



4-20-2011

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Recommended Citation

Morton, Martin L. and Pereyra, Maria E. (2011) "Mining patterns of the aspen leaf miner, *Phyllocnistis populiella*, on its host plant, *Populus tremuloides*," *Western North American Naturalist*: Vol. 71 : No. 1 , Article 5.

Available at: <https://scholarsarchive.byu.edu/wnan/vol71/iss1/5>

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MINING PATTERNS OF THE ASPEN LEAF MINER, *PHYLLOCNISTIS POPULIELLA*, ON ITS HOST PLANT, *POPULUS TREMULOIDES*

Martin L. Morton^{1,2} and Maria E. Pereyra¹

ABSTRACT.—Feeding behavior of aspen leaf miner (*Phyllocnistis populiella*) larvae on quaking aspen (*Populus tremuloides*) leaves was examined, by using image-analysis software, to determine the pattern and distance of the larvae's mining trails and measure the surface area of infested leaves and consumed leaf tissue. Mining pathways between hatching and pupation sites were serpentine and generally followed predictable, sequential changes in orientation. This pattern was most obvious in smaller leaves, those with a surface area of about 700 mm² or less. In leaves larger than 700 mm², plasticity in mining behavior increased. The serpentine pattern was initiated but became truncated when larvae reached full growth without having to consume all available leaf material. The final phase of the behavioral program was still executed, however, and larvae entered pupation at the usual leaf-edge location. We hypothesize that the serpentine pattern is genetically controlled and that it has been selected for because it promotes efficient consumption of a fixed food supply and ensures a favorable location for pupation, especially when reproductive females oviposit on small leaves.

RESUMEN.—Se examinó el comportamiento alimenticio de la larva del minero de hoja de álamo (*Phyllocnistis populiella*) en las hojas del álamo temblante (*Populus tremuloides*) utilizando software de análisis de imágenes para determinar el patrón y la longitud del rastro de las larvas y medir la superficie de las hojas infestadas y del tejido de hoja consumido. Los rastros del minero entre los sitios de eclosión y los de pupación fueron serpentinos, y en general sus cambios de orientación fueron predecibles y secuenciales. Esto fue más obvio en las hojas pequeñas, aquellas con una superficie de menos de 700 mm². En las hojas con una superficie mayor de 700 mm² aumentó la plasticidad en el comportamiento minero. El patrón serpentino apareció al inicio pero se truncó cuando las larvas alcanzaron su pleno desarrollo sin tener que consumir todo el material herbáceo disponible. Sin embargo, la última fase del patrón conductual aún se llevó a cabo, y comenzaron la pupación en el borde de la hoja como de costumbre. Planteamos la hipótesis de que el patrón serpentino tiene origen genético y que ha sido seleccionado a favor porque facilita el consumo eficiente de un suministro fijo de alimento y garantiza un lugar favorable para la pupación, especialmente cuando las hembras reproductoras ovipositan en hojas pequeñas.

Leaf-mining insects comprise a large, diverse group that is distributed throughout both tropical and temperate areas. Most species spend a major part of their life as larvae, feeding and developing within the confines of a mine. These mines are highly visible, even after the larvae depart, and provide a complete and quantifiable record of feeding behavior with known ecological context (Boomsma et al. 1987, Papaj and Prokopy 1989, Hespeneheide 1991). Thousands of leaf-mining species occur within the paraphyletic assemblage of moth families known as the microlepidoptera; studies of this group have made important contributions to the understanding of community organization and coexistence in folivorous insects (Boomsma et al. 1987, Doak et al. 2007). Some leaf-mining microlepidoptera lend themselves to studies of behavioral and phenotypic plasticity because their developing larvae exhibit marked changes in feeding mode (Gaston et al. 1991, Auerbach and Alberts 1992).

Endophytic herbivores tend to have narrow host preferences and many mining species are oligophagous or even monophagous (Hespeneheide 1991), suggesting that mining behavior has been optimized (Egan and Funk 2006). An example is the aspen leaf miner (*Phyllocnistis populiella*), a microlepidopteran that specializes on quaking aspen (*Populus tremuloides*) and produces mines with a characteristic weaving serpentine pattern. Unlike most other leaf miners, elements of larval morphology, developmental stages, and life history have been reported for *P. populiella* (Condrashoff 1962, 1964). Recently, investigations have focused on the effects of *P. populiella* herbivory on the physiology and growth of *P. tremuloides* (Doak et al. 2007, Wagner et al. 2008). To our knowledge, however, neither consumed leaf area nor functional significance of mining pathways, including their orientation upon the leaf, have been reported in detail. Herein, we quantify the larval feeding pattern of *P. populiella* as revealed from analysis

