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No Evidence for Enemy Release During Range Expansion of an Evergreen Tree in Northern Europe

ANNE-MARIE T. SKOU,1,2 BO MARKUSSEN,3 LENE SIGSGAARD,1 AND JOHANNES KOLLMANN1,4

ABSTRACT 1. Plant distributions are dynamic but the role of plant–insect interactions in controlling range dynamics is not well understood. Enemy release, for example, could facilitate plant range expansion under climate change. 2. We conducted a transplant experiment with the evergreen tree Ilex aquifolium L. in both the historical and the expanding range in Denmark to study possible effects of geographical position, small-scale distance, and plant types on presence and performance of the monophagous insect leaf-miner Phytomyza ilicis Curtis. 3. The leaf miner was present in the entire range of I. aquifolium in Denmark, and there were no differences in emergence success depending on geographical position. Small-scale distance to existing adult plants influenced the activity of the insect on the transplants, and oviposition density was negatively correlated with distance to adult plants. 4. Plant type had an effect on leaf miner feeding, oviposition and mining, and the native provenance of I. aquifolium supported higher densities than two cultivars. 5. There was no evidence that enemy release facilitates the current range expansion of I. aquifolium.

KEY WORDS  holly leaf-miner, Ilex aquifolium, geographical distribution, host-parasitoid system

Most plant species have restricted ranges, and it is generally accepted that climate is the most important large-scale factor determining species distributions (Gaston 2003). Temperature is the main variable controlling distribution of many species (Grace 1987, Jeffree and Jeffree 1994), but other factors like precipitation can also play a significant role in defining suitable habitats (Thuiller et al. 2003). Research in the past decades has shown increasing evidence for climate change affecting species and ecosystems (Hughes 2000, Thomas et al. 2001, Walther et al. 2002), with range expansion and contraction being among the most obvious effects (Bakkenes et al. 2002, Walther 2004). The impact of climate change on the dynamics of plant distributions is most visible at range limits where species grow under marginal ecological conditions (Walther 2004).

Although climate is accepted to strongly influence the present and future distribution of species at a large scale, the role of biotic interactions on species distribution is less well understood (Araújo and Luoto 2007, Van der Putten et al. 2010). One example of this deficit are ‘bioenvelope’ models that correlate current distribution of species with climatic variables and estimates of future climate to predict changed distributions (Huntley et al. 1995, Carey 1996, Bakkenes et al. 2002, Berry et al. 2002). It is, however, recognized that this model type fails to include species interactions and these are also neglected in many studies on global change (Brooker et al. 2007, Berg et al. 2010), especially intracontinental range expansions (Morrien et al. 2010). One way to identify and to disentangle the factors affecting species distributions is to carry out transplant experiments. For species limited by multiple factors, transplants outside their current range can show an interesting response that helps understand the controlling abiotic and biotic factors (Hennenberg and Bruelheide 2003), although the relative importance of these factors may vary among regions (Gaston 2003).

The lack of historical data on a regional scale is a challenge for studies of shifting range margins. Regarding data on past and present distributions, the woody evergreen tree Ilex aquifolium L. is an exception (Bañuelos et al. 2004, Walther et al. 2005). This species reaches its northeastern distribution border in Denmark, where it was present in Jutland and Funen but not on the eastern islands (Iversen 1944). This relatively sharp distribution border was correlated with a January isotherm of −0.5°C. Thus, I. aquifolium has been used as a classical example of a climatically-limited species (Gaston 2003). However, recent field observations have revealed presence of I. aquifolium in most eastern parts of Denmark (Hartvig 2004), and this range expansion seems to be related to changes in climate and land use (Bañuelos et al. 2004), whereas potential biotic limitations have not been studied. Phytomyza ilicis Curtis (Diptera: Agromyzidae), the holly leaf-miner, is the most common insect herbivore on I. aquifolium. It is strictly monophagous, and thus, its
distribution is restricted by the availability of holly trees (Brewer and Gaston 2003). Many aspects of this close plant–animal interaction have been studied (Heads and Lawton 1983, James and Pritchard 1988, Valladares and Lawton 1991, McGeoch and Gaston 2000, Bonsall and Eber 2001, Eber 2001, Klok et al. 2003, Banuelos and Kollmann 2011), but there is no published information on the response of the leaf miner to the expanding range of its host species. *P. ilicis* can cause premature leaf fall in *I. aquifolium*, with the probability of being shed double for a mined leaf compared with an unmined leaf (Owen 1978, Brewer and Gaston 2002), and thus a possible enemy release from the leaf miner may have positive effects on the range expansion of this plant species. Growth rates of *I. aquifolium* have also been shown to be negatively affected by *P. ilicis* (Ogilvy et al. 2006), with decreasing levels of photosynthesis in highly-infested plants (Hespenheide 1991). Eber (2004) showed that small-scale distance had a negative effect on colonization of the leaf miner but distant populations of hollies (>100 m away) were still located by the adult flies. Thus, when comparing leaf miner activity inside and outside the historical range of *I. aquifolium*, small-scale variation in leaf mining should be investigated as well. *I. aquifolium* reaches its north-eastern range margin within Denmark. Several natural populations are found in eastern Jutland, some on the island of Funen, and few on Lolland and Zealand (Odum 1968).

