

Leaf variegation in *Caladium steudneriifolium* (Araceae): a case of mimicry?

Ulf Soltau · Stefan Dötterl · Sigrid Liede-Schumann

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Abstract The leaves of *Caladium steudneriifolium* (Araceae) of the understorey of a submontane rainforest in the Podocarpus National Park (South East Ecuador, 1,060 m a.s.l.) are plain green or patterned with whitish variegation. Of the 3,413 individual leaves randomly chosen and examined in April 2003, two-thirds were plain green, whereas one third were variegated (i.e., whitish due to absence of chloroplasts). Leaves of both morphs are frequently attacked by mining moth caterpillars. Our BLAST analysis based on Cytochrome-*c*-Oxidase-subunit-1 sequences suggests that the moth is possibly a member of the Pyraloidea or another microlepidopteran group. It was observed that the variegated leaf zones strongly resemble recent damages caused by mining larvae and therefore may mimic an attack by moth larvae. Infestation was significantly 4–12 times higher for green leaves than for variegated leaves. To test the hypothesis that variegation can be interpreted as mimicry to deter ovipositing moths, we first ruled out the possibility that variegation is a function of canopy density (i.e., that the moths might be attracted or deterred by factors unrelated to the plant). Then plain green leaves were artificially variegated and the number of mining larvae counted after 3 months. The results on infestation rate (7.88% of green leaves, 1.61% of the variegated leaves, 0.41% of white manipulated leaves and 9.12% of uncoloured manipulated leaves) suggest that ovipositing moths are deterred by the miner-infestation mimicry. Thus, variegation might be beneficial for the plants despite the implicated loss of photosynthetically active surface.

Keywords Araceae · Herbivory · Mimicry · Mining moths · Understorey · Variegation

Introduction

Variegated leaves are characteristic of many species of Angiosperms, in particular among understorey herbs in tropical and temperate forests (Givnish 1990). The partial loss of photosynthetically active surface in variegated leaves affects absorption and utilization of

U. Soltau · S. Dötterl · S. Liede-Schumann (✉)
Department of Plant Systematics, University of Bayreuth, 95440 Bayreuth, Germany
e-mail: sigrid.liede@uni-bayreuth.de

light and therefore net photosynthesis, and as a consequence growth and reproduction. The stable co-occurrence of discrete leaf colour forms within plant populations therefore implies that there have to be particular selective pressures that support variegation despite the energetic handicap compared to plain leaves (Smith 1986).

Smith (1986) lists seven potential hypotheses that offer explanations for the occurrence of leaf-colour polymorphisms: (1) the Temporal Heterogeneity Hypothesis posits that different leaf morphs are adaptations to changing light conditions during the seasons; (2) the Spatial Heterogeneity Hypothesis posits that the production of different ecotypes adapted to sites of different canopy openness has an evolutionary advantage; (3) the Leaf Apparency Hypothesis posits that variegated leaves might better evade herbivores than plain leaves do; (4) the Frequency or Density-Dependent Herbivory Hypothesis posits that the morph that is dominant at a given moment experiences the most intense herbivore pressure; (5) the Mimicry Hypothesis posits that variegated morphs mimic herbivory damage; (6) the Aposematic Coloration Hypothesis posits that a distinct leaf colour pattern is linked to a certain chemical defence; and (7) the Neutral Hypothesis posits that leaf colour polymorphism is not in itself adaptive but might persist as a relic trait. So far, only few studies have tested these hypotheses.