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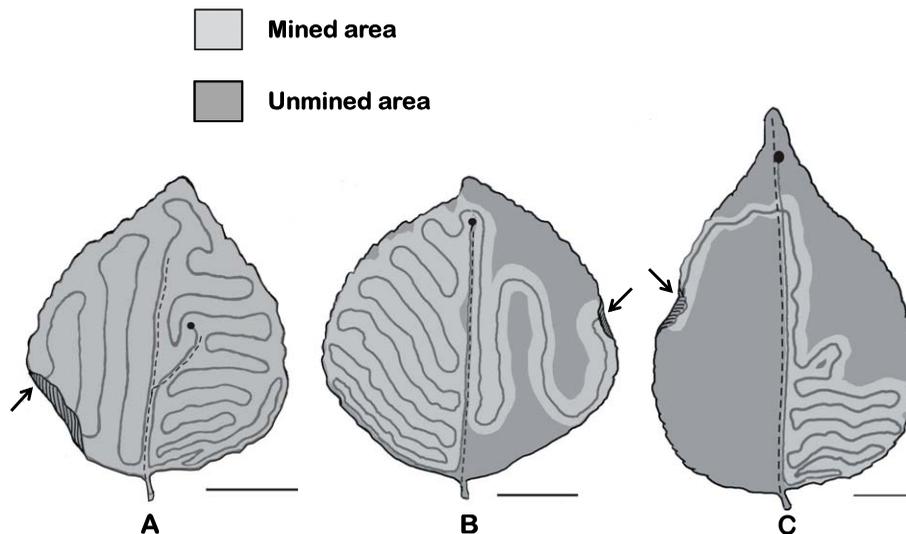


Fig. 1. Mining pattern of *Phyllocnistis populiella* larvae in 3 differently sized *Populus tremuloides* leaves. Oviposition sites are marked with a black dot and pupation sites by an arrow. Dashed lines show locations of midveins, and one lateral vein in leaf A. Scale bars beneath each leaf indicate 1 cm. Surface area and percentage mined: leaf A, 582 mm² and 100%; leaf B, 1019 mm² and 71%; leaf C, 1915 mm² and 31%.

of mines of specimens that had reached pupation. The wide range of leaf sizes chosen by ovipositing females within our sample, permitted us to examine relationships between leaf area and larval mining behavior and to hypothesize about the importance and ultimate causation of the serpentine pattern.

METHODS

The study area was at 1000–1500 m altitude on the east-facing slope of Steamboat Mountain (Zone 8N, 6626400 N, 585186 E, NAD 27) about 25 km north of Atlin, British Columbia. This slope burned in August 1986, and aspen ramets, now about 2–4 m in height, are being utilized for reproduction by *P. populiella*. During the summers of 2005–2007, while conducting studies of avian reproduction in the Steamboat burn, we sometimes paused to collect 5–10 mined aspen leaves that held pupae. These were placed in a plastic bag and transferred to a plant press upon our return to camp. Later, in the laboratory, dried leaves ($n = 217$) were digitized, and their features measured with the image analysis program SPOT Advanced Software, version 4.6 (Diagnostic Instruments, Inc.; www.spotimaging.com). Within our sample, 143 leaves had been mined on the ventral side (stomata present) and 74 on the dorsal side. Data

from the 2 sides were combined because they did not differ significantly in any of our analyses ($P > 0.05$).

RESULTS

The Basic Mining Pattern

The most compact and complete version of the mining pattern, including its sequence of orientations was as follows. Upon hatching, usually near the leaf apex, a typical *P. populiella* larva began feeding on the epidermal cells located just beneath the cuticle. The larva swung its head from side to side as it moved forward and grew, leaving behind a slightly widening mine or trail. The mine was easily observed because the transparent cuticle was lifted up and became grayish or silvery in appearance against the green of the leaf and because a brownish trail of excreta (frass) was deposited within it after the first instar (Condrashoff 1964). Initially, the 1-mm-long larva mined to the midvein, then traveled alongside it all the way to the base of the leaf. It then turned and began to travel along the leaf margin, sometimes as far as half-way up the leaf or even more before turning back on a parallel route toward the midvein. A serpentine, back-and-forth pathway, roughly at right angles to the midvein (the horizontal pattern), was then established. Eventually, the larva