The holly leaf-miner *Phytomyza ilicis* Curtis (Diptera: Agromyzidae) has a simple life cycle (Cameron 1939, Lewis and Taylor 1967). Briefly, the adult fly lays eggs in May–June on the fresh leaves at the base of the underside of the midrib. The female flies leave characteristic scars because of the insertion of the ovipositor into the midrib. The young larvae eat through the midrib, and by autumn enter the central tissue and create blotch mines (Fig. 1). Feeding continues over winter, and then the larvae pupate to produce adults in May and June. The flies also feed on the leaves and leave visible punctuations.

During the months spent inside the leaves, the leaf miners can suffer from different kinds of mortalities. As larvae they can die from parasitism by *Chrysocharis*...
aquifolium, selected inside and outside the historical range of sites widely distributed over Denmark, haphazardly for unexplained causes (natural death; Brewer 2004). Pupal parasitism also occurs because of at least eight Kollmann 2011). Bird predation is mainly by thrushes.

gemma Walker (Hymenoptera: Eulophidae) or other parasites, pathogens, and predators (Bañuelos and Kollmann 2011). Bird predation is mainly by thrushes. Pupal parasitism also occurs because of at least eight species of hymenopteran parasitoids. Finally some larvae die for unexplained causes (natural death; Brewer and Gaston 2003).

Study Sites. The transplant study was done in 18 sites widely distributed over Denmark, haphazardly selected inside and outside the historical range of I. aquifolium, with contrasting climatic conditions but little geomorphological or geological variation (Fig. 2; Table 1). In western Denmark, most sites were in mid-Jutland except 'Randbøldal' and 'Haderslev' in southern Jutland. The sites on Zealand represented the expanding range of the species and they were all located in the eastern part of this island.

Half of the sites in Jutland were natural populations of I. aquifolium ('NaturalJ'). The other six sites in Jutland were identified as garden-escaped populations ('EscapeJ') from a previous survey (Atlas Flora Danica; Hartvig 2004). The six populations on Zealand were all garden-escape populations ('EscapeZ'). These three categories: NaturalJ, EscapeJ, and EscapeZ represent location types. All study sites were open forests with most of them dominated by beech (Fagus sylvatica L.), some by conifers and others by a mix of common deciduous trees. Every site contained at least one adult I. aquifolium (population size 1-100 plants).

Plant Material and Planting Design. I. aquifolium transplants from two common garden cultivars ('Blue Angel', 'Madame Briot') and from a native population ('Randbøldal') in SE-Jutland were used. The rooted cuttings were produced by the nursery 'Vornaes', on Tåsinge near Funen in central Denmark in spring 2007 and 2008. Upon arrival at the gardens at the University of Copenhagen in June, the cuttings were transplanted into 2-l pots by using a standard peat-based substrate (N, 120 g m⁻³; P, 60 g m⁻³; K, 200 g m⁻³; pH 5.9). For acclimation to Danish climate the plants were kept in pots in the university gardens for 4 mo until transport to the field sites; plants were covered with an insect net to avoid infestation by the leaf miner, and tap water was provided when necessary. In the study sites, the transplants were taken out of their pots and planted in four groups with 1- and 10-m distance, respectively, to the edge of a focal adult I. aquifolium (Fig. 3). Within the groups, transplant distance was 0.3–0.6 m, and the order of the plant types was random. Care was taken not to damage the root balls of

Table 1. Site characteristics of the study populations of I. aquifolium (Location type, location type within the species’ range—see Figure 1; average temperature, mean temperature in 2009; minimum temperature, minimum temperature in 2009; population apparency, which is the number of plant individuals multiplied with average plant height)