In particular, the Mimicry Hypothesis has been insufficiently tested so far, as has the whole question of mimicry in plants as a mechanism to escape herbivory (Augner and Bernays 1998; Lev-Yadun et al. 2002). A summary of known cases and a classification of the different forms of mimicry in general are given in Wiens (1978) and Barret (1987). Batesian mimicry has been documented in a well-known study about egg mimicry in plants to avoid oviposition by Lepidoptera (Benson et al. 1975; Gilbert 1980; Shapiro 1981; Williams and Gilbert 1981). Lev-Yadun (2003) explains variegation in the desert-rosette-plant genus *Silybium* (Asteraceae) mainly as vegetal aposematic warning to mammalian herbivores that the plant is spiny. However, Lev-Yadun (2003) observed that the tunnels of larvae of Agromyid flies closely resemble the white variegation patterns of *Silybium*. This suggests that the variegation of *Silybium* might also serve as mimicry of an already infected leaf to deter female Agromyidae flies from laying eggs, and thus to protect the plant against insect herbivores. The present paper focuses on testing the Mimicry Hypothesis in a species of Araceae in the understorey of a tropical mountain forest.

Leaf variegation is widespread in different genera of Araceae such as *Caladium* Vent., *Dieffenbachia* Schott or *Homalomena* Schott (T. Croat, pers. comm.). Wiens (1978) mentioned that leaf colour patterns of the poisonous *Caladium* and *Dieffenbachia* may have aposematic functions on primarily visually orienting predators. However, he also discussed a possible mimicry of these patterns by non-protected associated plants. In this case the colour patterns are predicted to act as classical Batesian mimicry.

Niemelä and Tuomi (1987) described irregular hollows on leaf blades of Moraceae as potential caterpillar damage mimicry. Egg-laying female herbivores may avoid leaves with visible feeding marks implying that damage-mimicry may reduce oviposition. Lev-Yadun et al. (2004) mentioned that leaf colour polymorphism of many plants enables predators of herbivores to find their prey more successfully on the leaf surface (“the enemy of my enemy is my friend”). Furthermore, Lev-Yadun and Inbar (2002) described three cases of animal mimicry in plants (ant-, aphid- and caterpillar-mimicry) serving as herbivore repellent cues. Overall, there is suggestive evidence in the support of the Mimicry hypothesis, but experimental evidence is still rare.

On the other hand, Smith (1986) discussed that leaf colour polymorphism of tropical *Byttneria aculeata* (Jacq.) Jacq. (Malvaceae-Byttnerioideae) may act as an adaptation to high light habitats (i.e., the Spatial Heterogeneity Hypothesis). Smith (1986), however, also

noticed that both morphs were attacked by an unknown miner, but with the variegated morphs being less susceptible. As in his study the association of the variegated morph with more open habitats was evident, he could not rule out that mining frequency was influenced by abiotic conditions favoured by the miner rather than by the differences in leaf variegation itself. Finally, Lev-Yadun et al. (2002) suggested that different leaf colour patterns might also represent non-adaptive traits that have neither advantageous nor detrimental effects and exist because of developmental or physiological constraints (i.e., the Neutral Hypothesis).

To test the hypothesis that leaf variegation in *C. steudneriifolium* Engl. has a mimicry function, we first established that openness of the forest, as described by Smith (1986), has no influence on the frequency of plain and variegated plants. Then two experiments on *C. steudneriifolium* plants were conducted. In the first experiment, conducted in March 2004, one group consisted of plants with plain green leaves, a second group consisted of plants with variegated leaves and a third group consisted of plants with plain leaves that we had painted with white correction fluid in a pattern mimicking the natural variegation. In the second experiment, conducted in March 2005, we also corrected for potential side effects of the correction fluid by adding a fourth group of plants with plain leaves that we had painted with a correction fluid without any pigments. After 3 months the number of leaves attacked by moth larvae in each group was counted.

Material and methods

Study species and study site

Caladium is a New World Araceae genus of the tribe Caladieae Schott. In the past, the tribe was assigned to Araceae-Colocasioideae (Bogner and Nicolson 1991; Grayum 1990), but Mayo et al. (1997) have recently moved the genus into the subfamily Aroideae. Madison (1981) recognized only seven species in *Caladium*, but his circumscription especially of *C. bicolor* (Aiton) Vent. is extremely broad, including even *C. steudneriifolium*. Later (Croat 1994; Mayo et al. 1997), *C. steudneriifolium* has been considered as a species independent from *C. bicolor*, and the total number of species in the genus is now given as 12–17. Ressler (1999: http://facultystaff.vwc.edu/~presslar/greenhouse/caladium/Genus_C.htm) presents an overview of the genus and Croat (1988) describes its habitats. Lately, Maia and Schindwein (2006) have shown that *C. bicolor* from Brazil engages in a specialized plant-pollinator relationship with the Cyclocephalini beetle *Cyclocephala rustica* (Olivier).

