also found that a leafminer in the branch or cross mine is more time-consuming than in simpler advancing mine. Because when the parasitoid chooses the wrong side at branching and cross points, the parasitoid must repeatedly walk along vacant branches and loops before locating a leafminer. In his model he suggested that parasitoids have a giving-up distance over which the parasitoid stops searching and leaves the leaf.

In 2008, another study was done in practice to test Kato's model (Ayabe *et al.*, 2008). They looked at the mining pattern of the leafminer *Liriomyza trifolii* and the searching time of its parasitoid *Hemiptarsenus varicornis*. This study demonstrated the adverse effect of mining patterns of *L. trifolii* on parasitoid searching efficiency and the importance of the retracking strategy adopted by *H. varicornis* in terms of searching time. Behavioural observations of *H. varicornis* showed that, first, mines with crosses increased the number of mine trackings and consequently increased total searching time of the parasitoid, and, second, each mine-tracking time was independent of both behavioural events during tracking and the complexity of mines. They also performed computer simulations which indicated that the retracking of a mine with a constant mine-leaving rate seems advantageous in terms of total searching time. Mines of *L. trifolii* with crosses prolonged the total searching time of *H. varicornis*. This is so

far consistent with Kato's model, however, according to Ayabe et al, a tortuous mining pattern functions only partially as a defence against parasitoid attack because it does not lead to an unsuccessful search. This study therefore only partially supports the hypothesis that complexity of mining patterns may function to defend leafminers from parasitoid attack. But despite the incomplete defence provided by the tortuous mining pattern, the increase in parasitoid search cost through mine complexity might be an important component influencing antagonistic interactions between leafminers and their parasitoids (Ayabe *et al.*, 2008).

So while host plants have evolved stunning defence mechanisms, leafminers have adapted to these defences and have also evolved stunning defence mechanisms of their own. Through discriminate oviposition behaviour they can avoid the host plants which attract the most parasitoids. And even if they are eventually found by parasitoids they have evolved their complex mining pattern in such a way that the parasitoid gives up, or at least increases the cost of the search for the parasitoid, which in turn is a benefit for leafminers.

Discussion

There is already a lot of research done on the tritrophic interactions in general as well as on leafminers specifically. Multiple researches have revealed that plants have evolved a wide array of chemical defenses against herbivores both direct defenses as well as indirect defenses. Direct defenses include chemical deterrents such as vegetative volatiles (De Moraes *et al.*, 2001; Kessler & Baldwin, 2001; Wang *et al.*, 2008) and physical deterrents such as premature leaf abscission (Addicott & Lyon, 1973; Williams & Whitham, 1986). A lot of studies gathered evidence to support adaptations of leafminers to these direct defenses. Through discriminate oviposition behaviour, leafminers can avoid the host plant which uses the most chemical deterrents or attracts the most parasitoids (Videla *et al.*, 2006; Uesugi, 2008). Evidence for adaptation of leafminers to premature leaf abscission is provided by Oishi, who recently found that leafminers may be able to prevent premature leaf abscission, although the direct mechanism for this is still unknown and further evidence is still limited (Oishi & Sato, 2007).

In addition to direct defenses, plants have also evolved a unique indirect defense mechanism against leafminers, the tritrophic interaction. Herbivore damage evoke the release of chemical volatiles, which attract parasitoids and predators. A lot is known about these tritrophic interactions and about specific blends of chemicals different plants use and also about their effect on both herbivorous and carnivorous insects. Quite recently they have discovered plants differ in their ability to attract parasitoids (Barbosa *et al.*, 2001). Evidence suggests leafminers have adapted to these differences in tritrophic interactions of different host plants through discriminate oviposition behaviour (Videla *et al.*, 2006; Uesugi, 2008). These findings suggest that leafminers do prefer plants with lower levels of parasitoids over plants with higher levels of parasitoids. This may play an important role in understanding host plant selection by parasites and in turn may provide useful information for the protection of agricultural crops. In addition to discriminate oviposition behavior, leafminers have also evolved another, more direct way to avoid parasitoids and predators. Kato's findings suggest that the complex structure of the mine acts as a direct defense against parasitism by enhancing the search time and so the search costs for parasitoids (Kato, 1985). Although a later research suggests this does not lead to an ineffective search (Ayabe *et al.*, 2008), it does enhance the search cost for the parasitoid and is thus considered an effective adaptation.

A lot of research has already been done on the interactions between leafminers and their host plants, and a lot of examples of tritrophic interactions in many species of leafminers and plants can be found. However, more research on differences in ability of plants to attract parasitoids needs to be done, because even though there is compelling evidence for this difference, it is still poorly understood and needs to be further examined. Thus far, there are only a couple of studies done on this subject and therefore it is limited to a few plant and parasitoid species. With all the different plant and parasitoid species, these studies provide some evidence, but not enough to provide an overall picture of plants and parasitoid species in general. Therefore, future studies may provide more evidence in more diverse plant and parasitoid species and may present a more general picture.

Also, the tortuous mining patterns of leafminers as a defense against parasitoids is not clear. One study suggests that these complex mining patterns prevent parasitoids from finding the leafminer completely, while another study suggests that it only enhances the searching time and thus the searching costs of the parasitoid. Either way, it is an adaptation of leafminers to prevent parasitism, so it does provide an advantage, but to what extent is still not known. Therefore, more studies should be done on this adaptation, to find out to what extent the tortuous mining pattern is an effective adaptation.

Another point of attention is the recently discovered adaptation of leafminers to prevent premature leaf abscission. Although there is some evidence supporting these findings, there is still little evidence for this adaptation and the mechanisms around it are not clear. Thus far the theory is based on a single study done by Oishi and Sato (2007) and therefore provide an interesting theory, but it certainly does not provide an overall picture of leafminer and plant species in general. Future studies may provide more evidence to support this theory, but for now it is too early to draw general conclusions from these findings. It does however, suggest a most interesting adaptation of leafminers to their host plants.

Because of the enormous diversity in leafminer and host plant species and their worldwide distribution, the same holds true for the discriminate ovipositing behaviour. Even though there are multiple studies that provide evidence for this behaviour, it may be too easy to draw any general conclusions from these findings. However, all of these different adaptations do provide some interesting examples of the many evolutionary strategies employed by leafminer and host plant species and should not be ignored because of the little studies done so far. On the contrary; more studies should be done on the different adaptations in more diverse leafminer, plant and parasitoid species to provide more evidence for the existing theories and to find new ones.

In addition to scientific interests, future studies can also be a great help for finding a biological repellent against leafminers in agricultural crops. Leafminers are still considered a pest species and a useful biological repellent still has not been developed. New studies may provide insight into a biological control of leafminers, through the natural chemical attractants of parasitoids or other natural chemical repellants used by host plants. This way there is no need for the use of artificial chemical repellants which are harmful to the environment and the biodiversity present.

All in all, I hope this paper has improved the understanding of the tritrophic interaction. I hope it has shown that leafminers are entangled in an evolutionary arms race with their host plants and their parasitoids, and have well adapted to these threats. Because of the enormous diversity in leafminer and host plant species it is still difficult to provide a general picture, but

it is clear that leafminers have evolved multiple adaptations to face their threats. For future researchers still a lot of studies can be done and a lot of exciting interactions can be found.

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