<table>
<thead>
<tr>
<th>Loc. type</th>
<th>Site</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Av. temp. (°C)</th>
<th>Min. temp. (°C)</th>
<th>Forest type</th>
<th>Geological substrate</th>
<th>Pop. app.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaturalJ</td>
<td>Dorup</td>
<td>56° 1' 44.45&quot; N</td>
<td>9° 43' 56.67&quot; E</td>
<td>9.5</td>
<td>-9.0</td>
<td>Beech</td>
<td>Sand/gravel</td>
<td>300</td>
</tr>
<tr>
<td>NaturalJ</td>
<td>Frueing</td>
<td>56° 2' 22.99&quot; N</td>
<td>9° 59' 27.87&quot; E</td>
<td>8.9</td>
<td>-8.0</td>
<td>Beech, conifers</td>
<td>Clay</td>
<td>150</td>
</tr>
<tr>
<td>NaturalJ</td>
<td>Gjern Bakker</td>
<td>56° 13' 21.45&quot; N</td>
<td>9° 41' 17.74&quot; E</td>
<td>9.0</td>
<td>-8.5</td>
<td>Pine, spruce</td>
<td>Sand/gravel</td>
<td>500</td>
</tr>
<tr>
<td>NaturalJ</td>
<td>Gudenåvej</td>
<td>56° 0' 35.47&quot; N</td>
<td>9° 40' 6.42&quot; E</td>
<td>9.5</td>
<td>-9.0</td>
<td>Beech, spruce</td>
<td>Sand/gravel</td>
<td>6.25</td>
</tr>
<tr>
<td>NaturalJ</td>
<td>Haderslev</td>
<td>55° 10' 21.73&quot; N</td>
<td>9° 31' 45.47&quot; E</td>
<td>8.7</td>
<td>-9.5</td>
<td>Old beech</td>
<td>Clay</td>
<td>13.5</td>
</tr>
<tr>
<td>NaturalJ</td>
<td>Randbøldal</td>
<td>55° 40' 27.91&quot; N</td>
<td>9° 17' 36.74&quot; E</td>
<td>9.0</td>
<td>-10.0</td>
<td>Old beech</td>
<td>Sand/gravel</td>
<td>500</td>
</tr>
<tr>
<td>EscapeJ</td>
<td>Bjerringbro</td>
<td>56° 23' 0.75&quot; N</td>
<td>9° 39' 16.88&quot; E</td>
<td>8.9</td>
<td>-8.5</td>
<td>Beech, pine</td>
<td>Sand/gravel</td>
<td>38</td>
</tr>
<tr>
<td>EscapeJ</td>
<td>Fjøstrup</td>
<td>56° 3' 57.29&quot; N</td>
<td>10° 14' 42.71&quot; E</td>
<td>8.9</td>
<td>-7.5</td>
<td>Beech</td>
<td>Clay</td>
<td>10</td>
</tr>
<tr>
<td>EscapeJ</td>
<td>Neder Romalt</td>
<td>56° 27' 24.22&quot; N</td>
<td>10° 7' 54.23&quot; E</td>
<td>8.7</td>
<td>-7.0</td>
<td>Beech</td>
<td>Clay</td>
<td>5</td>
</tr>
<tr>
<td>EscapeJ</td>
<td>Overholt Plantage</td>
<td>56° 12' 19.48&quot; N</td>
<td>9° 42' 34.12&quot; E</td>
<td>9.0</td>
<td>-7.5</td>
<td>Fir plantation</td>
<td>Sand/gravel</td>
<td>200</td>
</tr>
<tr>
<td>EscapeJ</td>
<td>Risskov</td>
<td>56° 10' 54.21&quot; N</td>
<td>10° 13' 36.20&quot; E</td>
<td>8.7</td>
<td>-6.5</td>
<td>Beech, larch</td>
<td>Clay</td>
<td>36</td>
</tr>
<tr>
<td>EscapeJ</td>
<td>Stokkebro</td>
<td>56° 30' 15.73&quot; N</td>
<td>10° 49' 9.00&quot; E</td>
<td>8.7</td>
<td>-7.0</td>
<td>Old beech</td>
<td>Clay</td>
<td>76</td>
</tr>
<tr>
<td>EscapeZ</td>
<td>Folehave</td>
<td>55° 52' 14.38&quot; N</td>
<td>12° 31' 50.44&quot; E</td>
<td>9.0</td>
<td>-10.0</td>
<td>Old beech</td>
<td>Clay</td>
<td>5</td>
</tr>
<tr>
<td>EscapeZ</td>
<td>Gammelmosen</td>
<td>55° 5' 11.33&quot; N</td>
<td>12° 30' 16.44&quot; E</td>
<td>8.9</td>
<td>-12.0</td>
<td>Beech</td>
<td>Clay</td>
<td>27</td>
</tr>
<tr>
<td>EscapeZ</td>
<td>Hornby Sand</td>
<td>56° 5' 30.21&quot; N</td>
<td>12° 23' 15.06&quot; E</td>
<td>8.6</td>
<td>-10.5</td>
<td>Beech</td>
<td>Sand/gravel</td>
<td>7.5</td>
</tr>
<tr>
<td>EscapeZ</td>
<td>Hesterkøb</td>
<td>55° 51' 17.08&quot; N</td>
<td>12° 28' 22.99&quot; E</td>
<td>8.5</td>
<td>-10.5</td>
<td>Old beech</td>
<td>Clay</td>
<td>114</td>
</tr>
<tr>
<td>EscapeZ</td>
<td>Jøggersborg Hegn</td>
<td>55° 49' 24.24&quot; N</td>
<td>12° 32' 50.38&quot; E</td>
<td>8.8</td>
<td>-10.0</td>
<td>Old beech</td>
<td>Sand/gravel</td>
<td>10</td>
</tr>
<tr>
<td>EscapeZ</td>
<td>Stenholtsvang</td>
<td>55° 56' 38.41&quot; N</td>
<td>12° 18' 42.31&quot; E</td>
<td>8.5</td>
<td>-11.0</td>
<td>Old beech</td>
<td>Sand/gravel</td>
<td>5</td>
</tr>
</tbody>
</table>
the transplants when planting. The transplant study used the same plant types and planting design in all 18 sites, but half of the transplants were planted in September 2007 and half in September 2008.