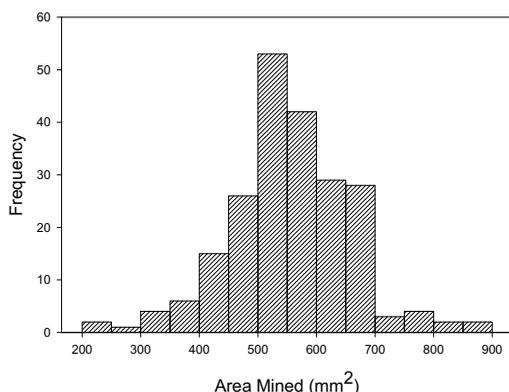


Fig. 2. Frequency distribution of leaf area mined by *Phyllocnistis populiella* larvae that had reached pupation ($n = 217$).

crossed the midvein to the other half of the leaf, usually near the apex, where it turned and again followed the midvein to the leaf base. Mining continued on parallel pathways, but these were now aligned with the long axis of the leaf (the vertical pattern). This behavior was followed until the larva, now grown to about 5 mm in length, mined to the edge, usually near the widest part of the leaf, where it rolled over a portion of the edge and pupated beneath it (Fig. 1).

Effects of Leaf Size

Total surface area (one side of the leaf only) of leaves with pupae varied between 205 mm² and 3698 mm², an 18.0-fold difference ($\bar{x} = 935.8$, $SD = 479.2$). The actual area mined on those same leaves varied between 205 mm² and 880 mm² (Fig. 2), a 4.3-fold difference ($\bar{x} = 556.4$, $SD = 103.5$). This decrease in ratio occurred because smaller leaves, those with surface areas less than about 700 mm², were often mined completely, but larger leaves were not. The percentage of leaf area mined decreased steadily from 100% down to about 20% as total leaf area increased (Fig. 3; linear regression, log of percentage mined as a function of total leaf area: $\beta = -0.915$, $t = -33.2$, $P < 0.001$). Mine length, the distance traveled between hatching and pupation sites, varied between 164 mm and 429 mm and was correlated with mined leaf area ($r^2 = 0.662$, $P < 0.001$).

Variation in leaf size affected mining pattern as well as percentage of leaf consumed. This change in behavior is illustrated with 2 infested leaves that were both larger than the one in

Figure 1A. In the first leaf, with a surface area of 1019 mm², the larva terminated its vertical pattern and entered pupation after mining 723 mm² or 71% of the total leaf area (Fig. 1B). In an even larger leaf, one with a surface area of 1915 mm², the larva terminated serpentine mining while still in the horizontal pattern, then mined along the midvein toward the apex before traveling across to enter pupation. In this case, 591 mm² or 31% of the total leaf area was mined (Fig. 1C). Larvae sometimes produced horizontal mines that were at a shallow angle to the midvein. Although these pathways were also effective in providing access to leaf area, they tended to lie parallel to lateral veins, as in Figure 1B, rather than across them, as in Figures 1A and 1C.

Supplementary Observations

From examinations of many hundreds of infested, uncollected leaves, we noted numerous variations in mining behavior. The underlying basic mining pattern was detectable, but each trail was unique. For instance, larvae sometimes mined aimlessly around the hatching spot for several millimeters before becoming oriented and heading for the midvein. While traveling alongside the midvein toward the base, they frequently mined out along one side of a lateral vein, then crossed to its other side before mining back to the midvein and continuing on with the usual path. Newly hatched larvae were also observed mining alongside the midvein, but toward the leaf apex instead of the base. When this happened, and upon becoming oriented, they would not turn back within their own mine (this was true for larvae of all ages); rather they crossed the midvein, then turned toward the base. This suggests that the midvein serves as a reference point for hatchlings and not as a barrier.

Females sometimes oviposited well away from the apex and out toward the leaf margin. Larvae hatched from those eggs typically encountered a lateral vein, mined along it to the midvein, then mined the rest of the way to the base along the midvein (Fig. 1A). When females laid more than one egg on the same leaf, larval responses varied. If oviposition occurred on opposite sides of the leaf, larvae mined as though unaware of the other's presence. This was not the case when both were on the same side of the leaf, but from eggs on opposite sides of the midvein. In this rare situation they did not cross the midvein, mined only on their half

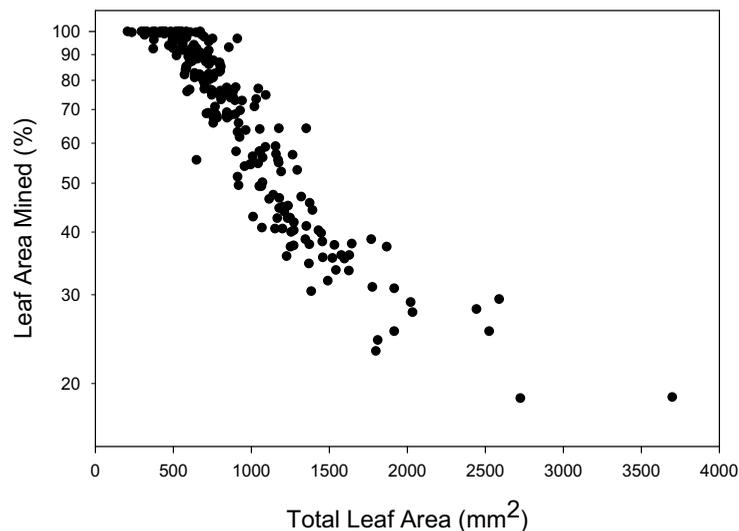


Fig. 3. Log of percentage leaf area mined by *Phyllocnistis populiella* larvae against total leaf area available. Data are for larvae that had reached pupation ($n = 217$).