Caladium steudneriifolium is a common member of Araceae of the east Andean rainforests occurring in the eastern and western subandine provinces in Colombia, Ecuador and Peru from 40 to 1,524 m a.s.l. It is well adapted to wet areas and disturbance, and is frequently found near creeks at the edge of the forest in partial shade and along waysides. It is a small understorey herb (30–50 cm height) with 1–4 erect, peltate and plain (Fig. 1a) or, frequently, variegated leaves (Fig. 1c), and a subglobose, subterranean tuber. Plants with plain leaves and those with variegated leaves usually occur in the same population. The variegation is caused by irregularly shaped patches of cells without chlorophyll occurring in some distance to the midrib (own obs.).

The study was carried out in Bombuscaro, the east entrance of the Podocarpus National Park, Province of Zamorra-Chinchipe, South East Ecuador (4°4.5' S, 78°58.5' W), in a tropical submontane rainforest (1,060 m about sea level).

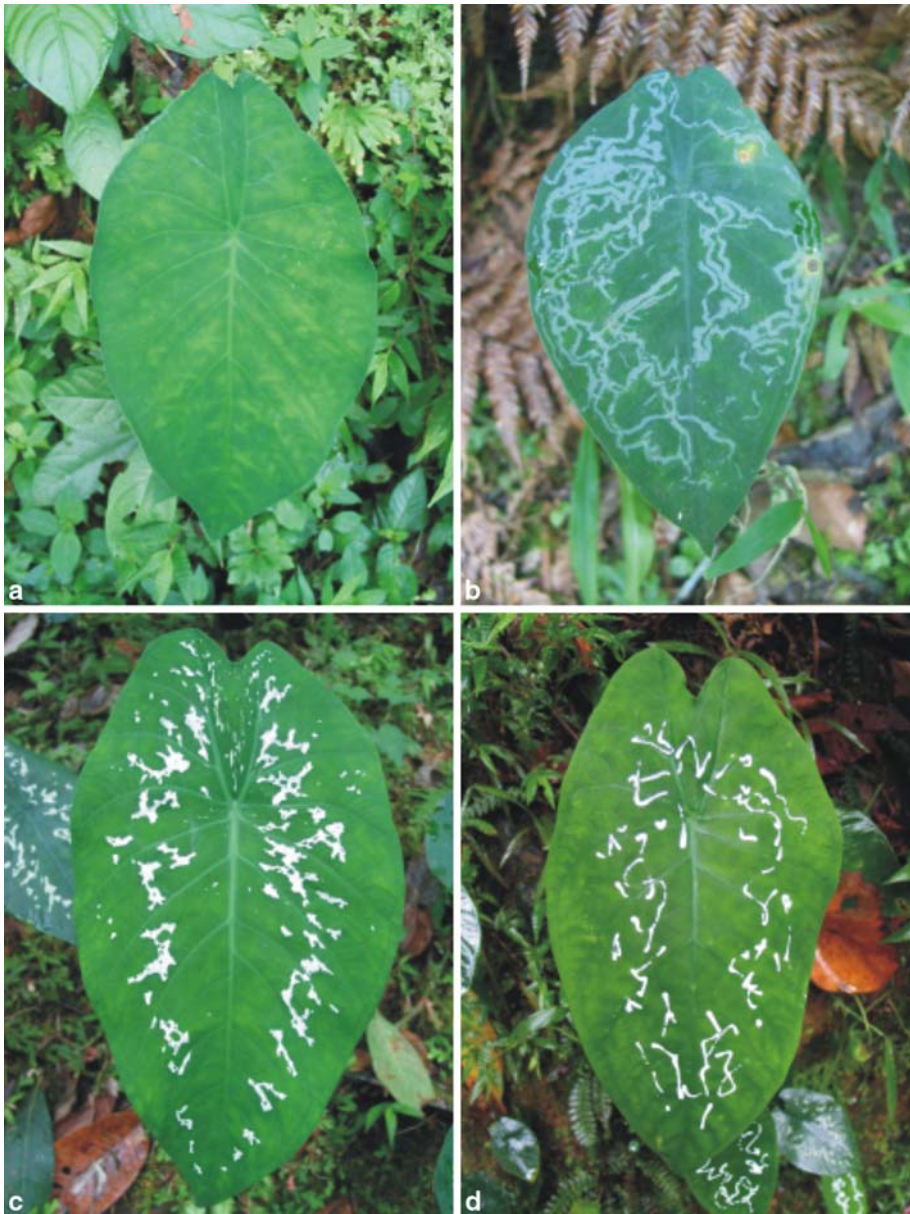


Fig. 1 Leaves of *Caladium steudnerifolium*. (a) Plain leaf. (b) Plain leaf with infestation of leaf-mining moth larvae. (c) Variegated leaf. (d) Plain leaf painted with white correction fluid in a pattern mimicking the natural variegation

Measurements and analysis

On April 2, 2003 three transects were established, two (46 m × 2 m, 44 m × 2 m) along the pathway between the parking area and the entrance building of Bombuscaro and one (55 m × 2 m) behind the entrance building parallel to the river (Río Bombuscaro). One

leaf each of 3,413 individuals of *C. steudneriifolium* was randomly chosen to calculate the ratio of plain and variegated leaves within the population and the ratio of leaf-mining-moth attacks respectively in both morphs.

In April 2005, the forest overstorey density was measured every 3 m along a new transect (246 m × 2 m, established along the pathway between the parking area and the entrance building) using a Spherical Densimeter (Model-C; Robert E. Lemmon, FOREST DENSIOMETERS, 5733 SE Cornell Dr., Bartlesville, OK 74006, (918) 333-2830). The number of green and variegated leaves (totalling 3,956 leaves) was also counted in these 3 m subtransects to verify whether canopy density influences the occurrence of one or the other colour morph. These data were analysed with Pearson's correlation test.