**Data Collection.** The 18 sites were revisited in June–July 2009, when all *P. ilicis* adults from the 2008 generation had emerged and mines from the 2009 generation had not yet developed. All leaves of the transplanted *I. aquifolium* were carefully examined for signs of adult feeding, oviposition and developed mines. The mines were also characterized as: 1) ‘successful’, leaving a D-shaped hole where the leaf miner had emerged; 2) ‘bird predation’ with a V-shaped mark; 3) ‘parasitized’ indicated by a small round hole; or 4) ‘miscellaneous deaths’ (refer to Field Studies Council 2009 for images).

The transplants from September 2008 contained no mines in summer 2009 because of protection against infection by *P. ilicis* within the university gardens of Copenhagen. These plants were therefore disregarded for the analysis of mines. In addition to examination of transplants, the focal adult *I. aquifolium* plants at each site were examined for feeding scars, oviposition, and mines at ten preselected branches (random choice 0–2 m height). In total, 310 transplants and 18 adult plants were investigated. To include variation in size of the 18 *I. aquifolium* populations an ‘apparency’ estimate was calculated for each site (Feeny 1976). This was done by estimating the number of *I. aquifolium* individuals and multiplying this value with the average height of the plants.

**Data Analyses.** Feeding scars: The large proportion of leaf observations without feeding scars made a joint analysis of responses necessary. The analysis contained a binary response, i.e., no feeding (0) versus feeding (1), and a count response on feeding (0). These variables are both modeled with a log-link (cf. Forslund et al. 2011 for methodology). The effects of location type, distance to adult plant, plant type, plant size, and population apparency of *I. aquifolium* (fixed effects) were tested together with the specific location name and plant (random effects) on the feeding response of the leaf miner. A χ² test was performed on the likelihood ratio (LR) statistic (SAS Institute 2004).

**Oviposition Scars.** To determine if there was an effect of location type, distance to adult plant, plant type, plant size, and population apparency (fixed effects) and their interactions on oviposition scars, data were analyzed for responses after the Poisson distribution (SAS Institute 2004). Specific location name and label were included as random effects. LR tests were also performed.

**Mines.** The entire data set was analyzed for mines using the same procedure as for oviposition scars with the same fixed effects. There are several possible outcomes of mines (successful emergence of leaf miner, attack from parasites, bird predation, natural death), and therefore the data only concerning mined leaves
were analyzed separately for the effect of the same fixed effects as mentioned for oviposition scars using the same statistical procedure.