of the leaf, and pupated there if they survived that long. The data reported in Figs. 1–3 are from leaves that were mined by one larva.

DISCUSSION

Female *P. populiella* oviposit on aspen leaves from time of opening until they are about two-thirds grown (Condrashoff 1964). Eggs are usually cemented close to the midvein at leaf tip, most often one egg per leaf, and on either the upper or lower surface (Henson 1959, Condrashoff 1964). From this starting position on the unfurling leaf, newly hatched larvae begin a regime of epidermal mining that should provide sufficient nutrients for completing growth and pupation. Also, in the interest of time and efficiency, larvae should not mine over their own trails or dead-end at the leaf edge unless they are fully developed. The supply of nutrients is finite, being limited to the epidermis on one side of the natal leaf, a layer that is only one cell thick (Condrashoff 1964). Larvae cannot exit or reenter the leaf or tunnel deeper into the mesophyll because of limitations imposed by their prognathus mouthparts (Wagner et al. 2008). Thus, a certain precision in mining behavior, one that maximizes consumption of all available epidermis if necessary, is to be expected.

In our study area, infested leaves varied 18-fold in surface area when fully expanded, and

adjusting to this variation in size and available food posed a behavioral challenge to larvae. For example, in the smallest leaves, usually from the upper part of the canopy, it was necessary for the larvae to mine all or nearly all of the leaf surface (Figs. 1A, 3). In the largest leaves, usually from the lower canopy, only a fraction of the available nutrients were utilized (Figs. 1B, 1C, 3). Although the mean of leaf area consumed was 556 mm², some larvae, those from the smallest leaves, were able to reach pupation on less than half of that (Fig. 2). This raises some unanswered questions that might be pursued. For example, did pupating larvae differ in size or sex in relation to leaf size? Did nutritional quality of leaves vary inversely with leaf size, especially since there was a 4-fold difference in area mined (Fig. 2)? Relevant to the latter question, leaves from the upper canopy of *P. tremuloides* appear to have a greater concentration of nutrients than those from the lower canopy (Bartos and Johnston 1978, Mandre et al. 1998).

Insect behavior is generally described as being stereotyped and under genetic control (Hammer and Menzel 1995, Wada and Kanzaki 2005). There is evidence, however, of plasticity in behavioral patterns due to responses to plant chemistry (Renwick 2001, Vickerman and De Boer 2002, Haribal et al. 2006) and feedback from various types of peripheral receptors (Bässler and Büchges 1998, Yang et al. 2008). Mining

behavior of *P. populiella* larvae has repetitive, stereotypical components, suggesting guidance by a genetically-based program. While responding to this program, larvae seem to use the midvein, leaf margins, and edges of their own mines for guidance. They also appear to incorporate other cues, such as those derived from leaf chemistry. But why does mining proceed in a serpentine pattern, the defining trait of this species, and why does the pattern generally unfold in horizontal fashion from leaf base to apex, then switch immediately by 45° to the vertical when the midvein is crossed? We hypothesize that this behavioral sequence has been favored by natural selection because it maximizes larval success when females oviposit on small leaves, ones that are 700 mm² or less in area. In those situations, larvae are able to consume all available food without searching or backtracking and also end at a site favorable for pupation (Fig. 1A). In larger leaves, where more food was available than needed, the same fundamental pattern was exhibited, although portions were deleted prior to pupation (Figs. 1B, 1C). In contrast, selective pressure on feeding behavior may be more relaxed on other microlepidopterans that specialize on *P. tremuloides*. The aspen blotch leafminer (*Phyllonorycter tremuloidiella*), for example, likely obtains more nutrition per unit area of leaf than *P. populiella* because it feeds on both mesophyll and palisade layers rather than on the epidermis only (Kopper and Lindroth 2003). The aspen blotch leafminer appears to mine aimlessly in one general location (the blotch) and does not consume more than about 200 mm² of leaf area before pupating (Auerbach and Alberts 1992).

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Received 26 April 2010
Accepted 2 December 2010