Both colour morphs were frequently attacked by larvae of an unknown moth species. It was observed that the variegated leaf zones strongly resemble recent damages caused by mining larvae, and therefore may mimic a moth attack (compare Fig. 1b and c). To test whether the two different leaf colour patterns have any influence on the abundance of mining moth attacks, two experiments were established in the same transect of 246 m × 2 m.

Experiment 1 was started March 22, 2004. Three groups of 200 leaves each of *C. steudneriifolium* were established randomly along the transect. Group one was represented by plants with plain green leaves (Fig. 1a), group two with variegated leaves (Fig. 1c) and group three with plain green leaves that were painted with white correction fluid (Tipp-Ex[®]) in a pattern mimicking the natural variegation (Fig. 1d). Only young leaves were used in order to minimize potential previous oviposition by moths. Young leaves are easy to recognize by their bright green colour and their water-repellent (hydrophobic) surface. After 3 months (June 18, 2004), the number of leaves attacked by moth larvae in each group was counted. However, only 381 of the 600 leaves could be relocated in the field.

To test whether not the colour but the chemistry or texture of the Tipp-Ex[®] fluid had an influence on moth behaviour, we repeated the experiment in 2005 (experiment 2, starting March 15) and added a fourth treatment group with 200 leaves of *C. steudneriifolium* that were painted at the upper and lower surfaces of the plain green leaves with uncoloured correction fluid (Tipp-Ex[®] thinner). After 3 months (June 15, 2005), the number of leaves attacked by moth larvae in each group was counted again, with 660 of the 800 marked leaves relocated in the field.