Population Apparenccy. To determine the effect of apparenccy of the *I. aquifolium* on feeding scars, oviposition scars and mines, data were analyzed with SAS JMP 6.0 (JMP). Before analysis the data were log\[arcsine(square root)] transformed to meet the requirements for a parametric analysis.

Results

General Patterns in Leaf Miner Activity. The holly leaf-miner was present in all 18 sites within the historical and expanding range of *I. aquifolium* and mining was observed in 15 sites. The two sites without feeding, oviposition, or mining by *P. ilicis* were within the historical (Natural) and expanding range (EscapeZ), respectively, whereas another EscapeZ site showed only feeding scars. For several sites of both escape-location types, high levels of mining activities equal to the natural populations were found. Thus, some form of leaf miner activity was observed at all location types with no marked difference between historical and expanding range of *I. aquifolium*.

On average, 253 feeding scars per 100 leaves were found at the 18 sites, and 24 oviposition scars per 100 leaves. Although multiple ovipositions per leaf were frequent, most mined leaves developed only one mine (93.8%); in a few cases two (5.6%) or three (0.6%) mines were found. In the cases of two mines, 66% of the leaves had two successful emergences and for the single leaf with three mines, one emergence was successful. In total, 160 mines were found on the 328 *I. aquifolium* studied for mines.

Effect of Location Type. With considerable variation among sites, no significant differences in leaf miner activities related to geographical location were also found. The natural plants from the escape populations in the historical range (Escape1) had the highest levels of both oviposition scars and number of mines per 100 leaves, however, successful emergence of *P. ilicis* was not significantly different among the three location types \(X^2 = 3.10, df = 2, P = 0.21\). The density of feeding scars and mines varied among sites in all three location types with particularly high variation among the EscapeZ sites (11–649 feeding scars per 100 leaves; 0–24 mines per 100 leaves). High variation was also observed for oviposition, but here Escape1 showed the largest variation among sites (2–102 oviposition scars per 100 leaves).

Effect of Small-Scale Distance. The number of oviposition scars was significantly influenced by distance to adult plants \(X^2 = 6.6, df = 1, P = 0.010\). The highest density of oviposition scars was observed on adult plants (0-m distance class). Intermediate oviposition density was found on transplants 1 m from the adult plant and lowest density on transplants with 10 m from the adult plant (Fig. 4a).

The level of parasitism assessed as proportion of mines with parasitoid emergence holes, was also significantly affected by small-scale distance \(X^2 = 10.5, df = 2, P = 0.0054\), and the leaf miner was more often killed by parasites on transplants at 10 m than at 1 m distance from the adult plant (Fig. 4b). Parasitism was similar on the mines of the adult plant and the transplants 1 m away.

Effect of Plant Type. Plant type had an effect on all three leaf miner activities investigated, i.e., feeding, oviposition, and mining. There was a marginally significant effect of plant type and distance classes regarding feeding \(X^2 = 4.5, df = 1, P = 0.033\); Fig. 5). If we remove the interaction, then the parallel likelihood ratio tests for the main effects of distance \(X^2 = 13.9, df = 1, P = 0.0002\) and genotype \(X^2 = 18.0, df = 1, P = 0.00002\). Thus, both main effects are highly significant. The adult plant of *I. aquifolium* had significantly more oviposition scars per leaf than the two transplanted cultivars ‘Blue Angel’ and ‘Madame Briot’ \(X^2 = 11.7, df = 1, P = 0.0006\); Fig. 6a). The same pattern was observed regarding mine density, which was significantly different for the three plant types \(X^2 = 33.0, df = 2, P < 0.0001\); Fig. 6b). When analyzed for all leaves examined \(n = 3,503\), population ap-
transparency had a significant positive effect on the number of feeding scars (linear regression; $R^2 = 0.32$, $P = 0.015$, $N = 18$), oviposition scars ($R^2 = 0.26$, $P = 0.03$) and mines ($R^2 = 0.26$, $P = 0.03$). Moreover, among the 160 mines investigated, the occurrence of natural death of the leaf miner in the leaf was significantly affected by the interaction of plant type and overall plant height ($F_{1,113} = \infty$, $P < 0.0001$).

Discussion

No Evidence for Enemy Release From the Leaf Miner. As the holly leaf-miner ($P. ilicis$) was present and abundant in both the historical and expanding range of $I. aquifolium$ in Denmark, there is no evidence for enemy release from this herbivore. Thus, hypothesis 1 of our study could not be supported. One could speculate that leaf miners arrived later to Zealand, thereby having fewer natural enemies than in their original area. Enemy release is frequently advocated as an explanation for the success of introduced species (Keane and Crawley 2002, Cincotta et al. 2008, Hartley et al. 2010), but studies of this hypothesis as a general rule in plant invasions are equivocal, suggesting its importance in some invasions, whereas other mechanisms like community disturbance are more important in others (Colautti et al. 2004).

Average oviposition density was 21–29 egg scars per 100 leaves for the three location types and this is somewhat similar to the density (around 20 oviposition scars per 100 leaves) for the same latitude extrapolated by Brewer and Gaston (2003). However, Bañuelos and Kollmann (2011) found on average 60 oviposition scars per 100 leaves for Danish populations in a different year. Mine density (average 4.9 mines per 100 leaves) was smaller than the 10–15 mines per 100 leaves calculated by Brewer and Gaston (2003) and the 15.7 mines per 100 leaves calculated by Bañuelos and Kollmann (2011), both for Denmark, perhaps because in both studies they only counted mines on 1-yr-old leaves. An even higher density of 28 mines per 100 leaves was reported from another study in central United Kingdom (Heads and Lawton 1983). Annual and climatic differences can also affect $P. ilicis$ and hence mine densities (Gaston 2003), and as the study shows there can be quite a variation in mine density, with the highest found in the EscapeZ sites (0–24 mines per 100 leaves).

The presence of $P. ilicis$ in naturalized populations could be related to natural dispersal of the insect or to the use of $I. aquifolium$ as an ornamental plant and hence co-introduction of the leaf miner unrelated to the leaf miners’ natural dispersal capacities. Although enemy release has been investigated in relation to range expansion of host species caused by climate change (Menendez et al. 2008), the use of $I. aquifolium$ as an ornamental may thus complicate the observations of natural mechanisms because of increased possibility of co-introductions of pathogens and herbivores, which may very well be the case for the interaction between $I. aquifolium$ and $P. ilicis$. A number of agromyzids are pests of ornamental plants and most of them have been spread inadvertently to locations beyond their original geographical range, coincident with the increase in global trade (Lambkin et al. 2008). Another possible explanation could be that the plant defends itself well against the miner’s eggs.
and larvae, but the low percentage of successful emergence could also be because of natural enemies. **Performance of the Leaf Miner at the Range Margin.** Oviposition scars resulting in mines (successful hatching) varied greatly among sites with an average of 19% successful hatching for oviposition scars in our study. Báñuelos and Kollmann (2011) also found low percentages of successful hatching for both female and male shrubs in the same region. However, this value is lower than the successful hatching of 48% found by Brewer and Gaston (2003), of 50% found by Valladares and Lawton (1991), and of 40% found by Eber (2004). Mortality of eggs and young larvae (indicated by an egg scar without any visible leaf mine), also called midrib mortality, however, was the most important source of mortality, as in other studies (Valladares and Lawton 1991, Brewer and Gaston 2003, Eber 2004). Brewer and Gaston (2003), when studying the whole range of *I. aquifolium* distribution, found the levels of successful emergence of adults from mines to be higher at the edge of the range of the holly leaf-miner distribution. Our observations in Denmark at the limit of the range of expansion with relatively high levels of emergence are consistent with these findings as they are notably higher than the 3.8% successful emergence found in southern United Kingdom (Valladares and Lawton 1991). The general pattern of higher levels of successful emergence in Denmark than in England is likely to be related to the absence of the larval parasitoid *Chrysocharis gemma* from the north-eastern part of the leaf miner range.

**Effect of Short-Scale Distance on Leaf Miner.** Eber (2004) showed that adult *P. ilicis* select specific trees for oviposition and are able to cover several 100 m between host plants. Thus, the higher rates of egg scars on adult plants are most likely not an effect of dispersal limitation but are because of low ‘apparency’ of the small-sized transplants, as feeding, oviposition, and mining were affected by this factor. Also, the values for oviposition density on the transplants are quite similar compared with the focal adult plant at 0 m, and a higher infestation level at the 1 m than 10 m distance would be expected if the leaf miner suffered from dispersal limitations over short distances. The same tendency can be observed for the interaction between plant type and distance regarding feeding scars. These results support hypothesis 2 of the study.