To test for differences in the mining rates between plain and variegated leaves, a χ^2 -test was calculated in STATISTICA (Statsoft Inc. 2005). Logistic regression was used to test whether the categorical factors "variegation" (yes or no) and "manipulation" (yes or no) influenced the infestation rate. The logistic regression alone was calculated for experiment 2, where all treatment combinations were available.

Attempts were made to identify the mining moth. Larvae (Fig. 2) were collected and stored in alcohol. Because identification by morphological characters failed, an identification via isolated DNA was attempted. DNA was extracted from three larvae with the DNeasy Tissue Kit (Qiagen) following the protocol of the manufacturer. Cytochrome *c* Oxidase subunit 1 (COX1) sequences from the mitochondrial genome were obtained using the primers TY-J-1460 and C1-N-2191 (Simon et al. 1994) and sequencing the products on an ABI310 capillary sequencer. The resulting consensus sequences were subjected to several BLAST (Altschul et al. 1990) searches. Furthermore, ten attacked plants were separated and cultivated in cages to capture the adult moths, but all larvae died and none reached the pupal or adult stage.

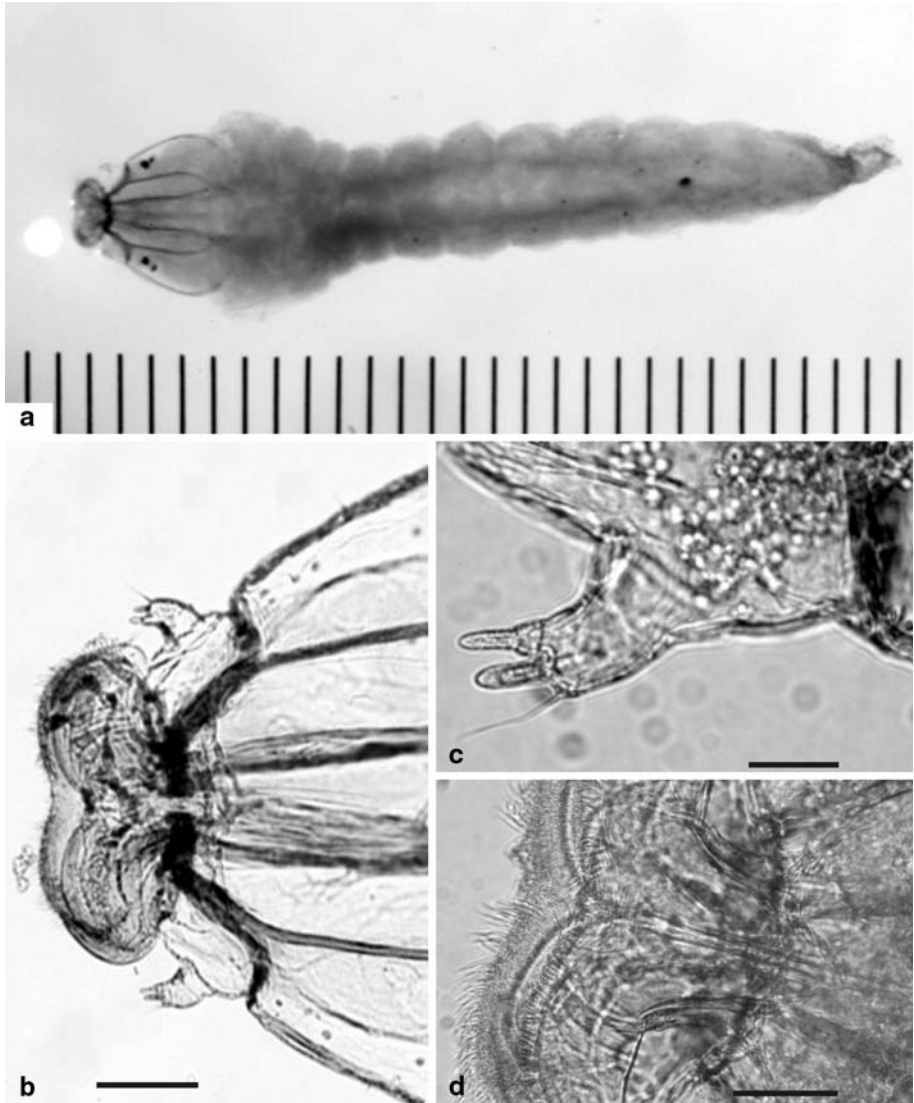


Fig. 2 The mining moth. (a) whole animal, (a, d) head, (c) leg of first segment (graduation marks in (a) represent $100\mu\text{m}$, bars represent $200\mu\text{m}$ in (b), $50\mu\text{m}$ in (a) and $120\mu\text{m}$ in (d))

Results

Identity of the miner

The 10 observed larvae were about 2.7–3.2 mm long (Fig. 2). Observations in the field and further studies under the microscope showed that they were not feeding in the mesophyll but in the upper epidermis of the leaves. In the field, no imagoes could be found. Identification of the larvae from morphological characteristics also failed. The COX1 sequence (AM940020) that we analysed was 642 bp long. However, it did not yield an exact

determination either, most likely due to the lack of sequences from closely related species. A mega-BLAST search yielded a closest match of 86% similarity with several *Dioryctria* (Pyraloidea–Pyralidae–Phycitinae), *Glyphodes* (Pyraloidea–Crambidae–Pyraustinae) and *Lycaena* (Lycaenidae) species as well as one *Rhodinia* (Saturniidae) species. *Glyphodes* occurs in Ecuador, but is not a miner. *Dioryctria* are miners in conifers, but so far not known in Ecuador (K. Fiedler, Vienna, pers. comm.). Lycaenidae are not known as miners. The COX1 sequence therefore only confirms that the larvae belong to a Lepidoptera and that its closest relatives should be in the Pyraloidea or a closely related microlepidoptera group.