Parasite attack on the leaf miner was positively affected by increasing distance from the focal natural plant. Parasitization is not caused by *C. gemma* that, as previously described, is absent from the northeastern part of the range (Brewer and Gaston 2003), but by other larval and pupal parasitoids. Not knowing the composition of parasitoids, we can speculate that more generalists are active in the eastern populations and more specialists are active in the native holly area (Grabenweger et al. 2010). Pupal parasitism can be a significant source of mortality for leaf miners, and a single species of leaf miner can be associated with a complex parasitoid community, as eight species of pupal parasitoids were found in England (Brewer and Gaston 2003).

The actual number of parasite attacks per leaf was seven times larger on the adult plant compared with the transplants at 10-m distance. This could be an effect of density dependence or affected by a response to host plant size. Parasitoid response to host densities has been shown to be species-specific. For 171 host-parasitoid systems reviewed, 25% of the systems were density-dependent and 23% density-independent (Stiling 1987), so the species in studies like ours must be identified to examine this further.

Short distances of 1–10 m as in our study do not affect the level of mining or successful emergence of mines, indicating that the adult leaf miners were able to disperse from the host plant and to reproduce on the transplants, but did so to a lesser extent than on the adult plant.

**Effect of Plant Type on the Leaf Miner.** The highest oviposition density was observed on the adult (native) plant compared with the two other plant types, supporting hypothesis 3. This could, however, be an effect of size, as the native plant was considerably larger than the transplants. As shown by Valladares and Lawton (1991), oviposition density and number of mines per 100 leaves are strongly correlated, so the results regarding plant type and number of mines are consistent with their findings.

The specialization of *P. ilicis* as monophagous leaf miner might be the reason for higher infestation rates on the natural plant. Selection of individual bushes by leaf miner females was shown by Valladares and Lawton (1991), although the clues used by females remain unknown. Trees susceptible to attack tend consistently to be so, whereas those that are not remain largely free of mines (Brewer and Gaston 2002). Leaf color polymorphism has been shown to affect herbivory of leaf miners on *Byttneria;* here mine density varied with frequencies of green- and white-variegated leaf morphs and was lower on the variegated morph (Hespenheide 1991). This could have affected the values found on the *I. aquifolium* ‘Madame Briot’. Moreover, Eber (2004) showed that leaf morphology traits like leaf spines and flatness of leaves may have an impact on oviposition, and could affect response of *P. ilicis* to the three plant types used in our study.

Although not tested, there are indications of preferences related to number of newly flushed leaves per plant. Báñuelos and Kollmann (2011) found a trend toward more palatable leaves on male versus female plants. As we did not distinguish between male and female plants in our study, this could have affected the results. Eber (2004) found increasing infestation with extended periods of leaf flush and a greater number of leaves per shoot, and our survey also revealed an effect of host plant ‘apparency’ as all three leaf miner activities were affected by this factor. Host apparency related to size and morphological characteristics has been shown to affect searching efficiency of insects (Parmesan 1991), whereas other studies have shown contrasting results (Wiklund 1984, Courtney 1985). It is possible that the significant lower levels of infestations on the transplants are an effect of these factors. A study from Japan found similar densities of leaf
miners on naturalized and cultivated woody plants (Sugiura 2010). Although leaf miners in that study were not monophagous, the lack of any difference between results concerning miner activity on adult cultivated plants is interesting. It is necessary to test different plant types of *I. aquifolium* with similar heights to disentangle any effect of these factors and preferentially for several generations of the leaf miner to see whether or not the statistically significant effects of plant types persist over time to produce a biological relevance.

**Conclusions**

Our study found no evidence of enemy release for the host plant *I. aquifolium* from the leaf miner *P. ilicis* as the plant species has expanded its range. Mine density and successful emergence of leaf miners are not correlated with population size of holly leafminer, which appears to have considerable populations in both the historical and expanding range of *I. aquifolium* with high variation among sites. Regarding leaf miner mortality, there was also no indication that it is related to location type. The study shows the usefulness of transplant experiments to detect effects of biotic interactions on distribution edges and during small-scale dispersal. The transplant experiments should persist for several years to observe any differences with older, i.e., taller, plants. More detailed studies on the chemical constituents of the different plant types could also help to determine if any preferences exist with leaf miner responses.

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