Infestation of *Caladium steudneriifolium* leaves

The initial count of 3,143 randomly chosen leaves of different individuals in the transects in April 2003 revealed that 64% of the plants had plain leaves (2,189) and 36% (1,224) variegated leaves. The rate of infestation by mining moths in this first count differed significantly between plain and variegated leaves ($\chi^2_{df=1} = 9.3$; $P = 0.002$). It was 0.96% (21 leaves) in plain leaves and 0.08% (one leaf) in variegated leaves, and was therefore 12 times higher in plain than in variegated leaves.

Canopy density (percentage cover) of most subtransects varied between 85% and 95%, and there was no correlation between canopy density and the relative frequency of the plain morph ($r = -0.09$, $P = 0.43$, Fig. 3). Therefore, there was little variation in overstorey density along the transect, and this indicates that this variation was not responsible for the observed differences in mining rates between the two plant morphs.

The results of experiments 1 and 2 are presented in Table 1. In experiment 1 only uniformly green leaves were infested, whereas in experiment 2 mining moths were found in each treatment. In experiment 2, the mining rates differed between variegated and non-variegated leaves, and no effect of the manipulation treatment was found. Further, there was no significant effect of the variegation-by-manipulation interaction, indicating that the magnitude of the mining rate was not dependent on whether the variegation was natural or made by white Tipp-Ex[®] and that the chemistry of Tipp-Ex[®] did not influence the behaviour of moths (see Table 2). These results indicate that the mining rate in variegated leaves was less compared to uniformly green leaves, and that this is due to visual cues.

Fig. 3 Distribution of morphs along a light gradient (given as percentage cover) in the field

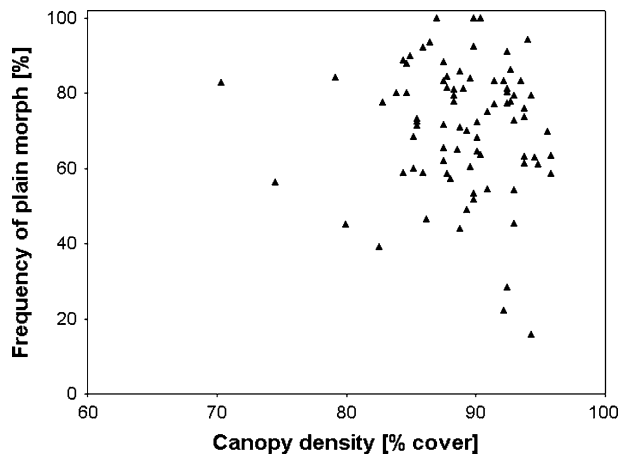


Table 1 Number of uninfested and infested leaves in the different experimental groups

	Uniformly green leaves	Variegated leaves	White manipulated leaves	Uncoloured manipulated leaves
Experiment 1				
March 22–June 18, 2004	7/126 (5.6%)	0/114 (0.0%)	0/141 (0.0%)	–
Experiment 2				
March 15–June 15, 2005	18/164 (11.0%)	5/174 (2.9%)	11/169 (6.5%)	14/153 (9.1%)

The figures indicate infested leaves / total leaves (percentage of infested leaves)

Table 2 Results of the logistic regression analysis calculated on the basis of experiment 2

	d.f.	Wald statistics	<i>P</i>
Intercept	1	251.7258	0.000000
Variegation	1	7.2665	0.007025
Manipulation	1	0.9617	0.326747
Variegation × manipulation	1	2.5199	0.112415

Discussion

The observation that the whitish areas of variegated leaves strongly resemble the leaf damages caused by the larvae of mining moths suggested that the colour patterns of the variegated leaves mimic these damages to escape oviposition by adult female moths. Among other chemical, tactile or visual cues, insects can visually detect and assess previous infestation during the process of host plant selection (Benson et al. 1975; Gilbert 1980; Lev-Yadun and Inbar 2002; Lev-Yadun 2003; Prokopy and Owens 1983; Shapiro 1981; Smith 1986; Williams and Gilbert 1981).

However, Smith (1986) has shown that the frequency of colour morphs also may be correlated with light environment. The frequency of variegated leaves in *Bytneria aculeata* (Jacq.) Jacq. is much higher in open sites than under a dense canopy. However, in the present case of *C. steudneriifolium*, the canopy density data acquired by using a Spherical Densimeter did not correlate with the frequency of variegated and plain leaves (Fig. 3). Therefore, differences in infestation rate between plain and variegated leaves of *C. steudneriifolium* cannot be explained by canopy-openness preferences of ovipositing moths.

The first count and the first experiment show that the rate of infestation by mining moths is 4–12 times higher in plain leaves than in variegated leaves. These results implicate that the occurrence of variegated leaves within the population of *C. steudneriifolium* is associated with the presence of mining moth attacks. Plain leaves that were painted with white colour in a pattern mimicking natural variegation had a similar low rate of moth attacks as leaves with natural variegation. Thus, the hypothesis that leaf variegation in *C. steudneriifolium* reduces the likelihood of attacks by leaf-mining moths is supported by our experiments and can be interpreted as mimicry.

To exclude misleading effects caused by the chemistry of texture of the white correction fluid, which may irritate female mining moths and deter them from oviposition, an

additional experiment was started with the same correction fluid without any pigments. However, there was no significant difference in infestation rates between untreated plain leaves and plain leaves partially painted with uncoloured correction fluid. Therefore, the reduced infestation rate of the Tipp-Ex[®] painted leaves has to be attributed to the white pigment and not to the chemistry of the fluid.

Plants with variegated leaves have less photosynthetically active leaf area than plants with plain leaves. The persistence of the presumed handicap of variegated plants is only comprehensible by considering the consequences of a mining moth attack. Infested leaves were observed to have a much shorter life span than not infested ones; infested leaves which were found in June after the first check in experiment 2 did not survive the next 2 months. These leaves were attacked by fungi using the epidermal damages caused by the moth infestation for successful attack (own obs.). Our study therefore shows in the presence of herbivores, leaf variegation can be of high selective advantage despite the loss of photosynthetically active leaf area compared to plain leaves. This can explain the stable coexistence of variegated and plain morphs.

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